

Molecular characterisation of the monogenea parasites of blue mackerel *Scomber australasicus* (Perciformes: Scombridae) in Australian waters

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ABSTRACT

This study describes the occurrence and molecular identification of Monogenea from blue mackerel *Scomber australasicus* (Cuvier) (Perciformes: Scombridae), an edible fish, from Australian waters. Previous studies have provided either morphological or genetic results, whereas this study combines both methods of species identification. A total of 50 fish sourced from the waters off the south-eastern Australian coastline were examined and 71 Monogenea were recovered from the gills. The overall prevalence, mean intensity, and mean abundance were 64%, 2.22, and 1.42, respectively. Monogenea were initially classified morphologically as five species belonging to two families. Family Mazocraeidae was represented by *Kuhnia scombri* (Kuhn, 1829) Sproston, 1945, *K. scombercolias* Nasir & Fuentes Zambrano, 1983 and *Pseudokuhnia minor* (Goto, 1894) Rohde & Watson, 1985 and family Gastrocotylidae by *Gastrocotyle kurra* Unnithan, 1968 and *Allogastrocotyle bivaginalis* Nasir & Fuentes Zambrano, 1983. Molecular identification of Monogenea was conducted through sequencing of the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene. The host *S. australasicus* was barcoded (*cox1*) to confirm the specific identity. There was no comparable sequence available in GenBank for *K. scombercolias*. Also, limited sequences were available in GenBank for the gastrocotylid Monogenea identified in this study. However, phylogenetic analyses of *cox1* sequences of the Monogenea identified in this study clustered according to their familial groups. *Gastrocotyle kurra* and *A. bivaginalis* were identified for the first-time on *S. australasicus* in Australian waters. This study provides the first sequencing of *cox1* gene for *K. scombercolias*. The outcomes of the study provide a basis for future Monogenea research in Australian waters, as well as for other *Scomber* spp.

1. Introduction

Blue mackerel *Scomber australasicus* (Cuvier) (Perciformes: Scombridae) is a small to medium-sized, schooling, teleost fish, which feed on plankton, copepods and crustaceans while young and, as adults, may predate small fish and squids (Froese and Pauly, 2018). The distribution of *S. australasicus* includes the western Pacific Ocean, waters of Taiwan and Japan and extends to Australia and New Zealand waters (Chen and Shih, 2015; Chou et al., 2011; Froese and Pauly, 2018; Quiazon et al., 2011). In Australia, *S. australasicus* is fished commercially and by recreational anglers (FRDC, 2020; Ward et al., 2009). The charter boat industry also uses *S. australasicus* as live bait for pelagic sports fishing and this species is also fed to maricultured tuna (Lowry et al., 2006; Neira and Keane, 2008).

Monogenea are ectoparasitic platyhelminthes that mainly parasitise

the gills of fish (Whittington and Chisholm, 2008). Approximately 3500 Monogenea species have been described from marine fish (Rohde, 2005). In general, these parasites cause severe damage to the gills due to the invasiveness of their clamps and hooks at the site of attachment (Hutson et al., 2007; Whittington and Chisholm, 2008). Serious pathology and marked pathogenicity may lead to the death of the fish (Deveney et al., 2001).

To date, extensive surveys have been conducted to identify Monogenea infection in four *Scomber* (Linnaeus) species of mackerel from multiple locations in the Indo-Pacific and Atlantic Oceans (Rohde, 1986, 1989b; Rohde and Watson, 1985a, b). In studies by Rohde (1986, 1989b) and Rohde and Watson (1985a, 1985b), large numbers of *Scomber* hosts from several locations were examined. The authors concluded that geographical variation in morphology is common in the mazocraeid Monogenea. Therefore, populations of Monogenea from

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different geographical areas that differ only slightly in morphological features are not necessarily different species (Rohde, 1989b).

At the present time, five Monogenea species (*Kuhnina scombri* (Kuhn, 1829) Sproston, 1945, *K. scombercolias* Nasir & Fuentes Zambrano, 1983, *K. sprostonae* Price, 1961, *Pseudokuhnina minor* (Goto, 1894) Rohde & Watson, 1985 and *Grubea australis* Rohde, 1987), have been reported to infect *S. australasicus* in Australian waters (Table 1 and Rohde (1989b)). Previous studies on the parasites of *Scomber* species were based on morphological species identification and earlier morphological identification of Monogenea species from *Scomber* hosts showed ambiguity and many challenges according to Rohde (1989b) and Rohde and Watson (1985a, 1985b). These authors concluded that serious consideration should be given before naming species based on small morphometric and meristic variations. There have been no studies that have used combined morphological and molecular methods to identify and describe Monogenea species from *S. australasicus*. Therefore,

Table 1

Previous reports of Monogenea belonging to the family Mazocraeidae found on the gills of blue mackerel *Scomber australasicus* in Australian waters and other parts of the world. Abbreviations: NSW=New South Wales, VIC= Victoria, SA=South Australia, QLD = Queensland, WA=Western Australia.

Parasite taxa	Localities	Reference
<i>Kuhnina scombri</i>	Cape Moreton, QLD; Gulf St.	Schmarr et al. (2011)
<i>K. scombercolias</i>	Vincent, SA; Fremantle, WA; and New Zealand	
<i>Pseudokuhnina minor</i>		
<i>Grubea australis</i>		
<i>K. scombri</i>	Location unspecified	Korotaeva (1974)
<i>P. minor</i> (syn. <i>K. minor</i>)		
<i>Kuhnina</i> sp.		
<i>K. scombri</i>	Jervis Bay and Sydney Fish Market, NSW; Golden Bay, WA; and New Zealand	Rohde (1989)
<i>K. sprostonae</i> ¹	Sydney Fish Market, Eden, NSW; Perth, WA	Rohde (1989)
<i>K. scombercolias</i> ²	Coffs Harbour, NSW, Northern NSW; Tasmania; WA; Hawaii, USA	Rohde (1989)
<i>Grubea australis</i>	Coast of NSW, Australia	Rohde (1986)
<i>K. scombri</i>	Jervis Bay and Sydney Fish Market, NSW, Australia	Rohde (1987)
<i>K. sprostonae</i>		
<i>P. minor</i>		
<i>Grubea australis</i>		
<i>P. minor</i>	Jervis Bay and Sydney Fish Market, NSW, Australia; Philippines; Amoy, China	Rohde and Watson (1985a)
<i>K. scombri</i>	Eden, NSW, Australia	Hayward et al. (1998)
<i>K. sprostonae</i>		
<i>K. scombercolias</i>		
<i>Grubea australis</i>		
<i>P. minor</i>		
<i>K. scombri</i>	Eden, NSW, Australia	Perera (1993)
<i>K. sprostonae</i>		
<i>K. scombercolias</i>		
<i>Grubea australis</i>		
<i>P. minor</i>		
A microcotylid Monogenea ³		
Mazocraeidae III, IV, V, VI (species name not mentioned in this publication)	South-eastern Australia	Rohde (1988)
<i>K. scombri</i>	Jervis Bay and Sydney Fish Market, NSW, Australia; Golden Bay, WA; New Zealand; Alumahan Bato, Philippines	Rohde and Watson (1985b)
<i>K. sprostonae</i>	NSW, Australia; Hawaii, USA	Rohde and Watson (1985b)

Note: ¹Microhabitat of this Monogenea parasite is pseudobranchs. ²According to Rohde (1989), *Kuhnina sprostonae* species identified and described in the publications of Rohde and Watson (1985a, 1985b) would be considered as the combination of *K. sprostonae* and *K. scombercolias*. ³Family name of this parasite is Microcotylidae.

specific identification for every species of Monogenea from *S. australasicus* using combined morphology and molecular tools is warranted.

Hebert et al. (2003) and Ward et al. (2005) refer to the amplification of the mitochondrial *cox1* gene as “the core of a global bio identification system for animals”. The molecular method has been extensively used for the accurate identification of many parasite species (McManus and Bowles, 1996). The molecular characterisation may also lead to the discovery of new or cryptic species (Ayadi et al., 2017; Bouguerche et al., 2019a; Oliva et al., 2014). For example, the *cox1* gene has been used to differentiate among species of Monogenea (Catalano et al., 2010; Jovelín and Justine, 2001; Oliva et al., 2014). In previous studies, the characterisation of many Monogenea species was based on small sample numbers and amplification of a highly conserved region of the nuclear gene (28S rRNA, for example), which results in a lower degree of apparent genetic diversity. Therefore, the aim of the present study was to morphologically identify Monogenea species from *S. australasicus* and to characterise the species genetically, based on partial mitochondrial *cox1* gene, in order to validate their taxonomic status.

2. Materials and methods

2.1. Fish collection

A total of 50 fish were purchased from fishermen from two retail fish markets in Australia. The fish had been caught from two separate locations off the coast of New South Wales (NSW; n = 20; July 23, 2018) and off the coast of Victoria (VIC; n = 30; September 29, 2018). Fish were transferred fresh to the Parasitology Laboratory of Charles Sturt University, Wagga Wagga Campus, in an insulated box filled with ice. All fish from each batch were examined on the day of arrival at the University. Fish were identified using morphological keys provided by Gommon et al. (2008).

2.2. Parasite collection

Each fish was dissected to remove the gills and the gills were separated and placed in an individual Petri dish containing salt water (1000 ml of water to 35 g of salt). The surfaces of all gills were thoroughly inspected under a dissecting microscope (Leica EZ4 Stereo Microscope, China) for the presence of Monogenea. Parasites were removed from gills using fine dissection needles. Collected parasites were counted and preserved in 70% ethanol for further morphological and molecular analyses.

2.3. Morphological examination

Mature Monogenea that were neither shattered, folded, or twisted were chosen for morphological identification. The specimens were rehydrated (70 percent ethanol to 50 percent ethanol to distilled water for 10–15 min each time), dyed with acetocarmine, then dehydrated in an increasing graduated ethanol series (50 percent, 70 percent, 90 percent, 95 percent, and 100 percent for 10–15 min each immersion). After that, the specimens were xylene-cleared and mounted in Canada balsam (Barton et al., 2009). Initial morphological analyses were conducted using a compound microscope (Upright Motorized Microscope ECLIPSE Ni-E, Nikon, Japan) fitted with a computer screen. Monogenea were initially grouped based on their key morphological traits including body shape and size; morphology and morphometry of the sucker, haptor and genital atrium; number and organisation of clamps; shape, size, number of anchors and marginals according to Nasir and Fuentes Zambrano (1983), Rohde (1986, 1989b), Rohde and Watson (1985a, 1985b), Sproston (1945), Unnithan (1968), and Bouguerche et al. (2019b). Characteristics were measured directly with an eyepiece micrometre (BX-43 Olympus Microscope, Olympus Corporation, Japan). All measurements are in micrometres and are given as the range

followed by the mean in parentheses unless otherwise stated. A dash (–) indicates that measurements could not be made or were not available. A microscope (BX-43 Olympus Microscope, Olympus Corporation, Japan) with a drawing tube (Camera Lucida) was used for drawings. Voucher material (specimens) were deposited in the South Australian Museum under the accession numbers 36952 – 36966.

2.4. Molecular barcoding of host and parasite

A small piece of host muscle tissue and a small piece from each parasite were transferred into separate 1.5ml autoclaved Eppendorf tubes for molecular analyses. The remaining anterior and posterior regions of the parasites were processed for microscopy and morphological studies.

DNA was extracted using DNeasy Blood & Tissue Kits (Qiagen, Hilden, Germany), with some modifications to the manufacturer's instructions (Shamsi et al., 2019), and eluted in 40 µl of elution buffer. PCR amplification of the fragment of the mitochondrial *cox1* gene of both mackerel and parasite was carried out using the following primer sets. For mackerel, FishF1 (forward: 5'- TCA ACC AAC CAC AAA GAC ATT GGC AC-3') and FishR1 (reverse: 5'- TAG ACT TCT GGG TGG CCA AAG AAT CA -3') were used (Ward et al., 2005). For Monogenea, COI-ASmit1 (forward: 5'-TTT TTT GGG CAT CCT GAG GTT TAT-3') and COI-ASmit2 (reverse: 5'-TAA AGA AAG AAC ATA ATG AAA ATG-3')

were used (Littlewood et al., 1997). The cycling conditions to amplify the host's *cox1* gene was an initial 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s, and extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. The *cox1* gene of Monogenea was amplified according to protocol provided in Hossen et al. (2020). An aliquot (3 µl) of each amplicon was examined on a 1.5% w/v agarose gel stained with GelRed® and photographed using a gel documentation system.

Representative samples from hosts and parasites were sent to the Australian Genome Research Facility (AGRF), Queensland, Australia, and were subjected to Sanger sequencing using the same primer sets as for PCR. Sequence data including chromatograms were observed initially through Sequence Scanner Software (Applied Biosystems Genetic Analysers). The host and parasite sequences were compared to the GenBank database content with BLAST and deposited in GenBank. A single alignment file was created for all the sequences and then aligned by the MUSCLE (in MEGA v.10) (Kumar et al., 2016). The evolutionary (pairwise) genetic distance was calculated using MEGA v.10 (Kumar et al., 2016).

2.5. Phylogenetic analysis

Almost all mazocreid and gastrocotylid species sequences available in GenBank were included in a preliminary phylogenetic analyses. The

Table 2

Details of the sequences used in the present study to construct the phylogenetic trees based on *cox1* data. Abbreviations: NSW=New South Wales, VIC= Victoria.

Monogenea species	Monogenea family	Host species	Host family	Geographical origin	Species morphology	GenBank ID <i>cox1</i>	Reference
<i>Gastrocotyle kurra</i> Unnithan, 1968	Gastrocotylidae	<i>Scomber australasicus</i> (Cuvier)	Scombridae	Australia: Off the coast of NSW	Yes	MZ273876–78	Present study
<i>Allogastrocotyle bivaginalis</i> Nasir & Fuentes Zambrano, 1983	Gastrocotylidae	<i>S. australasicus</i>	Scombridae	Australia: Off the coast of VIC	Yes	MZ273879–81	Present study
<i>Kuhnia scombri</i> (Kuhn, 1829) Sproston, 1945	Mazocraeidae	<i>S. australasicus</i>	Scombridae	Australia: Off the coast of VIC	Yes	MZ273888–91	Present study
<i>K. scombercolias</i> Nasir & Fuentes Zambrano, 1983	Mazocraeidae	<i>S. australasicus</i>	Scombridae	Australia: Off the coast of VIC	Yes	MZ273882–87	Present study
<i>Pseudokuhnia minor</i> (Goto, 1894) Rohde & Watson, 1985	Mazocraeidae	<i>S. australasicus</i>	Scombridae	Australia: Off the coast of VIC	Yes	MZ273892–93	Present study
<i>Allogastrocotyle bivaginalis</i>	Gastrocotylidae	<i>Trachurus picturatus</i> (Bowdich)	Carangidae	Algeria	Yes	MN192391–92	Bouguerche et al. (2019)
<i>Pellonicola elongatus</i> Unnithan, 1968	Gastrocotylidae	<i>Ilisha megaloptera</i> (Swainson)	Pristigasteridae	India	No	KU872043*	Unpublished
<i>Engraulicola thrissocles</i> (Tripathi, 1959) Lebedev, 1971	Gastrocotylidae	<i>Thryssa hamiltonii</i> (Gray)	Engraulidae	India	No	KU872046*	Unpublished
<i>Pseudaxine trachuri</i> Parona & Perugia, 1889	Gastrocotylidae	<i>Trachurus trachurus</i> (Linnaeus)	Carangidae	France	No	AY009168	Jovelin and Justine (2001)
<i>Pseudaxine trachuri</i>	Gastrocotylidae	<i>Trachurus trachurus</i>	Carangidae	Algeria	Yes	MN192393	Bouguerche et al. (2019)
<i>Gotocotyla sawara</i> Ishii, 1936	Gotocotylidae	<i>Scomberomorus nipponius</i> (Cuvier)	Scombridae	China: Eight localities along the coast of China	No	KF739594	Shi et al. (2014)
<i>Kuhnia scombri</i>	Mazocraeidae	<i>Scomber japonicus</i>	Scombridae	China: Ten localities along the coast of China	No	KU380080–82	Yan et al. (2016)
<i>Pseudokuhnia minor</i>	Mazocraeidae	<i>Scomber japonicus</i> (Houttuyn)	Scombridae	China	No	KU379830–32	Yan et al. (2016)
<i>Mazocraeoides gonialosae</i> Tripathi, 1959	Mazocraeidae	<i>Konosirus punctatus</i> (Temminck & Schlegel)		China: Seven localities along the coast of China	No	JF773397	Li et al. (2011)
<i>Gastrocotyle kurra</i>	Gastrocotylidae	–	–	–	No	KF804042*	Unpublished
<i>Gastrocotyle trachuri</i> Van Beneden & Hesse, 1863	Gastrocotylidae	<i>Trachurus trachurus</i>	Carangidae	France: Sete	No	AY009167**	Jovelin and Justine (2001)
<i>Choricotyle australiensis</i> Roubal, Armitage & Rohde, 1983 (Outgroup)	Diclidophoridae	<i>Chrysophrys auratus</i> (Forster)	Sparidae	Australia: New South Wales	Yes	MT783686	Hossen et al. (2020)

Note: *Sequences published in GenBank only. **The sequence deposited for *Gastrocotyle trachuri* in GenBank under the accession number of AY009167 has later been considered as *Pseudaxine trachuri* by Bouguerche et al. (2020).

phylogenetic tree was constructed from the sequences generated in this study with sequences from GenBank which had both morphological and *cox1* molecular data (Table 2). All sequences were then aligned with MUSCLE in MEGA v. 7 (Kumar et al., 2016). The phylogenetic relationships among species were determined using the Bayesian method using MrBayes v.3.2 (Ronquist and Huelsenbeck, 2003). The HKY+G model was applied for mitochondrial genes as suggested by jModelTest 2 (Darriba et al., 2012). *Choricotyle australiensis* Roubal, Armitage & Rohde, 1983 (Sequence ID: MT783686) identified from snapper *Chrysophrys auratus* (Förster) in Australian waters was used as the outgroup for phylogenetic analyses (Hossen et al., 2020). Sample frequency was set at 1000 and calculated for 1,500,000 generations for *cox1* regions until the p-value < 0.01. After the ‘mcmc’ run, the first 25% samples were discarded, and the ‘sumt’ command was used to summarise the phylogenetic trees. Figtree v.1.4.3 was used to visualise the phylogenetic trees (Rambaut, 2014).

2.6. Epidemiological data analyses

The prevalence, mean intensity and mean abundance of the Monogenea were calculated as follows: Prevalence (%) = Number of infected fish/Total number of examined fish × 100; Mean intensity = Number of parasites/Number of infected hosts; and Mean abundance = Number of parasites/Total number of examined hosts (Bush et al., 1997).

3. Results

3.1. Molecular identification of fish

The preliminary identification of the host species using morphological characteristics was ascertained by the sequencing of *cox1* gene. A search in GenBank for the representative sequence generated (Sequence ID: MZ274049–50) in this study showed 100% similarity with *S. australasicus* (Sequence ID: DQ107708) identified from Australian waters (Ward et al., 2005), thus confirming the host’s taxonomic status.

3.2. Morphological identification of monogenea

Microscopic examination (which included morphological, morphometric and meristic data) revealed five species of Monogenea belonging to the families Mazocraeidae (*Kuhnina scombri* (Kuhn, 1829) Sproston, 1945, *K. scombercolias* Nasir & Fuentes Zambrano, 1983, and *Pseudokuhnina minor* (Goto, 1894) Rohde & Watson, 1985) and Gastrocotylidae (*Gastrocotyle kurra* Unnithan, 1968 and *Allogastrocotyle bivaginalis* Nasir & Fuentes Zambrano, 1983).

A total of 71 Monogenea individuals were collected from 32 of the 50 fish examined. All Monogenea were recovered from the gills of *S. australasicus*. The overall prevalence, mean intensity, and mean abundance were 64%, 2.22 and 1.42, respectively. The intensity of infection of individual species of Monogenea was highest in the fish collected from off the coast of Victoria. Fish sourced from Victorian

waters had a Monogenea prevalence of 73%, which was the highest overall. The diversity of Monogenea morphotypes was highest in Victorian samples with four species identified. *Kuhnina scombri* had the highest overall intensity at 2.64. The most prevalent Monogenea was found to be *Gastrocotyle kurra* at 50%. *Allogastrocotyle bivaginalis* was the least prevalent Monogenea species. The prevalence, mean intensity, and mean abundance of Monogenea from *S. australasicus* are presented in Table 3.

Parasite species collected in this study corresponded in morphology and measurements to previous descriptions as provided by Nasir and Fuentes Zambrano (1983), Rohde (1986, 1989b), Rohde and Watson (1985a, 1985b), and Sproston (1945), Unnithan (1968), and Bouguerche et al. (2019b).

3.2.1. *Kuhnina scombri*

Seventeen specimens were observed in Victorian waters (Fig. 1a–g and Table 4). Body lanceolate. Maximum width near mid-line, tapering to narrow anterior and posterior regions up until the haptor. Copulatory organ consists of a central pad with two rows of 5 genital hooks, and two smaller pads anterolaterally, each with one large hook (Fig. 1c). Haptor well separated from body proper, heart-shaped, contains eight clamps in two opposing rows of four clamps (Fig. 1a). Distance between clamp

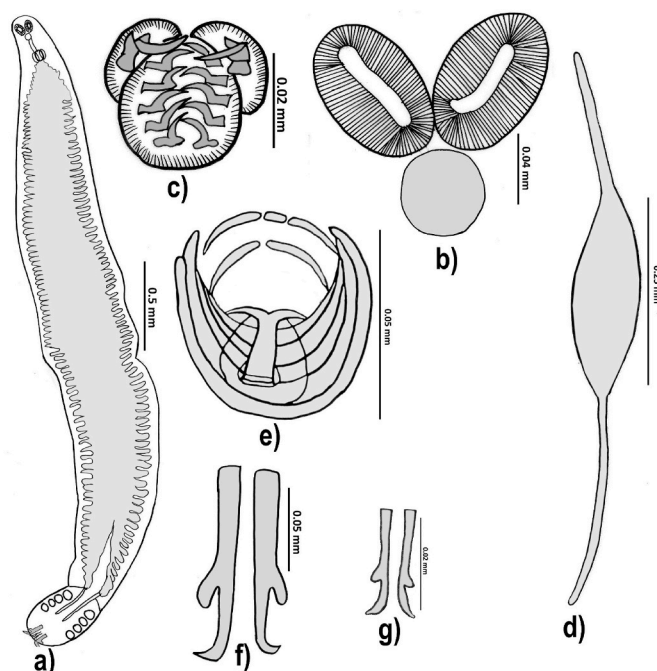


Fig. 1. *Kuhnina scombri* ex *Scomber australasicus*. a) Whole body; b) Oral suckers with pharynx; c) Copulatory organ; d) A typical egg; e) A typical clamp; f) Large anchors; g) Small anchors.

Table 3

Prevalence and abundance of Monogenea in *Scomber australasicus* (Cuvier) examined in the present study. Abbreviations: NSW=New South Wales, VIC= Victoria.

Source of fish (number examined)	Name of parasites	Fish infected	Range in infected fish	Prevalence (%)	Total number found	Mean intensity	Mean abundance
Off the coast of NSW, Australia (n = 20)	<i>Gastrocotyle kurra</i>	10	1–4	50	22	2.2	1.1
Date: 23-07-2018	Total	10	1–4	50	22	2.2	1.1
Off the coast of VIC, Australia (n = 30)	<i>Allogastrocotyle bivaginalis</i>	3	1–1	10	3	1	0.1
Date: 29-09-2018	<i>Kuhnina scombri</i>	11	1–5	37	29	2.64	0.97
	<i>K. scombercolias</i>	7	1–4	23	11	1.58	0.37
	<i>Pseudokuhnina minor</i>	5	1–2	17	6	1.2	0.2
	Total	22	1–7	73	49	2.28	1.63
	Grand Total (n = 50)	32	1–7	64	71	2.22	1.42

Table 4

Comparative measurements of mazoceid Monogenea. Measurements are in micrometres unless otherwise stated and indicated as the range followed by the mean. Abbreviations: NSW=New South Wales, VIC= Victoria, WA=Western Australia.

Source	Present study	Rohde and Watson (1985b)	Present study	Nasir and Fuentes Zambrano (1983)	Rohde (1989)	Present study	Rohde and Watson (1985a)
Monogenea	<i>Kuhnia scombri</i>	<i>K. scombri</i>	<i>K. scombercolias</i>	<i>K. scombercolias</i>	<i>K. scombercolias</i>	<i>Pseudokuhnia minor</i>	<i>P. minor</i>
Hosts (Scientific name)	<i>Scomber australasicus</i>	<i>S. australasicus</i>	<i>S. australasicus</i>	<i>S. colias</i>	<i>S. australasicus</i>	<i>S. australasicus</i>	<i>S. australasicus</i>
Hosts (Common name)	Blue mackerel	Blue mackerel	Blue mackerel	Atlantic chub mackerel	Blue mackerel	Blue mackerel	Blue mackerel
Locality	Off the coast of VIC, Australia	Jervis Bay and Sydney Fish Market, NSW, Australia	Off the coast of VIC, Australia	Gulf of Cariaco, Venezuela	Sydney fish market and Eden, NSW; Perth, WA	Off the coast of VIC, Australia	NSW, Australia
No. of specimens	Seventeen (n = 17)	Not specified	Eight (n = 8)	Three (n = 3)	Not specified	Six (n = 6)	Not specified
Total body length (mm)	2.25–4.28 (3.30)	1.49–4.33, 2.43 (13)	1.85–2.65 (2.12)	1120–1400	0.82–344, 1.55 (6)	1.20–2.65 (1.86)	0.70–1.45, 1.09 (8)
Maximum body width (mm)	0.20–0.65 (0.48)	0.16–0.73, 0.43 (13)	0.40–0.65 (0.48)	378–585	0.28–0.73, 0.42 (6)	0.18–0.45 (0.32)	0.17–0.35, 0.25 (8)
Haptor (opisthaptor) length	275–400 (331.67)	250–420, 310 (13)	175–320 (242.50)	120–130	160–450, 240 (6)	220–300 (250)	170–330, 240 (7)
Oral sucker length	65–90 (75.53)	–	75–100 (86.89)	22–26	–	63–73 (67)	–
Oral sucker width	40–60 (50.35)	–	48–50 (49.11)	21–24	–	30–38 (33.83)	–
Buccal suckers (diameter)	–	36–52, 41 (10)	–	–	–	38–65, 54 (5)	21–44, 32 (7)
Pharynx length	36–50 (44.31)	–	40–50 (44.33)	23–30	–	35–42 (39.33)	–
Pharynx width	36–45 (40.31)	–	40–46 (42.78)	20–27	–	27–33 (29.17)	–
Pharynx (diameter)	–	24–37, 31 (10)	–	–	–	22–37, 31 (5)	17–30, 25 (8)
Clamps number	8	8	8	8	8	8	8
Clamp length (large)	43–55 (48.29)	–	43–60 (48.11)	26–34	–	45–50 (47.17)	–
Clamp width (large)	35–46 (42.14)	–	40–50 (44.22)	35–40	–	38–43 (40.17)	–
Largest clamp skeleton (diameter)	–	35–59, 43 (13)	–	–	–	35–66, 44 (6)	30–39, 34 (8)
Genital atrium length	55–75 (65.86)	–	35–50 (45)	–	–	45–80 (65)	–
Genital atrium width	50–60 (53.21)	–	35–50 (44.11)	–	–	45–75 (62.17)	–
Large genital hooks length	15–25 (19)	20–27, 23.2 (21)	14–16 (15)	–	–	13–21, 16 (5)	26–35 (30.33)
Small genital hooks length	12–18 (15.81)	14–18, 15.7 (21)	8–12 (9.78)	–	–	10–14, 11.2 (6)	14–20 (16.50)
Number of genital atrial hooks	8–12 (10.50)	8–11, 9.9 (20)	10–14 (11.71)	–	–	10–11, 10.8 (4)	7–8
Distance genital atrium-anterior end	200–250 (230)	–	150–180 (165)	–	–	140–220 (180)	–
Distance vitellaria-anterior end	275.00	–	200–350 (251.88)	–	–	370–450 (400)	–
Large hamulus length	90–114 (103.53)	106–110	40–50 (44.33)	32–40	31–35	42–52 (46.17)	26–36
Small hamulus (marginal's) length	15–27 (21.82)	21–24, 22.5 (21)	16–20 (17.11)	07–09	17–21, 19 (2)	13–18 (15)	14–18
Egg length (without filament)	275–350 (300)	–	250–320 (273.33)	130.00	–	275 (n = 1)	–
Egg width	80–90 (86.67)	–	65–95 (75)	30.00	–	82 (n = 1)	–

rows decreases towards anterior portion of haptor. The caeca posteriorly connected and nearly reach to the end of body. Anchors long, strong, stout, with a hook and ridged shaft (Fig. 1f and g). No vitellaria observed in haptor. Eggs elongated-ellipsoid, operculated, filaments at each pole (Fig. 1d).

3.2.2. *Kuhnia scombercolias*

Eight specimens were observed in Victorian waters (Fig. 2a–f and Table 4). Body elongated and looked dorsoventrally flattened but comparatively shorter than *K. scombri*. Copulatory organ similar to that

of *K. scombri*, with an additional strong hook on central pad (Fig. 2b). Haptor not clearly separated from body proper. Haptor contains eight clamps and organised in two opposing separate rows of four clamps on each side and distance between each row of clamps decreases posteriorly (Fig. 2a). Anchors smaller than those observed in *K. scombri* (Fig. 2e and f).

3.2.3. *Pseudokuhnia minor*

Six specimens were observed in Victorian waters (Fig. 3a–f and Table 4). Body proper lanceolate and dorsoventrally flattened (Fig. 3a).

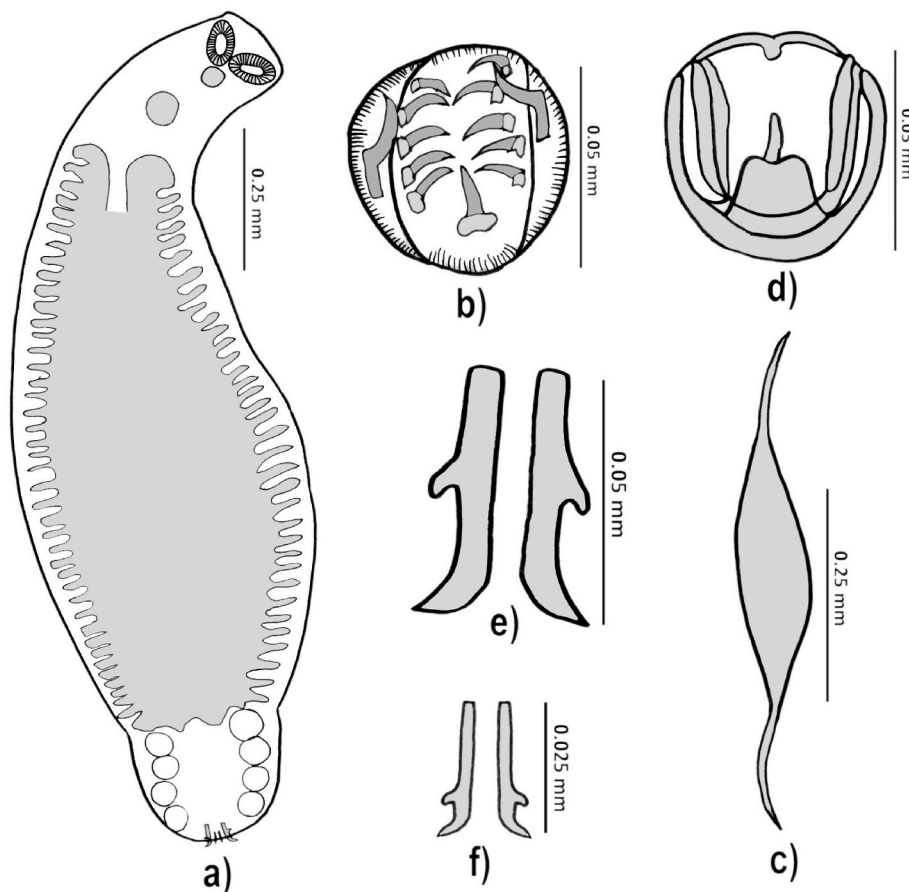


Fig. 2. *Kuhnia scombercolias* ex *Scomber australasicus*. a) Whole body; b) Copulatory organ; c) A typical egg; d) A typical clamp; e) Large anchors; f) Small anchors.

Haptor ventral and well separated from body proper. Oral suckers separated, and pharynx suckers smaller than the buccal suckers (Fig. 3b). Copulatory organ consists of a central muscular pad with 10 (may vary) median smaller genital hooks and two muscular pads anterolaterally, each with a single curved large hook (Fig. 3c). Terminal lappet contains one pair of anchors and one pair of marginals. Large shape and structure of anchors also differ from other *Kuhnia* species being distinctly hook-like at posterior end and forked anteriorly (Fig. 3f). Haptor containing two approximately parallel rows of clamps of equal size with four clamps in each row. Caeca terminating at the level of the posterior part of the haptor. Species contains two dorsolateral vaginas, or two vaginal cavities (posterior to gonopore) supported by numerous transverse ridges.

3.2.4. *Gastrocotyle kurra*

Ten specimens were observed in NSW waters (Fig. 4a–f and Table 5). Body typically gastrocotylid and body proper divided into two regions. Anterior (neck) region narrow and contains pharyngeal or post-pharyngeal area while main part of body proper (comparatively wider/leaf-like) contains a haptor frill extending forwards from base of the terminal lappet to the centre and almost straight (Fig. 4a). Division between neck and body proper always clearly visible even in immature specimens. Maximum body width at level of the haptor frill. Number of clamps 18–27 and arranged in a single row along the margin of the unilateral frill (Fig. 4a). Terminal lappet (Fig. 4f) in the form of a terminal knob containing three symmetrical pairs of oblique anchors: proximal anchors (largest), middle anchors (smallest), and distal anchors (intermediate between largest and smallest). Spindle-shaped eggs with a filament at each pole observed in a few specimens (Fig. 4d). Anterior filament slightly longer than the posterior filament.

3.2.5. *Allogastrocotyle bivaginalis*

Three specimens were observed in Victorian waters (Fig. 5a–f and Table 5). Body typically gastrocotylid, elongated and anterior extremity constricted. Two ventrolateral vaginal openings present. Haptors long, unilateral, and asymmetrical and armed with 26–28 clamps and arranged in a single row (Fig. 5a). Single terminal lappet and armed with two pairs of anchors; large (Fig. 5e) and small (Fig. 5f). Large anchors sickle-shaped (Fig. 5e). Oesophagus bifurcates posterior to the genital atrium. Genital atrium (gonopore) armed with hooks and obliquely oriented (Fig. 5c).

3.3. Molecular characterisation of monogenea

A total of 18 specimens (representatives from each species and each collection location) were sequenced for their *cox1* regions.

3.3.1. *Mazocraeid monogenea*

Four specimens (voucher numbers 319-2, 319-3, 319-5 and 322-1) belonging to *K. scombri* from Victoria were subjected to sequencing. The length of the *cox1* sequences generated for the specimens was 396 bp. However, the sequences were not identical. The pairwise genetic comparison between the sequences revealed 0.76–1.56% nucleotide variability. A search in GenBank for one of the four sequences (Sequence ID: MZ273888–91) generated in this study showed 99% similarity (349/350 with no nucleotide gaps) with *K. scombri* (Sequence ID: KU380242) identified from the chub mackerel *S. japonicus* (Houttuyn) in ten locations along the coast of China (Yan et al., 2016).

Two specimens (voucher numbers 317-1 and 321-1) belonging to *P. minor* from Victorian fish were sequenced. The length of the *cox1* sequences generated for the specimens was 396 bp but were not identical. The pairwise genetic comparison between the sequences

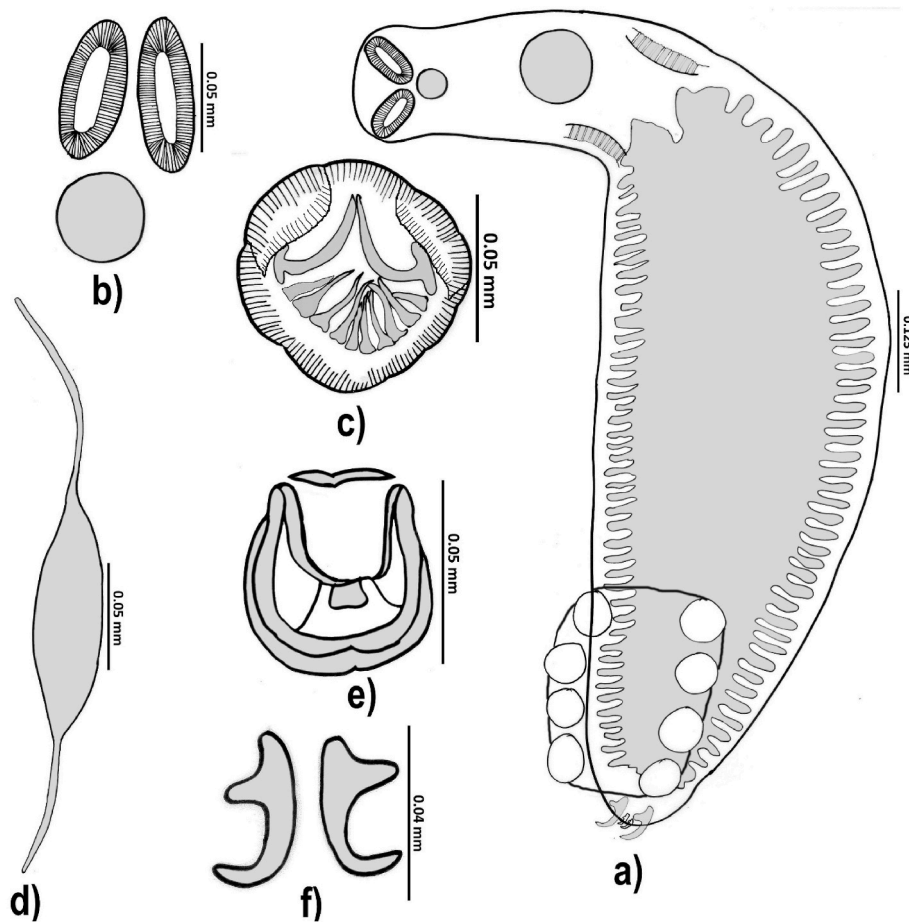


Fig. 3. *Pseudokuhnia minor* ex *Scomber australasicus*. a) Whole body; b) Oral suckers with pharynx; c) Copulatory organ; d) A typical egg; e) A typical clamp; f) Large anchors.

(Sequence ID: MZ273892 & MZ273899) revealed 0.25% nucleotide variability. A search in GenBank for the representative sequence showed 96% similarity (302/315 with no nucleotide gaps) with the *P. minor* (Sequence ID: KU379915) identified from the chub mackerel *S. japonicus* in ten locations along the coast of China (Yan et al., 2016).

Six specimens (voucher numbers 315-1, 315-2, 318, 319-4, 322-2 and 331-2) belonging to *K. scombercolias* from Victoria were subjected to sequencing. The length of the *cox1* sequences generated for the specimens was 396 bp. However, the sequences were not identical. The pairwise genetic comparison between the sequences (Sequence ID: MZ273882–87) generated in the present study revealed 0.77% nucleotide variability. There is no comparable sequence data available in GenBank for this parasite. Therefore, no similarity index is provided for this species.

3.3.2. Gastrocotylid monogenea

Three specimens (voucher numbers 190, 194 and 199) belonging to *G. kurra* from NSW fish were subjected to sequencing. The length of the *cox1* sequences generated for the specimens was 396 bp but not identical. The pairwise genetic comparison between the sequences (Sequence ID: MZ273876–78) revealed 1.54% nucleotide variation. A search in GenBank for one of the three sequences showed 90% similarity (255/282 with no nucleotide gaps) with the only sequence available for *G. kurra* (Sequence ID: KF804042).

Three specimens (voucher numbers 324-2, 331-1 and 335) belonging to *A. bivaginalis* from Victorian fish were subjected to sequencing. The length of the *cox1* sequences generated for the specimens was 396 bp but not identical. The pairwise genetic comparison between the sequences (Sequence ID: MZ273879–81) revealed 0.25–0.51% nucleotide

variation. A search in GenBank for one of the three sequences generated in this study showed 98% similarity (389/396 with no nucleotide gaps) with the *A. bivaginalis* (Sequence ID: MN192392) identified from the blue jack mackerel *Trachurus picturatus* (Bowdich) in Algeria, Mediterranean Sea (Bouguerche et al., 2019b).

3.4. Phylogenetic analyses

The Bayesian phylogenetic analyses grouped the Monogenea of Mazocraeidae and Gastrocotylidae independently. *Kuhnia scombri* clustered with *K. scombri* and *P. minor* grouped with *P. minor*. The sequences for *K. scombercolias* clustered with closely related *Kuhnia* species but with a clear distinction and a strong bootstrap value. The gastrocotylid Monogenea *G. kurra* clustered with *G. kurra*. *Allogastrocotyle bivaginalis* displayed a clear distinction from *G. kurra* and other gastrocotylid Monogenea species and the phylogenetic tree grouped with *A. bivaginalis*. The phylogenetic relationship of Monogenea species is shown in Fig. 6.

4. Discussion

This study confirmed the presence and specific identification of three mazocraeid Monogenea species (*K. scombri*, *K. scombercolias*, and *P. minor*) and the first evidence of the occurrence and identification of two gastrocotylid Monogenea, *G. kurra* and *A. bivaginalis*, infecting *S. australasicus* from Australian waters. The studies conducted of *S. australasicus* by Rohde (1986, 1989b) and Rohde and Watson (1985a, 1985b) showed that five Monogenea species (*K. scombri*, *K. scombercolias*, *K. sprostonae*, *P. minor*, and *Grubea australasicus*)

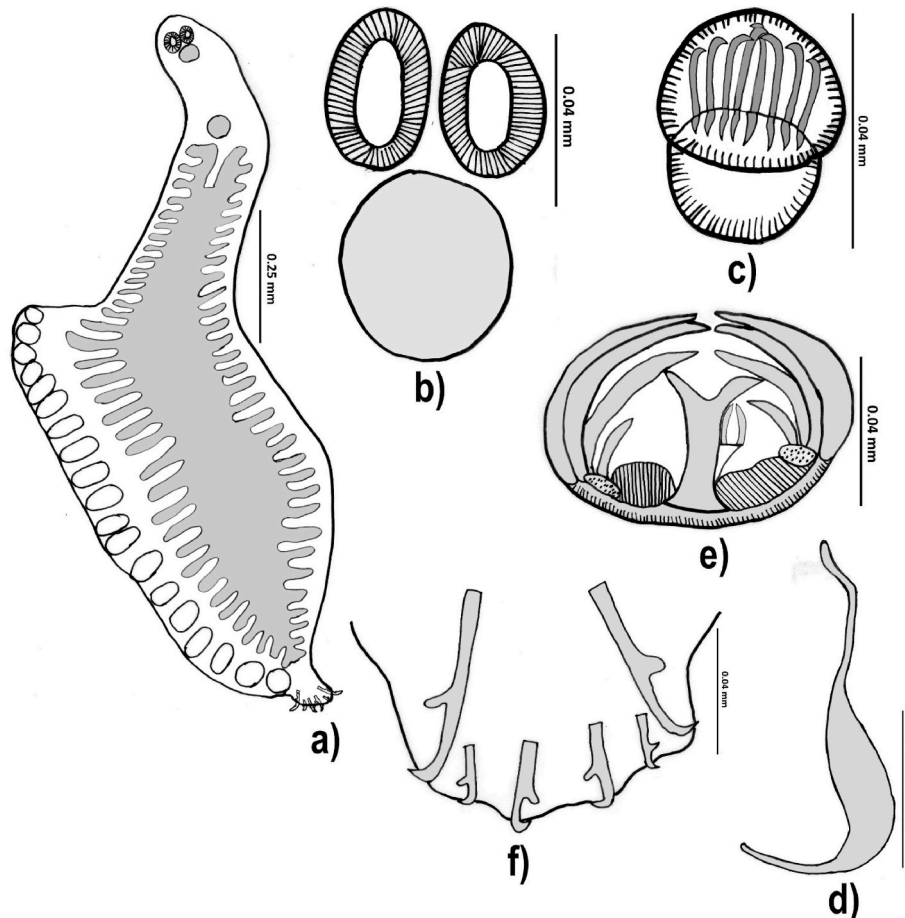


Fig. 4. *Gastrocotyle kurra* ex *Scomber australasicus*. a) Whole body; b) Oral suckers with pharynx; c) Copulatory organ; d) A typical egg; e) A typical clamp; f) Terminal lappet with anchors.

commonly occur in *S. australasicus* from Australian waters.

Kuhnia scombri (Kuhn, 1829) Sproston, 1945 was first described by Kuhn in 1829 as *Octostoma scombri*. Goto (1894) followed by providing a detailed account of the internal anatomy of this species. To date, *K. scombri* has been identified from four species of *Scomber* fish: *Scomber scombrus* (Linnaeus) in the waters of North America, North Sea, Bay of Biscay, Mediterranean, east and west Atlantic and Guernsey; *S. japonicus* in the waters of Brazil, Mediterranean, Japan, Galapagos Islands, Ecuador, South Africa and Argentina; *S. colias* (Gmelin) from the Mediterranean; *S. australasicus* in NSW (south-east Australia), New Zealand and Philippines (where only a single specimen was observed) (Rohde and Watson, 1985b). Sproston (1945) also identified this parasite from *S. scombrus* from waters of Skagerrak, North Sea, English Channel, east and west Atlantic, and the Mediterranean; *S. colias* from the Mediterranean; and *S. japonicus* from Vladivostok and Misaki (Japan). Rohde and Watson (1985b) found extensive morphological variation of this species in different *Scomber* hosts from disparate geographical locations.

There are 164 *cox1* sequences available in GenBank for *K. scombri* from a single study conducted in ten locations along the coast of China in 2016 (Yan et al., 2016). The parasite was identified from chub mackerel *S. japonicus*; however, no morphology data was provided in this publication and there was 0.1–5.6% (mean 2.4%) sequence divergence (Yan et al., 2016). The pairwise genetic distance between the sequences generated in the present study with representative GenBank sequences (KU380080–82 and KU380242) showed 0.57–3.88% nucleotide variability (Table 6). Due to the low genetic diversity between the present data and GenBank sequences, this variation was not considered as interspecific variation. Also, the morphometric and meristic data of the species showed a close or complete similarity with the earlier studies

(Table 4). Three sequences of *K. scombri* for nuclear genes (two 28S and one 18S) were also available in GenBank; however, no morphological data were provided (Littlewood et al., 1999; Mollaret et al., 2000; Olson and Littlewood, 2002). This study explores, for the first time, the *cox1* sequences of *K. scombri* from the *S. australasicus* in Australian waters in conjunction with the morphological examination.

Kuhnia scombercolias Nasir & Fuentes Zambrano, 1983, was first described by Nasir and Fuentes Zambrano (1983) from the Atlantic chub mackerel *S. colias* in Venezuelan waters. To date, the species *K. scombercolias* has been identified from *S. australasicus* in waters of Australia and Hawaii, USA; from *S. colias* in Venezuela; from *S. japonicus* in Ecuador, Brazil and the Arabian Sea (Table 1 and Rohde (1989b)). Morphologically, *K. scombercolias* is difficult to distinguish from other *Kuhnia* species, particularly *K. sprostonae* (Rohde, 1989b; Rohde and Watson, 1985b), as both species share several morphological characteristics (Rohde, 1989b) including: i) the haptor is not clearly distinguished from the rest of the body; ii) the copulatory organ has the same structure; iii) the number, organisation and shape/structure of clamps are similar; and iv) the anchor shape/structure is the same. These similarities created challenges and ambiguities during morphological species identification in the current study, making it difficult to differentiate these two species. Rohde and Watson (1985b) partially explain the greater morphological variations of *K. sprostonae* associated with hosts and geographical origins. However, in a subsequent publication, Rohde (1989b) concluded that some of the *K. sprostonae* species identified and described in Rohde and Watson (1985b) should be synonymised with *K. scombercolias*. Hence, morphological species identification of small mazocraeid Monogenea species is sometimes problematic. Rohde (1989a, 1989b) stated that “populations of Monogenea from the same

Table 5

Comparative measurements of gastrocotylid Monogenea. Measurements are in micrometres unless otherwise stated and indicated as the range followed by the mean. Abbreviations: NSW=New South Wales.

Source	Present study	Unnithan (1968)	Present study	Bouguerche et al. (2019)	Bouguerche et al. (2019)	Nasir and Fuentes Zambrano (1983)
Monogenea	<i>Gastrocotyle kurra</i>	<i>G. kurra</i>	<i>Allogastrocotyle bivaginalis</i>	<i>A. bivaginalis</i>	<i>A. bivaginalis</i>	<i>A. bivaginalis</i>
Hosts (Scientific name)	<i>Scomber australasicus</i>	<i>Caranx kurra</i> (Syn. now as <i>Decapterus kurroides</i>)	<i>Scomber australasicus</i>	<i>Trachurus picturatus</i>	<i>Trachurus lathami</i>	<i>Trachurus lathami</i>
Hosts (Common name)	Blue mackerel	Redtail scad	Blue mackerel	Blue jack mackerel	Rough scad	Rough scad
Locality	Off the coast of NSW, Australia	Trivandrum and near places, Kerala, India	Off the coast of NSW, Australia	Off Algeria, Mediterranean Sea	Off Venezuela, Atlantic	Off Venezuela, Western Atlantic
No. of specimens	Ten (n = 10)	Seven (n = 7)	Three (n = 3)	Thirty-three (n = 33)	Holotype	Two specimens measured
Total body length (mm)	1.60–2.10 (1.84)	1.50–2.87	2.05–3.00 (2.54)	1.18–2.68 (2.03; n = 26)	1.76	1.75–2.21
Body width (mm)	0.32–0.60 (0.40)	0.45–0.64	0.38–0.45 (0.43)	0.31–1.93 (0.48; n = 26)	0.42	0.46–0.50
Anterior narrow neck (mm)	0.70–1.00 (0.86)	–	–	–	–	–
Haptor length	750–1200 (990)	1880	730–925 (801.67)	310–1870 (1143; n = 26)	945.00	945.00
Terminal lappet	100–160 (122) x 75–100 (84)	225 × 135	500 (n = 1)	–	–	100–130
Oral sucker length	30–40 (34.50)	–	38–40 (38.67)	20–40 (29; n = 28)	20	16–26
Oral sucker width	20–32 (25.40)	–	35–40 (36.67)	15–50 (29; n = 28)	20	18–22
Pharynx length	42–50 (45.80)	40–44	33–43 (37)	30–50 (38; n = 24)	30	54
Pharynx width	33–46 (38.50)	–	32–40 (35.33)	20–52 (36; n = 24)	27	58
Clamps number	18–27 (23)	16–29	26–28 (27)	23–36 (32 ± 3; n = 29)	32	32–33
Clamp length (large)	63–85 (77.50)	60 × 76	55–58 (56)	37–68 (55 ± 7; n = 48)	55	50–56
Clamp width (large)	55–76 (63.60)	–	45–48 (46.67)	31–68 (42 ± 6; n = 46)	33	41–44
Genital atrium length	40–48 (42.70)	–	45–50 (46.67)	25–50 (38 ± 6; n = 30)	37	–
Genital atrium width	29–38 (32.40)	–	40–45 (42.67)	25–50 (39 ± 5; n = 30)	30	–
Number of atrial hooks	17–19 (18)	–	11–13 (12)	11–13 (12; n = 20)	12	12
Atrial hooks length (large)	14–17 (15.30)	–	17–20 (18.50)	13–25 (21 ± 3; n = 41)	30	17–20
Atrial hooks length (small)	5–7 (5.70)	–	10–14 (11.67)	–	–	–
Large hamuli (proximal anchor) length	45–55 (50.30)	40–44	33–35 (34)	24–32 (29; n = 20)	33	30–34
Small hamuli (middle anchor) length	17–20 (18)	16	–	–	–	–
Distal anchor (marginal's) length	25–30 (26.50)	20	10–15 (12.33)	9–13 (11; n = 19)	13	–
Distance genital atrium-anterior end	150–275 (233.57)	–	160–200 (174.33)	125–310 (206; n = 27)	160	134–216
Egg length	300 (n = 1)	225 × 75 to 255 × 90	–	–	–	80
Egg width	80 (n = 1)	–	–	–	–	28
Anterior filament	250 (n = 1)	300–375	–	–	–	–
Posterior filament	150 (n = 1)	285–375	–	–	–	–

host species or genus in different geographical areas are likely to be conspecific and should not be described as different species if they differ only slightly from each other". In the current study, however, the similar genetic sequences resolved the issues. As a result, it is suggested that both species (*K. sprostonae* and *K. scombercolias*) be reviewed using genetic data to ensure that they are indeed two distinct species.

There is no comparable genetic sequence data available for either *K. sprostonae* or *K. scombercolias* and sequence data is available for only one *Kuhnina* species, *K. scombri*. A search in GenBank for one of the representative sequences generated for *K. scombercolias* showed 85% similarity (295/346 with no nucleotide gaps) with *K. scombri* (Sequence ID: KU380140) identified from the chub mackerel *S. japonicus* in ten locations along the coast of China (Yan et al., 2016). Our sequences are novel for *K. scombercolias*.

Pseudokuhnina minor (Goto, 1894) Rohde & Watson, 1985, was first described by Goto (1894) from the gills of chub mackerel *S. japonicus* from Japan as *Octocotyle minor* (approximately 2 mm in length). Sproston (1945) synonymised *Kuhnina* spp. with *Octocotyle* spp. The

species *Kuhnina minor*, since its original description by Goto (1894), has been included in this genus by other parasitologists. Rohde and Watson (1985a) described *Kuhnina minor* as having two vaginas. This important characteristic was overlooked by previous parasitologists. The absence of vagina (no vagina) is an important feature of the genus *Kuhnina*. Subsequently, a new genus, *Pseudokuhnina*, was established for *Kuhnina minor* under the family Mazocraeidae (Rohde and Watson, 1985a).

Pseudokuhnina minor has previously been identified from *S. australasicus* in NSW (south-east Australia), Philippines and China; *S. japonicus* in Atlantic South Africa (Capetown), Atlantic Spain, Haifa (Mediterranean Israel) and Japan; Indian Ocean (host not mentioned); *S. scombrus* in the west English Channel area (Table 1 and Rohde and Watson (1985a)). Rohde and Watson (1985a) found the extensive morphological variation of this species from the different hosts and geographical locations.

There are 250 *cox1* sequences available in GenBank for *P. minor* from a single study conducted in ten locations along the coast of China in 2016 (Yan et al., 2016). The parasite was identified from chub mackerel

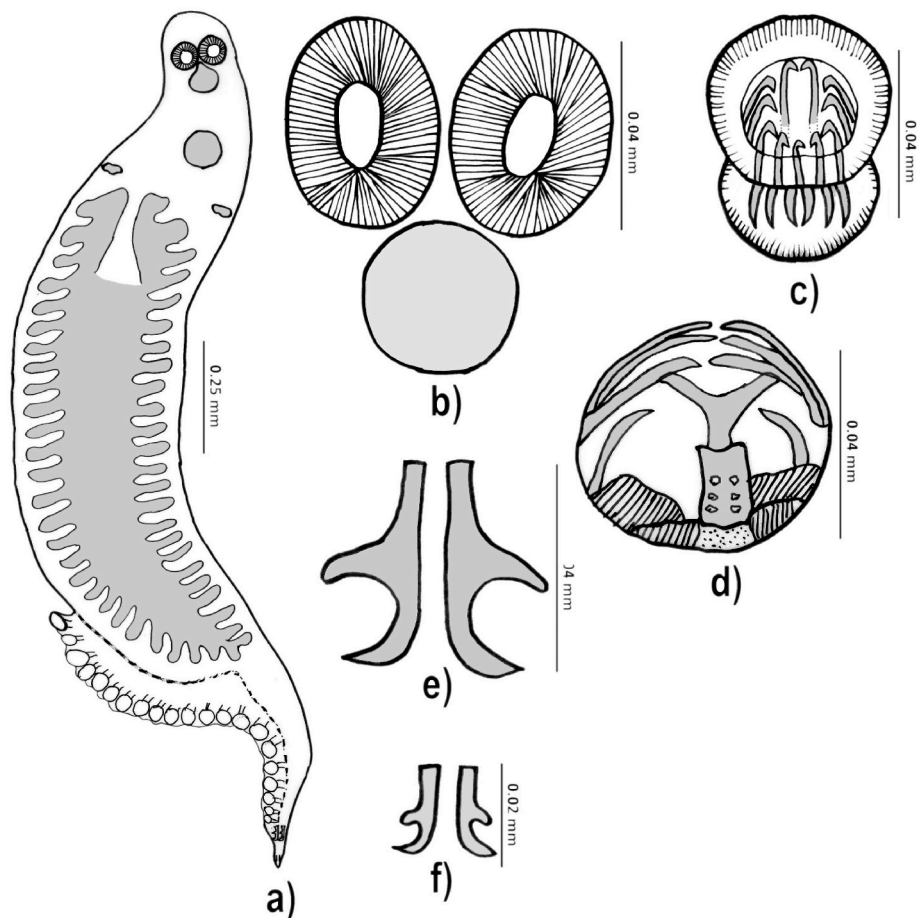


Fig. 5. *Allogastrocotyle bivaginalis* ex *Scomber australasicus*. a) Whole body; b) Oral suckers with pharynx; c) Copulatory organ; d) A typical clamp; e) Large anchors; f) Small anchors.

S. japonicus however, no morphology data of the parasite was provided in this publication. No sequence data are available in GenBank for the nuclear genes of *P. minor*. In Yan et al. (2016), 0.1–4.8% (mean 1.6%) divergence was observed among the sequences. The pairwise genetic distance between the sequences generated in the present study with representative GenBank sequences (KU379830–32 and KU379915) showed 0.32–6.54% nucleotide variability (Table 7). Due to the low genetic diversity between the present data and GenBank sequences, the variation is not considered as interspecific variation. Although, the morphology, morphometric, and meristic data of the species showed close or complete similarity with earlier studies (Fig. 3a–f and Table 4) additional molecular work is required for definitive identification of this species. This study explores, for the first time, the *cox1* sequences in combination with morphological characteristics of *P. minor* from *S. australasicus* in Australian waters. Rohde and Watson (1985a) observed greater morphological and geographical variations of *P. minor* from four *Scomber* fish species.

Gastrocotyle kurra Unnithan, 1968 was first described by Unnithan (1968) from redtail scad *Decapterus kurroides* (Bleeker) (Syn. *Caranx kurra*) in Indian waters. No updated morphological description of *G. kurra* is available in the literature. The morphometric and meristic data for *G. kurra* in the present study closely or completely matched with the earlier descriptions (Fig. 4a–f and Table 5). There is a single *cox1* sequence available in GenBank for *G. kurra* as a direct submission. However, the sequence obtained from GenBank did not have morphological data and information regarding the host and locality was not provided. One sequence for the nuclear 28S gene is available in GenBank; however, again, no morphological description is included (Tambireddy et al., 2016). As the pairwise genetic distance between the

sequences generated in the present study and the GenBank sequence (Sequence ID: KF804042) showed 11.21% nucleotide variability, the GenBank sequence must be a misidentification. Without morphological description, the identity of this specimen will not be able to be determined. The present study describes, for the first time, the *cox1* sequence and morphological identification of *G. kurra* from *S. australasicus* in Australian waters. This study also provides a new host record of this Monogenea species.

Allogastrocotyle bivaginalis Nasir & Fuentes Zambrano, 1983, was first described by Nasir and Fuentes Zambrano (1983) from rough scad *Trachurus lathami* (Nichols) from the waters of Venezuela, Western Atlantic. *Allogastrocotyle bivaginalis* found in the present study is recorded for the third time after its original description by Nasir and Fuentes Zambrano (1983). Previously, Bouguerche et al. (2019b) morphologically redescribed this species from blue jack mackerel *T. picturatus* from waters of the Algerian coast, and two *cox1* sequences were deposited in GenBank to provide molecular validation for this species. The only two *cox1* sequence data available in GenBank are from Bouguerche et al. (2019b). The pairwise genetic distance between the sequences of the present study and two GenBank sequences (Sequence ID: MN192391–92) showed 2.33% nucleotide variability, which is within the range of intraspecific variation (Bouguerche et al., 2019a). The specimens of *A. bivaginalis* that has been collected from *S. australasicus* in the present study morphologically slightly differ from those collected on *T. lathami* (by Nasir and Fuentes Zambrano, 1983) by body length, length of terminal lappet, dimensions of oral suckers and of pharynx; they differ from those collected on *T. picturatus* (by Bouguerche et al., 2019b) by haptor length, remarkably by clamp number and dimensions of clamps (Fig. 5a–f and Table 5). However, their genetic data

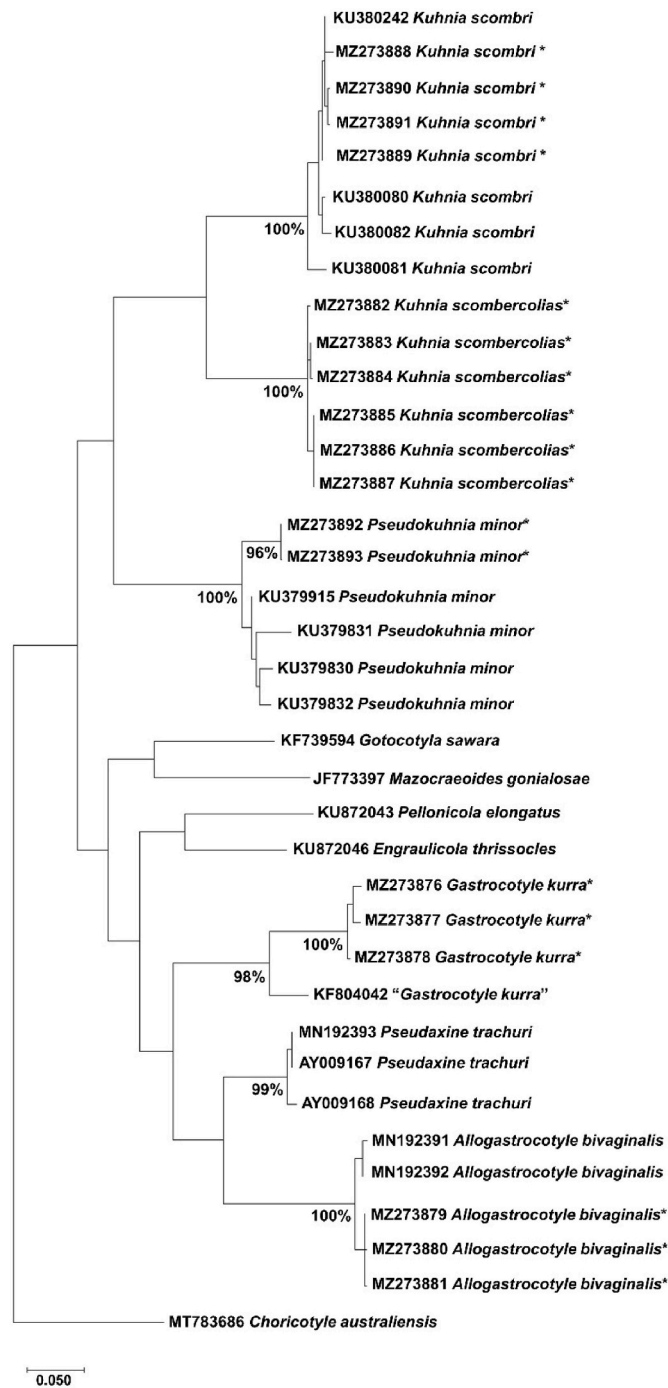


Fig. 6. The Phylogenetic relationship of Monogenea identified from blue mackerel *Scomber australasicus* in this study compared with closely related species *cox1* sequences available in GenBank (Table 2 for details). The tree has been constructed/inferred using the Bayesian method. *indicates the *cox1* sequences generated in the present study. The Bayesian posterior probability values more than 95% were shown on the node.

supporting the identification *A. bivaginalis*, for the first time, in Australian waters from *S. australasicus*.

In the present study, the interspecific genetic variation for a species was found to be up to 10% for the *cox1* sequences. According to Hebert et al. (2003), the *cox1* sequence diversity greater than 2% indicates different species, and sequence diversity less than 2% indicates intra-specific variation. However, several studies have shown that the threshold values may not be applicable to all species and that this value

Table 6

Pairwise genetic distance (%) of *Kuhnina scombri* between the sequences generated in the present study with four representative GenBank sequences; *indicates the *cox1* sequences generated in the present study.

Sequence ID, Monogenea and host	1	2	3	4	5	6	7
1 *MZ273888 <i>Kuhnina scombri</i> ex <i>Scomber australasicus</i>							
2 *MZ273889 <i>K. scombri</i> ex <i>S. australasicus</i>	0.87						
3 *MZ273890 <i>K. scombri</i> ex <i>S. australasicus</i>	1.15	0.86					
4 *MZ273891 <i>K. scombri</i> ex <i>S. australasicus</i>	1.16	0.86	0.57				
5 KU380080 <i>K. scombri</i> ex <i>S. japonicus</i>	1.79	0.87	1.75	1.76			
6 KU380081 <i>K. scombri</i> ex <i>S. japonicus</i>	3.27	2.95	3.27	3.30	3.27		
7 KU380082 <i>K. scombri</i> ex <i>S. japonicus</i>	1.77	1.46	1.74	1.75	1.16	3.88	
8 KU380242 <i>K. scombri</i> ex <i>S. japonicus</i>	0.58	0.29	0.57	0.57	1.17	3.27	1.16

Table 7

Pairwise genetic distance (%) of *Pseudokuhnia minor* between the sequences generated in the present study with four representative GenBank sequences; *indicates the *cox1* sequences generated in the present study.

Sequence ID, Monogenea and host	1	2	3	4	5
1 *MZ273892 <i>Pseudokuhnia minor</i> ex <i>Scomber australasicus</i>					
2 *MZ273893 <i>P. minor</i> ex <i>S. australasicus</i>	0.32				
3 KU379830 <i>P. minor</i> ex <i>S. japonicus</i>	6.11	6.50			
4 KU379831 <i>P. minor</i> ex <i>S. japonicus</i>	6.14	6.54	4.39		
5 KU379832 <i>P. minor</i> ex <i>S. japonicus</i>	5.33	5.70	2.28	4.65	
6 KU379915 <i>P. minor</i> ex <i>S. japonicus</i>	4.32	4.68	1.95	3.65	1.63

is group-specific (Meyer and Paulay, 2005; Collins and Cruickshank, 2013). In a comparison of intraspecific variation for various representatives of the Polyopisthocotylea, Bouguerche et al. (2019a) found the range to be 0–5.6%, with the majority under 2%. Increasing the range of samples, from a wide range of Monogenea species, hosts and geographical distributions, as well as markers, are needed in future investigations to gain a deeper understanding of the evolutionary relationship between these Monogenea.

5. Conclusion

The overall prevalence of Monogenea in the study was 64% and the diversity and intensity of parasitic infection were highest in the fish sourced from Victorian waters. Five species of Monogenea from two families were identified and a new host record was established for *G. kurra* and *A. bivaginalis*. This study highlights that for species of Monogenea from *S. australasicus* of NSW and Victorian waters, geographical variation is common and should be considered when identifying other groups of Monogenea. This study further shows that populations of Monogenea from the same genera of hosts in different geographical areas are likely to be conspecific and should not be described as novel species unless genetic material from the original identification is also examined. Further morphological and molecular analyses of Monogenea parasites from different hosts and locations are

required to explore the distribution of this understudied group.

Ethical approval

All applicable institutional, national, and international guidelines for the care and use of animals were followed.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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