

Molecular and morphological evidence for the widespread distribution of *Laticola paralatesi* infecting wild and farmed *Lates calcarifer* in Australia

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ABSTRACT: Infections with monogeneans of the Diplectanidae can limit productivity of and cause considerable health issues for fish in aquaculture. To date, 9 species of diplectanids have been reported from the Asian sea bass or barramundi *Lates calcarifer* (Perciformes: Latidae) in the Asia-Pacific region. This study characterised the diplectanid parasite fauna found infecting wild and farmed barramundi from 5 localities in tropical Australia, including north Queensland and Western Australia. A combination of morphometric and comparative genetic analyses of partial 28S ribosomal RNA (28S rRNA) from specimens recovered were used to confirm their identity and to explore relationships with other diplectanids. These data revealed that a single, dominant species of diplectanid, *Laticola paralatesi*, infects wild and farmed *Lates calcarifer* in tropical Australia. *Laticola lingaoensis* Yang, Kritsky, Sun, Jiangying, Shi & Agrawal, 2006 is synonymised with *L. seabassi* (Wu, Li, Zhu & Xie, 2005) Domingues & Boeger, 2008 based on the combination of the host infected (*Lates calcarifer*), geographic distribution, distinct morphological similarity, and identical 28S rRNA sequence data identified here. *Laticola seabassi* is now designated as the type species of *Laticola* due to nomenclatural priority.

KEY WORDS: Diplectanidae · *Laticola paralatesi* · Monogenea · Barramundi · *Lates calcarifer* · Latidae · Aquaculture · Aquatic animal health · Species diagnostics

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INTRODUCTION

Diplectanidae (Platyhelminthes: Monogenea) impact finfish production due to high infection prevalence, intensity, and resulting pathological impacts (Leong & Wong 1990, Liang & Leong 1991). Pathogenicity is thought to be the result of parasite attachment and feeding activities. Open lesions from rubbing or ‘flashing’ behaviour of infected fish also increase rates of opportunistic infection from protozoa, bacteria and viruses (Hoa & Ut 2007, Isshiki et al. 2007). Most species of Diplectanidae are distin-

guished from other gill- or skin-infecting monogeneans by the possession of a distinct structure, called the squamodisc or lamellocdisc, which is a disc-like organ with multiple rows of sclerotised rodlet structures arranged in a concentric U-shape (Buchmann & Bresciani 2006, Sigura & Justine 2008). Squamodiscs are generally present on both ventral and dorsal surfaces and function primarily as a friction pad, preventing dislodgement of parasites by gill currents (Whittington 2005). The haptor anchors, which are composed of sclerotised proteins, help in attachment by penetrating deep into the basal membrane and

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connective tissue of gill lamellae (Dezfuli et al. 2007). The feeding activity of diplectanids can destroy epithelial cell layers, reduce the number of chloride cells on gills, cause over-production of mucus and ultimately reduce respiratory performance of host fish (Thoney & Hargis 1991, Buchmann & Lindstrøm 2002, Whittington 2005).

Losses or reduced productivity of commercially important fish stocks due to diplectanid infection have been well documented. Dezfuli et al. (2007) estimated that *Diplectanum aequans* induced up to 10% of the annual mortality (worth US\$700 000) of juvenile European sea bass *Dicentrarchus labrax* cultured in the Mediterranean. In Asia, *Pseudorhabdosynochus* species plague grouper (*Epinephelus* spp.) culture in the South China Sea (Yang et al. 2005, Luo & Yang 2010). Nine diplectanid species, representing 3 genera, have been reported from barramundi, *Lates calcarifer* (including *Diplectanum narimeen*, *D. penangi*, *D. setosum*, *Laticola latesi*, *L. lingaensis*, *L. paralatesi*, *L. seabassi*, *Pseudorhabdosynochus epinepheli*, and *P. lantauensis*), some which develop high prevalences and intensities on cultured fish (Leong & Wong, 1990).

Barramundi farming is a rapidly expanding, high value aquaculture industry in tropical Australia (production was 4352 tonnes, valued at AUD\$35.7 million in 2011; Skirtun et al. 2013). There is a surprising paucity of research on the identification of diplectanids infecting *Lates calcarifer* in Australia, considering that the industry is plagued with persistent infections in many aquaculture facilities. The only diplectanid that has been previously reported from Australia is *Laticola paralatesi*, obtained from wild *L. calcarifer* off Bathurst Island, Northern Territory (Yang et al. 2006). The aim of this study was to conduct a survey of the diplectanid fauna infecting wild and farmed *L. calcarifer* from a wide range of localities around Australia. A combination of morphological and molecular analyses were used to diagnose the putative taxa present in the region.

Knowledge of the species present will assist further investigations of species-specific pathological effects, host–pathogen interactions and strategies to prevent and eliminate persistent infections impacting the industry.

MATERIALS AND METHODS

Parasite collection

Diplectanids were collected from the gills of farmed barramundi *L. calcarifer* obtained from Mainstream Aquaculture Barramundi stock, Townsville, Queensland (19° 19' S, 146° 15' E), Ponderosa Farm, Cairns, Queensland (16° 55' S, 145° 46' E) and Marine Produce Australia, Cone Bay, Western Australia (16° 28' S, 123° 32' E). Specimens were also collected from wild *L. calcarifer* captured at Cleveland Bay, Townsville, Queensland (19° 15' S, 146° 49' E) and Trinity Inlet, Cairns, Queensland (16° 55' S, 146° 46' E) (Table 1). Fish were sampled opportunistically, which limited robust prevalence and intensity data. Diplectanids were gently removed from gills of infected *L. calcarifer* using fine forceps (or small needles) and pipettes under a dissecting microscope. Specimens were heat fixed in near boiling seawater (or water at the same salinity the host fish were held) and placed immediately into 70% or 98% ethanol; a few individuals were fixed in 10% formalin. Voucher specimens mounted on microscope slides were deposited in the Queensland Museum (QM), Brisbane, Queensland, Australia. All procedures were approved by the James Cook University Animal Ethics Committee (A1649).

Morphological description

Parasite samples for morphological examination were stained in Mayer's haematoxylin or left un-

Table 1. Diplectanid gill fluke specimens collected from the gills of *Lates calcarifer* showing origin and numbers examined in morphological and molecular analyses

Location	Fish origin	Date (dd/mm/yy)	No. of specimens studied	
			Morphology	Molecules
Mainstream Aquaculture, Townsville, QLD	Farmed	05/06/2013	37	4
Ponderosa, Cairns, QLD	Farmed	29/04/2014	8	1
Marine Produce Australia, Cone Bay, WA	Farmed	07/03/2011	2	2
Cleveland Bay, Townsville, QLD	Wild	09/04/2011	21	4
Trinity Inlet, Cairns, QLD	Wild	03/2014	2	2

stained, dehydrated through a graded ethanol series (i.e. 50, 75, 95, 100 and 100% ethanol), cleared in methyl salicylate and mounted in Canada balsam. Specimens mounted on slides were then examined using an Olympus BX53 compound microscope, equipped with a UC50 camera and drawing tube. Morphometric measurements were obtained using LabSens software (Olympus Soft Imaging Solutions) according to Mizelle & Klucka (1953). Measurements are reported in μm with the mean followed by ranges in parentheses. Line drawings were created using a drawing tube attached to a compound microscope, and digitised in Adobe Illustrator CS6.

Genetic analyses

Thirteen specimens from 5 different localities were included for comparative molecular analyses (Table 1). Genomic DNA was isolated separately for each specimen using QIAGEN® DNeasy® Blood & Tissue kit according to the manufacturer's protocol. The partial 28S rRNA region examined here was amplified and sequenced using the forward primer C1 (5'-ACC CGC TGA ATT TAA GCA T-3') and reverse primer D2 (5'-TGG TCC GTG TTT CAA GAC-3') (Šimková et al. 2003). Polymerase chain reactions (PCR) were conducted in PCR tubes containing 10 μl of 5×Phusion® GC buffer, 1 μl of 10 mM dNTPs, 2.5 μl of each primer (10 mM), 1.5 μl of DMSO, 0.5 μl of Phusion® *Taq* DNA polymerase and 8 μl of DNA template. The final volume of 50 μl was achieved with the addition of Invitrogen™ ultraPURE™ distilled water. PCR cycling involved an initial 95°C denaturation for 4 min, followed by 30 cycles of 95°C denaturation for 1 min, 56°C annealing for 1 min and 72°C extension for 2 min, followed by a single cycle of 95°C denaturation for 1 min, 55°C annealing for 45 s and a final 72°C extension for 4 min on a MJ Research PTC-150 thermocycler. Successfully amplified PCR products were purified using a QIAGEN® QIAquick® PCR purification kit, according to the manufacturer's protocol. Purified products were then sent to the Australian Genome Research Facility (AGRF) in Brisbane for sequencing with an AB3730xl capillary sequencer.

Sequences were edited, and forward and reverse sequence contigs were created using Geneious v. 5.4 software. Edited sequences were then examined for comparative purposes using the BLAST-n algorithm against data available on GenBank (Altschul et al. 1997). The partial 28S rRNA dataset was aligned for comparative purposes using MUSCLE v. 3.7 (Edgar 2004) with ClustalW sequence weighting and UPGMA

clustering for iterations 1 and 2. The resultant alignments were refined by eye using MESQUITE (Maddison & Maddison 2009). After alignment of the final 28S dataset was edited, the ends of each fragment were trimmed to match the shortest sequence in the alignment.

The software jModelTest v. 0.1.1 (Guindon & Gascuel 2003, Posada 2008) was used to estimate the best nucleotide substitution models for these 2 datasets. Bayesian inference analyses of the small (SSU) and large subunit (LSU) rDNA datasets were performed using MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003) run on the CIPRES portal (Miller et al. 2009) to explore relationships among these taxa. Bayesian inference analysis was conducted on the 28S rRNA dataset using the TVM+I+G model predicted as the best estimator for both datasets by Akaike's information criterion (AIC) and Bayesian's information criterion (BIC) in jModelTest. Bayesian inference analyses were run over 10 000 000 generations (ngen = 10000000) with 2 runs each containing 4 simultaneous Markov Chain Monte Carlo (MCMC) chains (nchains = 4) and every 1000th tree saved (samplefreq = 1000). Bayesian analyses used the following parameters: nst = 6, rates = invgamma, 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 = 3000' and 'sumt burnin = 3000'. These 'burnin' parameters were chosen because the log likelihood scores 'stabilised' well before 3 000 000 replicates in the Bayesian inference analyses.

Maximum likelihood analysis was performed on the 28S dataset using the RAxML algorithm (Stamatakis et al. 2008) on the CIPRES portal with the gamma rate model of heterogeneity and maximum likelihood search estimating the proportion of invariable sites parameters. Nodal support was inferred based on 100 bootstrap replicates.

RESULTS

Molecular analyses

Alignment of the 28S rRNA dataset yielded 1010 characters for analysis. Comparative phylogenetic analyses of the partial 28S rRNA dataset using Bayesian inference and maximum likelihood resulted in phylograms with identical topologies (Fig. 1). Comparative analyses of the partial 28S rRNA dataset from all of the host/parasite/locality isolates

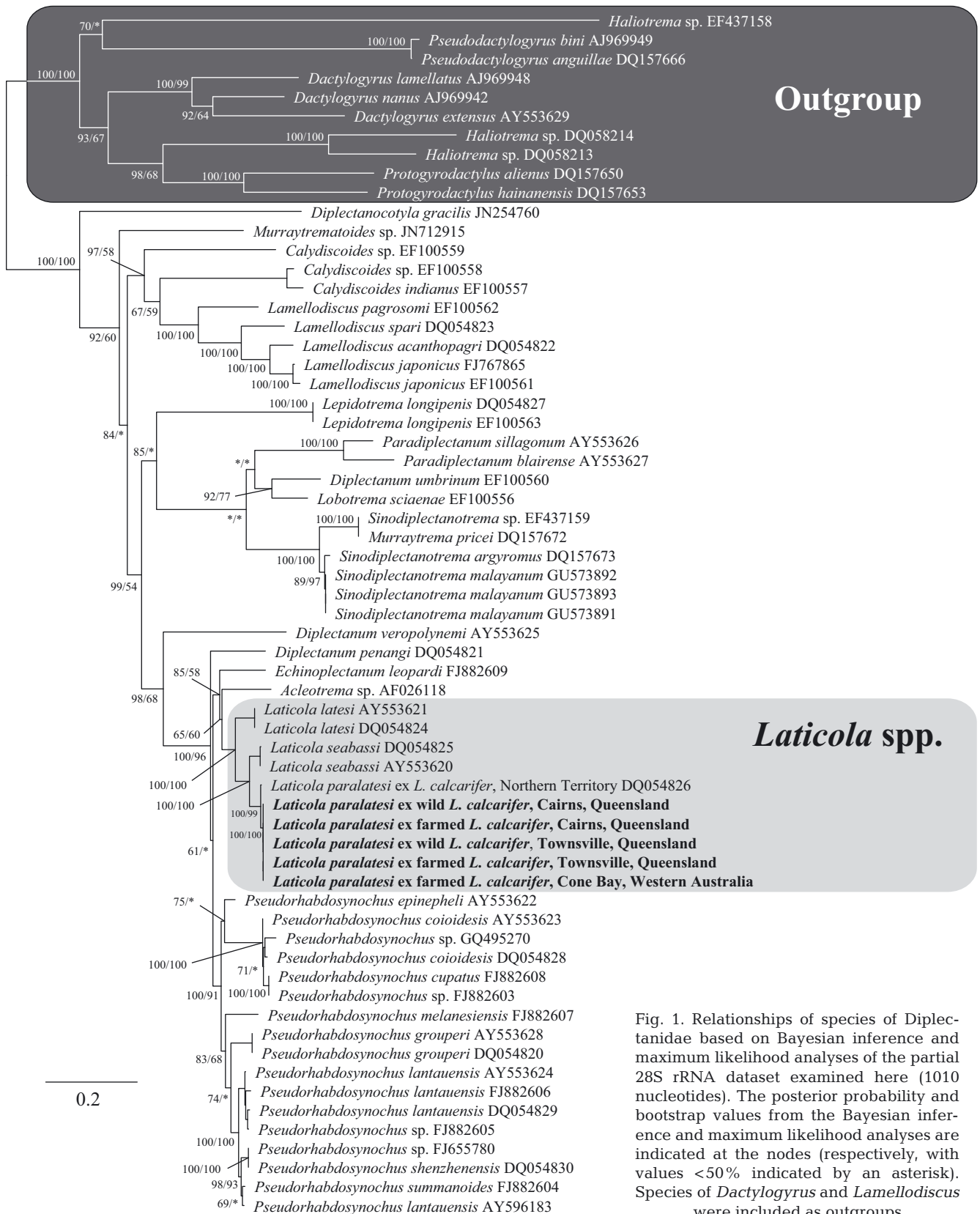


Fig. 1. Relationships of species of Diplectanidae based on Bayesian inference and maximum likelihood analyses of the partial 28S rRNA dataset examined here (1010 nucleotides). The posterior probability and bootstrap values from the Bayesian inference and maximum likelihood analyses are indicated at the nodes (respectively, with values <50% indicated by an asterisk). Species of *Dactylogyrus* and *Lamellogyrus* were included as outgroups

obtained in this study showed 99.8% similarity to sequences of *Laticola paralatesi* reported from *Lates calcarifer* from the Northern Territory by Yang et al. (2006). The genera *Laticola* and *Pseudorhabdosynochus* were each resolved as monophyletic groups with high posterior probability and bootstrap support in both analyses (Fig. 1). Partial 28S rRNA sequences for the type species of *Laticola*, *L. lingaoensis* Yang, Kritsky, Sun, Zhang, Shi & Agrawal, 2006, described from *Lates calcarifer* off Hainan Province, China by Yang et al. (2006) and *L. seabassi* (Wu, Li, Zhu & Xie, 2005) Domingues & Boeger, 2008 described from *Lates calcarifer* off Guangdong Province, China by Wu et al. (2005a), were identical. This suggests that *L. lingaoensis* and *L. seabassi* are synonymous.

Morphological analysis

All diplectanid specimens from Australian *Lates calcarifer* showed compelling morphological similarity to *Laticola paralatesi* (Nagibina, 1976) Yang, Kritsky, Sun, Zhang, Shi & Agrawal, 2006. The morphometric measurements of the specimens examined here were similar to those reported for *L. paralatesi* by Yang et al. (2006) (Table 2). Herein, we provide a description of *L. paralatesi* from specimens collected in this study and compare morphological measurements with specimens examined by Yang et al. (2006) previously collected from Bathurst Island, Northern Territory, Australia.

Taxonomic description

Monogenea

Diplectanidae

Laticola paralatesi (Nagibina, 1976) Yang, Kritsky, Sun, Zhang, Shi & Agrawal, 2006.

Synonyms: *Diplectanum paralatesi* Nagibina, 1976; *Pseudorhabdosynochus yangjiangensis* Wu & Li, 2005.

Host: *Lates calcarifer* (Bloch, 1790) (Latidae).

Localities: Mainstream Aquaculture Barramundi stock, Townsville, Queensland (19° 19' S,

Table 2. Comparison of morphometric measurements of *Laticola paralatesi* between this study and those examined by Yang et al. (2006). The average measurements are presented in μm followed by ranges and specimen number in parentheses. MCO = male copulatory organ

Feature	Measurements from studied specimens (μm)	Measurements from Yang et al. (2006) (μm)
Body length	512 (324–676; n = 43)	487 (424–600; n = 26)
Body width	149 (73–211; n = 44)	127 (89–147; n = 27)
Pharynx length	32 (19–89; n = 53)	Not measured
Pharynx width	33 (18–44; n = 53)	30 (24–39; n = 27)
Haptor length	Not measured	89 (78–103; n = 21)
Haptor width	Not measured	173 (153–196; n = 25)
Dorsal squamodisc length	81 (59–106; n = 43)	Not measured
Dorsal squamodisc width	92 (74–110; n = 52)	97 (85–110; n = 26)
Ventral squamodisc length	81 (67–95; n = 40)	Not measured
Ventral squamodisc width	91 (70–107; n = 54)	102 (81–115; n = 19)
Number of rodlet rows	12 (11–14; n = 43)	12 (11–13; n = not given)
Length of dorsal anchor	33 (22–46; n = 53)	29 (27–30; n = 5)
Base width of dorsal anchor	13 (9–20; n = 43)	10 (9–15; n = 5)
Length of ventral anchor	35 (27–48; n = 51)	36 (34–38; n = 4)
Base width of ventral anchor	16 (11–29; n = 43)	15 (13–17; n = 3)
Hooklet length	9 (9–10; n = 3)	10 (10–12; n = 12)
Dorsal bar length	50 (34–57; n = 58)	48 (45–50; n = 5)
Ventral bar length	117 (91–141; n = 58)	106 (97–125; n = 14)
Testis length	61 (32–74; n = 5)	28 (25–31; n = 2)
Testis width	8 (5–13; n = 3)	35 (33–36; n = 2)
MCO base length	22 (16–27; n = 57)	Not measured
MCO distal length	71 (66–85; n = 43)	74 (69–83; n = 14)
Germarium width	39 (23–51; n = 33)	35 (21–43; n = 23)
Vagina length	21 (17–25; n = 15)	Not measured

146° 15' E); Ponderosa Farm, Cairns, Queensland (16° 55' S, 145° 46' E); Marine Produce Australia, Cone Bay, Western Australia (16° 28' S, 123° 32' E); Cleveland Bay, Townsville, Queensland (19° 15' S, 146° 49' E); Trinity Inlet, Cairns, Queensland (16° 55' S, 146° 46' E).

Previous records: *Lates calcarifer*: South China Sea (Nagibina, 1976); (fish origin not identified); farmed *L. calcarifer*: off Lingao, Hainan Province, China (19° 57' N, 109° 47' E) (Yang et al. 2006); wild *L. calcarifer*: off Bathurst Island, Northern Territory, Australia (11° 22' S, 130° 41' E) (Yang et al. 2006); Adelaide River, Northern Territory, Australia (12° 14' S, 131° 15' E) (collected by K. S. Hutson, identified by Delane C. Kritsky, United States National Parasite Collection [USNPC], now held at the US National Museum of Natural History at the Smithsonian, accession no. 107282.00).

Site of infection: Gills.

Specimens deposited: Queensland Museum, 12 voucher specimens (QM G234503 to 234514).

GenBank accession numbers: KP313564–KP313568.

Morphological description: (Figs. 2 & 3, Table 2).

Body long and slender, 512 (324–676, n = 43) long,

with tapered anterior trunk. Body dorso-ventrally flattened. Greatest width 149 (73–211, $n = 44$), in posterior region at level of gonads. Anterior half of body covered with smooth cuticle. Tegumental scales anteriorly directed and conical, extending from peduncle to posterior half of body (infrequently extending to vaginal opening or male copulatory organ).

Anterior region with 3 pairs of head organs and 2 pairs of eye-spots present on head with smaller spherical/ellipsoid anterior pairs and larger ellipsoid/reniform posterior pair. Distance between outer margins of posterior eye-spot pair less than anterior pair. Pharynx 33 (18–44, $n = 53$) wide, 32 (19–59, $n = 53$)

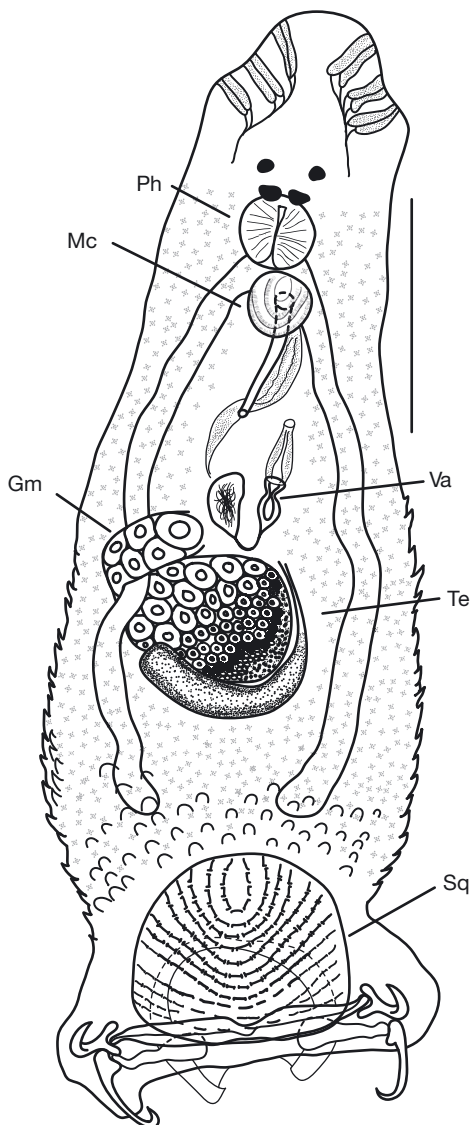


Fig. 2. Line drawing of *Laticola paralatesi* infecting *Lates calcarifer* off Townsville, Queensland. Mc: male copulatory organ; Gm: germarium; Ph: pharynx; Sq: squamodisc; Te: testis; Va: vagina. Scale bar = 100 μm

long, located in the median, middle indentation and lines of musculature visible. Oesophagus short, ill-defined, mostly absent. Intestinal caeca sac-like in shape, simple and unbranched. Diverticula from intestinal caeca absent. Intestinal caeca extend laterally from oesophagus to posterior of the gonad, terminating blindly prior to peduncle and squamodisc.

Gonads located close to mid-body, tandem, germarium immediately anterior and adjacent to testis. Single testis, 61 (32–74, $n = 5$) long and 9 (5–13, $n = 3$) wide, visible as long cylindrical crescent portion around posterior half of germarium. Testis appears infrequently as a small oblate ellipsoid mass posterior to germarium. Germarium 39 (23–51, $n = 33$) wide, appears as pyriform cell mass, looping around right intestinal caecum. Follicles and oocytes exist in different developmental stages; larger and more developed oocyte located anteriorly.

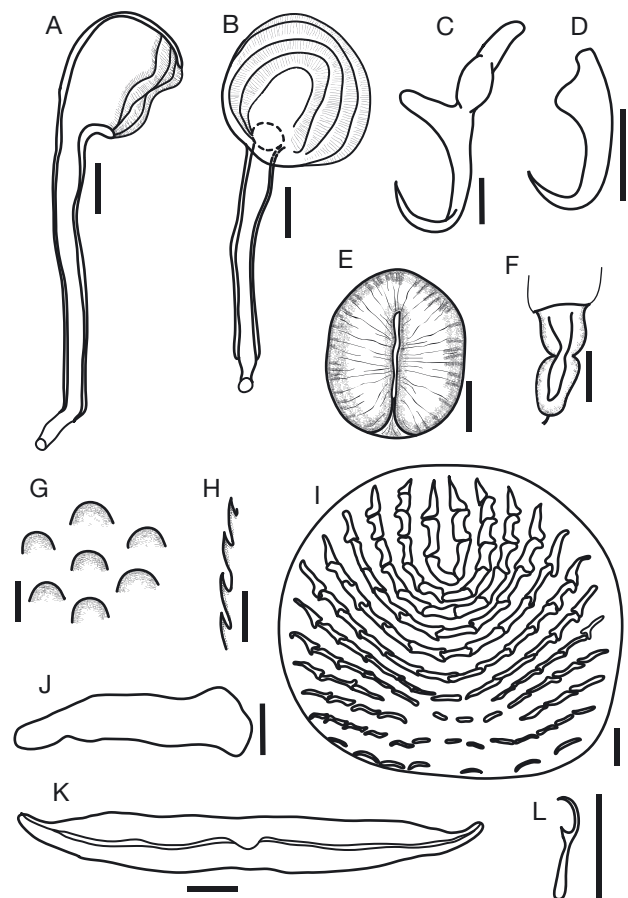


Fig. 3. Morphological diagnostic features of *Laticola paralatesi*. Male copulatory organ (A) lateral view, (B) ventral view; (C) ventral anchor; (D) dorsal anchor; (E) pharynx; (F) vaginal valve; (G, H) tegumental scales; (I) squamodisc; (J) dorsal bar; (K) transverse ventral bar; and (L) marginal hooklet. Scale bars = (A–C, E–L) 10 μm , (D) 20 μm

Genital organs located in anterior half of body, close to mid-line. Male copulatory organ (MCO) anterior of vagina. Genital pores of both organs open on ventral surface at similar level; male genital opening located to right of female genital opening. Male copulatory organ spoon-shaped, consists of anterior cone and straight posterior tube. Anterior cone of MCO contains 4 thin muscular ridges. Base 22 (16–27, n = 57) wide, distal section 71 (66–85, n = 43) long. Muscular, thick-walled uterus leads to thistle-shaped vaginal valve, 21 (17–25, n = 15) long. Vaginal valve connected to spherical seminal receptacle via narrow duct.

Haptor, perpendicularly directed from body posterior as bilateral lobes, contains 2 squamodiscs, 3 haptor bars, 7 pairs of marginal hooklets, and 2 pairs of anchors. Squamodiscs cover almost entire area of haptor except lateral lobe region. Ventral squamodisc 81 (67–95, n = 40) long and 91 (70–107, n = 52) wide. Dorsal squamodisc 81 (59–106, n = 43) long and 92 (74–110, n = 52) wide. Each squamodisc possesses 12 (11–14, n = 43) rodlet rows arranged in a concentric U-shape. Rodlets dumbbell-shaped, large and coarse on inner rows, becoming smaller and more delicate towards outer rows and squamodisc margin. Dorsal anchor is conical with hook-like curving tip, length 34 (23–46, n = 53). Base of dorsal anchor 13 (9–20, n = 43) wide, has short truncate superficial root protruding from narrow conical main root. Ventral anchor 35 (27–48, n = 51) long. Base of ventral anchor 16 (11–29, n = 43) wide, diverted into fork-like shape with 2 roots; main root with swelling along its length and under-developed superficial root protruding perpendicularly from main root. Each anchor connects to haptor bar at base around diverging point of main root and superficial root. Dorsal bar 50 (34–57, n = 58) long, displaying spatulate shape with tapering tip attached to dorsal anchor. Transverse ventral bar lip-like in shape, with longitudinal groove along its length 117 (91–141, n = 58); bar is widest near body mid-line and tapers off laterally toward the distal ends that attach to ventral anchors. Marginal hooklets 9 (9–10, n = 3).

Remarks

The specimens examined here were unambiguously identified as *Laticola paralatesi* based on the combination of morphological characteristics as detailed in the original description by Yang et al. (2006) and Sigura & Justine (2008), host infected (*Lates calcarifer*), and the identical 28S rDNA sequence data. Species of *Laticola* can be easily distinguished from

species of the morphologically similar diplectanid genus *Pseudorhabdosynochus* by the compartmentalisation and shape of the MCO. The MCO of *Pseudorhabdosynochus* spp. are characterised by their overall reniform shape and 4 compartments (loculi) that are limited by sclerotised walls, whereas the MCO in *Laticola* spp. has 4 thin muscular ridges (Domingues & Boeger 2008, Justine 2009). *L. paralatesi* has 2 squamodiscs, each on the ventral and dorsal surface, while *L. latesi* has only a single ventral squamodisc (Yang et al. 2006). *L. latesi* is also known synonymously in the literature as *P. latesi*, *P. monosquamodiscusi* and *Diplectanum latesi*. *P. yangjiangensis* reported from *Lates calcarifer* off Guangdong, China is considered to be the subjective synonym of *L. paralatesi* (Yang et al. 2006).

Laticola Yang, Kritsky, Sun, Zhang, Shi & Agrawal, 2006 currently contains 6 species, including *L. lingaoensis* Yang, Kritsky, Sun, Zhang, Shi & Agrawal, 2006 (type species) reported from *Lates calcarifer* from the South China Sea by Yang et al. (2006); *L. seabassi* (Wu, Li, Zhu & Xie, 2005) Domingues & Boeger, 2008 reported from *Lates calcarifer* from 3 different localities in China by Wu et al. (2005a); *L. latesi* (Tripathi, 1957) Yang, Kritsky, Sun, Zhang, Shi & Agrawal, 2006 reported from *Lates calcarifer* from the South China Sea and India by Tripathi (1957) and Yang et al. (2006); *L. paralatesi* (Nagibina, 1976) Yang, Kritsky, Sun, Zhang, Shi & Agrawal, 2006 reported from *Lates calcarifer* from the South China Sea by Nagibina (1976) and Australia by Yang et al. (2006); *L. cyanus* Sigura & Justine, 2008 described from *Epinephelus cyanopodus* off New Caledonia by Sigura & Justine (2008); and *L. dae* Journo & Justine, 2006 reported from *E. maculatus* off New Caledonia by Journo & Justine (2006). Sigura & Justine (2008) highlighted and discussed potential nomenclatural problems associated with species of *Laticola*, due primarily to the almost simultaneous multiple descriptions of species in this genus in 3 different publications (Wu & Li 2005, Wu et al. 2005a, Yang et al. 2006). The main issue that Sigura & Justine (2008) identified is the distinct morphological similarity (particularly in the shape of the vagina) between *L. seabassi* described by Wu et al. (2005a), the type species, and *L. lingaoensis*, described by Yang et al. (2006), which were both reported infecting *Lates calcarifer* near China. Sigura & Justine (2008) predicted that these 2 species are probably synonymous, and if so, International Commission for Zoological Nomenclature (ICZN) rules of priority of description would require the type-species of *Laticola* be designated *L. seabassi*. The combination of

the host infected, geographic distribution, distinct morphological similarity between *L. seabassi* and *L. lingaoensis*, and the identical 28S rDNA sequences identified here warrant the formal synonymy of these 2 species. Therefore, we synonymise *Laticola lingaoensis* Yang, Kritsky, Sun, Jiangying, Shi & Agrawal, 2006 with *L. seabassi* (Wu, Li, Zhu & Xie, 2005) Domingues & Boeger, 2008 and designate *L. seabassi* as the type species due to priority with *L. lingaoensis* Yang, Kritsky, Sun, Zhang, Shi & Agrawal, 2006 as the junior synonym.

DISCUSSION

The combined morphological and molecular investigation provides unequivocal evidence that *Laticola paralatesi* is the single, or dominant, species of diplectanid parasitising *Lates calcarifer* in tropical Australia. The remaining 7 species of Diplectanidae known to parasitise *L. calcarifer* in Asia may not be distributed in Australian waters, or alternatively, they may occur at a very low prevalence in Australia (Table 3).

L. calcarifer inhabiting regions of Southeast Asia host a high diversity of diplectanid species relative to tropical northern Australia. The observed species diversity could potentially be a consequence of host-switching events and/or co-evolution within discrete *L. calcarifer* populations (Kearn 1968, Poulin 2002, Ziętara & Lumme 2002). Host switching may have occurred in *Pseudorhabdosynochus coioidesis* and *P. serrani*, which can parasitise closely related grouper species, including *Epinephelus coioides*, *E. bruneus* and *E. awoara* (Luo & Yang 2010). High-density polyculture and sea cage farming, which are common practices in Southeast Asian marine aquaculture, can also increase interactions between potential hosts from different species (Ziętara & Lumme 2002, Luo & Yang 2010).

Yue et al. (2009) suggest that Australian and south-east Asian *L. calcarifer* populations have diverged into 2 distinct genetic groups, with Australian barramundi exhibiting less genetic diversity. Sea level rises during the Pleistocene, which decreased the area of coastline and estuary habitats, could have contributed to increased gene flow within Australian

Table 3. Species of Diplectanidae reported from the gills of barramundi *Lates calcarifer*

Species	Locality	Reference
<i>Diplectanum narimeen</i> (<i>incertae sedis</i> *)	Mandapam Camp, South India	Unnithan (1964)
<i>Diplectanum penangi</i> (<i>incertae sedis</i> *)	Zhanjiang, Guangdong Province, China Malaysia Thailand	Liang & Leong (1991)
<i>Diplectanum setosum</i> (<i>incertae sedis</i> *)	South China Sea	Nagibina (1976)
<i>Laticola latesi</i> (formerly known as <i>Pseudorhabdosynochus latesi</i> , <i>Pseudorhabdosynochus monosquamodiscusi</i> and <i>Diplectanum latesi</i>)	Lingao, Hainan Province, China Zhanjiang, Guangdong Province, China Malaysia Thailand Chilka, India Lamoung, Sumatra, Indonesia	Liang & Leong (1991); Balasuriya & Leong (1995); Yang et al. (2006)
<i>Laticola seabassi</i> (formerly known as <i>Pseudorhabdosynochus seabassi</i>)	Guangdong, China Hainan, China Yangjiang, China	Wu et al. (2005a)
Syn. <i>L. lingaoensis</i>	Lingao, Hainan Province, China	Yang et al. (2006)
<i>Laticola paralatesi</i> (formerly known as <i>Diplectanum paralatesi</i>)	Lingao, Hainan Province, China Bathurst Island, NT, Australia	Nagibina (1976); Yang et al. (2006)
<i>Pseudorhabdosynochus epinepheli</i> (formerly known as <i>Cycloplectanum hongkongense</i> , <i>Diplectanum epinepheli</i> and <i>Diplectanum hongkongense</i>)	Lampung Bay, South Sumatra, Indonesia	Rückert et al. (2008)
<i>Pseudorhabdosynochus lantauensis</i> (formerly known as <i>Cycloplectanum lantauense</i> and <i>Diplectanum lantauense</i>)	Lampung Bay, South Sumatra, Indonesia	Rückert et al. (2008)

* *Incertae sedis* status indicates 'uncertain position or validity' of the species

barramundi populations (Chenoweth et al. 1998). Thus, the high diversity of co-evolved diplectanids found in Southeast Asia may reflect the evolutionary history of *L. calcarifer* based on the high genetic variation observed between different populations in the region. However, a recent molecular study using 16 microsatellite loci identified 21 genetically distinguishable sub-populations of *L. calcarifer* across the species' distribution within Australia (Jerry et al. 2013).

Although most of the measurements for the specimens recovered in this study fall well within the ranges for *Laticola paralatesi* reported by Yang et al. (2006), morphometric analysis of the male copulatory organ and vaginal valve demonstrated notable deviation (Table 2). Therefore, morphometric analyses may not be the most effective method for identifying diplectanid species. Justine (2005) demonstrated that morphometrics of diplectanids from the same species which were preserved using different treatment and fixation techniques could result in statistically significant differences. For instance, *P. cupatus* and *P. melanesiensis* showed significant differences in measurement of body dimension, haptor bars, male copulatory organ and vaginal valve between 'carmine' and 'picrate' fixations (Justine 2005). Measurements affected by specimen preparation are not only those of structures dominantly composed of soft tissue, but also hollow sclerotized structures (Justine 2005, 2009). However, Justine (2009) noted that the overall shapes of sclerotized structures in diplectanids were maintained relatively well despite varying fixation procedures and can still be used for species diagnoses with confidence.

The fine details of some internal organs of *Laticola* spp. have been described by Yang et al. (2006), including the vaginal valve, male prostatic reservoir and marginal hooklets. For the vaginal valve, Yang et al. (2006) described the presence of 2 lobe-like structures within the proximal valve adjacent to connection with seminal receptacles as well as 2 delicate tubes connecting proximal and distal ends. They also noted the presence of a vas deferens that 'dilates to form fusiform seminal vesicle' and the prostatic reservoir located on the dorsal side of the vas deferens (Yang et al. 2006).

Molecular analyses combined with morphological study have helped to resolve phylogenetic relationships and species identity in diplectanid monogeneans, which are frequently subjected to changes in taxonomic classification (Domingues & Boeger 2006, 2008). For example, analysis of partial sequences of the internal transcribed spacer 1 (ITS1) and 28S

rDNA demonstrated that *P. lantauensis* infecting *E. coioides* was actually comprised of 2 separate species (Mollaret et al. 2000, Chisholm et al. 2001, Wu et al. 2005b). Comparison of partial 28S rRNA confirmed that diplectanid specimens from wild and farmed *L. calcarifer* in north Queensland and Western Australia are the same taxon as reported previously from the Northern Territory by Yang et al. (2006).

Wild *L. calcarifer* infected with *Laticola paralatesi* could act as reservoirs of infection for farmed fish. These diplectanids most likely enter farming systems through intake of natural water resources containing eggs and oncomiracidia or from infected wild-caught brood stock (Woo 1995, Whittington et al. 1999). Since diplectanid infections tend to persist and amplify rapidly under farming conditions, preventive measures should be taken to minimise losses (Whittington 2005). This may include decreased reliance on wild-caught brood stock or applying appropriate chemotherapeutic (e.g. praziquantel) treatments to brood stock in quarantine (Schmahl & Mehlhorn 1985, Woo 1995). Where feasible, the industry should consider using re-circulated water and fine filtration to reduce parasite transfer.

Further study is needed to provide evidence for the absence of other diplectanid species infecting *L. calcarifer* in Australia. Examining the pathogenicity of *Laticola paralatesi* will help determine whether this taxon can cause significant production losses or chronic health deterioration.

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