Research Article

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Morphological and molecular data for three species of the Microphallidae (Trematoda: Digenea) in Australia, including the first descriptions of the cercariae of *Maritrema brevisacciferum* Shimazu et Pearson, 1991 and *Microphallus minutus* Johnston, 1948

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Abstract: Cercariae and metacercariae of three species of the Microphallidae Travassos, 1920 were found in snails and crustaceans from tributaries of the Brisbane River, Queensland, Australia. Specimens of *Maritrema brevisacciferum* Shimazu et Pearson, 1991 and *Microphallus minutus* Johnston, 1948, which have previously been reported in Queensland, were found as cercariae in the tateid gastropod *Posticobia brazieri* (Smith) and as metacercariae of *M. brevisacciferum* in the atyid shrimp *Caridina indistincta* Calman and of *M. minutus* in the parastacid crayfish *Cherax dispar* Reik. Combined analysis of morphological and molecular data, based on newly generated ITS2 and partial 28S rDNA data, linked cercariae and metacercariae for both species. This is the first report of the first intermediate hosts of *M. brevisacciferum* and *M. minutus*. Infections of another unidentified microphallid metacercariae, Microphallidae gen. sp., were found in *P. brazieri* and *C. indistincta*. The sequences of metacercarial isolates from both hosts were identical. The data on the Microphallidae from Australia and species that develop in freshwater invertebrates were examined in detail.

Keywords: larval stages, life cycle, morphology, rDNA sequences, freshwaters, Brisbane River

The Microphallidae Travassos, 1920 is a large and globally distributed family of digeneans that parasitise the intestine of most vertebrate classes (Deblock 2008), but have their greatest richness in birds and mammals. The life cycles of microphallids involve gastropods, mostly marine and brackish water, as first intermediate hosts and crustaceans as second intermediate hosts. Previous studies from Australia have reported 35 microphallid species from 12 genera (*Atriophallophorus* Deblock et Rosé, 1964; *Basantisia* Pande, 1938; *Endocotyle* Belopol'skaya, 1952; *Gynaecotyla* Yamaguti, 1939; *Levinseniella* Stiles et Hassall, 1901; *Maritrema* Nicoll, 1907; *Microphallus* Ward, 1901; *Mochliscotrema* Deblock et Pearson, 1986; *Pseudolevinseniella* Tsai, 1955; *Queenslandisia* Pearson et Deblock, 1987; *Rhyncostophallus* Deblock et Canaris, 1997; *Thulakiotrema* Deblock, Williams et Evans, 1990), mainly from birds, with few species known from mammals (Hickman 1955, Deblock and Pearson 1968, 1969, Mawson et al. 1986, Shimazu and Pearson 1991).

However, despite the numerous records of microphallids in their definitive hosts, there are few records of the larval stages from intermediate hosts from this region. Metacercariae of 12 species have been found in crustaceans; one of these also has been reported from the brackish water snail, *Coxiella badgerensis* Johnston (see Johnston 1948, Deblock and Pearson 1968, 1969, Smith 1974, 1983, Deblock et al. 1990, Shimazu and Pearson 1991, Deblock and Canaris 1996). The only study of microphallid cercariae in Australia was by Cannon (1978), who recorded cercariae of four unidentified species in a marine cerithiid gastropod, *Clypeomorus batillariaeformis* Habe et Kosuge (as *Cerithium moniliferum* Kiener) from the Great Barrier Reef. There are no previous reports in the literature of microphallid cercariae from freshwater snails in Australia. Recently, however, cercaria of *Maritrema poulini* Presswell, Blasco-Costa et Kostadinova, 2014 and metacercariae of *Microphallus* sp. 'livelyi' were described from a freshwater snail, *Potamopyrgus antipodarum*

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Table 1. Host data, geographical origin and GenBank accession numbers for digenean species used in phylogenetic analyses.

Species	Host	Country	GenBank No. 28S	Reference
<i>Maritrema</i> Nicoll, 1907				
<i>M. arenaria</i> Hadley et Castle, 1940	<i>Semibalanus balanoides</i> (Linnaeus)	Belfast Lough, Northern Ireland	AY220629	Tkach et al. (2003)
<i>M. brevisacciferum</i> Shimazu et Pearson, 1991	<i>Posticobia brazieri</i> (Smith)	Brisbane, Australia	KT355819	Present study
<i>M. brevisacciferum</i>	<i>Caridina indistincta</i> Calman	Brisbane, Australia	KT355818	Present study
<i>M. eroliae</i> Yamaguti, 1939	<i>Clypeomorus bifasciatus</i> (Sowerby)	Shuwaikh Bay, Kuwait	JF826247	Al-Kandari et al. (2011)
<i>M. heardi</i> (Kinsella et Deblock, 1994)	<i>Oryzomys palustris</i> (Harlan)	Florida, USA	AY220632	Tkach et al. (2003)
<i>M. neomi</i> Tkach, 1998	<i>Neomys anomalus</i> Cabrera	Zakarpatska Region, Ukraine	AF151927	Tkach et al. (2000)
<i>M. novaezealandense</i> Martorelli, Fredensborg, Mouritsen et Poulin, 2004	<i>Zeacumantus subcarinatus</i> (Sowerby)	Portobello Bay, Dunedin, New Zealand	KJ144178	Presswell et al. (2014)
<i>M. oocysta</i> (Lebour, 1907)	<i>Hydrobia ulvae</i> (Pennant)	Belfast Lough, Northern Ireland	AY220630	Olson et al. (2003)
<i>M. poulini</i> Presswell, Blasco-Costa et Kostadinova, 2014	<i>Paracorophium excavatum</i> (Thomson)	Lake Waiholo, Waiholo, New Zealand	KJ144177	Presswell et al. (2014)
<i>M. prosthetra</i> Deblock et Heard, 1970	<i>Oryzomys palustris</i>	Cedar Key, Florida, USA	AY220631	Tkach et al. (2003)
<i>M. subdolum</i> Jägerskiöld, 1909	<i>Hydrobia ulvae</i>	Kandalaksha Bay, White Sea, Russia	HM584135	Galaktionov et al. (2012)
<i>M. deblocki</i> Presswell, Blasco-Costa et Kostadinova, 2014	<i>Anas platyrhynchos</i> Linnaeus	Karitane Estuary, Otago, New Zealand	KJ144173	Presswell et al. (2014)
<i>Microphallus</i> Ward, 1901				
<i>M. abortivus</i> Deblock, 1974	<i>Hydrobia ulvae</i>	Belfast Lough, Northern Ireland	AY220626	Tkach et al. (2003)
<i>M. basodactylophallus</i> (Bridgman, 1969)	<i>Oryzomys palustris</i>	Cedar Key, Florida, USA	AY220628	Tkach et al. (2003)
<i>M. fusiformis</i> Reimer, 1963	<i>Hydrobia ulvae</i>	Belfast Lough, Northern Ireland	AY220633	Tkach et al. (2003)
<i>M. minutus</i> Johnston, 1948	<i>Posticobia brazieri</i>	Brisbane, Australia	KT355823	Present study
<i>M. minutus</i>	<i>Cherax dispar</i> Reik	Brisbane, Australia	KT355822	Present study
<i>M. primas</i> (Jägerskiöld, 1908)	<i>Hydrobia ulvae</i>	Belfast Lough, Northern Ireland	AY220627	Tkach et al. (2003)
<i>M. similis</i> (Jägerskiöld, 1900)	<i>Carcinus maenas</i> (Linnaeus)	Belfast Lough, Northern Ireland	AY220625	Tkach et al. (2003)
<i>M. triangulatus</i> Galaktionov, 1984	<i>Somateria mollissima</i> Bonaparte et Gray	Yamskaya Bay, Sea of Okhotsk, Russia	HM584139	Galaktionov et al. (2012)
<i>Microphallus</i> sp.	<i>Somateria mollissima</i>	Cape Taygonos, Sea of Okhotsk, Russia	HM584142	Galaktionov et al. (2012)
<i>Microphallus</i> sp.	<i>Austrolittorina cincta</i> (Quoy et Gaimard)	Weller's Rock, New Zealand	KJ868217	O'Dwyer et al. (2014)
Microphallidae gen. sp.	<i>Posticobia brazieri</i>	Brisbane, Australia	KT355820	Present study
Microphallidae gen. sp.	<i>Caridina indistincta</i>	Brisbane, Australia	KT355821	Present study
Outgroup				
<i>Paraleithodendrium chilostomum</i> (Mehlis, 1831)	<i>Viviparus viviparus</i> (Linnaeus)	Kyiv, Ukraine	KJ126725	Kudlai et al. (2015)

(Gray), in New Zealand (Hechinger 2012, Presswell et al. 2014).

During a study of parasites infecting freshwater invertebrates of the Brisbane River and its tributaries, specimens of the tateid gastropod *Posticobia brazieri* (Smith) and the crustaceans *Caridina indistincta* Calman and *Cherax dispar* Reik were found to be naturally infected with the larval stages of species belonging to the family Microphallidae. Detailed morphological study and comparative analyses based on newly-obtained sequences of the nuclear ribosomal ITS2 region and partial 28S rDNA gene revealed the presence of three species, identified as *Maritrema brevisacciferum* Shimazu et Pearson, 1991, *Microphallus minutus* Johnston, 1948 and *Microphallidae* gen. sp. Metacercariae of *M. brevisacciferum* and *M. minutus* have previously been recorded in prawns *Caridina nilotica* (Roux) and crayfish *C. dispar*, respectively, from the Brisbane River (Shimazu and Pearson 1991), but the first intermediate host of neither species has been reported. This paper provides morphological descriptions and genetic characterisation of the larval stages of the three microphallid species discovered from the Brisbane River and its tributaries.

MATERIALS AND METHODS

Sample collection

Sampling of snails and crustaceans was carried out in February 2015 at three sites on tributaries of the Brisbane River: Moggill Creek (27°30'16"S; 152°55'50"E), Churchbank Weir (27°46'16"S; 152°41'02"E) and Fairnie Brook (27°28'58"S; 152°40'04"E). A total of 4480 *Posticobia brazieri* were examined. Additionally, 38 *Caridina indistincta*, 34 *Macrobrachium tolmerum* Reik and one *Cherax dispar* were examined from Moggill Creek and Churchbank Weir.

Morphological data

In the laboratory, snails were placed individually in plastic containers with fresh pond water and examined daily for the presence of naturally emerged cercariae. Infected gastropods were subsequently dissected to determine the nature of the intramolluscan stages. Cercariae were examined alive unstained and then following staining with neutral red, under slight coverslip pressure. Photomicrographs of live isolates (cercariae and metacercariae) were taken with a Nikon Digital Sight DS-LI digital camera (Nikon Corporation, Tokyo, Japan) mounted on an Olympus BH-2 microscope. Measurements were taken from pictures of live material

with SPOTTM imaging software. All measurements are in micrometres and are given as a range followed by the mean and the number of measurements taken (n) in parentheses. Drawings are based on digital images of live cercariae and metacercariae.

Molecular data

Total genomic DNA was isolated using phenol/chloroform extraction techniques (Sambrook and Russell 2001). The partial D1–D3 fragment of the 28S nuclear ribosomal DNA region was amplified using the primers LSU5 (5'-TAGGTCGACCCGCTGAAYTTAAGC-3'; Littlewood et al. 2000) and 1500R (5'-GC-TATCCTGAGGGAACTTCG-3'; Snyder and Tkach 2001) and the ITS2 region was amplified using the primers 3S (5'-GGTACCGGTGGATCACGTGGCTAGTG-3'; Bowles et al. 1993) and ITS2.2 (5'-CCTGGTTAGTTTCTTTCTCCGC-3'; Cribb et al. 1998). PCR for both the 28S and ITS2 regions was performed with a total volume of 20 µl consisting of 5 µl of 5× MyTaq Reaction Buffer (Bioline), 0.75 µl of each primer (10 pmols), 0.25 µl of Taq polymerase (Bioline MyTaqTM DNA Polymerase), 2 µl of DNA template (approximately 10 ng), made up to 20 µl with InvitrogenTM ultraPURETM distilled water. Amplification was carried out on a MJ Research PTC-150 thermocycler.

The following profile was used to amplify the 28S region: an initial 95 °C denaturation for 4 min, followed by 30 cycles of 95 °C denaturation for 1 min, 56 °C annealing for 1 min, 72 °C extension for 2 min, followed by a single cycle of 95 °C denaturation for 1 min, 55 °C annealing for 45 s and a final 72 °C extension for 4 min. The following profile was used to amplify the ITS2 region: an initial single cycle of 95 °C denaturation for 3 min, 45 °C annealing for 2 min, 72 °C extension for 90 s, followed by 4 cycles of 95 °C denaturation for 45 s, 50 °C annealing for 45 s, 72 °C extension for 90 s, followed by 30 cycles of 95 °C denaturation for 20 s, 52 °C annealing for 20 s, 72 °C extension for 90 s, followed by a final 72 °C extension for 5 min.

Amplified DNA was purified using a Bioline ISOLATE II PCR and Gel Kit according to the manufacturer's protocol. Cycle sequencing of purified DNA was carried out using ABI Big DyeTM v.3.1 chemistry following the manufacturer's recommendations, using the same primers used for PCR amplification as well as the additional 28S rDNA primers 300F (5'-CAAGTACCGTGAGGGAAAGTT-3'; Littlewood et al. 2000) and ECD2 (5'-CTT GGTCCGTGTTTCAAGACGGG-3'; Littlewood et al. 2000), and the additional ITS2 primer GA1 (5'-AGAACATCGA-CATCTTGAAC-3'; Anderson and Barker 1998). Cycle sequencing was carried out at the Australian Genome Research Facility using an AB3730xl capillary sequencer. SequencherTM version 4.5 (GeneCodes Corp.) was used to assemble and edit contiguous sequences. GenBank accession numbers for taxa sequenced in this study are shown in Table 1.

Newly generated 28S rDNA data from larval stages of *Maritrema brevisacciferum*, *Microphallus minutus* and Microphallidae gen. sp., together with sequences of other microphallids available on GenBank, were used in phylogenetic analyses (Table 1). A sequence of the lecitodendriid *Paralecithodendrium chilostomum* (Mehlis, 1831) (KJ126725) was used as an outgroup based on the topologies in the phylogenetic trees of the Digenea published by Olson et al. (2003).

Sequences were aligned using ClustalW as implemented in the BioEdit program, version 7.0.1 (Hall 1999). The alignment

was then trimmed to the length of the shortest sequence. Phylogenetic analysis was carried out using Bayesian inference (BI) and Maximum Likelihood (ML) analyses. The nucleotide substitution model, the general time reversible model, with estimates of invariant sites and gamma distributed among-site rate variation (GTR + I + G) was determined using jModelTest 2 software (Darriba et al. 2012). BI analysis was performed using MrBayes software (ver. 3.2.3) (Ronquist et al. 2012) run on the CIPRES portal (Miller et al. 2010) with the following nucleotide substitution model settings: lset nst = 6, rates = invgamma, samplefreq = 100, ncat = 4, shape = estimate, inferrates = yes and basefreq = empirical. Markov chain Monte Carlo (MCMC) chains were run for 10 000 000 generations, log-likelihood scores were plotted and only the final 75% of trees were used to produce the consensus trees by setting the 'burn-in' parameter at 2 500. This number of generations was considered sufficient because the standard deviation dropped well below 0.01 at the end of the run. ML analysis was performed using PhyML version 3.0 (Guindon et al. 2010) run online on the ATGC bioinformatics platform, Next generation Sequencing [http://www.atgc-montpellier.fr/ngs]. Nodal support in the Maximum Likelihood analysis was estimated by performing 100 bootstrap pseudoreplicates. Trees were visualised using the FigTree ver. 1.4 software (Rambaut 2012).

RESULTS

Examination of 4 480 individuals of *Posticobia brazieri* from the Brisbane River tributaries at three localities in Queensland revealed infections with digeneans from eight families. Nine infections consistent with species of the family Microphallidae were identified based on naturally emerged cercariae. Metacercariae of *Maritrema brevisacciferum* and Microphallidae gen. sp. were found in *Caridina indistincta*; metacercariae of Microphallidae gen. sp. were also found in huge numbers in the tissues of a single *P. brazieri*. Metacercariae of *Microphallus minutus* were recovered from *Cherax dispar*. No specimen of *Macrobrachium tolmerum* was infected by digenean metacercariae. Descriptions of the mature cercariae and metacercariae of these digeneans are provided below.

Family Microphallidae Travassos, 1920

Genus *Maritrema* Nicoll, 1907

Maritrema brevisacciferum Shimazu et Pearson, 1991 Fig. 1

Description of cercaria (based on 20 live naturally emerged specimens from *P. brazieri* from Moggill Creek): Small monostome xiphidiocercariae developing in elongate sporocysts. Body elongate-oval, 82–119 (98; n = 19) long, with maximum width at midbody 38–59 (48). Tegument covered with minute spines. Tail simple, 69–107 (98; n = 13) long, 12–15 (13; n = 15) wide at base, slightly shorter than body. Oral sucker subterminal, spherical, muscular, 17–25 × 18–25 (21 × 22). Stylet straight, lanceolate, slightly wider at base, 14–18 × 2.5–3 (16 × 2.9) with lateral pointed thickenings at first third of length. Digestive system not developed. Penetration gland-cells in two pairs,

Fig. 1. *Maritrema brevisacciferum* Shimazu et Pearson, 1991. **A** – cercaria from *Posticobia brazieri* (Smith), ventral view; **B** – stylet, ventral and lateral view; **C** – metacercaria from *Cardina indistincta* Calman.

filled with secretory material in form of small granules, located in third quarter of body, at 52–90 (66; $n = 18$) from anterior extremity of body. Posterior pairs of penetration gland-cells not observed. Ducts open ventrolaterally to stylet. Genital primordium an oval mass of cells in posterior third of body. Excretory vesicle Y-shaped. Flame-cell formula $2[(2 + 2) + (2 + 2)] = 16$.

Description of metacercaria (based on 28 live encysted specimens from Moggill Creek – 18 and Churchbank Weir – 10): Metacercaria in elongate-oval cyst, 161–215 $\times$ 113–141 (186 $\times$ 126). Cyst wall consisting of two hyaline layers; third, external layer also present, probably formed by encapsulation process induced by host haematocytes. Entire tegument covered with dense spines. Oral sucker subterminal; ventral sucker, 33–36 $\times$ 35–38 (35 $\times$ 37; $n = 6$); oesophagus long; caeca short, reaching to ventral sucker. Reproductive system well-developed. Testes two, lateral, oval, symmetrical; left testis 30–35 $\times$ 29–31 (33 $\times$ 30; $n = 4$), right testis 31–39 $\times$ 28–32 ($n = 2$). Cirrus-sac long, 93–106 (99; 6), occupying almost entire body width, maximum width at base 16–28 (24; $n = 6$), with thick muscular wall located between intestinal caeca and ventral sucker, enclosing seminal vesicle and ejaculatory duct. Seminal vesicle elongate, occupies one third of length of cirrus-sac. Ejaculatory duct long, convoluted. Ovary oval, 26–30 $\times$ 21–27 (28 $\times$ 24; $n = 4$). Vitellarium in hindbody, comprised of few small follicles forming two symmetrical

ribbons encircling testes; ribbons converge anteriorly to testes, interrupted posteriorly. Excretory vesicle Y-shaped; arms reach to mid-level of testes; pore terminal.

First intermediate host: *Posticobia brazieri* (Smith) (Gastropoda: Tateidae).

Locality: Moggill Creek and Fairnie Brook, Queensland, Australia.

Prevalence of emergence: 1 of 2160 (Moggill Creek), 7 of 2080 (Fairnie Brook).

Second intermediate host: *Cardina indistincta* Calman (Decapoda: Atyidae).

Locality: Moggill Creek and Churchbank Weir, Queensland, Australia.

Prevalence: 71% (Moggill Creek), 42% (Churchbank Weir).

Representative sequences: Cercaria KT355819 (28S rDNA), KT355825 (ITS2), metacercaria KT355818 (28S rDNA), KT355824 (ITS2).

Remarks. Specimens of the metacercaria described above possess a cirrus-sac enclosing the cirrus, seminal vesicle and ejaculatory duct, which is characteristic of the Maritreminae Nicoll, 1907 (see Deblock 2008). This subfamily is represented in Australia by nine species of the genus *Maritrema*; three of these have been recorded in second intermediate hosts (Table 2).

A comparison with the known metacercariae from fresh waters in Australia shows striking similarities between the

Table 2. Representatives of the subfamilies Maritreminae Nicoll, 1907 and Microphallinae Ward, 1901 and data on their hosts and distribution in Australia.

Species	Second intermediate host	Definitive host	Region	Source
Subfamily Maritreminae Nicoll, 1907				
<i>Maritrema</i> Nicoll, 1907				
<i>M. eroliae</i> Yamaguti, 1939	<i>Paragrapsus gainardii</i> (Milne Edwards)	<i>Charadrius mongolus</i> Pallas; <i>Thalasseus bergii</i> (Lichtenstein); <i>Arenaria interpres</i> (Linnaeus); <i>Charadrius ruficapillus</i> Temminck	Queensland; Tasmania	Smith (1983); Deblock and Canaris (1996)
<i>M. oocysta</i> (Lebour, 1907)	-	<i>Hydromys chrysogaster</i> Geoffroy; <i>Tachybaptus novaehollandiae</i> (Stephens); <i>T. ruficollis</i> (Pallas); <i>Anhinga melanogaster</i> Pennant; <i>Egretta novaehollandiae</i> (Latham)	Queensland	Mawson et al. (1986)
<i>Maritrema</i> sp.	-	<i>Charadrius leschenaultii</i> Lesson; <i>Tringa hypoleucos</i> (Linnaeus); <i>Limosa lapponica</i> (Linnaeus); <i>Thalasseus bergii</i>	Queensland	Mawson et al. (1986)
<i>Maritrema</i> sp.	-	<i>Calidris acuminata</i> (Horsfield)	Queensland	Deblock and Canaris (1996)
<i>M. brevisaciferum</i> Shimazu et Pearson, 1991	<i>Caridina nilotica</i> (Roux)	Chickens (exp.)	Queensland	Shimazu and Pearson (1991)
<i>M. spinosulum</i> Deblock et Canaris, 1996	-	<i>Charadrius ruficapillus</i>	King Island; Tasmania	Deblock and Canaris (1996)
<i>M. rubeum</i> Deblock et Canaris, 1996	-	<i>Charadrius mongolus</i> ; <i>Calidris acuminata</i>	Queensland	Deblock and Canaris (1996)
<i>M. ornithorhynchi</i> Hickman, 1955	-	<i>Ornithorhynchus anatinus</i> Blumenbach	Tasmania	Hickman (1955)
<i>M. caberensis</i> Smith, 1974	<i>Coxiella badgerensis</i> Johnston, <i>Austrochiltonia australis</i> (Sayce)	<i>Anas castanea</i> (Eyton); <i>Thinornis cucullatus</i> (Vieillot); <i>Elseyaornis melanops</i> (Vieillot)	Tasmania	Smith (1974)
Subfamily Microphallinae Ward, 1901				
<i>Rhyncostophallus</i> Deblock et Canaris, 1997				
<i>R. insularegii</i> Deblock et Canaris, 1997	-	<i>Charadrius ruficapillus</i>	King Island; Tasmania	Deblock and Canaris (1997)
<i>Microphallus</i> Ward, 1901				
<i>M. papillormatus</i> Deblock et Pearson, 1969	-	<i>Pluvialis dominica</i> (Statius Muller); <i>Charadrius mongolus</i> ; <i>Chroicocephalus novaehollandiae</i> (Stephens)	South Australia; Deblock and Pearson (1969)	Deblock and Pearson (1969)
<i>M. vaginosus</i> Deblock et Pearson, 1969	-	<i>Pluvialis dominica</i>	Queensland	Deblock and Pearson (1969)
<i>Microphallus</i> sp.	-	<i>Charadrius mongolus</i>	Queensland	Deblock and Pearson (1969)
<i>M. minus</i> Ochi, 1928	<i>Metapenaeus bennettiae</i> Racek et Dall	<i>Hydromys chrysogaster</i>	Queensland	Johnston (1948); Deblock and Pearson (1969); Shimazu and Pearson (1991)
<i>M. minutus</i> Johnston, 1948	<i>Cherax dispar</i> Reik	<i>Arenaria interpres</i>	King Island; Tasmania	Deblock and Canaris (1997)
<i>M. pearsoni</i> Deblock et Canaris, 1997	-	<i>Anas platyrhynchos</i> (Linnaeus) (exp.)	Brunei Island; Tasmania	Smith (1983)
<i>M. paragrapsi</i> Smith, 1983	<i>Paragrapsus gainardii</i>		Tasmania	Smith (1974)
<i>Atriophallophorus</i> Deblock et Rose, 1964				
<i>A. coxiellae</i> Smith, 1974	<i>Coxiella badgerensis</i>	<i>Poliococephalus poliocephalus</i> (Jardine et Selby); <i>Fulica atra</i> (Linnaeus); <i>Charadrius rubricollis</i> (Vieillot); <i>C. ruficapillus</i>	Queensland	Deblock and Pearson (1968)
<i>Endocotyle</i> Belopol'skaya, 1952	-	<i>Charadrius mongolus</i> ; <i>Calidris acuminata</i>		
<i>E. incana</i> Belopol'skaya, 1952	-			

Table 3. Comparative metrical data for metacercariae of *Maritrema brevisacciferum* Shimazu et Pearson, 1991 and *Microphallus minutus* Johnston, 1948. Measurements are in micrometres.

Species	<i>Maritrema brevisacciferum</i>		<i>Maritrema brevisacciferum</i>	<i>Microphallus minutus</i>		<i>Microphallus minutus</i>
Host	<i>Caridina indistincta</i> Calman		<i>Caridina nilotica</i> (Roux)	<i>Cherax dispar</i> Reik		<i>Cherax dispar</i>
Source	Present study		Shimazu and Pearson (1991)	Present study		Shimazu and Pearson (1991)
	Range	Mean	Range	Range	Mean	Range
Cyst length	161–215	186	150–230	238–320	297	310–540
Cyst width	113–141	126	120–140	235–297	278	290–540
Body length	-	-	232–288	-	-	376–408*
Body width	-	-	144–168	-	-	256–312*
Oral sucker length	-	-	24–27	50	-	32–40*
Oral sucker width	-	-	30–32	55	-	51–56*
Pharynx length	-	-	17–19	18	-	22–24*
Pharynx width	-	-	11–13	15	-	16–17*
Oesophagus length	-	-	19–29	-	-	48–80*
Ventral sucker length	33–36	35	33–40	44	-	-
Ventral sucker width	35–38	37	40–43	55	-	51–56*
Right testis length	30–35	-	33–40	77–85	82	72–96*
Right testis width	29–31	-	32–36	49–60	55	40–64*
Left testis length	31–39	33	33–40	80–83	81	72–96*
Left testis width	28–32	30	32–36	58–60	59	40–64*
Cirrus-sac length	93–106	99	96–104	-	-	45–76**
Cirrus-sac width	21–27	24	24–29	-	-	40**
Ovary length	26–30	28	32	61–70	64	56–72*
Ovary width	21–27	24	29–40	38–45	41	48–80*

* measurements for two-day-old adults; ** measurements of the phallus.

present metacercaria and that of *M. brevisacciferum* described by Shimazu and Pearson (1991) from *C. nilotica* in the Brisbane River with both possessing the characteristic oval shape and size of cyst, the presence of a long cirrus-sac with a thick muscular wall, long ejaculatory duct and both parasitising freshwater prawns, *Caridina* spp. The metrical data for the present metacercariae generally fall within the range for *M. brevisacciferum*, but include slightly smaller measurements for testes and ovary, and smaller lower limits for the width of cyst, width of the ventral sucker and length and width of the cirrus-sac (see Table 3).

Comparative sequence analysis of cercarial isolates from the snail *P. brazieri* and metacercarial isolates from *C. indistincta* revealed identical sequences for each of the 28S and ITS2 rDNA regions (Fig. 4). This result allowed us to assign this cercaria to *M. brevisacciferum*. This species was described from experimentally infected chickens (Shimazu and Pearson 1991) and has not yet been recorded from a natural definitive host. This is the first report of the first intermediate hosts of *M. brevisacciferum* and the first description of its cercaria.

Genus *Microphallus* Ward, 1901

Microphallus minutus Johnston, 1948

Fig. 2

Description of cercaria (based on 18 live naturally emerged specimens): Small monostome xiphidiocercariae developing in elongate sporocysts. Body elongate-oval, 87–122 (101) long, with maximum width at level of oral sucker, 40–66 (53). Tegument covered with minute spines. Tail simple, 60–96 (84) long, 10–13 (11) wide at base, shorter than body. Oral sucker subterminal, muscular, sub-spherical, 23–30 × 20–31 (26 × 27; n = 15). Stylet straight,

sharply-pointed, 16–17 × 3–4 (16 × 3.2; n = 16), slightly wider at level of lateral pointed thickenings at about mid-length. Digestive system not developed. Penetration gland-cells in four pairs: two anterior pairs filled with secretory material in form of large granules, extend to slightly posterior to oral sucker; two posterior pairs equatorial, filled with secretory material in form of small granules; gland ducts extend around oral sucker and open ventrolaterally to stylet. Genital primordium an oval mass of cells in posterior third of body. Excretory vesicle V-shaped. Other details of excretory system not observed; flame-cell formula not determined.

Description of metacercaria (based on 13 live encysted specimens): Metacercaria in subspherical thin-walled cyst, 238–320 × 235–297 (297 × 278). Entire tegument covered with dense spines. Oral sucker subterminal, 50 × 55; ventral sucker 44 × 55; prepharynx short; pharynx 18 × 15; oesophagus long; caeca short. Reproductive system well-developed. Testes lateral, oval, symmetrical; left testis 77–85 × 49–60 (82 × 55; n = 4); right testis 80–83 × 58–60 (81 × 59; n = 3). Cirrus-sac absent. Seminal vesicle inter-caecal in mid-body. Phallus large, muscular, sucker-like, spherical, with polygonal ejaculatory duct and muscular walls, sinistral to ventral sucker. Ovary dextral, transversely oval, 38–45 × 61–70 (41 × 64; n = 3). Vitellarium in two symmetrical clusters of few large follicles, at testicular level in hindbody. Excretory vesicle V-shaped, filled with large granules.

First intermediate host: *Posticobia brazieri* (Smith) (Gastropoda: Tateidae).

Locality: Moggill Creek, Queensland, Australia.

Prevalence of emergence: 1 of 2160.

Second intermediate host: *Cherax dispar* Reik (De-

Fig. 2. *Microphallus minutus* Johnston, 1948. **A** – cercaria from *Posticobia brazieri* (Smith), ventral view; **B** – stylet, ventral and lateral view; **C** – metacercaria from *Cherax dispar* Reik.

capoda: Parastacidae).

Locality: Moggill Creek, Queensland, Australia.

Prevalence: 1 of 1.

Representative sequences: Cercaria KT355823 (28S rDNA), KT355829 (ITS2), metacercaria KT355822 (28S rDNA), KT355828 (ITS2).

Remarks. The metacercariae from our collection exhibit features typical for the Microphallinae Ward, 1901, in particular the absence of a cirrus-sac and the seminal vesicle and pars prostatica lying free in the parenchyma (Deblock 2008).

Seven species of *Microphallus* have been reported in Australia and three of them are known from second intermediate hosts (Table 2). The present metacercaria is strongly consistent with the morphological description of *M. minutus* by Shimazu and Pearson (1991) from *C. dispar* in the shape and structure of the cyst, whereby the outer layer in young metacercariae is very thick. Although no metrical data for the metacercariae are available, we compared the encysted metacercariae with a well-developed reproductive system with the metrical data given for adults of *M. minutus* by Shimazu and Pearson (1991) (see Table 3). Comparisons revealed that the metrical data of our description fall within the range for *M. minutus*, but our material exhibits slightly lower limits for the size of the pharynx ($18 \times 15 \mu\text{m}$ vs $22\text{--}24 \times 16\text{--}17 \mu\text{m}$) and the width of the ovary ($38\text{--}45 \mu\text{m}$ vs $48\text{--}80 \mu\text{m}$). Since the thick outer layer of the cyst was included in measurements by Shimazu and Pearson (1991), the size of the cyst differs from our data in distinctly higher upper limits ($238\text{--}320 \mu\text{m} \times 235\text{--}297 \mu\text{m}$ vs $310\text{--}540 \mu\text{m} \times 290\text{--}540 \mu\text{m}$).

Fig. 3. Microphallidae gen. sp., metacercaria from *Posticobia brazieri* (Smith).

The species was described based on material from *Hydromys chrysogaster* Geoffroy in the Murray River (South Australia) by Johnston (1948) who suggested *Cherax destructor* Clark as potential second intermediate host of *M. minutus*. Deblock and Pearson (1969) reported *M. minutus* from *H. chrysogaster* in Moggill and Palmwood, Queensland, Australia. Shimazu and Pearson (1991) found metacercariae of this species in *C. dispar* from the Brisbane River (Queensland), confirming the suggestion of Johnston (1948). Notably, *P. brazieri* has been reported widely in eastern Australia (Clark 2009), including from

Fig. 4. Phylogenetic relationships among the taxa of the Microphallidae resulting from Bayesian inference (BI) and Maximum likelihood (ML) analyses based on partial 28S rDNA data. Nodal support associated with the branches are listed as BI posterior probability/ML bootstrap support; support values lower than 0.90 (BI) and 70 (ML) are not shown. The scale-bar indicates the expected number of substitutions per site. Sequences obtained in the present study indicated by bold font. *Abbreviations:* C – cercaria; M – metacercaria.

the type locality of *M. minutus*, Tailem Bend on the Murray River in South Australia; presumably it is also involved in transmission there.

The sequences of cercarial isolates from the snail *P. brazieri* and metacercarial isolates from *C. dispar* were identical for each of the 28S and ITS2 rDNA regions. We, therefore, consider that they belong to the same species. This is the first report and description of the cercaria of *M. minutus*.

Microphallidae gen. sp.

Fig. 3

Description of metacercaria (preliminary description based on 24 live encysted specimens from *P. brazieri* from Fairnie Brook – 19 and *C. indistincta* from Churchbank Weir – 5): Metacercaria in subspherical cyst, 115–129 × 105–123 (123 × 114). Cyst wall consisting of two hyaline layers. Entire tegument covered with dense spines. Oral sucker subterminal; ventral sucker large, muscular. Reproductive system well-developed. Testes lateral, oval, symmetrical. Structure resembling phallus large, almost equal to ventral sucker in size, sinistral to ventral sucker. Vitellarium in two symmetrical clusters of few large follicles, post-testicular in hindbody. Excretory vesicle Y-shaped.

Second intermediate host: *Posticobia brazieri* (Smith) (Gastropoda, Tateidae), *Caridina indistincta* Calman

(Decapoda, Atyidae).

Locality: Fairnie Brook and Churchbank Weir, Queensland, Australia.

Prevalence: *C. indistincta* 8% (Churchbank Weir).

Representative sequences: KT355820, KT355821 (28S rDNA), KT355826, KT355827 (ITS2).

Remarks. Further study on the morphology of this species is required. This species may well be closely related to, or even conspecific with, *Microphallus* sp. ('livelyi') of Hechinger (2012) from *Potamopyrgus antipodarum* from New Zealand, which also develops directly to metacercariae in the tissues of the snail. Abbreviation of microphallid life cycles by formation of metacercariae in the first intermediate host is common (see Galaktionov and Skirnisson 2007). However, in addition to such development in the present case, our recording of genetically and morphologically identical metacercariae from atyid shrimps shows that the present form has two potential pathways of transmission. We are unaware of previous reports of such a dichotomy.

Phylogenetic analysis

The specimens of *M. brevisacciferum*, *M. minutus* and *Microphallidae* gen. sp. showed no intraspecific sequence variability for either the 28S or ITS2 rDNA regions. The alignment used in the phylogenetic analysis included the

newly-generated partial 28S rDNA sequences for the three species obtained during this study together with sequences for 18 representatives of the Microphallidae available on GenBank and the lecithodendriid outgroup *Paralecithodendrium chilostomum* (Table 1). The final alignment was 1166 bp long including several introduced gaps. BI and ML analyses produced fully resolved phylogenetic trees comprising three major clades with strong support (Fig. 4). The first clade included nine species of the genus *Microphallus*. Sequences for *M. minutus* generated during this study clustered with strong support together with other species of *Microphallus*. The second strongly supported clade included 12 species of *Maritrema* with which the new sequences for *M. brevisacciferum* formed a monophyletic lineage associated with strong support with sequences for *Maritrema* spp. from a freshwater snail *P. antipodarum* and a bird *Anas platyrhynchos* (Linnaeus), described from New Zealand (Presswell et al. 2014).

Our phylogenetic analyses clearly indicated that Microphallidae gen. sp. forms a third distinct clade, together with a species identified as *Microphallus fusiformis* Reimer, 1963, sister to, or even a member of *Maritrema* (see below). Obtaining rDNA sequences from additional representatives of the Microphallidae would be essential for clarifying its generic affiliation.

DISCUSSION

Using combined morphological and molecular characteristics, we were able to establish links between cercariae and metacercariae of *Maritrema brevisacciferum* and *Microphallus minutus* for which complete life cycles were previously unknown; we provide the first descriptions of their cercariae. *M. minutus* has a three-host life cycle with cercariae developing in the tateid snail *Posticobia brazieri* and metacercariae encysting in parastacid crayfish, *Cherax dispar*. Adults develop in mammals, water rats (*Hydromys chrysogaster*; natural host) and mice (experimental host). Cercariae of *M. brevisacciferum* also develop in *P. brazieri* and metacercariae develop in atyid shrimps, *Caridina indistincta*; the natural definitive host is unknown. Microphallids frequently lack strict specificity, with final hosts being dependent on trophic habits of predators for crustacean or, occasionally, molluscan second intermediate hosts (Deblock 2008); thus, the natural host of this species is difficult to predict. For both species the morphology of the cercariae does not differ significantly from that of other congeners.

The new sequences of the metacercariae of Microphallidae gen. sp. formed a well-supported clade within the Microphallidae, but not with species of *Microphallus* and *Maritrema*. The position of this species as a sister group to the *Maritrema* clade is not unexpected from the viewpoint of morphology. Microphallidae gen. sp. differs from species of *Maritrema* in the absence of cirrus-sac and in having the vitellarium in two post-testicular clusters of follicles in the hindbody. Based on phylogenetic analysis, this species appeared closely related to *Microphallus fusiformis*, but the identification of the material sequenced as *M. fusiformis* is unclear. The annotation in GenBank (Tk-

ach et al. 2003) indicates ‘chick eggs’ as a ‘laboratory host’ and the United Kingdom as the country of provenance for the isolate. However, the sequence has been published as Microphallidae gen. sp. from *Hydrobia ulvae* (Pennant) in Northern Ireland by Tkach et al. (2003) and as *M. fusiformis* with the same provenance by Olson et al. (2003) (see table 1 in both papers). The separation of this species from the remaining species of *Microphallus* in its own genus-level group demonstrated in our phylogenetic analyses has been reported previously (Olson et al. 2003, Tkach et al. 2003, Presswell et al. 2014). Therefore, we prefer to consider this result with caution until the re-identification of the material sequenced by Tkach et al. (2003) and Olson et al. (2003).

Life cycles of microphallids take place in brackish, marine or fresh waters, in decreasing order of frequency (Deblock 2008). The vast majority of known first intermediate hosts are littoral gastropods of the genera *Hydrobia* Hartmann and *Littorina* Férussac (see Belopol'skaya 1963, Deblock 1980, Galaktionov and Bustnes 1999, Granovich et al. 2000, Galaktionov et al. 2012). At least 31 microphallid species are currently known to develop in fresh water. They are mainly reported from second intermediate hosts and described from experimentally obtained adults (Table 4). In our analyses, three species of *Maritrema* that develop in freshwater invertebrates (*Maritrema neomi* Tkach 1998, *M. brevisacciferum* and *Maritrema poulini*), clustered in a strongly supported subclade nested among species developing in marine invertebrates; the life cycle of the fourth species in the subclade, *Maritrema deblocki* Presswell, Blasco-Costa et Kostadinova, 2014, remains unknown.

Posticobia brazieri has traditionally been included in the Hydrobiidae Stimpson (e.g. Clark 2009). However, we have here recognised it as belonging to the Tateidae in line with the phylogenetic analysis of ‘rissooid’ gastropods proposed by Wilke et al. (2013). This analysis does not specifically refer to *P. brazieri*, but it is clear that the scope of the family is thought to incorporate it. The report of three species of the Microphallidae from *P. brazieri* here adds to the remarkable list of trematodes known to infect this species in southern Queensland. Further records include two species of the Aporocotylidae Odhner, 1912 (only one named – Nolan and Cribb 2004), one species of the Cryptogonimidae Ward, 1917, one species of the Haploporidae Nicoll, 1914, two species of the Lepocreadiidae Odhner, 1905, one species of the Opecoelidae Ozaki, 1925 and one species of the Transversotrematidae Wittenberg, 1944 (see Cribb 1985, 1986, 1988a, Martin 1973, Watson 1984). In addition, Cribb (1988b) mentioned the occurrence of two cystophorous cercariae (Hemiuroidea Looss, 1899) and in the present study we have also detected infections of at least one pronoccephaloid, one renicoloid and one psilostomid. We are thus aware of a minimum of 16 trematode species infecting *P. brazieri*. Among at least the Pronoccephaloidea Looss, 1899 and Haploporidae, it seems likely that more than one species is involved.

Two aspects of this richness are noteworthy. First, as the only rissooid gastropod that we have seen in fresh water in south-east Queensland, it is clear that this species,

Table 4. Species of the Microphallidae with freshwater life cycles.

Species	First intermediate host	Second intermediate host	Definitive host	Region	Source
<i>Pseudolevinseniella cheni</i> Tsai, 1955	-	<i>Macrobrachium nipponense</i> (De Haan)	<i>Anas platyrhynchos domesticus</i>	China	1
<i>P. ananiteron</i> Deblock et Pearson, 1968	-	<i>Macrobrachium australiense</i> Holthuis, <i>M. australe</i> (Guérin-Mèneville)	Mice (exp.)	Australia	2, 3
<i>Maritrema brevisaciferum</i> Shimazu et Pearson, 1991	-	<i>Caridina nilotica</i> (Roux)	Chicken (exp.)	Australia	3
<i>Maritrema erpobdellicola</i> Timon-David, 1962	-	<i>Erpobdella testacea</i> (Savigny)	-	France	4
<i>Maritrema felui</i> Gracenea, Montoliu et Deblock, 1993	-	<i>Mercuria confusa</i> (Frauenfeld)	<i>Crocidura russula</i> (Hermann)	Spain	5
<i>Maritrema galloprovinciale</i> Timon-David, 1960	-	<i>Gammarus aequicauda</i> (Martynov)	-	France	1
<i>Maritrema huillini</i> Flores, Brugni et Pozzi, 2012	-	<i>Gammarus pulex</i> (Linnaeus)	<i>Lontra provocax</i> (Thomas)	Argentina	6
<i>Maritrema inusitata</i> Leonov et Tsimbalyuk, 1963	-	<i>Obesogammarus crassus</i> (Sars), <i>O. obesus</i> (Sars), <i>Dikrogammarus haemobaphes</i> (Eichwald)	<i>Clangula hyemalis</i> (Linnaeus) (natural host), chicken (exp.)	Far East, Ukraine	7, 8
<i>Maritrema mapensis</i> (Chen, 1957)	-	<i>Neocaridina denticulata</i> (De Haan)	<i>A. platyrhynchos</i> (Linnaeus) (natural host), rat (exp. host)	China	1
<i>Maritrema neomi</i> Tkach, 1998	-	<i>Gammarus balcanicus</i> Schälterna	<i>Neomys anomalus</i> Cabrera	Ukraine	9
<i>Maritrema parainusitata</i> Kulkina et Beljakova, 1983	-	<i>Gammarus balcanicus</i>	Mice (exp.)	Trans-Ili Alatau	10
<i>Maritrema obsipum</i> (Van Cleave et Mueller, 1932)	-	<i>Asellus communis</i> Say	<i>Ambloplites rupestris</i> (Rafinesque) (natural host), <i>Anas platyrhynchos</i> , hamster, white mice, chicken, pigeon, canaries (all exp. hosts)	USA	11
<i>Maritrema patagonica</i> Rauque, Flores et Brugni, 2013	-	<i>Aegla neuquensis</i> Schmitt, <i>A. riolmayana</i> Schmitt	<i>Gallus gallus domesticus</i> (Linnaeus)	Argentinean Patagonia	12
<i>Maritrema poulini</i> Presswell, Blasco-Costa et Kostadinova, 2014	-	<i>Austridotea annexiens</i> Nicholls, <i>A. lacustris</i> (Thomson), <i>Paracalliope fluviatilis</i> (Thomson), <i>Paracorphium excavatum</i> (Thomson)	-	New Zealand	13
<i>Maritrema pyrenaica</i> Deblock et Combes, 1965	-	<i>Gammarus pulex</i>	<i>Galemys pyrenaicus</i> (Geoffroy), <i>Neomys fodiens</i> Pennant	Spain	14
<i>Maritrema</i> sp.	-	<i>Cambaroides japonicus</i> (De Haan)	-	USSR	1
<i>Quasimaritremps medius</i> (Van Cleave et Mueller, 1932)	-	<i>Cambarus propinquus</i> Girard, <i>C. viridis</i> Moenkhaus	Mice (exp.)	USA	15
<i>Quasimaritrema caridinae</i> (Yamaguti et Nisimura, 1944)	-	<i>Neocaridina denticulata</i>	<i>Rostratula benhalensis</i> (Linnaeus)	Japan	1
<i>Megalophallides apamhorayi</i> Ching et Ibáñez, 1976	-	<i>Pseudohelphusa chilensis</i> Smith	-	Peru	16
<i>Microphallus fontis</i> Overstreet, Heard et Lotz, 1992	-	<i>Procamburus clarkii</i> (Girard)	-	USA	17
<i>Microphallus minus</i> Ochi, 1928	-	<i>Macrobrachium asperulus</i> (von Martens), <i>M. nipponense</i> (De Haan), <i>Metapenaeus bennettiae</i> Racek et Dall	White mice, cat, dog, humans (exp.)	China, Australia	18, 19
<i>Microphallus minutus</i> Johnston, 1948	-	<i>Cherax dispar</i> Riek	<i>Hydromys chrysogaster</i> Geoffroy	Australia	3, 19, 20
<i>Microphallus opacus</i> (Ward, 1894)	-	<i>Cambarus propinquus</i>	Natural hosts: <i>Ania cava</i> (Linnaeus), <i>Micropterus dolomieu</i> (Lacepède), <i>Ictalurus punctatus</i> (Rafinesque), <i>Perca flavescens</i> (Mitchill); experimental hosts: <i>Chrysomys picta</i> (Schneider), <i>Emys blandingii</i> (Holbrook), <i>Thamnophis s. sauritus</i> (Linnaeus), <i>Regina septemvittata</i> (Say), <i>Didelphis virginiana</i> (Kerr), <i>Procyon lotor</i> (Linnaeus)	USA	15, 21, 22
<i>Microphallus</i> sp. ('lively')	-	<i>Amnicola limosa</i> (Say)	-	New Zealand	23
<i>Microphallus szidati</i> Martorelli, 1986	-	<i>Potamopyrgus antipodarum</i>	<i>Himantopus melanurus</i> Vieillot, <i>Rallus sanguinolentus</i> (Swainson)	Argentina	24
<i>M. tauricus</i> Stenko, 1973	-	<i>Palaeonetes argentinus</i> Nobili, <i>Cyrtograpus angulatus</i> Dana	<i>Anas platyrhynchos domesticus</i> (exp.)	Ukraine	25
<i>Levinseniella amnicolae</i> Eiges, 1953	-	<i>Gammarus balcanicus</i>	<i>Anas platyrhynchos domesticus</i> (exp.)	USA	11
<i>Levinseniella cruzi</i> Travassos, 1920	-	<i>Asellus communis</i>	<i>Himantopus melanurus</i> Vieillot, <i>Rollandia rolland chilensis</i> (Lesson)	Argentina	26
<i>Levinseniella minuta</i> Price, 1934	-	<i>Palaeonetes argentinus</i>	<i>Aythya affinis</i> (Eyton)	USA	27
<i>Levinseniella ophidea</i> Nicol, Demaree et Wootton, 1985	-	<i>Amnicola limosa</i> (Say)	<i>Thamnophis elegans</i> Baird et Girard, <i>Rana catesbeiana</i> Shaw	USA	28
<i>Sogandarema progeneticus</i> (Sogandares-Bernal, 1962)	-	<i>Dina parva</i> Moore, <i>Glossiphonia complanata</i> (Linnaeus), <i>Helobdella stagnalis</i> (Linnaeus), <i>Erpobdella punctata</i> (Leidy)	-	USA	29, 30

1 – Belopolskaya (1963); 2 – Deblock and Pearson (1968); 3 – Shimazu and Pearson (1991); 4 – Timon-David and Pearson (1991); 5 – Gracenea et al. (1993); 6 – Flores et al. (2012); 7 – Leonov and Tsimbalyuk (1963); 8 – Kurandina (1978); 9 – Tkach (1998); 10 – Kulkina and Beljakova (1983); 11 – Eiges (1953); 12 – Raue et al. (2013); 13 – Presswell et al. (2014); 14 – Casanova et al. (1998); 15 – Belopolskaya (1952); 16 – Ching and Ibáñez (1976); 17 – Overstreet et al. (1992); 18 – Wu (1951); 19 – Deblock and Pearson (1969); 20 – Johnston (1948); 21 – Rausch (1947); 22 – Caveny and Etges (1971); 23 – Hechinger (2012); 24 – Martorelli (1986 a,b); 25 – Stenko (1973); 26 – Martorelli (1988); 27 – Stunkard (1958); 28 – Nicol et al. (1985); 29 – Sogandares-Bernal (1962); 30 – Lotz and Corkum (1983); (exp.) – experimentally obtained adults.

which often occurs in huge densities, plays a pivotal role in a wide variety of the trematode cycles in the region. Secondly, we note that this role is also filled by rissoids elsewhere. Hechinger (2012) summarised knowledge of 20 species of trematodes that infect the New Zealand tateid, *P. antipodarum*. The parallels with the fauna of *P. brazieri* are striking. Both harbour Aporocotylidae (both two species), Cryptogonimidae, Lepocreadiidae, Microphallidae, Opecoelidae, Pronocephaloidea, and (possibly) Haploporidae and Psilostomidae Looss, 1900. The only family level distinctions between the two are the strigeid and 'lecithodendriid-like families' in *P. antipodarum* and the presence of hemiuroid, renicolid and transversotrematids in *P. brazieri*. Further study may increase the level of similarity. Similarly, Deblock (1978) summarised the fauna of

three species of *Hydrobia* from salt marshes and estuaries in France; he reported a remarkable 40 trematode species from 14 families. Microphallids occupy a central position in the list with 12 species. Species of *Hydrobia* are currently known as an intermediate host for about 19 species of the Microphallidae (Deblock 1978, 1980, Field and Irwin 1999, Bartoli and Gibson 2007, Bordalo et al. 2011).

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