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The occurrence and clinical importance of infectious stage of *Echinocephalus* (Nematoda: Gnathostomidae) larvae in selected Australian edible fish

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ABSTRACT

Cases of gnathostomiasis, an infection caused by consuming infected seafood, have been reported in Australia. However, doubt exists over the validity of these diagnoses as there are no reports of *Gnathostoma* spp. in Australian teleost fish. Also, the diagnoses in human cases were based on a serological test developed in Thailand. The specificity and sensitivity of this test in non-endemic areas are uncertain. Interestingly, parasites belonging to the genus *Echinocephalus*, which morphologically are very similar to *Gnathostomum*, are commonly found in Australian fish and shellfish and can potentially infect humans. The aim of this study was to determine the occurrence of these zoonotic nematodes within commercial fish and to characterise nematode larvae in order to provide insights into the specific identity of the potential causative agents of gnathostomiasis in Australia. Six edible fish species (n = 163) were examined. Gnathostomid-type larvae were found only in *Acanthopagrus australis* and *Rhabdosargus sarba*. Detailed examination and sequence data suggested parasite larvae belonged to the genus *Echinocephalus*. Further investigation of the occurrence of zoonotic nematodes within marine environments and observation of their spatial and temporal patterns will help raise awareness of the significance of this food safety issue within global fishing industries and health sectors. The accurate identification of zoonotic nematodes is a key component of disease surveillance and control. This information can also be used to develop specific and sensitive diagnostic test.

1. Introduction

In Australia, seafood is a popular source of dietary protein and its regular consumption as part of a healthy diet is widely recommended by health practitioners. Research shows that knowledge and awareness of the risk of transmissible parasites from edible fish to humans is poor among Australian medical practitioners [1]. Of the seafood-borne parasites reported in Australia, knowledge about gnathostomid nematodes is scarce. In a 2011 case report, an Australian couple was diagnosed with gnathostomiasis caused by a nematode parasite belonging to the genus *Gnathostoma* after consuming freshly caught fish that had been cooked over a campfire [2]. Due to the lack of a standard diagnostic test for seafood-borne parasites in Australia, the diagnosis was made overseas, based on a serological test developed in Thailand [3] with unknown specificity and sensitivity. Later, Shamsi and Sheorey [4] questioned the diagnosis as the cross reactivity was unknown. Whether antibodies to these two parasites cross react has not been tested, meaning it is unclear if the Australian couple was infected by *Gnathostoma* or *Echinocephalus*.

Gnathostoma parasites in Australian teleost fish have not been reported, although several reports exist of larval *Echinocephalus* Molin, 1858 in fish across the country (Table 1). Interestingly, parasites belonging to the genus *Echinocephalus*, which morphologically are very similar to *Gnathostomum* and belong to the same family (Gnathostomatidae), are commonly found in Australian fish and shellfish [5]. To investigate their ability to infect humans, Ko [6] experimentally infected 37 kittens, 11 monkeys and two puppies with *Echinocephalus sinensis* retrieved from oysters in Hong Kong. In the kittens, the worms were found to be highly infectious and were found in most organs. Severe clinical signs, resulting in the death of three kittens 16–30 h post infection, were demonstrated. Degenerating worms were found in a fibrous nodule in the stomach wall of a monkey examined 14 days post-infection; one worm was also recovered from the lung. Symptoms of infection were severe but varied between host types; after penetrating the gastrointestinal tract the parasites underwent a random visceral migration. Furthermore, there was a seasonal variation with infection only occurring during August to October, inferring that mammalian infection may be dependent on

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Table 1
Listing of the reported of *Echinocephalus* spp. from Australian Waters. Abbreviation: WA: Western Australia; NSW: New South Wales; SA: South Australia; Tas: Tasmania; Qld: Queensland, NT: Northern Territory.

Parasite species	Host scientific name	Host common name(s)	Locality	Publication
E. overstrstreeti	*Heterodontus portusjacksoni* (Meyer)	Port Jackson shark	Bunbury, WA	Beveridge (1991)
			Kangaroo Island, SA	Beveridge (1987)
			Stanley, Tas	Beveridge (1991)
	Myliobatis australis Macleay	Australian bull ray	Bunbury, WA	Beveridge (1991)
	Pastinachus sephen (Forsskal) *Syn: Dasyatis sephen* (Forsskal)	Cowtail stingray	Fog Bay, NT	Beveridge (1991)
			Nickol Bay, WA	Beveridge (1991)
	Aptuchotrema vincentiana[L] (Haacke)	Western shovelnose ray	SA	Beveridge (1987)
	Bathytoshia brevicaudata (Hutton)[L] Syn: *Dasyatis brevicaudatus* (Hutton)	Short-tail stingray	Kangaroo Island, SA	Beveridge (1987)
	B. lata (Garman)[L] Syn: *Dasyatis thetidis* (Waite)	Brown stingray	Kangaroo Island, SA	Beveridge (1987)
	Callorhynchus milii Bory de St Vincent[L]	Ghost shark	Kangaroo Island, SA	Beveridge (1987)
	Equichlamys bifrons (Lamarck)[L] Syn: *Chlamys bifrons*	Queen Scallop	Northaven, Edithburg, SA	Beveridge (1987)
	Hypnos monopterygium (Shaw & Nodder)[L]	Coffin ray	Kangaroo Island, SA	Beveridge (1987)
	Myliobatis australis Macleay[L]	Australian bull ray	SA	Beveridge (1987)
	Orectolobus maculatus (Bonnaterre)[L]	Spotted wobbegong	Kangaroo Island, SA	Beveridge (1987)
	Parascyllium ferrugineum McCulloch[L]	Rusty carpetshark	Kangaroo Island, SA	Beveridge (1987)
	Pecten albus Tate[L]	invertebrate	Northaven, SA	Beveridge (1987)
	Spiniraja whitleyi (Iredale)[L] Syn: *Raja whitleyi* Iredale	Melbourne skate	Kangaroo Island, SA	Beveridge (1987)
	Trygonorrhina fasciata Müller & Henle[L] Syn: *Trygonorhina guanerius* Whitley	Eastern fiddler ray	Kangaroo Island, SA	Beveridge (1987)
	Urolophus mucosus (Whitley)[L]	Western shovelnose stingaree	Kangaroo Island, SA	Beveridge (1987)
E. sinensis	*Hemitrygon fluviorum* (Ogilby) Syn: *Dasyatis fluviorum* Ogilby	Estuary stingray	Off southern Queensland coast; Qld	Beveridge (1991)
E. spinosissimus	*Heterodontus portusjacksoni* Syn: *Heterodontus philippi*	Port Jackson Shark	Port Willunga, SA	Johnston and Mawson (1943); Beumer et al. (1982)
			Hobart, Tas	Johnston and Mawson (1945a)
	Trygonoptera testacea (Mueller and Henley) Syn: *Urolophus testaceus*	Common Stingaree	NSW	Johnston and Mawson (1943); Beumer et al. (1982)
E. uncinatus	*Pseudolabrus rubicundus* (Macleay)[#]	Rosy wrasse	Port Noarlunga, SA	Johnston and Mawson (1945b); Beumer et al. (1982)
E. uncinatus	*Psuedolabrus psittaculus*[*]	–	–	Johnston and Mawson (1945b)
	Chrysophrys auratus[L] Syn: *Pagrosomus auratus*	Australasian Snapper	Glenelg, SA	Johnston and Mawson (1945b); Beumer et al. (1982)
	Foetorepus calauropomus[L] Syn: *Callionymus calauropomus*	Common Stinkfish	Port Lincoln, SA	Johnston and Mawson (1945a); Beumer et al. (1982)
	Katelysia scalarina Lamarck[L]	Sand Cockle	St Vincent Gulf, SA	Johnston and Mawson (1945a)
	Platycephalus bassensis Cuvier[L]	Southern Sand Flathead	Hobart, Tas	Johnston and Mawson (1945a)
	Platycephalus fuscus Cuvier[L]	Dusky/Black Flathead	Glenelg, SA	Johnston and Mawson (1945b); Beumer et al. (1982)
			St Vincent Gulf, SA	Johnston and Mawson (1945a)
	Platycephalus laevigatus Cuvier[L]	Black/Rock Flathead	Hobart, Tas	Johnston and Mawson (1945a); Beumer et al. (1982)
	Conuber conicum	Gastropod	St Vincent Gulf, SA	Johnston and

(*continued on next page*)

Table 1 (*continued*)

Parasite species	Host scientific name	Host common name(s)	Locality	Publication
	(Lamarck, 1822)[L] **Syn:** *Polinices conica*			Mawson (1945a)
	Sillaginodes punctatus (Cuvier)[L]	King George Whiting	St Vincent Gulf, SA	Johnston and Mawson (1945a)
			Glenelg, SA	Johnston and Mawson (1945b)
Echinocephalus sp. (larva)	*Aetobatus narinari* (Euphrasen)[L]	Whitespotted eagle ray	Fog Bay, NT	Beveridge (1991)
	Carcharinus plumbeus (Nardo)[L]	Sandbar shark	Bunbury, WA	Beveridge (1991)
	Himantura uarnak (Frosskal)[L]	Honeycomb stingray	Fog Bay, NT	Beveridge (1991)
	Rhynchobatus djiddensis (Frosskal)[L]	Giant guitarfish	Flat Top Island, Qld	Beveridge (1991)

[*] According to FishBase (Froese et al. 2019), *Pseudolabrus psittaculus* (Richardson, 1840) is not a valid scientific name anymore and possible scientific name would be *Pseudolabrus rubicundus* (Macleay, 1881).

[#] Reported as *Pseudolabrus miles* (common name: Scarlet Wrasse) in Beumer's checklist (Beumer et al. 1982); however, the scientific name has undergone through new combination. See FishBase (Froese et al. 2019).

[L] Indicates larval stage of the parasite was reported in the host.

environmental conditions and ambient temperatures. Ko's study indicated that under the right circumstances, *Echinocephalus* spp. is likely to cause human infection. The diagnosis of human infection with *Gnathostoma*, where parasites invade host tissue, is difficult. As mentioned above, morphologically, *Gnathostoma* and *Echinocephalus* look similar in larval stages, which makes accurate differentiation difficult [4]. Sequence data are useful for the specific identification of nematode larvae [7]. However, so far, no sequence data is available for these larval types in Australia. Specific identification of parasite larvae in fish is important for seafood safety and human health and for understanding the biodiversity of aquatic parasitic fauna in Australian waters. With the increased consumption of seafood and global warming, which generally favours the successful completion of parasite lifecycles, the risk of infection with these parasites is likely to increase. Therefore, the accurate identification of fish parasites is crucial for the rapid and successful diagnosis of seafood-borne parasitic disease. However, due to an increasing loss of expertise in taxonomists of aquatic parasites, this may be an issue [1]. The aim of this study is to provide evidence that gnathostomid larva found in Australia belong to the genus *Echinocephalus* through a taxonomic approach. The outcome of this study is important to developing an accurate diagnostic tool for humans.

2. Materials and methods

Fish specimens were collected from Moreton Bay, Queensland, on the east coast of Australia. A total of 163 fish belonging to six edible species (Table 3), were available to be examined for infection with parasites. Each fish was thoroughly examined for the presence of parasites, according to the method of Shamsi and Suthar [8]. When found, parasites were preserved in 70% ethanol and transported to the Parasitology Laboratory at Charles Sturt University, Wagga Wagga, New South Wales. A small section (less than a millimetre) of the mid-body from each gnathostomid nematode collected was removed using a scalpel blade and sections were frozen at −20 °C for DNA extraction. The remaining posterior and anterior sections of each specimen were mounted onto a microscope slide and cleared in lactophenol for morphological examination followed by detailed measurements of important features. Illustrations have been produced and are drawn to scale with the aid of a microscope equipped with a drawing tube – all measurements are given in millimetres unless stated otherwise. Mean measurements are given, followed by the range in parentheses. Genomic DNA was isolated from individual larvae by a DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA, USA). Extraction was based on the modified version of the maufacturer protocol[1] and eluted into 40 μl of water. Host DNA was isolated from the musculature of fish using the same method. The ITS1 region was amplified and sequenced using primer pair Lim1657 (forward) 5-CTGCCCTTTGTACACACCG-3 and ITS1-RIXO (Reverse) 5-TGGCTGCGTTCTTCATCG-3 [9] and PCR conditions described previously [10]. 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. The amplicons were purified over mini-columns (Wizard™ PCR Prep, Promega, WI, USA), eluted in 30 μl H_2O and then subjected to automated sequencing, in both directions, using the same primers as for PCR. Following a manual quality check of the sequence chromatograms, the primer sequences were removed from the sequences for further analysis. The sequences were aligned using the computer program ClustalX [11] and then adjusted manually. Available ITS sequences of the family of Gnathostomatidae were obtained from the GenBank (Table 2). *Anisakis simplex* was used as an outgroup (JX237370). The sequences were aligned with the MAFFT online service [12], using the default setting followed by manual adjustment. The alignment was subjected to phylogenetic analysis using MrBayes V 3.2 [13] and the HKY + G model as suggested by the Jmo-

Table 2
Details of sequences used in the present study for phylogenetic analyses.

Parasites	Localities	Host	Accession	Citation
Echinocephalus sp.	Australia	*Rhabdosargus sarba*	MW136167	This study
Echinocephalus sp.	Australia	*Rhabdosargus sarba*	MW136168	This study
Echinocephalus sp.	Australia	*Acanthopagrus australis*	MW136169	This study
Spiroxys japonica	China	*Pelophylax nigromaculatus*	KF530321.1	Li et al. (2014)
S. japonica	China	*Pelophylax nigromaculatus*	KF530322.1	Li et al. (2014)
S. japonica	China	*Pelophylax nigromaculatus*	KF530323.1	Li et al. (2014)
S. japonica	Japan	*Pelophylax nigromaculatus*	KF530324.1	Li et al. (2014)
S. japonica	Japan	*Lithobates catesbeianus*	KF530325.1	Li et al. (2014)
S. hanzaki	Japan	*Andrias japonicus*	KF530326.1	Li et al. (2014)
Gnathostoma spinigerum	Thailand	*Fluta alba*	AB181155.1	Ando et al. (2006)
G. doloresi	Japan	*Sus scrofa*	AB181156.1	Ando et al. (2006)
G. nipponicum	Japan	*Mustela sibirica itasi*	AB181157.1	Ando et al. (2006)
G. hispidum	China	*Sus scrofa*	AB181158.1	Ando et al. (2006)
G. binucleatum	Ecuador	*Rhamdia cinerascens*	AB181159.1	Ando et al. (2006)
Anisakis simplex	Copenhagen	*Clupea harengus*	JX237370.1	(Bahlool et al. 2012)

[1] Shamsi S, Briand MJ, Justine J-L. Occurrence of *Anisakis* (Nematoda: Anisakidae) larvae in unusual hosts in Southern hemisphere. Parasitology International. 2017;66:837-40.

delTest 2.0 [14]. The analysis was run for 2,000,000 generations with the standard deviation of split frequencies lower than 0.05. The phylogenetic tree was visualised using Figtree [15].

3. Results

Of all the fish examined, gnathostomid larvae were found in only two species, *Acanthopagrus australis* and *Rhabdosargus sarba*, both belonging to the family Sparidae (Table 3). Interestingly, the infected fish were not infected with any other parasite. Gnathostomid larvae were found within the digestive tract and encapsulated within the mesenteric tissues of the infected fish. Larvae were gently excised from the surrounding thick membrane. Morphological examination revealed the specimens as third stage larvae, with an unarmed body, finely striated cuticle and a bilaterally symmetrical mouth compartment (Fig. 1). The following morphometrics and morphological examination allowed their identification as *Echinocephalus* larva. Nematode larvae possessed two medium sized pseudolabia, orientated dorsoventrally with paired papillae, equal in size, wider 0.05 (0.03–0.07; n = 3) than the length 0.03 (0.02–0.04; n = 3), with smooth posterior-lateral edgings at either side to the anterior extremity of the oesophagus, a prominent cephalic bulb armed with six transverse rows of spines, 0.20 (0.017–0.24; n = 11) long, 0.29 (0.20–0.35; n = 11) wide, each cephalic spinal row separated by a ring of unarmed cuticle, with rows slightly more compact towards its anterior and maximal separation observed towards the midline and posterior regions, and uncinate cephalic hooks, larger in size towards the posterior rows. Characteristic of their development, hook sizes exhibited from the anterior extremity were as follows; the first two rows were similar with small sized spines while the third, fourth and fifth rows exhibited an enlargement of hook sizes per posterior row and the final (sixth) row was similar sized to the fifth row. The maximum spinal tooth size was 0.03 (0.02–0.04; n = 20) long. The cephalic region contains four ballonets. A cuticular collar is present. The oesophagus is muscular, 2.13 (1.28–2.78; n = 8) long and expands in width posteriorly. The maximum width is 1.48 (0.94–2.25; n = 4) and rounded posteriorly. Four cervical sacs are present and attached at the anterior to ballonet structures. The length of the adjoined ballonet and caudal sacs from the anterior extremity is 1.43 (0.89–2.12; n = 7), and approximately 0.08 (0.06–0.09; n = 7), respectively. The nerve ring is located 0.39 (0.30–0.45; n = 11) from the anterior extremity, occurring within the first 5–7% (n = 9) of total body length. The larval tail is tapered to a sharply pointed terminus. The tail length and maximum width is 0.23 (0.13–0.36; n = 8) and 0.13 (0.10–0.16; n = 9), respectively. The cuticle of the tail is strongly annulated and accounts for 2.89% (1.79–4.40%; n = 8) of total body length. Representative specimens from each species of fish were selected to obtain the ITS sequences. The sequences (GenBank accession numbers MW136167-MW136169) were identical except for one polymorphism in alignment position 165 (Table 4). A comparison of the sequence data showed that up to 58% of pairwise genetic difference can be found between *Gnathostoma* spp. and *Echinocephalus* spp. (Table 4). Similarly, the base pair (bp) difference between other genera (*Gnathostoma* spp. and *Spiroxys* spp.) of the family Gnathostomidae was significant (Table 4). The phylogenetic tree (Fig. 2) showed that all specimens in the present study formed a distinct group with 100% branch support and were significantly different from *Gnathostoma* spp. and other gnathostomid nematode sequences that were available in the GenBank.

4. Discussion

All gnathostomid larvae in edible fish examined in this study were in the third developmental stage, which is the infective stage of these nematodes. All specimens were covered in a thick membrane, which did not look like a worm to untrained eyes. Morphologically, *Echinocephalus* larvae can be differentiated from *Gnathostoma* larvae based on the number of rows of hooks on their anterior (being six [16–18] and three to four [19–22], respectively). Additionally, the tail is pointed in *Echinocephalus* larvae [16,17], whereas the tail is rounded in *Gnathostoma* larvae [21,22]. Sequence data generated in our study and the phylogenetic analysis supported the distinction of *Echinocephalus* larvae found in the present study from *Gnathostoma* spp. Future research on the suitability of other DNA regions, such as 18S rRNA and 28S rRNA, for detailed differentiation between gnathostomid nematodes, would be of value. Due to the absence of comparable sequences belonging to *Echinocephalus* spp., it was not possible to identify these larvae to a species level; however, their sequences will provide a useful tool for their identification in the future when well identified adults are genetically fully characterised. *Echinocephalus* comprises 12 species (World Register of Marine Species; http://www.marinespecies.org/aphia.php?p=taxlist; sighted 12/09/2020), which according to Hoberg et al. [23] only infect marine and freshwater stingrays. Of taxa belonging to the family Gnathostomidae, *E. overtreeti* and *E. sinensis* were recovered from Australian eastern waters. However, there is no known morphological criteria which reliably distinguish the larvae 191 of *E. overstreeti* and *E. sinensis* [5]. In recent years, there has been a growing global health concern relating to fish-borne zoonosis [24]. However, in Australia, awareness about seafood-borne parasites among health practitioners, the public and authorities in charge of seafood safety protocols is low [25–27], with no standard diagnostic test available [4]. The findings of the present study support previous doubt about the diagnosis of Gnathostomiasis caused by *Gnathostoma* larvae [2]. In addition, this study highlights the need for reliable diagnostic tests for seafood-borne parasites occurring in Australian waters, many unique to Australian marine species, rather than relying on tests developed overseas with unknown specificity and sensitivity. Many cases have probably gone undiagnosed due to a lack of awareness and unreliable diagnostic tests. As a result, it is not known whether the two genera cause different symptoms in infected patients and/or whether treatment should be different. Considering the popularity of seafood in Australia and increased exotic cuisines that include serving raw and undercooked seafood, it is important to develop standard diagnostic tools specifically for the

Table 3
An outlined taxon of selected commercial fish species (Order: Perciformes) from Moreton Bay and the examination number within this study.

Family	Scientific name	Common name	No. of fish examined	No of *Echinocephalus* larvae in the infected fish	Total no of *Echinocephalus* larvae found in the infected fish
Mugilidae	*Mugil cephalus*	Sea mullet	35 (0)	0	0
Sparidae	*Acanthopagrus australis*	Surf Bream	66 (3)	3, 1, 2	6
	Rhabdosargus sarba	Gold-lined Sea Bream	29 (2)	11, 3	14
Teradontidae	*Arothron hispidus*	White spotted puffer	10 (0)	0	0
	Arothron manilensis	Narrow Lined puffer	9 (0)	0	0
Triacanthidae	*Tripodichthys angustifrons*	Yellow Fin Tripod Fish	14 (0)	0	0
Total			163		20

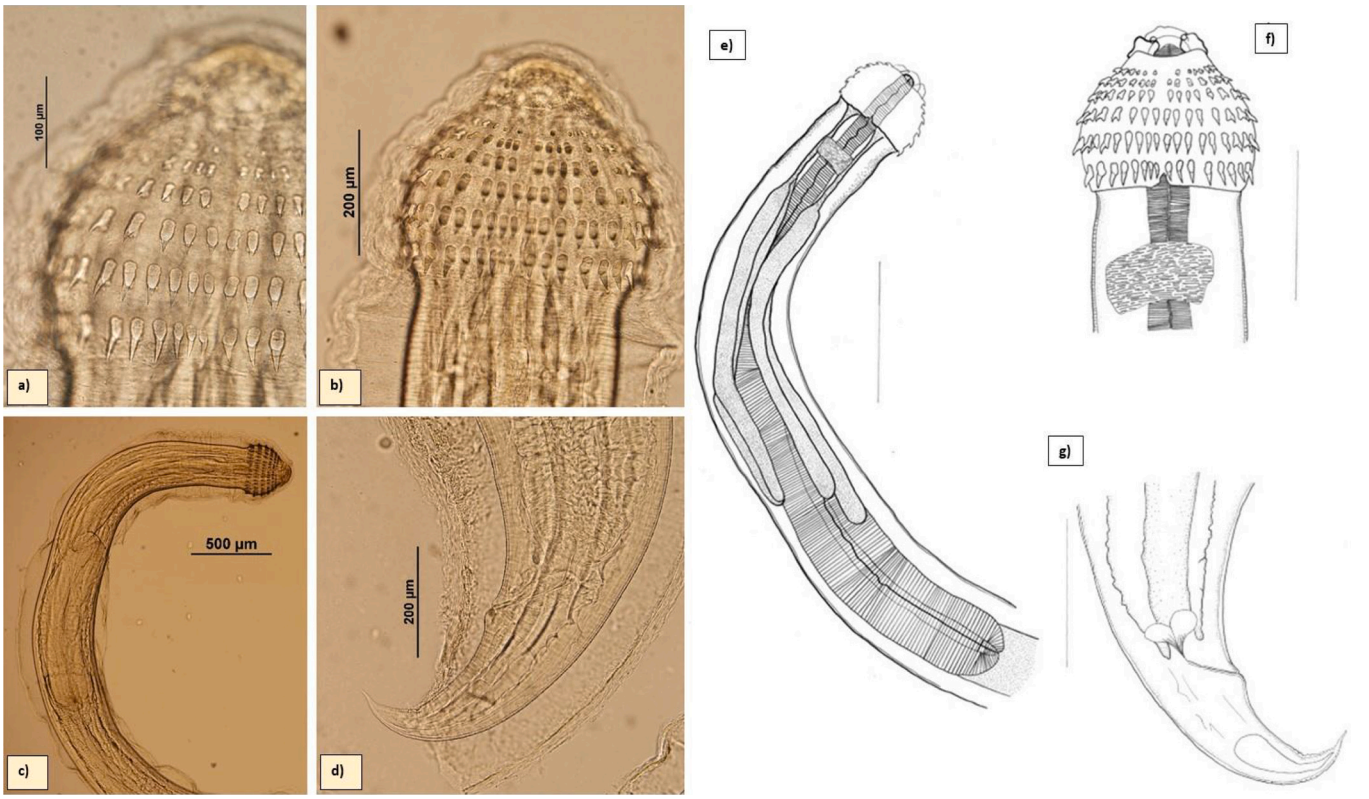

Fig. 1. Light microscopy images (a to d) and line drawing (e to g) of the *Echinocephalus* larvae found in the presents study; a, b and e) cranial part of the parasite, including cephalic bulb, pseudolabial structure and spinal rows; c and e) anterior features; d and g) posterior end exhibiting tapering of tail and fine tip; Scale bars; 200 µm in e and g and 100 µm in f.

Table 4
Pairwise genetic distance matrix of the ITS regions obtained from this study compared to other ITS sequences of Gnathostomidae species from GenBank, alignment gaps are pairwise deleted for analysis, shown as p-difference (%) (above the diagonal) and number of differences (below the diagonal). *Anisakis simplex* is used as an outgroup.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Present study (MW136169)		0.18	0.00	56.85	55.90	57.46	57.84	56.31	48.30	48.09	48.09	48.09	48.09	51.22	54.49
2	Present study (MW136168)	1		0.18	56.85	55.90	57.65	58.03	56.31	48.30	48.09	48.09	48.09	48.09	51.22	54.49
3	Present study (MW136167)	0	1		56.85	55.90	57.46	57.84	56.31	48.30	48.09	48.09	48.09	48.09	51.22	54.49
4	*G. spinigerum* (AB181155)	307	307	307		11.43	19.61	16.86	16.59	45.92	45.92	45.48	45.48	45.66	48.91	46.85
5	*G. binucleatum* (AB181159)	303	303	303	114		18.04	13.30	11.93	46.62	46.62	46.18	46.18	46.36	48.76	47.29
6	*G. doloresi* (AB181156)	308	309	308	193	175		12.19	20.70	46.50	46.65	46.36	46.36	46.10	48.25	46.93
7	*G. hispidum* (AB181158)	306	307	306	161	125	118		16.63	47.08	47.08	46.79	46.79	46.83	48.39	45.13
8	*G. nipponicum* (AB181157)	308	308	308	175	120	206	161		46.38	46.38	46.23	46.23	46.12	48.06	47.73
9	*S. japonica* (KF530321)	227	227	227	315	317	319	323	320		0.71	0.85	1.00	0.14	28.08	41.74
10	*S. japonica* (KF530322)	226	226	226	315	317	320	323	320	5		1.28	1.42	0.57	28.68	41.90
11	*S. japonica* (KF530324)	226	226	226	312	314	318	321	319	6	9		0.14	0.72	27.93	41.44
12	*S. japonica* (KF530325)	226	226	226	312	314	318	321	319	7	10	1		0.86	27.79	41.44
13	*S. japonica* (KF530323)	226	226	226	310	312	313	318	315	1	4	5	6		27.48	41.58
14	*S. hanzaki* (KF530326)	251	251	251	337	333	330	331	335	189	193	188	187	183		42.84
15	*Anisakis simplex* (JX237370)	273	273	273	350	349	344	329	358	273	274	271	271	269	281	

diagnosis of Australian gnathostomids (i.e., *Echinocephalus* spp.). Accurate diagnosis gives an opportunity to develop specific treatment for these zoonotic parasites.

All fish examined in the present study are popular edible fish and only from the east coasts of Australia. Identifying the extent of infection with zoonotic larvae among other edible fish and from other regions are another areas for urgent investigation in thefuture. The recently reported human cases in Australia were due to locally acquired parasites [2,28,29] where infection occurred after the consumption of recreationally caught fish. Nevertheless, this has raised concerns for the biosecurity of Australian seafood and the occurrence, prevalence and detection of zoonotic nematodes within commercial fish species from Australian coastal waters [30]. Fish parasite infections are not only significant for their adverse health reactions, they are also important economically. Parasites can affect the quality of seafood, particularly when they are found within the musculature of fish. This may lead to serious financial losses within the Australian fisheries industry. Moreover, nematode infection can affect the health of their teleost host. The symptoms and severity of parasitic infection in fish are variable and dependent on several factors, including the host (age, previous health issues), the parasite in question, the organ(s) they infect, the intensity of host infection and the extent of tissue destruction and nutrient loss [31]. However, many zoonotic parasites may cause no evident signs of diseases or symptoms in aquatic organisms, making their presence difficult

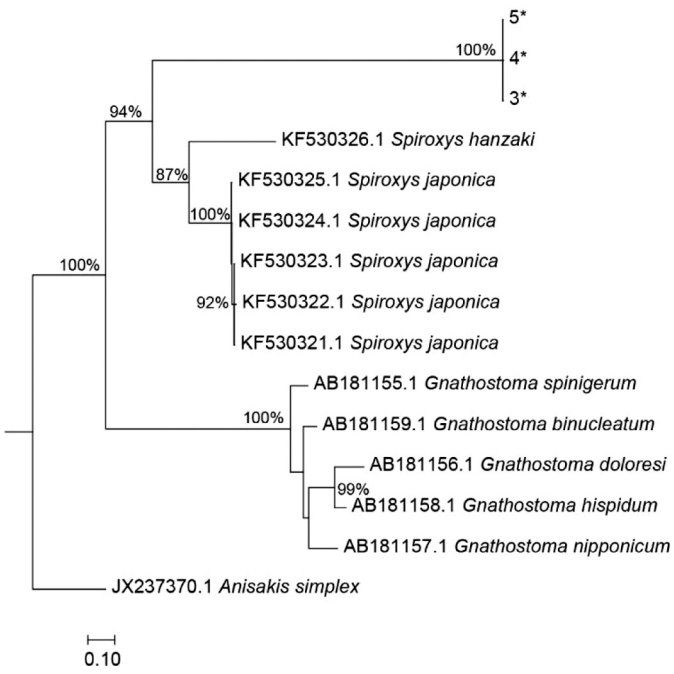

Fig. 2. Bayesian phylogenetic analysis using ITS sequences obtained from this study (marked with *) and sequences from GenBank. Branch supports (>90%), shown as posterior probabilities, are indicated on the branches. Numbers 3 to 5 corresponds to MW136167-MW136169, respectively, obtained in the present study.

to detect, especially if the larvae are small and few in number [32]. Although aquatic nematodes have a worldwide distribution, little is known about their lifecycles and ecology, particularly within Australia. Therefore, future studies are essential to determine the full distribution, range of hosts and prevalence of aquatic nematodes within Australian teleost species. Furthermore, mechanisms affecting intermediate host infections should be investigated through concerted efforts by all relevant stakeholders to minimise the economic losses and health risks associated with this zoonotic group of parasites [33].

Authors' contributions

S Shamsi: Team leader; study design; fish examination; parasite collection; DNA examination; data analyses; writing the manuscript.

E Steller: Parasite preparation; morphological examination.

X Zhu: Molecular work; phylogenetic analyses.

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Ethical approval

All applicable institutional, national and international guidelines for the care and use of animals were followed (AEC approval number SBS/248/15/ABRS/ARC). Most fishes examined during this study were obtained from a commercial fishery; those not obtained from this source were collected under Queensland General Fisheries Permit 187264.

Declaration of Competing Interest

None.

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