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Two species of *Phyllodistomum* Braun, 1899 (Trematoda: Gorgoderidae) from Moreton Bay, Australia

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Abstract Two species of the trematode genus *Phyllodistomum* Braun, 1899 (Gorgoderidae) are reported infecting teleost fishes from Moreton Bay, Queensland, Australia. *Phyllodistomum hyporhamphi* n. sp. is described from two species of garfishes (Hemiramphidae), *Hyporhamphus regularis ardelio* (Whitley) and *H. australis* (Steindachner). The new species differs from other marine species of *Phyllodistomum* in possessing a forebody length less than half that of the body, a body length to width ratio < 4:1, an oral sucker width to ventral sucker width ratio > 1:1 and < 2:1, 7–9 strong, marginal undulations on each side of the body and large, slightly lobed vitelline masses. *Phyllodistomum pacificum* Yamaguti, 1951 is reported, for the first time in Australian waters, from *Pantolabus radiatus* (MacLeay) (Carangidae). The new material agrees closely with the original description of *P. pacificum*, in *Carangoides equula* (Temminck & Schlegel) off Hamazima, Mie Prefecture, Japan, although the specimens from Moreton Bay are larger than those of the original description (4,575–5,338 × 1,111–1,328 *vs* 2,200–3,100 × 570–930 μm). *Cetiotrema carangis* (Manter, 1947) Manter, 1970 is found to be a synonym of *Cetiotrema carangis* (MacCallum, 1913) Williams & Bunkley-Williams, 1996 and the species is formally moved to *Phyllodistomum* as *P. carangis* (MacCallum, 1913) n. comb. Phylogenetic analyses of 28S rDNA data showed that the six marine species of *Phyllodistomum* for which molecular data are available form a strongly-supported clade.

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Introduction

Phyllodistomum Braun, 1899 is the most speciose gorgoderid genus, with species infecting marine and freshwater teleost fishes globally. The phylogenetic analysis of Cutmore et al. (2013) demonstrated that species of *Phyllodistomum* form several different clades, with those infecting marine fishes forming a clade distinct from freshwater species. As the type-species of *Phyllodistomum* was described from a freshwater fish, Cutmore et al. (2013) speculated that the species infecting marine fishes should ultimately be considered a distinct genus. Species of *Phyllodistomum* from marine fishes infect the urinary bladder and ureters, with 27 species reported from fishes of 17 teleost families (Ho et al., 2014). Reports

incorporating molecular data have shown that marine species of this genus infect at least fishes of the Hemiramphidae, Labridae, Mullidae and Serranidae (Cutmore et al., 2013; Ho et al., 2014), and, although there are no molecular data for the majority of species, there is little evidence to doubt the validity of the other reported marine host families. The richness of this genus is likely to be far greater than presently known, because the infection location means species of this group are seldom sought specifically and are often only collected by chance. Here we report two *Phyllodistomum* species from Moreton Bay infecting fishes of two teleost families, the Carangidae and the Hemiramphidae.

Materials and methods

Specimen collection and morphological analysis
Teleosts were collected by seine and tunnel netting in Moreton Bay, Queensland, Australia. Fish were euthanased and examined for trematodes as described by Cribb & Bray (2010). The urinary bladder was removed from the body cavity, placed in saline solution (0.85% NaCl solution) and opened. Host bodies were then soaked in saline solution for a minimum of 30 min, the supernatant decanted and the resulting sediment examined under a stereomicroscope. Trematodes were washed in vertebrate saline, fixed by pipetting into near-boiling saline, and preserved in 70% ethanol for parallel morphological and molecular characterisation. Some individual worms were processed for both morphological and molecular analysis (hologenophores *sensu* Pleijel et al., 2008).

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

microscope and drawing tube. Type- and voucher specimens are lodged in the Queensland Museum (QM), Brisbane. Specimens representing *Phyllodistomum* sp. 2 of Cutmore et al. (2013) were borrowed from the QM for morphological characterisation.

Molecular sequencing and phylogenetic analysis
Specimens for molecular analysis were processed according to the protocols used by Sun et al. (2014) and Wee et al. (2017). As recommended by Blasco-Costa et al. (2016), three genetic markers were sequenced in this study. The complete ITS2 rDNA region was amplified and sequenced using the primers 3S (Morgan & Blair, 1995) and ITS2.2 (Cribb et al., 1998), the partial D1-D3 28S rDNA region using LSU5 (Littlewood, 1994), 300F (Littlewood et al., 2000), ECD2 (Littlewood et al., 1997) and 1500R (Snyder & Tkach, 2001) and the partial *cox*1 region using Dig_cox1Fa (Wee et al., 2017) and Dig_cox1R (Wee et al., 2017). Geneious® version 10.2.3 (Kearse et al., 2012) was used to assemble and edit contiguous sequences and the start and end of the ITS2 rDNA region were determined by annotation through the ITS2 Database (Keller et al., 2009; Ankenbrand et al., 2015) using the 'Metazoa' model.

ITS2 rDNA sequence data generated during this study were aligned with those of other adult marine species of *Phyllodistomum* (Table 1) using MEGA version 6 (Tamura et al., 2013) and pairwise differences estimated using the following conditions: "variance estimation method = none", "model/method = no. of differences" and "substitutions to include = d: transitions + transversions".

The partial 28S rDNA sequences generated during this study were aligned with sequences of related species from GenBank using MUSCLE version 3.7 (Edgar, 2004) run on the CIPRES portal (Miller et al., 2010), with ClustalW sequence weighting and UPGMA clustering for iterations 1 and 2. The resultant alignment was refined by eye using MESQUITE (Maddison & Maddison, 2017) and the ends of each fragment were trimmed to match the shortest sequence. Bayesian inference and maximum likelihood analyses of the 28S rDNA dataset were conducted to explore relationships among these taxa. Bayesian inference analysis was performed using MrBayes version 3.2.6 (Ronquist et al., 2012) and maximum likelihood analysis using RAxML version 8.2.10 (Stamatakis, 2014), both run on the CIPRES

Table 1 Collection data and GenBank accession numbers for gorgoderid species analysed in this study

Species	Host species	GenBank ID		Reference
		ITS2 rDNA	28S rDNA	
Phyllodistomum hoggettae Ho, Bray, Cutmore, Ward & Cribb, 2014	*Plectropomus leopardus* (Lacepède)	KF013148	KF013191	Cutmore et al. (2013)
Phyllodistomum hyporhamphi n. sp.	*Hyporhamphus australis* (Steindachner)	KF013150	KF013190	Cutmore et al. (2013)
Phyllodistomum pacificum Yamaguti, 1951	*Pantolabus radiatus* (MacLeay)	MG845601	MG845599	This study
Phyllodistomum vaili Ho, Bray, Cutmore, Ward & Cribb, 2014	*Mulloidichthys flavolineatus* (Lacepède)	KF013155	KF013173	Cutmore et al. (2013)
Phyllodistomum vaili	*Mulloidichthys vanicolensis* (Valenciennes)	KF013164	KF013187	Cutmore et al. (2013)
Phyllodistomum sp. 4	*Cephalopholis urodeta* (Forster)	KF013151	KF013183	Cutmore et al. (2013)
Phyllodistomum sp. 4	*Epibulus insidiator* (Pallas)	KF013165	KF013179	Cutmore et al. (2013)
Phyllodistomum sp. 5	*Cephalopholis boenak* (Bloch)	KF013161	KF013175	Cutmore et al. (2013)
Phyllodistomum sp. 5	*Epibulus insidiator* (Pallas)	KF013147	KF013181	Cutmore et al. (2013)
Asexual stages	*Lioconcha castrensis* (Linnaeus)	–	KF013193	Cutmore et al. (2013)
Outgroup taxa				
Gorgodera cygnoides (Zeder, 1800)	*Rana ridibunda* Pallas	–	AY222264	Olson et al. (2003)
Phyllodistomum brevicecum Steen 1938	*Umbra limi* (Kirtland)	–	KC760208	Razo-Mendivil et al. (2013)
Phyllodistomum lacustri (Loewen, 1929)	*Ameiurus melas* (Rafinesque)	–	EF032692	Curran et al. (2006)
Phyllodistomum spinopapillatum Pérez-Ponce de León, Pinacho-Pinacho, Mendoza-Garfias & García-Varela, 2015	*Profundulus punctatus* Ahl	–	KM659382	Pérez-Ponce de León et al. (2015)

portal. The best nucleotide substitution model was estimated using jModelTest version 2.1.10 (Darriba et al., 2012). The Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) predicted the GTR+Γ and TPM3uf+Γ models as the best estimators, respectively; Bayesian inference and maximum likelihood analyses were conducted using the closest approximation to these models. Nodal support in the maximum likelihood analysis was estimated by performing 100 bootstrap pseudoreplicates. Bayesian inference analysis was run over 10,000,000 generations (ngen = 10,000,000) with two runs each containing four simultaneous Markov Chain Monte Carlo (MCMC) chains (nchains = 4) and every 1,000th

tree saved. Bayesian inference analysis used the following parameters: nst = 6, rates = gamma, ngammacat = 4, 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 = 3,000 and sumt burnin = 3,000. Species of *Phyllodistomum* and *Gorgodera* Looss, 1899 that infect freshwater fishes and amphibians were designated as functional outgroup taxa, *sensu* Cutmore et al. (2013).

Results

Gorgoderid trematodes were collected from three species of teleost fishes, *Hyporhamphus australis* (Steindachner), *Hyporhamphus regularis ardelio* (Whitley) and *Pantolabus radiatus* (MacLeay). Specimens from *H. australis* were previously characterised genetically by Cutmore et al. (2013) as part of a phylogenetic analysis of the family, in which they were reported as an undescribed species, *Phyllodistomum* sp. 2. These specimens, together with those from *H. regularis ardelio*, represent a species new to science which is formally described here. The specimens from *Pantolabus radiatus* are identified as *Phyllodistomum pacificum* Yamaguti, 1951. This is the first report of this species from *Pantolabus radiatus* and the first from Australian waters; novel molecular data were generated for this species and a description based on the new specimens is provided.

Family Gorgoderidae Looss, 1899
Genus *Phyllodistomum* Braun, 1899

Phyllodistomum hyporhamphi n. sp.
Syn. *Phyllodistomum* sp. 2 of Cutmore et al. (2013)

Type-host: *Hyporhamphus regularis ardelio* (Whitley), river garfish (Beloniformes: Hemiramphidae).
Other host: *Hyporhamphus australis* (Steindachner), eastern sea garfish (Beloniformes: Hemiramphidae).
Type-locality: Western Moreton Bay, Queensland, Australia (27°22′S, 153°13′E).
Other locality: Eastern Moreton Bay, Queensland, Australia (27°26′S, 153°24′E).
Site in host: Urinary bladder.
Type-material: Holotype (QM G236796) and 15 paratypes (QM G234204–10, G234212, G236797–03; one hologenophore QM G236800.
Representative DNA sequences: KF013190 (28S rDNA) and KF013150 (ITS2 rDNA) of Cutmore et al. (2013) ex *H. australis*; ITS2 rDNA, three replicates ex *H. regularis ardelio* (one submitted to GenBank: MG845600); *cox*1 mtDNA, one replicate ex *H. regularis ardelio* (submitted to GenBank: MG845602).
ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Phyllodistomum hyporhamphi* n. sp. is urn:lsid:zoobank.org:act:AAE30852-A3DC-404F-9228-69DB40539AEC.
Etymology: This species is named after the genus of fishes it infects, *Hyporhamphus*.

Description (Figs. 1A, 2, 3)

[Based on 16 whole-mounted gravid specimens.] Body spatulate, widest at level of ovary, 1,830–5,080 × 874–2,928 (3,402 × 1,716). Forebody narrow and tapering, 662–1,912 (1,240) long, occupying 31–45 (37)% of body length. Hindbody with 7–9 strong marginal undulations on each side produced by distinct lateral muscular loops, most pronounced in largest specimens. Body length to width ratio 1.72–2.45:1 (2.05:1); ratio smallest in most gravid specimens. Oral sucker subspherical, opens subterminally, with consistent shallow depression anterior to aperture, 259–529 × 198–449 (410 × 346). Ventral sucker far smaller than oral sucker, 135–286 × 145–310 (224 × 235). Oral sucker width to ventral sucker width ratio 1.15–1.80:1 (1.51:1). Intestinal bifurcation midway between oral and ventral suckers. Oesophagus 116–481 (287) long. Caeca blind, slightly sinuous, terminating 344–799 (580) from posterior extremity.

Testes entire, smooth, oblique, in mid-hindbody; anterior testis 135–429 × 145–433 (229 × 264); posterior testis 161–535 × 149–435 (268 × 267). Seminal vesicle saccular, bipartite, with chambers of similar size; dorsal, postero-dorsal chamber dorsal to genital pore, constricting anteriorly to antero-ventral chamber; antero-ventral chamber anterior to genital pore. Cirrus-sac absent. Prostatic chamber and surrounding cells ventral and posterior to antero-ventral chamber of seminal vesicle. Ejaculatory duct short. Genital pore median, 65–283 (192) anterior to ventral sucker.

Ovary subspherical, almost entire but with slight irregular indentations, posterior to ventral sucker and lateral to anterior testis, dextral in 6 specimens and sinistral in 10, 140–328 × 13–440 (231 × 231). Oviduct expands to form distinct chamber prior to entering Mehlis' gland. Vitelline masses two, entire with slightly irregular outline, immediately posterior to ventral sucker; left mass 100–281 × 129–290 (197 × 185); right mass 114–301 × 125–322 (198 × 194).

Fig. 1 *Phyllodistomum* spp. from Moreton Bay. A, *Phyllodistomum hyporhamphi* n. sp. ex *Hyporhamphus regularis ardelio*, whole worm (holotype, QM G236796); B, *Phyllodistomum pacificum* Yamaguti, 1951 ex *Pantolabus radiatus*, whole worm (voucher, QM G236804). *Scale-bars*: 1000 μm
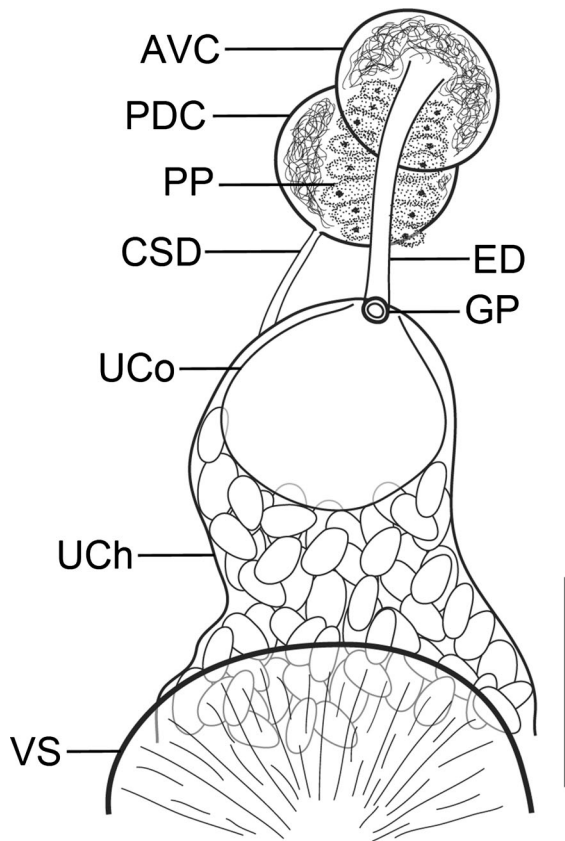
Fig. 2 *Phyllodistomum hyporhamphi* n. sp., terminal genitalia (holotype, QM G236796). *Abbreviations*: AVC, antero-ventral chamber of seminal vesicle; CSD, common sperm duct; ED, ejaculatory duct; GP, genital pore; PDC, postero-dorsal chamber seminal vesicle; PP, pars prostatica; UCh, uterine chamber; UCo, uterine constriction; VS, ventral sucker. *Scale-bar*: 100 μm

Laurer's canal opens dorsally at level of vitelline masses, opposite ovary. Canalicular seminal receptacle absent. Uterus extensive in hindbody, inter- and extracaecal; coils extending posteriorly beyond posterior testis or to just beyond intestinal caeca; uterine chamber distinct and prominent dorsal to ventral sucker and genital pore, filled with eggs in gravid specimens, constricted prior to genital pore, up to 736 in diameter in fully gravid specimens. Eggs develop *in utero*; largest eggs 33–49 × 17–26 (39 × 21).

Excretory vesicle tubular, extending anteriorly to level of ovary; main collecting ducts extending laterally between vitelline masses and anterior testis and ovary. Excretory pore dorsally subterminal, close to posterior end of body.

Phyllodistomum pacificum Yamaguti, 1951

Type-host: *Carangoides equula* (Temminck & Schlegel), whitefin trevally (Perciformes: Carangidae).
Type-locality: Off Hamazima, Japan.
New host: *Pantolabus radiatus* (MacLeay), Fringefin trevally (Perciformes: Carangidae).
New locality: Western Moreton Bay, Queensland, Australia (27°22′S, 153°13′E).
Site in host: Urinary bladder.
Voucher material: Five voucher specimens (QM G236804–8, including one hologenophore QM G236805).
Representative DNA sequences: 28S rDNA, one replicate (submitted to GenBank: MG845599); ITS2 rDNA, one replicate (submitted to GenBank: MG845601); *cox*1 mtDNA, one replicate (submitted to GenBank: MG845603).

Description (Fig. 1B)

[Based on 4 whole-mounted gravid specimens.] Body lanceolate, widest at level of anterior testis, tapering anteriorly and posteriorly, 4,575–5,338 ×1,111–1,328 (4,950 × 1,226). Forebody 1,443–1,960 (1,709) long, occupying 35–37 (36)% of body length. Hindbody with 4–5 weak marginal undulations on each side, produced by distinct lateral muscular loops. Body length to width ratio 3.44–4.52:1 (3.94:1). Oral sucker subspherical, opens subterminally, with consistent shallow depression anterior to aperture, 312–410 × 310–384 (356 × 337). Ventral sucker slightly smaller than oral sucker, 260–340 × 268–321 (293 × 288). Oral sucker width to ventral sucker width ratio 1.13–1.21:1 (1.17:1). Intestinal bifurcation between oral and ventral suckers, closer to oral than ventral. Oesophagus 301–525 (436) long. Caeca simple, blind, terminating 628–791 (713) from posterior extremity.

Testes slightly lobed, oblique, in mid-hindbody; anterior testis 320–489 × 247–423 (396 × 307); posterior testis 418–598 × 272–487 (501 × 353). Seminal vesicle saccular, bipartite; postero-dorsal chamber large, dorsal to genital pore, constricting anteriorly to antero-ventral chamber; antero-ventral chamber anterior to genital pore. Prostatic chamber and surrounding cells ventral to postero-dorsal chamber of seminal vesicle. Genital pore median, 196–301 (256) anterior to ventral sucker.
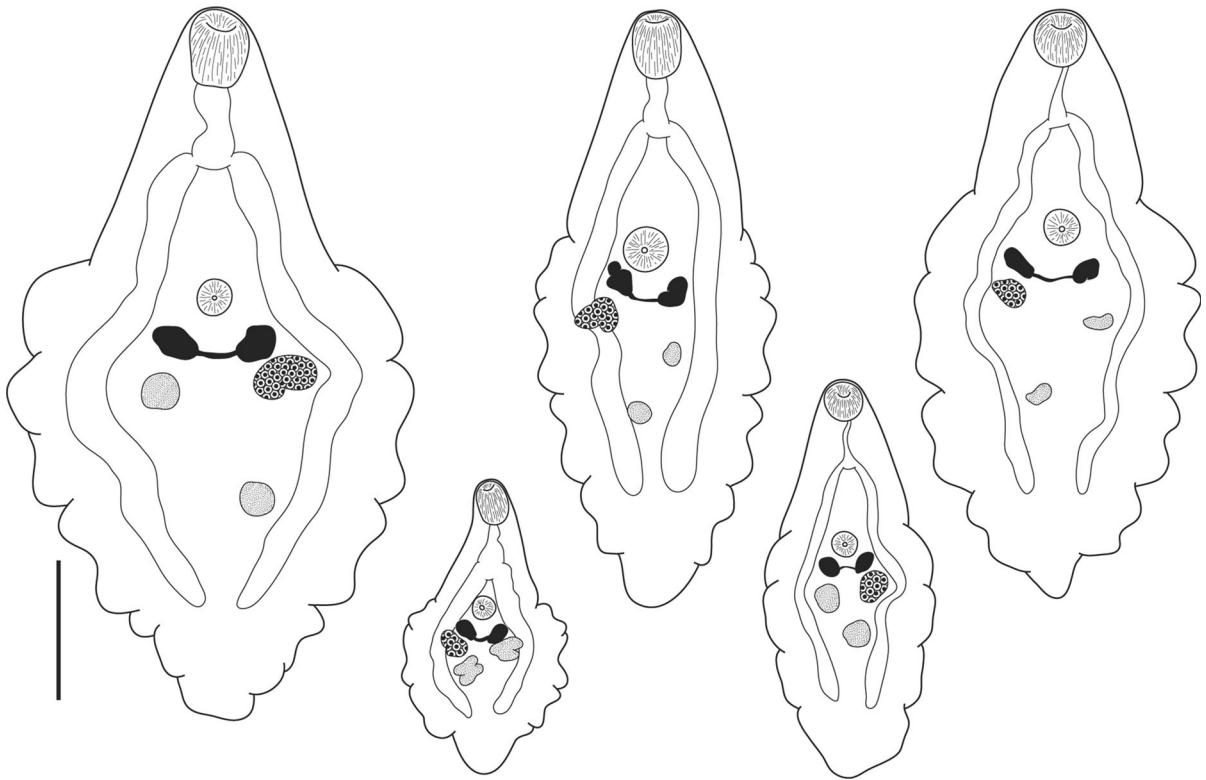
Fig. 3 *Phyllodistomum hyporhamphi* n. sp., gravid paratypes, showing variability in body size, body shape and arrangement of gonads. *Scale-bar*: 1000 μm

Ovary subspherical, entire, posterior to ventral sucker and slightly anterior to anterior testes, dextral in 2 specimens and sinistral in 2, 163–308 × 122–246 (209 × 167). Oviduct expands to form distinct chamber prior to entering Mehlis' gland. Vitelline masses two, entire, immediately posterior to ventral sucker; left mass 95–174 × 116–186 (126 × 140); right mass 87–160 × 126–192 (113 × 146). Laurer's canal not detected. Uterus almost entirely intercaecal in hindbody, with extensive coils extending posteriorly to just beyond ends of intestinal caeca; uterine chamber distinct and prominent dorsal to ventral sucker and genital pore, filled with eggs in gravid specimens, constricted prior to genital pore, up to 457 in diameter in fully gravid specimens. Eggs develop *in utero*; largest eggs 32–51 × 21–32 (42 × 26).

Excretory vesicle tubular, extends anteriorly to level with anterior testis. Excretory pore dorsally subterminal, close to posterior end of body.

Phyllodistomum carangis (MacCallum, 1913) n. comb.

Syns *Distomum carangis* MacCallum, 1913; *Gorgoderina carangis* (MacCallum, 1913) Yamaguti, 1971; *Cetiotrema carangis* (MacCallum, 1913) Williams & Bunkley-Williams, 1996; *Phyllodistomum carangis* Manter, 1947; *Cetiotrema carangis* (Manter, 1947) Manter, 1970

Type-host: *Caranx crysos* (Mitchill), Blue runner (Perciformes: Carangidae).
Type-locality: Off Woods Hole, Massachusetts, USA.

Remarks

There has been some confusion relating to gorgoderids described with the species name '*carangis*'. MacCallum (1913) described *Distomum carangis* MacCallum, 1913 infecting *Caranx crysos* (Mitchill) off Woods

Hole, Massachusetts, USA. This species was later transferred to a genus of frog-infecting gorgoderids, *Gorgoderina* Looss, 1902, by Yamaguti (1971). *Phyllodistomum carangis* Manter, 1947 was described by Manter (1947) in *Caranx ruber* (Bloch) from the Dry Tortugas, off Florida. There have been several subsequent reports of specimens identified as this species (Bravo-Hollis & Manter, 1957; Winter, 1958; Parukhin, 1976; Violante-González et al., 2016). It was transferred to *Cetiotrema* Manter, 1970 by Manter (1970) as the second species of that genus. The issue of the two species was considered briefly by Williams & Bunkley-Williams (1996), who considered the two forms to be synonyms. These authors recognised *C. carangis* (MacCallum, 1913); in our view the full authority for this combination should be *C. carangis* (MacCallum, 1913) Williams & Bunkley-Williams, 1996.

We agree with Williams & Bunkley-Williams (1996) in considering the specimens reported by Manter (1947) to be synonymous with those reported by MacCallum (1913), however consider the assignment to *Cetiotrema* erroneous. Manter (1970) argued that the possession of broadly rounded posterior and anterior ends, tri-lobed vitelline masses, narrow, intercaecal uterine coils and a seminal vesicle anterior to the genital pore placed this species in the new genus. However, many of these features are shared by other marine species of *Phyllodistomum*, and do not justify a position in *Cetiotrema*. The broadly rounded posterior and anterior ends that are apparent in the type-species, *Cetiotrema crassum* Manter, 1970, are not homologous with the body shape seen in the illustration of *C. carangis*, and the uterine coils appear to be restricted to the hindbody in *P. carangis*, whereas they are well developed in the forebody in *C. crassum*; the possession of uterine coils in the forebody was noted as one of the key features distinguishing *Cetiotrema* by Campbell (2008). Furthermore, the forebody is exceptionally short in *C. crassum*, whereas in *P. carangis* it resembles the longer forebody seen in all other marine species of *Phyllodistomum*. Thus, we consider this species as a member of *Phyllodistomum* [Group C *sensu* Ho et al. (2014)] and consider the two named species to be subjective synonyms. As a result, we propose the new combination *Phyllodistomum carangis* (MacCallum, 1913) n. comb.

Phyllodistomum carangis appears to be restricted to the Atlantic and the eastern Pacific (Manter, 1947;

Bravo-Hollis & Manter, 1957; Winter, 1958; Violante-González et al., 2016), with just a single report from the Tropical Indo-west Pacific (Parukhin, 1976). Parukhin (1976) reported this species from *Scomberoides lysan* (Forsskål) [as *Chorinemus lysan* (Forsskål)] in the South China Sea and the Gulf of Mannar. However, based on the limited data reported by Parukhin (1976) it is impossible to discern whether this record represents *P. carangis* or the morphologically similar species *P. pacificum*. Given the locations of the report being in the west Pacific and Indian Ocean, and the type-locality of *P. pacificum* being off Hamazima, Japan, it seems likely this report actually represents *P. pacificum*.

Phylogenetic analysis

A preliminary analysis incorporating all available gorgoderid taxa (not shown) identified that all marine species of *Phyllodistomum* formed a strongly-supported clade to the exclusion of freshwater species of *Phyllodistomum*, as previously recognised by Cutmore et al. (2013), Pérez-Ponce de León et al. (2015) and Urabe et al. (2015). Consequently, we show here a reduced analysis of the marine *Phyllodistomum* clade relative to selected freshwater gorgoderid outgroup taxa.

Alignment of the 28S rDNA dataset (Table 1) yielded 1,143 characters for analysis (including indels). Bayesian inference and maximum likelihood analyses resulted in phylograms with almost identical topologies (Fig. 4). In both analyses all marine species of *Phyllodistomum* formed a strongly-supported clade to the exclusion of the freshwater gorgoderid taxa. In the phylogram resulting from the maximum likelihood analysis, *Phyllodistomum* sp. 4 and *Phyllodistomum* sp. 5 formed a clade with *P. hoggettae* Ho, Bray, Cutmore, Ward & Cribb, 2014 and the asexual stages taken from an infection in the bivalve *Lioconcha castrensis* (Linnaeus), whereas in the phylogram from the Bayesian inference analysis they formed a clade with *P. hyporhamphi*, *P. pacificum* and *P. vaili* Ho, Bray, Cutmore, Ward & Cribb, 2014. In both analyses, *P. pacificum* formed a strongly-supported clade with *P. vaili*, which was sister to *P. hyporhamphi*. *Phyllodistomum pacificum* and *P. vaili* appear to be closely related, differing by just seven bases in the D1-D3 28S analysis; however, these two species differed by 13

bases in the ITS2 region, a level consistent with species-level distinction for trematodes (e.g. Diaz et al., 2015; Cutmore et al., 2016).

Discussion

Morphology

Given the genetic distance between clades of marine and freshwater species of *Phyllodistomum* found by Cutmore et al. (2013), marine species are likely to represent a distinct genus. Therefore, morphological comparisons here are made relative only to marine representatives of the genus. Ho et al. (2014) considered 28 species of *Phyllodistomum* infecting marine fishes to be valid. They also included *P. centropomi* Mendoza-Garfias & Pérez-Ponce de León, 2005 because it infects a host known from freshwater, estuarine and marine systems; however, Pérez-Ponce de León et al. (2015) used genetic data to show that

this species belongs to one of the freshwater clades, and we thus do not consider the species as part of the marine clade.

Phyllodistomum hyporhamphi n. sp. belongs to *Phyllodistomum* Group C *sensu* Ho et al. (2014) in that it possesses a forebody length of less than half of the body length (31–45%) and a body length to width ratio < 4:1 (1.72–2.45:1). The new species is smaller than *P. acceptum* Looss, 1901, *P. carangis*, *P. lancea* Mamaev, 1968, *P. marinae* Bravo-Hollis & Manter, 1957, *P. parukhini* Yamaguti, 1971 and *P. thalassomum* Soheir & Ahmed, 2000 (1,830–5,080 *vs* 7,200, 13,000, 12,000, 7,060–7,400, 7,500–13,000 and 6,400–10,800 μm, respectively) and has a smaller body length to width ratio than *P. carangis*, *P. hoggettae*, *P. leilae* Nagaty, 1956, *P. marinae*, *P. pacificum*, *P. thalassomum* and *P. vaili* (1.72–2.45:1 *vs* 3.25, 2.39–3.60, 3.44, 3.00, 3.33, 3.08 and 2.85–3.85, respectively). The new species has a larger oral sucker width to ventral sucker width ratio than *P. acceptum*, *P. borisbychowskyi* Caballero y Caballero, 1969,
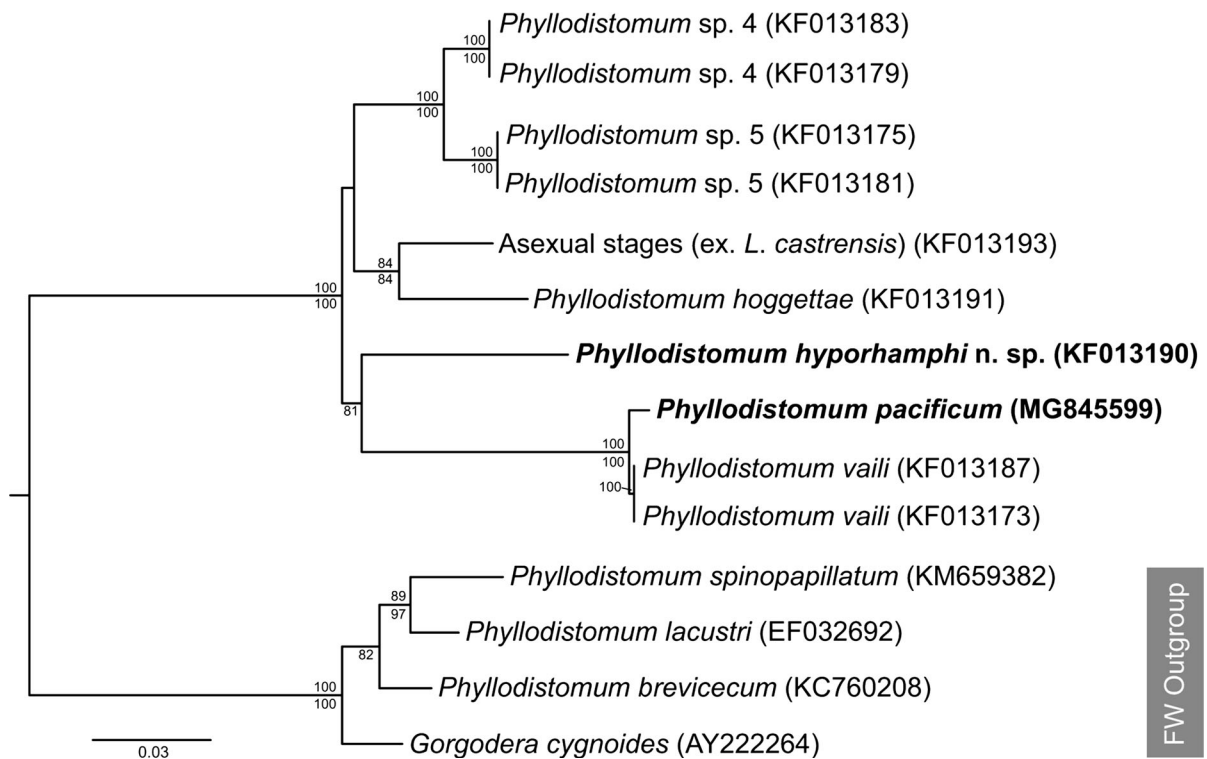

Fig. 4 Relationships between marine species of *Phyllodistomum* based on maximum likelihood analysis of the partial 28S rDNA dataset. Maximum likelihood bootstrap support values are shown above the nodes. Bayesian inference posterior probabilities are shown below for relationships also found in the Bayesian inference analysis. Support values < 80 not shown

P. crenilabri Dolgikh & Naidenova, 1968, *P. lancea*, *P. leilae*, *P. mamaevi* Parukhin, 1971, *P. pacificum*, *P. pomacanthi* Nahhas & Cable, 1964, *P. scrippsi* Brooks & Mayes, 1975, *P. sobolevi* Parukhin, 1979 and *P. unicum* Odhner, 1902 (1.15–1.80:1 *vs* 0.95, 0.82, 1.03, 0.87, 0.88, 0.91, 0.95, 0.71, 0.80, 0.89 and 0.95:1, respectively) and a smaller oral sucker width to ventral sucker width ratio than *P. mirandai* Lamothe-Argumedo, 1969 (1.15–1.80:1 *vs* 2.6–2.8:1). The new form differs from *P. tongaatense* Bray, 1985 and *P. trinectes* Corkum, 1961 in the possession of strong marginal undulations (*versus* absent or indistinct), and the possession of entire vitelline masses with a slightly irregular outline differentiates it from *P. lewisi* Srivastava, 1938, which has strongly lobed vitelline masses. *Phyllodistomum hyporhamphi* differs from the five other species for which molecular data are available by 41–68 bases in the ITS2 rDNA alignment.

The new specimens of *P. pacificum* agree with the original description of Yamaguti (1951) in all morphological features, especially in the possession of similar-sized oral and ventral suckers, lobed testes, weak marginal undulations and a bipartite seminal vesicle that is antero-dorsal to the genital pore. The specimens from *Pantolabus radiatus* differ from those infecting *Carangoides equula* (Temminck & Schlegel) only in overall size, with the new specimens being larger than those described by Yamaguti (1951) (body length 4,575–5,338 × 1,111–1,328 *vs* 2,200–3,100 × 570–930 μm), and having a larger oral (312–410 × 310–384 *vs* 160–210 × 170–250 μm) and ventral sucker (260–340 × 268–321 *vs* 150–200 × 150–230 μm); however, the size of the testes (320–598 × 247–487 *vs* 260–450 × 170–380 μm), ovary (163–308 × 122–246 *vs* 150–270 × 100–195 μm) and eggs (32–51 × 21–32 *vs* 39–51 × 24–39 μm) were similar to those described by Yamaguti (1951).

Phyllodistomum pacificum is morphologically similar to *P. carangis*, with both species infecting carangids, but the two species differ dramatically in overall size. The type-specimen of *P. carangis* (from MacCallum, 1913) is much larger than all specimens of *P. pacificum* (13,000 *vs* 2,200–5,338 μm), and possesses a larger oral sucker (1,500 × 800 *vs* 312–410 × 310–384 μm), ventral sucker (900 × 700 *vs* 260–340 × 268–321 μm), testes (450–850 × 550–570 *vs* 320–598 × 247–487 μm), ovary (340 × 350 *vs* 163–308 × 122–246 μm) and vitelline masses (470–520 × 270–370 *vs* 87–174 × 116–192 μm).

Specimens of *P. carangis* reported by Winter (1958) from the Pacific coast of Mexico were also much larger than any specimens of *P. pacificum* (total length 10,013–12,665 μm), as is the specimen reported by Manter (1947) (8,061 μm), despite appearing to be not yet fully gravid (having smaller gonads, a less extensive uterus and a uterine chamber not filled with eggs).

Host range of *Phyllodistomum* spp.

An interesting aspect of *Phyllodistomum* is the remarkable breadth of teleost families infected. Marine species of *Phyllodistomum* have been reported from 18 teleost families belonging to multiple feeding guilds. Although comparably wide host distributions have been reported for some other trematode genera, recent genetic studies have shown that some of these patterns are erroneously inflated groups of morphologically similar species (e.g. *Neolebouria* Gibson, 1976, *Prosorhynchoides* Dollfus, 1929, *Podocotyloides* Yamaguti, 1934 and *Rhipidocotyle* Diesing, 1858) rather than distinct monophyletic clades of related species (see Nolan et al., 2015; Martin et al., 2017, 2018). Interestingly, marine species of *Phyllodistomum* show no pattern of being linked by their ecology or diet; those that have been shown to be definitively part of the same clade by genetic analysis infect the Carangidae (pelagic piscivores), Hemiramphidae (epipelagic omnivores), Labridae and Mullidae (benthic invertivores) and Serranidae (benthic piscivores).

We speculate that the nature of the urinary bladder as a habitat explains the diverse range of hosts reported for species of *Phyllodistomum*. Compared with infection locations of other trematode families (i.e. intestinal tract or circulatory system), the urinary bladder is less variable between host families and orders, and we predict that this homogeneity has enabled extensive host switching. Cutmore et al. (2010) also showed that species of another gorgoderid genus have low specificity, reporting *Staphylorchis cymatodes* (Johnston, 1913) Travassos, 1922 from four families and three orders of elasmobranchs. This is again most likely due to the infection location (i.e. body cavity) being relatively homogenous across elasmobranch lineages.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

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