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Morphological disparity despite genetic similarity; new species of *Lobosorchis* Miller & Cribb, 2005 (Digenea: Cryptogonimidae) from the Great Barrier Reef and the Maldives

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Abstract

Examination of the humpback red snapper, *Lutjanus gibbus* (Perciformes: Lutjanidae), from Lizard Island off the Great Barrier Reef and Rasdhoo Atoll, Maldives revealed the presence of a new species of *Lobosorchis* (Digenea: Cryptogonimidae), *L. polygongylus* **n. sp.** *Lobosorchis polygongylus* **n. sp.** is distinguished from the type- and only other species, *L. tibaldiae* by the combination of body size, oral spine number (60–81 in *L. polygongylus*, 47–56 in *L. tibaldiae*) and number of testes (13–25 in *L. polygongylus*, 9 in *L. tibaldiae*). Bayesian inference analysis using data from the internal transcribed spacers 1 and 2 (ITS1 and ITS2), 5.8S and the large subunit (LSU) nuclear ribosomal DNA of *L. polygongylus*, *L. tibaldiae* and species of *Beluesca*, *Caulanus*, *Chelediadema*, *Neometadena*, *Latuterus*, *Retrovarium* and *Siphoderina* was performed to explore phylogenetic relationships of species of *Lobosorchis* with other cryptogonimid taxa. Despite the significant morphological differences between *Lobosorchis polygongylus* and *L. tibaldiae*, these two species differed consistently by only 5 base pairs (bp) over the entire ITS region (3 bp in ITS1, 0 bp in 5.8S and 2 bp in ITS2) and 1 bp in the LSU rDNA regions examined. The ITS2 rDNA region was sequenced from metacercariae obtained from the fins, flesh or body cavities of a number of fishes belonging to the Blenniidae, Pomacentridae and Tetraodontidae and analysed using minimum evolution analysis with *L. polygongylus* and *L. tibaldiae*. This revealed the presence of two additional genotypes (putative *Lobosorchis* sp. A and B), which consistently differed from *L. polygongylus* by 1 and 4 bp, *L. tibaldiae* by 1 and 4 bp and from each other by 3 bp over the ITS2 dataset. Although these genetic differences are relatively small, when evaluated in light of the differences observed between *L. polygongylus* and *L. tibaldiae* (which are morphologically quite distinct) and differences seen in other congeneric cryptogonimid taxa, the ITS2 rDNA data alone suggest that at least two more species of *Lobosorchis* are present at Lizard Island. These data also suggest that the ITS2 rDNA region alone is suitable for resolving operational taxonomic units (OTUs) at the species level within the Cryptogonimidae based on what was observed in this and other cryptogonimid systems. A morphological description of metacercariae of *L. tibaldiae* obtained from two species of Pomacentridae at Heron Island, off the Great Barrier Reef is also provided.

Key words: Biodiversity; Blenniidae; Cryptogonimidae; Digenea; Great Barrier Reef; Lizard Island; *Lobosorchis*; Lutjanidae; *Lutjanus gibbus*; *Lutjanus*; Maldives; Opisthorchiata; Opisthorchioidea; Pomacentridae; ribosomal DNA; Tetraodontidae

Introduction

The humpback red snapper, *Lutjanus gibbus* (Forsskål) (Lutjanidae), is a relatively common inhabitant of Indo-West Pacific coral reef ecosystems from the Red Sea and East Africa to the Line and Society islands, north to southern Japan and south to Australia (Allen 1985). The diet of this long-lived species includes a variety of fishes, benthic crustaceans, echinoderms and mollusks, similar to its sister taxon, the two-spot red snapper, *L. bohar* (Forsskål) (Miller & Cribb 2007a; Froese & Pauly 2008). *Lutjanus gibbus* is known to harbour a

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number of digenetic trematode species. An unidentified species of *Stephanostomum* Looss, 1899 (Acanthocolpidae Lühe, 1906) was reported from *L. gibbus* off Kuwait by Al-Yamani and Nahhas (1981). Parukhin (1971) reported the faustulid *Bacciger bacciger* (Rudolphi, 1819) Nicoll, 1914 from the Red Sea. Unidentified sclerodistomid (*Prosogonotrema* Vigueras, 1940 sp.) and lepocreadiid (*Pseudocreadium* Layman, 1930 sp.) species were reported from the Persian Gulf and Gulf of Oman by El-Naffar *et al*. (1992). Rigby *et al*. (1999) reported an unidentified species of Hemiuridae Looss, 1899 from French Polynesia. A few species of Opecoelidae Ozaki, 1925 have also been reported from *L. gibbus*: *Hamacreadium lethrini* Yamaguti, 1934 was reported by Fischthal and Kuntz (1964) off the Philippines; *H. mutabile* Linton, 1910 was reported from the Red Sea by Parukhin (1970), French Polynesia by Rigby *et al*. (1999) and the Great Barrier Reef by Bray and Cribb (1989); an unidentified species of *Helicometra* Odhner, 1902 was reported off French Polynesia by Rigby *et al*. (1999). The only cryptogonimid known from *L. gibbus* is *Retrovarium sphericum* (Nahhas, Sey & Nishimoto, 1998) Miller & Cribb, 2007, which was reported off Kuwait by Nahhas *et al*. (1998).

The monotypic *Lobosorchis* Miller & Cribb, 2005 (Digenea: Cryptogonimidae) was proposed by Miller and Cribb (2005) for *L. tibaldiae* Miller & Cribb, 2005 recovered from the Spanish flag snapper, *Lutjanus carponotatus* (Richardson), and the dory snapper, *L. fulviflamma* (Forsskål), off Heron and Lizard Islands on the Great Barrier Reef and New Caledonia and distinguished from all other cryptogonimid genera based on its combination of having oral spines, multiple testes in two symmetrical groups, and vitelline follicles that are distributed mainly in the forebody. A recent survey of the digenetic trematode fauna inhabiting Indo-West Pacific lutjanids revealed the presence of a new species of *Lobosorchis* from *Lutjanus gibbus* off Rasdhoo Atoll in the Maldives and Lizard Island off the Great Barrier Reef, Australia. Here we describe this new species and provide an amended diagnosis of *Lobosorchis*. We also compare morphometric and molecular data obtained from the internal transcribed spacer (includes ITS1, 5.8S and ITS2) and the large subunit (LSU) ribosomal DNA (rDNA) regions of this new taxon with the type-species *L. tibaldiae* and putative *Lobosorchis* metacercariae recovered from three species of Blenniidae, three Pomacentridae spp., and one species of Tetraodontidae off the Great Barrier Reef. Bayesian Inference analysis of a combined ITS and LSU dataset was performed to explore the phylogenetic relationships of species of *Lobosorchis* with other recently reported cryptogonimid taxa (Miller & Cribb 2007b; c; d). Minimum evolution analysis was also conducted on the ITS2 dataset (which included data from metacercariae obtained from intermediate hosts at Lizard Island) to attempt to elucidate the life-cycles of and explore the diversity of *Lobosorchis* species on the Great Barrier Reef (GBR).

Material and methods

Host and parasite collection

Fish were collected using baited line, clove oil, seine or spear from the following localities: Heron Island (23°26'S; 151°54'E) in the southern GBR, Lizard Island (14°40'S; 145°27E) in the northern GBR and Rasdhoo Atoll (4°16'N; 72°58'E), Maldives. Total numbers of species of Lutjanidae collected during this study are listed in Miller and Cribb (2007b). Fish were killed by neural pithing and the intestine immediately removed, washed in vertebrate saline (0.85%), and examined for the presence of endohelminths. Adult trematodes obtained from the intestine or pyloric caeca were washed in saline and killed by pipetting them into nearly boiling saline. Specimens for morphological analysis were then stored in 10% formalin and specimens for DNA extraction and analysis were stored in 95–100% ethanol at -20°C. Metacercariae were obtained from the musculature and fins of fish examined using fine-tipped forceps. When possible, metacercariae were gently excised from their cysts using forceps for putative identification using a dissecting microscope, then heat fixed in nearly boiling saline and stored in 10% formalin for morphological analysis or 95–100% ethanol at -20°C for molecular analysis. If metacercariae were damaged or excision unsuccessful, they were immediately stored in ethanol for molecular analysis.

Morphological and molecular sample processing

Specimens for morphological and molecular analysis (DNA extraction, amplification of the entire internal transcribed spacer (ITS1, 5.8S and ITS2) and large subunit (LSU) nuclear ribosomal DNA regions, and sequencing) were processed according to the protocols reported by Miller and Cribb (2007b; d). The ITS and LSU regions were sequenced for the type-species of *Lobosorchis*, *L. tibaldiae*, from the type-host and locality and submitted to GenBank under the following accession numbers: ITS (FJ154899), LSU (FJ154901). GenBank accession numbers for the remaining species of *Lobosorchis* sequenced in this study are provided under the appropriate specimens in the Results section. The consensus sequences for each taxon utilized in this study were constructed from multiple replicates (each replicate contig being both a forward and reverse sequence from a single individual). Additional replicate contigs were sequenced from specimens obtained from different host/parasite/location combinations whenever possible. All morphometric measurements were made using an ocular micrometer and are in micrometers, with the mean followed by range in parentheses. Length-width or sucker-width ratios in the diagnoses refer to the length divided by the width or oral sucker width divided by the ventral sucker width expressed as a whole number. Type- and voucher specimens were deposited in the Queensland Museum, Brisbane, Australia.

Comparative DNA analyses

Three rDNA datasets were analysed independently in this study. These included the entire ITS (ITS1, 5.8S and ITS2), ITS2 only (because only ITS2 data was obtained for some metacercariae included here) and LSU regions. The three rDNA region datasets from taxa sequenced in this study were initially aligned using ClustalX version 2.0.9 (Larkin *et al.* 2007) under the following parameters: pairwise alignment parameters = gap opening 10.00, gap extension 0.10, DNA weight matrix International Union of Biochemistry (IUB); multiple alignment parameters = gap opening 10.00, gap extension 0.20, delay divergent sequences 30%, DNA weight matrix IUB. The resulting sequence alignments were exported from ClustalX in FASTA and NEXUS formats, and refined by eye using MacClade version 4.08 (Maddison & Maddison 2005). After alignments of the rDNA regions were edited, the ends of each fragment were trimmed to match the shortest sequence in the alignment. Distance matrices for the three rDNA region datasets (entire ITS, ITS2 and LSU) were constructed with the absolute pairwise character difference and the percentage of uncorrected "p" pairwise character differences. Pairwise comparisons of absolute sequence divergence for all taxa were calculated with gaps treated as missing data.

The entire ITS, ITS2 only and LSU rDNA regions for species of *Lobosorchis* were also aligned with those reported for species of the cryptogonimid genera *Beluesca* Miller & Cribb, 2007, *Caulanus* Miller & Cribb, 2007, *Chelediadema* Miller & Cribb, 2007, *Latuterus* Miller & Cribb, 2007, *Neometadena* Hafeezullah & Siddiqi, 1970, and *Retrovarium* Miller & Cribb, 2007 by Miller and Cribb (2007b; c; d) for comparative purposes and to explore levels of interspecific variation. The ITS and LSU rDNA regions were then combined and assigned partitions in a single NEXUS file. Minimum evolution and Bayesian inference analyses of the combined (entire ITS and LSU) dataset of these taxa were performed using PAUP* version 4.0b10 (Swofford 2003) and MrBayes version 3.1.2 (Ronquist & Huelsenbeck 2003). Modeltest version 3.7 (Posada & Crandall 1998) was used to estimate the best substitution model for the combined dataset. Bayesian inference analysis was conducted on the combined dataset using the GTR+I+G model predicted as the best estimator by the Akaike Information Criterion (AIC) in Modeltest. The ITS and LSU regions were partitioned in the combined dataset to allow estimates of each model parameter for each rDNA region. Bayesian inference analysis was run over 1,000,000 generations (ngen=1000000) via four simultaneous Markov Chain Monte Carlo (MCMC) chains (nchains=4) and every 100th tree saved (samplefreq=100). Bayesian analyses used the following parameters: nst=6, rates=invgamma, ngammacat=4, and the MCMC parameters were left at the default settings, and the priors parameters of the combined dataset were set to ratepr=variable. Samples of substitution model parameters, and tree and branch lengths were summarized using the parameters 'sump burnin=3000',

'sumt burnin=3000'. These 'burnin' parameters were chosen because the log likelihood scores 'plateaued' well before 300,000 replicates in the BI analyses.

Minimum evolution analysis was also conducted for comparative purposes on the ITS2 only dataset independently (which included putative *Lobosorchis* spp. sequences obtained from metacercariae off the GBR) using PAUP*. Nodal support for minimum evolution analyses of the combined (ITS and LSU) and ITS2 only datasets were inferred by bootstrap analysis using a heuristic search of 10,000 replicates.

Results

Morphological data

Lobosorchis Miller & Cribb, 2005

Type-species: *L. tibaldiae* Miller & Cribb, 2005.

Diagnosis: Body oval; length/width ratio *c*. 1.4–1.9. Tegument armed with small to minute spines. Oral sucker distinctly wider than long, with enlarged oral spines, opens nearly terminally. Ventral sucker unspecialised, embedded in ventrogenital sac. Ratio oral/ventral sucker width *c*. 2.5–3. Forebody occupies *c*. 30–40% of body length. Prepharynx short. Pharynx wider than ventral sucker. Oesophagus short. Intestinal bifurcation between ventral sucker and pharynx. Caeca blind, terminate close to posterior end of body. Testes multiple, in two symmetrical groups of 4–5 or more per group, total number of testes 9–25, in mid-hindbody. Seminal vesicle tubulosaccular, between ovary and ventral sucker. Common genital pore immediately anterior to ventral sucker. Gonotyl a small lobe arising from anterior wall of ventrogenital sac. Ovary deeply lobed, ventral to or immediately anterior and adjacent to testes. Laurer's canal present. Seminal receptacle saccular, dextral or sinistral to seminal vesicle, between ovary and ventral sucker. Vitelline follicles confined almost entirely to forebody, confluent dorsally, extend from slightly posterior to ventral sucker to near posterior margin of oral sucker. Uterine coils restricted to hindbody, extend from posterior end of body to ventral sucker. Excretory vesicle Y-shaped; arms reach pharynx.

It is to be treated as masculine.

Lobosorchis polygongylus n. sp.
(Fig. 1)

Type host: *Lutjanus gibbus* (Forsskål), Perciformes, Lutjanidae, humpback red snapper.

Type locality: Rasdhoo Atoll (4º16'N; 72º58'E), Maldives.

Additional localities: Lizard Island, Great Barrier Reef (14º40'S; 145º27'E), Queensland, Australia.

Site: Intestine and pyloric caeca.

Prevalence: 5 of 5 (100%) at Rasdhoo Atoll; 2 of 7 (29%) at Lizard Island.

Molecular sequence data: ITS, 3 individuals from Lizard Island, 4 individuals from the Maldives; LSU, 3 individuals from Lizard Island, 3 individuals from the Maldives.

Deposited specimens: holotype G230563, 12 paratypes G230564-G230575.

GenBank accession numbers: ITS (FJ154900), LSU (FJ154902).

Etymology: The epithet *polygongylus* is derived from the Greek *poly*, meaning many and the Greek *gongylos*, meaning ball-shaped, round or spherical, referring to the multiple testicular follicles present in this species.

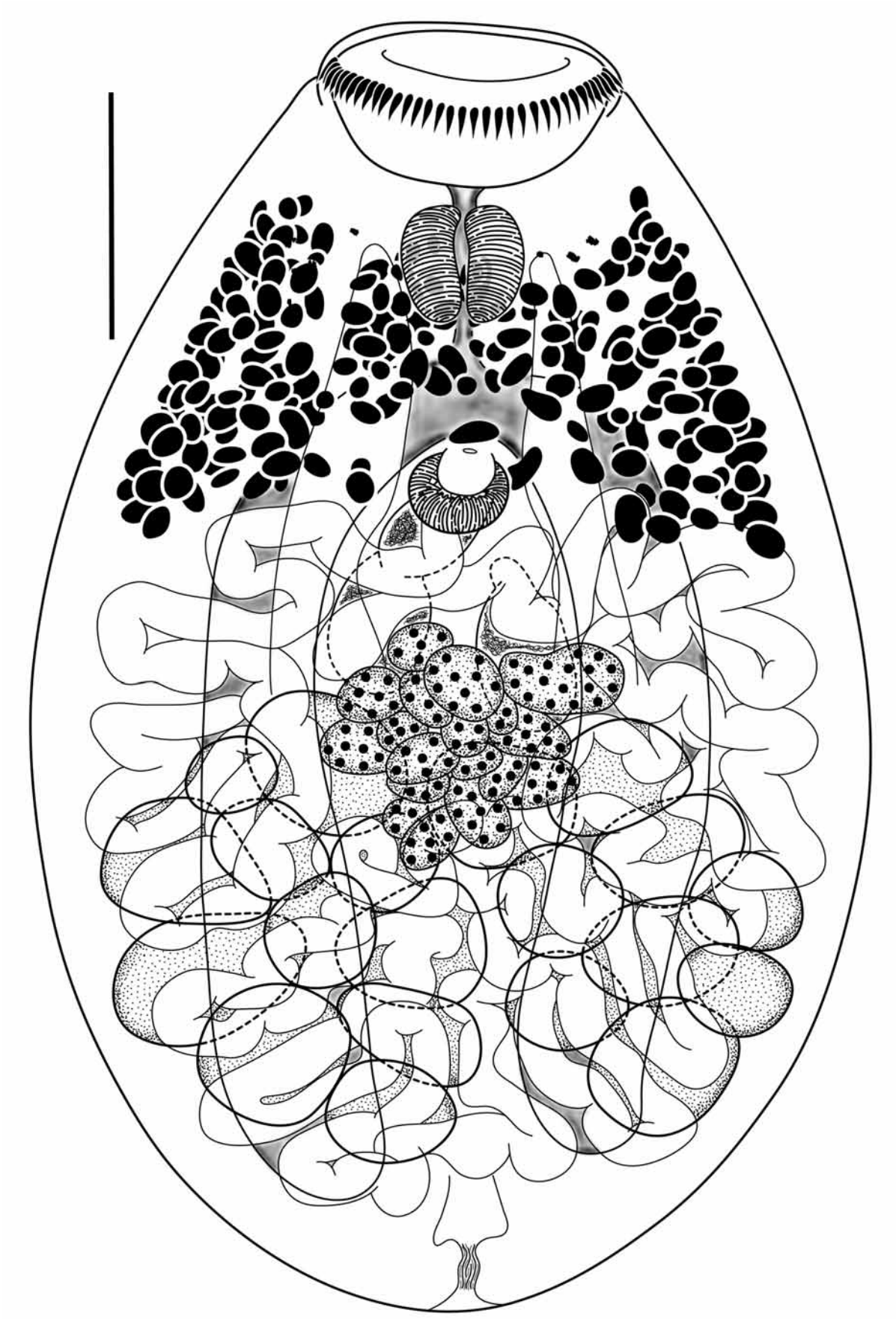

FIGURE 1. *Lobosorchis polygongylus* **n. sp.** from the intestine of *Lutjanus gibbus* off Rasdhoo Atoll, Maldives. Ventral view of holotype. Scale bar = 200 µm.

Description: Based on 13 specimens. Body oval, longer than wide, 959 (598–1,176) long by 580 (374–688) wide; length/width ratio 1.7 (1.4–1.9). Oral sucker 123 (78–154) long by 217 (165–254) wide. Oral spines 70 (60–81), length 19 (8–27). Ventral sucker 68 (53–80) long by 78 (59–88) wide. Ratio oral/ventral sucker width 2.8 (2.5–3). Forebody occupying 34 (31–36)% of body length. Prepharynx shorter than oesophagus, 15 (6–22) long. Pharynx 92 (62–115) long by 93 (70–109) wide. Ventral sucker/pharynx width ratio 0.8 (0.8–0.9). Oesophagus 36 (19–48) long. Intestinal bifurcation between ventral sucker and pharynx. Intestinal caeca blind, 668 (390–852) long, terminate close to posterior end of body. Testes multiple, in two symmetrical groups, total number 19 (13–25), in mid-hindbody, individual testes 87 (45–131) long by 99 (43–179) wide. Seminal vesicle tubulosaccular, between ovary and ventral sucker. Gonotyl a small lobe arising from anterior wall of ventrogenital sac. Genital pore immediately anterior to ventral sucker. Ovary deeply lobed, immediately anterior and adjacent to testes, 171 (122–202) long by 190 (134–256) wide. Laurer's canal present. Seminal receptacle saccular, dextral or sinistral to seminal vesicle, between ovary and ventral sucker. Vitelline follicles confined almost entirely to forebody, confluent dorsally, extend from slightly posterior to ventral sucker to near posterior margin of oral sucker. Uterine coils restricted to hindbody, extend from posterior end of body to ventral sucker. Eggs small, darkly tanned, 18 (15–21) long by 8 (7–9) wide. Excretory vesicle Y-shaped, bifurcates dorsal to ovary; arms extend to pharynx, 757 (442–944) long. Excretory pore opens at posterior end of body.

***Lobosorchis tibaldiae* Miller & Cribb, 2005 Metacercariae**
(Fig. 2)

Hosts: *Neoglyphidodon melas* (Cuvier), Perciformes, Pomacentridae, bowtie damselfish; *Pomacentrus melanochir* (Bleeker), Perciformes, Pomacentridae, Indonesian damsel.

Locality: Heron Island, Great Barrier Reef (23º26'S; 151º54'E), Queensland, Australia.

Site: Flesh.

Deposited specimens: vouchers G230576–G230578.

Description: Based on 3 specimens. Body oval, longer than wide, 388 (328–426) long by 238 (163–289) wide; length/width ratio 1.7 (1.5–2). Oral sucker 64 (63–65) long by 111 (80–128) wide. Oral spines 60 (54–65), length 11 (8–15). Ventral sucker 42 (40–45) long by 42 (38–43) wide. Ratio oral/ventral sucker width 2.6 (2.1–3). Forebody occupying 37 (35–40)% of body length. Prepharynx 11 (6–13) long. Pharynx 39 (35–45) long by 49 (45–53) wide. Ventral sucker/pharynx width ratio 0.9 (0.8–0.9). Oesophagus 21 (16–26) long. Intestinal bifurcation dorsal to or immediately anterior to ventral sucker. Intestinal caeca blind, 398 (286–494) long, terminate midway between ventral sucker and posterior end of body or close to posterior end of body. Testes multiple, in two symmetrical groups of 4–5, in mid-hindbody, individual testes 21 (14–29) long by 22 (14–29) wide. Gonotyl a small lobe arising from anterior wall of ventrogenital sac, often undeveloped. Genital pore immediately anterior to ventral sucker. Ovary deeply lobed, immediately anterior and adjacent to testes, 22 (16–26) long by 26 (18–35) wide. Laurer's canal present. Vitelline system undeveloped. Excretory vesicle Y-shaped, bifurcates immediately posterior to or dorsal to ovary; arms extend to pharynx, 275 (230–307) long. Excretory pore opens at posterior end of body.

Molecular data

***Lobosorchis* sp. A Metacercariae**

Hosts: *Ecsenius stictus* (Springer), Perciformes, Blenniidae, Great Barrier Reef blenny; *Salarias alboguttatus* (Kner), Perciformes, Blenniidae, white-spotted blenny; *Salarias fasciatus* (Bloch), Perciformes, Blenniidae,

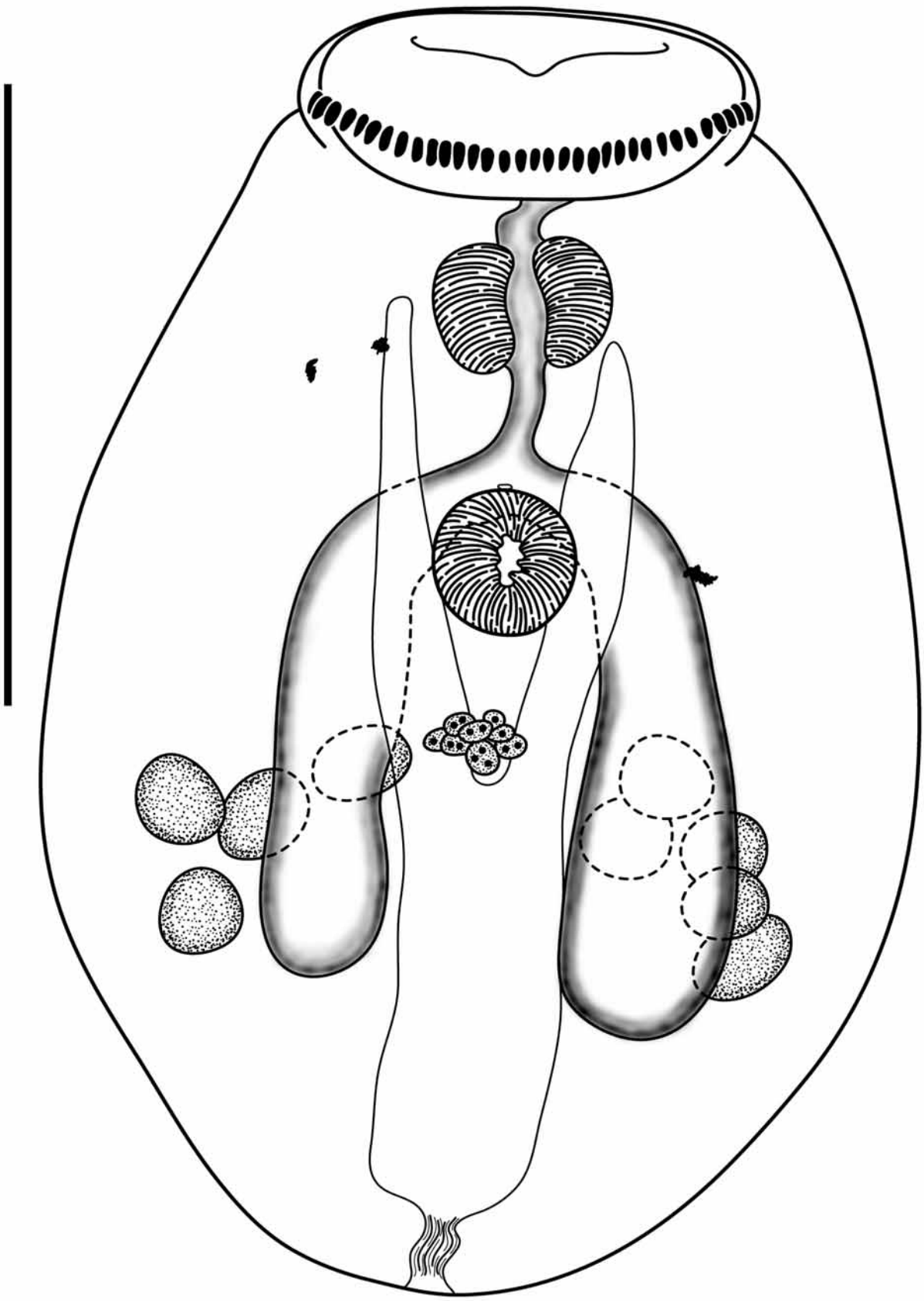

FIGURE 2. *Lobosorchis tibaldiae* Miller & Cribb, 2005 metacercaria from the flesh of *Neoglyphidodon melas* off Heron Island, Great Barrier Reef, Australia. Scale Bar = 200 µm.

jewelled blenny; *Canthigaster bennetti* (Bleeker), Tetraodontiformes, Tetraodontidae, Bennett's sharpnose puffer.

Locality: Lizard Island, Great Barrier Reef (14º40'S; 145º27'E), Queensland, Australia.

Site: Flesh.

Molecular sequence data: ITS2, 5 individuals.

GenBank accession number: ITS2 (FJ154903).

***Lobosorchis* sp. B Metacercariae**

Hosts: *Dischistodus perspicillatus* (Cuvier), Perciformes, Pomacentridae, white damsel.

Locality: Lizard Island, Great Barrier Reef (14º40'S; 145º27'E), Queensland, Australia.

Site: Flesh.

Molecular sequence data: ITS2, 1 individual.

GenBank accession number: ITS2 (FJ154904).

ITS rDNA

Alignment of the ITS rDNA region (ITS1, 5.8S and ITS2) between *L. polygonylus* and *L. tibaldiae* only revealed that these two taxa differed by 5 bp in total (3 bp in ITS1, 0 bp in 5.8S and 2 bp in ITS2). The three base differences in the ITS1 between *L. polygongylus* and *L. tibaldiae* were due to (in consecutive order) an adenine insertion, an adenine – guanine transition and a cytosine – thymine transition. The two base pair differences in the ITS2 dataset were both due to adenine – cytosine transversions. No intraspecific variation was observed in any of the taxa sequenced over the ITS rDNA region.

Variation in the 5' half of the ITS1 made alignment in this region between the all of the cryptogonimid taxa examined here impossible, so only the 3' half of the ITS1 was included for comparative purposes as in Miller and Cribb (2007b; c; d), because this region was easily alignable. Alignment of the ITS dataset containing the other cryptogonimid taxa, which included the 3' end of the ITS1, the entire 5.8S and ITS2 and 13 bp of the 5' end of the LSU yielded 874 characters for analysis. *Lobosorchis polygongylus* differed from *L. tibaldiae* by 3 bp in the 5' trimmed ITS dataset (1 bp in the ITS1 and 2 bp in the ITS2).

LSU rDNA

Sequencing of the LSU rDNA yielded an average of approximately 870 bp for all taxa. The aligned and trimmed sequences incorporated a total of 859 characters (base pairs and gaps) for analysis. *Lobosorchis polygongylus* differed from *L. tibaldiae* by 1 bp in the LSU dataset. This single base difference was due to a cytosine – thymine transition. No intraspecific variation was observed in any of the taxa sequenced over the LSU rDNA region.

Combined ITS and LSU rDNA dataset analyses

Bayesian inference analysis of the combined ITS (included 3' end of ITS1, 5.8S and ITS2) and LSU rDNA dataset resulted in a phylogram in which *Lobosorchis polygongylus* and *L. tibaldiae* form a well-supported clade sister to *Neometadena ovata* (Hafeezullah & Siddiqi, 1970) Miller & Cribb, 2008 (Fig. 3). All genera were well resolved with high posterior probabilities in the Bayesian analysis. Species of *Retrovarium* formed

a clade sister to *Chelediadema marjoriae* Miller & Cribb, 2007. *Caulanus thomasi* Miller & Cribb, 2007 formed a strong clade with species of *Latuterus* and species of *Beluesca* and *Chelediadema* (both found in haemulids) were distinctly genetically distant as observed in Miller and Cribb (2007c; d).

Minimum evolution analysis (ME score = 796.87) of the combined ITS and LSU dataset resulted in a phylogram identical in topology to that observed in the Bayesian inference analysis. Nodal support was also relatively high for the observed clades (Fig. 3).

ITS2 only dataset analysis

Alignment of the ITS2 rDNA region for *Lobosorchis polygongylus*, *L. tibaldiae*, putative *Lobosorchis* sp. metacercariae obtained from the body cavity or flesh of intermediate hosts on the GBR, and the remainder of the cryptogonimid taxa yielded 309 bp (base pairs and gaps) for analysis. This alignment revealed the presence of two additional genotypes (no 'intraspecific' variation was observed within these genotypes), one associated with metacercariae obtained from three species of Blenniidae and one species of Tetraodontidae (putative *Lobosorchis* sp. A) and the other genotype associated with the specimen obtained from the pomacentrid *Dischistodus perspicillatus* (putative *Lobosorchis* sp. B). These two additional genotypes consistently differed from *L. polygongylus* by 1 and 4 bp, *L. tibaldiae* by 1 and 4 bp and from each other by 3 bp over the ITS2 dataset. *Lobosorchis polygongylus* and *L. tibaldiae* differed from one another by 2 bp in the ITS2 rDNA region. The three base pair differences between putative *Lobosorchis* sp. A and B were all due to cytosine – thymine transitions.

Minimum evolution analysis (ME score = 208.42) of the ITS2 dataset resulted in a phylogram with species of *Lobosorchis* forming a well-resolved clade (Fig. 4). All genera were well resolved, similar to Bayesian inference analysis of the combined ITS and LSU dataset, but intergeneric relationships were poorly resolved as evidenced by the low nodal support observed.

Discussion

Differential diagnosis

Lobosorchis polygongylus **n. sp.** can be easily distinguished from *L. tibaldiae* by the combination of body size, oral spine number (60–81 in *L. polygongylus*, 47–56 in *L. tibaldiae*) and number of testicular follicles (13–25 in *L. polygongylus*, 9 in *L. tibaldiae*). Species of a few cryptogonimid genera have multiple (= 9) testes (*Acanthosiphodera* Madhavi, 1976, *Iheringtrema* Travassos, 1947, *Novemtestis* Yamaguti, 1942, *Polyorchitrema* Srivastava, 1939 and *Siphodera* Linton, 1910), but are all easily distinguished from *L. polygongylus* based on testes number (all of the species in these genera except for species of *Polyorchitrema* have only 9 testes), presence or absence of oral spines (species of *Iheringtrema* and *Siphodera* lack oral spines) and vitelline follicle distribution (species of *Acanthosiphodera* and *Novemtestis* have vitelline follicles confined almost entirely or entirely posterior to the ventral sucker). The only other cryptogonimids that have been reported with more than approximately 9 testes are species of *Polyorchitrema*, which may have 8–50 testes (Miller & Cribb 2008), but these differ significantly from *L. polygongylus* in lacking oral spines, the vitelline follicles are distributed in lateral groups posterior to the ventral sucker, the ovary is entire to slightly lobed and the uterus is confined mainly anterior to the testes.

Morphological disparity in light of genetic similarity

Despite the extensive morphological differences between *L. polygongylus* and *L. tibaldiae*, these two species differed from each other by only 5 bp (0.5% sequence divergence) in the ITS and 1 bp (0.1%) in the LSU

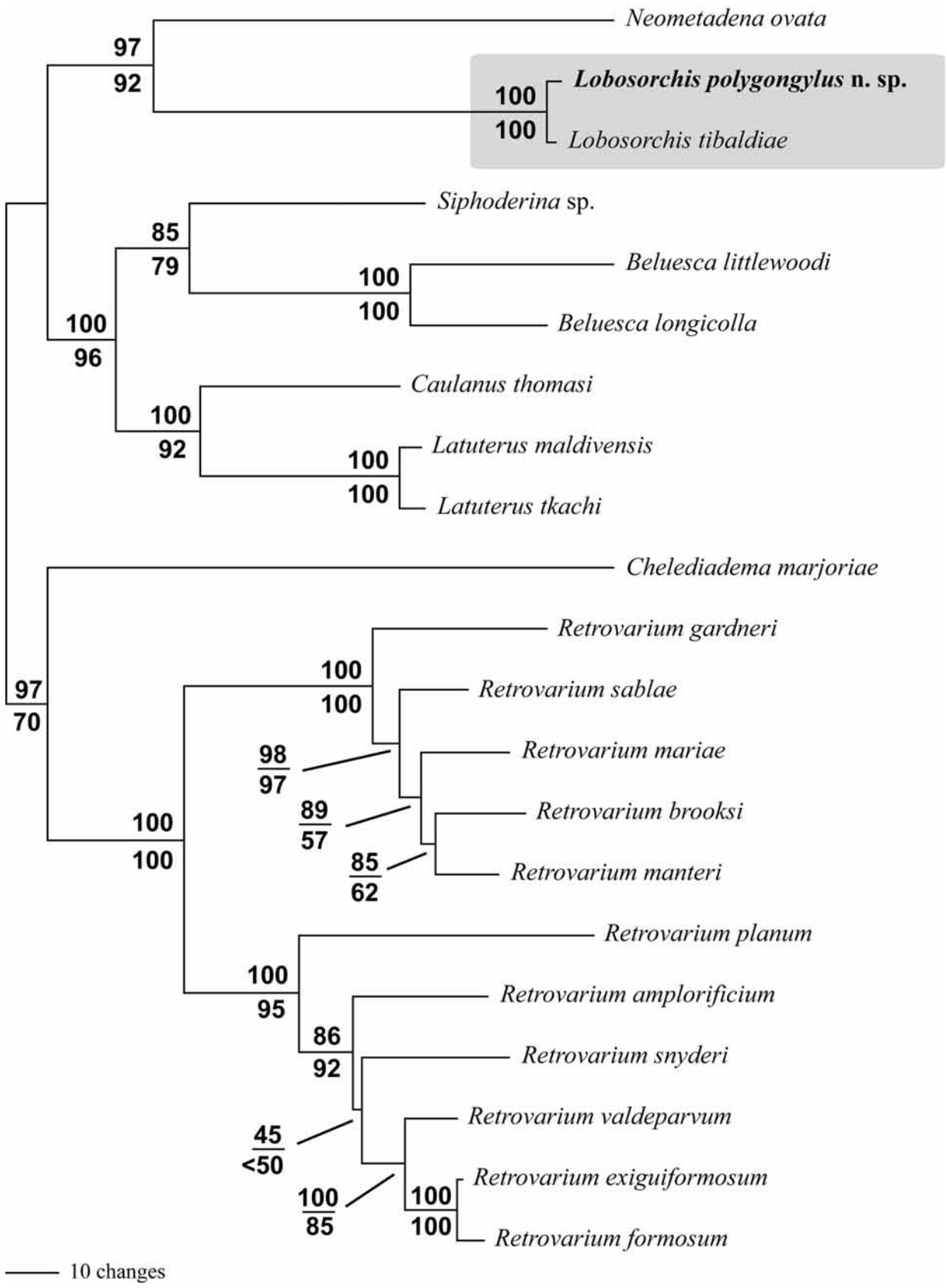

FIGURE 3. Phylogram of relationships between species of *Lobosorchis* Miller & Cribb, 2005 and the other cryptogonimid taxa included based on Bayesian inference analysis of the combined ITS (includes partial ITS1 and complete 5.8S and ITS2) and LSU rDNA dataset. Posterior probabilities are shown above the nodes and bootstrap support values shown below. Phylogram is midpoint rooted.

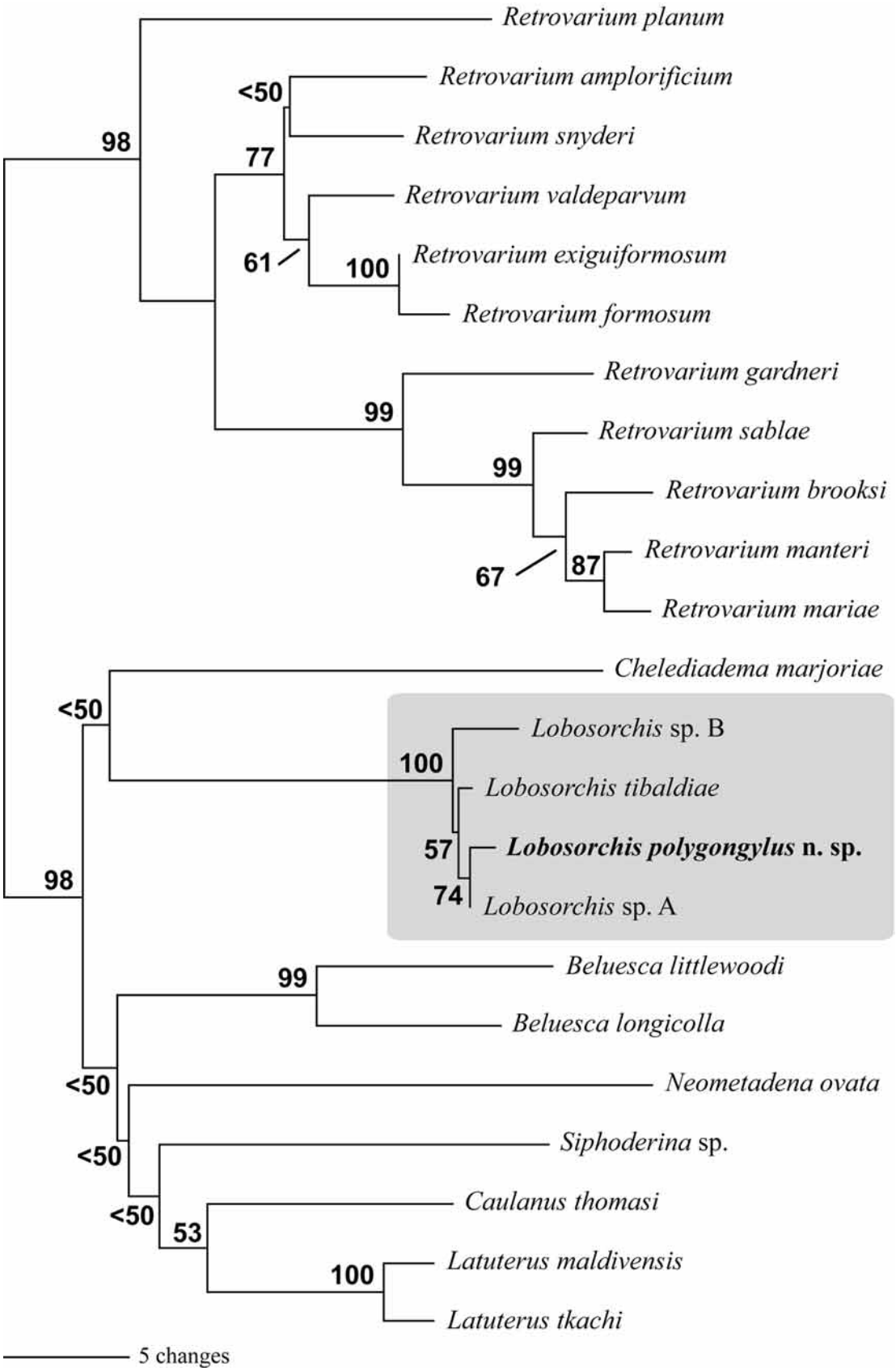

FIGURE 4. Phylogram of relationships between species of *Lobosorchis* Miller & Cribb, 2005 (including the two putative species A and B from metacercariae obtained from the flesh of species of Blenniidae, Pomacentridae and Tetraodontidae off Lizard Island, Great Barrier Reef) and the other cryptogonimid taxa included based on minimum evolution analysis of the ITS2 rDNA dataset. Bootstrap values are indicated at the nodes. Phylogram is midpoint rooted.

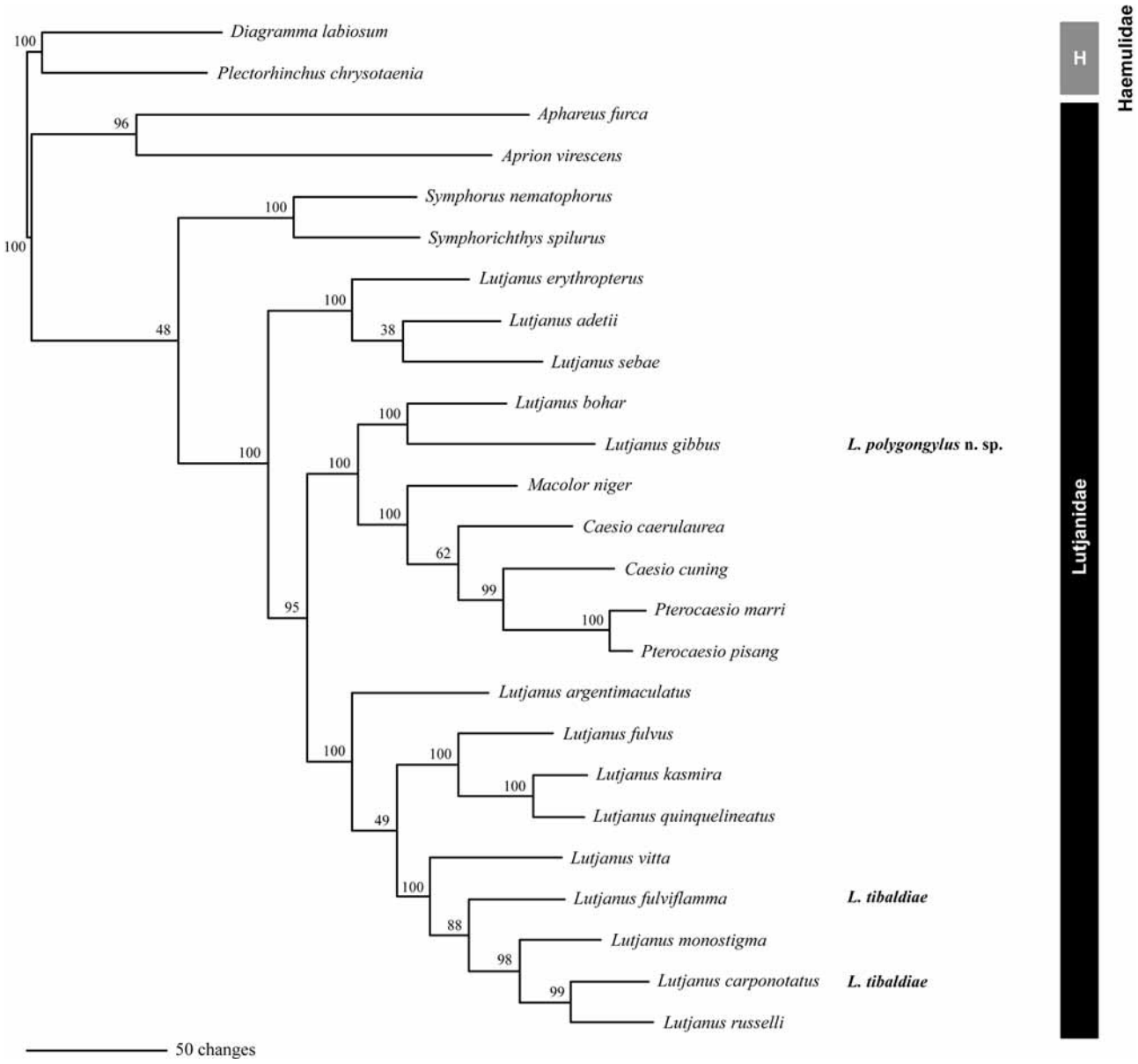

FIGURE 5. Host distribution of species of *Lobosorchis* Miller & Cribb, 2005 mapped onto the phylogeny of Indo-West Pacific Lutjanidae produced by Miller and Cribb (2007a; b).

regions. Some cryptic pairs of species of cryptogonimids such as *Latuterus tkachi* Miller & Cribb, 2007 and *L. maldivensis* Miller & Cribb, 2007 and *Retrovarium exiguiformosum* Miller & Cribb, 2007 and *R. formosum* Miller & Cribb, 2007 have been reported as having as few as 2 bp differences over the same rDNA regions (Miller & Cribb 2007b; c), but this is the first example of two easily distinguished and host specific species differing genetically by such a small amount over these regions. Differences of as little as 1–3 base pairs over the ITS2 region have been reported between morphologically and ecologically distinct species of Didymozoidae Poche, 1907, Echinostomatidae Looss, 1899, Haematoloechidae Odening, 1964, Schistosomatidae Stiles & Hassall, 1898, and Strigeidae Railliet, 1919 (Bowles *et al.* 1995; Morgan & Blair 1995; Anderson & Barker 1998; Agatsuma *et al.* 2001; Bell *et al.* 2001; Snyder & Tkach 2001). These data in addition to that observed in this system further support the conclusion that even a small amount of variation in the rDNA regions among cryptogonimid taxa is significant. This also suggests that the ITS2 rDNA region alone is suitable for resolving operational taxonomic units (OTUs) at the species level within the Cryptogonimidae based on what was observed in this and other cryptogonimid systems (Miller & Cribb 2007b; c; d).

Intriguingly, when viewed in light of the small genetic differences observed between *L. polygongylus* and *L. tibaldiae*, the discovery of two additional genotypes (which differed genetically in similar measure) in various intermediate hosts at Lizard Island suggests that at least two more undescribed species of *Lobosorchis* are present at this locality (unfortunately morphological voucher specimens of the metacercariae of these specimens were not obtained). Although rare, intraspecific variation over the ITS2 rDNA region has been reported for trematodes (Nolan & Cribb 2005), but the combination of lack of intraspecific variation observed in other cryptogonimids and the relatively large morphological disparity coupled with the small genetic differences observed between *L. polygongylus* and *L. tibaldiae* supports the hypothesis that these two metacercarial genotypes represent distinct species. This will need to be confirmed by morphometric and phylogenetic analysis of adult representatives of these genotypes when found.

The conserved nature of the ITS2 region in cryptogonimids (no intraspecific variation has yet been observed within this family despite multiple replicates being sequenced for many taxa and identical sequences have been found at localities separated by over 9,000 kilometres) makes it an ideal candidate for 'barcode region' status as suggested by Nolan and Cribb (2005), at least within the Cryptogonimidae and possibly wider within the superfamily Opisthorchioidea. It is well accepted that a large proportion of the biodiversity in the oceans remains unknown (Knowlton 1993; Reaka-Kudla 1997; Barber & Boyce 2006), particularly the cryptic parasite fauna (Cribb 1998), so the discovery of these two additional genotypes that probably represent distinct species is not surprising. Because the ITS2 rDNA region appears to be an ideal biomarker with the ability to resolve species level differences, sequencing of this region from putative cryptogonimid species recovered from their first or second intermediate hosts in addition to adults will assist future studies in elucidating life-cycles (Cribb *et al.* 1998; Nolan & Cribb 2004) and in exploring the biodiversity of the system within specific localities.

Biogeographic and host distribution

The identical rDNA sequences (ITS and LSU) obtained from specimens of *L. polygongylus* from the Great Barrier Reef and the Maldives, indicates that this species has a biogeographic range of over 9,600 kilometres, similar to that observed in other cryptogonimids (e.g. *Caulanus thomasi* and *Retrovarium brooksi* Miller & Cribb, 2007) reported recently (Miller & Cribb 2007b; c). How these parasites have dispersed so widely despite their limited vagility is a central question in marine trematode biogeography. Future population genetic studies of these cryptogonimids throughout their ranges using various informative mitochondrial and nuclear markers may help to reveal the origin of dispersal or the direction of gene flow within these widely separated Indo-Pacific populations.

In contrast to the wide biogeographic distribution of *L. polygongylus*, the host distribution of this species appears to be restricted to *Lutjanus gibbus*, as this is the only host from which it has been recovered despite the large number of lutjanid individuals and species examined at the same localities (Miller & Cribb 2007b). Despite the close genetic relatedness of *Lobosorchis polygongylus* and *L. tibaldiae*, the hosts they are found in are relatively distantly related; *Lutjanus gibbus* is phylogenetically distant to *Lutjanus carponotatus* and *L. fulviflamma* (Fig. 5), the hosts from which *Lobosorchis tibaldiae* was reported (Miller & Cribb 2005; 2007a). Although more work is obviously needed to uncover the hosts harbouring adults of the putative *Lobosorchis* species A and B, which were characterised molecularly here, we predict that, based on the host distributions of *L. polygongylus* and *L. tibaldiae*, they will be species of *Lutjanus*.

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