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Gynichthys diakidnus n. g., n. sp. (Digenea: Cryptogonimidae) from the grunt *Plectorhinchus gibbosus* (Lacépède, 1802) (Perciformes: Haemulidae) off the Great Barrier Reef, Australia

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Abstract *Gynichthys diakidnus* n. g., n. sp. (Digenea: Cryptogonimidae) is described from the fish *Plectorhinchus gibbosus* (Lacépède) (Perciformes: Haemulidae) off Heron and Lizard Islands on the Great Barrier Reef, Australia. The monotypic *Gynichthys* n. g. is distinguished from all other cryptogonimid genera by the combination of a fusiform body, the lack of oral spines, a forebody that occupies approximately half or more of the body length, a deeply lobed ovary, opposite to slightly oblique testes, a seminal vesicle that is confined mainly in the forebody and the presence of multiple gonotyls in the form of two small slightly muscular pores or pseudosucker-like structures in the mid-line well anterior to the ventral sucker. Bayesian inference analysis of LSU rDNA data revealed that *G. diakidnus* n. sp. grouped relatively distant to species of the cryptogonimid genus *Oligogonotylus* Watson, 1976, which also have multiple gonotyls, suggesting that the presence of multiple gonotyls is homoplasious and has thus at least evolved twice in the family. The secondary structure of the internal transcribed spacer 2 (ITS2) rDNA region was inferred for *G. diakidnus* using minimum free energy and homology modelling algorithms. A four helix model was inferred with helices I and IV being relatively short (<30 nucleotides) and helix three being the longest; this structure is homologous with that observed for other digeneans and eukaryotes in general.

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Introduction

Plectorhinchus gibbosus (Lacépède) is a relatively large haemulid that is widely distributed throughout the Indo-West Pacific from the Red Sea to South Africa in the west, north to the Ryukyu Islands, south to Australia and east to the Caroline and Mariana Islands of Micronesia (Froese & Pauly, 2009). Its diet consists mainly of benthic macroinvertebrates (Kulbicki et al., 2005), but, like other haemulids, individuals prey opportunistically on teleosts, making them susceptible to infection by cryptogonimid trematodes. This species has recently been reported harbouring four species of the Cryptogonimidae Ward, 1917 (Platyhelminthes: Digenea) from three genera, *Beluesca littlewoodi* Miller & Cribb, 2007, *B. longicolla* Miller & Cribb, 2007, *Chelediadema marjoriae* Miller & Cribb, 2007 and *Siphoderina grunnitus* Miller & Cribb, 2008 (Miller & Cribb, 2007c, 2008). The only other digenetic trematode reported from this host is the lecithasterid *Weketrema hawaiiense* (Yamaguti, 1970) by Bray & Cribb (2001) off the Great Barrier Reef, Australia. Here

we describe and characterise a new cryptogonimid genus and species, *Gynichthys diakidnus* n. g., n. sp., recovered from *P. gibbosus* off Heron and Lizard Islands on the Great Barrier Reef (GBR) using a combined morphological and molecular approach.

We augment our morphologically based taxonomic approach to the taxon recovered here with an analysis of genetic data from the large subunit (LSU) nuclear ribosomal DNA region with the aim of exploring the integrity of the new species and its phylogenetic relationships with other cryptogonimid taxa. The LSU rDNA data obtained for the specimens reported here were aligned with data available from previously reported cryptogonimids (Miller & Cribb, 2007a, b, c, 2008; Miller et al., 2009; Olson et al., 2003; Razo-Mendivil et al., 2008) and analysed using Bayesian inference analysis to explore intergeneric relationships between these species. In addition, we infer the secondary structure of the internal transcribed spacer 2 (ITS2) rDNA region to explore whether the structure is homologous with that reported for other digeneans and eukaryotes in general.

Materials and methods

Host and parasite collection

Individuals of the fish *Plectorhinchus gibbosus* were collected by spear from Heron Island (23°26′S; 151°54′E) in the southern GBR and Lizard Island (14°40′S; 145°27′E) in the northern GBR. Fish were killed by neural pithing and the intestine immediately removed, washed in vertebrate saline (0.85%) and examined for endohelminths. Digeneans were collected live and immediately fixed in hot 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.

Morphological samples

Preserved digenean specimens for morphological analysis were placed in Mayer's haematoxylin for staining. The specimens were overstained and then destained by placing them in 1% acid solution. Stained specimens were then dehydrated through a graded series of ethanol for at least half an hour at each dehydration step, cleared in methyl salicylate and mounted in Canada balsam. Drawings were made with the aid of a drawing tube on an Olympus BH-2 microscope. All measurements were made using an ocular micrometer and are quoted in micrometres with the mean followed by the range in parentheses. For two-dimensional measurements, length is given before breadth. Type-specimens were deposited in the Queensland Museum (QM), Brisbane, Australia. Line drawings of the holotype of *Gynichthys diakidnus* were deposited in MorphBank.

Molecular sample processing

Specimens for 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 & Cribb (2007b, c). The consensus sequences for each taxon utilised 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.

Comparative DNA analyses

The LSU rDNA region for *Gynichthys diakidnus* was aligned with data reported for species of the cryptogonimid genera *Beluesca* Miller & Cribb, 2007, *Caecincola* Marshall & Gilbert, 1905, *Caulanus* Miller & Cribb, 2007, *Chelediadema* Miller & Cribb, 2007, *Latuterus* Miller & Cribb, 2007, *Lobosorchis* Miller & Cribb, 2005, *Mitotrema* Manter, 1963, *Neometadena* Hafeezullah & Siddiqi, 1970, *Oligogonotylus* Watson, 1976, *Retrovarium* Miller & Cribb, 2007, *Siphodera* Linton, 1910 and *Siphoderina* Manter, 1934 by Miller & Cribb (2007a, b, c, 2008), Miller et al. (2009), Olson et al. (2003) and Razo-Mendivil et al. (2008) for comparative purposes and to explore levels of intergeneric variation. The LSU rDNA fragments for species of *Oligogonotylus* deposited on GenBank by Razo-Mendivil et al. (2008) overlap the LSU region for the remainder of the cryptogonimid taxa listed above by only approximately 440 bp, so two LSU alignments were constructed for analysis. The first alignment included approximately 860 bp from the taxa listed above, but omitted species of *Oligogonotylus*. The second, shorter alignment, was only approximately 440 bp long and included all of the taxa listed above

and species of *Oligogonotylus*. These two alignments were analysed independently because the larger dataset included more characters, allowing for a robust inference of the generic status and integrity of *Gynichthys*, whereas the shorter dataset allowed for phylogenetic comparison of species of *Gynichthys* and *Oligogonotylus*, the only taxa with multiple gonotyls for which genetic data are available for comparative analysis. The two LSU rDNA datasets 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); and 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 alignment of the LSU datasets were edited, the ends of each fragment were trimmed to match the shortest sequence in the alignment. Bayesian inference analysis of the LSU datasets were performed using MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003) to explore relationships between these taxa. Modeltest version 3.7 (Posada & Crandall, 1998) was used to estimate the best substitution model for the LSU datasets. Bayesian inference analysis was conducted on the LSU rDNA dataset not containing species of *Oligogonotylus* using the TVM+I+G model and on the LSU dataset including species of *Oligogonotylus* using the GTR+I+G model predicted as the best estimators by the Akaike Information Criterion (AIC) in Modeltest. Bayesian inference analyses were 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 summarised using the parameters 'sump burnin = 3000' and 'sumt burnin = 3000'. These 'burnin' parameters were chosen because the log likelihood scores 'plateaued' well before 300,000 replicates in the Bayesian inference analyses.

ITS2 rDNA secondary structure modelling

The secondary structure of the ITS2 rDNA region for *Gynichthys diakidnus* was predicted using minimum free energy folding algorithms with Mfold software version 3.2 (Zuker, 2003) and by homology modelling using the ITS2 database (Selig et al., 2008). The ITS2 sequence was treated as linear and the folding temperature set at 37°C for analysis using Mfold. The ITS2PAM50 matrix was used to predict the secondary structure of *G. diakidnus* using the ITS2 database. The predicted folding results from the Mfold and ITS2 database analyses were viewed with Pseudoviewer version 3 (Byun & Han, 2006).

Family Cryptogonimidae Ward, 1917

Gynichthys n. g.

Diagnosis

Body fusiform, longer than wide, widest between ventral sucker and intestinal bifurcation; length: width ratio 1:0.36–0.44. Tegument armed with small spines. Eyespot pigment present in anterior forebody between ventral sucker and pharynx. Oral sucker nearly spherical, without enlarged oral spines, opens terminally. Ventral sucker unspecialised. Oral/ventral sucker width ratio 1:0.47–0.68. Forebody occupies *c*.50–60% of body length. Prepharynx short. Oesophagus short. Intestinal bifurcation slightly posterior to pharynx. Caeca blind, terminate close to posterior end of body. Testes 2, opposite to slightly oblique, in anterior hindbody. Cirrus and cirrus-sac absent. Seminal vesicle tubular, predominantly in forebody, extends well anterior to ventral sucker. Common genital pore immediately anterior to ventral sucker. Multiple gonotyls present as 2 small slightly muscular pores or pseudosucker-like structures in mid-line well anterior to ventral sucker; each gonotyl separated by approximate diameter of ventral sucker. Ovary deeply lobed, immediately anterior and adjacent to testes. Laurer's canal present. Seminal receptacle saccular, dorsal and dextral or sinistral to seminal vesicle, between ovary and ventral sucker, may reach slightly anterior to ventral sucker. Vitelline follicles in 2 lateral groups, almost confluent in mid-line, extend from level of anterior half of ovary to midway between ventral sucker and intestinal bifurcation. Uterine coils restricted to hindbody, extend from posterior end of body to ventral sucker.

Excretory vesicle Y-shaped; arms extend ventral to caeca in forebody and terminate anterior to pharynx; excretory pore terminal at posterior end of body. Type-species: *G. diakidnus* n. sp.

Etymology: The name *Gynichthys* is derived from the Greek *gyne*, meaning woman or female and the Greek *ichthys* meaning fish. It is proposed in reference to the reef off Mermaid Beach, Lizard Island, where the type-species of this genus was discovered. It is to be treated as masculine.

Differential diagnosis

Gynichthys n. g. is distinguished from all other cryptogonimid genera by the combination of a fusiform body, the lack of oral spines, a forebody that occupies approximately half or more of the body length, a deeply lobed ovary, opposite to slightly oblique testes, a seminal vesicle that is confined mainly in the forebody and the presence of multiple gonotyls in the form of two small slightly muscular pores or pseudosucker-like structures in the mid-line well anterior to the ventral sucker. Like most cryptogonimid genera, *Gynichthys* is not diagnosed by any single character, but a combination of characters, although the presence of two well-separated gonotyls anterior to the ventral sucker and the forebody occupying half or more of the body length in species of this genus immediately distinguish them from other cryptogonimids.

Gynichthys diakidnus n. sp.

Type-host: *Plectorhinchus gibbosus* (Lacépède) (Haemulidae).
Type-locality: Lizard Island, Great Barrier Reef (14°40′S; 145°27′E), Queensland, Australia.
Other localities: Heron Island, Great Barrier Reef (23°26′S; 151°54′E), Queensland, Australia.
Site: Intestine and pyloric caeca.
Type-material: Holotype QM (G231321), 19 paratypes QM (G231322-G231340).
Prevalence: 1 of 1 (100%) at Lizard Island; 1 of 5 (20%) at Heron Island.
Molecular sequence data: ITS, 3 replicates from Heron Island, 3 replicates from Lizard Island; LSU, 3 replicates from Heron Island, 3 replicates from Lizard Island.
GenBank accession numbers: ITS (FJ907332); LSU (FJ907333).
MorphBank specimen number: 471559.
Etymology: The epithet *diakidnus* is derived from the Greek *di*, meaning two and the Greek *akidnos*, meaning weak, feeble or faint, referring to the two weakly muscular gonotyls found in this species.

Description (Figs. 1–2)

[Based on 20 specimens.] Body fusiform, longer than wide, widest between ventral sucker and intestinal bifurcation, 1071 (808–1376) × 422 (320–560); length/width ratio 1:0.39 (1:0.36–0.44). Eyespot pigment present in anterior forebody between ventral sucker and pharynx. Oral sucker 86 (77–99) × 132 (109–163). Oral spines absent. Ventral sucker 69 (45–86) × 79 (51–94). Oral/ventral sucker width ratio 1:0.6 (1:0.47–0.68). Forebody occupies 52 (48–59)% of body length. Prepharynx 31 (10–38) long. Pharynx 78 (64–93) × 84 (70–99). Ventral sucker/pharynx width ratio 1:1.07 (1:0.93–1.38). Oesophagus 25 (16–48) long. Intestinal bifurcation slightly posterior to pharynx. Intestinal caeca blind, 815 (631–1151) long, terminate close to posterior end of body. Testes 2, opposite to slightly oblique, in anterior hindbody, 118 (88–166) × 116 (86–144). Cirrus and cirrus-sac absent. Seminal vesicle tubular, predominantly in forebody, extends well anterior to ventral sucker. Gonotyls present as 2 small slightly muscular pores or pseudosucker-like structures in mid-line well anterior to ventral sucker; each gonotyl separated by approximate diameter of ventral sucker. Genital pore immediately anterior to ventral sucker. Ovary deeply lobed, immediately anterior and adjacent to testes, 103 (77–157) × 140 (99–170). Laurer's canal present. Seminal receptacle saccular, dorsal and dextral or sinistral to seminal vesicle, between ovary and ventral sucker, may reach slightly anterior to ventral sucker. Vitelline follicles in 2 lateral groups, almost confluent in mid-line, extend from level of anterior half of ovary to midway between ventral sucker and intestinal bifurcation. Uterine coils restricted to hindbody, extend from posterior end of body to ventral sucker. Eggs small, darkly tanned, 19 (13–24) × 10 (8–12). Excretory vesicle Y-shaped, bifurcates dorsal to ovary; arms extend slightly anterior to pharynx, 904 (688–1152) long; excretory pore terminal at posterior end of body.

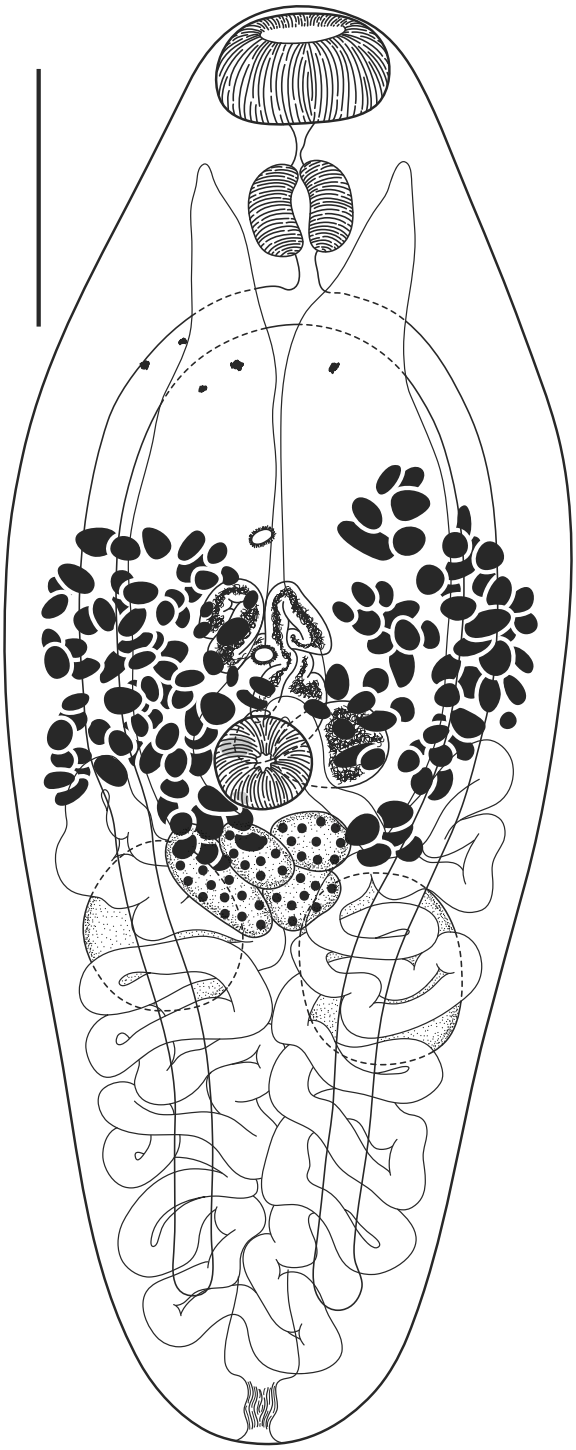

Fig. 1 *Gynichthys diakidnus* n. g., n. sp. from the intestine of *Plectorhinchus gibbosus* off Lizard Island, Great Barrier Reef, Australia. Ventral view of holotype. *Scale-bar*: 200 μm

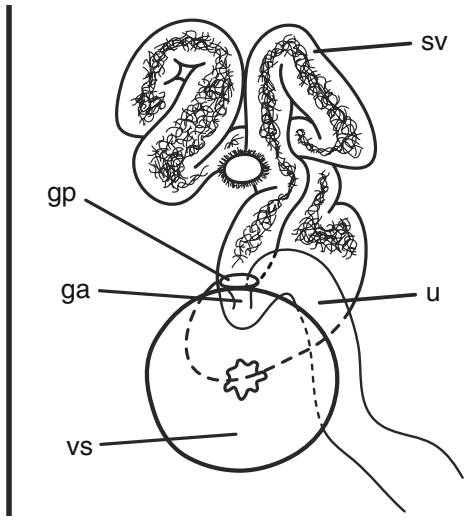

Fig. 2 Terminal genitalia of the holotype of *Gynichthys diakidnus* n. g., n. sp. from the intestine of *Plectorhinchus gibbosus* off Lizard Island, Great Barrier Reef, Australia. Ventral view. *Abbreviations*: ga, genital atrium; gp, genital pore; sv, seminal vesicle; u, uterus; vs, ventral sucker. *Scale-bar*: 200 μm

Comparative DNA analysis

Alignment of the larger LSU rDNA dataset for *Gynichthys diakidnus* n. sp. and the remainder of the cryptogonimid taxa examined (excluding species of *Oligogonotylus*) yielded 863 characters for analysis. No intraspecific variation was observed in *G. diakidnus* over the LSU rDNA region sequenced here. Bayesian inference analysis of this LSU rDNA dataset resulted in a phylogram with *G. diakidnus* forming a poorly-supported clade with *Neometadena ovata* (Yamaguti, 1952) (Fig. 3a). All genera were resolved with strong posterior probability support in this Bayesian analysis, although many intergeneric relationships were not well supported. A well-supported clade was formed between species of *Beluesca*, *Caulanus*, *Latuterus* and *Siphoderina*. Another clade resolved with a high posterior probability was that between the North American freshwater species *Caecincola parvulus* Marshall & Gilbert, 1905 and the Atlantic Ocean inhabiting *Siphodera vinaledwardsii* (Linton, 1901). These taxa were nested closely with Indo-Pacific taxa, but the relationships were not well resolved in this analysis.

Alignment of the second LSU rDNA dataset for *Gynichthys diakidnus* n. sp. and the remainder of the

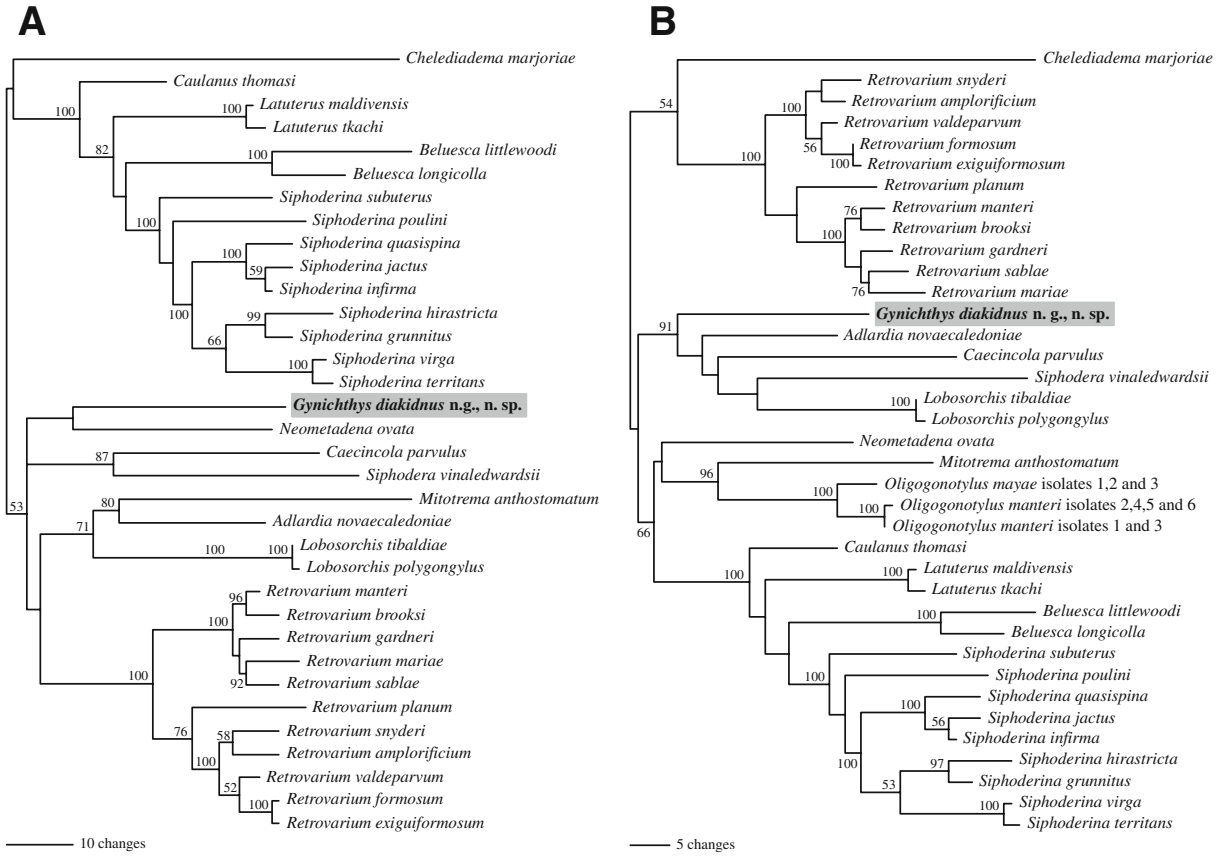

Fig. 3 Relationships between *Gynichthys diakidnus* n. g., n. sp. and the remainder of the cryptogonimid taxa examined here based on Bayesian inference analysis of the LSU rDNA dataset. A. Phylogram based on analysis of the longer LSU rDNA alignment excluding species of *Oligogonotylus*; B. Phylogram based on analysis of the shorter (approx. 440 bp total) LSU rDNA alignment including species of *Oligogonotylus* for comparative purposes. Posterior probabilities are shown at the nodes with values <50 not shown. Phylograms are mid-point rooted

cryptogonimid taxa examined (including species of *Oligogonotylus*) yielded 444 characters for analysis. Bayesian inference analysis of this LSU dataset resulted in a phylogram with taxa possessing multiple gonotyls, *G. diakidnus* and *Oligogonotylus* spp. grouping relatively distant to each other (Fig. 3b). As with the analysis of the larger LSU dataset, all genera were well resolved, although intergeneric relationships were not well supported. Species of *Beluesca*, *Caulanus*, *Latuterus* and *Siphoderina* again formed a strongly supported clade, as in the previous analysis.

Secondary structure of *Gynichthys diakidnus* n. g., n. sp.

The putative secondary structure predicted for the ITS2 rDNA region of *Gynichthys diakidnus* using Mfold software resulted in a four helix model with a calculated free-energy minimum of −96.7 kcal/mol (Fig. 4a). Helices I and IV of this putative model are both relatively short, each consisting of fewer than 30 nucleotides. Helix III was the longest, comprising over 150 nucleotides.

Homology modelling of the ITS2 sequence for *G. diakidnus* on the ITS2 database resulted in the optimal structural being modelled using the template structure of *Caulanus thomasi* Miller & Cribb, 2007 (whose structure is deposited in the database). The predicted ITS2 structure of *G. diakidnus,* using *C. thomasi* as a template, resulted in a high quality model with all helices present (percentages of helix similarity I–IV respectively, 100/95/92/100), an E value of 9.1e-90 and a calculated free energy of −67.80 kcal/mol, according to the ITS2 database.

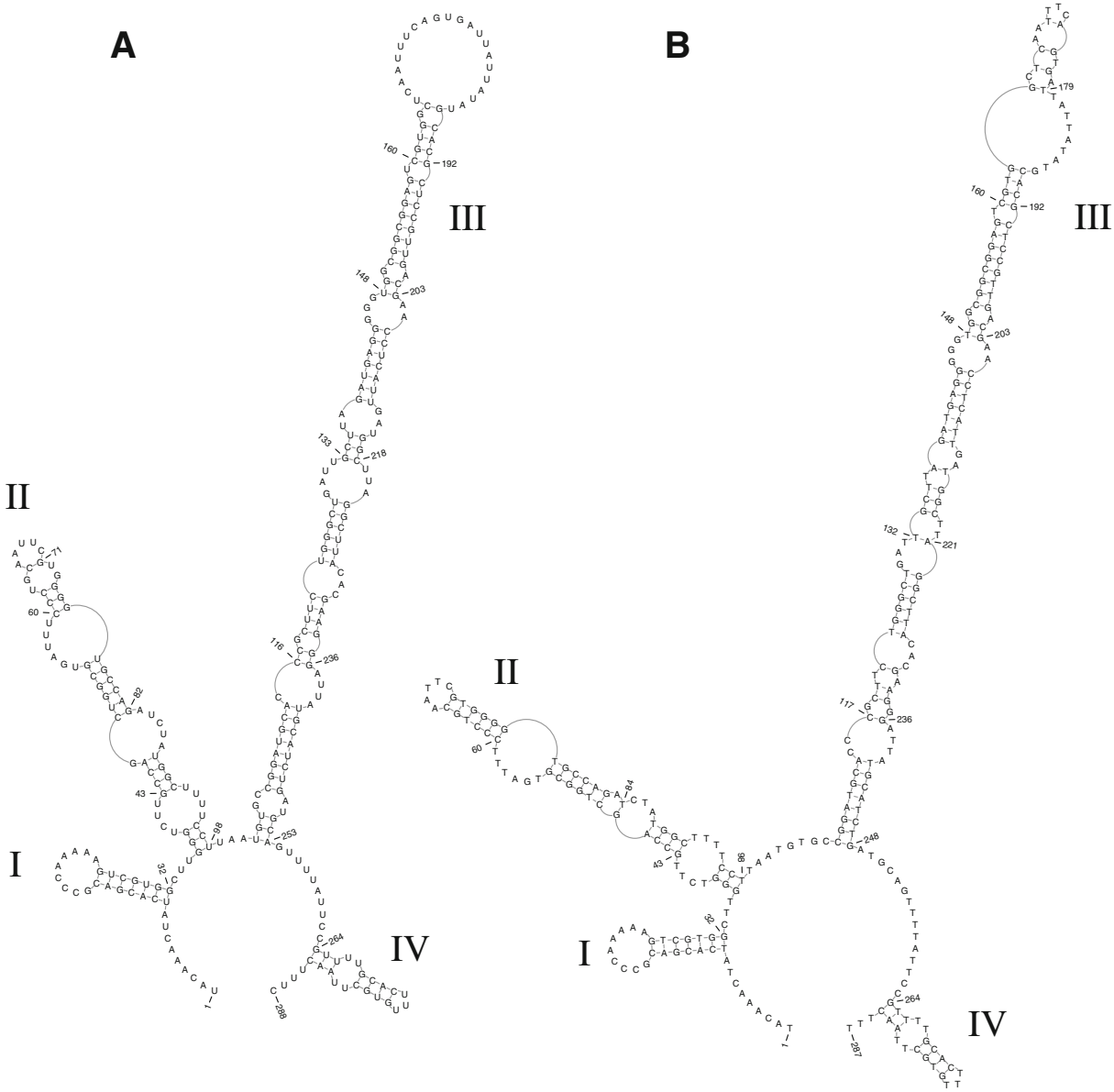

Fig. 4 Inferred secondary structure of the ITS2 rDNA region for *Gynichthys diakidnus* n. g., n. sp. based on: A. minimum free energy modelling using Mfold software; B. Homology modelling on the ITS2 database. Helices one through four are indicated with Roman numerals

The ITS2 secondary structure of *G. diakidnus* predicted using the ITS2 database was very similar to the structure predicted by Mfold (Fig. 4b). Helix II of these two optimal models were the same length, but differed by three nucleotide pairings; helices I and IV were identical in structure between these two models. The most significant secondary structural differences in the inferred models of *G. diakidnus,* using minimum free energy and homology modelling, were found in helix III, which was 10 nucleotides longer in the free energy analysis. Also, the distal end of helix III displayed more pairing in the homology based model than in the minimum free energy predicted structure.

Discussion

Systematics

Gynichthys diakidnus n. g., n. sp. is the only cryptogonimid that has been reported with two small slightly muscular pores or pseudosucker-like gonotyls anterior to the ventral sucker and a forebody that comprises half or more of the body length. These two characters, independent of the combination of other distinguishing morphological features, immediately distinguishes this species from other cryptogonimids. Three other cryptogonimid genera have species with multiple gonotyls, *Multigonotylus* Premvati, 1967, *Oligogonotylus* Watson, 1976 and *Polycryptocylix* Lamothe-Argumedo, 1970. *G. diakidnus* is easily distinguished from the type- and only species of *Multigonotylus*, *M. micropteri* Premvati, 1967, which has more than five gonotyls present as transverse muscular bands that extend from immediately anterior to the ventral sucker to the intestinal bifurcation, testes that are strongly oblique to nearly tandem, a seminal vesicle that is mainly restricted to the hindbody and vitelline follicles that do not extend posteriorly to the ventral sucker; it is also is restricted to North American freshwater fishes. Species of *Oligogonotylus* differ from *G. diakidnus* in that they have more than five gonotyls which are not separated by more than one gonotyl diameter, tandem testes, a seminal vesicle that is restricted mainly to the hindbody and lateral vitelline follicles that are restricted to the hindbody; they are also restricted to cichlid hosts. Species of *Polycryptocylix* are distinguished from *G. diakidnus* in that they distinctly muscular gonotyls, oral spines and a forebody that occupies no more than 40% of the body length.

Seven cryptogonimid genera have species with a forebody that occupies nearly half or more of the body length: *Acanthocollaritrema* Travassos, Freitas & Bührnheim, 1965, *Beluesca*, *Chelediadema*, *Multigonotylus*, *Olmeca* Lamothe-Argumedo & Pineda-López, 1990, *Textrema* Dronen, Underwood & Suderman, 1977 and *Stemmatostoma* Cribb, 1986. Species of *Acanthocollaritrema*, *Beluesca*, *Chelediadema* and *Stemmatostoma* are immediately distinguished from *G. diakidnus* because they all have oral spines and lack multiple gonotyls. The type- and only species of *Olmeca*, *O. laurae* Lamothe-Argumedo & Pineda-López, 1990, differs from *G. diakidnus* by having strongly oblique testes, a uterus that extends well into the forebody and vitelline follicles that do not extend posteriorly to the ventral sucker. The type- and only species of *Textrema*, *T. hopkinsi* Dronen, Underwood & Suderman, 1977, is distinguished from *G. diakidnus* by having tandem testes, a seminal vesicle that is confined mainly to the hindbody, vitelline follicles that extend to the level of the pharynx and the presence of a single, muscular gonotyl with spine- or rod-like structures arising from the anterior wall of the ventrogenital sac.

Relationships between *Gynichthys diakidnus* n. sp. and other cryptogonimid taxa

Specimens of *Gynichthys* n. g. have not been recovered from any of the many lutjanid taxa examined in surveys (Miller & Cribb, 2007a, c) off Australia, so it is likely that specimens of this genus do not infect lutjanids, at least on the Great Barrier Reef. It is certainly possible that other species of haemulid harbour specimens of *Gynichthys*, as much of the haemulid fauna of the GBR has yet to be explored comprehensively (for the numbers of haemulids sampled on the GBR, see Miller & Cribb, 2007c). The discovery of *G. diakidnus* in *Plectorhinchus gibbosus* makes it the fifth cryptogonimid species reported from this haemulid, which rivals the species richness observed inhabiting lutjanids in the Indo-West Pacific (Miller & Cribb, 2007a, b, c, 2008).

Phylogenetic analysis of the taxa examined here showed that *G. diakidnus* nested within a clade of cryptogonimids known exclusively from lutjanids (Fig. 3). These results agree with previous analyses showing that other species of the Cryptogonimidae known only from haemulids nested closely with species known only from lutjanids (Miller & Cribb, 2007a, c). Species of *Oligogonotylus*, which, similar to *G. diakidnus,* have multiple gonotyls and lack oral spines, grouped relatively distant to *G. diakidnus* in Bayesian inference analysis, suggesting that the presence of multiple gonotyls is homoplasious and has evolved at least twice in the evolutionary history of the family. The phylogenetic disparity observed between species of *Gynichthys* and *Oligogonotylus* also reflects differences in their biogeographical and host distributions; *Oligogonotylus* spp. are restricted

to cichlids in or near freshwaters in southern Mexico and Central America, whereas *G. diakidnus* is found in an Indo-West Pacific reef-associated fish.

Biogeographical distribution

Gynichthys diakidnus n. sp. is apparently widely distributed on the Great Barrier Reef, as it was recovered from localities in the northern and southern regions that are separated by over 1,100 kilometres. Given that *Plectorhinchus gibbosus* occurs widely in the Indo-West Pacific, it is possible that *G. diakidnus* is also distributed widely throughout the Indo-West Pacific, particularly in view of the wide biogeographical distributions reported recently for other cryptogonimid taxa in this region (Miller & Cribb, 2007a, c; Miller et al., 2009).

Inferred secondary structure of the ITS2 rDNA region

The inferred secondary structure of the ITS2 rDNA region for *G. diakidnus,* using both minimum free energy and homology modelling, agrees with the core four helix domain structure (with helix III being the longest) that has been recently identified as common to almost all eukaryote taxa (Coleman, 2003, 2007; Schultz et al., 2005). The optimal secondary structures for *G. diakidnus* inferred here also agree with the four domain model of the ITS2 rDNA region using minimum free energy folding algorithms reported by Morgan & Blair (1998) for other digeneans. All of the helices in the models for *G. diakidnus* are of similar length and structure to the general digenean structure reported by Morgan & Blair (1998).

The highly conserved core secondary structure of the ITS2 rDNA region makes using inferred structure data in addition to sequence data a 'double-edged' tool for use in systematic and taxonomic studies (Coleman, 2003, 2007, 2009; Schultz et al., 2005, 2006). Secondary structure data can be used to generate reliable alignments between divergent ITS2 sequences that cannot be aligned based on sequence data alone (Schultz et al., 2005; Seibel et al., 2008; Siebert & Backofen, 2005; Wolf et al., 2007), resulting in more robust phylogenetic inferences based on ITS data. It has also been shown recently that differences of as little as a single compensatory base change in the secondary structure pairing of the ITS2 region is strongly correlated with sexual incompatibility and is, therefore, indicative of distinct "biological species" within a system (Coleman, 2009; Müller et al., 2007; Wolf et al., 2005). While it has been reported recently that differences of as little as one nucleotide change in the ITS2 sequence alone can be used to distinguish closely related species of digeneans (Nolan & Cribb, 2005), it is clear that also incorporating analyses of secondary structure along with analysis of raw sequence data will certainly aid platyhelminth taxonomy and systematics. Given that many species of the Cryptogonimidae are morphologically similar or cryptic, using rDNA secondary structure data in addition to sequence data will help resolve many of the taxonomically difficult groups of taxa within the family, while also possibly helping to elucidate phylogenetic relationships among the group as a whole.

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