Dactylogyrus oryziasi n. sp. (Monogenea: Dactylogyridae) from *Oryzias latipes* (Beloniformes: Adrianichthyidae) in Japan

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Dactylogyrus oryziasi n. sp. (Monogenea: Dactylogyridae) is described from the gills of the southern medaka *Oryzias latipes* (Temminck and Schlegel, 1846) (Beloniformes: Adrianichthyidae) collected from an irrigation canal, Tokushima city, Tokushima Prefecture, Shikoku, Japan. This new species resembles *Dactylogyrus rysavyi* Ergens, 1970 in having a coiled penis passing through a tube of an accessory piece associated with a sigmoid rod, and shapes of haptor parts, but these species differ from each other by turns of the penis and a connection point between the rod and tube of the accessory piece. This is the first record of *Dactylogyrus* from beloniform fish.

Key Words: *Dactylogyrus oryziasi* n. sp., Monogenea, Dactylogyridae, *Oryzias latipes*, Beloniformes, Adrianichthyidae, Japan.

Introduction

The southern medaka *Oryzias latipes* (Temminck and Schlegel, 1846) (Beloniformes: Adrianichthyidae) is a freshwater fish endemic to the Japanese Archipelago ranging from Honshu Island to the Ryukyu Islands (Senou 2013). Recently, the wild and pure population of the species has greatly decreased because of the habitat loss, introduction of invasive alien species, and hybridization with other geographical races of the same species, and the fish has thus been designated as “vulnerable” in Japan (Hosoya 2015). While about 20 species of protistan and metazoan parasites have been reported from the fish in Japan (Nagasawa *et al.* 2012), much remains unstudied. An undescribed dactylogyrid monogenean was collected from the fish in an irrigation canal in Tokushima Prefecture, Shikoku, Japan, and is herein described as *Dactylogyrus oryziasi* n. sp.

Materials and Methods

Twenty individuals of the southern medaka were collected by a hand net in an irrigation canal connected to the So-nose River (34°02′17.1″N, 134°31′15.4″E) at Hirota, Kami-Hachiman-cho, Tokushima city, Tokushima Prefecture, Shikoku, Japan, on 18 September 2013. They were transported alive to the laboratory of Hiroshima University, killed by icing, identified based on Senou (2013), and then examined for gill parasites under a dissecting microscope. Monogeneans were removed from the gills using small needles.

For molecular analysis, one specimen was identified under slight coverslip pressure and preserved in 99% ethanol. For morphological analysis, some specimens were fixed in 70% ethanol under coverslips and stained with Heidenhain’s iron hematoxylin, or fixed in ammonium picrate glycerin (APG) under coverslip pressure (Lim 1991).

All specimens except the one for molecular study were dehydrated through a graded ethanol series, cleared in xylene, and mounted in Canada balsam. The drawings were made with the aid of a drawing tube fitted on an Olympus BX51 light microscope. The method of measuring sclerotized structures are presented in Fig. 1. All measurements except lengths of penis and vagina were made with a calibrated ocular micrometer on an Olympus BX51 light microscope. Lengths of penis and vagina were measured from drawings using ImageJ software (version 1.45i). Measurements, in micrometers, are expressed as the mean $\pm$ standard deviation followed in parentheses by the range of structure measurements and the number (*n*) of specimens examined. Numbering of marginal hook pairs follows Llewellyn (1963). Type specimens are deposited in the Platyhelminthes collection at the National Museum of Nature and Science (NSMT-PI), Tsukuba city, Ibaraki Prefecture, Japan.

DNA was extracted from one specimen with the use of a DNeasy blood and tissue kit (Qiagen, Valencia, California) in accordance with the manufacturer’s instructions. The DNA was amplified by polymerase chain reaction (PCR) using the primer pair C1 (5′-ACCCGCTGAATTTAA GCA T-3′) and D2 (5′-TGGTCCGTGTTTCAA GAC-3′) (Hassouna *et al.* 1984) to amplify partial 28S rDNA. An amplification reaction was performed in a final volume

Fig. 1. Measurements of hard parts. A, anchor; B, bar; C, marginal hook; D, male copulatory organ; E, vagina. Abbreviations: al, accessory piece length; atl, anchor total length; bmw, bar median width; btl, bar total length; btw, bar total width; irl, inner root length; ln, length to notch; mhl, marginal hook length; orl, outer root length; pl, penis length; pol, point length; vl, vagina length.

of 25 μ L containing 3 μ L of DNA template, 10 $\times$ Titanium Taq PCR Buffer (Clontech), 0.2 mM of each dNTP, 1 μ M of each primer, and 1 $\times$ Titanium Taq DNA Polymerase (Clontech). PCR was carried out with the following protocol: 94°C for 5 min followed by 35 cycles of 94°C for 60 sec, 56°C for 60 sec and 72°C for 60 sec, and 5 min of final hold at 72°C. PCR products were electrophoresed on a 2% agarose gel and stained with ethidium bromide. Fragments were cut from the gel and purified using QIAquick Gel Extraction Kit (Qiagen, Valencia, California), and the product was sequenced with a 3130X Genetic Analyzer (Applied Biosystems, Foster, California) with the same primers used for PCR. The sequence was compared with the all available sequences of dactylogyrids using BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>) software on 19 July 2016.

***Dactylogyrus oryziasi* n. sp.**

[New Japanese name: medaka-yubigata-mushi]
(Fig. 2)

Holotype. NSMT-PI 6291 fixed in APG.

Paratypes. Three specimens stained in Heidenhain's iron hematoxylin (NSMT-PI 6292) and six specimens fixed in APG (NSMT-PI 6293).

Description. Body (Fig. 2A) including haptor length 459 ± 99.0 (290–628; $n=9$), width at mid-body 95 ± 12.9 (75–113; $n=9$). Three pairs of head organs. Two pairs of

eye-spots with some dissociated eyes. Alimentary systems consisting of spherical pharynx: diameter 44 ± 5.3 (33–50; $n=9$), esophagus and bifurcated intestine confluent just posterior to testis. Testis ovate to pyriform, postero-dorsal to ovary. Vas deferens arising from anterior part of testis, looping around left intestinal caeca onto ventral, extending seminal vesicle located dorsal to copulatory organ (Fig. 2N), entering base of copulatory organ. Two prostatic reservoirs saccate (Fig. 2N). Male copulatory organ (Fig. 2M, N) consisting of penis and accessory piece. Penis length 185 ± 24.0 (155–226; $n=7$), clockwise coiling two and half and counterclockwise coiling one and half toward point; its tip passing through tube of accessory piece; its base touching base of sigmoid rod. Sclerotized accessory piece, length 23 ± 1.6 (21–26; $n=10$), consisting of bended funnel-shaped tube and sigmoid rod connected to middle of tube. Ovary in mid-body, elongate. Oviduct arising anterior part of ovary, continuing as oötype surrounded by Mehlis' gland. Vagina (Fig. 2O) thin sclerotized tube, length 75 ± 15.3 (56–94; $n=5$), arising from right side of oviduct; vaginal pore located at mid-body on right ventral body surface. Vitellaria co-extensive with intestinal caeca.

Haptor length 48 ± 7.2 (34–55; $n=8$), width 90 ± 5.2 (85–98; $n=8$). Haptoral glands developed at base of haptor. Anchor (Fig. 2B) total length 23 ± 0.9 (22–25; $n=10$), length to notch 19 ± 0.7 (18–20; $n=10$), outer root length 3 ± 0.5 (2–3; $n=10$), inner root length 8 ± 0.9 (7–10; $n=10$), point length 7 ± 0.7 (6–8; $n=10$). Dorsal bar (Fig. 2C) broad U-shape, total length 25 ± 0.8 (24–26; $n=10$), total width 9 ± 0.8 (8–10; $n=10$), median width 3 ± 0.7 (2–4; $n=10$). Ventral bar (Fig. 2D) broad V-shape, total length 26 ± 2.1 (24–31; $n=10$), total width 5 ± 0.4 (4–5; $n=10$), median width 3 ± 0.5 (2–3; $n=10$). Marginal hooks in 7 pairs; hook length: pair I (Fig. 2E) 16 ± 0.5 (16–17; $n=9$); pair II (Fig. 2F) 16 ± 0.4 (16–17; $n=9$); pair III (Fig. 2G) 17 ± 0.7 (16–18; $n=8$); pair IV (Fig. 2H) 17 ± 0.7 (16–18; $n=9$); pair V (Fig. 2I) 17 ± 0.4 (16–17; $n=9$), pair VI (Fig. 2J) 17 ± 0.5 (16–17; $n=9$); pair VII (Fig. 2K) 17 ± 0.5 (16–17; $n=9$). Pair of needles (Fig. 2L), length 8 ± 0.7 (7–9; $n=9$), located near top of second hooks.

Host. *Oryzias latipes* (Beloniformes: Adrianichthyidae).

Site of infection. Gill filaments.

Prevalence and intensity range (mean). Seven (35%) of 20 fish examined were infected by 1–4 (mean: 1.6) worms.

Sequence data. The partial 28S rDNA (696 bp) sequence was submitted to DDBJ (accession no. LC190737). A BLAST search of the sequence did not have any identical hit. The closest hits for this sequence are *Dactylogyrus inexpectatus* Isjumova in Gussev, 1955 (AJ969945, 88% similarity with 93% coverage), *D. petruschewskyi* Gussev, 1955 (AY548927, 87% similarity with 83% coverage), *D. extensus* Mueller and Van Cleave, 1932 (AY553629, 84% similarity with 93% coverage), *D. hemiamphibothrium* Ergens, 1956 (AJ969946, 84% similarity with 97% coverage) and *D. lamellatus* Achmerow, 1952 (AY307019, 84% similarity with 93% coverage).

Remarks. This new species corresponds to the diag-

Fig. 2. *Dactylogyrus oryziasi* n. sp., paratype (NSMT-PI 6292) for A, N, holotype (NSMT-PI 6291) for B–M, O. A, whole mount (ventral view); B, anchor; C, dorsal bar; D, ventral bar; E, marginal hook pair I; F, marginal hook pair II; G, marginal hook pair III; H, marginal hook pair IV; I, marginal hook pair V; J, marginal hook pair VI; K, marginal hook pair VII; L, needle; M, male copulatory organ (dorsal view); N, male genital organs (ventral view); O, vagina (dorsal view). Scale bars: A, 50 μ m; B–O, 10 μ m. Abbreviations: an, anchor; ap, accessory piece; e, eye; h, haptor; hg, haptoral grand; ho, head organ; i, intestine; mg, Mehlis' gland; mh, marginal hook; n, needle; o, oötype; od, oviduct; ov, ovary; p, penis; ph, pharynx; pr, prostatic reservoir; sv, seminal vesicle; t, testis; v, vagina; vd, vas deferens; vl, vitellaria; vo, vaginal opening.

nostic characteristics of the genus *Dactylogyrus* Diesing, 1850 (Beverley-Burton 1984; Gussev *et al.* 2010), and the BLAST searches support the generic position of the species. *Dactylogyrus oryziasi* n. sp. resembles *Dactylogyrus rysavyi* Ergens, 1970 in a coiled penis passing through a tube of an accessory piece associated with a sigmoid rod, and the shape of the anchor (Ergens 1970). The new species, however, differs from *D. rysavyi* which has the penis making right-hand one and half turns and left-hand one and half turns toward the point and the rod of the accessory piece connecting to the end of its tube (Ergens 1970; Gussev *et al.* 2010).

Etymology. The new specific name is delivered from the generic name of the type host.

Japanese name. The new Japanese name is a combination of “medaka” and “yubigata-mushi”, which are the Japanese name of *Oryzias* and *Dactylogyrus* (as “yubigata-mushi-zoku”), respectively.

Discussion

Over 900 nominal species of *Dactylogyrus* are known over the world, and about 95% of these species have been reported from cypriniform fishes (Gibson *et al.* 1996). This is the first record of *Dactylogyrus* parasitic on beloniform fish. Fishes of the genus *Oryzias* Jordan and Snyder, 1906 are distributed ranging from Japan to India through mainland China and in a nearby region of Indonesia (Parenti 2008). This genus currently consists of about 33 species (Parenti 2008; Magtoon 2010; Herder and Chapuis 2010; Parenti and Hadiaty 2010; Asai *et al.* 2011; Herder *et al.* 2012; Parenti *et al.* 2013; Mokodongan *et al.* 2014). In Japan, *O. latipes* is separated into nine geographic populations (Sakaizumi 2000), and the fish examined in this study belongs to “the East Seto inland Sea Group.” It would be interesting to clarify the dactylogyrid fauna of different species of *Oryzias* and different geographic populations of *O. latipes* which may demonstrate that the timing and frequency of host-switching of *Dactylogyrus* from cyprinids as major hosts to adrianichthyids.

Recently, *O. latipes* has been used in laboratories as one of the most marked vertebrate model organisms to study genetics, normal body function, and diseases (Wittbrodi *et al.* 2002; Iwamatsu 2006; Kinoshita *et al.* 2009). Nagasawa *et al.* (2012) suggested that this species has potential for investigating various aspects of a host-parasite relationship, and if *Dactylogyrus oryziasi* n. sp. can be kept using *O. latipes* as its host in aquaria, much information on such a relationship of these animals may be obtained, like guppy *Poecilia reticulata* Peters, 1859 (Cyprinodontiformes: Poeciliidae) and gyrodactylid monogeneans *Gyrodactylus* spp. (e.g., Scott 1982; Scott and Anderson 1984; Harris 1989).

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