

A new genus for *Diplectanum setosum* Nagibina, 1976 (Monogenea: Dipletanidae), a parasite of *Psammoperca waigiensis* (Cuvier) (Perciformes: Latidae) from Okinawajima Island, Japan

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Abstract The diplectanid monogenean Diplectanum setosum Nagibina, 1976 is redescribed based on newly collected specimens from Psammoperca waigiensis (Cuvier) (Perciformes: Latidae) from the Okinawa-jima Islands, Okinawa Prefecture, Japan, and transferred to a new genus, Latiphagum n. g., herein proposed based on the results of the morphological and molecular analysis. This new genus is closely related with Pseudorhabdosynochus Yamaguti, 1958, Echinoplectanum Justine & Euzet, 2006, and Laticola Yang, Kritsky, Sun, Zhang, Shi, & Agrawal, 2006, but distinguished from them by the male copulatory organ (MCO) of the new genus devoid of cirrus, the tube supported both sides by two plates with long bristles, and the presence of the prostatic reservoir in the expanded base of the MCO.

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Introduction

Diplectanum setosum Nagibina, 1976 (Diplectanidae), collected from the fish species *Psammoperca waigiensis* (Cuvier) (as *Psamoperca* [*sic*] *waigiensis*) and *Lates calcarifer* (Bloch) (Perciformes: Latidae) in the South China Sea off Hainan, China (Nagibina, 1976), was previously described as *D. setosus*. However, *D. setosum* was recently considered as *incertae sedis* by Domingues & Boeger (2008) based on a morphological analysis. During the examination of monogeneans from marine fishes from the Okinawa-jima Island, some diplectanids were recovered from gills of *P. waigiensis*. In this paper, based on the results of morphological and molecular analysis, these diplectanids are redescribed as *D. setosum* and transferred to a new genus herein proposed.

Materials and methods

One specimen of *Psammoperca waigiensis* captured off Okinawa-jima Island was purchased at the Awase fish market in Okinawa City, Okinawa Prefecture, Japan on 19 December 2018. It was brought on ice to the laboratory of Ryukyus University Museum (Fujukan) and examined for gill parasites under a dissecting microscope. Monogeneans were collected from this fish using small needles and forceps. The bodies of three monogenean specimens were cut from the haptors using needles and preserved in 99%

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ethanol. The separated haptors and some whole specimens were flattened under coverslip pressure and fixed in modified picrate glycerine (Nitta & Nagasawa, 2018). The remaining specimens were fixed in acetic acid-formalin alcohol between a slide and a coverslip and stained with Heidenhain's iron haematoxylin.

All specimens used for morphological analysis were dehydrated through a graded ethanol series, cleared in xylene, and mounted in Canada balsam. Drawings were made using a drawing tube fitted on an Olympus BX60 microscope. All measurements are in micrometres and are given as the range followed by the mean and number (n) of specimens examined in parentheses. The numbering of hook pairs follows Mizelle (1936). Fish identification was based on Hatooka (2013). The specimens are deposited in the Platyhelminthes Collection of the National Museum of Nature and Science (NSMT-Pl), Tsukuba City, Ibaraki Prefecture, Japan.

DNA was extracted using the NucleoSpin Tissue XS (Macherey-Nagel) kit in accordance with the manufacturer's instructions. Partial 28S rDNA fragment was amplified from the extracted DNA 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') (Li et al., 1994). The PCR was performed in a total volume of 20 µl containing 0.1 µl Takara Ex Taq DNA polymerase (TaKaRa), 2.0 µl PCR buffer (TaKaRa), 1.6 µl dNTP mixture (2.5 mM of each dNTP) (TaKaRa), 0.6 µl of each 10 µM primer, 1.6 µl of extracted DNA, and 13.5 µl of distilled water. Cycling conditions included initial denaturation at 94°C for 5 min, followed by 30 cycles at 94°C for 30 s, at 54°C for 30 s, and at 72°C for 30 s, and 5 min at 72°C for a final extension. The amplified PCR products were purified using the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel) and sequenced using a 3130X Genetic Analyzer (Applied Biosystems) with the same PCR primers.

The phylogenetic position of the species was estimated by aligning the newly obtained sequence and 28S rDNA sequences for 27 diplectanid species retrieved from GenBank (Table 1) and by performing analyses using maximum likelihood (ML) and Bayesian inference (BI). The alignment was performed with MAFFT version 7 (Katoh et al., 2019) using the default parameters. The ML phylogeny was constructed under the TIM3+F+G4 model, which was determined to be the best-fit model based on the Bayesian information criterion using IQ-TREE version 1.6.8. (Nguyen et al., 2015; Kalyaanamoorthy et al., 2017), and the phylogeny was tested by 1,000 bootstrap repeats. BI and Bayesian posterior probabilities were estimated using MrBayes 3.2.6 (Ronquist et al., 2012), and the best-fit model, the GTR+G model, was selected based on the Bayesian information criterion using jModeltest 2.1.7 (Guindon & Gascuel, 2003; Darriba et al., 2012). Two independent runs of four Markov chains were conducted for 1,000,000 generations and the tree was sampled every 100 generations. Parameter estimates and convergence were checked using Tracer v. 1.6.0 (Rambaut & Drummond, 2013); the first 10,000 samples from each run were discarded as 'burn-in'and the remaining were analysed.

Family Diplectanidae Monticelli, 1903 Subfamily Diplectaninae Monticelli, 1903

Latiphagum n.g.

Diagnosis

Body fusiform, comprising body proper and haptor. Peduncle slender, smooth. Eyespots two pairs. Bilateral head organs three pairs. Muscular pharynx, oesophagus, and bifurcate intestinal caeca present. Common genital pore midventral, posterior to male copulatory organ (MCO). Gonads tandem. Testis pyriform, posterior to germarium, intercaecal. Vas deferens arising from anterior margin of testis, extending from intercaecal portion, forming seminal vesicle, entering the expanded base of MCO. Single saccate prostatic reservoir present in expanded base of MCO. MCO sclerotised, base with three concentric incomplete ridges, tube coiled, and supported on both sides by two curved plates with long bristles. Germarium in mid-body, pyriform, pretesticular, encircling right intestinal caecum dorsoventrally, then forming oviduct. Oviduct receiving duct from seminal receptacle, continuing as oötype. Mehlis' gland connecting base of oötype. Vaginal pore opening ventral, left of common genital pore. Vagina comprising distal thick-walled and muscular tube, delicate vaginal duct then leading to saccate seminal receptacle lying ventrally to oviduct. Vitelline fields approximately co-extensive with intestinal caeca. Cement glands

Table 1 List of diplectanid monogenean species used in this study with GenBank accession numbers

Monogenean species	Synonymised name registered in GenBank	Accession number
Subfamily Diplectaninae Monticelli, 1903		
Diplectanum penangi		DQ054821
Diplectanum umbrinum		EF100560
Diplectanum veropolynemi		AY553625
Echinoplectanum leopardi		FJ882609
Furcohaptor brevis		LC270951
Laticola latesi	Pseudorhabdosynochus latesis	AY553621
Laticola latesi		DQ054824
Laticola paralatesi		DQ054826
Laticola paralatesi		KP313568
Laticola seabassi	Pseudorhabdosynochus seabassi	AY553620
Laticola seabassi	Laticola lingaoensis	DQ054825
Latiphagum setosum n. comb.		LC494521 ^a
Murraytrema pricei		DQ157672
Paradiplectanum blaiense	Diplectanum blaiense	AY553627
Paradiplectanum sillagonum	Diplectanum sillagonum	AY553626
Pseudorhabdosynochus coioidesis		AY553623
Pseudorhabdosynochus cupatus		FJ882608
Pseudorhabdosynochus epinepheli		AY553622
Pseudorhabdosynochus grouperi	Diplectanum grouperi	DQ054820
Pseudorhabdosynochus lantauensis		DQ054829
Pseudorhabdosynochus melanesiensis		FJ882607
Pseudorhabdosynochus shenzhenensis		DQ054830
Sinodiplectanotrema argyrosomus	Sinodiplectanotrema argyromus [sic]	DQ157673
Sinodiplectanotrema malayanum		GU573891
Teraplectanum angustitubus		KY008488
Subfamily Lamellodiscinae Oliver, 1969		
Lamellodiscus acanthopagri		DQ054822
Lamellodiscus spari		DQ054823

^aNewly obtained sequence

present. Haptor with bilateral lobes, dorsal and ventral squamodiscs. Squamodiscs with concentric rows of rodlets, first inner row closed. Ventral anchor with well-developed roots. Dorsal anchor with subtriangular base. Ventral bar with ventral longitudinal groove. Dorsal bar paired with enlarged medial end. Two pairs of hooks on ventral side of mid-haptor, three lateral pairs ventrally and two dorsal pairs in haptor laterally. On gills of latid fishes.

Type-species: Diplectanum setosum Nagibina, 1976.

ZooBank registration: To comply with the regulations set in Article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN, 2012), details of the new name have been submitted to ZooBank. The Life Science Identifier (LSID) for *Latiphagum* n. g. is urn:lsid:-zoobank.org:act: 6B6064AE-5E7E-4941-A819-607F85DCD53A.

Etymology: The genus name is a combination of the Latin word *lateo* from the host family, Latidae, and *phagein*, which means eating. Feminine.

Remarks

This new genus is distinguished from other genera of diplectanids, except for Pseudorhabdosynochus Yamaguti, 1958, Echinoplectanum Justine & Euzet, 2006, and Laticola Yang, Kritsky, Sun, Zhang, Shi, & Agrawal, 2006, by its MCO having the expanded base with muscular ridges, lacking the accessory copulatory organ, and the ventral anchor having the developed superficial root (Yamaguti, 1958; Justine & Euzet, 2006; Yang et al., 2006; Domingues & Boeger, 2008). Latiphagum n. g. can be readily separated from Pseudorhabdosynochus, which has a characteristic male quadriloculate organ (Yamaguti, 1958). The gonads and vagina of the new genus are tandem and muscular, whereas those of Echinoplectanum are parallel and sclerotised, respectively (Justine & Euzet, 2006). The MCO of Laticola is spoon-shaped, whereas that of Latiphagum n. g. is not. Furthermore, the MCO is devoid of cirrus, the tube is supported both sides by two curved plates with long bristles, and the prostatic reservoir is present in the expanded base of the MCO in the new genus, but not in its closely related genera.

Latiphagum setosum (Nagibina, 1976) n. comb.

Syn. *Diplectanum setosum* Nagibina, 1976, originally described as *D. setosus*.

Type-host: Psammoperca waigiensis (Cuvier) (Perciformes: Latidae).

Other host: Lates calcarifer (Bloch) (Perciformes: Latidae).

Type-locality: South China Sea, off Hainan, China (Nagibina, 1976; Zhang et al., 2001).

Other locality: Off Okinawa-jima Island, Okinawa Prefecture, southern Japan (this study).

Site in host: Gill filaments.

Material examined: 13 specimens (NSMT-Pl 6396). *Intensity*: 19 monogeneans infected one host fish.

Representative DNA sequence: Partial 28S rDNA sequence was submitted to DNA Data Bank of Japan (DDBJ) under the accession number LC494521 (863 bp).

Redescription (Fig. 1)

Body fusiform, 653-759 (714, n = 3) long including haptor, 128–152 (139, n = 3) wide in mid-body. Peduncle slender and smooth. Eyespots 2 pairs.

Bilateral head organs 3 pairs. Pharynx muscular, 33-43 (38, n = 3) long, 35-35 (35, n = 3) wide. Oesophagus and bifurcate intestinal caeca present. Cement glands present.

Common genital pore midventral, posterior to MCO. Gonads tandem. Testis pyriform, posterior to germarium, intercaecal, 60–75 (68, n = 3) long, 53–55 (54, n = 3) wide. Vas deferens arising from anterior margin of testis, extending from intercaecal portion, forming seminal vesicle, entering expanded base of MCO. Single saccate prostatic reservoir present in expanded base of MCO. MCO sclerotised, 84–107 (96, n = 13) in total length, with 3 concentric incomplete ridges on base, 43–60 (51, n = 10) long, 35–66 (55, n = 10) wide, and coiled tube, 49–79 (68, n = 13) long, supported on both sides by 2 curved plates with long bristles; plates attached at middle of MCO and expanded slightly with pressure.

Germarium in mid-body, pyriform, pretesticular, encircling right intestinal caecum dorsoventrally, 29–40 (34, n = 3) in width, then forming oviduct. Oviduct receiving duct from seminal receptacle, continuing as oötype. Mehlis' gland connecting base of oötype. Vaginal pore opening ventral, left of common genital pore. Vagina comprising distal thick-walled and muscular tube, 71–90 (78, n = 3) long, 49–61 (55, n = 3) wide, delicate vaginal duct then leading to saccate seminal receptacle lying on ventral to oviduct. Vitelline fields approximately co-extensive with intestinal caeca.

Haptor with bilateral lobes, 53-75 (68, n = 3) long, 112–142 (127, n = 3) wide. Dorsal squamodisc, 30–37 $(33, n = 10) \log_{10}(29-39)(33, n = 10)$ wide, with 10-12 (11, n = 10) concentric rows of rodlets, first inner row closed. Ventral squamodiscs, 35-42 (37, n = 10) long, 36-42 (39, n = 10) wide with 11-12 (11, n = 10) concentric rows of rodlets, first inner row closed. Dorsal anchor with short truncate superficial root, total length 30-33 (32, n = 12), shaft length 26-29 (27, n = 12), deep root length 3-6 (4, n = 11), superficial root length 3-5 (4, n = 11), point length 8-12 (11, n = 11). Ventral anchor with developed roots, total length 29–38 (35, n = 13), shaft length 25–31 (29, n = 13), deep root length 7–14 (9, n = 12), superficial root length 8-10 (9, n = 10), point length 9-11 (10, n = 10). Dorsal bar paired, with enlarged medial end, 41-47 $(43, n = 12) \log_{10}(10-14) (12, n = 12)$ wide. Ventral bar with ventral longitudinal groove, tapered ends and slight medial constriction, length 45-59 (54, n = 12),



Fig. 1 Latiphagum setosum (Nagibina, 1976) n. comb., parasitic on a Psammoperca waigiensis (Cuvier) specimen collected off the Okinawa-jima Island, southern Japan (NSMT-PI 6396). A, Whole body (ventral view); B, Dorsal anchors; C, Ventral anchors; D, Dorsal bars; E, Ventral bar; F, Hooks (pair I to VII); G, Dorsal squamodisc; H, Ventral squamodisc; I, Male copulatory organ (MCO) of slightly flatted and stained specimen (ventral view); J, MCO of heavily flatted and unstained specimen (dorsal view); K, Vagina (ventral view)



Fig. 2 Bayesian inference (BI) tree for the Diplectaninae based on partial 28S rDNA data using two species of *Lamellodiscus* (Lamellodiscinae) as the outgroup. The species newly sequenced in this study is indicated in bold. The corresponding GenBank accession numbers are shown. The tree includes results for BI and Maximum Likelihood with PP/bootstrap branch support

total width 10–15 (13, n = 12), median width 5–10 (7, n = 12). Hooks 7 pairs; pairs I and V on ventral side of mid-haptor, pairs II–IV lateral ventrally, pairs VI and VII dorsal on haptor laterally; pair I 9–11 (10, n = 7) long, pair II 10–11 (10, n = 6) long, pair III 10–11 (10, n = 6) long, pair V 9–11 (10, n = 6) long, pair V 9–11 (10, n = 6) long. Pair VII 9–11 (10, n = 6) long.

Remarks

Nagibina (1976) did not designate this species' type host, but Yang et al. (2006) conjectured that *P. waigiensis* is the natural host of *D. setosum*, while its occurrence on *L. calcarifer* is accidental. Nagibina (1976) and Zhang et al. (2001) described the sclerotised structures of *D. setosum*, but they overlooked the expanded base of MCO probably because this part is slightly difficult to recognise on unstained or heavily flatted specimens. The measurements and morphology of sclerotised parts of the specimens collected in the present study are almost identical to the descriptions of *D. setosum* by Nagibina (1976) and Zhang et al. (2001), but the measurements of the haptor provided by Zhang et al. (2001) are most likely misprints because of the extremely small measurements.

Phylogenetic analysis

Two species of *Lamellodiscus* (Lamellodiscinae) were used as the outgroup following Domingues & Boeger (2008), Lim et al. (2010), and Nitta & Nagasawa (2017). ML and BI analyses resulted in similar tree topologies (Fig. 2), and the tree conformed with the analyses by Lim et al. (2010) and Nitta & Nagasawa (2017). Latiphagum setosum n. comb. was assigned to the Diplectaninae and has a sister group with the clade consisting of species of Echinoplectanum, Pseudorhabdosynochus and Laticola. Sequences of Paradiplectanum spp., Sinodiplectanotrema spp., *Pseudorhabdosynochus* spp. and *Laticola* spp. formed clades with high branch support.

Discussion

Pseudorhabdosynochus and Laticola, have common morphological features according to the analysis of Domingues & Boeger (2008): MCO with distal tube enclosing eversible cirrus and base of the MCO expanded. The MCO of Latiphagum n. g. had no enclosing eversible cirrus, and L. setosum was not part of the clade comprising representatives of these three genera. The results of the morphological analysis are consistent with those of the molecular phylogeny (Fig. 2). The molecular analysis showed high support rates for the monophyletic clade of monogeneans including Diplectanum (sensu lato) penangi Liang & Leong, 1991. Two early-diverged species, D. penangi and L. setosum n. comb., and Laticola spp. use latid fishes as hosts (Nagibina, 1976; Liang & Leong, 1991; Yang et al., 2006), and this may indicate that Echinoplectanum and Pseudorhabdosynochus may have derived from monogenean transfer from latid hosts to serranids.

Diplectanum is considered as a paraphyletic group and the official affiliation of c.40 species have not been determined (Domingues & Boeger, 2008). Some of these species groups have already been rearranged; however, Kritsky & Diggles (2015) mentioned that it is necessary to transfer some species to other existing genera or to propose new genera to accommodate these species. The affiliation of some species of Diplectanum (sensu lato), for instance, cannot be determined because their original descriptions lack information on anatomy and on details of MCO; as Latiphagum setosum n. comb., some species require redescriptions based on newly collected materials. Furthermore, molecular analysis is a useful tool that can help solve taxonomical issues of diplectanids (e.g. Lim et al., 2010; Nitta & Nagasawa, 2017) and will provide supporting data for the taxonomic treatment of Diplectanum.

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Compliance with ethical standards

Conflict of interest The author declares that he has 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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