Article

Parasites in Imported Edible Fish and a Systematic Review of the Pathophysiology of Infection and the Potential Threat to Australian Native Aquatic Species

Michelle Williams *[ID], Marta Hernandez-Jover [ID] and Shokoofeh Shamsi [ID]

School of Agricultural, Environmental and Veterinary Sciences & Graham Centre for Agricultural Innovation, Charles Sturt University, Wagga Wagga, NSW 2650, Australia
* Correspondence: miwilliams@csu.edu or shellparell@gmail.com

Abstract: Previous research has shown that certain types of edible fish imported into Australia are infected with *Euclinostomum*/*Isoparorchis* digenetic trematodes. In the present study, imported *Channa* fish were examined for parasites which were then morphologically identified to the lowest taxonomic unit possible. Here we provide the first Australian report of *Pallisentis* sp. Van Cleave, 1928 (Prevalence (P) 35.9%) of family Quadrigyridae; *Genarchopsis* sp. Ozaki, 1925 (P. 16.5%), family Derogenidae and *Senga* sp. Dollfus, 1934 (P. 4.8%) in edible imported *Channa* fish (n = 103). *Pallisentis* sp. and *Senga* sp. have invasive hold fast organs which cause significant mechanical damage to fish intestinal structures and *Euclinostomum*/*Isoparorchis* cause severe pathology and loss of marketability in infected fish. These exotic parasites, if introduced into Australia, have the potential to negatively impact the health, fecundity, resilience and marketability of native and commercial fish species. Biosecurity is a constant ontogenesis of novel hypothesis based on current scientific discoveries. To further increase understanding of how parasitism impacts fish health, a systematic literature review was conducted and the pathophysiology of infection described. Potential exposure pathways and parasite host associations in Australia are discussed.

Keywords: imported fish; Australian biosecurity; native aquatic species

1. Introduction

Fish-borne parasites introduced to regions where the parasite is unrecognized have the potential to inflict significant economic hardship on the commercial fisheries industry [1] through detrimental retardation of growth, fecundity and increased mortality [2–4]. Additionally, countries with phylogenetically homogenous native aquatic fauna to imported fish may be at high risk for co-introduction of parasites which may impact the health and survival of native aquatic species [5]. Transported invasive parasite species which become established in the environment are a high biodiversity risk across ecosystems for the emergence of aquatic diseases and decline in native animal populations [6,7].

It is generally accepted that the trade and release of ornamental fish into Australian waterways has been responsible for the introduction of many aquatic parasite species [5]; the cestode *Bothriocephalus acheilognathi* Yamaguti, 1934 syn. *Schyzocotyle acheilognathi* Brabec, Waeschenbach, Scholz, Littlewood & Kuchta, 2015 [8], monogeneans *Gyrodactylus bullatarudis* Turnbull, 1956, *G. macracanthus* Hukuda, 1940, *Dactylogyrus extensus* Mueller & Van Cleave, 1932, *D. anchoratus* (Dujardin, 1845) Wagener, 1857 [9] and the parasitic copepod *Lernaea cyprinacea* syn. *Lernaea cyprinacea cyprinacea* Linnaeus, 1758 are now established in many species of native Australian fish [10,11]. However, the risk posed by parasites in imported edible fish which, due to weaknesses in the supply chain, may find their way into the aquatic environment, is rarely considered (Figure 1). Australian biosecurity is considered exemplary and the nation's import conditions for edible fish stringent [12,13]. However, Australian importation commodity codes and trade data indicate that some fresh

or chilled fish for human consumption originates from countries with endemic infection of parasites alien to Australia [14,15] and many of these fish-borne parasites have been demonstrated to cause significant damage to fish and other aquatic or native species [16–20].
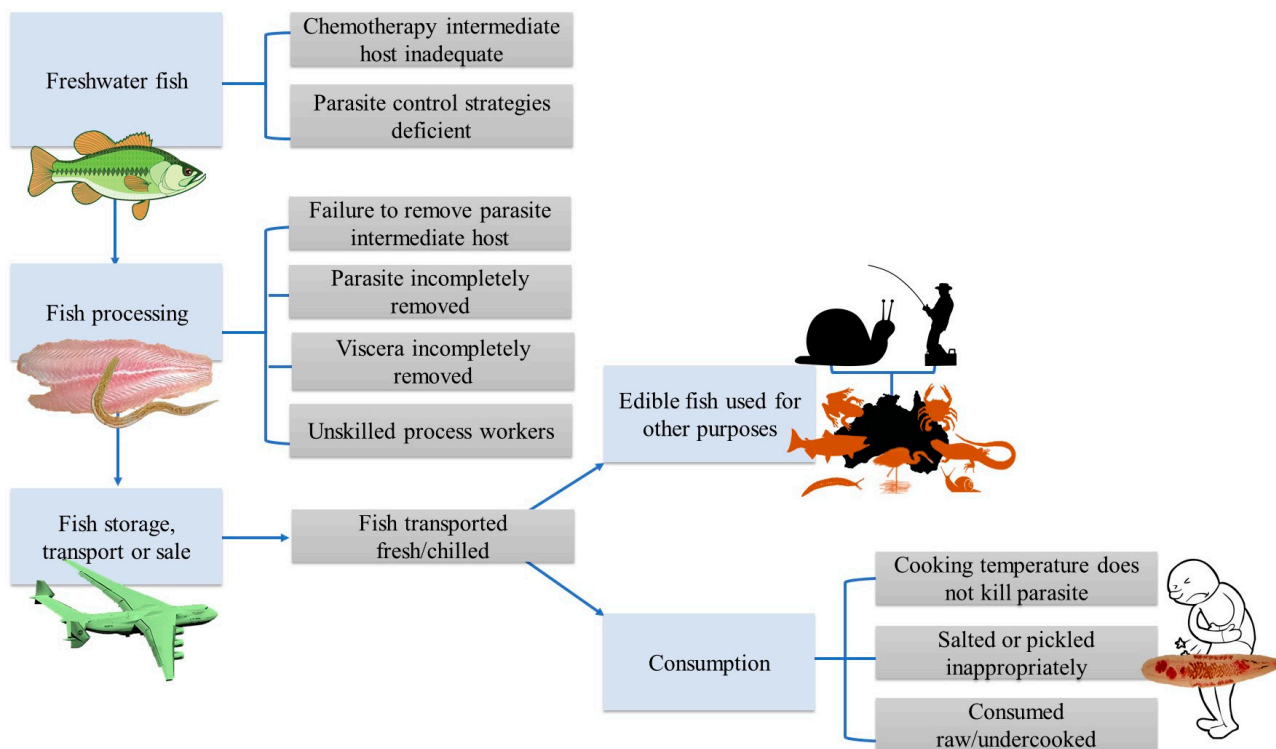

Figure 1. The fish supply chain, risk points and possible outcomes for Australian aquatic and native species. In light blue are the basic steps in the fish supply chain and in grey the risk factors at each step which may contribute to parasites or intermediate hosts of parasites being introduced into the Australian environment. Inadequate cooking at the consumption step may cause illness but in some cases parasites may mature in humans who may then introduce immature parasite stages into the environment. If infected fish are used for other purposes such as bait, in the presence of a suitable intermediate, paratenic or definitive host, parasites may successfully complete their lifecycle by involving native aquatic or other species.

Metacercariae of many parasitic flukes and larval nematodes can be very resilient. These infectious stages of a parasite can remain viable for long periods in refrigerated, salted, pickled and frozen fish products [21–26]. The introduction of white spot syndrome virus (WSSV) into Australia, from frozen imported edible shrimp [27], used by recreational fishers as bait or repackaged in Australia as bait [28], highlighted this possible but previously little-considered exposure pathway. It also provided a salient reminder of the social, environmental and economic repercussions associated with a biosecurity breach [29]. Although oxymoronic, the introduction of WSSV into Australia was a lesson in biosecurity needing to anticipate the unexpected.

In Williams, et al. [30] six predictor variables were used for a risk scoring system to identify countries which may be at high risk of seafood supply chain breaches. All countries scored were given an anonymous and unique numerical identifier and 200 parasites were collected from fish imported to Australia from Country 22 (Country 22 = unique numerical identifier). In Williams, et al. [31], zoonotic/potentially zoonotic parasites, *Isoparorchis* sp. Southwell, 1913 and *Euclinostomum* sp. Travassos, 1928 were identified from the 200 parasites previously collected. Non-zoonotic parasites in the present study were collected from *Channa* Scopoli, 1777 species fish originating from Country 22.

Channa species are freshwater predatory fish which are distributed from Asia to the Middle East [32]. Member species are an important source of dietary protein in many

countries and are commercially farmed and exported (food and aquarium trade) [33] from some regions. The preferred habitat of *Channa* species are ponds/ditches and, as adults, swamps, stagnant and muddy streams/water [34]. Due to the carnivorous and voracious predation of frogs, immature turtles and small fish/fish fry, *Channa* species are vulnerable to heavy intestinal parasite infection [35].

To further the understand the risk posed by parasites present in imported edible fish, the aim of the present study was to identify 'non-zoonotic' parasites from *Channa* fish collected in Williams, et al. [30] to the lowest taxonomic unit possible using morphological methodology. The secondary aim was to explore the pathophysiology of fish parasitism associated with non-zoonotic species identified in this study and *Isoparorchis* sp./*Euclinostomum* identified in Williams, et al. [31] through a systematic review of published literature. The tertiary aim was to explore and discuss the availably of suitable host species and the potential of each parasite to develop a successful lifecycle in Australia.

In the present study, we investigate parasitic helminths *Pallisentis* sp. (Acanthocephala: Quadriguyridae), *Genarchopsis* sp. (Trematoda: Derogenidae), *Senga* sp. (Cestoda: Bothriocephalidae), *Isoparorchis* sp. (Trematoda: Isoparorchiidae) and *Euclinostomum* sp. (Trematoda: Clinostomidae). The potential exposure pathways discussed include the availability of both suitable intermediate, if applicable, and final hosts in Australia according to the lifecycle of each parasite.

The purpose of this study was to identify non-zoonotic parasites from *Channa* fish imported into Australia, not to disadvantage the fish export market of any developing country. Therefore, information which may lead to the identification of any country is absent from this manuscript. This may include auxiliary tables, figures, fish species and citations. Citation omitted from this manuscript to maintain country confidentiality are indicated with (*).

2. Materials and Method

2.1. Parasite Collection and Preparation

Zoonotic/potentially zoonotic parasite species, *Isoparorchis* sp. and *Euclinostomum* sp. were previously identified in Williams, et al. [31] and non-zoonotic parasite species were identified in the present study. All non-zoonotic parasites were obtained from edible *Channa* fish (n = 103 individual *Channa* fish of the same species). The method for parasite collection and preparation is described in detail in Williams, et al. [30] and included the methods for fish examination found in Shamsi and Suthar [36] and the pepsin digestion method found in Bier, et al. [37]. Parasites were stored in 70% ethanol until morphological identification. In the present study, whole specimens were slide mounted in lactophenol and morphological examination followed descriptions in published literature.

2.2. Morphological Identification

The morphometric characteristics of importance were measured for selected specimens, using a calibrated eyepiece micrometre (BX-43 Olympus Microscope, Olympus Corporation, Tokyo, Japan) and compared with descriptions in published literature. Unless otherwise stated, all measurements are given in millimetres. Measurement ranges are given in length x width mm format or as length or width only. An Upright Motorized Microscope ECLIPSE Ni-E, Nikon, Tokyo, Japan was used for image capture of specimens.

2.3. Parasite Population Calculations

The prevalence (P), mean intensity (MI) and mean abundance (MA) of the parasites *Genarchopsis*, *Pallisentis* and *Senga* genera, described in the present study, followed calculations in Bush, et al. [38]:

P = (number of infected fish/total number of examined fish) × 100;
MI = (number of parasites/number of infected hosts);
MA = number of parasites/total number of examined hosts.

Parasite population calculations have been provided for parasite genera *Genarchopsis*, *Pallisentis* and *Senga* (Table 1). For *Pallisentis* (B) sp. 1, *Pallisentis (P) gomptii*, *Genarchopsis* sp. 1 and *Genarchopsis paithanensis* the number of parasites and the number of infected fish only have been provided (Table 1).

Table 1. List and number of parasites found in Channa sp. from Country 22. The total number of parasites found for *Pallisentis* (B) sp. 1, *Pallisentis (P) gomptii*, *Genarchopsis paithanensis* and *Genarchopsis* sp. 1 in this Table are included in and not additional to the total number of parasites found for *Pallisentis* sp. or *Genarchopsis* sp.

Fish and Number (N=)	Parasite Species	Site of Infection	No. of Fish Infected	Range in Infected Fish	Prevalence (%)	Total No. of Parasites Found	Mean Intensity	Mean Abundance
Channa species (n = 103)	*Pallisentis* sp. Van Cleave, 1928 in total	96 *Pallisentis (P)* identified intestinal mesentery and intestinal wall. One *Pallisentis (B)* embedded in fish musculature	37	0–11	35.9	97	2.62	0.94
	Pallisentis (B.) sp. 1 Amin, Heckmann, Nguyen, Pham & Pham, 2000		1			1		
	Pallisentis (P.) gomptii Gupta & Verma, 1980		5			7		
	Genarchopsis sp. Ozaki, 1925 total	Free in abdominal cavity	17	0–4	16.5	36	2.1	0.34
	Genarchopsis paithanensis Pardeshi & Hiware, 2012		3			3		
	Genarchopsis sp. 1		1			1		
	Senga sp. Dollfus, 1934	Intestinal lumen	5	0–1	04.8	5	1.0	0.04

2.4. Literature Search

For each parasite species *Pallisentis* (*Brevitritospinus*) sp., or *Pallisentis* (*Pallisentis*) sp., *Senga* sp. and *Genarchopsis* sp. identified in the present study and *Euclinostomum* sp. and *Isoparorchis* sp. identified in Williams, et al. [31], a literature search for English text articles was conducted via Google Scholar and Charles Sturt University (CSU) PRIMO search engines. Where full text articles were not available, CSU interlibrary loan was used to obtain a copy of the publication wherever possible. The Charles Sturt University PRIMO search engine maintains an account with all major scholarly journals. There were no time limitations for publications sourced. The search terms used for the literature search included: "each parasite name" AND histopathology OR pathophysiology OR histochemical. Results from review articles were excluded. For each paper, we compiled datasets on hosts infected, anatomical site of infection, geographic locality where hosts were identified infected and observations of the physiological consequences of infection in order to provide a general overview of the risks to fish health.

3. Results

3.1. Prevalence of Helminths in Fish

In this study, *Pallisentis* sp. (n = 97 in total), *Genarchopsis* sp. (n = 36 in total) and *Senga* sp. (n = 5 in total) parasites were recovered from 103 imported *Channa* species fish. All *Channa* fish examined in this study were the same species. Many of the parasite specimens

were damaged, therefore, provisional identification to a species level has only been made in some instances. Of the 97 *Pallisentis* sp., 7 females were identified as *Pallisentis (P) gomptii* (n = 7/97) and 1 adult male identified as *Pallisentis (B)* sp. 1 (n = 1/97). There were 36 *Genarchopsis* sp. in total identified, inclusive of 3 *Genarchopsis paithanensis* (n = 3/36) and 1 *Genarchopsis folliculata* (n = 1/36) herein identified as *Genarchopsis* sp. 1. A total of 5 cestodes were identified as *Senga* species.

Pallisentis sp. was the most prevalent parasite infecting *Channa* fish (P 35.9%), followed by *Genarchopsis* sp. (P 16.5%) and *Senga* sp. with a prevalence of 4.8% (Table 1). Adult and cystacanths of *Pallisentis* (*Pallisentis (P.)*) sp. were recovered. Cystacanths without exception were encysted in clear capsules attached to the intestinal mesentery. Adult *Pallisentis* (*P.*) sp. were all found with the proboscis embedded in the intestinal wall or intestinal mesentery. The proboscis of one adult male specimen of *Pallisentis* (*Brevitritospinus (B.)*) sp. was embedded in fish musculature. *Genarchopsis* sp. were found free in the abdominal cavity. All *Senga* sp. were obtained from the lumen of the intestine. The exterior colour of the tapeworms reflected the fish intestinal contents.

3.2. Morphological Identification of Helminths from Consumer Ready Channa Fish

3.2.1. *Genarchopsis* Species Ozaki, 1925, Family Derogenidae Nicoll, 1910, Class Trematoda

Genarchopsis Species General Observations

Many specimens were damaged and identification to a species level was not possible. A total of 36 specimens were identified as *Genarchopsis* species based on descriptions in Shimazu, et al. [39] and Urabe, et al. [40]. General observation of damaged *Genarchopsis* species are as follows: Specimens range in size and width between different species. The posterior is bluntly pointed. The acetabulum surrounding the ventral and oral suckers is muscular. Ventral suckers range from slightly post equatorial to situated at the anterior section of the posterior third of the body. The posterior body angles away at the ventral sucker from the anterior body by ~10–45° depending on the species. In general, the ventral sucker is approximately twice as long and one and a half times as wide as the oral sucker. Vitellaria number varies from one to four between species and occurs at the posterior end of the body.

Genarchopsis paithanensis

Three reproducing adult specimens (n = 3/36) were identified as *Genarchopsis paithanensis* based on descriptions in Pardeshi and Hiware [41]. *Genarchopsis paithanensis* (Figure 2A–C) are 1.5–3.85 mm in length. Width at the ventral sucker (VS) is 0.4–0.5 mm, at the anterior body 0.4–0.9 mm and the posterior body 0.25–0.32 mm. The oral sucker is 0.2–0.25 × 0.52–0.45 mm and the VS 0.4–0.62 × 0.37–0.55 mm. The body angles away by approximately 45° at the ventral sucker. The vitelline gland is a single heart-shaped granular mass and is situated at the posterior extremity of the body. The right arm of the vitelline gland is 0.05 × 0.03–0.035 mm and the left arm 0.045 × 0.035 mm. The uterus is distinctive and multi coiled. Egg is engorged from the anterior body above the VS. Mature eggs are oval in shape (0.04–0.05 mm long) and have a distinct polar filament. There are two testes (T) situated in the posterior third below the ventral sucker along the opposing sides of the lateral body line which are 0.08 × 0.09 mm (T1) and 0.08 × 0.08 mm (T2).
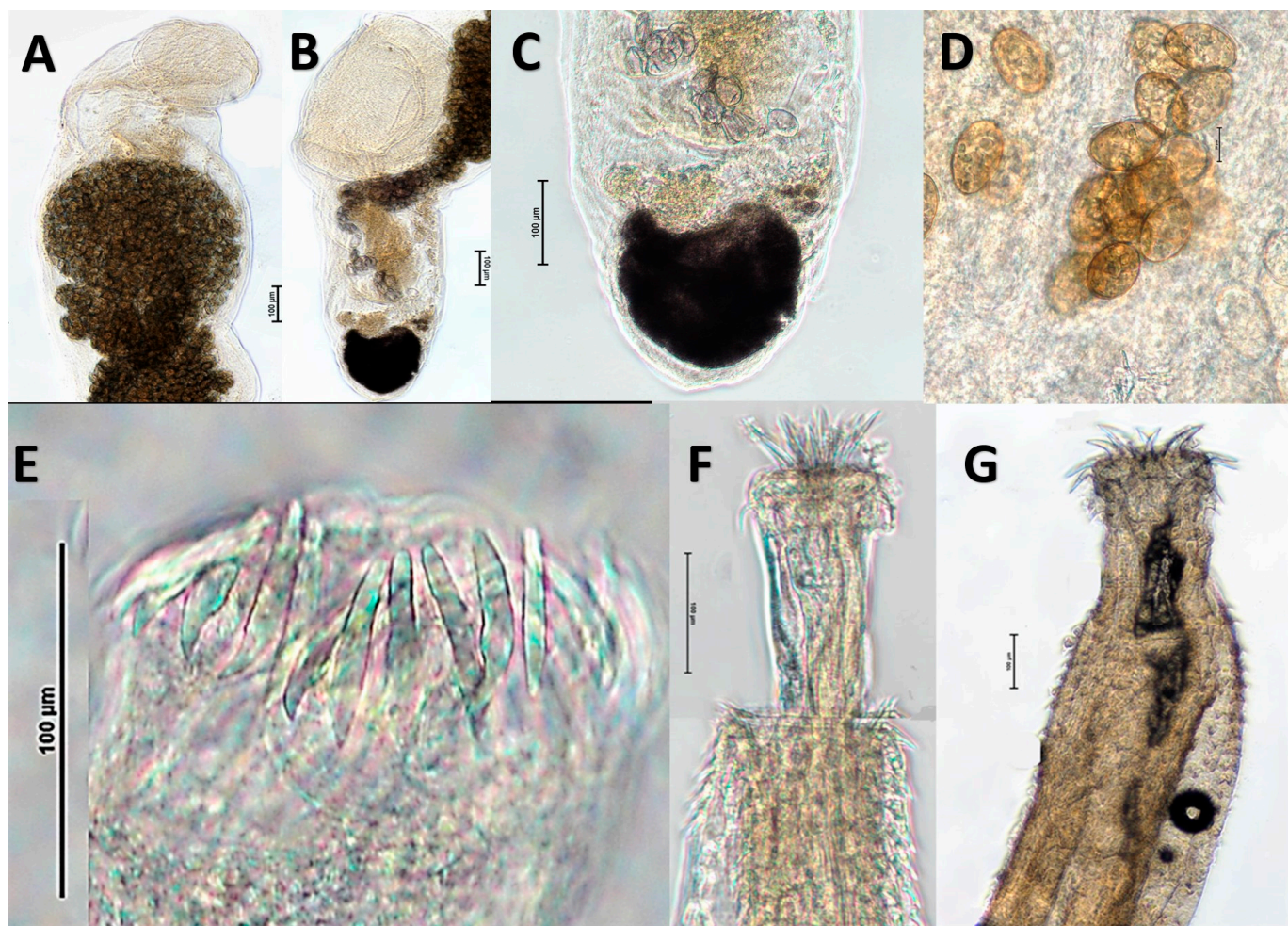
Figure 2. (**A**–**G**). **Parasites identified in imported fish.** (**A**–**C**) an egg-engorged mature digenean *Genarchopsis paithanensis*. The dark area in (**A**) and surrounding the ventral sucker in (**B**) is made up of hundreds of eggs. (**C**) heart shaped vitelline gland. (**D**) eggs in a mature segment of *Senga* sp. tapeworm. (**E**) shows the multiple invasive scolex hooks of *Senga* sp. which are situated in a circular crown on the scolex (**F**) the invasive proboscis hooks and collar spines of *Pallisentis* (*Brevitritospinus*) sp. and (**G**) *Pallisentis* (*Pallisentis*) sp.

Genarchopsis sp. 1

One reproducing adult specimen (n = 1/36) was close to descriptions for *G. folliculata* in Bhadauria and Dandotia [42] and identification was based on four vitelline glands which are of similar size; vitelline 1 (V1) (0.065 × 0.06 mm); V2 (0.66 × 0.05 mm); V3 (0.065 × 0.04 mm) and V4 (0.055 × 0.05 mm). Many other morphological features are indistinct or damaged. The specimen is 1.30 mm in length and 0.16 mm wide. The oral and VS are not able to be accurately measured. The uterus is multi coiled and contains many oval eggs (0.045 × 0.02 mm).

3.2.2. *Senga* sp. Dollfus, 1934, Family Bothriocephalidae Blanchard, 1849, Class Cestoda

Two reproducing adult specimens were identified as *Senga* sp. (Figure 2D–E) according to descriptions in Koiri and Roy [43], Majid and Shinde [44] and Pardeshi and Hiware [45]. The scolex is rounded and crowned with a single circle of 46–50 elongated hooks which attach to a short neck. Hooks are pointed at the posterior end and are 0.06–0.062 mm long and 0.01 mm wide. Mature proglottids are wider than long (0.15–0.22 × 1.0–1.5 mm). Testes are very small, round and evenly distributed in each segment. Eggs are numerous within the uterus and are oval with no operculate (0.045 × 0.022–0.025 mm). The cirrus

pouch is oval in shape. Three reproducing adult other specimens with damaged scolex were identified as *Senga* species based upon morphology of the undamaged scolex/hooks and the mature proglottids.

3.2.3. *Pallisentis* Species Van Cleave, 1928, Family Quadrigyridae Van Cleave, 1920, Class Eoacanthocephala

Ninety-seven individuals of *Pallisentis* sp. in total were recovered. Amin, et al. [46] established *Pallisentis* and *Brevitritospinus* as a subgenus of *Pallisentis* based on proboscis hook sizes. The armature of the proboscis of *Pallisentis* (*B.*) has posterior two rows of proboscis hooks approximately half as long as the hooks in the top two rows. The armature of *Pallisentis* (*P.*), however, has proboscis hooks which decline gradually in size from anterior to posterior. Selected specimens which were measured were allotted to either subgenera according to proboscis hook size or for damaged specimens, as *Pallisentis* sp.

Pallisentis (*Brevitritospinus*) sp. 1

One adult male specimen only (n = 1/97) in the present study was identified as belonging to *Pallisentis* (*B.*) (Figure 2F). The internal structures of this specimen were indistinct; however, all other features followed descriptions in Gupta and Verma [47] and Gautam, et al. [48] for *Pallisentis* (*B.*) *cavasii*. No female *Pallisentis* (*B.*) specimens were identified. The measurements for the male specimen in the present study are as follows: body 2.97 mm long, 0.25 mm wide at the collar, 0.27 mm at the anterior trunk and 0.10 mm wide at the posterior trunk. Proboscis is globular and first row of hooks are very robust and only slightly recurved with others appearing to be relatively straight. First (0.05 mm) and second (0.045 mm) row of proboscis hooks are close in length. Third (0.026 mm) to fourth (0.02 mm) row of hooks are similar in length and approximately half the length of hooks in the first two rows. The base of the proboscis hook appears deeply embedded in the proboscis wall. There are 15 collar spines with 10–11 spines per row and 0.015–0.02 mm distance between each spine. Collar spines measure 0.025 × 0.02 mm. There is a very small gap between the end of the collar spines and commencement of trunk spines (0.065 mm). Trunk spines are arranged in 18 rows and there are 14–20 spines per row which decrease at the posterior end of trunk to 2–3 spines. Trunk spines (0.025 × 0.01 mm) are forked at the proximal end and there is 0.02–0.025 mm between each spine.

Pallisentis (*Pallisentis*) *gomptii*

Seven adult female specimens (n = 7/97) were very close to descriptions in Gautam, et al. [48] and Gupta and Verma [47] for *Pallisentis* (*P.*) *gomptii* (Figure 2G). No male specimens were examined to confirm species identity. The body length range is 6.5–10.0. The body width range is 0.32–0.60 at the collar and 0.22–0.50 at the posterior trunk. Immediately following the collar, the width at the anterior body flares slightly (0.45–0.70). There are four circles of proboscis hooks with ten hooks per row. Hooks in the first row are 0.06–0.08, the second row 0.045–0.075, the third row 0.035–0.06 and in the fourth row 0.025–0.045. Collar spines are arranged in 14–15 rows with 15–17 spines per row. Collar spines are 0.02–0.04 in length and there is 0.02–0.09 distance between each spine. A spineless area (0.1–0.22) separates trunk and collar spines. Trunk spines are only conical in shape. There is no cuticular thickening at the base of proboscis hooks or trunk spines. Trunk spines do not extend to the posterior end of the trunk and are arranged in 60–70 rows each with 14–15 spines per circle. Trunk spines are 0.015–0.035 in length with 0.03–0.08 between each adjacent spine.

3.3. Literature Search Results

The literature search results have been included in Figure 3 and information obtained from the search has been included in Table 2 and the manuscript text.
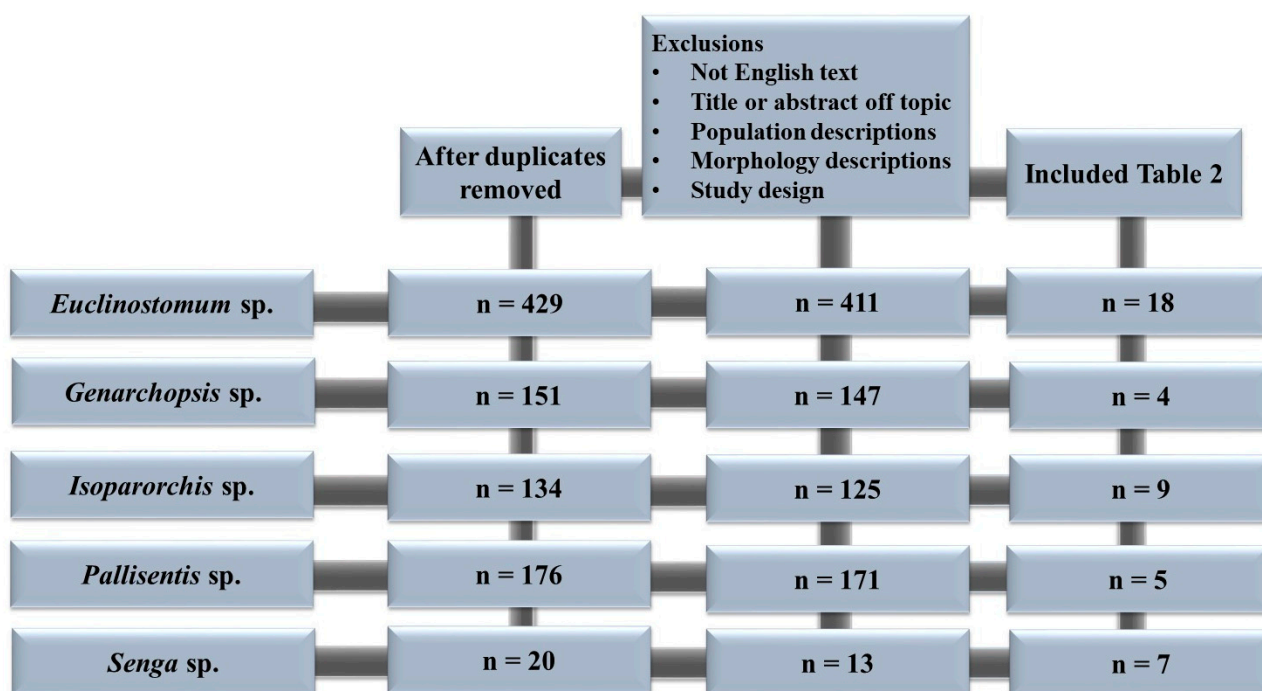
		Exclusions • Not English text • Title or abstract off topic • Population descriptions • Morphology descriptions • Study design	
	After duplicates removed		Included Table 2
Euclinostomum sp.	n = 429	n = 411	n = 18
Genarchopsis sp.	n = 151	n = 147	n = 4
Isoparorchis sp.	n = 134	n = 125	n = 9
Pallisentis sp.	n = 176	n = 171	n = 5
Senga sp.	n = 20	n = 13	n = 7

Figure 3. Literature search flow, exclusion criteria and results from the literature search which was conducted via CSU PRIMO which maintains an account with all major scholarly journals.

3.3.1. *Euclinostomum* sp., Travassos, 1928, Pathophysiology

Euclinostomum species of family Clinostomidae are haemophagic digenean parasites [49] which are able to infect an extensive species range [50]. *Euclinostomum* sp. feed on host blood both directly from the internal organs of the host as well as extracorporeally [49]. The pathogenicity to the host is dependent on the site where metacercariae encyst. Skin encystation has an irritating effect/thickens skin and fish are very likely to self-mutilate [21,50]. Encystation of the host muscle is associated with abnormal swimming behaviours and mass mortality [51,52]. Gross histopathological changes have been observed in the host kidney and liver when metacercariae encyst in these organs [1].

The teleostean kidney and liver are metabolically critical organs for gaseous exchange, excretion of toxins, hemopoiesis and osmoregulation [53]. In addition, the liver is important for metabolism of proteins, carbohydrates, lipids and functions to store glycogen, to catabolise fatty acids and synthesise amino acids [54]. It is considered the significant damage to both the liver and kidney resultant of parasitism would limit fish survival. In addition, impairment of hemopoiesis in conjunction with a very efficient haemophagic parasite may induce anaemia which has been demonstrated to impair fish growth, diminish health and increase mortality [55,56].

Table 2. **Previous reports of host parasitism and pathophysiology identified following literature review.** Fish pathophysiology column includes histopathology and any perceived or measured effects. N/D indicates not discussed.

Species of Parasite	Host Infected	Site of Infection	Geographical Locality	Fish Pathophysiology	Reference
Euclinostomum ardeolae El-Naffar & Khalifa, 1981	Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758)	Kidney	Egypt	Grey/black cysts	Ahmed, et al. [57]
Euclinostomum heterostomum (Rudolphi, 1809) Travassos, 1928	Spotted snakehead, *Channa punctata* (Bloch, 1793)	Liver, kidney & viscera	India	Heavily parasitized fish lethargic	Bhargavi, et al. [58]
Euclinostomum heterostomum	Mozambique tilapia, *Oreochromis mossambicus* (Peters, 1852)	High in muscle	Multiple locations, Venda and Lebowa, southern Africa	Loss of consumer confidence.	Britz, et al. [51]
Euclinostomum heterostomum	Redbelly tilapia, *Tilapia zillii* syn. *Coptodon zillii* (Gervais, 1848)	Body cavity, skin, eye	Opi Lake, Nigeria	Pronounced inflammation & roughened skin. Ex-cysted metacercariae associated damage from burrowing through host organs. Fish blindness, myositis, muscle bumps. Decreased fish marketability.	Echi, et al. [21]
Euclinostomum heterostomum	Redbelly tilapia *T. zillii* syn. *Coptodon zillii*	Skin	Nigeria	Co-infection with other clinostomatids causes cysts, ulcers, degeneration of skin/muscle, necrosis.	Echi, et al. [59]
Euclinostomum heterostomum	Striped snakehead, *Channa striata* (Bloch, 1793)	Body cavity, muscles, liver, gill opening, intestine, kidneys & ovaries	Bhopal, India	Fish with reduced glomeruli size, severe degeneration/necrosis of hemopoietic tissue and tubule cells with hypertrophied nuclei & epithelial cells detached. Occlusion of tubular lumen.	Kaur, et al. [60]
Euclinostomum heterostomum	Spotted snakehead, *C. punctata*	Liver	Bhopal, India	Degeneration & necrosis of liver tissue with enucleated hepatocytes.	Kaur, et al. [61]
Euclinostomum heterostomum	*Apistogramma ramirezi* syn. *Mikrogeophagus ramirez* (Myers & Harry, 1948)	Encysted skin	Imported from Hong Kong to Purdue University, USA	Irritating effect, rubbing against rocks & self-trauma.	Kazacos and Appel [50]
Euclinostomum heterostomum	Guppy, *Poecilia reticulata* Peters, 1859	Musculature	Chonburi Province, Thailand	Localised degeneration & necrosis where parasite present.	Laoprasert, et al. [62]
Euclinostomum heterostomum	Striped snakehead, *C. striata* and Spotted snakehead, *C. punctata*	Liver	N/D	Rupture & loss of hepatocyte distinct shape. Vacuolation of cytoplasm. Hypertrophy of hepatocytes. Perilobular space of liver shows vacuolation, loosening of hepatic tissue & necrosis.	Laxma Reddy, et al. [63]

Table 2. *Cont.*

Species of Parasite	Host Infected	Site of Infection	Geographical Locality	Fish Pathophysiology	Reference
Euclinostomum heterostomum	Redbelly tilapia, *T. zillii* syn. *Coptodon zillii*	Encapsulated mostly in peritoneum of the kidney some musculature	Nile, Giza governorate, Egypt	Parasite cyst wall merged with renal interstitium & glomerular structures. Intense inflammatory cells extending into surrounding renal tissue. Significant glomerular & interstitial congestion, tubular epithelium with haemorrhages, vacuolization & necrosis.	Mahdy, et al. [64]
Euclinostomum heterostomum	African catfish, *Clarias gariepinus* (Burchell, 1822)	Muscle	Buffeldoorn Dam & Seshego Dam Lebowa, South Africa	Heavy infestations likely to occur in ponds with high fish density if intermediate snail host is present. Loss consumer confidence.	Mashego and Saayman [65]
Euclinostomum heterostomum	Nile tilapia, *O. niloticus*	Kidney	Commercial markets Cairo & fisherman Kafr El Sheikh, Egypt	Thick fibrous area surrounding parasite. Degenerative renal tissue, tubules & congested blood vessels. Some sections showed changes to glomeruli and Bowman's capsule.	Mohamed, et al. [66]
Euclinostomum clarias (Dubois, 1930) Dollfus, 1932	African catfish, *C. gariepinus*	Liver	Nigeria	Hepatic degeneration, necrosis/fibrosis, inflammation of bile duct, severe damage result of larval migration.	Onucha [67]
Euclinostomum heterostomum	Wild caught croaking gourami, *Trichopsis vittata* (Cuvier, 1831), Siamese fighting fish, *Betta splendens* Regan, 1910 and crescent betta, *Betta imbellis* Ladiges, 1975	Musculature	Southern Thailand	Tubercle-like thickened areas on skin.	Pinky, et al. [68]
Euclinostomum heterostomum	Spotted snakehead, *C. punctata*	Liver, kidney, peritoneum, muscle, and ovary	Local fish market, Aligarh, North India	Tissue damage, infiltration immune cells cyst wall, chronic inflammation, granulomas. Liver degeneration hepatocytes, cytoplasmic vacuolation, nuclear alterations, mallory body formation, fibrosis, necrosis. Kidney distortion/dilation renal tubules, vacuolar degeneration, hypertrophy/hyperplasia tubular epithelial cells, occlusion tubules, fibrosis, haemorrhage, congestion glomeruli.	Shareef and Abidi [1]
Euclinostomum heterostomum	Guppy, *P. reticulata* cultured	Muscle	Kidchakan Supamattaya Aquatic Animal Health Research Center, Songkhla, southern Thailand	Abnormal swimming behaviour. Fish death severe infection.	Suanyuk, et al. [52]

Table 2. *Cont.*

Species of Parasite	Host Infected	Site of Infection	Geographical Locality	Fish Pathophysiology	Reference
Euclinostomum ardeolae	Nile tilapia, *O. niloticus*	Kidney	The Nile, Egypt	Cysts embedded kidney exerting pressure on tissue, black discolouration.	Tayel, et al. [69]
Isoparorchis hypselobagri (Billet, 1898) Ejsmont, 1932 (probably *Isoparorchis trisimilitubis*)	Wallago, *Wallago attu* (Bloch & Schneider, 1801)	Swim bladder	India	Infected fish unsuitable for human consumption. Patches of black pigments in the muscles and viscera of its hosts. Causes mortality and great economic loss. Adult parasites excrete poisonous metabolic substances within swim bladder. Ammonia is converted to urea. Urea high depending on parasite number.	Adak and Manna [70]
Isoparorchis hypselobagri (probably *Isoparorchis trisimilitubis*)	Wallago, *W. attu*	Swim bladder	India	Ammonia major excretory product. Amount of excreted ammonia differs depending on parasite number. Ammonotelic and ammonia can be formed by the action of several enzymes in *Schistosoma mansoni* as well.	Adak and Manna [71]
Isoparorchis hypselobagri (*probably Isoparorchis trisimilitubis* or *Isoparorchis* sp. 3)	Wallago, *W. attu*	Swim bladder	Dhaka, Bangladesh	Juvenile forms caused massive tissue damage, resulting erosions and tunnels in musculature, exudate, discoloration connective tissue, extreme melanisation, mixed inflammatory responses.	Alam [72]
Isoparorchis hypselobagri (probably *Isoparorchis trisimilitubis*)	Wallago, *W. attu*	Air bladder	Kakraiya lake, Jahangirabad, India	Inkspot disease.	Choudhary, et al. [73]
Isoparorchis hypselobagri (probably *Isoparorchis trisimilitubis*)	Long-whiskered catfish, *Mystus aor* syn. *Sperata aor* (Hamilton, 1822)Day's mystus, *Mystus bleekeri* (Day, 1877)	Muscles, swim bladder, visceral organs, body cavity, viscera, some in the mouth, urinary system, biliary system, ovaries	Kuliarchar & Upazila rivers, India	Extensive tissue damage including inflammation, necrosis, and empty spaces with fragmented blood capillaries, tissue debris, lymphocytes and fluids. Infected liver, swim bladder and kidney showed vacuolation and massive melanisation.	Farhana and Khanum [74]
Isoparorchis hypselobagri (probably *Isoparorchis trisimilitubis*)	Spotted snakehead, *C. punctata*	Fins, liver, ovaries, abdominal cavity	Khookas bundh, Jaipur, India	Necrosis of fin tissues, scale loss. Necrotic areas with extensive inflammatory exudate formation were seen throughout the viscera. Liver reduced in size. Haemorrhage of intestinal wall.	Mahajan, et al. [18]
Isoparorchis hypselobagri (probably *Isoparorchis trisimilitubis*)	Red-crowned roofed turtle *Kachuga kachuga* syn. *Batagur kachuga* (Gray, 1831)	Body cavity	Hyderabad, India	N/D however this turtle is critically endangered in India and likely extinct Bangladesh.	Simha [75], Praschag, et al. [76]

Table 2. *Cont.*

Species of Parasite	Host Infected	Site of Infection	Geographical Locality	Fish Pathophysiology	Reference
Isoparorchis hypselobagri	*Pungtungia herzi,* Herzenstein, 1892 *Acheilognathus koreensis* syn. *Tanakia koreensis* (Kim & Kim, 1990), *Squalidus japonicus coreanus* syn. *Squalidus japonicus* (Sauvage, 1883) and *Odontobutis platycephala* Iwata & Jeon, 1985	Muscle & lesions skin	Saengbiryang-myeon, Sancheong-gun, Gyeongsangnam-do, Korea	Inkspot disease, muscle and skin swellings and lesions.	Sohn and Na [77]
Isoparorchis hypselobagri (probably *Isoparorchis trisimilitubis*)	*Mystus seenghala,* syn. *Sperata seenghala* (Sykes, 1839)	Swim bladder	River Godavari, Rajahmundry, India	"Ink spot disease".	Vankara, et al. [78]
Genarchopsis goppo Ozaki, 1925	Striped snakehead, *C. striata*	Intestine	Warangal, India	Histopathological changes include shortening and destruction of villi, vacuolation of sub mucous cells, dilation of blood vessels thickening of muscles and necrosis. In the infected fish carbohydrates, glycogen, protein and lipid contents are increased significantly to compensate for parasite presence.	Laxmareddy and Benarjee [17]
Genarchopsis paithanensis Pardeshi & Hiware, 2012	Zig-zag eel, *Mastacembelus armatus* (Lacepède, 1800)	Intestine	India	Damage sub and mucosal layer and dilation blood vessels, destruction and extrusion of intestinal villi, inframammary and hyperplastic fibrosis.	Pardeshi and Hiware [79]
Genrachopsis goppo	Striped snakehead, *C. striata*	Intestine	Warangal, India	Severe damage to villi and other layers of intestine. Infections interfere with digestion and absorption of food material causing metabolic disturbances. Excretory products and metabolic end products excreted into intestine produce toxicity, interfere with protein metabolism of host. Host tissue may show decrease in protein content.	Reddy and Benarjee [16]
Genrachopsis goppo	Spotted snakehead, *C. punctata*	Intestine	Kakatiya, India	Glycogen content increased during infections to compensate for parasite needs.	Vinatha, et al. [80]
Pallisentis (*P.*) *nagpurensis* Bhalerao, 1931	Gibelion catla, *Catla catla* syn. *Labeo catla* (Hamilton, 1822) and roho labeo, *Labeo rohita* (Hamilton, 1822)	N/D	Hyderabad, India	Overall protein in liver and intestine by 17%–26%. Amino acids increased by 14%–48.8% with highest increase in liver.	Kumar [81]

Table 2. *Cont.*

Species of Parasite	Host Infected	Site of Infection	Geographical Locality	Fish Pathophysiology	Reference
Pallisentis punctatin (misspelling likely *Pallisentis (Brevitritospinus) punctati*)	Spotted snakehead, *C. punctata*	Digestive tract	Hyderabad, India	Metabolic enzymes, succinate dehydrogenase and lactate dehydrogenase activity higher in infected fish. Oxidative stress enzymes lipid peroxidation, glutathione peroxidase and superoxide dismutase increased in infected fish.	Latha, et al. [82]
Pallisentis (P) *nagapurensis*	Striped snakehead, *C striata*	Intestine	Warangal district, Andhra Pradesh, India	Complete disruption intestinal mucosa and submucosa, thickened lamina propria, damage epithelial cells, mucosal folding & clumps. Villi shrunken, infected intestine enlarged and slightly inflamed.	Laxma Reddy and Benarjee [83]
Pallisentis (P) *celatus* (Van Cleave, 1928) Baylis, 1933	Asian swamp eel, *Monopterus albus* (Zuiew, 1793)	Intestine	N/D	Mechanical damage to intestinal epidermis & muscle layer.	LI Chun-tao, et al. [84]
Acanthocephalan species not specified	Spotted snakehead, *C. punctata*	Intestine	River Gomti, Lucknow, India	Damage of intestinal tissues, shortening of villi, granuloma site of attachment. Erosion villi tip, necrosis & hyperplasia.	Verma and Saxena [85]
Senga sp. Dollfus, 1934	Zig-zag eel, *M. armatus* & Snakehead, *Channa* sp. Scopoli, 1777	Intestine	Maharashtra State, India	Damage intestinal villi, granuloma site of attachment.	Bhure and Nanware [86]
Senga mastacembelusae sp. nov. (not a valid species but *Senga* sp. likely)	Zig-zag eel, *M. armatus*	Intestine	Godavari Basin, India	Significant mechanical damage. Scolex deeply penetrating intestinal layers & damage mucosa, submucosa, muscularis mucosa. Intestinal villi architecture destruction & granuloma at scolex attachment.	Fartade and Fartade [87]
Senga rostellarae (probably *Senga pahangensis* or *Senga filiformis*)	Indonesian snakehead, *Channa micropeltes* (Cuvier, 1831)	Intestine	Kenyir Lake, Malaysia	Intestine with severe villus damage, destruction of villi epithelium and necrosis. Cross section of cestode showed increase of goblet cells and generated necrosis and severe damage to fish intestine. Conditions likely cause of death in fish due to haemorrhage and malabsorption of nutrients.	Hassan, et al. [88]
Senga species (probably *Senga malayana* for *C. striata* and *Senga vishakapatnamensis* for *C. punctata*)	Striped snakehead, *C. striata* & spotted snakehead, *C. punctata*	Intestine	Unknown	Excess mucus secretion, severe degeneration and necrosis in mucosal, submucosal, serosa layer and muscular layers at attachment. Ruptured serosa layer, vacuolization in tunica muscularis and lamina propria, shortened, fused and irregular shaped villous processes.	Kaur [89]

Table 2. *Cont.*

Species of Parasite	Host Infected	Site of Infection	Geographical Locality	Fish Pathophysiology	Reference
Senga sp.	Zig-zag eel, *M. armatus*	Intestine	India	Shortening, flattening and damage of villi and cyst formation in the intestine of fish.	Nanware and Bhure [90]
Senga sp.	Striped snakehead, *C. striata*	Intestine	Kaigaon Toka, India	Mechanical damage to intestinal tissue including shortening & damage to villi, thickening of the muscle layer, destruction of villi, hold fast penetration of the mucosa & damage to mucous & submucous membranes.	Shirsat, et al. [91]
Senga sp.	Siamese fighting fish, *Betta splendens*	Intestine	Aurangabad district, India	Destruction & extrusion of intestinal villi, fibroblast cell & plasma cell.	Wankhede, et al. [92]

3.3.2. *Genarchopsis* sp. Pathophysiology

Genarchopsis sp. are digenetic trematodes of family Derogenidae which infect freshwater fish distributed throughout Japan, South East Asia [93] and the Indian sub-continent [17]. Lifecycle information may be incomplete due to possible misidentification of *Genarchopsis* sp. Supplementary Table S1 (S1) includes synonymised species. Severe histopathology has been described in both striped and spotted snakehead, *Channa* sp. Scopoli, 1777 in India. Reddy and Benarjee [16] demonstrated infection of the intestine with *G. goppo* resulted in severe intestinal damage which limited food digestion and protein absorption and Laxmareddy and Benarjee [17] observed intestinal necrosis. In infected snakehead, carbohydrate, glycogen, protein and lipid production are increased significantly to compensate for parasite needs [80] and host tissue showed a decrease in protein [17].

The liver is the principal organ of glucose homeostasis and lipid storage [54] and the cascade of increased metabolic changes described by Vinatha, et al. [80] is indicative of parasite-induced physiological challenges in fish [94]. Lipids are crucial for fish growth, reproduction, vision, osmoregularity, thermal adaptation and immune response [95]. If a consequence of fish parasitism by *Genarchopsis* spp. is mobilisation of lipid reserves and increased glucose homeostasis it is expected that aquaculture and native fish species will exhibit decreased growth, compromised immune function and reduced fecundity. Reddy and Benarjee [16] comment that *G. goppo* excretory products produce toxicity in the host intestine which aligns with reports of ammonia excretion in other digenetic species *Isoparorchis* sp. and *Schistosoma mansoni* [71].

3.3.3. *Isoparorchis* sp., Southwell, 1913, Pathophysiology

Isoparorchis species are digenetic trematodes of family Isoparorchiidae. Shimazu, et al. [39], using a combined morphological and molecular method, provided clarity to the species within the genus *Isoparorchis* and conclusions in the study point to member species being regionally endemic. Infection with *Isoparorchis* spp. may result in "Ink spot disease" [73,78] which manifests in characteristic patches of black pigment in host fish muscle, viscera and fins often causing scale loss [70,71]. Migrating juvenile metacercaria cause massive tissue erosion and tunnels in fish musculature and necrosis at the site of infection with abundant inflammatory exudate [72]. Infection may lead to economic loss due to the death of aquacultured fish [18]. Adult parasites excrete ammonia in infected fish [70,71] and ammonia is recognised as extremely toxic to fish if allowed to accumulate in the body [96]. The teleostean gills are the major site of ammonia excretion; however, smaller quantities of ammonia are excreted by the kidneys [97]. It is assumed the significant physical damage to the kidney described by Mahajan, et al. [18] and Farhana and Khanum [74] would limit the ability of infected fish to effectively excrete ammonia. Parasitisation of the spleen has also been reported with a significant decrease in mean corpuscular haemoglobin (MCH) [18,74].

3.3.4. *Pallisentis* (*P.*) and *Pallisentis* (*B.*) Pathophysiology

Pallisentis (*B.*) and *Pallisentis* (*P.*) are acanthocephalan parasites which infect freshwater fish. The pathogenicity of adult acanthocephalans is determined by the magnitude of infection and the extent of mechanical damage exerted by proboscis hooks and the collar and trunk spines which penetrate at the site of attachment [98,99]. Figure 2F,G illustrate the invasive potential of the proboscis hooks and collar spines and in cases of heavy fish infection, it seems clear that mechanical damage may result at the site of attachment. Significant damage to intestinal structure and villi accompanied by necrosis and hyperplasia is associated with *Pallisentis* (*P.*) and *Pallisentis* (*B.*) in fish [83–85]. In a study of cultivated fresh water fish, Catla (*Gibelion catla*) and roho labeo (*Labeo rohita*) infected with *Pallisentis nagpurensis* (syn. (*P.*) *nagpurensis*) Bhalerao, 1931 the pathological damage observed in the fish hosts correlated with appreciable changes to protein and amino acid metabolism. Both of these metabolic pathways are associated with tissue repair mechanisms following parasitism [81]. This is supported in Latha, et al. [82] who found carbohydrate metabolism

and lipid peroxidation significantly increased in spotted snakehead infected with *Pallisentis* (*B.*) *punctati* Gupta, Gupta & Singhal, 2015 in response to parasite-induced damage and physiological stress. Verma and Saxena [85] found acanthocephalan infection in spotted snakehead damaged intestinal digestive and absorptive efficacy and affected fish general health and growth. Plasma loss from the intestine at the site of parasite attachment has also been widely reported in fish infected with acanthocephalan parasites [100,101]. Fish infection correlated with a decrease in fish body lipids [102] and stored energy [103]. Lipids in fish are significant influencers of reproduction, growth, immune response, osmoregularity behaviours, vision and thermal conversion [95]. It is expected that a depletion in lipid reserves would have a great impact on production of commercial species and survival of native fish populations.

3.3.5. *Senga* Species Pathophysiology

Senga species of family Bothriocephalidae [104] are cestodes of freshwater fish [105]. Great taxonomic uncertainty exists in genera *Senga* with many species identified morphologically as novel based on extremely minor differences in morphological and morphometric characteristics. Table S1 includes species which have been synonymised. There are still a number of new species of *Senga* yet to be confirmed as valid. At present, there are 16 valid *Senga* sp.

Senga sp. attach to the submucosal intestinal surface of the fish with a scolex which has ~30–70 rostellar hooks depending on the *Senga* species [45]. Intestinal mechanical damage described in fish hosts [87,91] appears commensurate with such an invasive hold fast organ. Excess mucus secretion degenerating to necrosis in the intestinal mucosal, submucosal and serosa layer has been reported [89,91]. Histopathology reports of infected intestine describe ruptured serosal layer, vacuoles in both the lamina propria and tunica muscularis, significant damage and necrosis to intestinal villi [88–90]. Destruction of intestinal villi is conspicuous in fish infected with *Senga* sp. [86–88,106].

The integrity of the fish intestine is fundamental to maintaining fish health. The interaction between a healthy intestinal microbial population is essential to a functional innate and adaptive immune system [107]. Feed digestibility and absorption through intestinal barriers is a consequence of the absorptive area of villi [108]. This is supported in Hassan, et al. [88], Shaharom [106] and Shirsat, et al. [91] who concluded that the damage to the intestine caused by *Senga* sp. was consistent with an outcome of fish death due to haemorrhage and/or malabsorption of nutrients. In aquaculture systems economic loss due to growth retardation, increased mortality, increased pathogen susceptibility and reduced quality of edible flesh have been described [86,87].

4. Discussion

In the present study *Pallisentis*, *Senga* and *Genarchopsis* species were identified in imported edible consumer ready *Channa* fish from Country 22. Together with *Euclinostomum* and *Isoparorchis* species identified in a previous study [31] of imported fish, all parasite species have been demonstrated to cause severe pathophysiology in infected fish. It must be clearly stated that the fish examined in both studies were frozen and providing appropriate freezing temperature was maintained along the supply chain the parasite risk has been effectively negated. However, at the time this study was conducted (2020), fish were permitted entry into Australia fresh or chilled and this affects the infective potential of all parasites described. However, presence of a parasite in imported edible fish is only a threat to Australian native and commercial aquatic species if a viable exposure pathway can be identified and there are suitable hosts for parasites to successfully complete their life cycle and become established. In Sections 3.3.1–3.3.5 the pathophysiology of fish infection with each respective parasite is detailed and in Section 4, viable exposure pathways and aquatic creatures present in Australia which may be vulnerable to infection are discussed.

Parasites endemic to Australia share evolutionary pathways with native fish, are a natural component of the ecosystem, and interactions between parasite and host fish are

considered to produce non-clinical or non-pathogenic infections [109]. However, alien parasites introduced to a new geographical location may cause disastrous clinical outcomes in indigenous fish species, the hypothesis being that naïve fish lack innate immunity to the alien parasite [7,110,111]. Extreme clinical outcomes and mortality in indigenous fish populations can be exacerbated when introduced parasites have low host specificity/host switch and are able to infect multiple indigenous fish species [7,112,113], are introduced to countries with phylogenetically homogenous native/introduced aquatic fauna [5] and are highly fecund and reproduce rapidly [111,114]. Alien parasites, for example *Isoparorchis* species, which have as yet unrecognised potential to reach maturity in humans/other mammals/aquatic creatures may also cause widespread environmental contamination, exposing indigenous fish species to infection.

There are ~280 species of Australian native fish and 22 of these are at various stages of population vulnerability [115,116]. The concomitant health impacts on fish infected with parasites discussed in this study may include retardation of fish growth, increased mortality [55,56,91,95], impaired fecundity, decreased thermal adaption, compromised immune response, poor food conversion [17,74,80,85,94,95], ammonia accumulation, decreases in blood MCH [18,71,74], loss of marketability [78,80] and other negative health indicators. The pathophysiology of infection described in this manuscript pertains, in the main, to fish species which are the natural hosts for each parasite. Evidence therefore suggests that the clinical outcomes for naïve Australian fish would be devastating if these parasites were to be introduced.

Table 2 and Figure 4 include host and parasite lifecycle information relevant to the discussion. At present *Euclinostomum* has not been reported in native or aquacultured fish in Australia. However, co-infection of *E. heterostomum* and *Clinostomum tilapiae* is reported in Mozambique tilapia (*O. mossambicus*) from South Africa [51]. Co-infection of *E. heterostomum*, *C. tilapiae* and *Clinostomum complanatum* (Rudolphi, 1814) Braun, 1899 has been reported in redbelly (*Tilapia zillii*) from Iran [21]. The demonstrated co-infection potential of *Euclinostomum* and *Clinostomum* is supported by conclusions in Shareef and Abidi [49] who describe a shared "functional and evolutionary significance" between the closely related genera. Co-infection of *Euclinostomum* and *Clinostomum* has also been identified in the piscivorous little egret [117]. Both Mozambique tilapia (*O. mossambicus*) and redbelly tilapia (*Tilapia zillii* syn. *Coptodon zillii*) are highly suitable hosts and have been introduced into Australia. Successful breeding populations have been established at a number of localities in Queensland, Victoria and Western Australia [118]. Of particular concern for Australian native fish and regional aquaculture are populations of Mozambique tilapia (*O. mossambicus*) within 3 km of the Murray–Darling Basin (MDB) [118]. *Clinostomum complanatum* was recorded in the body cavity of Mozambique tilapia (*O. mossambicus*) from Queensland waters [119]; however, so far *E. heterostomum* has not been identified. Tilapia (*Oreochromis* species) and other cichlid fish belong to family Cichlidae. Cichlids are also vulnerable to infection with *E. heterostomum* [120–122]. Approximately 17 species of cichlids have been introduced to Australian waterways [123]. *Euclinostomum* is considered to have little host specificity [50] and although Cichlidae fish appear to be the preferred host this genus of parasite has also been identified in gourami *Trichopsis* Canestrini, 1860 species, crescent betta *Betta imbellis* [124] of family Osphronemidae, the guppy (*Poecilia reticulata*, family Poeciliidae) [52] and air-breathing catfish (*Clarias* species, family Clariidae) [67].
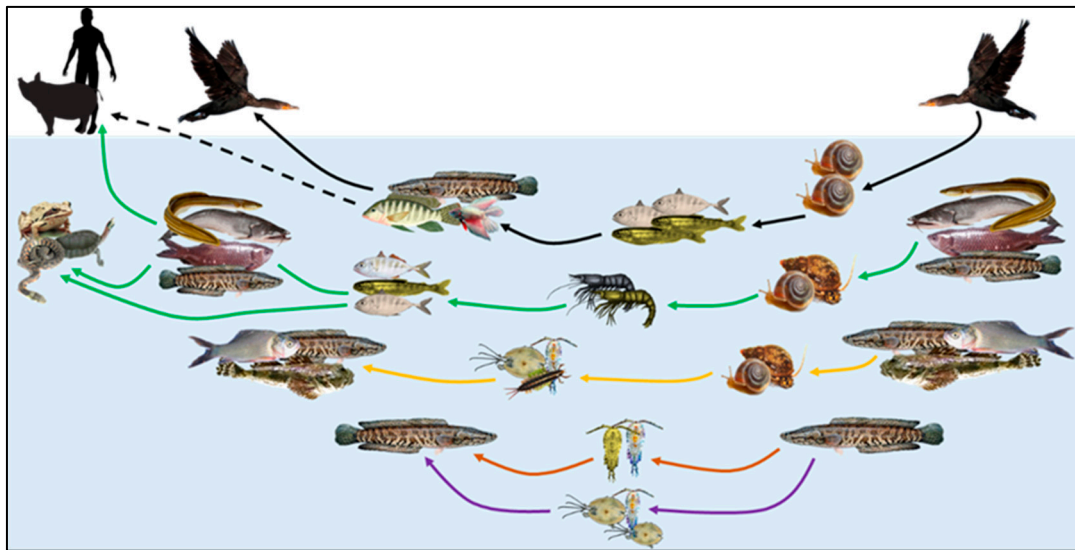
Figure 4. The respective basic life cycles for each parasite species. Black arrows indicate *Euclinostomum* sp. with a broken black line for zoonotic potential without proven cases of human infection; green arrows show *Isoparorchis* sp.; yellow arrows indicate *Genarchopsis* sp.; tan is *Senga* sp. and purple *Pallisentis* sp.

Neither of the freshwater snails which serve as intermediate hosts to *E. heterostomum* have been identified in Australia. However, *Indoplanorbis* sp. Annandale & Prashad, 1921 and *Lymnaea* sp. Lamarck, 1799 snails were collected from gill mud of edible fish imported into Australia in Williams, et al. [30]. In Australia, the temperate indigenous freshwater snail, *Lymnaea tomentosa* syn. *Austropeplea tomentosa* (L. Pfeiffer, 1855), the introduced *Lymnaea viridis* syn. *Orientogalba viridis* (Quoy & Gaimard, 1832) and *Lymnaea columella* syn. *Pseudosuccinea columella* Say, 1817 serve as suitable intermediate hosts for *Fasciola hepatica* Linnaeus, 1758 [125]. It is considered, if viable *Euclinostomum* metacercariae were introduced into Australia, there could be suitable host fish/snail species to establish a lifecycle. Co-introduction of the snail intermediate hosts may enhance this potential. Certainly, Australia hosts numerous species of heron and cormorants as well as cattle egrets [126] which are recognised as suitable definitive hosts for this parasite [127–130]. Mashego and Saayman [65] concluded heavy infestations are likely to occur in ponds where a suitable snail intermediate is present, making impounded native and aquaculture species vulnerable. As noted by Bhargavi, et al. [58] fish become lethargic when heavily infected and this may facilitate the transmission potential to piscivorous birds. In a study of little egrets captured at a fish farm in Egypt *Euclinostomum* was the most prevalent (44%) [117] parasite identified. In addition, metacercariae encysted in fish may result in loss of consumer confidence and market value [1,21,64].

Genarchopsis species have not been described in Australia. Only one species of fish identified as a suitable host in Bangladesh, the tank goby *Glossogobius giuris* (Hamilton, 1822), is found in Australia [131]. However, Australia does host eight other species belonging to the genus *Glossogobius* Gill, 1859 [132–134] which may be suitable hosts of *Genarchopsis* sp. In Japan, *Tridentiger* sp. are susceptible to infection. *Tridentiger trigonocephalus* (Gill, 1859) was introduced into Australia via eggs/and or larvae in ship ballast water or adhered to oyster shells [135]. It is unknown if a brackish water species of fish could be a suitable host for this parasite. Fish of family Channidae from the Indian sub-continent have been widely described as suitable hosts for *Genarchopsis* sp. Abol-Munafi, et al. [136] indicates that snakehead of *Channa* genus have been released into the Australian environment by fish enthusiasts. However, the veracity of this report could not be confirmed. There is also an anecdotal report of striped snakehead (*Channa striata*) found in QLD [137]. The freshwater snail *Gabbia travancorica* (W. H. Benson, 1860), according to Bagni and Galli [138], has not

been identified in Australia. However, there are 21 species of *Gabbia* Tryon, 1865 widely distributed around Australia which may prove to be suitable intermediate hosts. There is only one record of the second intermediate ostracod host *Stenocypris malcolmsoni* (Brady, 1886) in Australia [139] and no record of *Eucypris capensis* Daday, 1910 (sensu Martens 1986). However, *Eucypris virens* (Jurine, 1820) [140], *Eucypris lateraria* (King, 1855), *Eucypris crinita* (Henry, 1923), *Eucypris pratensis* Eagar, 1970 and *Eucypris thomsoni* Chapman, 1963 have been identified in the Australasian Region [141]. Australia has availability of closely related first and second intermediate hosts and fish species susceptible to infection. *Channa* species in all Australian states apart from Northern Territory are restricted species. The potential threat of *Channa* sp. illegally brought into Australia and propagated for food [142] should be noted particularly as species within this genus in previous studies were identified as highly parasitised with a great diversity of parasites [30].

Only *Isoparorchis tandani* Johnston, 1927 is present in Australia and it is unknown if intermediate hosts within the lifecycle of *I. tandani* would be suitable for imported species of *Isoparorchis*. *Isoparorchis* sp. in Bangladesh is the exception to regional endemicity [39] with less host specificity demonstrated. In Bangladesh, host fish include two species of family Channidae, two from family Schilbeidae, and one representative each from families Bagridae and Siluridae [39,143,144]. This may be partially accounted for by the identification of two species of this parasite in Bangladesh, *Isoparorchis hypselobagri* (Billet, 1898) Ejsmont, 1932 redescribed as *Isoparorchis trisimilitubis* Southwell, 1913 or *Isoparorchis* sp. 3 [39]. *Isoparorchis* sp. have also been identified in the stomach of a crocodile (Assam, India) [75], the body cavity of the red-crowned roofed turtle (*Kachuga kachuga* syn. *Batagur kachuga* (Hyderabad State, India)) [75,76], the intestine of a checkered keelback snake *Tropidonotus piscator* syn. *Xenochrophis piscator* (Schneider, 1799) (Hyderabad, India) [145] and encysted in the liver of the Indian bullfrog *Rana tigrina* syn. *Hoplobatrachus tigerinus* (Daudin, 1802) (India) [146]. *Isoparorchis* sp. identified in the stomach or intestine of species other than fish is considered to be a result of consumption of parasitized fish. However, where metacercariae have encysted in the liver or migrated to the body cavity it suggests these species may be suitable hosts for this parasite. The reports of aberrant hosts in India and Bangladesh may support a hypothesis that *Isoparorchis* sp. in these countries are less host specific and may pose a threat to Australian fish, freshwater turtles or frogs if introduced. Species of bullfrogs belonging to *Hoplobatrachus* and turtles of *Batagur* species are not identified in Australia. However, there are many freshwater frogs and turtles with a critical, near threatened or vulnerable conservation status [147,148]. In addition, viable reproducing adult *Isoparorchis* sp. have been recovered from humans in India [18,149,150], China [151] and a pig in India [152]. No record exists in Australia of human infection from *I. tandani*. It is possible that species of *Isoparorchis* from India or China are able to reach maturity, or survive for prolonged periods, in mammals. A viable reproducing adult digenean has been retrieved from the gall bladder of the pig in India [152] which shows that migration from the stomach has occurred and in Manipur, India reproducing adult *Isoparorchis* have often been retrieved from human patients after treatment [149]. Ashford and Crewe 2003 also report expelled adult worms from humans following treatment [151]. Should mammalian infection occur broad environmental contamination may result from parasite eggs shed in faeces. Recovery of *Isoparorchis* eggs from human faeces has also been reported [151,153].

Infestation renders the fish visually unacceptable for human consumption [71]. Compounds such as ammonia generated through fish spoilage is a significant problem in the food industry and is associated with human health problems which include diarrhoea, vomiting, oedema and hypotension [154]. It is unknown how ammonia, the major excretory product of *Isoparorchis* in fish [71], would affect spoilage of commercial species and if this compound may be responsible for cases of human infection described in literature.

Smales, et al. [155] conducted a comprehensive study of acanthocephalans infecting Australian freshwater fish. Species of *Pallisentis* (*B.*) and *Pallisentis* (*P.*) were not identified nor were any acanthocephalans belonging to the same order. *Cyclops strenuous* Fischer,

1851 is also absent from Australia; however, there are many freshwater invertebrate species introduced or native to Australia which may have potential to become suitable hosts. According to Smales, et al. [155] acanthocephalans introduced into Australia may be unable to establish in native freshwater fish species due to the dry climate, and absence of suitable invertebrate intermediate hosts. Many acanthocephalans manipulate host behaviours to ensure infected intermediate hosts are preferentially consumed [156–158]. In addition, acanthocephalans have unique adaptions which place their eggs in the most advantageous place to be consumed by intermediate hosts [159,160]. The egg of *Pallisentis* (*P.*) *nagpurensis* demonstrates this type of adaption [161]. In the absence of a suitable fish host in Australia is seems unlikely these genera of acanthocephalans would develop a successful life cycle if introduced.

There has so far been only one report of *Senga* species in Australia. *Senga scleropagis* was identified by Blair [162] infecting the intestine of freshwater fish, Southern saratoga *Scleropages leichardti* Günther, 1864, from the Wenlock River, Cape York peninsula, Australia. This species is considered valid in Kuchta [104]. It is unknown if *Senga* sp. are widespread amongst Australian freshwater fish or if the lack of any report since Blair in 1978 is a reflection of fewer studies in the parasite fauna of freshwater fish. However, *M. leuckarti* syn. *M. leuckarti leuckarti* (Claus, 1857) has been described as the dominant copepod zooplankton in various freshwater bodies of Australia [163–165]. *Thermocyclops crassus* syn. *T. crassus crassus* (Fischer, 1853) along with four other *Thermocyclops* Kiefer, 1927 copepod species have been described in Northern Queensland and in the same study *T. rylovi* an East African/Central and South Asian species was described for the first time in Australia [166]. All are drought and salinity tolerant copepod species [167].

The Bonylip barb *Osteochilus hasseltii* syn. *O. vittatus* (Valenciennes, 1842), a cyprinid fish species, Malaysia, has been identified highly infected with plerocercoids of *Senga* species. Several cyprinid species of fish have been introduced to Australia via the aquarium trade; the common goldfish, *Carassius auratus* (Linnaeus, 1758), Common carp, *Cyprinus carpio* Linnaeus, 1758 [5], Rosy Barb, *Puntius conchonius* syn. *Pethia conchonius* (Hamilton, 1822), Roach, *Rutilus rutilus* (Linnaeus, 1758) and Tench *Tinca tinca* (Linnaeus, 1758) [168]. It is therefore possible that a successful lifecycle may establish more broadly across Australia if other *Senga* species were introduced. Bothriocephalideans are in general stenoxenous [104]. It is important that a suitable copepod and fish host be present in Australia for a *Senga* sp. successful lifecycle to establish.

5. Conclusions

There seems little doubt that the costs to aquatic, human and animal health of introduced parasites can be enormous. The challenge to Australian biosecurity is to anticipate and respond to a myriad of threats posed by parasites and the commensals hidden in imported seafood products and packaging. Australian importation commodity codes (2020) indicate these edible parasitised fish may be imported fresh or chilled. Mud, snails, other debris, vegetation and food remains have been previously identified in fish packaging of infected fish [30] and parasites identified in viscera, which was required to be removed, in consumer-ready fish imported into Australia [30]. The Australian biosecurity risks could certainly be mitigated with greater support for fish processors in the exporting country to reach food safety compliance.

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