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# Zoonotic nematode parasites infecting selected edible fish in New South Wales, Australia

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ABSTRACT

Despite increases in the annual consumption of seafood in Australia, studies on the occurrence and prevalence of zoonotic parasites in fish and the risk they may pose to human health are limited. The present study was aimed at determining the occurrence of zoonotic nematodes in commonly consumed fish in New South Wales, Australia's most populous state. Three species of fish, including the Australian pilchard, Australian anchovy, and eastern school whiting, were purchased from a fish market and examined for the presence of nematode parasites. All Australian pilchards examined in this study were infected (100%; n = 19), followed by the eastern school whiting (70%; n = 20) and Australian anchovy (56%; n = 70). Nematodes were in the larval stage and, therefore, classified by morphotype, followed by specific identification through sequencing of their internal transcribed spacer (ITS) regions. Seven different larval types with zoonotic potential, belonging to the families Anisakidae (*Contracaecum* type II and *Terranova* type II) and Raphidascarididae (*Hysterothylacium* types IV [genotypes A and B], VIII, XIV and a novel *Hysterothylacium* larval type, herein assigned as type XVIII), were found. The new larval type was identified as *Hysterothylacium thalassini*, based on ITS sequence data. The presence of the infective stage of a range of zoonotic parasites in fish commonly consumed in New South Wales is important, particularly as, in some dishes, these fish are used whole, raw or undercooked. This study provides the basis for future research on other aspects of these parasites, in regards to public health.

## 1. Introduction

Seafood consumption has increased globally, due to its health benefits. In Australia, seafood has now surpassed the consumption of sheep and lamb protein in popularity (Savage and Hobsbawn, 2015). In particular, the consumption of raw or undercooked seafood has become more common and, as a result, seafood-borne parasites are of public health concern (Sumner et al., 2015).

The Australian pilchard, Australian anchovy and Eastern school whiting are among popularly consumed table fish that are rich in omega 3 and 6 fatty acids and readily available for purchase all year round in New South Wales (NSW). These fish may be prepared in a variety of ways, including raw, marinated, smoked, steamed, poached, pan-fried and grilled. If the fish is infected with parasites and prepared raw or undercooked, zoonotic infection may occur after consumption (Shamsi and Sheorey, 2018). Infection with parasites in these fish is important for other reasons, too. For example, being pelagic fish and on the lower levels of the food chain, they play an important role in transferring parasites and other pathogens to their predators, such as tuna and mackerel, which are also popular edible fish. In Australia, our knowledge about parasites of these important fish, particularly in NSW, the most populous state of the country, is poor (Table 1). Therefore, the aim of the present study is to determine the occurrence of nematode larvae in these fish in NSW.

## 2. Materials and methods

### 2.1. Fish collection

Three fish species, including Australian pilchard (*Sardinops sagax*; n = 19), Australian anchovy (*Engraulis australis*; n = 70) and Eastern school whiting (*Sillago flindersi*; n = 20), were purchased from a fish market in Sydney, New South Wales, Australia. All fish were purchased on the same day, in August 2017, and were transferred to the Parasitology Laboratory of the School of Animal and Veterinary Sciences, Charles Sturt University, in Wagga Wagga, about 500 km away on the same day, in ice and an insulated box. They were all caught within a 50 km radius of Sydney. All fish were then examined within the next 5 days. The common name of fish examined in this study is in accordance with Gommon et al. (2008).

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**Table 1**
Previous reports of nematodes from Australian pilchard, Australian anchovy and Eastern school whiting in Australia.

| Common name of the host | Scientific name of the host | Nematode larvae | Localities | Reference |
|---|---|---|---|---|
| Australian anchovy | *Engraulis australis* | *Hysterothylacium* larval type VIII | Heron Island, Queensland; Victoria; and Greenly Is, South Australia | Shamsi et al. (2013) |
| | | *Terranova* larval type II | Queensland; NSW; Victoria; South Australia; and Western Australia | Shamsi and Suthar (2016b) |
| Australian pilchard | *Sardinops sagax* | *Hysterothylacium* larval type VIII | Melbourne and Lakes Entrance, Victoria | Shamsi et al. (2011a) |
| | | *Terranova* larval type II | Queensland; NSW; Victoria; South Australia; and Western Australia | Shamsi and Suthar (2016a) |
| Eastern school whiting | *Sillago flindersi* | *Hysterothylacium* larval type IV genotype B | Port Phillip Bay, Victoria | Shamsi et al. (2013) |
| | | *Hysterothylacium* larval type VIII | Heron Island, Queensland; Victoria; and Greenly Is, South Australia | Shamsi et al. (2013) |
| | | Anisakids seven genotypes (A-G) | Bass Strait, Melbourne, Victoria | Jabbar et al. (2012) |
| | | *Anisakis pegreffii* | | |
| | | *Hysterothylacium* larval type IV | | |
| | | *Hysterothylacium* larval type VIII | | |
| | | *Terranova* larval type II | Queensland; NSW; Victoria; South Australia; and Western Australia | Shamsi and Suthar (2016b) |
| | | *Anisakis* larval type | Fish market, Sydney, NSW | Shamsi and Suthar (2016a) |
| | | *Hysterothylacium* larval types | | |

### 2.2. Parasite collection

Fish were dissected and examined for the presence of nematodes, in accordance with Shamsi and Suthar (2016a). In brief, the surfaces of all inner organs were thoroughly inspected for the presence of nematodes. The alimentary canal, from mouth to anus, was then examined under a dissecting microscope (Leica EZ4 Stereo Microscope; 10 times magnification) for the presence of parasites and this was followed by overnight incubation of internal organs at room temperature, to allow deeply embedded and encysted larvae to emerge from the tissue (Shamsi and Suthar, 2016a). All nematodes were found alive. They were collected and then preserved in 70% ethanol. A small piece from the mid-body of each nematode larvae was excised for molecular study and the rest of the body was cleared with lactophenol for morphological study.

### 2.3. Morphological examination

Lactophenol-cleared larvae were initially identified according to genus and classified into various morphotypes (Cannon, 1977; Shamsi et al., 2011b; Shamsi et al., 2013; Shamsi et al., 2015) which were then subjected to being drawn. All drawings were made to scale, with the aid of a camera lucida, and measurements were made directly with an eyepiece micrometer. All measurements are given in millimetres (mm) as the mean, followed by the range in parentheses.

### 2.4. Molecular analyses

Genomic deoxyribonucleic acid (DNA) was extracted by DNeasy Blood & Tissue Kits (QIAGEN, Germany), according to the manufacturer's instruction, and eluted by 40 µl of elution buffer. Polymerase Chain Reaction (PCR) method was conducted to amplify the ITS-1 and ITS-2 regions using the primer sets SS1 & NC13R and SS2 & NC2, respectively (Shamsi and Suthar, 2016b). Representative samples from each morphotype and each fish host were selected for sequencing, using the same primers as PCR. Sequence data, including chromatogram, were observed initially through Sequence Scanner Software (Applied Biosystems® Genetic Analysers), then aligned by ClustalX (Thompson et al., 1997) and adjusted manually, wherever necessary.

A neighbour-joining method was used to construct a phylogenetic tree, based on the best-fit model of evolution (Kimura-2-parameter model), from the sequences generated in this study, along with the representative sample sequences from the GenBank (Table 2). *Anisakis simplex* was used as an outgroup. The reliability of the neighbour-joining tree was assessed by the bootstrap method, with 1000 replications. These analyses were performed using the Molecular Evolutionary Genetics Analysis (MEGA7®) software (Kumar et al., 2016).

### 2.5. Parasite data analyses

The prevalence (P), mean intensity (MI) and mean abundance (MA) of nematode larvae were calculated as below:

Prevalence (P) = (number of infected fish/total number of examined fish) × 100;

Mean intensity (MI) = (number of parasites/number of infected fish);

Mean abundance (MA) = (number of parasites/total number of examined fish).

## 3. Results

### 3.1. Prevalence, mean intensity and mean abundance

Detailed information, on the occurrence and prevalence of larval types found in the examined fish, is presented in Table 3. Nematode parasites found in the present study were all in larval stages (i.e., third (L3) and fourth (L4) developmental stage) belonging to three genera,

**Table 2**
Details of specimens used to construct the phylogenetic tree.

| Nematode specimen | GenBank accession number (ITS-1 & ITS-2) | Host species | Geographical origin of the sample | Reference |
|---|---|---|---|---|
| *Anisakis simplex* (outgroup) | KM273046 | *Gadus morhua* | Southwestern Baltic Sea, east of the island of Bornholm near the islet Christiansø | Mehrdana et al. (2014) |
| *Hysterothylacium aduncum* | MF693084 & MF693100 | *Micromesistius poutassou* | West Mediterranean (FAO zone 37.1.1) and the North-East Atlantic (FAO zone 27.8), Spain | Roca-Geronès et al. (2018) |
| *H. auctum* | AF115571 | *Zoarces viviparous* | South Baltic Sea, Poland | Szostakowska et al. (2001) |
| *H. australe* | HE862216 & HE862223 | *Seriola lalandi* | Port Augusta, South Australia | Shamsi (2016) |
| *H. bidentatum* | AY603539 | *Acipenser ruthenus* | Danube river, Poland | Kijewska et al. (2008) |
| *H. brucei* | HE86222 & HE862230 | *Kajikia audax* | Nelson Bay, NSW, Australia | Shamsi (2016) |
| *H. deardorffoverstreetorum* | JF730202 | *Paralichthys isosceles* | Littoral area, Angra dos Reis, Rio de Janeiro, Brazil | Knoff et al. (2012) |
| *H. kajikiae* | HE862220 & HE862226 | *Kajikia audax* | Nelson Bay, NSW, Australia | Shamsi (2016) |
| *H. liparis* | KF601900 | *Liparis tanakae* | Yellow Sea, China | Guo et al. (2014) |
| *H. longilabrum* | JQ520159 | *Siganus fuscescens* | The South China Sea, off Sanya, Hainan Province, China | Li et al. (2012) |
| *H. persicum* | LT576366 & LT576369 | *Scomberomorus commerson* | Bandar Abbas Fish market, Hormozgan, Iran | Shamsi et al. (2016) |
| *H. reliquens* | LT717080 & LT717085 | *Otolithes ruber* | Khor Abdulla, Iraq | Ghadam et al. (2017) |
| *H. thalassini* | JX982126 | *Priacanthus tayenus* | South China Sea, China | Liu et al. (2013) |
| *H. zhoushanense* | JX028281 | *Pseudorhombus oligodon* | The East China Sea, off Zhoushan Island, Zhejiang Province, China | Li et al. (2012) |
| *Hysterothylacium* type V | FN811738 & FN811699 | *Lutjanus carponotatus* | Heron Island, Queensland, Australia | Shamsi et al. (2013) |
| *Hysterothylacium* type VI | FN811740 & FN811701 | *Chaetodon lineolatus* | Heron Island, Queensland, Australia | Shamsi et al. (2013) |
| *Hysterothylacium* type VII | FN811749 & FN811709 | *Caesio cunning* | Heron Island, Queensland, Australia | Shamsi et al. (2013) |
| *Hysterothylacium* type XV | LT576354 & LT576363 | *Otolithes ruber* | Bandar Abbas Fish market, Hormozgan, Iran | Shamsi et al. (2016) |
| *Hysterothylacium* type XVII | MG594313 & MG594336 | *Acanthopagrus australis* | Moreton Bay, Queensland, Australia | Shamsi et al. (2018) |
| *Hysterothylacium* type IV-A | MK161414-7 & MK161439-42 | *Engraulis australis* | Sydney, NSW, Australia | Present study |
| *Hysterothylacium* type IV-B | MK161418-20 & MK161443-5 | *Sardionops sagax*, *Sillago flindersi*, *Engraulis australis* | Sydney, NSW, Australia | Present study |
| *Hysterothylacium* type VIII | MK161421-3 & MK161446-8 | *Sardionops sagax*, *Sillago flindersi*, *Engraulis australis* | Sydney, NSW, Australia | Present study |
| *Hysterothylacium* type XIV | MK161424 & MK161449 | *Engraulis australis* | Sydney, NSW, Australia | Present study |
| *Hysterothylacium* type XVIII | MK161425-7 & MK161450-2 | *Engraulis australis* | Sydney, NSW, Australia | Present study |

*Contracaecum*, *Terranova* and *Hysterothylacium* (Figs. 1 and 2). All three species of fish were infected with at least one of these genera (Table 3). As Table 3 shows, the maximum number of parasites were found in the Australian pilchard (151 larvae in 19 fish) followed by the Australian anchovy (98 larvae in 70 fish) and the Eastern school whiting (21 larvae in 20 fish). Australian anchovies were infected with the most diverse group of nematode larvae (seven different larval types) followed by the Australian pilchard (three different larval types) and Eastern school whiting (two different larval types) (Table 3). All Australian pilchards were found to be infected with nematode larvae. In the present study, all nematode larvae were collected from the digestive system, gonad, and liver.

### 3.2. Larval identification

#### 3.2.1. Genus Contracaecum

Larvae belonging to this genus were the least abundant in the examined fish. All *Contracaecum* larvae were morphologically classified as type II, based on the ratio of ventricular appendix to intestinal caecum and the morphology of the tail, being rounded at the tip. They were found in the Australian pilchard and anchovy. Based on identical ITS sequences, *Contracaecum* larval type II in the present study (accession numbers MK161408-11 and MK161433-6) were identified as *Contracaecum ogmorhini* (accession numbers FM177542 and FM177549).

#### 3.2.2. Genus Terranova

*Terranova* larvae in the present study were found only in the Australian anchovy, and were morphologically classified as type II, based on an intestinal caecum longer than the ventriculus. There was no comparable sequence from a well-identified adult in GenBank, therefore, the specific identity of this larval type remains unknown.

#### 3.2.3. Genus Hysterothylacium

As a group, *Hysterothylacium* larval types were the most abundant and diverse group of nematode larvae found in the examined fish. In particular, *Hysterothylacium* type VIII was found in all fish and was the most abundant larval type. Based on morphology of the tail and digestive system, *Hysterothylacium* larval types found in the present study were classified under four types, namely type IV, VIII, XIV and XVIII, with the latter being found for the first time. Therefore a description is provided (Fig. 2) and biometrical data have been presented in Table 4.

For specific identification, three representatives (isolate numbers: 80-3, 84-1, and 126-2) were subjected to sequencing of ITS region. All specimens demonstrated identical ITS-1 (accession numbers: MK161425-7) and ITS-2 (accession numbers: MK161450-2) sequences. Alignment of ITS-1 and ITS-2 sequence data with those formerly deposited in the GenBank showed that they are identical with *Hysterothylacium thalassini* (accession numbers JX982129 and JX982127 for ITS-1 and ITS-2, respectively).

**Table 3**
List and number of ascaridoid nematode larvae found in the present study.

| Fish (number examined) | Nematode larvae | Number of fish infected | Minimum and maximum number of larvae in infected fish | Prevalence (%) | Total number of parasites found | Mean intensity (MI) | Mean abundance (MA) | Museum accession number | Gene Bank accession number (ITS-1 & ITS-2 respectively) |
|---|---|---|---|---|---|---|---|---|---|
| Australian pilchard (19) | *Contracaecum* type II | 2 | 1–2 | 10.5 | 3 | 1.50 | 0.16 | AHC48513 | MK161408-10 & MK161433-5 |
| | *Hysterothylacium* type IV (genotype B) | 4 | 1–5 | 21.05 | 8 | 2.00 | 0.42 | AHC48518 | MK161418 & MK161443 |
| | *Hysterothylacium* type VIII | 18 | 1–14 | 94.7 | 140 | 7.78 | 7.37 | AHC48521 | MK161421 & MK161446 |
| | Total | 19 | 1–14 | 100 | 151 | 7.95 | 7.95 | | |
| Australian anchovy (70) | *Contracaecum* type II | 1 | 1–1 | 1.43 | 2 | 2 | 0.03 | AHC48514 | MK161411 & MK161436 |
| | *Terranova* type II | 5 | 1–5 | 7.14 | 12 | 2.40 | 0.17 | AHC48515 | MK161412-3 & MK161437-8 |
| | *Hysterothylacium* type IV (genotype A) | 1 | 1–2 | 1.43 | 2 | 2.00 | 0.03 | AHC48516 | MK161414-7 & MK161439 - 42 |
| | *Hysterothylacium* type IV (genotype B) | 3 | 1–1 | 4.3 | 3 | 1.00 | 0.04 | AHC48517 | MK161420 & MK161445 |
| | *Hysterothylacium* type VIII | 30 | 1–8 | 42.86 | 72 | 2.40 | 1.03 | AHC48520 | MK161423 & MK161448 |
| | *Hysterothylacium* type XIV | 1 | 1–1 | 1.43 | 1 | 1 | 0.01 | AHC48523 | MK161424 & MK161449 |
| | *Hysterothylacium* type XVIII | 4 | 1–2 | 5.71 | 6 | 1.50 | 0.09 | AHC48524 | MK161425-7 & MK161450-2 |
| | Total | 39 | 1–8 | 56 | 98 | 2.51 | 1.4 | | |
| Eastern school whiting (20) | *Hysterothylacium* type IV (genotype B) | 10 | 1–2 | 50 | 11 | 1.10 | 0.55 | AHC48519 | MK161419 & MK161444 |
| | *Hysterothylacium* type VIII | 7 | 1–2 | 35 | 10 | 1.43 | 0.50 | AHC48522 | MK161422 & MK161447 |
| | Total | 14 | 1–2 | 70 | 21 | 1.5 | 1.05 | | |

There were no identical ITS sequence data belonging to a well identified adult in the GenBank for other *Hysterothylacium* larval types found in the present study, except for *Hysterothylacium* larval type XIV, which was identified as *H. persicum*. Phylogenetic analyses of *Hysterothylacium* larval types found in the present study (Fig. 3) showed that they are distinct taxa and this supported the specific identification of *Hysterothylacium* larval types XIV and XVIII.

## 4. Discussion

One significant finding of our study is that the nematodes found in the present study all belonged to ascaridoids, in larval stage and alive, suggesting that they all were in their infective stage and could be potentially transferred to consumers if the fish were consumed raw or undercooked. Indeed, human infection with all these three larval types, including *Contracaecum*, *Terranova*, and *Hysterothylacium*, has been previously reported (Arizono et al., 2011; Jofré et al., 2008; Shamsi and Butcher, 2011; Yagi et al., 1996).

In our study, the *Contracaecum* larval type is reported for the first time in the examined fish. Although the number of *Contracaecum* larval type in the present study was low, they were alive when the fish were examined (approximately 5 days after the fish were caught). These larvae were identified as *C. ogmorhini*, which were previously reported by Shamsi et al. (2009) as coming from marine mammals (Australian and New Zealand fur seals), *Arctocephalus pusillus doriferus* and *Arctocephalus forsteri*, in Victoria, Australia. The infection of humans with *Contracaecum* larval type has also been reported in Australia (Shamsi and Butcher, 2011) as well as from the Baltic region (Schaum and Müller, 1967), France (Dei-Cas et al., 1986), the Republic of Korea (Im et al., 1995) and Japan (Nagasawa, 2012), which have shown *Contracaecum* larvae cause a severe and painful condition in humans, following ingestion of raw or undercooked fish carrying third-stage larvae; however, human cases due to the *Contracaecum* larval type seems to be less abundant compared to human cases due to the *Anisakis* spp. However, *Contracaecum* larvae cannot be identified to species level without the aid of molecular tools and in all the human cases mentioned above, morphological identification was to genus level only. Additionally, in some cases *Contracaecum* larvae were reported as *C. osculatum* based only on the prevalence of the natural definitive host in the region and the assumption that humans and marine mammals can share similar parasites (Shamsi, 2019).

Another larval type identified in this study was *Terranova* type II. Larval nematodes belonging to the genera *Pseudoterranova*, *Terranova* and *Pulchrascaris* are usually grouped as *Terranova* larval type (Shamsi and Suthar, 2016b). Although the specific identity of *Terranova* larval types in Australian waters is unknown (due to the lack of comparable sequence data from a well-identified adult), based on phylogenetic analyses it has been argued that they most likely belong to *Terranova* larval type rather than *Pseudoterranova* larval type (Shamsi and Suthar, 2016b), with the latter known to be zoonotic.

The most abundant larval nematodes observed in the present study were *Hysterothylacium* larval types. Since some larvae of *Hysterothylacium* taken from marine fish cannot be differentiated morphologically from other nematode larvae, such as *Paraheterotyphlum*, *Heterotyphlum*, *Iheringascaris* and *Lapetascaris*, a phylogenetic tree has been constructed to confirm their taxonomic status, which suggests that larvae found in the present study belong to the genus *Hysterothylacium*. Although the zoonotic potential of *Hysterothylacium* species has been under debate, there have been two published cases of human infection caused by *Hysterothylacium* sp., in Japan (Yagi et al., 1996) and in Spain (González-Amores et al., 2015). As *Hysterothylacium* spp. have been shown to infect a broad range of marine and freshwater fish species (Gazzonis et al., 2017; Ghadam et al., 2017; Jabbar et al., 2012; Shamsi et al., 2013; Zhao et al.,

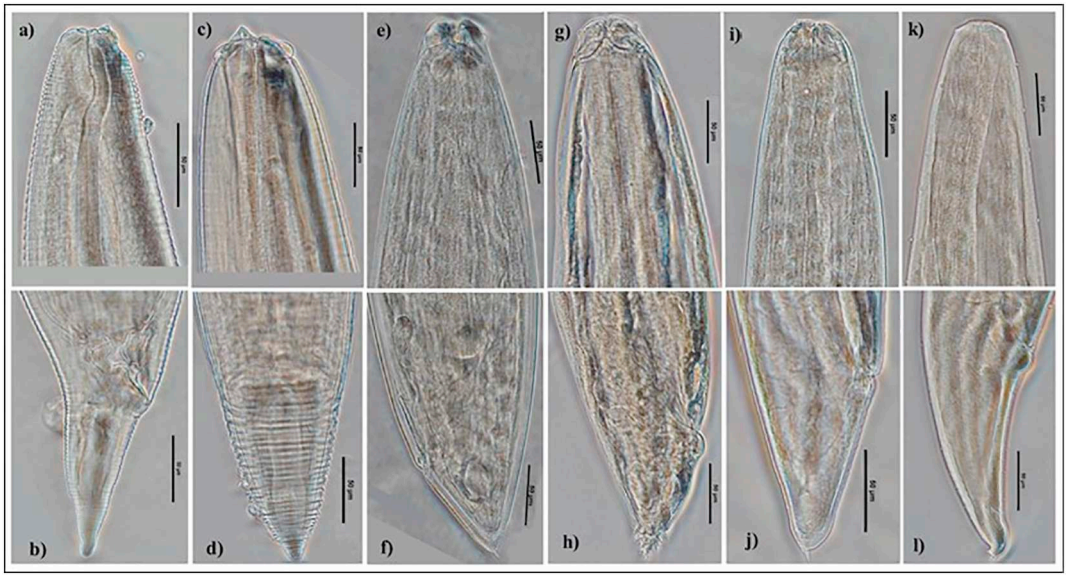


**Fig. 1.** Morphology of six larval types found in this study: a) and b) anterior and posterior end of *Contracaecum* larval type II (scale-bars = 50 μm); c) and d) anterior and posterior end of *Terranova* larval type II; e) and f) anterior and posterior end of *Hysterothylacium* larval type IV-A; g) and h) anterior and posterior end of *Hysterothylacium* larval type IV-B; i) and j) anterior and posterior end of *Hysterothylacium* larval type VIII; k) and l) anterior and posterior end of *Hysterothylacium* larval type XIV. All scale-bars = 50 μm.

2016), including edible species, assessment of their risk to humans may need to be revised. Although *Hysterothylacium* larval types are believed to be less important, in terms of zoonotic potential, their higher abundance may increase the risk they pose to consumers.

One of the novelties of this study is the identification of a new *Hysterothylacium* larval type (type XVIII), which was identified as *H. thalassini*. This larval type can be differentiated from other *Hysterothylacium* larval types based on the strong annulation on the outer surface of the body (Fig. 2a, b & c) and with no spine on the tail.

Interestingly, no *Anisakis* larval type was found in the specimens examined in the present study, despite several reports of *Anisakis* spp. larvae in fish collected in NSW waters in the past (e.g., Hooper, 1983; Shamsi and Suthar, 2016a). *Anisakis* larvae have a broad host specificity. Their definitive hosts (whales and dolphins) are commonly found in NSW waters during June to November (Stockin and Burgess, 2005), with increased population due to conservation efforts (EPBC, 1999). Therefore, a significant prevalence is expected (Clers and Andersen, 1995; Lile, 1998). The absence of *Anisakis* larvae in our study is an unusual finding worth further investigation, as this may be due to environmental changes or dramatic decline in the population of first intermediate hosts, such as zooplanktons experience in their life cycle due to seasonality (Dione et al., 2013; Shamsi et al., 2018; Torres et al., 2007) or pollution (Valtonen et al., 1997), which in turn influences the density of nematode populations (Marcogliese, 2008; McClelland, 2005).

Nematode larvae were isolated from the digestive system, gonad, and liver of each tested fish species and their presence in the internal organs of commonly consumed fish is significant. These ascaridoid larvae are capable of post-mortem migration, from visceral organs to fish musculature, as demonstrated in herrings of the Clupeidae family. The Australian pilchard, tested in the present study, belongs to the same family. Post mortem larval migration has also been observed in the European pilchards, *Sardina pilchardus* and *Engraulis* sp., which are from the same family as the Australian anchovy (Buselic et al., 2018; Cipriani et al., 2016; Cipriani et al., 2018; Smith and Wootten, 1975). Smith (1984) found *Anisakis simplex* L3 migrated from the body cavity into the flesh in mackerel, *Scomber scombrus*, but not in other less oily species of fish, and concluded that post-mortem larval migration into flesh was a phenomenon of 'fatty' species, such as herring and mackerel. Both *Engraulis* spp. and *Sardinops* spp. are considered oily-fleshed fish (Moffat and McGill, 1993), and it is considered that the presence of ascaridoid larvae in the internal organs may be indicative of a significant risk of post-mortem larval migration, as described by Buselic et al. (2018); Cipriani et al. (2016). While muscle tissue was not tested for the presence of nematode larvae in the present study, the prevalence of infection and the number of parasites present in small fish that are commonly consumed whole, such as anchovy and pilchard, is significant, and warrants education of the public and various stakeholders.

The global food standards, which are of relevance to the safety of fish for human consumption, are developed and reviewed by Codex Alimentarius. In CAC/RCP52-2003 (2016) 'Code of practice for fish and fishery products', candling, trimming belly flaps and physically removing the parasites are recommended to reduce parasite hazards, however, the method is not considered to completely eliminate them. The marketable anchovy and pilchard products in Australia are frequently purchased by the consumer un-eviscerated and, in the case of anchovy, generally consumed whole (FSANZ, 2004; Shamsi and Sheorey, 2018). In Australia, the Mediterranean diet, recommended for optimum cardiovascular health (Estruch et al., 2013), is inclusive of the weekly consumption of oily fish, such as sardines (RACGP). Additionally, the Mediterranean diet is part of the cultural heritage of Italy, Greece and Spain (RACGP); immigrants from these countries comprise a significant portion of the Australian population (ABARES, 2018). In the present study, three potentially zoonotic genera of

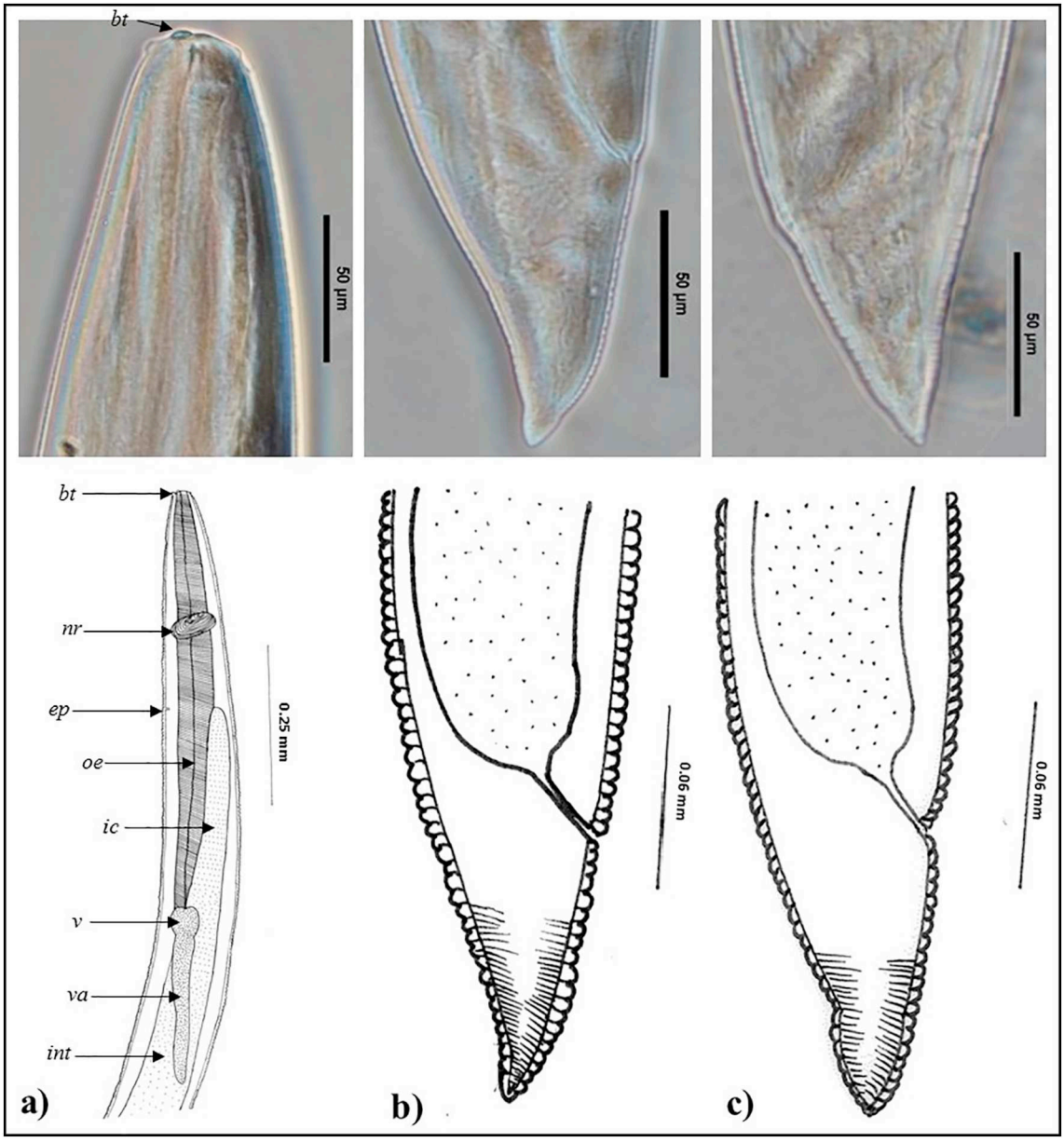


**Fig. 2.** Morphology of the novel *Hysterothylacium* larval type XVIII isolated from Australian anchovy in the present study. They were all in third stage development which is the infective stage, medium sized nematodes with distinct annulation on the outer surface for the entire body, a rare characteristic among *Hysterothylacium* larval types. Note presence of boring tooth (a) and tail tapering and the rounded tip (b & c). Absence of any spine or extension at the tip of the tail is also a rare feature among *Hysterothylacium* larval types. The top row represents the microscopic view (scale-bars = 50 µm) and corresponding bottom row indicates the drawing view (scale-bars = 0.25 mm and 0.06 mm, respectively). br: boring tooth; nr: nerve ring; ep: excretory pore; oe: oesophagus; ic: intestinal caecum; v: ventriculus; va: ventricular appendix; int: intestine.

nematodes were identified in the fish examined. While many food safety agencies globally recommend freezing all fish which is to be consumed raw, including cured, salted and pickled fish products (EFSA, 2018), and in Switzerland it is against the law to sell fresh fish intended to be consumed raw or semi-raw without having been stored at $\leq -20$ °C (De Marval et al., 2013), the *Australia New Zealand Food Standards Code*, Standard 3.2.1 – 'Food Safety Programs', Standard 3.2.2 – 'Food safety practices and general requirements' and Standard 4.2.1 – 'Primary production and processing standard for seafood' (Australia Only), do not include information on parasites in local fish or provide any recommendations regarding freezing fish intended for consumption as raw, pickled or cured (FSANZ, 2004). In Appendix 1 of the '*The Compendium of Microbiological Criteria for Food (2018)*' *from Food Standards Australia and New Zealand*, parasites are mentioned as

**Table 4**
Biometry of the new *Hysterothylacium* larval type XVIII found in the present study. (n = 5; materials examined: 80-3, 84-1, 100-4, 100-5, 126-2) All measurements are given in millimetres. Mean measurements are given, followed by the range in parentheses. Abbreviations: BL: Body Length, BW: Body Width, NR: Nerve Ring (from anterior end), EP: Excretory Pore (from anterior end), Oe: Oesophagus length, V: Ventriculus length, VA: Ventricular Appendix length, IC: Intestinal Caecum length, TL: Tail Length, TW: Tail Width.

| Feature | Measurement |
|---|---|
| BL | 3.12 (2.70–3.70) |
| BW | 0.12 (0.10–0.13) |
| NR | 0.19 (0.18–0.20) |
| EP | 0.33 (0.31–0.34) |
| Oe | 0.62 (0.58–0.66) |
| V | 0.04 (0.03–0.04) |
| VA | 0.22 (0.20–0.24) |
| IC | 0.30 (0.29–0.32) |
| OE:BL | 0.20 (0.17–0.25) |
| IC:V | 8.78 (7.41–11.5) |
| IC:VA | 1.38 (1.30–1.44) |
| IC:Oe | 0.49 (0.45–0.53) |
| VA:Oe | 0.35 (0.32–0.38) |
| TL | 0.09 (0.08–0.10) |
| TW | 0.06 (0.05–0.06) |
| TL:BL | 0.03 (0.02–0.03) |

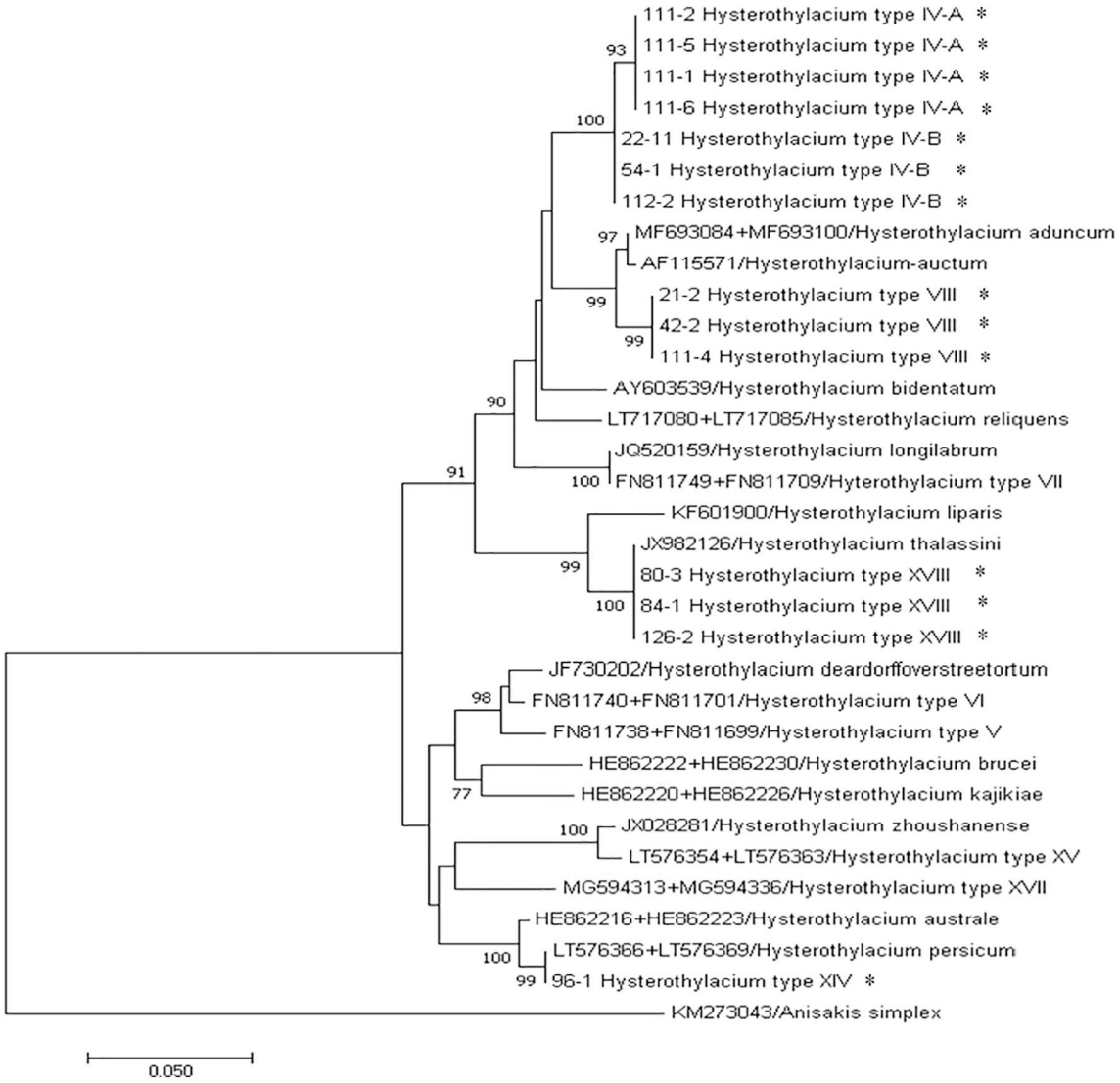


**Fig. 3.** Neighbour-joining phylogenetic tree based on the combined ITS-1 and ITS-2 sequence data for *Hysterothylacium* spp. with *Anisakis simplex* as an outgroup. Asterisks represent *Hysterothylacium* larvae identified in the present study. Bootstrap (1000 replicates) support values are indicated. The evolutionary distances were computed using the Tamura-Nei method. Only one GenBank accession number indicates whole ITS sequence data.

possible pathogenic microorganisms which can cause foodborne illness. However, this is mentioned only once in the document (FSANZ, 2018). SafeFish is funded by the FRDC and is concerned with Australian seafood safety and trade (Safefish:www.safefish.com.au). The 'seafood safety fact sheets' produced by Safefish make no mention of parasites in seafood (Safefish, 2016). It may be prudent to evaluate the current seafood safety standards in Australia against those adopted by other nations.

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