Parasitology International 67 (2018) 768–775

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Aporocotylids from batoid and elopomorph fishes from Moreton Bay, Queensland, Australia, including a new genus and species of blood fluke infecting the Giant shovelnose ray, *Glaucostegus typus* (Rhinopristiformes: Glaucostegidae)

Scott C. Cutmore*, Thomas H. Cribb, Russell Q.-Y. Yong

The University of Queensland, School of Biological Sciences, St Lucia, Queensland 4072, Australia

ARTICLE INFO

Keywords:
Ogawaia
Elopicola
Glaucostegus
Elops
Batoidea
Elopomorpha
Moreton Bay
Blood fluke

ABSTRACT

Fishes of the elasmobranch superorder Batoidea and the basal teleost superorder Elopomorpha were assessed for blood flukes (Digenea: Aporocotylidae) during a parasitological survey conducted in Moreton Bay, Queensland, Australia. A new blood fluke genus and species, *Ogawaia glaucostegi* n. gen., n. sp., is described from the Giant shovelnose ray, *Glaucostegus typus* (Anonymous [Bennett]) (Rhinopristiformes: Glaucostegidae). *Ogawaia glaucostegi* differs from species of all other aporocotylid genera in the combination of the absence of anterior caeca and oral sucker, having a pronounced distal oesophageal chamber, a strongly coiled testis and a common genital pore. The new species most closely resembles *Myliobaticola richardheardi* Bullard & Jensen, 2008, from which it differs in lacking an oral sucker and in possessing a straight (rather than coiled) oesophagus, longer caeca in proportion to the oesophageal and total body length, and a much longer testis relative to body length. *Ogawaia glaucostegi* is just the eighth aporocotylid described from chondrichthyans, of which four belong to monotypic genera. This is the first description of a blood fluke from the order Rhinopristiformes, and the first of a chondrichthyan-infecting aporocotylid from Australian waters. *Elopicola bristowi* Orélis-Ribeiro & Bullard, 2017 is reported from Australia for the first time, from the type-host, *Elops hawaiensis* Regan (Elopiformes: Elopidae). This species is identified by morphological and molecular data and distinctions between our specimens and those of the original description are discussed.

1. Introduction

Knowledge of the systematics and taxonomy of the fish blood flukes (Aporocotylidae Odhner, 1912) from Australian waters has progressed rapidly in the last 15 years, largely due to two intense periods of focused study, the first by Nolan & Cribb [1–5] and the second by Yong et al. [6–10]. These works have resulted in Australia being the most well-studied region globally for marine aporocotylids [11, 12], with 43 species (approximately 27% of the global fauna) now known from the region. However, there are major gaps in the knowledge; just three aporocotylids are known from basal teleosts in Australian waters (two from ostariophysan gonorynchiforms and one from elopomorph anguilliforms), and those infecting chondrichthyans are completely unknown in the region.

Of the 159 known aporocotylid species, just seven have been reported from chondrichthyans: two species from lamniforms [13, 14], two from carcharhiniforms [15, 16], two from myliobatiforms [17, 18] and one from a chimaeriform [19]. Recently, Cribb et al. [20] incorporated novel sequence data for an unidentified aporocotylid from *Glaucostegus typus* (Anonymous [Bennett]) (Glaucostegidae) in a phylogenetic analysis of the Aporocotylidae, which they identified as "cf. *Myliobaticola* sp.". In this study we formally describe this form, which we conclude represents a genus and species new to science. We also record a species of the elopomorph-infecting genus *Elopicola* Bullard, 2014, from Australian waters for the first time, in the Hawaiian ladyfish *Elops hawaiensis* Regan. Only one aporocotylid species was previously known from Australian elopomorph fishes; *Paracardicoloides yamagutii* Martin, 1974, which infects three species of freshwater eels (Anguillidae) [21–23].

* Corresponding author.
E-mail address: scott.cutmore@uqconnect.edu.au (S.C. Cutmore).

https://doi.org/10.1016/j.parint.2018.08.003
Received 14 May 2018; Received in revised form 8 August 2018; Accepted 13 August 2018
Available online 14 August 2018
1383-5769/

2. Materials and methods

2.1. Specimen collection

Batoid and elopomorph fishes were collected by seine, tunnel netting, spear and hand-nets in various locations throughout Moreton Bay, Queensland, Australia. The heart (including, in the case of batoid specimens, the conus arteriosus and ventral aorta) was removed, placed in saline solution (0.85% NaCl solution) and each section opened separately. Gill filaments were removed and examined for the presence of eggs following Yong et al. [8]. Aporocotylids were washed in vertebrate saline, fixed by pipetting into near-boiling saline, and preserved in 70% ethanol for parallel morphological and molecular characterisation. Some individual worms were processed for both morphological and molecular analysis (hologenophores *sensu* Pleijel et al. [24]).

2.2. Morphological analysis

Specimens for morphological analysis were washed in fresh water, stained in Mayer's haematoxylin, destained in a solution of 1.0% HCl and neutralised in 1.0% ammonium hydroxide solution. Specimens were then dehydrated through a graded ethanol series, cleared in methyl salicylate and mounted in Canada balsam. Measurements were made using an Olympus SC50 digital camera mounted on an Olympus BX-53 compound microscope using cellSens Standard imaging software. Measurements are in micrometres (μm) and given as a range followed by the mean in parentheses. Where length is followed by breadth, the two measurements are separated by '×'. Drawings were made using an Olympus BX-53 compound microscope and drawing tube. Type- and voucher specimens are lodged in the Queensland Museum (QM), Brisbane.

2.3. Molecular sequencing and phylogenetic analysis

As recommended by Blasco-Costa et al. [25], four genetic markers were sequenced; specimens for molecular analysis were processed according to protocols used by Cutmore et al. [26] for the ITS2 and 28S rDNA regions, Huston et al. [27] for the 18S rDNA region and Wee et al. [28] for the *cox*1 mtDNA region. The complete ITS2 rDNA region was amplified and sequenced using the primers 3S [29] and ITS2.2 [30]; the partial D1-D3 28S rDNA region using LSU5 [31], 300F [32], ECD2 [33] and 1500R [34]; complete 18S rDNA using 18S-E [35], 300F [36], 1100F [35], Cestode-1 [35], 1270R [35] and WormB [35]; and partial *cox*1 mtDNA region using Dig_cox1Fa [28] and Dig_cox1R [28]. Geneious® version 10.2.3 [37] was used to assemble and edit contiguous sequences and the start and end of the ITS2 rDNA region were determined by annotation through the ITS2 Database [38, 39], using the 'Metazoa' model.

3. Results

3.1. General results

Aporocotylids were found in nine of 16 *G. typus* examined from Moreton Bay; no aporocotylids were found in any of the eight other batoid species assessed (Table 1). Within *G. typus*, aporocotylids were typically found intertwined in the connective tissue of the valves of the conus arteriosus, and less commonly in the ventricle. These specimens represent a new genus and species, just the seventh blood fluke to be described from an elasmobranch host. Aporocotylids were found in a single *Elops hawaiensis* examined from Moreton Bay; these were identified as *Elopicola bristowi* Orélis-Ribeiro & Bullard, 2017, which is here reported from Australia for the first time. No aporocotylids were found in four other elopomorph species assessed from Moreton Bay (Table 1).

Table 1
Batoid and elopomorph species assessed for blood flukes from Moreton Bay in this study. Those infected by aporocotylids shown in bold.

Host	No. examined (infected)
BATOIDEA	
MYLIOBATIFORMES	
Dasyatidae	
Hemitrygon fluviorum (Ogilby)	3 (0)
Himantura uarnak (Gmelin)	1 (0)
Maculabatis cf. astra	1 (0)
Maculabatis toshi (Whitley)	4 (0)
Neotrygon trigonoides (Castelnau)	4 (0)
Pastinachus ater (Macleay)	1 (0)
Myliobatidae	
Aetobatus ocellatus (Kuhl)	6 (0)
RHINOPRISTIFORMES	
Glaucostegidae	
***Glaucostegus typus* (Anonymous [Bennett])**	**16 (9)**
Rhinobatidae	
Aptychotrema rostrata (Shaw)	6 (0)
ELOPOMORPHA	
ANGUILLIFORMES	
Muraenesocidae	
Muraenesox bagio (Hamilton)	2 (0)
Muraenidae	
Gymnothorax eurostus (Abbott)	3 (0)
Gymnothorax pseudothyrsoideus (Bleeker)	22 (0)
ELOPIFORMES	
Elopidae	
***Elops hawaiensis* Regan**	**1 (1)**
Megalopidae	
Megalops cyprinoides (Broussonet)	1 (0)

3.2. Ogawaia n. gen

Diagnosis: Body overall narrowly lanceolate, flat in forebody, becoming more cylindrical and narrow in midbody before broadening and flattening again at level of terminal genitalia, broadest at levels of oesophagus and uterine coils. Tegumental spines absent. Oral sucker absent. Mouth a simple pore, just ventrally subterminal. Oesophagus mostly straight, occasionally slightly sinuous. Oesophageal chamber prominent at distal extremity of oesophagus. Anterior caeca absent. Posterior caeca mostly straight, occasionally with some sinuosity at distal ends, sub-equal to unequal in length, with ends variably swollen. Testis occupying nearly half of total body length, strongly coiled, with smooth margins, entirely post-caecal. Vas deferens originates medially from posterior margin of testis, simple and uncoiled for much of length. Seminal vesicle an indistinctly-defined expansion of vas deferens, leads directly to common genital pore; cirrus-sac and cirrus absent. Common genital pore dorso-sinistral. Ovary broadly trigonal, with margins crenulated to distinctly lobed, medio-dextral, entirely post-testicular. Oviduct originates dextrally from posterior margin of ovary, passes sinistrally and posteriorly to meet oötype. Oötype well-defined, dextral. Vitelline follicles finely granular, largely confined by nerve cords, evenly distributed from dorsal nerve commissure to level of common genital pore, overlapping oesophagus, caeca, testis and ovary, without division into lateral fields. Vitelline duct runs dextrally parallel to and overlapping lateral nerve cord, straight for most of traceable length, convoluted at level of oviduct, dorsal to remainder of genitalia. Uterus strongly convoluted, passing sinistral to oötype anteriorly to parallel sinistral ovarian margin, then posteriorly to meet common genital pore. Distal uterine coils not distinctly muscularised or modified to form metraterm. Confluence of male and female genital systems at site of genital pore not expanded to form genital atrium or similar. Uterine coils often filled with sperm, with one coil consistently expanded to form uterine seminal receptacle. Uterine seminal receptacle oblong, varying in turgidity according to egg and sperm content. Eggs oblong, unspined and anoperculate, often numerous. Excretory system not