Journal of Helminthology

cambridge.org/jhl

Isorchis cannoni n. sp. (Digenea: Atractotrematidae) from Great Barrier Reef rabbitfishes and the molecular elucidation of its life cycle

D.C. Huston[1], S.C. Cutmore[1] and T.H. Cribb[1]

[1]School of Biological Sciences, The University of Queensland, St Lucia, QLD 4072, Australia

Research Paper

This article was registered in the Official Register of Zoological Nomenclature (ZooBank) as 9E6C6CA1-B94B-4F6A-9679-AD898704C84D.

Cite this article: Huston DC, Cutmore SC, Cribb TH (2018). *Isorchis cannoni* n. sp. (Digenea: Atractotrematidae) from Great Barrier Reef rabbitfishes and the molecular elucidation of its life cycle. *Journal of Helminthology* **92**, 604–611. https://doi.org/10.1017/S0022149X17000906

Received: 21 May 2017
Accepted: 28 August 2017
First published online: 2 November 2017

Author for correspondence:
D.C. Huston, E-mail: Daniel.Huston@uqconnect.edu.au

Abstract

We describe *Isorchis cannoni* n. sp. from the rabbitfishes *Siganus fuscescens* (Houttuyn) and *Siganus lineatus* (Valenciennes) (Siganidae) collected off Heron Island, southern Great Barrier Reef, Australia and, using molecular data, demonstrate that '*Cercariae queenslandae* II' of Cannon (1978) from the gastropod *Clypeomorus batillariaeformis* Habe & Kosuge (Cerithiidae) is the larval form of this new species. The cercariae of *I. cannoni* n. sp. develop in rediae, encyst in the environment after emergence, and are inferred to then be consumed by grazing rabbitfish. Additionally, we provide a new report of *Isorchis currani* Andres, Pulis & Overstreet, 2016 from the type host, *Selenotoca multifasciata* (Richardson) (Scatophagidae) collected in Moreton Bay, south-east Queensland, Australia, greatly expanding the known geographical range of this species. Molecular sequence data (ITS1, ITS2 and 28S rDNA) generated for *I. cannoni* n. sp. and the new specimens of *I. currani*, confirm the identification of *I. currani* and demonstrate a distinct genotype for *I. cannoni* n. sp. relative to other species of *Isorchis* Durio & Manter, 1969, for which molecular data are available. *Isorchis cannoni* n. sp. is morphologically distinct from all other species in the genus, and is further distinguished by utilizing species of Siganidae as definitive hosts, rather than species of Chanidae or Scatophagidae. Because haploporid and atractotrematid cercariae have well-developed reproductive organs, we find cercariae of these closely related families morphologically distinguishable in the same way as adult trematodes: atractotrematids have two symmetrical testes and haploporids have a single testis or, rarely, two tandem or oblique testes.

Introduction

It has been nearly 50 years since the study of Pearson (1968), which elucidated the first trematode life cycle from the Great Barrier Reef. Despite the great inherent value in understanding trematode life cycles (Thompson *et al.*, 2005; Blasco-Costa & Poulin, 2017) and significant advances in our understanding of the diversity, richness and host specificity of adult trematodes in this ecosystem since that time (e.g. Miller *et al.*, 2011; Cribb *et al.*, 2014, 2016), relatively little life-cycle information has been added. The studies of Rohde (1973, 1977) and Huston *et al.* (2016) each elucidated a single trematode life cycle. Downie & Cribb (2011) connected an adult trematode with a cercaria described by Lucas *et al.* (2005), and a few studies have connected metacercariae from various hosts with adult trematodes (Cribb *et al.*, 1996, 1998; Miller *et al.*, 2009; Kudlai *et al.*, 2016). Additionally, several studies have reported larval trematodes from molluscan hosts which remain unconnected with adults (Cannon, 1978; Rohde, 1981; Shelley *et al.*, 1988; Beuret & Pearson, 1994; Bott *et al.*, 2005).

Cannon (1978) found 11 distinct trematode species exploiting the cerithiid gastropod *Clypeomorus batillariaeformis* Habe & Kosuge from Heron Island, southern Great Barrier Reef, and provided high-quality descriptions of new cercariae, giving each a numerical placeholder name (i.e. *Cercariae queenslandae* I–X). Of these, Cannon (1978) identified *C. queenslandae* II as a member of the Haploporidae Nicoll, 1914 and noted that these cercariae encysted in the environment about 12 h after emergence. Cannon (1978) also mentioned a morphological similarity between this cercaria and a species considered a haploporid at the time, *Atractotrema sigani* Durio & Manter, 1969, a parasite of rabbitfishes (Siganidae). As siganids are common off the beachrock of Heron Island where *C. battilariaeformis* is found, Cannon (1978) speculated that *C. queenslandae* II was likely the larva of *A. sigani*. No further study has explored this idea.

Current classification based upon morphological and molecular data recognizes two families, the Atractotrematidae Yamaguti, 1939 and Haploporidae, within the superfamily Haploporoidea Nicoll, 1914 (Jones 2005; Overstreet & Curran 2005a, b; Blasco-Costa *et al.*, 2009; Pulis & Overstreet, 2013; Andres *et al.*, 2014, 2016). Atractotrematids possess two symmetrical testes and utilize only estuarine and marine fishes as hosts, whereas haploporids

CAMBRIDGE UNIVERSITY PRESS

usually have a single testis or, rarely, two tandem or oblique testes, and utilize marine, estuarine and freshwater fishes as hosts (Jones, 2005; Overstreet & Curran 2005a, b; Andres *et al.*, 2016). While the Haploporidae includes many genera and species, the Atractotrematidae contains just 11 species in four genera (Overstreet & Curran, 2005b; Andres *et al.*, 2016). Two of the four atractotrematid genera, *Pseudisorchis* Ahmad, 1985 and *Pseudomegasolena* Machida & Kamiya, 1976 are monotypic, *Atractotrema* Goto & Ozaki, 1929 includes three species and *Isorchis* Durio & Manter, 1969 includes six (Cribb, 2010). Andres *et al.* (2016) described three new species of *Isorchis* from Australian waters, including the first known species from non-chanid hosts, and provided the first molecular data for the genus. Here, we describe an additional species of *Isorchis* from rabbitfish from the Great Barrier Reef, and using molecular data, demonstrate that *C. queenslandae* II of Cannon (1978) is the larval form of this new species, elucidating the first confirmed life cycle for a species of the family Atractotrematidae. In addition, we provide a new record of *Isorchis currani* Andres, Pulis & Overstreet, 2016 collected from Moreton Bay in south-east Queensland.

Materials and methods

Specimen collection

Between 2015 and 2016, large numbers of the cerithiid gastropod *C. batillariaeformis* were collected from beach rock at Heron Island, southern Great Barrier Reef, Queensland, Australia (23° 27′S, 151°55′E). Snails were isolated in a small amount of seawater in individual 10-ml wells and left for 24–48 h to observe natural emergence of cercariae. Snails from which no cercariae had emerged within 48 h were released at the site of capture. Upon discovery, emerged cercariae were fixed in near-boiling saline and preserved in 70% ethanol for parallel morphological and molecular analyses. Snails from which cercariae had emerged were either retained to acquire additional emerged cercariae and then released, or dissected for collection of intramolluscan trematode larvae, which were subsequently fixed and preserved as above. In 2016, as part of a larger parasitological survey and in collaboration with local commercial fishermen, specimens of the scat *Selenotoca multifasciata* (Richardson) were collected in Moreton Bay (27°28′S, 153°18′E) using a tunnel net. In 2017, the rabbitfishes *Siganus fuscescens* (Houttuyn) and *Siganus lineatus* (Valenciennes) were collected by spear off Heron Island. The gut of each fish was excised and examined following the recommendations of Cribb & Bray (2010). Trematodes collected were fixed and preserved as above.

Morphological analyses

Trematode specimens used for morphological examination were washed in fresh water, overstained in Mayer's haematoxylin, destained in a solution of 1.0% hydrochloric acid and neutralized in 0.5% ammonium hydroxide solution. Specimens were then dehydrated in a graded ethanol series, cleared in methyl salicylate and mounted in Canada balsam. Drawings were made using an Olympus BX-53 compound microscope with attached drawing tube (Olympus, Notting Hill, Australia), and illustrations were digitized in Adobe Illustrator. Measurements were made with cellSens standard imaging software paired with an Olympus SC50 digital camera mounted on an Olympus BX-53 compound microscope. Measurements are provided in micrometres (μm) and given as the range followed by the mean in parentheses. Where length is followed by breadth, the two measurements are separated by '×'. All vouchers are lodged in the Queensland Museum (QM), Brisbane, Australia.

Molecular sequencing

Total genomic DNA was extracted from trematodes using phenol/chloroform extraction techniques (Sambrook & Russell, 2001). Polymerase chain reaction (PCR) for the internal transcribed spacers ITS1 and ITS2, and 28S regions was performed using Bioline MyTaq™ DNA Polymerase and Reaction Buffer following the manufacturer's recommendations. The entire ITS2 rDNA region and partial 28S rDNA were amplified with the primers and cycling conditions used by Huston *et al.* (2016). The ITS1 region was amplified using the primers S20T2 (5′-GGT AAG TGC AAG TCA TAA GC-3′) (Jousson *et al.*, 1999) and 5.8S1 (5′-GCT GCG CTC TTC ATC GAC A-3′) (Bartoli *et al.*, 2000), using the following cycling conditions: 40 cycles of 30 s at 94°C, 30 s at 53°C and 120 s at 72°C, then a 5 min final extension at 72°C.

Amplified DNA was purified using a Bioline ISOLATE II PCR and gel purification kit (Bioline, Alexandria, Australia), per the manufacturer's protocol. Cycle sequencing of purified DNA was carried out using ABI Big Dye™ v.3.1 chemistry (Thermo Fisher Scientific) following the manufacturer's recommendations at the Australian Genome Research Facility, using an AB3730 × 1 capillary sequencer. Primers used for sequencing the ITS1 region were those used in amplification. Primers used for sequencing the ITS2 and 28S regions are listed in Huston *et al.* (2016). Sequencher™ version 4.5 (GeneCodes Corp., http://www.genecodes.com/sequencher) was used to assemble and edit contiguous sequences. For each newly generated sequence of ITS1 rDNA, the start and end of the ITS1 region was determined through comparison with previously published atractotrematid and haploporid sequences that include the ITS1 region. For each newly generated sequence of ITS2 rDNA, the start and end of the ITS2 region was determined by annotation using the ITS2 Database Metazoa model (Keller *et al.*, 2009; Ankenbrand *et al.*, 2015). Collection data and GenBank accession numbers for sequenced taxa are presented in the taxonomic section of this manuscript.

Results

Molecular results

ITS1, ITS2 and partial 28S rDNA sequence data were generated for the specimens of adult *Isorchis* collected from *S. lineatus* and *S. fuscescens* from Heron Island and *S. multifasciata* from Moreton Bay. Additionally, ITS1 and ITS2 sequences were generated for *C. queenslandae* II from infected *C. batillariaeformis* collected at Heron Island. The ITS1 and ITS2 sequences from infected *C. batillariaeformis* were identical to those of adult *Isorchis* from Heron Island siganids. Comparison of these new sequence data with that available on GenBank indicated that the Heron Island material represented an uncharacterized genotype, which, with the inclusion of morphological data, is described here as *Isorchis cannoni* n. sp. Sequences of adult *Isorchis* from Moreton Bay *S. multifasciata* matched those of *I. currani* reported by Andres *et al.* (2016) from Darwin, Northern Territory.

https://doi.org/10.1017/S0022149X17000906 Published online by Cambridge University Press

All ITS1 sequences were 703 bp long, comprising 156 bp of flanking 18S rDNA, 513 bp of ITS1 rDNA and 34 bp of flanking 5.8S rDNA. The ITS2 sequences generated for *I. cannoni* n. sp. were 437 bp long, comprising 123 bp flanking 5.8S rDNA, 265 bp ITS2 rDNA and 49 bp flanking 28S rDNA. The ITS2 sequences generated for *I. currani* from Moreton Bay were 439 bp long, comprising 123 bp flanking 5.8S rDNA, 267 bp ITS2 rDNA and 49 bp flanking 28S rDNA. The ITS1 and ITS2 sequences of *I. cannoni* n. sp. differed from those of *Isorchis anomalus* Andres, Pulis & Overstreet, 2016, *I. currani* and *Isorchis megas* Andres, Pulis & Overstreet, 2016 (the only other species of *Isorchis* for which molecular data are available) by 12 and 6, 2 and 3, and 5 and 3 base positions, respectively (table 1). The ITS1 and ITS2 sequences of *I. currani* from Moreton Bay were identical to those of *I. currani* provided by Andres *et al.* (2016) from Darwin, Northern Territory, whereas the 28S sequence from Moreton Bay differed from those of Darwin by one base. The 28S sequences of *I. cannoni* n. sp. differed from those of *I. anomalus*, *I. currani* isolate 1, *I. currani* isolate 2 and *I. megas* by 16, 4, 3 and 5 base positions, respectively (table 1).

Family Atractotrematidae Yamaguti, 1939; Genus Isorchis *Durio & Manter, 1969;* Isorchis cannoni *n. sp.*

Taxonomic summary

Type host. Siganus lineatus (Valenciennes), golden-lined spinefoot (Perciformes: Siganidae).

Other definitive hosts. Siganus fuscescens (Houttuyn), mottled spinefoot (Perciformes: Siganidae).

Intermediate host. Clypeomorus batillariaeformis Habe & Kosuge (Cerithiidae).

Type locality. Off Heron Island, southern Great Barrier Reef, Queensland, Australia (23°27′S, 151°55′E).

Prevalence. Three of six *S. lineatus* and six of six *S. fuscescens*.

Type material. Holotype (QM G236318) and 19 paratypes (QM G236319–236337) including two hologenophores (QM G236338–236339).

Larval voucher material. Excised rediae and cercariae, five slides (QM G236340–236344).

Site in definitive host. Intestine.

Site in intermediate host. Digestive gland.

Representative DNA sequences. For both ITS1 and ITS2 rDNA, nine identical replicates, three from adult worms ex *S. lineatus*, three from adults ex *S. fuscescens* and three from intramolluscan stages ex *C. batillariaeformis* (one replicate of ITS1 and ITS2 submitted to GenBank, ITS1: MF803155; ITS2: MF803156). For 28S rDNA, three identical replicates, two from adult worms ex *S. lineatus*, and one from an adult ex *S. fuscescens* (one submitted to GenBank: MF803154).

Zoobank registration. The Life Science Identifier for *Isorchis cannoni* n. sp. is urn:lsid:zoobank.org:act:121143A3-D7CF-4F31-AFA3-4B83A26DAB1C.

Etymology. This species is named for Dr Lester R.G. Cannon, in recognition of his contributions to marine parasitology and knowledge of the larval digenean fauna of the Great Barrier Reef.

Description

Based on 20 gravid whole mounts, ten from *S. lineatus*, ten from *S. fuscescens* (fig. 1A, B). Body fusiform, 378–492 × 180–251 (436 × 223). Body length/width 1.7–2.2 (1.9). Tegument finely spinose throughout. Forebody 144–189 (170), occupying 35–43 (39)% of body length; hindbody 139–244 (187), occupying 37–46 (43)% of body length. Eyespot pigment mostly restricted to forebody, profuse, most prominent dorsally. Oral sucker terminal, subglobular, 57–74 × 74–94 (64 × 84). Ventral sucker in midbody, globular, 72–94 × 73–96 (80 × 84); aperture oval. Ventral sucker length/oral sucker length 1.14–1.45 (1.3); ventral sucker width/oral sucker width 0.91–1.10 (1.0). Prepharynx 9–23 (15) long; pharynx ovoid, 42–58 × 46–60 (48 × 54). Pharynx width/oral sucker width 0.56–0.72 (0.64). Oesophagus relatively straight, 34–65 (50) long. Intestinal bifurcation occurring between region just anterior to ventral sucker and mid-level of ventral sucker. Caeca blind, 136–197 × 26–60 (168 × 37), terminating 79–128 (99) from posterior extremity; post-caecal space representing 18–27 (23)% of body length.

Testes two, opposite, in anterior to mid-hindbody, ventral to caeca, generally slightly asymmetrical, ovoid; sinistral testis 62–100 × 48–76 (85 × 63); dextral testis 65–102 × 50–78 (85 × 62). Post-testicular space 86–160 (121), representing 23–33 (28)% of body length. External seminal vesicle dorsal to ventral sucker, ovoid, 35–45 × 36–49 (41 × 39). Hermaphroditic sac dorsal to ventral sucker, ventral to caeca, 53–77 × 47–74 (68 × 57), occupying 13–19 (16)% of body length. Internal seminal vesicle 28–37 × 29–36 (33 × 33); prostatic bulb elongate to ovoid with male duct a short tube uniting with female duct at about mid-level of hermaphroditic sac; hermaphroditic duct occupying approximately half the length of sac; diverticula two, joined to anterior portion of hermaphroditic duct. Genital pore ventral, just anterior to ventral sucker.

Ovary in anterior hindbody, intercaecal and intertesticular, with anterior margin dorsal to posterior margin of ventral sucker or nearly so, subglobular, 32–49 × 30–44 (40 × 36). Seminal receptacle subglobular, dorsal to and slightly smaller than ovary. Ootype anterior to seminal receptacle and ovary. Mehlis' gland

Table 1. Base pair differences between the ITS1, ITS2 and 28S rDNA regions for all species of *Isorchis* for which molecular data are available.

	I. cannoni n. sp.			*I. anomalus*			*I. currani*		
	ITS1	ITS2	28S	ITS1	ITS2	28S	ITS1	ITS2	28S
I. anomalus	12	6	16						
I. currani	2	3	3–4	4	7	11			
I. megas	5	3	5	9	7	13	4	0	2–3

https://doi.org/10.1017/S0022149X17000906 Published online by Cambridge University Press

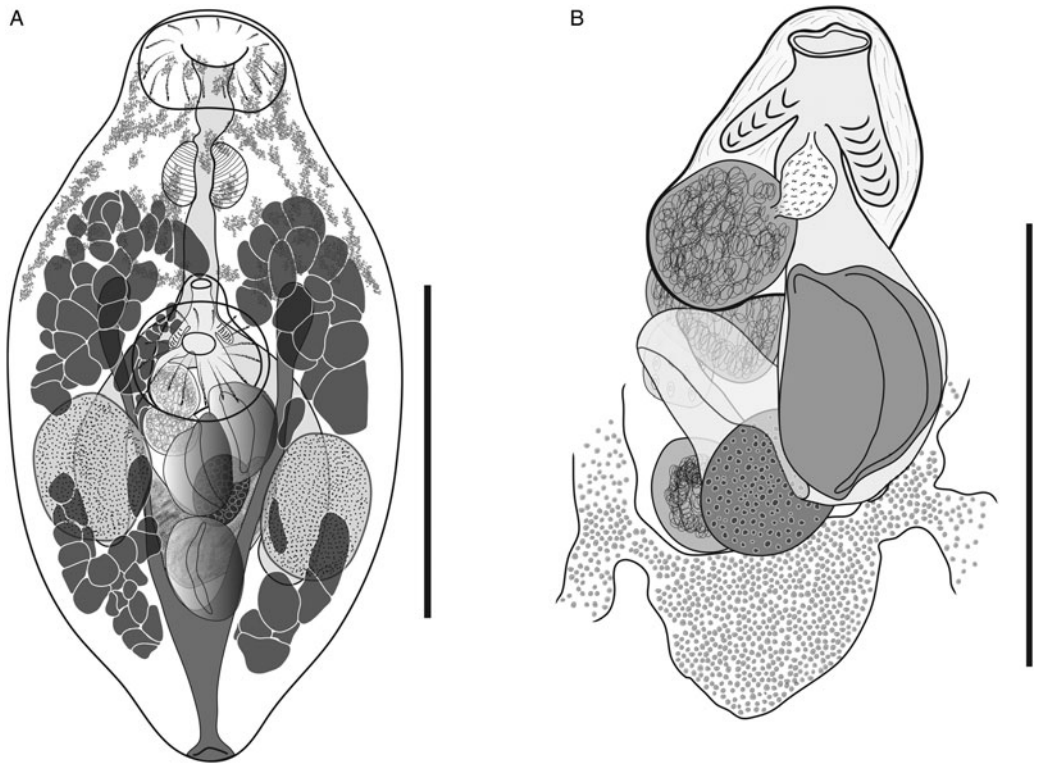

Fig. 1. (A) *Isorchis cannoni* n. sp., ventral view, holotype, from *Siganus lineatus*, Heron Island, Great Barrier Reef. (B) *Isorchis cannoni* n. sp., ventral view, hermaphroditic sac and ovarian complex, paratype, from *Siganus fuscescens*, Heron Island. Scale bars: (A) 200 μm; (B) 100 μm.

indistinct. Laurer's canal not observed. Vitellarium follicular, arranged in two fields confluent dorsally or not, interrupted ventrally at level of testes; anterior field wrapping from dorsal longitudinal median bilaterally to sinistral and dextral body regions, not confluent ventrally, 68–119 (97) from anterior body extremity, representing 17–29 (22)% of body length; posterior field not confluent dorsally, wrapping bilaterally from dorsal sinistral and dextral body regions to ventral sinistral and dextral body regions, 47–74 (58) from posterior body extremity, representing 10–19 (13)% of body length. Vitelline reservoir crescentiform to elliptical, with anterior margin contiguous with posterior margin of ovary; arms of collecting ducts passing anteriorly from reservoir bilaterally around ovary. Uterus between posterior margins of hermaphroditic sac and vitelline reservoir. Eggs 1–5 in number, 62–87 × 40–61 (79 × 49); egg length representing 13–22 (18)% of body length.

Excretory vesicle Y-shaped, extending 64–74 (69)% of body length, bifurcating 108–123 (96) from posterior extremity; arms extending into mid-forebody to 108–165 (140) from anterior extremity. Excretory pore terminal.

Measurements of the holotype

Body 450 × 237. Body length/width 1.8. Forebody 176, occupying 39% of body length; hindbody 202, occupying 45% of body length. Oral sucker 63 × 87. Ventral sucker 72 × 79. Ventral sucker length/oral sucker length 1.14; ventral sucker width/oral sucker width 0.91. Prepharynx 19 long; pharynx 42 × 52. Pharynx width/oral sucker width 0.60. Oesophagus 65 long. Caeca 171 × 33, terminating 85 from posterior extremity; post-caecal space representing 19% of body length. Sinistral testis 90 × 69; dextral testis 87 × 61. Post-testicular space 106, representing 24% of body length. External seminal vesicle 39 × 38. Hermaphroditic sac 76 × 57–74, occupying 17% of body length. Internal seminal vesicle 35 × 29. Ovary 41 × 34–44. Vitellarium 111 from anterior body extremity, representing 24% of body length; 48 from posterior body extremity, representing 11% of body length. Eggs 4 in number, 66–81 × 56–44 (74 × 50); egg length representing 15–18 (17)% of body length. Excretory vesicle extending 64% of body length, bifurcating 90 from posterior extremity; arms extending to 161 from anterior extremity.

Remarks

All ITS1 and ITS2 rDNA sequences generated from larval digeneans from three separate *C. batillariaeformis* were identical to those ITS1 and ITS2 sequences generated for *I. cannoni* n. sp. from *S. lineatus* and *S. fuscescens*. All these hosts were collected in the same area of Heron Reef. Cannon (1978) noted that this cercaria encysts in the environment, and would thus readily encyst upon algae growing on the beachrock found along Heron Island. Siganids are a major group of grazers along this beachrock (Stephenson & Searles, 1960), therefore this is undoubtedly the route of metacercarial ingestion in the life cycle of *I. cannoni* n. sp.

Isorchis cannoni n. sp. is morphologically distinct from all other species of *Isorchis*. From *I. anomalus*, *I. cannoni* n. sp. differs in having a genital pore margin that is ovoid without folds or creases, a smaller body (378–492 × 180–251 vs. 523–709 × 232–394), smaller testes (sinistral 65–100 × 48–76, dextral 65–102 × 50–78 vs. sinistral 122–203 × 91–144, dextral 127–189 × 90–116), smaller hermaphroditic sac, both absolutely (53–77 × 47–74 vs. 93–151 × 50–134) and relative to body length (13–18 vs. 17–23%), and a 12–20% larger oral sucker to pharynx width ratio. In addition,

https://doi.org/10.1017/S0022149X17000906 Published online by Cambridge University Press

the eggs of *I. cannoni* n. sp. are shorter but broader than those of *I. anomalus* (62–87 × 40–61 vs. 63–101 × 37–55). From *Isorchis chanosi* (Zhukov, 1972) Ahmad, 1985, *I. cannoni* n. sp. differs in having tegumental spines that extend to the posterior end of the body rather than to only the mid-hindbody, shorter testes (sinistral 65–100 × 48–76, dextral 65–102 × 50–78 vs. 100–167 × 41–83), caeca that terminate posterior to the testes rather than mid-testes, a vitellarium that does not extend to the posterior end of the body and by possessing a uterus that does not extend to the posterior end of the body. From *I. currani*, *I. cannoni* n. sp. differs in having a smaller body (378–492 × 180–251 vs. 591–695 × 192–353), testes (sinistral 65–100 × 48–76, dextral 65–102 × 50–78 vs. sinistral 134–200 × 70–125, dextral 118–198 × 70–125), shorter post-caecal space (17–29 vs. 27–36% of body length), an 18–36% larger oral sucker to pharynx width ratio and shorter, broader eggs (62–87 × 40–61 vs. 75–94 × 32–47). Furthermore, *I. currani* possesses a larger oral sucker than ventral sucker, whereas *I. cannoni* n. sp. possesses a ventral sucker that is slightly larger than the oral sucker (longer and of nearly equal width). From *I. megas*, *I. cannoni* n. sp. differs in having a larger ovary (32–49 × 30–44 vs. 19–35 × 15–35), a vitellarium that extends anteriorly to the level of the pharynx rather than the posterior forebody, an oral sucker that is smaller than the ventral sucker and a ∼40% greater oral sucker to ventral sucker width ratio. Additionally, *I. cannoni* n. sp. has more eggs, which are shorter and broader than those of *I. megas* (62–87 × 40–61 vs. 83–92 × 37–44), and are less than 20% of body length. From *Isorchis parvus* Durio & Manter, 1969, *I. cannoni* n. sp. differs in having a shorter body length (378–492 vs. 567–912), lacking radial muscles surrounding the genital pore, and gut bifurcation in the region of the ventral sucker rather than mid-way between the oral and ventral suckers. Additionally, the eggs of *I. cannoni* n. sp. are smaller than those of *I. parvus* (62–87 × 40–61 vs. 72–88 × 43–51), but larger relative to body size (13–22 vs. ∼12% body length). From *Isorchis skrjabini* Ahmad, 1985, *I. cannoni* n. sp. differs in having a smaller body (378–492 × 180–251 vs. 665–1065 × 370–600), a terminal, subglobular oral sucker rather than a subterminal cup-shaped oral sucker, an oral sucker smaller than the ventral sucker, vitellarium extending anteriorly to the level of the pharynx rather than to the level of caecal bifurcation, a ventral sucker posterior to the caecal bifurcation, an ovary that is oval rather than triangular, and longer excretory vesicle arms that extend into the forebody rather than terminating in the hindbody.

Isorchis cannoni n. sp. is further distinguished from all other species of *Isorchis* by utilization of a siganid as a definitive host, rather than a chanid or scatophagid. Molecular data also demonstrate that *I. cannoni* n. sp. is distinct from those other species for which such data are available: *I. anomalus*, *I. currani* and *I. megas*.

Isorchis currani *Andres, Pulis & Overstreet, 2016*

Taxonomic summary

Type host. *Selenotoca multifasciata* (Richardson), spotbanded scat (Perciformes: Scatophagidae).

Type locality. Fannie Bay, Northern Territory, Australia (12° 26′S, 130°49′E).

New material: taxonomic summary

Host. *Selenotoca multifasciata*.

Locality. Moreton Bay, Queensland, Australia (27°28′S, 153° 18′E).

Prevalence. Two of 28 *S. multifasciata*.

Voucher material. Twelve whole mounts (QM G236345–236356).

Site in host. Intestine.

Representative DNA sequences. For both ITS1 and ITS2 rDNA, two identical replicates (one of each submitted to GenBank, ITS1: MF803158; ITS2: MF803159). For 28S rDNA, one replicate (MF803157).

New measurements

Based on 12 gravid whole mounts, all from *S. multifasciata* from Moreton Bay. Body 564–727 × 250–364 (673 × 316). Body length/width 1.9–2.4 (2.2). Forebody 220–305 (267), occupying 36–44 (40)% of body length; hindbody 266–337 (307), occupying 41–51 (46)% of body length. Oral sucker 88–134 × 125–149 (98 × 140). Ventral sucker 85–131 × 80–120 (105 × 102). Ventral sucker length/oral sucker length 0.79–1.26 (1.1); ventral sucker width/oral sucker width 0.64–0.81 (0.73). Prepharynx 8–18 (12) long; pharynx 40–67 × 53–68 (51 × 64). Pharynx width/oral sucker width 0.42–0.48 (0.46). Oesophagus 56–106 (88) long. Caeca 196–307 × 48–64 (247 × 53), terminating 134–224 (185) from posterior extremity; post-caecal space representing 24–32 (27)% of body length. Sinistral testis 132–186 × 80–122 (162 × 95); dextral testis 127–192 × 76–153 (164 × 103). Post-testicular space 126–230 (180), representing 18–35 (27)% of body length. Hermaphroditic sac 82–116 × 70–97 (92 × 83), occupying 12–17 (14)% of body length. Ovary 44–70 × 45–67 (58 × 53). Vitellarium 125–175 (149) from anterior body extremity, 35–92 (66) from posterior body extremity. Eggs 4–16 in number, 75–86 × 50–67 (81 × 57); egg length representing 11–15 (12)% of body length. Excretory vesicle extending 72–77 (75)% of body length, bifurcating 109–216 (166) from posterior extremity, arms extending into mid-forebody to 156–201 (172) from anterior extremity.

Remarks

These specimens are morphologically indistinguishable from those representing *I. currani* described by Andres *et al.* (2016) and are from the same host species. Furthermore, ITS1 and ITS2 sequences generated for the new material are identical to sequences of *I. currani* provided by Andres *et al.* (2016), and the 28S sequences generated for the new material differ by only one base. There is little doubt that the *Isorchis* specimens recovered from Moreton Bay are *I. currani*. This new report represents a significant range extension for the species.

Discussion

The molecular data generated in this study demonstrate that *I. cannoni* n. sp. is a member of the Atractotrematidae and is closely related to existing species in the genus *Isorchis*. The differences between *I. cannoni* n. sp. and those other species of *Isorchis* for which molecular data are available are slight, but these differences are sufficient to consistently distinguish *I. cannoni* n. sp. from *I. anomalus, I. currani* and *I. megas*. Sequences generated for *I. cannoni* n. sp. differ from those of *I. currani* and *I. megas* at only a few base positions. Sequence variation increases when comparing *I. cannoni* with *I. anomalus,* a species that utilizes *Chanos chanos* (Forsskål) as a definitive host. *Isorchis anomalus* was resolved as basal to *I. currani* and *I. megas* in the molecular phylogeny of Andres *et al.* (2016). This may provide some insight into the

https://doi.org/10.1017/S0022149X17000906 Published online by Cambridge University Press

relationship between *I. cannoni* and other species of *Isorchis* that utilize *C. chanos*, and might lend support to the speculation of Andres *et al.* (2016) that chanids are the ancestral host group for species of *Isorchis*. However, we face the same limitation as Andres *et al.* (2016) in that additional molecular evidence from *Isorchis* species exploiting *C. chanos* will be required to test this theory.

Isorchis cannoni n. sp. is the first species of *Isorchis* known from the fish family Siganidae. Of the other known species of *Isorchis*, *I. anomalus*, *I. chanosi*, *I. parvus* and *I. skrjabini* utilize *C. chanos* (Chanidae) as a definitive host, whereas *I. currani* and *I. megas* utilize *S. multifasciata* (Scatophagidae). Species of the monotypic genera *Pseudisorchis* and *Pseudomegasolena* utilize species of Mugilidae and Scaridae, respectively. All species of *Atractotrema* utilize siganids as definitive hosts. In the molecular phylogeny of the Haploporoidea Nicoll, 1914 of Andres *et al.* (2016), *Atractotrema* is resolved as the most basal lineage of the Atractotrematidae. Thus, although chanids might represent the ancestral host group of *Isorchis*, siganids might represent the ancestral host group of all atractotrematids. If this were the case, then the utilization of a siganid host by *I. cannoni* n. sp. would represent a recolonization host-switch. Although theories on the ancestral state of atractotrematids remain strictly speculative, further molecular exploration in this family may reveal an interesting evolutionary narrative.

All our *Isorchis* specimens from *S. multifasciata* from Moreton Bay were consistent with *I. currani*; no specimens consistent with *I. megas* were found. Andres *et al.* (2016) found only *I. megas* in Western Australia, whereas they found *I. currani* and *I. megas* occurring sympatrically in the Northern Territory. Considering the molecular similarities between *I. currani* and *I. megas*, and utilization of the same definitive host in both these species, perhaps our collecting only *I. currani* in Moreton Bay is consistent with the existence of two species that have diverged based on geography, or have geographically restricted intermediate hosts. This seems plausible, as phylogenetic differentiation has been observed between eastern and western Australia for multiple animal groups (Benzie *et al.,* 1992; Chenoweth *et al.,* 1998; Gopurenko & Hughes, 2002; Reid *et al.,* 2006). This differentiation is thought to be a result of lowering sea levels exposing the Sahul Shelf during the Pleistocene, breaking the connection between the Indian and Pacific Oceans (Voris, 2000). Much of the Sahul Shelf is now under the shallow Timor and Arafour Seas, a distinct ecoregion, which includes the coastal region of the Northern Territory, and separates the marine regions of Western Australia and Queensland (Spalding *et al.,* 2007).

Our molecular pairing of Cannon's (1978) *C. queenslandae* II with *I. cannoni* n. sp. has provided the first confirmed atractotrematid cercaria and intermediate host. Cannon (1978) recognized the strong similarities between his cercaria and those of haploporids. Haploporid cercariae are all ocellate, of the gymnocephalus type and have long, simple tails (for a list of known haploporid cercariae, see Overstreet & Curran, 2005a). The cercaria of *I. cannoni* n. sp. agrees with haploporid cercariae in all these respects. However, the cercariae of *I. cannoni* n. sp. can be differentiated from those of the Haploporidae much in the same manner as adults of atractotrematids and haploporids are differentiated. The cercariae of haploporids and those of *I. cannoni* n. sp. have well-developed reproductive organs. Thus, the presence of two symmetrical testes is indicative of the Atracotrematidae, whereas a single testis, or two tandem or oblique testes, would suggest the Haploporidae. These testes are easily detectible in our stained and mounted specimens of *I. cannoni* n. sp. cercariae, and the reproductive organs are well characterized in most descriptions of haploporid cercariae. Although not explicitly stated, Cannon (1978) must have based his suggestion that *C. queenslandae* II was the larva of *A. sigani* partially on the symmetrical testes present in both. Although we cannot know with certainty if this morphological differentiation will hold as more atractotrematid and haploporid cercariae are described, it seems likely.

The first intermediate hosts of atractotrematids had been predicted to be marine gastropods in the superfamilies Rissooidea or Truncatelloidea, as these groups serve as intermediate hosts for haploporids (Andres *et al.,* 2016). The discovery of *C. batillariaeformis* as the intermediate host in the life cycle of *I. cannoni* n. sp. has defied prediction, as this gastropod is a member of the superfamily Cerithioidea. In the taxonomy of the Gastropoda of Bouchet & Rocroi (2005), the superfamilies Rissooidea and Truncatelloidea are related Hypsogastropoda lineages within the greater clade Caenogastropoda. Although the Cerithioidea is also within the Caenogastropoda clade, it is outside the Hypsogastropoda. Thus, the intermediate host lineages utilized by atractotrematids and haploporids are distantly related. Such a pattern might at first seem improbable, but parallels are known from the Lepocreadioidea Odhner, 1905 lineages. Most known intermediate hosts for lepocreadioid species are within the Hypsogastropoda, and yet the only known intermediate host for the Gyliauchenidae Fukui, 1929 is a cerithiid, and intermediate hosts of the Deropristidae Cable & Hunnien, 1942 are reported from both cerithiid and truncatelloid gastropods (Huston *et al.,* 2016). Additional evidence of broad molluscan host utilization can be found in the Schistosomatidae Poche, 1907, species of which are known from caenogastropods, pulmonates and opisthobranchs (Blair *et al.,* 2001).

Because of these precedents observed for the Lepocreadioidea and Schistosomatidae, a single known atractotrematid intermediate host is insufficient to draw deeper conclusions about the evolutionary history of the Haploporoidea. However, efforts should be made to discover additional atractotrematid intermediate hosts as these data could provide profound insights. Considering that species of the Cerithiidae are exclusively marine (Bouchet & Rocroi, 2005), atractotrematid intermediate host utilization may restrict definitive host availability. Furthermore, if additional cerithiid species are found as hosts of atractotrematids, then it may be possible to use the geological timing of the diversification of the Caenogastropoda lineages to determine the timing of the split between the Atractotrematidae and the Haploporidae. In addition, as atractotrematids and haploporids use both phylogenetically and ecologically related hosts, further life-cycle data would be useful in determining whether intermediate, definitive or both host groups are major drivers of speciation in the Haploporoidea.

Acknowledgements. The authors thank the staff of the Heron Island and Moreton Bay (University of Queensland) research stations for their ongoing support of our field expeditions. We thank John Page and Dave Thompson for assistance with specimen collection in Moreton Bay. We would also like to thank our anonymous reviewers for constructive criticisms which greatly improved the content of this manuscript.

Financial support. D.C.H. is funded through grants from the PADI Foundation, Linnean Society of New South Wales, Systematics Research Fund (Systematics Society in partnership with the Linnean Society of London), and a Moreton Bay Research Station Scholarship in partnership with SIBELCO Australia. Collections in Moreton Bay were part of a larger project funded by an ABRS National Taxonomy Research Grant RF215–40 awarded to S.C.C. and T.H.C.

Conflict of interest. None.

Ethical standards. All applicable institutional, national and international guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

References

Andres MJ, Pulis EE, Cribb TH and Overstreet RM (2014) Erection of the haploporid genus *Litosaccus* n. g. and its phylogenetic relationship within the Haploporidae Nicoll, 1914. *Systematic Parasitology* **89**, 185–194.

Andres MJ, Pulis EE and Overstreet RM (2016) Description of three species of *Isorchis* (Digenea: Atractotrematidae) from Australia. *Acta Parasitologica* **61**, 590–601.

Ankenbrand MJ, Keller A, Wolf M, Schultz J and Förster F (2015) ITS2 database V: Twice as much. *Molecular Biology and Evolution*, **32**, 3030–3032.

Bartoli P, Jousson O and Russell-Pinto F (2000) The life cycle of *Monorchis parvus* (Digenea: Monorchiidae) demonstrated by developmental and molecular data. *Journal of Parasitology* **86**, 479–489.

Benzie J, Frusher S and Ballment E (1992) Geographical variation in allozyme frequencies of populations of *Penaeus monodon* (Crustacea: Decapoda) in Australia. *Marine and Freshwater Research* **43**, 715–725.

Beuret J and Pearson J (1994) Description of a new zygocercous cercaria (Opisthorchioidea: Heterophyidae) from prosobranch gastropods collected at Heron Island (Great Barrier Reef, Australia) and a review of zygocercariae. *Systematic Parasitology* **27**, 105–125.

Blair D, Davis G and Wu B (2001) Evolutionary relationships between trematodes and snails emphasizing schistosomes and paragonimids. *Parasitology* **123**, 229–243.

Blasco-Costa I and Poulin R (2017) Parasite life-cycle studies: a plea to resurrect an old parasitological tradition. *Journal of Helminthology*. https://doi.org/10.1017/S0022149X16000924.

Blasco-Costa I, Balbuena JA, Kostadinova A and Olson PD (2009) Interrelationships of the Haploporinae (Digenea: Haploporidae): a molecular test of the taxonomic framework based on morphology. *Parasitology International* **58**, 263–269.

Bott NJ, Healy JM and Cribb TH (2005) Patterns of digenean parasitism of bivalves from the Great Barrier Reef and associated waters. *Marine and Freshwater Research* **56**, 387–394.

Bouchet P and Rocroi J-P (2005) Classification and nomenclature of gastropod families. *Malacologia* **47**, 1–397.

Cannon LRG (1978) Marine cercariae from the gastropod *Cerithium moniliferum* Kiener at Heron Island, Great Barrier Reef. *Proceedings of the Royal Society of Queensland* **89**, 45–57.

Chenoweth SF, Hughes JM, Keenan CP and Lavery S (1998) When oceans meet: a teleost shows secondary intergradation at an Indian–Pacific interface. *Proceedings of the Royal Society of London B: Biological Sciences* **265**, 415–420.

Cribb TH (2010) Atractotrematidae Yamaguti, 1939. Accessed through: World Register of Marine Species. Available at http://www.marinespecies.org/aphia.php?p=taxdetails&id=468971 (accessed 18 May 2017).

Cribb TH and Bray RA (2010) Gut wash, body soak, blender and heat-fixation: approaches to the effective collection, fixation and preservation of trematodes of fishes. *Systematic Parasitology* **76**, 1–7.

Cribb TH, Bray RA, Barker SC and Adlard RD (1996) Taxonomy and biology of *Mitrotrema anthostomatum* Manter, 1963 (Digenea: Cryptogonimidae) from fishes of the southern Great Barrier Reef, Australia. *Journal of the Helminthological Society of Washington* **63**, 110–115.

Cribb TH, Adlard RD and Bray RA (1998) A DNA-based demonstration of a three-host life-cycle for the Bivesiculidae (Platyhelminthes: Digenea). *International Journal for Parasitology* **28**, 1791–1795.

Cribb TH, Bott NJ, Bray RA, McNamara MK, Miller TL, Nolan MJ and Cutmore SC (2014) Trematodes of the Great Barrier Reef, Australia: emerging patterns of diversity and richness in coral reef fishes. *International Journal for Parasitology* **44**, 929–939.

Cribb TH, Bray RA, Diaz PE, Huston DC, Kudlai O, Martin SB, Yong RQ-Y and Cutmore SC (2016) Trematodes of fishes of the Indo-west Pacific: told and untold richness. *Systematic Parasitology* **93**, 237–247.

Downie A and Cribb TH (2011) Phylogenetic studies explain the discrepant host distribution of *Allopodocotyle heronensis* sp. nov. (Digenea, Opecoelidae) in Great Barrier Reef serranids. *Acta Parasitologica* **56**, 296–300.

Gopurenko D and Hughes JM (2002) Regional patterns of genetic structure among Australian populations of the mud crab, *Scylla serrata* (Crustacea: Decapoda): evidence from mitochondrial DNA. *Marine and Freshwater Research* **53**, 849–857.

Huston DC, Cutmore SC and Cribb TH (2016) The life-cycle of *Gorgocephalus yaaji* Bray & Cribb, 2005 (Digenea: Gorgocephalidae) with a review of the first intermediate hosts for the superfamily Lepocreadioidea Odhner, 1905. *Systematic Parasitology* **93**, 653–665.

Jones A (2005) Superfamily Haploporoidea Nicoll, 1914. pp. 127–128 *in* Jones A, Bray RA and Gibson DI (*Eds*) *Keys to the Trematoda. Volume 2*. Wallingford, CABI International.

Jousson O, Bartoli P and Pawlowski J (1999) Molecular identification of developmental stages in Opecoelidae (Digenea). *International Journal for Parasitology* **29**, 1853–1858.

Keller A, Schleicher T, Schultz J, Müller T, Dandekar T and Wolf M (2009) 5.8S–28S rRNA interaction and HMM-based ITS2 annotation. *Gene* **430**, 50–57.

Kudlai O, Cribb TH and Cutmore SC (2016) A new species of microphallid (Trematoda: Digenea) infecting a novel host family, the Muraenidae, on the northern Great Barrier Reef, Australia. *Systematic Parasitology* **93**, 863–876.

Lucas T, O'Brien EK, Cribb TH and Degnan BM (2005) Digenean trematodes infecting the tropical abalone *Haliotis asinina* have species-specific cercarial emergence patterns that follow daily or semilunar spawning cycles. *Marine Biology* **148**, 285–292.

Miller TL, Bray RA and Cribb TH (2011) Taxonomic approaches to and interpretation of host specificity of trematodes of fishes: lessons from the Great Barrier Reef. *Parasitology* **138**, 1710–1722.

Miller TL, Downie AJ and Cribb TH (2009) Morphological disparity despite genetic similarity; new species of *Lobosorchis* Miller & Cribb, 2005 (Digenea: Cryptogonimidae) from the Great Barrier Reef and the Maldives. *Zootaxa* **1992**, 37–52.

Overstreet RM and Curran SS (2005a) Family Haploporidae Nicoll, 1914. pp. 129–165 *in* Jones A, Bray RA and Gibson DI (*Eds*) *Keys to the Trematoda. Volume 2*. Wallingford, CABI International.

Overstreet RM and Curran SS (2005b) Family Atractotrematidae Yamaguti, 1939. pp. 167–174 *in* Jones A, Bray RA and Gibson DI (*Eds*) *Keys to the Trematoda. Volume 2*. Wallingford, CABI International.

Pearson J (1968) Observations on the morphology and life-cycle of *Paucivitellosus fragilis* Coil, Reid & Kuntz, 1965 (Trematoda: Bivesiculidae). *Parasitology* **58**, 769–788.

Pulis EE and Overstreet RM (2013) Review of haploporid (Trematoda) genera with ornate muscularisation in the region of the oral sucker, including four new species and a new genus. *Systematic Parasitology* **84**, 167–191.

Reid DG, Lal K, Mackenzie-Dodds J, Kaligis F, Littlewood DTJ and Williams ST (2006) Comparative phylogeography and species boundaries in *Echinolittorina* snails in the central Indo-West Pacific. *Journal of Biogeography* **33**, 990–1006.

Rohde K (1973) Structure and development of *Lobatostoma manteri* sp. nov. (Trematoda: Aspidogastrea) from the Great Barrier Reef, Australia. *Parasitology* **66**, 63–83.

Rohde K (1977) The bird schistosome *Austrobilharzia terrigalensis* from the Great Barrier Reef, Australia. *Zeitschrift für Parasitenkunde* **52**, 39–51.

Rohde K (1981) Population dynamics of two snail species, *Planaxis sulcatus* and *Cerithium moniliferum*, and their trematode species at Heron Island, Great Barrier Reef. *Oecologia* **49**, 344–352.

Sambrook J and Russell D (2001) *Molecular cloning: A laboratory manual*. 234 pp. Cold Spring Harbor, Cold Spring Harbor Laboratory Press.

Shelley C, Glazebrook J, Turak E, Winsor L and Denton G (1988) Trematode (Digenea: Bucephalidae) infection in the burrowing clam *Tridacna crocea* from the Great Barrier Reef. *Diseases of Aquatic Organisms* **4**, 143–147.

Spalding MD, Fox HE, Allen GR, Davidson N, Ferdana ZA, Finlayson M, Halpern BS, Jorge MA, Lombana A and Lourie SA (2007) Marine

ecoregions of the world: a bioregionalization of coastal and shelf areas. *BioScience* **57**, 573–583.

Stephenson W and Searles R (1960) Experimental studies on the ecology of intertidal environments at Heron Island. I. Exclusion of fish from beach rock. *Marine and Freshwater Research* **11**, 241–268.

Thompson RM, Mouritsen KN and Poulin R (2005) Importance of parasites and their life cycle characteristics in determining the structure of a large marine food web. *Journal of Animal Ecology* **74**, 77–85.

Voris HK (2000) Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *Journal of Biogeography* **27**, 1153–1167.

https://doi.org/10.1017/S0022149X17000906 Published online by Cambridge University Press