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Occurrence and characterisation of *Eustrongylides* species in Australian native birds and fish

Shokoofeh Shamsi [a,*], Nidhish Francis [a], Juliet Masiga [a,e], Diane P. Barton [a], Xiaocheng Zhu [a,b], Luke Pearce [c], Matthew McLellan [d]

[a] *School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Wagga Wagga, Australia*
[b] *NSW Department of Primary Industries, Wagga Wagga Agricultural Institute, Wagga Wagga, NSW, Australia*
[c] *NSW Department of Primary Industries, Fisheries, Habitat & Threatened Species Unit, Freshwater Environment Branch, Australia*
[d] *NSW Department of Primary Industries, Fisheries and Aquaculture Management, Narrandera Fisheries Centre, Narrandera, NSW 2700, Australia*
[e] *Kenya Veterinary Vaccines Production Institute(KEVEVAPI), Road A off Enterprise Road, Nairobi, Kenya*

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ABSTRACT

In Australia, nematodes belonging to the genus *Eustrongylides* were believed to be endemic species until the late 20th century when they were all considered to be *E. excisus*, invalid or *inquirendae*. Although these nematodes have frequently been reported in Australian fish, reptiles, and birds and cause disease or mortality among them, there has been no attempt to date to characterise them genetically. Globally, also, no one has validated or defined suitable genetic markers to distinguish between species of *Eustrongylides*. In this study, adult *Eustrongylides* from little black cormorant (*Phalacrocorax sulcirostris*; $n = 3$) and larvae from mountain galaxias (*Galaxias olidus,* $n = 2$) and a Murray cod (*Maccullochella peelii*, $n = 1$), and a Murray cod-trout cod hybrids (*Maccullochella peelii* x *Maccullochella macquariensis*, n = 1) were available for morphological examination and molecular characterisation. The adult nematodes from cormorants were identified as *E. excisus*. Sequences of the 18S and ITS regions were then obtained for all nematodes, which were identical among all specimens (larvae and adults) and also identical to those of *E. excisus* available in the GenBank. However, only one base pair difference exists between the 18S sequences of *E. excisus* and *E. ignotus,* with limited sequences available in GenBank accompanied with proper morphological data for the nematodes. With that limitation in mind, identifying our specimens as *E. excisus* suggests spill-over – that it is an introduced parasite species that has successfully established its life cycle among Australian native species – may have occurred. Our study is the first report of *E. excisus* in the little black cormorant, *P. sulcirostris.* Our results do not exclude the possibility of the occurrence of other species of *Eustrongylides,* either native or exotic, in Australia. This parasite is zoonotic and with increasing demand for fish and changing dietary preferences, such as the consumption of raw or undercooked fish, its occurrence in the flesh of the fish is concerning. This parasite is also associated with anthropogenic habitat alteration affecting the reproductive success of the infected hosts. Therefore, awareness among the relevant authorities of the presence of the parasite in Australia and its adverse impact on native animals is crucial for the success of conservation plans such as fish recovery and relocation efforts.

* Corresponding author.
E-mail addresses: sshamsi@csu.edu.au (S. Shamsi), dibarton@csu.edu.au (D.P. Barton).

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1. Introduction

Introduced pathogenic parasites have long threatened Australia's biodiversity. Unlike research and investment on introduced parasites affecting farming systems, our knowledge of introduced parasites that impact wildlife in Australia is scarce (Spratt and Beveridge, 2019). Australia's inland has unique biodiversity that needs to be protected from the harm of introduced parasites. However, the impacts of invisible parasites in establishing biosecurity guidelines and protection efforts, such as the relocation of threatened species, have been largely overlooked or underestimated due to an absence of parasitologists in decision-making (Anantanawat et al., 2012; Harnwell, 2019; Timi and Poulin, 2020). Recent studies suggest that parasites harmful to terrestrial and freshwater animals still bypass Australia's strict quarantine controls and enter the country through the importation of commodities and live animals (Trujillo-González et al., 2018; Shamsi et al., 2020; Zhu et al., 2021; Williams et al., 2022). Recently we showed that a number of introduced parasites entered the country and now are established in Australia, infecting native and protected species. For example, the European tongue worm, *Linguatula serrata*, was found in red-necked wallabies and spotted-tail quolls (Barton et al., 2020; Barton et al., 2021), and anchor worm, *Lernaea cyprinacea*, was found in wild Murray cod (Zhu et al., 2021).

Nematodes belonging to the genus *Eustrongylides* have been spread to all continents (Moravec, 2008; Menconi et al., 2020). They have a vast geographical distribution and complex, indirect life cycles (Anderson, 2000). Definitive hosts are piscivorous birds, with aquatic oligochaetes and fish being the first and second intermediate hosts, respectively. Mortality due to infection has been reported in both fish and bird hosts (Wiese et al., 1977; Sutherland et al., 2018). *Eustrongylides* spp. are also of zoonotic importance (Eberhard and Ruiz-Tiben, 2014), causing symptoms such as gastritis and intestinal perforation (Deardorff and Overstreet, 1991; Rusconi et al., 2022). The genus *Eustrongylides* belongs to the Family Dioctophymatidae and has been reported from humans in Sudan as well as in the US after consuming raw fish (Eiras et al., 2018).

The taxonomic status of species within genus *Eustrongylides* has been subjected to constant change. In brief, in her revision, Measures (1988) considered three species within the genus as valid: *E. tubifex* (Rudolphi, 1819), *E. ignotus* Jägerskiöld, 1909, and *E. excisus* Jägerskiöld, 1909. However, the number of specimens examined was limited with considerable overlap among morphometrics (Supplementary Table 1). Although molecular taxonomy has been useful in determining the taxonomic status of numerous parasitic nematodes, no comprehensive sequence data is available to assist with the taxonomic status of *Eustrongylides* species. Therefore, the value of various DNA regions for the specific identification of these parasites is unknown.

In Australia, *Eustrongylides* spp. were believed to be endemic and distinct from those reported elsewhere in the world (Johnston and Mawson, 1941, 1944), all of which were subsequently considered to be *E. excisus*, invalid or *inquirendae* by Measures (1988). *Eustrongylides gadopsis* has been reported from a wide range of freshwater fish throughout South Australia and New South Wales, with scattered reports from Western Australia and Queensland (Supplementary Table 2). This species was originally reported as *Filaria sanguinea*, collected from *Galaxias scriba* (syn. *Galaxias maculatus*), from the Murray River in 1861 (see Johnston and Mawson, 1940). Johnston and Mawson (1940) described these nematodes as *E. gadopsis*, which was subsequently determined to be the larval stage for *E. phalacrocoracis*, reported from various species of cormorants (Johnston and Mawson, 1944), although host records for *E. gadopsis* were still being reported by Johnston and Mawson (1951). *Eustrongylides phalacrocoracis* Johnston and Mawson, 1941 has been reported from Black-faced cormorant cited in Johnston and Mawson (1944) referring to a 1942 paper, but none of their 1942 papers contains any record of the host species (Supplementary Table 2). *Eustrongylides galaxias*, described from *Galaxias olidus* from Adelaide by Johnston and Mawson (1940) was determined to be a synonym of *E. gadopsis* (Johnston and Mawson, 1944). Adult specimens of *Eustrongylides plotinus* were described from *Anhinga novaehollandiae* in northern Queensland by Johnston and Mawson (1941). None of these species has been genetically characterised. *Eustrongylides* larvae were also referred as "red worms of trout" in Australia and were considered the most common nematode infecting introduced fish in Australia (Ashburner, 1978). *Eustrongylides* spp. were found to infect the muscle and organs of trout and various Australian native fish in larval form and also been reported from a range of other hosts in the country, including birds and reptiles (Baird, 1861b; Baird, 1861a; Krefft, 1871; Linstow, 1899; Sweet, 1909; Young, 1939; Johnston and Mawson, 1940, 1941, 1942, 1944, 1945, 1947, 1951; Lake, 1957; Mackerras, 1962; Pollard, 1974; Beumer, 1976; Backhouse and Gooley, 1979; Mawson et al., 1986; Kennedy, 1995; McDowall, 2000; Chapman et al., 2006; Mulder and Smales, 2009; Mulder and Smales, 2015; Barton and Jones, 2018). Pathogenicity due to infection with *Eustrongylides* has been reported in both intermediate and definitive hosts in Australian native species (Kennedy, 2007; Sutherland et al., 2018). Pollard (1974) showed the inhibition of egg laying by female *Galaxias maculatus* when *Eustrongylides* larvae encysted in the ovary or vent.

To date, the specific identity of this highly pathogenic and zoonotic parasite has not been resolved. In this study, we provide morphological and sequence data for larvae and adult *Eustrongylides* from Australian native fish and bird species.

2. Materials and methods

2.1. Hosts

In, 2017, 18 little black cormorants, *Phalacrocorax sulcirostris*, were collected from the Narrandera Fisheries Centre, which is a part of the New South Wales Department of Primary Industries. The centre culls cormorants under licence from the New South Wales National Parks and Wildlife Service to protect fish stocks held at the centre. The birds had been frozen to preserve them for research purposes and were transported to the Parasitology Laboratory at Charles Sturt University and kept frozen until post-mortem examinations were performed.

In December 2019, two fish (mountain galaxias, *Galaxias olidus*) with visible lesions on the body surface were submitted to the Parasitology Laboratory at Charles Sturt University. The fish came from the New South Wales Department of Primary Industries sample

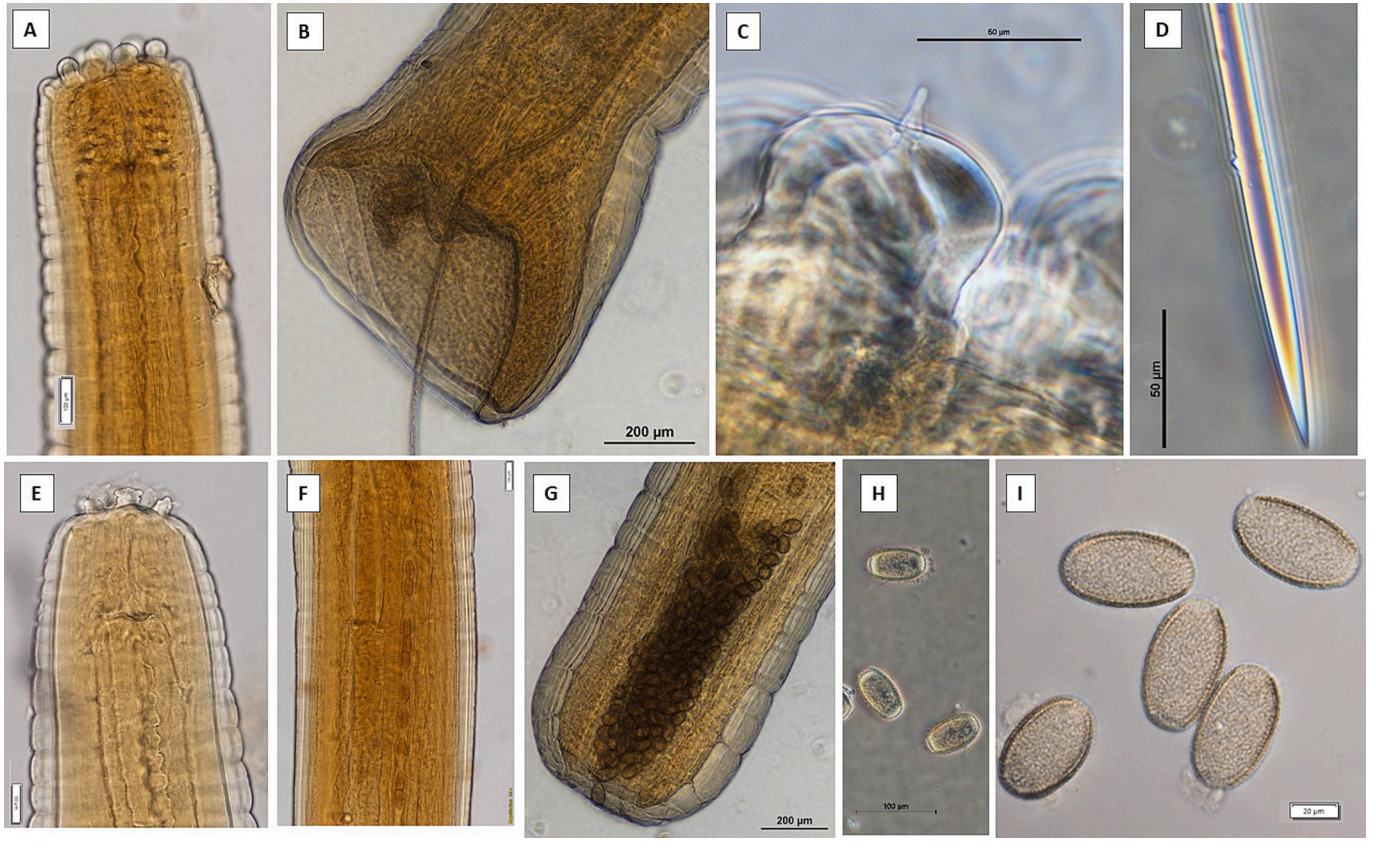

Fig. 1. Adult *Eustrongylides excisus* collected from cormorants in the present study. A anterior end of male nematode (lateral view), B Posterior end of a male nematode (ventral view), C magnified view of inner labial papilla, D tip of the spicule, E anterior end of a female nematode, F, lateral view of a female, uterus containing eggs, G posterior end of a female nematode, H and I eggs.

site called The Sink (−34.097660° 149.659160°) on the Retreat River, which is a tributary of the Abercrombie River north of Goulburn. It is an unregulated stream with a largely intact and protected catchment that is predominantly native forest and National Park, with smaller areas of pine plantations and cleared farmland used mainly for grazing livestock. Additionally, two fish (a Murray cod (*Maccullochella peelii*) and a Murray cod-trout cod hybrid (*Maccullochella peelii* x *Maccullochella macquariensis*) were collected in 2019 from the Cataract Dam, which forms part of the Sydney water supply network and is located on the escarpment above Wollongong. All animals were examined for parasites according to protocols described previously (Fernando et al., 1972; Shamsi and Suthar, 2016; Shamsi et al., 2021).

2.2. Parasites

All parasites were kept in 70% ethanol. After removing a small amount of mid body for DNA extraction, the rest of the body was placed on a slide and cleared in lactophenol (Shamsi et al., 2017) for morphological examination. After completion of the morphological examination, all specimens were deposited in Natural History Division, South Australian Museum, Adelaide (Accession numbers: AHC 49214 (ex cormorants), AHC 49213 (ex galaxids) and AHC 49215 (ex Murray cod and hybrid Murray cod x trout cod).

DNA extraction was performed using a DNeasy Blood and Tissue Kit (QIAGEN) according to the manufacturer's protocol and as modified by Shamsi et al. (2019). The 18S and ITS regions of nuclear ribosomal DNA were amplified using primer sets Sobo18s F and R (Koehler et al., 2009) for the former and primer sets 81_f & ITS2·S_r (Gustinelli et al., 2010) for the latter region. Samples that were found to be positive and of sufficient strength were sent to the Australian Genome Research Facility for Sanger sequencing. Sequence quality was checked using SeqMan v8.0 (DNASTAR). The consensus sequence (assembling from forward and 100 reverse reads) was built using the BioEdit software v7.2.5 (Hall, 1999).

Comparable sequences of closely related species were obtained from GenBank for phylogenetic analyses (Supplementary Table 3). The only available sequence in Australia was from imported fish (Williams et al., 2022) which was included in the tree. Sequences were aligned with the Clustal W program built in BioEdit (Hall, 1999), manually checked and trimmed according to the shortest sequence. Alignment gaps were excluded for analyses. Pairwise genetic distances, shown as a percentage of difference, were calculated using MEGA X (Kumar et al., 2018). A phylogenetic tree was built using comparable sequences (as much as possible from published work) of the taxa belonging to the family Dioctophymatidae available in the GenBank. The phylogeny of selected sequences was calculated using MrBayes 3.2 using the GTR + G model as suggested by jModelTest 2 (Darriba et al., 2012). The Markov chain Monte Carlo algorithm was run for 2,000,000 generations until the average standard deviation was lower than 0.005. The tree was visualised using

Fig. 2. A galaxid fish infected with larval stage of *E. excisus*, B whole larva, C posterior end of larval nematode (lateral view), D anterior end of larval nematode (lateral view).

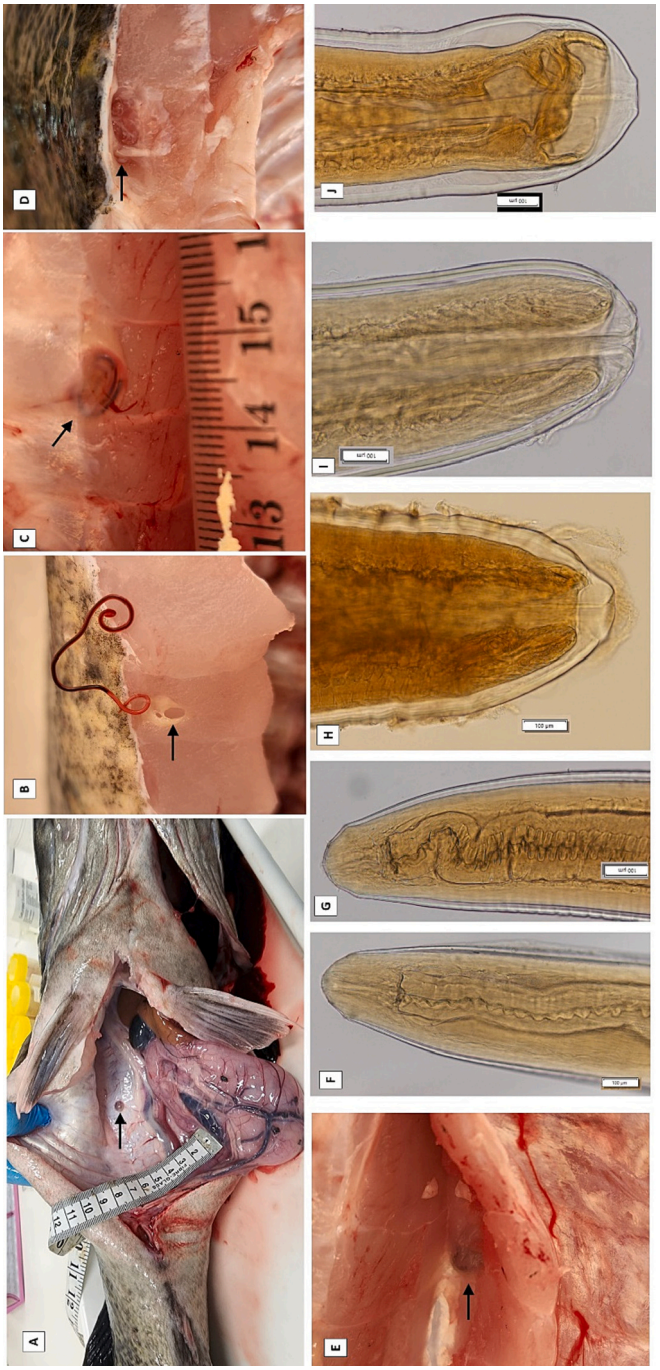

Fig. 3. Larvae of *E. excisus* from Murray cod: A) an encysted larva (see arrow) in the body cavity which was easily detectible, B) emerged larva from the flesh of the fish (see arrow; photo taken 12 h after refrigeration in 4 °C), C to E) coiled larvae in the flesh of the fish in various parts of the fish body (see arrows), F and G) anterior end of larvae, H to J) posterior end of larval nematodes. Larvae were found to be alive in refrigerated fish after 5 days in 4 °C.

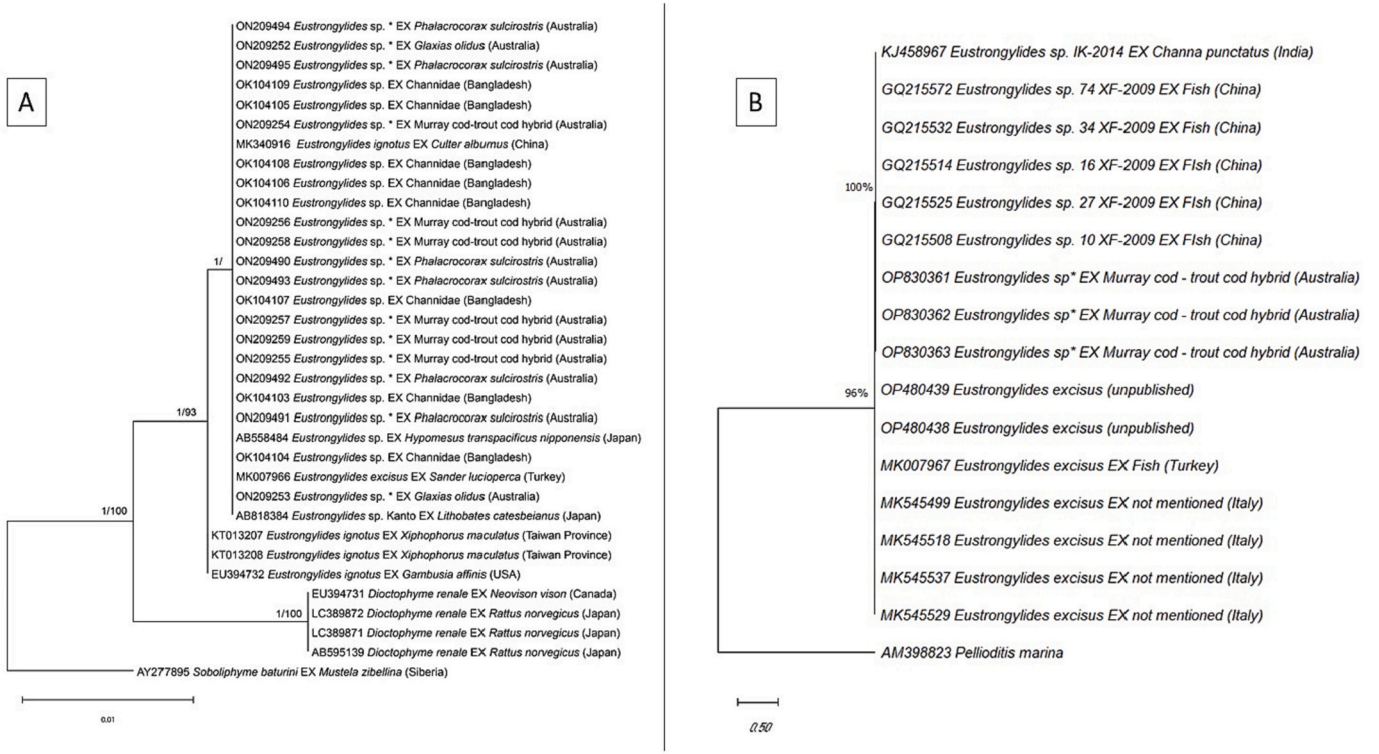

Fig. 4. Phylogenetic tree based on the sequences of the 18 S (A) and ITS (B) regions. GenBank accession number MK340916 has been labelled as *E. ignotus* in the GenBank, however it belongs to larval nematodes identified as *E. ignotus* based on its morphology and might be erroneous.

Figtree v 1.4.3 (Rambaut, 2014).

3. Results

Adult parasites (Fig. 1) were found in the three of 18 little black cormorants. The nematodes were found penetrating through the stomach wall with their anterior and posterior ends in the lumen of the stomach and also in the body cavity. The total number of nematodes found in one bird was 13, and the other two birds were infected with one nematode each. The nematodes were reddish brown. Of the nematodes found, only one was male. On the anterior end, double rings of 12 papillae (6 × 2) were counted with the inner papillae being larger. The posterior end of the single male specimen was expanded with a visible cleft. Bosses were present on the inner surface of the caudal bursa. The spicule was long with simple distal point (Fig. 1). An identification key was developed based on Measures (1988) as below, which led to their identification as *E. excisus*:

1.1)	Inner circle cephalic papillae smaller than outer circle cephalic papillae.	*E. tubifext*
1.2)	Inner circle cephalic papillae larger than outer circle cephalic papillae.	2
2.1)	Outer perimeter of posterior sucker in male nematode with wide cuticular hem; the inner perimeter lacks cuticular projections; the caudal sucker lacks ventral cleft.	*E. ignotus*
2.2)	Outer perimeter of posterior sucker in male nematode with reduced cuticular hem; the inner perimeter of sucker with row of cuticular projections; deep ventricular cleft.	*E. excisus*

We found two nematodes in each mountain galaxias examined (a total of four nematodes from this species of fish). We selected 3 and 4 nematodes from the flesh of the Murray cod and hybrid Murray cod x trout cod. The parasites in the fish examined in this study were found to be larval stage (Figs. 2 and 3). Morphologically, they only could be identified to genus level as *Eustrongylides* larvae. Some morphological variations were observed in the anatomy and size of the anterior end, cephalic papillae, oesophagus, and posterior end of larvae (Figs. 2 and 3). The Murray cod and the hybrid fish were heavily infected, with up to 75 and 82 larvae collected from them, respectively. Except for a couple of larvae that were found in the body cavity of the Murray cod, the rest of them were embedded deeply in the flesh of the fish. The latter larvae were missed during the visual examination and were accidentally found later (when disposing them) after about 12 h being kept in 4 °C refrigerator) when they started to emerge from the fish fillet.

Representative samples from each infected animal were selected for molecular analyses (Supplementary Table 3). At the time of conducting this study, sequences of the 18 s and ITS were available in GenBank. Sequences of the 18S region were successfully obtained for both larval and adult specimens collected from fish and birds in this study (Supplementary Table 3; accession numbers ON209252–60 and ON209490–5), whereas for the ITS region, we only managed to obtain sequences from three larval specimens from the hybrid fish (accession numbers OP830361–3). For each region, the sequences were identical among all specimens, suggesting larvae and adult nematodes in this study belong to the same species (Fig. 4). When the sequences obtained from the present study aligned with available sequences in GenBank, we found that our sequences are identical with sequences assigned to *E. excisus* in GenBank. Our sequences were also identical with a sequence (GenBank accession number MK340916), labelled as *E. ignotus* in the GenBank; however, accession number MK340916 belongs to larval nematode, identified as *E. ignotus* based on morphology (therefore, the identification is not reliable).

4. Discussion

In this study, we genetically characterised larvae and adult specimens of *Eustrongylides* nematodes which were identified to be *E. excisus*. *Eustrongylides excisus* is a cosmopolitan parasite that has been reported from all continents. The identification of nematodes in this study as *E. excisus* is of ecological significance and raises the question on its endemicity in Australia. On one hand, cormorants have been present in Australia for a long time, since the Pleistocene (Rich and van Tets, 1984). They also seem to be infected with parasites previously regarded as cosmopolitan (Mawson et al., 1986), including *Contracaecum rudolphii* sensu *lato* Hartwich, 1964 (Nematoda: Anisakidae) and *Paradilepsis scolecina* (Rudolphi, 1819) (Cestoda: Gryporhynchidae). These may lead to the simplest hypothesis for the presence of *E. exisus* in Australia that *E. exisus* arrived with cormorants in the distant past (since the Pleistocene). In Australia, the term "introduced" generally means a species that was not present before 1770, i.e., European contact/settlement (Thomson et al., 1989; Christides and Bowles, 2008). On the other hand, recent studies showed that *Contracaecum rudolphii* in Australian cormorants consists of genotypes which are distinct from the rest of the world (Shamsi et al., 2009; Zhang et al., 2009a; Zhang et al., 2009b; D'Amelio et al., 2012; Lin et al., 2013). Similarly, *Paradilepsis* larvae found in Australian native fish (Rochat et al., 2020) are distinct genetically from those reported in other continents reported by Scholz et al. (2018). Therefore, it is possible that *E. excisus* is an introduced parasite to Australia, and if so, then the parasite has successfully established its life cycle among Australian native species (spill-over). This then leads to the next question which is what was its route of entry to Australia?

It is difficult to determine the route of entry for this parasite into the country. An unknown species of *Eustrongylides* has been reported in fish in Japan and Papua New Guinea, which are destinations for migrating Australian cormorants (Llewellyn, 1983; Brandis, 2010; Ljubojevic et al., 2015). It might also be that like many other parasites, *E. excisus* has entered along with an imported fish to Australia, or through the entry of oligochaetes. Several species of fish have been introduced to Australia in the last couple of centuries. The first report of *Eustrongylides* in Australia was by Baird (1861b), in a single *Galaxias scriba* (syn. *Galaxias maculatus*), which died one week after arriving in the UK from Australia. However, as Baird (1861b) stated, it is possible that the infection occurred during or after importation. Other than the report by Baird (1861b), the oldest report of *Eustrongylides* in Australia that we could find is

E. gadopsis (now an invalid species) in *Galaxias maculatus* sent to Berlin from Adelaide (Linstow, 1899). Therefore, it is possible that introduced fish in Australia such as redfin perch (introduced to New South Wales in the 1860s) or carp (introduced about the late 1850s) were the first carriers of the parasite to the country.

It is noteworthy that our results do not exclude the possibility of the occurrence of other species of *Eustrongylides* in Australia. Since no *Eustrongylides* from South Australia, where most Australian *Eustrongylides* have been described, have been genetically characterised, it is unknown whether some native *Eustrongylides* may occur in Australia. It is also possible that in addition to *E. excisus*, other *Eustrongylides* spp. have entered the country. Mulder and Smales (2015) reported *E. acrochordi* (a species that has been considered as *E. excisus* by Measures (1988)) from the water python in Australia and argued that if *E. acrochordi*, the Australian species of *Eustrongylides*, is actually *E. excisus* then the species could have arrived in Australia from South-east Asia where the adults are known from piscivorous birds (Anderson, 2000). Although the parasite has been reported from birds in Australia, including *Phalacrocorax fuscens, P. carbo* and *P. melanoleucos* (Supplementary Table 2), our study is the first report of the occurrence of *E. excisus* in the little black cormorant, *P. sulcirostris*.

Correct identification of the parasite is central to establishing useful biosecurity measures; however, for *Eustrongylides* spp., a limited number of sequences for comparison are available in the GenBank, including sequences of the ITS region for *E. excisus* and sequences of the 18S region for both *E. ignotus* and *E. excisus*. The reliability of the latter region for specific identification of the *Eustrongylides* spp. remains doubtful since only one base pair difference and sometimes no base pair difference exists between the two species (Supplementary Table 4).

Globally, disease, pathogenicity, and fatality due to infection with *Eustrongylides* spp. have been reported in birds (Bowdish, 1948; Locke, 1961; Mazzone et al., 2019), reptiles (Mihalca et al., 2007), and fish (Paperna, 1974). In Australia, there is also a reported *Eustrongylides* fatality in an Australian darter (Sutherland et al., 2018).

Research in other parts of the world suggests that infection of fish with the parasite is associated with anthropogenic habitat alteration (Spalding and Forrester, 1993) with a serious impact on the reproductive success of the infected hosts (Wiese et al., 1977; Spalding et al., 1993; Spalding and Forrester, 1993). Spalding et al. (1993) reported that sites affected by nutrient pollution directly correlated with infected fish due to the increase of oligochaete intermediate host populations with the organic enrichment of the sediment (Weisberg et al., 1986; Coyner et al., 2003). Guagliardo et al. (2019) found larval *Eustrongylides* infecting the inner body wall and skeletal musculature of the caudal peduncle of *G. maculatus*, with complete commitment of muscle tissues. In the small galaxiid fish, such as *G. maculatus*, the parasite generates a conspicuous, strongly melanised cyst exposed to the exterior and complete disappearance of the musculature, which could lead to the fish becoming more easily recognisable prey for piscivorius birds (Coyner et al., 2001; Guagliardo et al., 2019). It may also compromise the swimming ability of the fish by affecting the muscle mass of the caudal peduncle (Guagliardo et al., 2019). In our study, Murray cod and hybrid Murray cod/trout cod were found heavily infected with *E. excisus* larvae. In the future, it would be interesting to study the histopathological impacts of the parasite on these fish. The emergence of the larvae from the encapsulation followed by migration to the musculature have been reported to cause muscle haemorrhage in high temperature conditions (Cooper et al., 1978).

Pollard (1974) reported the inhibition/prevention of egg laying by female fish due to larvae encysted either in the vent region or within the ovary itself as the most noticeable effect of the nematode larvae on their hosts. Larval *Eustrongylides* have been reported encysted in the inner body wall of *G. maculatus* from southwestern Australia (Chapman et al., 2006). In Western Australia, Lymbery et al. (2010) found *Eustrongylides* larvae more frequently in rivers with greater human use scores. In most of the study areas considered in this study and previous reports (see Supplementary Table 2), livestock and agricultural operations are common and are likely point sources of nutrient pollution. The collection location for the *G. olidus* in the present study, however, was largely native forest and National Park, although there is some agriculture and livestock production and pine forest plantations in the upper reaches. This demonstrates the potential reach of introduced parasites and that all areas should be considered when surveying for parasites. As there is rapid development of agricultural activities or urbanization in many rural areas of Australia (https://www.statista.com/statistics/260498/degree-of-urbanization-in-australia/#:~:text=Since%20the%201960s%2C%20Australia; sighted 11/07/2022), native species would be highly vulnerable to epizootic mortality such as has occurred elsewhere in the world, for example in the USA (Spalding and Forrester, 1993). Therefore, awareness of the presence of this parasite in Australia and its potentially adverse impact on native animals is important for the success of risk assessments and conservation plans such as fish relocations, particularly as the full life cycle of this parasite is not understood in Australia. Nothing is known about the first intermediate hosts (the invertebrates responsible for fish infection) in the country and therefore fish relocation can have detrimental impacts on Australia's native species and public health by extending the distribution of the parasite in the country. This parasite is difficult to detect in larger live fish and cannot be removed by usual bathing methods used to remove external parasites.

Further research is required to fully understand the life cycle of *Eustrongylides* in Australia and the factors that led to its emergence. This knowledge is essential to establish effective preventive and monitoring procedures to maintain public and native animals. Understanding the genetic make up of the *Eustrongylides* in Australia is also of high importance; however, there are no suitable genetic markers yet available for population genetic analyses.

To date, there has been several reports of human infection with *Eustrongylides* larvae (Eiras et al., 2018) after consuming sushi (Wittner et al., 1989), and eating raw minnow fish during fishing (Guerin et al., 1982; Eberhard et al., 1989; Narr et al., 1996). Except for one case, large worms were recovered surgically from the peritoneal cavity of the patients. There have also been report of cutaneous emergence of *Eustrongylides* from patients' skin (Eberhard and Ruiz-Tiben, 2014) in which the source of infection was not known. In Australia, where we found the parasites, there is no known reported case of *Eustrongylides* sp. related infection or fatality in humans (Shamsi, 2019), which could be due to a lack of awareness among medical doctors (Seal et al., 2020) and misdiagnosis (Shamsi and Sheorey, 2018). However, the increasing demand for fish and changing dietary preferences, such as the consumption of raw or

undercooked fish, are concerns (Sumner et al., 2015; Shamsi and Sheorey, 2018; Shamsi, 2020), particularly because this nematode inhabits the muscle of fish. Current protocols on seafood safety in Australia (Anantanawat et al., 2012; Authority, 2017) do not include many zoonoses (Shamsi, 2016), including *Eustrongylides* spp. Therefore, as previously pointed out, there is a need to assess the risks associated with consuming raw, undercooked or poorly prepared fish infected with these parasites (Ljubojevic et al., 2015; Food, Administration D, 2019; Shamsi, 2019).

Declaration of Competing Interest

The authors declare that they have no known competing financialinterestsor personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fawpar.2023.e00189.

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