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A new species of *Furcohaptor* Bijukumar & Kearn, 1996 (Monogenea: Diplectanidae) parasitic on *Cynoglossus robustus* Günther (Pleuronectiformes: Cynoglossidae) in the Seto Inland Sea, Japan, with comments on its systematic position and an amended generic diagnosis of *Furcohaptor*

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Abstract *Furcohaptor brevis* n. sp. is described from the gills of *Cynoglossus robustus* Günther caught in the Seto Inland Sea off Hiroshima Prefecture, Japan. Although *Furcohaptor* Bijukumar & Kearn, 1996 has been assigned to the Ancyrocephalinae Bychowsky, 1937 in the Dactylogyridae Bychowsky, 1933, this genus is transferred to the Diplectaninae Monticelli, 1903 in the Diplectanidae Monticelli, 1903 based on both morphological and molecular data. An amended generic diagnosis is provided.

Introduction

The monotypic *Furcohaptor* Bijukumar & Kearn, 1996 was erected for *Furcohaptor cynoglossi* Bijukumar & Kearn, 1996, a gill parasite of cynoglossids, *Cynoglossus macrostomus* Norman, and *C. puncticeps* (Richardson), from India, and assigned to the Ancyrocephalinae Bychowsky, 1937 in the Dactylogyridae Bychowsky, 1933 (see Bijukumar & Kearn, 1996). This genus is characterised by two long haptoral arms; one pair of typical anchors; one pair of rod-shaped hooks; five pairs of typical-shaped marginal hooks; and one pair of marginal hooks which are reduced in size and have no heels (Bijukumar & Kearn, 1996). Subsequently, Boeger et al. (2015) mentioned that the genus is most likely a member of the Diplectanidae Monticelli, 1903 based on the morphological features. However, the type-specimens of *F. cynoglossi* were in poor condition to make a detailed observation of their internal anatomy (Bijukumar & Kearn, 1996) and no further specimens of the species have been reported since the original description; thus its taxonomic position is still undetermined. During a survey of the parasites of *Cynoglossus robustus* Günther in the Seto Inland Sea off Hiroshima Prefecture, Japan, we collected specimens form gills identifiable as a species of *Furcohaptor*. This paper describes this material as a new species of *Furcohaptor* and amends the diagnosis of the genus to accommodate the new species.

Materials and methods

In total, 56 fresh specimens of *Cynoglossus robustus* were purchased at the Yokota fishery port, Fukuyama,

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This article is part of the Topical Collection Monogenea.

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Hiroshima Prefecture, Japan, on 25 January 2015 (n = 8; standard length, SL, 220.1–258.9 mm), 1 February 2015 (n = 17; SL 132.5–324.1 mm), 28 February 2015 (n = 8; SL 243.2–275.0 mm), 6 December 2015 (n = 10; SL 248.7–306.5 mm), 10 January 2016 (n = 10; SL 283.5–328.0 mm), and 27 March 2016 (n = 8, 236.0–272.0 mm). These fish were commercially caught by trawling in the Seto Inland Sea off Yoko-shima (34°21.5′N, 133°16.5′E), Hiroshima Prefecture, Japan. They were brought on ice to the laboratory of Hiroshima University and examined for gill parasites under the dissecting microscope. Specimens of monogeneans were picked up using small needles and forceps, and flattened between a slide and a coverslip. One specimen was identified under slight coverslip pressure and preserved in 99% ethanol for molecular analysis. Some specimens were fixed in ammonium picrate glycerin (Lim, 1991), while others were fixed in 70% ethanol or acetic acid-formalin-alcohol and stained in Heidenhain's iron hematoxylin.

All fixed specimens except the one for molecular study were dehydrated through a graded ethanol series, cleared in xylene, and mounted in Canada balsam. Drawings were made with the aid of a drawing tube fitted on an Olympus BX51 microscope. The method of measuring sclerotised structures is presented in Fig. 1. The penises were measured on images taken by a digital camera on an Olympus DP20 microscope at a magnification of ×1,000 using ImageJ software (version 1.48i). All measurements are in micrometres, and are given as the range followed by the mean and the number (n) of specimens examined in parentheses. The numbering of hook pairs follows Mizelle (1936). Fish identification was based on Yamada & Yagishita (2013). The type-specimens are deposited in the Platyhelminthes collection of the National Museum of Nature and Science (NSMT-Pl), Tsukuba, Ibaraki Prefecture, Japan.

DNA was extracted from one specimen using the DNeasy blood and tissue kit (Qiagen, Valencia, USA) in accordance with the manufacturer's instructions. The DNA was amplified by polymerase chain reaction (PCR) using the primer pair C1 (5′-ACC CGC TGA ATT TAA GCA T-3′) and D2 (5′-TGG TCC GTG TTT CAA GAC-3′) to amplify partial 28S rDNA (Hassouna et al., 1984). A total of 25 µl PCR reaction consisted of 1 µl of DNA template, 1× ExTaq Buffer (TaKaRa, Kusatsu, Japan), 0.2 mM of each dNTP, 1 µM of each primer, and 2.5 units of TaKaRa Ex Taq DNA Polymerase (TaKaRa). PCR was carried out with the following protocol: 94°C for 30 s followed by 35 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 2 min, and 10 min of final extension at 72°C. PCR products were purified using NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany) and sequenced with a 3130X Genetic Analyzer (Applied Biosystems, Foster City, USA) with the primers used in the PCR. The newly generated 28S rDNA sequence was aligned with sequences for 27 diplectanid species, one pseudomurraytrematid species, and two dactylogyrid species retrieved from the GenBank database. Alignment was performed with ClustalW using the default parameters. The phylogenic tree was constructed with the maximum likelihood (ML) method under the GTR + G model, which was determined to be the best-fit model using the Bayesian information criterion, with the phylogeny tested by 1,000 bootstrap repeats using MEGA 6 (Tamura et al., 2013).

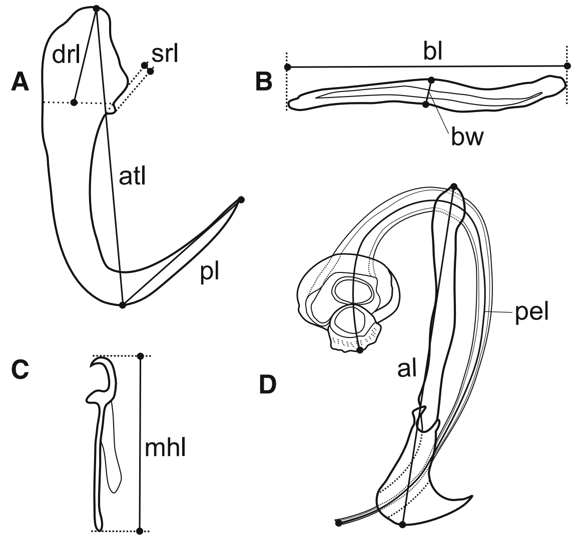

Fig. 1 Measurements of hard parts. A, Anchor; B, Bar; C, Marginal hook; D, Male copulatory organ. *Abbreviations*: al, accessory piece length; atl, anchor total length; bl, bar length; bw, bar width; drl, deep root length; ln, length to notch; mhl, marginal hook length; pel, penis length; pl, point length; srl, superficial root length

Family Diplectanidae Monticelli, 1903
Subfamily Diplectaninae Monticelli, 1903
Genus *Furcohaptor* Bijukumar & Kearn, 1996

***Furcohaptor brevis* n. sp.**

Type-host: *Cynoglossus robustus* Günther, 1873 (Pleuronectiformes: Cynoglossidae).
Type-locality: The Seto Inland Sea, off Yoko-shima (34°21.5′N, 133°16.5′E), Fukuyama, Hiroshima Prefecture, Japan.
Type-material: Holotype stained in Heidenhain's iron hematoxylin (NSMT-Pl 6294 collected on 1 February 2015), and four and one paratypes stained in Heidenhain's iron hematoxylin (NSMT-Pl 6296–6297) and fixed in ammonium picrate glycerin (NSMT-Pl 6295), respectively.
Site in host: Gill filaments.
Prevalence and intensity: 16.1% (9/56) [25% (2/8) on 25 January 2015; 29.4% (5/17) on 1 February 2015; 25% (2/8) on 27 March 2016], and a total of nine monogeneans infected nine fish.
Representative DNA sequence: Submitted to DNA Data Bank of Japan (DDBJ) under the accession number LC270915.
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 *Furcohaptor brevis* n. sp. is urn:lsid:zoobank.org:act: 80258204-E048-4183-BF50-75074A203E9C.
Etymology: The specific name of "*brevis*" means short in Latin and refers to the short arms of the bifurcate haptor.

Description (Figs. 2–3)

Body (Fig. 2A) elongate, length including haptor 405–709 (588; n = 6), width at mid-body 149–222 (189, n = 6). Three pairs of cephalic glands open on each anterolateral side of anterior extremity. Two pairs of eye-spots present. Pharynx subspherical (38–65 × 33–49 (51 × 41) (n = 6); oesophagus short; intestinal caeca extend to haptor. Testis pyriform, posterior to ovary. Vas deferens arising from anterior margin of testis, extending intercaecally, forming seminal vesicle. Single saccate prostatic reservoir located left to seminal vesicle. Male genital pore opening ventrally posterior to male copulatory organ. Male copulatory organ located posterior to oesophagus (Fig. 2K), consisting of penis and accessory piece. Penis tapered, curved, with flared proximal end, length along curve 58–66 (61, n = 6). Accessory piece rod-shaped, 29–39 (36, n = 6) long, its point growing wider, curved and covering tip of penis, non-articulated with penis. Ovary in mid-body, tubular, pretesticular, encircling right intestinal cecum dorsoventrally, then forming oviduct. Oviduct receiving duct from seminal receptacle, continuing as oötype surrounded by Mehlis' gland. Vagina (Fig. 2L) sclerotised, curved tubular, entering anterior side of seminal reservoir; vaginal pore funnel-shaped, opening posterior to male genital pore on left side of ventral body surface. Vitellarium approximately co-extensive with intestinal ceca.

Haptor bifurcates posteriorly to form 2 short haptoral arms. Dorsal anchor absent. Ventral anchor (Fig. 2B) with reduced superficial root, total length 30–32 (31, n = 5), deep root length 11–13 (12, n = 5), superficial root length 1–2 (1, n = 5), point length 16–19 (17, n = 5). Dorsal bar absent. One pair of ventral bars (Fig. 2C) with longitudinal groove, rod-shaped, associating with root of each ventral anchor, length 22–28 (26, n = 6), width 2–3 (3, n = 6). Marginal hooks in 7 pairs, pairs II, III, IV, VI, VII with typical marginal hook shape for diplectanids: pair I (Fig. 2D) small, lacking heel of sickle, protruding from haptor ventrally, 11–12 (11, n = 5) long; pair II (Fig. 2E) 17–19 (18, n = 6) long; pair III (Fig. 2F) 17–20 (19, n = 6) long; pair IV (Fig. 2G) 17–21 (19, n = 6) long; pair V (Fig. 2H) long, lacking heel of sickle, length 36–43 (40, n = 6), pair VI (Fig. 2I) 18–19 (18, n = 5) long; pair VII (Fig. 2J) 17–20 (18, n = 6) long. Pair of pyriform reservoirs located at tip of haptoral arm ventrally, ducts open at extrusion of ventral anchors. (Fig. 3). Pair of gland-cells located at mid-length of haptor dorsally associated with marginal hooks V (Fig. 3).

Phylogenetic analysis

The newly generated 28S rDNA sequence for *Furcohaptor brevis* n. sp. was 880 nt long. Three species, *Pseudomurraytrema* sp. (Pseudomurraytrematidae), *Dactylogyrus gotoi* Gussev, 1965 and *Dactylogyrus lamellatus* Achmerow, 1952 (Dactylogyridae) were used as the outgroup. The phylogenetic analysis based on 28S rDNA sequences showed that the diplectanids separated into two clades, the Diplectaninae and

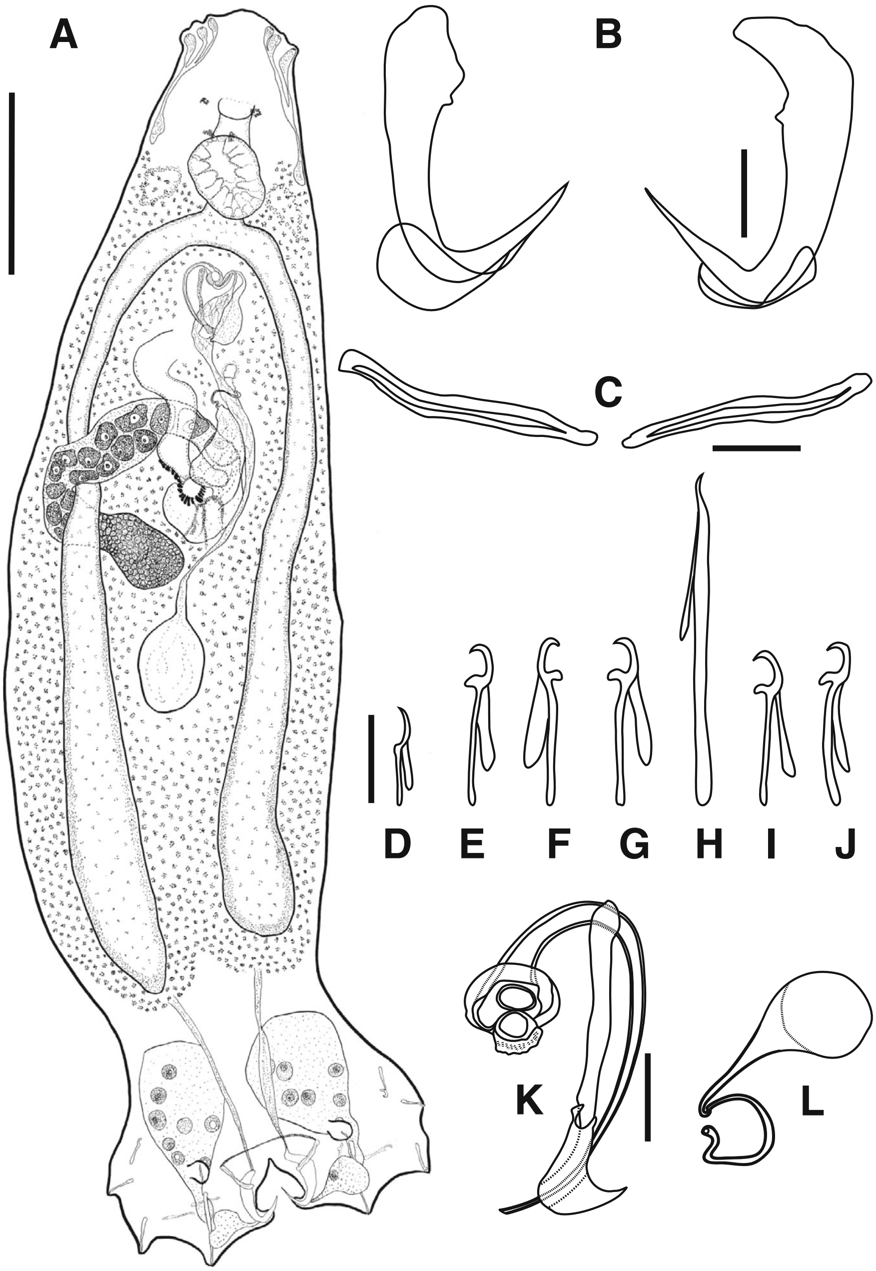

Fig. 2 *Furcohaptor brevis* n. sp. ex *Cynoglossus robustus*. A, Holotype (NSMT-Pl 6294), whole mount (ventral view). B–L, Paratype (NSMT-Pl 6295). B, Ventral anchors; C, Ventral bars; D, Marginal hook of pair I; E, Marginal hook of pair II; F, Marginal hook of pair III; G, Marginal hook of pair IV; H, Marginal hook of pair V; I, Marginal hook of pair VI; J, Marginal hook of pair VII; K, Male copulatory organ; L, Vagina. *Scale-bars*: A, 50 µm; B–L, 10 µm

Lamellodiscinae Oliver, 1969 (Fig. 4). The new species was assigned to the Diplectaninae in the Diplectanidae, and exhibited affinity with *Diplectanum veropolynemi* Nagibina, 1976 (Fig. 4). The sequences reported as *Pseudorhabdosynochus latesis* [sic] (AY553621) and *Laticola lingaoensis* Yang, Kritsky, Sun, Zhang, Shi & Agrawal, 2006 (DQ054825) should be referred to as *Laticola latesi* (Tripathi, 1955) and *L. seabassi* (Wu, Li, Zhu & Xie, 2005), respectively (Chotnipat et al., 2015).

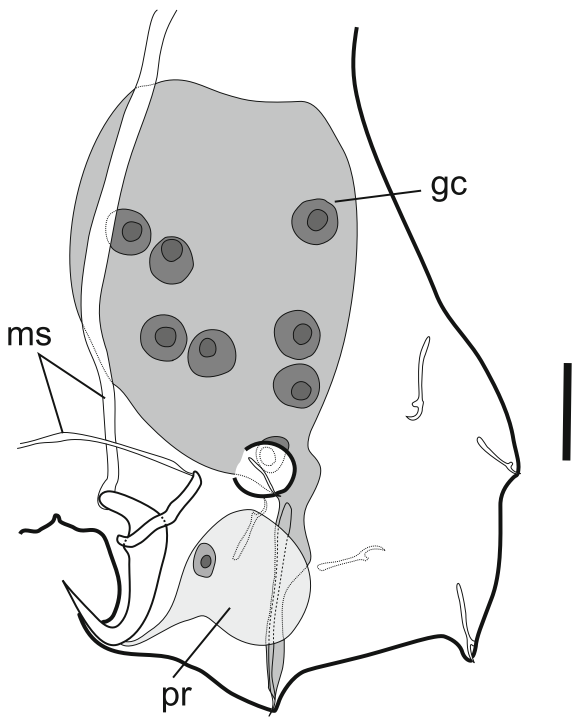

Fig. 3 Left half of haptor of *Furcohaptor brevis* n. sp. ex *Cynoglossus robustus* Holotype (NSMT-Pl 6294), ventral view. *Abbreviations*: gc, gland-cells; ms, muscle; r, reservoir. *Scale-bar*: 20 µm

Discussion

Furcohaptor brevis n. sp. resembles *F. cynoglossi* described by Bijukumar & Kearn (1996) in most of the morphological characters including a divided haptor; a pair of typical anchors with reduced superficial root; a pair of bars; a pair of rod-shaped hooks; five pairs of typical-shaped marginal hooks; a pair of marginal hooks reduced in size and lacking heels; and a penis with a flared proximal extremity. However, the new species differs from *F. cynoglossi* by the shape of the accessory piece and the length of the haptoral arms.

Furcohaptor has so far been assigned to Dactylogyridae, but based on the morphological features of *F. brevis* n. sp. (e.g. the ovary looping the right intestinal cecum; the distal part of the penis directed posteriorly; and the ventral bar having a longitudinal groove) and the phylogenetic analysis of the 28S rDNA sequences (Fig. 4), this genus evidently belongs to the Diplectaninae in the Diplectanidae. The sequences of *F. brevis* n. sp. and *Diplectanum veropolynemi* Nagibina, 1976 formed a sister group with a high bootstrap value (100%). *Diplectanum veropolynemi* was regarded as a species *incertae sedis* (Domingues & Boeger, 2008), and Kritsky & Diggles (2015) suggested that this monogenean is reassigned to *Acanthocercodes* Kritsky & Diggles, 2015. Further molecular data for species of *Acanthocercodes* will clarify the generic position of *D. veropolynemi* and show the affinities of *Furcohaptor* and *Acanthocercodes*.

Bijukumar & Kearn (1996) and Boeger et al. (2015) discussed the origin of typical anchors and rod-shaped hooks found in *Furcohaptor* spp. Based on the specimens of *F. brevis* n. sp., both the typical anchors and bars are identified as the ventral anchors and bars, respectively. This is supported by the bars located most ventrally on the typical anchors, the muscles running ventrally attached to the deep root of the typical anchors, and the duct of the reservoirs located ventrally opening the typical anchors (Fig. 3). Many monogeneans possess reservoirs of the haptor (e.g. dactylogyrids, tetraonchids and diplectanids), and most of these reservoirs are associated with the blades of the anchors (e.g. Lim, 1995; Lim & Gibson, 2009; Lim et al., 2010). The rod-shaped hook is identified as a fifth marginal hook because of its location on the ventral side of the tip of the haptor, and because it is enveloped by the ducts of the gland-cells (Fig. 3). The fifth marginal hook is commonly located ventrally at the tip of the haptor (e.g. Mizelle, 1936; Lim, 2015), and the gland-cells are associated with their handle of the marginal hooks in *Bravohollisia* Bychowsky & Nagibina, 1970 (see Wong et al., 2008). As Bijukumar & Kearn (1996) mentioned, the study on the ontogenetic development of *Furcohaptor* spp. reinforces the designation of the identification of haptoral structures.

Genus *Furcohaptor* Bijukumar & Kearn, 1996 (amend.)

Diagnosis

Diplectanidae, Diplectaninae. Body elongate. Two pairs of eye-spots present or absent. Cephalic glands open on each anterolateral side. Pharynx, oesophagus and bifurcate intestinal caeca present. Testis pyriform, posterior to ovary. Vas deferens arising from anterior margin of testis, extending intercaecal, forming

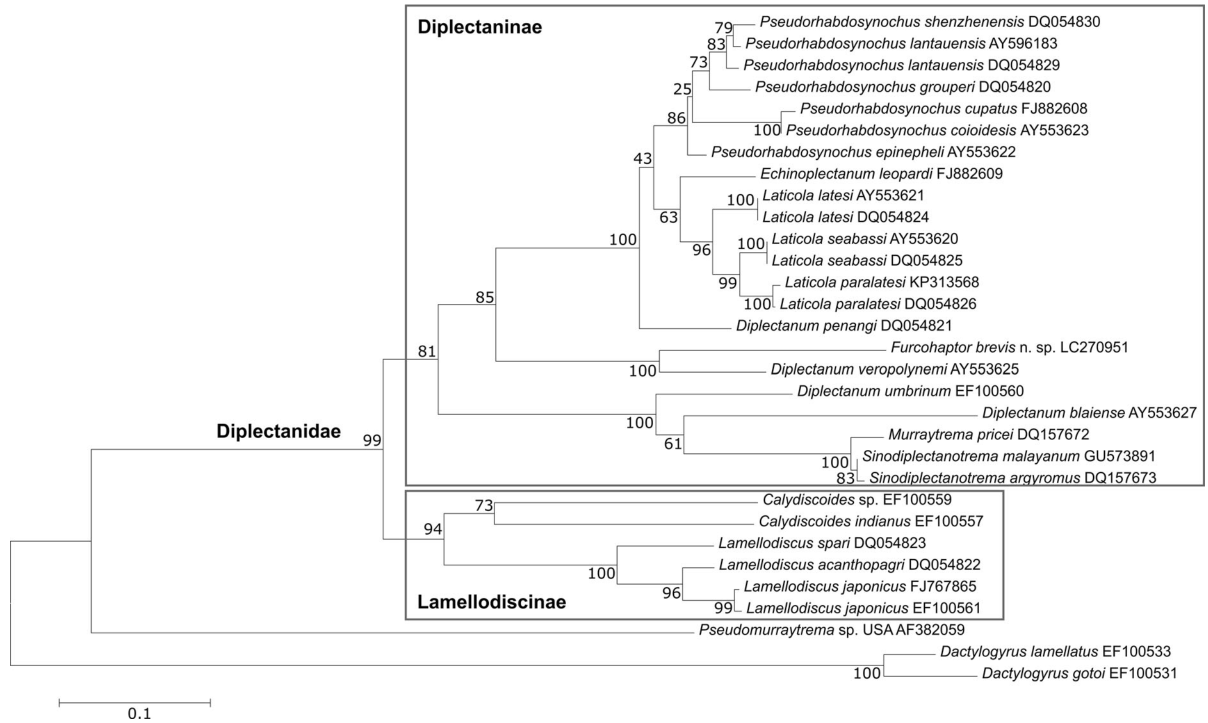

Fig. 4 Phylogenetic tree based on 28S rDNA partial sequences. The tree was constructed using the Maximum Likelihood method. The numbers along the branches indicate bootstrap values based on 1,000 bootstrap pseudoreplications

seminal vesicle. Single saccate prostatic reservoir present. Male genital pore opening ventrally posterior to male copulatory organ. Male copulatory organ consisting of penis and accessory piece. Penis tapered, curved, with flared proximal end. Accessory piece rod-shaped. Ovary in mid-body, tubular, pretesticular, encircling right intestinal caecum dorsoventrally, then forming oviduct. Oviduct receiving duct from seminal receptacle, continuing on as oötype surrounded by Mehlis' gland. Vagina, sclerotised, curved tubular, entering anterior side of seminal reservoir; vaginal pore funnel-shaped, posterior to male genital pore. Vitellaria approximately co-extensive with intestinal caeca. Haptor bifurcate posteriorly to form haptoral arms. Squamodiscs or lamellodiscs absent. Dorsal anchor and dorsal bar absent. Ventral anchor with reduced superficial root. One pair of ventral bars with longitudinal grooves. Seven pairs of marginal hooks present; pair I small, lacking heel of sickle; pair V long, lacking heel of sickle; other pairs typical marginal hook shape for diplectanids. On gills of cynoglossid fishes. *Type-species*: *Furcohaptor cynoglossi* Bijukumar & Kearn, 1996. *Other-species*: *Furcohaptor brevis* n. sp.

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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.

References

Bijukumar, A., & Kearn, G. C. (1996). *Furcohaptor cynoglossi* n. g., n. sp., an ancyrocephaline monogenean gill parasite with a bifurcate haptor and a note on its adhesive attitude. *Systematic Parasitology, 34,* 71–76.

Boeger, W. A., Pariselle, A., & Patella, L. (2015). *Susanlimae ianwhittingtoni* gen. nov., sp. nov. (Monogenoidea:

Dactylogyridae), a dweller of the gill rakers of *Pseudeutropius moolenburghae* (Siluriformes: Schilbeidae) from Sumatra. *Zoologia, 32,* 532–537.

Chotnipat, S., Miller, T. L., Knuckey, R. M., & Hutson, K. S. (2015). Molecular and morphological evidence for the widespread distribution of *Laticola paralatesi* infecting wild and farmed *Lates calcarifer* in Australia. *Diseases of Aquatic Organisms, 113,* 195–205.

Domingues, M. V., & Boeger, W. A. (2008). Phylogeny and revision of Diplectanidae Monticelli, 1903 (Platyhelminthes: Monogenoidea). *Zootaxa, 1698,* 1–40.

Hassouna, N., Michot, B., & Bachellerie, J.-P. (1984). The complete nucleotide sequence of mouse 28S rRNA gene. Implications for the process of size increase of the large subunit rRNA in higher eukaryotes. *Nucleic Acids Research, 12,* 3563–3583.

ICZN (2012). *International Commission on Zoological Nomenclature*: Amendment of articles 8, 9, 10, 21 and 78 of the International Code of Zoological Nomenclature to expand and refine methods of publication. *Zootaxa, 3450,* 1–7.

Kritsky, D. C., & Diggles, B. K. (2015). *Acanthocercodes* n. g. (Monogenoidea: Diplectanidae) for species parasitising threadfins (Perciformes: Polynemidae), with description of *Acanthocercodes bullardi* n. sp. from the Atlantic threadfin *Polydactylus octonemus* (Girard) and reassignment of three species of *Diplectanum* Monticelli, 1903 from the Indo-Pacific Ocean. *Systematic Parasitology, 91,* 191–201.

Lim, L. H. S. (1991). Three new species of *Bychowskyella* Achmerow, 1952 (Monogenea) from Peninsular Malaysia. *Systematic Parasitology, 19,* 33–41.

Lim, L. H. S. (1995). *Bravohollisia* Bychowsky & Nagibina, 1970 and *Caballeria* Bychowsky & Nagibina, 1970 (Monogenea: Ancyrocephalidae) from *Pomadasys hasta* (Bloch) (Pomadasyidae, with the description of a new attachment mechanism. *Systematic Parasitology*, *32*, 211–224.

Lim, L. H. S. (2015). *Teraplectanum* n. g. (Monogenea: Diplectanidae) from the banded grunter fish, *Terapon theraps* Cuvier (Perciformes: Terapontidae), off Peninsular Malaysia. *Journal of Helminthology, 89,* 307–316.

Lim, L. H. S., & Gibson, D. I. (2009). A new monogenean genus from an ephippid fish off Peninsular Malaysia. *Systematic Parasitology, 73,* 13–25.

Lim, L. H. S., Tan, W. B., & Gibson, D. I. (2010). Description of *Sinodiplectanotrema malayanum* n. sp. (Monogenea: Diplectanidae), with comments on the taxonomic position of the genus. *Systematic Parasitology, 76,* 145–157.

Mizelle, J. D. (1936). New species of trematodes from the gills of Illinois fishes. *American Midland Naturalist, 17,* 785–806.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution, 30,* 2725–2729.

Wong, W.-L., Brennan, G. P., Halton, D. W., Maule, A. G., & Lim, L. H. S. (2008). Secretory products of the haptoral reservoirs and peduncular glands in two species of *Bravohollisia* (Monogenea: Ancyrocephalidae). *Invertebrate Biology, 127*, 139–152.

Yamada, U., & Yagishita, N. (2013). Cynoglossidae. In: Nakabo, T. (Ed.) [*Fishes of Japan with pictorial keys to the species, Third edition*]. Hadano: Tokai University Press, pp. 1693–1698, 2233–2234 (In Japanese).