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Gerricola queenslandensis n. g., n. sp., a new monorchiid trematode from the eastern Australian coast and its life cycle partially elucidated

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Research Paper

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Abstract

Of over 250 species of Monorchiidae Odhner, 1911, just four are known from gerreid fishes. Here, we report adult specimens of a new species infecting *Gerres oyena* (Forsskål) and *Gerres subfasciatus* Cuvier from off Heron Island and North Stradbroke Island, Queensland, Australia. The species is morphologically most similar to the concept of *Lasiotocus* Looss, 1907, which currently comprises eight species, in the possession of an unspined genital atrium, bipartite terminal organ, round oral sucker and unlobed ovary. However, phylogenetic analyses of the 28S ribosomal DNA gene region shows the species to be distantly related to the two sequenced species of *Lasiotocus* – *Lasiotocus mulli* (Stossich, 1883) Odhner, 1911 and *Lasiotocus trachinoti* Overstreet & Brown, 1970 – and that it clearly requires a distinct genus; thus, we propose *Gerricola queenslandensis* n. g., n. sp. Morphologically, *G. queenslandensis* n. g., n. sp. differs significantly from *L. mulli* and *L. trachinoti* only in the possession of distinctly longer caeca, which terminate in the post-testicular region, and in the absence of a distinct gap in the terminal organ spines. The remaining species of *Lasiotocus* possess caeca that also terminate in the post-testicular region, which might warrant their transfer to *Gerricola* n. g. However, doubt about their monophyly due to a combination of significant morphological variation, a lack of information on some features and infection of a wide range of hosts, lead us to retain these taxa as species of *Lasiotocus* until molecular sequence data are available to better inform their phylogenetic and taxonomic positions. Sporocysts and cercariae of *G. queenslandensis* n. g., n. sp. were found in a lucinid bivalve, *Codakia paytenorum* (Iredale), from Heron Island. Sexual adult and intramolluscan stages were genetically matched with the ITS2 ribosomal DNA and *cox*1 mitochondrial DNA regions. This is the second record of the Lucinidae as a first intermediate host for the Monorchiidae. Additionally, we report sporocysts and cercariae of another monorchiid infection in a tellinid bivalve, *Jactellina clathrata* (Deshayes), from Heron Island. Molecular sequence data for this species do not match any sequenced species and phylogenetic analyses do not suggest any generic position.

Introduction

The Gerreidae (Perciformes), commonly known as mojarras or silverbiddies, are a family of primarily marine fishes comprising eight genera and 53 species. They typically inhabit the coastal waters of tropical seas, occasionally entering brackish waters, and are predators of benthic invertebrates (Froese & Pauly, 2021). Gerreids are host to a wide variety of digenean trematodes, including representatives of at least 13 families, including the Monorchiidae Odhner, 1911. Just four monorchiids have been reported from gerreids: *Alloproctorema gerres* Machida, 1973, *Hurleytrema shorti* (Nahhas & Powell, 1965) Overstreet, 1969, *Postmonorchis orthopristis* Hopkins, 1941 and *Pseudohurleytrema eucinostomi* (Manter, 1942) Yamaguti, 1954 (see Manter, 1942; Siddiqi & Cable, 1960; Nahhas & Cable, 1964; Overstreet, 1969; Machida, 1973; Wallet & Kohn, 1987; Machida, 2011). The overall paucity of monorchiids in gerreid fishes is perhaps surprising, considering that they prey on invertebrates, which are known monorchiid intermediate hosts.

Ten gerreid species are known in Australia, and there have been no reports of monorchiid infections in these species from the region. Of these, *Gerres oyena* (Forsskål) and *Gerres subfasciatus* (Cuvier) have the widest distribution and are the most commonly encountered species on the east coast of Australia. Here, we report a new monorchiid species from these gerreids and partly elucidate its life cycle. We also report an unidentified monorchiid infection from a tellinid bivalve and provide a description and molecular sequence data for it.

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Materials and methods

Host and parasite collection

Specimens of *G. oyena* and *G. subfasciatus* were collected from Moreton Bay (south-eastern Queensland) and off Heron Island (southern Great Barrier Reef), Queensland, Australia. In total, 80 specimens of *G. oyena* were collected from Heron Island and 41 from Moreton Bay, and 147 specimens of *G. subfasciatus* were collected from Moreton Bay. Fishes from Moreton Bay were collected via tunnel-netting and seine netting, and those from off Heron Island only via seine netting. The two species are morphologically similar and were differentiated by the presence of scales between the eyes and posterior to the nostrils in *G. subfasciatus* and the absence of these scales in *G. oyena* (Froese & Pauly, 2021). Fishes were euthanized via an overdose of anaesthetic (AQUI-S®, AQUI-S New Zealand Ltd, Lower Hutt, New Zealand). The gastrointestinal tract was removed and examined for parasites using the gut wash method described by Cribb & Bray (2010). Trematodes were washed in vertebrate saline, fixed by pipetting into near-boiling saline and preserved in 80% ethanol for parallel morphological and molecular characterization. Hologenophores and paragenophores (*sensu* Pleijel *et al.*, 2008) were prepared for several specimens.

Specimens of *Codakia paytenorum* (Iredale) and *Jactellina clathrata* (Deshayes) were collected from the reef flat on the southern side of Heron Island; 383 specimens of *C. paytenorum* and 208 specimens of *J. clathrata* were collected. Bivalves were collected by digging and sifting sand at low tide and identified according to Lamprell & Whitehead (1992). Bivalves were either shucked or cracked open with a hammer, and the viscera was carefully teased apart with forceps and examined for the presence of intramolluscan digenean stages. Trematode material was separated from bivalve host tissue and fixed by pipetting into near-boiling saline and preserved in 80% ethanol for parallel morphological and molecular characterization. Isogenophores (*sensu* Pleijel *et al.*, 2008) were prepared for some specimens.

Morphological analysis

Specimens were washed in fresh water, stained in Mayer's haematoxylin, destained in 1.0% hydrochloric acid and neutralized 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 (Olympus, Notting Hill, Australia) with cellSens Standard imaging software (https://www.olympus-lifescience.com/en/software/cellsens), and are in micrometres (µm) and presented as the range followed by the mean in parentheses. Where length is followed by breadth, the two measurements are separated by '×'. Line drawings were made with a drawing tube fitted to the same compound microscope and digitized in Adobe Illustrator CC 2018 software (https://www.adobe.com/au/products/illustrator.html). Type specimens are lodged in the Queensland Museum, Brisbane. To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of the new taxon have been submitted to ZooBank; the Life Science Identifier (LSID) is reported in the taxonomic summary.

Molecular sequencing

Genetic sequence data were generated for two barcoding regions: the complete second internal transcribed spacer unit (ITS2) ribosomal DNA (rDNA) noncoding region and the partial cytochrome *c* oxidase 1 (*cox*1) mitochondrial DNA (mtDNA) region, and one phylogenetically informative region: the partial large ribosomal subunit (28S) rDNA region. Amplification of the two rDNA regions was performed following the protocols of Wee *et al.* (2017b) using the primers 3S (5′-GGT ACC GGT GGA TCA CGT GGC TAG TG-3′; Morgan & Blair, 1995) and ITS2.2 (5′-CCT GGT TAG TTT CTT TTC CTC CGC-3′; Cribb *et al.*, 1998) for the ITS2 amplification, and LSU5 (5′-TAG GTC GAC CCG CTG AAY TTA AGC A-3′; Littlewood, 1994) and 1500R (5′-GCT ATC CTG AGG GAA ACT TCG-3′; Snyder & Tkach, 2001) for the 28S amplification. Amplification of the mtDNA region was performed following the protocols of Wee *et al.* (2017a) using the primers Dig_cox1Fa (5′-ATG ATW TTY YTD ATG CC-3′; Wee *et al.*, 2017a) and Dig_cox1R (5′-TCN GGR TGH CCR AAR AAY CAA AA-3′; Wee *et al.*, 2017a). All amplifications were conducted on a Takara TP-650 PCR Thermocycler (Takara, Otsu, Shiga, Japan). Sanger sequencing was conducted using the amplification primers for the ITS2 and *cox*1 regions, and an internal pair of primers for the 28S regions: 300F (5′-CAA GTA CCG TGA GGG AAA GTT G-3′; Littlewood *et al.*, 2000) and ECD2 (5′-CCT TGG TCC GTG TTT CAA GAC GGG-3′; Littlewood *et al.*, 1997). Contiguous sequences were assembled and edited with Geneious® version 11.0.5 (Kearse *et al.*, 2012). For ITS2 rDNA sequences, the start and end of the region were annotated via the ITS2 Database (Keller *et al.*, 2009; Ankenbrand *et al.*, 2015) using the 'Metazoa' model. GenBank accession numbers for novel sequence data are provided in the taxonomic summaries.

Alignments for the ITS2 and *cox*1 datasets were conducted in MEGA version X with MUSCLE algorithm and UPGMA clustering for iterations 1 and 2 (Kumar *et al.*, 2018). The *cox*1 alignment was checked for stop codons following translation with the echinoderm/flatworm mitochondrial code, and the correct reading frame was identified. The first column was then removed so that the reading frame began on position one, simplifying position-coding in downstream analyses. The alignment was then tested for non-stationarity and substitution saturation with a χ^2 test run on PAUP* (Swofford, 2002) and Xia's test run on DAMBE7 (Xia *et al.*, 2003; Xia & Lemey, 2009; Xia, 2018), respectively; no significant non-stationarity and substitution saturation was detected. Neighbour-joining analyses were also conducted in MEGA version X using the ITS2 and *cox*1 datasets to explore the number of base pair differences between samples and to determine species boundaries. The parameters used for both analyses were: 'test of phylogeny = bootstrap', 'no. of bootstrap replications = 10,000', 'model/method = no. of differences', 'substitutions to include = d: Transitions + Transversions' and 'rates among sites = Uniform rates'.

Phylogenetic analyses

The newly generated partial 28S rDNA sequences were aligned with sequences of other monorchiid taxa available on GenBank (table 1) using MUSCLE version 3.7 (Edgar, 2004) with ClustalW sequence weighting and UPGMA clustering for iterations 1 and 2. The resultant alignment was trimmed manually,

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Table 1. Collection data for 28S sequences from GenBank analysed in this study.

Species	Host	Location	GenBank ID	Reference
Family Monorchiidae				
Allobacciger annulatus Wee, Cutmore, Sasal & Cribb, 2019	*Centropyge tibicen* (Cuvier)	Off Heron Island, Australia	MK955782	Wee *et al.* (2020d)
Allobacciger brevicirrus Wee, Cutmore, Sasal & Cribb, 2019	*Scolopsis bilineata* (Bloch)	Off Heron Island, Australia	MK955781	Wee *et al.* (2020d)
Allobacciger polynesiensis Wee, Cutmore, Sasal & Cribb, 2019	*Centropyge flavissima* (Cuvier)	Off Moorea, French Polynesia	MK955780	Wee *et al.* (2020d)
Ancylocoelium typicum Nicoll, 1912	*Trachurus trachurus* (Linnaeus)	North Sea, UK	AY222254	Olson *et al.* (2003)
Cableia pudica Bray, Cribb & Barker, 1996	*Cantherhines pardalis* (Rüppell)	Off Heron Island, Australia	AY222251	Olson *et al.* (2003)
Diplomonorchis leiostomi Hopkins, 1941	*Leiostomus xanthurus* Lacepède	Off Ocean Springs, Mississippi, USA	AY222252	Olson *et al.* (2003)
Genolopa ampullacea Linton, 1910	*Haemulon macrostomum* (Günther)	Off Islamorada, Florida, USA	MN984474	Panyi *et al.* (2020)
Genolopa minuscula Panyi, Curran & Overstreet, 2020	*Anisotremus surinamensis* (Bloch)	Off Marathon, Florida, USA	MN984472	Panyi *et al.* (2020)
Genolopa vesca Panyi, Curran & Overstreet, 2020	*Haemulon sciurus* (Shaw)	Off Long Key, Florida, USA	MN984471	Panyi *et al.* (2020)
Helicometroides longicollis Yamaguti, 1934	*Diagramma labiosum* Macleay	Off Heron Island, Australia	KJ658287	Searle *et al.* (2014)
Hurleytrematoides chaetodoni (Manter, 1942) Yamaguti, 1954	*Chaetodon striatus* Linnaeus	Mona Passage, Puerto Rico	MH244116	Andres *et al.* (2018)
Hurleytrematoides galzini McNamara & Cribb, 2011	*Forcipiger flavissimus* Jordan & McGregor	Off Heron Island, Australia	MK501988	Wee *et al.* (2019)
Hurleytrematoides loi McNamara & Cribb, 2011	*Chelmon rostratus* (Linnaeus)	Moreton Bay, Australia	MK501989	Wee *et al.* (2019)
Infundiburictus arrhichostoma (Searle, Cutmore & Cribb, 2014) Wee, Cutmore, Pérez-del-Olmo & Cribb, 2020	*Diagramma labiosum*	Off Heron Island, Australia	KJ658289	Searle *et al.* (2014)
'Lasiotocus sp.'	*Menidia menidia* (Linnaeus)	Great Bay Estuary, New Jersey, USA	MN984477	Panyi *et al.* (2020)
Lasiotocus mulli (Stossich, 1883) Odhner, 1911	*Mullus surmuletus*	Off Santa Pola, Mediterranean Sea, Spain	MT669011	Wee *et al.* (2020c)
Lasiotocus trachinoti Overstreet & Brown, 1970	*Trachinotus carolinus* Linnaeus	Off Jacksonville, Florida, USA	MN984478	Panyi *et al.* (2020)
Madhavia fellaminuta Wee, Cutmore & Cribb, 2018	*Upeneus tragula* Richardson	Moreton Bay, Australia	MG920219	Wee *et al.* (2018)
Monorchis lewisi Cribb, Wee, Bray & Cutmore, 2017	*Acanthopagrus australis* (Günther)	Moreton Bay, Australia	MF503309	Cribb *et al.* (2018)
Monorchis (Stossich, 1890) Monticelli, 1893	*Diplodus vulgaris* (Geoffroy Saint-Hilaire)	near Corsica, France	AF184257	Tkach *et al.* (2001)
Ovipusillus geminus Wee, Cutmore & Cribb, 2019	*Gnathanodon speciosus* (Forsskål)	Moreton Bay, Australia	MF501987	Wee *et al.* (2019)
Ovipusillus mayu Dove & Cribb, 1998	*Gnathanodon speciosus*	Moreton Bay, Australia	MF503310	Cribb *et al.* (2018)
Parachrisomon delicatus (Manter & Prichard, 1964) Madhavi, 2008	*Upeneus tragula*	Moreton Bay, Australia	MG920218	Wee *et al.* (2018)
Postmonorchis orthopristis Hopkins, 1941	*Haemulon flavolineatum* Desmarest	Off Upper Matecumbe Key, Florida, USA	MN984475	Panyi *et al.* (2020)
Proctotrema addisoni Searle, Cutmore & Cribb, 2014	*Diagramma labiosum*	Off Heron Island, Australia	KJ658291	Searle *et al.* (2014)

(*Continued*)

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Table 1. (*Continued.*)

Species	Host	Location	GenBank ID	Reference
Provitellus chaometra Wee, Cutmore & Cribb, 2019	*Gnathanodon speciosus*	Moreton Bay, Australia	MK501984	Wee *et al.* (2019)
Provitellus infibrova Wee, Cutmore & Cribb, 2019	*Gnathanodon speciosus*	Moreton Bay, Australia	MK501986	Wee *et al.* (2019)
Provitellus infrequens Wee, Cutmore & Cribb, 2019	*Gnathanodon speciosus*	Moreton Bay, Australia	MK501985	Wee *et al.* (2019)
Provitellus turrum Dove & Cribb, 1998	*Pseudocaranx dentex* (Bloch & Schneider)	Off Heron Island, Australia	AY222253	Olson *et al.* (2003)
Pseudohurleytrema yolandae Wee, Crouch, Cutmore & Cribb, 2020	*Tripodichthys angustifrons* (Hollard)	Moreton Bay, Australia	MT649300	Wee *et al.* (2020b)
Retroporomonorchis pansho Wee, Cribb, Cutmore & Martin, 2020	*Lutjanus fulvus* (Forster)	Off Lizard Island, Australia	MT672340	Wee *et al.* (2020a)
Sinistroporomonorchis glebulentus (Overstreet, 1971) Wee, Cutmore, Pérez-del-Olmo & Cribb, 2020	*Mugil curema* Valenciennes	Off Beaufort, North Carolina, USA	MN984476	Panyi *et al.* (2020)
Sinistroporomonorchis lizae (Liu, 2002) Wee, Cutmore, Pérez-del-Olmo & Cribb, 2020	*Moolgarda perusii* (Valenciennes)	Tonkin Bay, Vietnam	LN831724	Atopkin *et al.* (2017)
Family Lissorchiidae				
Asaccotrema vietnamiense Sokolov & Gordeev, 2019	*Rasbora paviana* Tirant	Cat Tien National Park, Vietnam	MK863409	Sokolov & Gordeev (2019)
Asymphylodora perccotti Besprozvannykh, Ermolenko & Atopkin, 2012	*Perccottus glenii* Dybowski	Bolshaya Ussurka River Basin, Russia	FR822715	Besprozvannykh *et al.* (2012)
Asymphylodora progenetica Serkova & Bykhovskii, 1940	*Bithynia tentaculata* (Linnaeus)	Verkiai pond, Vilnius, Lithuania	MT103400	Petkevičiūtė *et al.* (2020)
Asymphylodora sp.	*Lithoglyphus naticoides*	Danube River, Hungary	MT153916	Petkevičiūtė *et al.* (2020)
Lissorchis kritskyi Barnhart & Powell, 1979	*Carpiodes cyprinus* (Lesueur)	Pascagoula River, Mississippi, USA	AY222250	Olson *et al.* (2003)
Palaeorchis incognitus Szidat, 1943	*Rutilus* (Linnaeus)	Kaunas water reservoir, Lithuania	MT103408	Petkevičiūtė *et al.* (2020)
Family Deropristidae				
Skrjabinopsolus nudidorsalis Sokolov, Voropaeva & Atopkin, 2020	*Acipenser ruthenus* Linnaeus	River Volga Basin, Russia	MN700996	Sokolov *et al.* (2020)

and indels larger than three bases and affecting >5% of sequences were removed; the removed bases amounted to less than 3% of the final alignment, which comprised 1275 base positions. Bayesian inference (BI) and maximum likelihood (ML) analyses were conducted using the implementations of MrBayes version 3.2.6 (Ronquist *et al.*, 2012) and RAxML version 8.2.6 (Stamatakis, 2014), respectively, in the CIPRES portal (Miller *et al.*, 2010). Both analyses were run with the closest estimation of the GTR + I+Γ model of evolution, based on implementation of the Akaike Information Criterion in jModelTest version 2.1.10 (Darriba *et al.*, 2012). The BI analysis was run over 10,000,000 generations (ngen = 10,000,000) with two runs each containing four simultaneous Markov chain Monte Carlo (MCMC) chains (nchains = 4) and every 1000th tree saved. The following parameters were used in the analysis: nst = 6, rates = invgamma, ngammacat = 4 and the priors parameters of the combined dataset were set to ratepr = variable. Samples of substitution model parameters were 'sump burnin' = 3000 and 'sumt burnin' = 3000. The ML analysis was run with 1000 bootstrap pseudoreplicates. Sequence data for the Lissorchiidae, the sister family to the Monorchiidae, were included in this dataset, and the deropristid *Skrjabinopsolus nudidorsalis* Sokolov, Voropaeva & Atopkin, 2020 was designated as the outgroup, based on phylogenetic findings of Sokolov *et al.* (2020).

Results

Of the examined gerreid specimens, 32 specimens of *G. oyena* from Heron Island, seven of *G. oyena* from Moreton Bay and 30 of *G. subfasciatus* were infected with adult trematodes consistent with the concept of the Monorchiidae, and all specimens appeared to conform morphologically to a single species. For the ITS2 rDNA region, eight identical sequences were generated (all from *G. oyena*, seven from Heron Island and one from Moreton Bay). For the *cox*1 mtDNA region, six sequences were generated (four from *G. oyena* from Heron Island, one from *G. oyena* from Moreton Bay and one from *G. subfasciatus* from Moreton Bay), representing three genotypes differing by 1–4 base pairs. We interpret these small differences as consistent with intraspecific variation. For the 28S rDNA region, three identical sequences were generated (all from *G. oyena*, two from Heron Island, one from Moreton Bay).

Of the examined specimens of the bivalve *C. paytenorum* from Heron Island, we found a single infection of monorchiid

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sporocysts and cercariae. ITS2 and *cox*1 sequence data generated for these intramolluscan stages were used to match them to the adult forms. The ITS2 sequence was identical to the sequences from sexual adults infecting *G. oyena* and *G. subfasciatus*, and the *cox*1 sequence was identical to two sequences from sexual adults infecting *G. oyena* from Heron Island. The 28S sequence for these intramolluscan stages differed from sequences from sexual adults at a single base position. Of the examined specimens of the bivalve *J. clathrata* from Heron Island, we found a single infection of monorchiid sporocysts and cercariae. ITS2, *cox*1 and 28S sequence data generated for this intramolluscan infection do not match any publicly available monorchiid sequence on GenBank.

Taxonomic summary

Family Monorchiidae Odhner, 1911
Subfamily Monorchiinae Odhner, 1911

Gerricola n. g.

Diagnosis

Body moderately elongate, pyriform. Tegument thin, spined. Eye-spot pigment present in forebody. Oral sucker terminal, almost round, with opening subterminal. Ventral sucker spherical, in middle third of body. Prepharynx short. Pharynx smaller than oral sucker, subspherical. Oesophagus simple, moderately long. Intestine bifurcates immediately anterior to ventral sucker. Intestinal caeca blind, long, extend into post-testicular region, near to posterior extremity of body. Testis single, entire, in posterior half of hindbody. Cirrus sac subcylindrical, in middle third of body, extends from anterior margin of testis to level of anterior portion of ventral sucker. Seminal vesicle unipartite, cylindrical. Pars prostatica short to moderately long, simple. Cirrus prominent, spined. Genital atrium aspinous. Common genital pore median, immediately anterior to ventral sucker. Ovary entire or weakly lobed, dextro-submedial, antero-dextral to and contiguous with testis. Uterine seminal receptacle present. Vitellarium composed of two, well-separated lateral clusters of regular, dense follicles in mid-hindbody. Uterus extensive in hindbody, extends beyond gonads posteriorly, without discernible metraterm, enters terminal organ at junction of spined and unspined sections. Terminal organ bipartite, smaller than cirrus sac; posterior section saccular, unspined; anterior section spined, without gap in spination. Eggs small, unfilamented. Excretory vesicle small, saccular, restricted to post-gonadal region. Excretory pore terminal. In intestine of gerreid fishes.

Type and only species. *Gerricola queenslandensis* n. g., n. sp.

ZooBank registration. The LSID for *Gerricola* n. g. is: urn:lsid:zoobank.org:act:F60871B3-9007-4F02-963B-1EC6A48DDD51.

Etymology. The name is derived from the definitive host genus, *Gerres*, and the Latin word '*cola*', for inhabiting. The genus is to be treated as masculine.

Gerricola queenslandensis n. g., n. sp.

Adults

Type host. Gerres oyena (Forsskål), the blacktip silverbiddy (Perciformes: Gerreidae).

Other host. Gerres subfasciatus Cuvier, the common silverbiddy (Perciformes: Gerreidae).

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

Other locality. Eastern Moreton Bay, Queensland, Australia (27°24′S, 153°20′E).

Site of infection. Intestine.

Prevalence. *Gerres oyena*, 32/80 (40.0%) (Heron Island), 7/41 (17.1%) (Moreton Bay); *G. subfasciatus*, 30/147 (20.4%) (Moreton Bay).

Deposition of specimens. Holotype (QM G239139) and 27 paratypes, comprising two hologenophores (QM G239165–6) and 25 paragenophores (QM G239140–63).

Molecular sequence data. ITS2 rDNA, eight identical replicates, all from *G. oyena* (seven from Heron Island, one from Moreton Bay), one submitted to GenBank (GB MZ271998); *cox*1 mtDNA, six sequences (four from *G. oyena* from Heron Island, and one each from *G. oyena* and *G. subfasciatus* from Moreton Bay), comprising three genotypes, one replicate of each host locality combination from each genotype submitted to GenBank (GB MZ295277, MZ295279–81); 28S rDNA, three identical replicates (all from *G. oyena*, two from Heron Island, one from Moreton Bay), one submitted to GenBank (GB MZ271999).

ZooBank registration: The Life Science Identifier (LSID) for *Gerricola queenslandensis* n. g., n. sp. is urn:lsid:zoobank.org:act:73AD8BD8-97D5-4EF5-8FF8-ACBA09A32F77.

Etymology. The specific name *queenslandensis* refers to the state of Australia where the species is found.

Description

Based on 28 gravid, unflattened specimens: 15 from *G. oyena* from Heron Island, six from *G. oyena* from Moreton Bay, seven from *G. subfasciatus* from Moreton Bay (fig. 1a–c).

Body small, pyriform, slightly tapering anteriorly and moderately rounded posteriorly, 442–789 (526) × 120–238 (163), 2.60–3.68 (3.15) times longer than wide, widest at middle of hindbody. Forebody 136–219 (171) long, occupying 29.6–39.9 (34.6)% of body length; hindbody 228–495 (299) long, occupying 51.6–66.4 (56.6)% of body length. Tegument thin, uniformly covered with small, fine, regular spines. Eye-spot pigment granules present, restricted to forebody.

Oral sucker terminal, almost round, with opening distinctly subterminal, 32–67 (48) × 40–70 (50), 0.90–1.28 (1.03) times wider than long. Ventral sucker roughly spherical, 40–65 (50) × 42–70 (52), 0.88–1.19 (1.04) times wider than long; ventral sucker 0.97–1.51 (1.08) times longer and 0.96–1.21 (1.05) times wider than oral sucker. Prepharynx short. Pharynx muscular, subspherical, 25–46 (32) × 29–49 (34), 0.85–1.06 (0.96) times longer than wide; pharynx length 61.9–78.7 (68.8)% of oral sucker length; pharynx width 57.1–82.2 (69.9)% of oral sucker width. Oesophagus moderately long, gently to strongly sinuous, 49–137 (84) long, occupies 10.7–20.9 (16.9)% of body length. Intestinal bifurcation immediately anterior to ventral sucker; pre-bifurcal zone occupies 26.7–41.3 (32.8)% of body length. Intestinal caeca blind, long, occupy 53.8–69.7 (61.1)% of body length, terminate in post-testicular zone, approximately at level of excretory vesicle, 7.6–16.8 (11.0)% of body length from posterior end of body.

Testis single, large, entire, subspherical to transversely or longitudinally ellipsoidal, mainly in posterior half of hindbody, median, can overlap either caecum, separated by 12.9–25.4 (19.6)% of body length from ventral sucker, 61–141 (96) × 71–150 (93); pre-testicular zone 52.1–71.2 (64.2)% of body length; post-testicular zone 9.7–29.5 (17.8)% of body length. Cirrus sac mainly in middle third of body, subcylindrical, mostly median,

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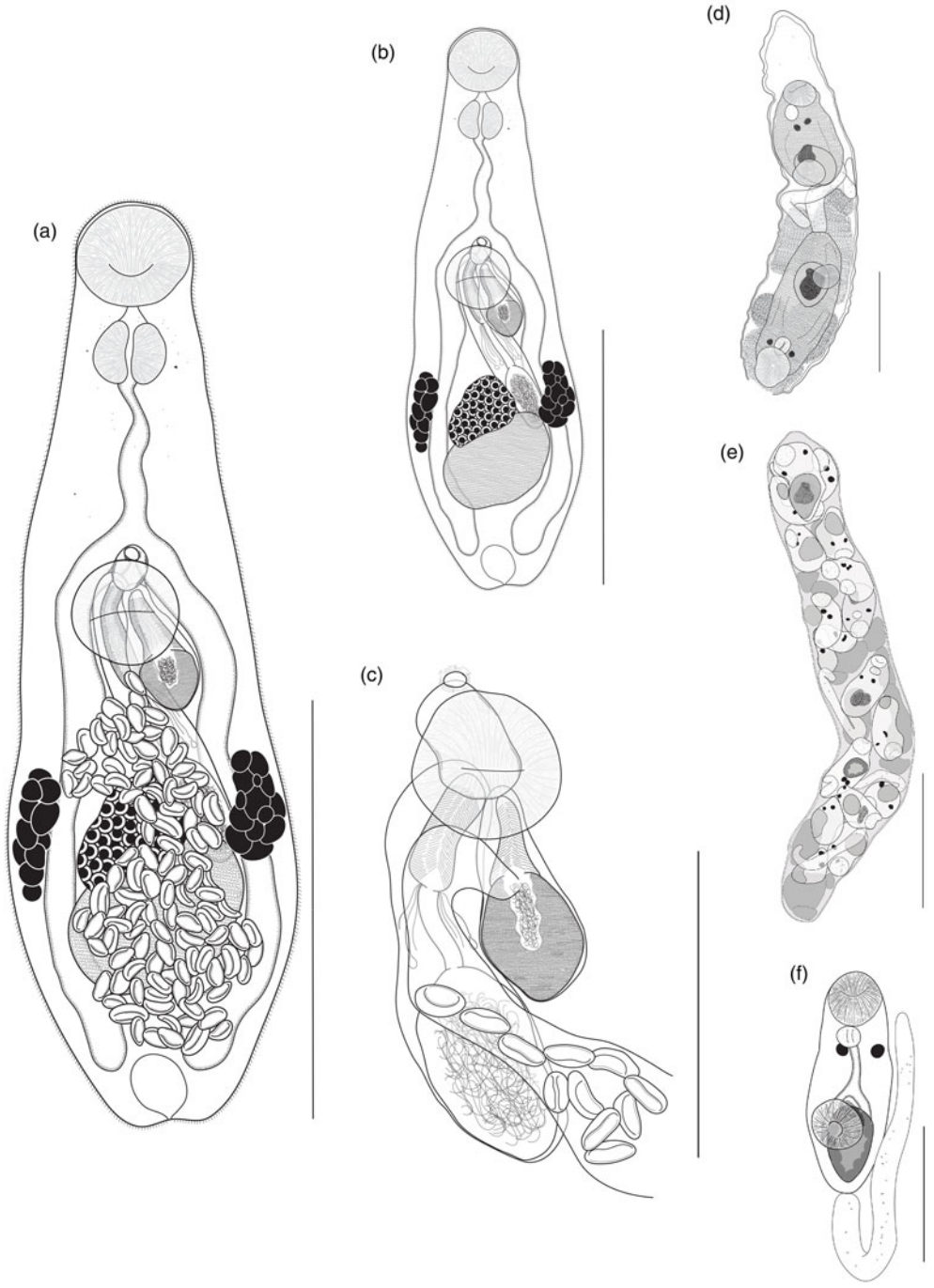

Fig. 1. Sexual and intramolluscan stages of *Gerricola queenslandensis* n. g., n. sp., and intramolluscan stages of Monorchiidae sp. from Heron Island, Queensland, Australia. (a) *Gerricola queenslandensis* n. g., n. sp., adult worm, ventral view; (b) *G. queenslandensis* n. g., n. sp., adult worm, eggs and uterus excluded, ventral view; (c) *G. queenslandensis* n. g., n. sp., terminal genitalia and part of uterus, ventral view; (d) *G. queenslandensis* n. g., n. sp., sporocyst containing cercariae; (e) Monorchiidae sp. ex *Jactellina clathrata*, sporocyst; (f) Monorchiidae sp. ex *J. clathrata*, cercaria. Scale bars: (a, b) 200 μm; (c–f) 100 μm.

typically mostly intercaecal, slightly overlapping both caeca, with posterior end typically slightly overlapping ovary and partially overlapping testis in some specimens, with posterior end typically slightly sinistro-submedian and anterior end slightly dextro-submedian, 108–198 (131) × 24–53 (35), occupies 21.2–32.1 (26.5)% of body length. Seminal vesicle ellipsoidal, unipartite, 33–77 (51) × 18–49 (31), occupies 27.4–52.1 (38.4)% of cirrus sac length. Pars prostatica short or moderately long, simple, with few prostatic cells observed, 17–51 (27) long. Cirrus relatively narrow, subcylindrical, armed with small acicular spines, tapers slightly anteriorly, 30–69 (44) × 11–27 (17), occupies 20.7–42.6 (33.5)% of cirrus sac length. Genital atrium prominent, aspinous, ellipsoidal, simple. Common genital pore median, immediately anterior to ventral sucker.

Ovary in middle of hindbody, anterodextral to and partially overlapping testis, partially overlaps right caecum, smooth,

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roughly triangular, 9.3–16.9 (12.7)% of body length from ventral sucker, 42–100 (55) × 38–81 (54); pre-ovarian zone occupies 46.7–62.8 (57.0)% of body length; post-ovarian zone occupies 25.6–40.8 (33.2)% of body length. Mehlis' gland large, dorsal to and easily mistaken as part of ovary, not observed in some specimens. Uterine seminal receptacle present. Vitellarium composed of two lateral masses of densely clustered follicles, at level of ovary and testis, may extend slightly anteriorly to ovary, never extends posteriorly beyond testis, ventral to and partially overlapping both caeca, mass length 42–101 (62), occupying 8.3–14.5 (12.0)% of body length. Uterus thin-walled, extensive, restricted to hindbody, ventral to ovary, testis, caeca and part of cirrus sac, coils mostly indiscernible, with ascending coil entering terminal organ at junction of spined and unspined sections. Terminal organ sinistro-ventral to and about a third the length of cirrus sac, bipartite, comprising unspined, saccular posterior chamber, and spined, tubular anterior section, 55–107 (72) × 26–46 (36). Saccular posterior chamber roughly spherical, contains fibrous mass, 30–65 (41) × 23–46 (32). Tubular anterior section with small acicular spines, approximately same size as those in cirrus, roughly subcylindrical, 16–44 (27) × 9–20 (14). Eggs small, lightly tanned, operculate, unfilamented, 13–20 (17) × 6–11 (8).

Excretory vesicle small, saccular, reaches to level of posterior ends of caeca. Excretory pore terminal.

Sporocysts and cercariae

Host. Codakia paytenorum (Iredale), Payten's Lucina Clam (Lucinidae).

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

Site of infection. Gonad.

Prevalence. One of 383 (0.26%).

Deposition of specimens. Isogenophores (QM G239167–71).

Molecular sequence data. One replicate each of ITS2 rDNA (GB MZ271997), 28S rDNA (GB MZ272000) and *cox*1 mtDNA (GB MZ295278).

Description

Sporocysts. Based on ten specimens (fig. 1d). Elongate, 313–469 (381) × 62–88 (75), mostly contain germinal balls, some with one or two recognizable cercariae in various developmental stages.

Cercariae. Based on five relatively well-developed specimens (fig. 1d). Oculate distome cercaria. Body elongate, 216–340 (260) × 57–70 (61), 3.26–5.96 (4.30) times longer than wide. Tegumental spines not observed. Forebody 72–89 (82) long, occupies 25.6–35.2 (31.8)% of body length. Oral sucker terminal, roughly round, 26–39 (31) × 28–33 (31), 0.81–1.27 (1.01) times wider than long. Ventral sucker roughly spherical, in posterior third of body, 27–33 (30) × 26–33 (29), 0.79–1.22 (0.99) times wider than long; 0.85–1.04 (0.97) times length and 0.79–1.18 (0.96) times width of oral sucker. Two eye-spots at pharyngeal or oesophageal zone, recognizable from early in development. Penetration glands not observed. Prepharynx not observed. Pharynx small, 17–22 (20) × 13–18 (16). Oesophagus not observed. Caeca still in early stages of development, reach to near posterior extremity of body. Genital primordium at level of ventral sucker. Excretory bladder ovoid, large, 37–43 (41) × 31–36 (34), occupies 12.6–18.9 (16.1)% of body length. Flame cell pattern not determined. Tail long, unspecialized, 110–181 (136) × 14–22 (17), 0.42–0.61 (0.53) times length of body.

Remarks

The infection here was not patent, given all cercariae were under-developed. The cercaria has most typical monorchiid characters – distome, a pharynx, eye-spots, long tail and lacking a stylet, but we were unable to detect any tegumental spines. Notably, excysted cercariae were poorly developed or damaged beyond any practical use, and the intrasporocyst cercariae depicted in fig. 1d were the best specimens available to us.

Cercariae and sporocysts ex *Jactellina clathrata* (Deshayes)

Host. Jactellina clathrata (Deshayes) (Tellinidae).

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

Site of infection. Gonad.

Prevalence. One of 208 (0.48%).

Deposition of specimens. Isogenophores (QM G239172–78).

Molecular sequence data. One replicate each of ITS2 rDNA (GB MZ271996), 28S rDNA (GB MZ272001) and *cox*1 mtDNA (GB MZ295282).

Description

Sporocysts. Based on ten specimens (fig. 1e). Elongate, 907–1165 (1006) × 116–168 (149), contain 13–26 recognizable cercariae in various developmental stages and germinal balls.

Cercariae. Based on eight relatively well-developed specimens (fig. 1f). Oculate distome cercaria. Body elongate, 134–183 (165) × 57–80 (72), 1.89–2.93 (2.28) times longer than wide. Tegumental spines not observed. Forebody 75–112 (95) long, occupies 50.0–69.4 (57.5)% of body length. Oral sucker terminal, roughly round, 37–41 (39) × 33–41 (38), 0.89–1.05 (0.99) times wider than long. Ventral sucker roughly spherical, 32–41 (38) × 34–42 (38), 0.90–1.06 (1.00) times wider than long; 0.78–1.05 (0.98) times length and 0.87–1.07 (0.99) times width of oral sucker. Two eye-spots at pharyngeal or oesophageal zone, recognizable from early development. Penetration glands not observed. Prepharynx not observed. Pharynx small, 16–29 (20) × 14–22 (20). Oesophagus short. Caeca mostly still in early stages of development, reach posterior third of body. Genital primordium at level of ventral sucker. Excretory bladder ovoid, large, lined with conspicuous cells, reaches to dorsal to ventral sucker, 49–66 (58) × 31–41 (37), occupies 29.3–38.7 (35.2)% of body length. Flame cell pattern not determined. Tail long, unspecialized, 192–264 (230) × 17–22 (19), 1.05–1.57 (1.41) times length of body.

Remarks

The single infection may not have been patent, given that most cercariae were under-developed. This cercaria has most typical monorchiid characters – distome, a pharynx, eye-spots, long tail and lacking a stylet – but we were unable to detect any tegumental spines. Sequence data did not match any known monorchiid species with sequence information publicly available on GenBank.

Molecular phylogenetic analyses

BI (fig. 2) and ML analyses of the 28S rDNA dataset produced similar phylograms in which *G. queenslandensis* n. g., n. sp. resolved deep within a large clade predominantly comprising

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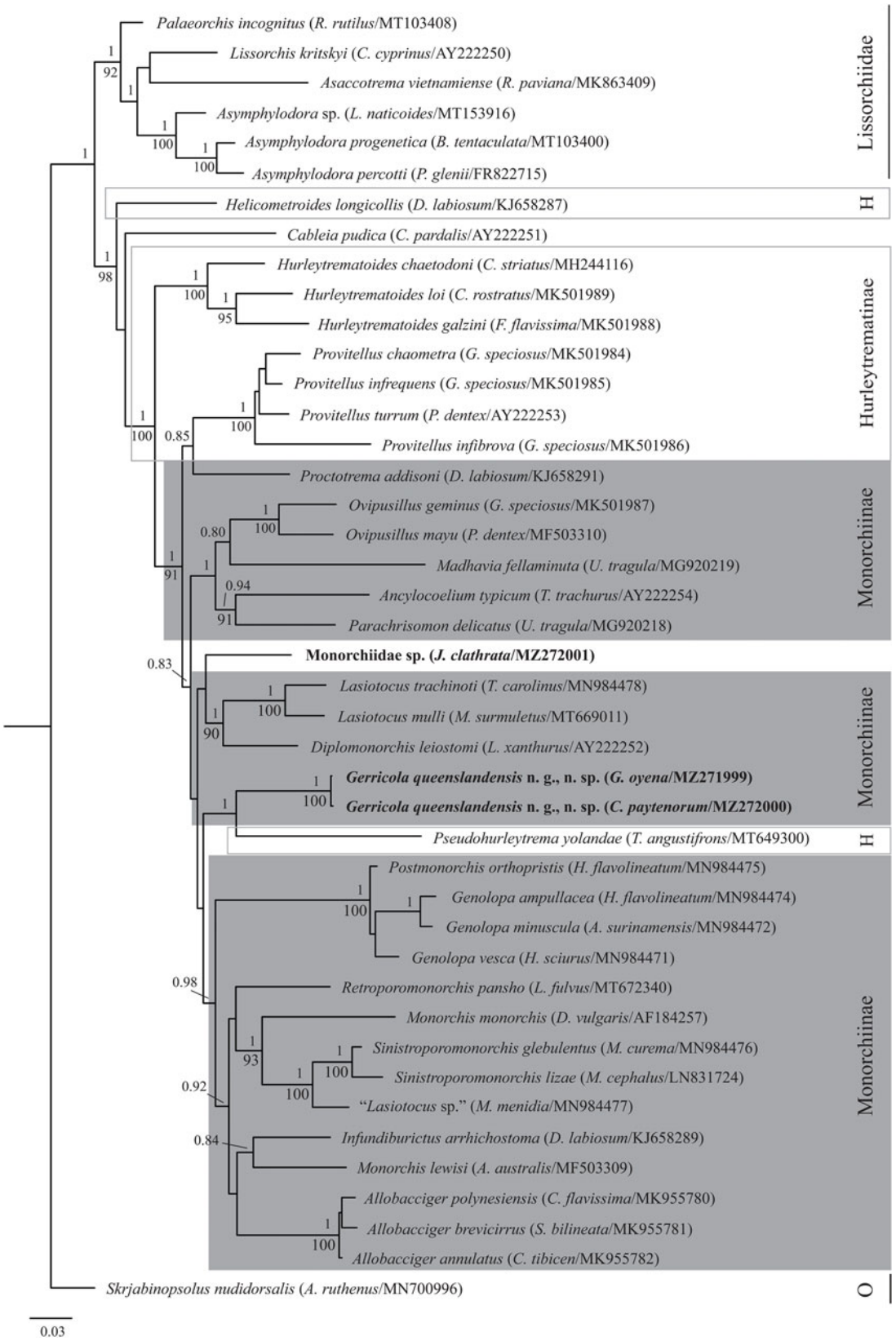

Fig. 2. Relationships of monorchiid taxa based on Bayesian inference analyses of 28S rDNA. Novel sequences for *Gerricola queenslandensis* n. g., n. sp. and the infection from *Jactellina clathrata* are indicated in bold. Host species and GenBank accession numbers are provided in parentheses. Monorchiid subfamilies are marked; the Hurleytrematinae is polyphyletic, the Monorchiinae is paraphyletic and *Cableia pudica* and the infection from *J. clathrata* are unassigned. Posterior probabilities are shown above the nodes, and where relationships were replicated in the maximum likelihood analysis, bootstrap values are shown below. Nodal support below 0.80/80 not shown. The scale bar represents the expected number of substitutions per site. Abbreviations: H, Hurleytrematinae; O, outgroup.

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monorchiine taxa. Within this large clade, however, the species formed a well-supported clade with the hurleytrematine species *Pseudohurleytrema yolandae* Wee, Crouch, Cutmore & Cribb, 2020, but with noticeably long branch lengths separating the two. This clade is sister to a clade comprising species of *Allobacciger* Hafeezullah & Siddiqi, 1970, *Genolopa* Linton, 1910, *Monorchis* Monticelli, 1893 and *Sinistroporomonorchis* Wee, Cutmore, Pérez-del-Olmo & Cribb, 2020, and *Infundiburictus arrhichostoma* (Searle, Cutmore & Cribb, 2014) Wee, Cutmore, Pérez-del-Olmo & Cribb, 2020, *P. orthopristis* Hopkins, 1941, *Retroporomonorchis pansho* Wee, Cribb, Cutmore & Martin, 2020 and '*Lasiotocus* sp.' The monorchiid from *J. clathrata* formed a poorly supported clade with *Diplomonorchis leiostomi* and two species of *Lasiotocus*, resolving sister to these recognized species. There were two differences with regards to the overall topology of the Monorchiidae between the two analyses, with the first involving the position of *R. pansho*. In the BI analysis, *R. pansho* resolves sister to a clade comprising *Monorchis monorchis* (Stossich, 1890) Looss, 1902, '*Lasiotocus* sp.' and species of *Sinistroporomonorchis*, whereas in the ML analysis, *R. pansho* resolves sister to a large clade comprising the same species as well as *I. arrhichostoma*, *Monorchis lewisi* Cribb, Wee, Bray & Cutmore, 2018 and species of *Allobacciger*; nodal support for its position in both analyses are poor. The second involves the positions of *Helicometroides longicollis* Yamaguti, 1934 and *Cableia pudica* Bray, Cribb & Barker, 1996, which were swapped in the two analyses, with the former the most basal monorchiid taxon in the BI analysis, and the latter the most basal in the ML analysis; the basal support of the derived taxon in both analyses were poor.

Discussion

Morphology and taxonomy of G. queenslandensis n. g., n. sp.

In the possession of a single testis, unfilamented eggs and a genital pore in the forebody, our specimens clearly belong to the subfamily Monorchiinae Odhner, 1911, as recognized by Madhavi (2008). According to the key to monorchiine genera of Wee *et al.* (2020c), the present species most closely conforms to the newly restricted concept of *Lasiotocus* Looss, 1907 in the possession of an unspined genital atrium, bipartite terminal organ, round oral sucker and unlobed ovary. However, as demonstrated by analysis of the 28S rDNA dataset, our new specimens are only distantly related to the two sequenced species of *Lasiotocus*, *Lasiotocus mulli* (Stossich, 1883) Odhner, 1911 (the type species) and *Lasiotocus trachinoti* Overstreet & Brown, 1970, and thus require a separate genus. *Gerricola queenslandensis* n. g., n. sp. is morphologically similar to these two *Lasiotocus* species, differing significantly only in the posterior extent of the caeca (terminating in the post-testicular region in *G. queenslandensis* n. g., n. sp. vs. at level of the testis in both *Lasiotocus* species) and spination of the terminal organ (no gap in terminal organ spines in *G. queenslandensis* n. g., n. sp. vs. distinct gap in terminal organ spines in both *Lasiotocus* species). The gap in terminal organ spines was not mentioned as an important taxonomic feature in the most recent revision of *Lasiotocus* (see Wee *et al.*, 2020c); however, Panyi (2020) speculated that the feature might be of generic level importance and our findings here support that view.

The remaining six species of *Lasiotocus* are morphologically similar to *G. queenslandensis* n. g., n. sp. in the possession of caeca that terminate in the post-testicular region, which might ultimately warrant their transfer to *Gerricola* n. g. However, in the recent revision of *Lasiotocus*, Wee *et al.* (2020c) cast doubt on the monophyly of these six species due to a combination of significant morphological variation, a lack of information on some features and their exploitation of fishes from a wide range of families. These issues will persist even if they are transferred to *Gerricola* n. g., and we think the conservative course of action is to leave these six species in *Lasiotocus* until molecular sequence data are available to better inform on their phylogenetic positions.

Phylogeny

Although *G. queenslandensis* n. g., n. sp. resolves deep within a large clade predominantly comprising other monorchiine taxa, it is surprising that it resolves sister to the hurleytrematine species *P. yolandae*, with strong nodal support. Although the position of *P. yolandae* within this large clade consisting primarily of monorchiine taxa was demonstrated by Wee *et al.* (2020b), the nodal support for its position at the time (sister to *Proctotrema addisoni* Searle, Cutmore & Cribb, 2014) was poor. Overall, the phylogenetic position of *P. yolandae* and species of *Provitellus* Dove & Cribb, 1998 relative to *G. queenslandensis* n. g., n. sp. and *P. addisoni*, respectively, in which a hurleytrematine taxon/taxa (*G. queenslandensis* n. g., n. sp. and *Provitellus* spp.) forms a clade with a monorchiine taxon, is evidence that both subfamilies require restructuring. However, given that so many genera are not yet represented by molecular sequence data, it remains premature to propose a major revision of either subfamilial structure.

Intramolluscan stages and their bivalve hosts

The sporocysts of both infections reported here are consistent with previously reported monorchiid sporocysts (Cremonte *et al.*, 2001; Gilardoni *et al.*, 2013; Bagnato *et al.*, 2016) in being unspecialized, immobile, thick-walled sacs containing cercariae of varying developmental stages. Cercariae from both infections exhibit typical monorchiid cercarial features – two suckers, a pharynx, eye-spots, a thick-walled excretory vesicle, a long, thin tail and lacking a stylet (Cable, 1956). However, as our specimens were under-developed, tegumental spines exhibited by other monorchiid cercariae were not observed in either species. As per the classification of monorchiid cercariae proposed by Cremonte *et al.* (2001), both new cercariae fall into Group 1, cercariae that possess eye-spots and a well-developed tail. The present infection in *C. paytenorum* is the second record of a monorchiid infection in a lucinid bivalve. The other record is of an unidentified monorchiid, *Cercaria caribbea* LXIV (and its sporocysts) from *Parvilucina pectinella* (C. B. Adams) from Jamaica (Cable, 1963), which also conforms to the concept of Group 1 cercariae. The unidentified infection from *J. clathrata* is the fourth record of monorchiids from tellinid bivalves, and the Tellinidae is the second most infected bivalve family, with more reports from only the Veneridae (five records). Two of the other three monorchiid infections from tellinids are unidentified, *Cercaria caribbea* XXXV Cable, 1956 infects *Macoma cerina* C.B. Adams from Puerto Rico (Cable, 1956), and *Cercaria caribbea* LXIII Cable, 1963 infects *Serratina martinicensis* (d'Orbigny) from Jamaica (Cable, 1963). The third, *Monorcheides cumingiae* (Martin, 1938) Martin, 1939, infects *Macoploma tenta* (Say) from the USA. These cercariae also conform to the concept of Group 1 cercariae.

Our study is the second report of bivalves as monorchiid intermediate hosts in Australia, following Bott *et al.* (2005). Bott *et al.*

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(2005) examined 47 bivalve species from 17 families off Queensland, including from off Heron Island. They reported monorchiids in five bivalve species, four from Heron Island: *C. paytenorum* (Lucinidae), *J. clathrata* (Tellinidae), *Scissulina dispar* (Conrad) (Tellinidae) and *Pinguitellina robusta* (Hanley) (Tellinidae). However, the study did not describe nor sequence the intramolluscan stages and the monorchiids were not formally identified. The infections in *C. paytenorum* and *J. clathrata* in the present study likely correspond to the same species reported by Bott *et al.* (2005).

Bivalves reported as the first intermediate hosts of monorchiids are relatively diverse, comprising 22 species from ten families from two superorders, the Anomalodesmata and Imparidentia, both of the infraclass Heteroconchia. Following the present study, these bivalves harbour 11 species of monorchiids matched to adults, as well as an additional 11 unidentified cercariae. Of the 11 matched monorchiids, only *G. queenslandensis* n. g., n. sp. is represented in our phylogenetic analysis. While there is molecular representation for four other genera that have had elucidated life cycles – *Lasiotocus*, *Monorchis*, *Postmonorchis* Hopkins, 1941 and *Proctotrema* Odhner, 1911 – the specific species for which the first intermediate host is known are not represented. It is noteworthy that the monophyly of some of these genera (particularly *Lasiotocus* and *Monorchis*) is doubtful; thus, it is uncertain if the species matched to adults will prove convincing congeners of the species in our phylogenetic analyses.

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Conflicts of interest. None.

Ethical standards. This study was conducted in compliance with all institutional, national and international guidelines on the care and use of animals.

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