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Gobioecetes longibasais n. sp. (Monogenea: Dactylogyridae) from *Rhinogobius similis* Gill (Perciformes: Gobiidae) from Okinawa-jima Island, the Ryukyu Archipelago, southern Japan, with a new host record for *Gobioecetes biwaensis* Ogawa & Itoh, 2017

Masato Nitta · Kazuya Nagasawa

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Abstract *Gobioecetes longibasis* n. sp. (Monogenea: Dactylogyridae) from the gills of the freshwater goby *Rhinogobius similis* Gill (Perciformes: Gobiidae) in the River Teima, Okinawa-jima Island, the Ryukyu Archipelago, southern Japan, is described. The new species is distinguished from two congeneric species of *Gobioecetes* Ogawa & Itoh, 2017, *G. rhinogobius* (Ling, 1973) and *G. biwaensis* Ogawa & Itoh, 2017, by having longer ventral hamuli, longer and wider internal process of the dorsal hamuli, and the ratio of dorsal hamulus length to base length. This new species is host-specific to *R. similis* and considered to be endemic to Okinawa-jima Island or the Ryukyu Archipelago. We also report *Rhinogobius* sp. OM from a tributary of Lake Biwa, Shiga Prefecture, central Japan, as a new host of *G. biwaensis*.

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M. Nitta (✉)
Graduate School of Science, Kobe University, 1-1 Rokkodai, Nada, Kobe 657-8501, Japan
e-mail: licht.bsn.mono@gmail.com

K. Nagasawa
Graduate School of Integrated Sciences for Life, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8528, Japan

Present Address:
K. Nagasawa
Aquaparasitology Laboratory, 365-61, Kusanagi, Shizuoka 424–0886, Japan

Introduction

Gobioecetes Ogawa & Itoh, 2017 (Monogenea: Dactylogyridae) was originally proposed for two species, *G. biwaensis* Ogawa & Itoh, 2017 and *G. rhinogobius* (Ling, 1973), parasitic on freshwater gobies *Rhinogobius* spp. and is characterised by having a tubular penis associated with the accessory piece and lacking the dorsal bar (Ogawa & Itoh, 2017). *Gobioecetes biwaensis* has been recorded from *Rhinogobius biwaensis* Takahashi & Okazaki from Lake Biwa, Shiga Prefecture, and an unidentified *Rhinogobius* specimen from Sento Imperial Palace, Kyoto Prefecture in the Yodo River system (Ogawa & Itoh, 2017). The latter goby was recently reported as a hybrid of *R. biwaensis* and *R. tyoni* Suzuki, Kimura & Shibukawa (as *Rhinogobius* sp. BF) (Akihito et al., 2019).

Okinawa-jima Island, the largest island of the Ryukyu Archipelago, is located in the southernmost and subtropical climate region of Japan, and about 100 species of fluvial and diadromous fishes are known from this island (Yoshigou, 2014). The monogenean

fauna of the island is poorly understood, and only 11 species of monogeneans have been reported from five species of freshwater fishes (Nitta & Nagasawa, 2018b). During a recent investigation into the monogenean fauna of freshwater fishes of the island, specimens of an undescribed dactylogyrid were collected from the diadromous freshwater goby, *Rhinogobius similis* Gill in the River Teima. We herein describe a new species of *Gobioecetes* from *R. similis* based on these specimens along with molecular data of the new species and *G. biwaensis*. To clarify the host specificity and geographical distribution of the new species, some other species of *Rhinogobius* from the River Teima and individuals of *R. similis* from seven localities in western and central Japan were examined for monogeneans of *Gobioecetes*. During this survey, we collected *G. biwaensis* from *Rhinogobius* sp. OM in the River Wadauchi, a tributary of Lake Biwa, and report on this collection as a new host record for *G. biwaensis*.

Materials and methods

Specimens of *R. similis* (n = 5; standard length, SL: 34.4–89.0 mm), *R. brunneus* (Temminck & Schlegel) (n = 10, SL: 32.3–49.5 mm), *R. nagoyae* Jordan & Seale (n = 17, SL: 26.5–48.7 mm), *Rhinogobius* sp. BB (n = 4, SL: 30.3–36.8 mm) and *Rhinogobius* sp. MO (n = 6, SL: 33.3–41.0 mm) were collected from the River Teima (26°33′29″N, 128°04′42″E) at Mihara, Nago City, Okinawa-jima Island, Okinawa Prefecture on 18 September 2012, 25 and 27 January 2013, 24–28 June 2015, and 23 May 2019. Specimens of *R. biwaensis* (n = 7, SL: 14.2–33.8 mm) and *Rhinogobius* sp. OM (n = 21, SL: 29.6–44.8 mm) were collected from the River Wadauchi (35°18′06″N, 136°00′50″E), a tributary of Lake Biwa, at Katsuno, Takashima City, Shiga Prefecture on 8 June 2017. In addition to these collections, a total of 45 specimens of *R. similis* was collected from seven localities in western and central Japan (Table 1). Gills were removed from each fish, and dactylogyrids were collected from the gills with needles under a dissecting microscope. Bodies of some specimens were cut from the haptors using needles and preserved in 99% ethanol for molecular analysis. The rest of the haptors and the other specimens were flattened under a coverslip and fixed in acetic acid-formalin-alcohol or modified picrate glycerin (Nitta & Nagasawa, 2018a). Some whole specimens were subsequently stained with Heidenhain's iron haematoxylin, and all flattened specimens were dehydrated through a graded ethanol series, cleared in xylene, and mounted in Canada balsam.

Illustrations were prepared with made with the aid of a drawing tube fitted on an Olympus BX60 microscope. Measurements except for penis length were obtained with the use of a calibrated filar micrometer, represented by straight-line distances between extreme points. Penis length was measured on drawings using ImageJ software (version 1.48i). All measurements are in micrometres and given as the range followed by the mean and the number (n) of specimens examined in parentheses. The fish identification was based on Akihito et al. (2013) and the scientific names of fishes mentioned in this paper follow Suzuki et al. (2015, 2019) and Takahashi & Okazaki (2017). Prevalence and intensity of infection are as defined by Bush et al. (1997). Voucher specimens of dactylogyrids are deposited in the Platyhelminthes collection of the National Museum of Nature and Science (NSMT-Pl), Tsukuba City, Ibaraki Prefecture, Japan.

DNA was extracted from bodies using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) or a NucleoSpin Tissue XS (Macherey-Nagel, Düren, Germany). The DNA was amplified by polymerase chain reaction (PCR) using the primer pairs C1 primer (5′-ACC CGC TGA ATT TAA GCA T-3′) and D2 primer (5′-TGG TCC GTG TTT CAA GAC-3′) to amplify partial 28S rDNA (Vân Le et al., 1993) and the primer pair S1 (5′-ATT CCG ATA ACG AAC GAG ACT-3′) (Sinnappah et al., 2001) and IR8 (5′-GCT AGC TGC GTT CTT CAT CA-3′) (Šimková et al., 2003) to amplify partial 18S rDNA, internal transcribed spacer 1 (ITS1), and partial 5.8S rDNA. PCR was performed in a total volume of 20 µl, containing 0.1 µl Takara Ex Taq DNA polymerase (TaKaRa, Kusatsu, Japan), 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. The 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, at 72°C for 30 s, and a final extension step at 72°C for 10 min. Amplified PCR products were purified using the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel) and were sequenced using a Big

Table 1 Sampling localities of *Rhinogobius similis*

Prefecture	Site	Longitude/latitude	n	SL range (mm)	Date
Okinawa	River Teima, Okinawa-jima Island	26°33′29″N, 128°04′42″E	5	34.3–89.0	18 September 2012; 25 January 2013; 23 May 2019
Kagoshima	River Koume, Tanegashima Island	30°42′53″N, 131°00′21″E	9	29.3–68.5	28 May 2019
	Lake Ikeda, Kyushu	31°15′04″N, 130°33′16″E	2	42.5–48.6	13 November 2014
Nagasaki	River Sasu, Tsushima Island	34°13′48″N, 129°12′23″E	6	42.3–77.5	22 June 2016
Shimane	River Masuda, Honshu	34°40′26″N, 131°50′58″E	5	40.9–68.7	29 January 2015
Hiroshima	River Kamo, Honshu	34°20′22″N, 132°54′04″E	11	23.7–46.3	19 November 2012
Wakayama	River Arida, Honshu	34°04′47″N, 135°09′01″E	10	32.3–45.7	11 November 2018; 9 September 2019
Aichi	An irrigation canal of the River Nagara, Honshu	35°11′00″N, 136°39′39″E	2	20.0–28.3	9 January 2019

Abbreviation: n, number of fish examined; SL, standard length

Dye Terminator V3.1 (Applied Biosystems, Foster, USA) and a 3130X Genetic Analyzer (Applied Biosystems) with the PCR primers. The sequences obtained were submitted to the DNA Data Bank of Japan Centre (DDBJ). The sequences of *Gobioecetes* spp. were aligned using MAFFT version 7 (Katoh et al., 2017), and the divergence was calculated with the use of a Kimura 2-parameter distance by MEGA7 (Kumar et al., 2016).

Results

Thirty-six specimens of dactylogyrid, which is described below as a new species, were collected from *R. similis* in the River Teima, Okinawa-jima Island, but no dactylogyrid was collected from the four other species of *Rhinogobius* (*R. brunneus*, *R. nagoyae*, *R.* sp. BB, and *R.* sp. MO) in this river and from *R. similis* in the seven localities of western and central Japan.

Seventeen specimens of *G. biwaensis* (NSMT-Pl 6403, 6404) were collected from *R. biwaensis* and *Rhinogobius* sp. OM in the River Wadauchi, central Japan, and this identification was morphologically and molecularly confirmed (Ogawa & Itoh 2017). *Rhinogobius* sp. OM represents a new host record for *G. biwaensis*. Prevalence and intensity of *G. biwaensis* on *R. biwaensis* were 14.3% (1/7) and 1, whereas those on *Rhinogobius* sp. OM were 38.1% (8/21) and 1–3, respectively.

Order Dactylogyridea Bychowsky, 1937
Family Dactylogyridae Bychowsky, 1933
Genus *Gobioecetes* Ogawa & Itoh, 2017

Gobioecetes longibasis n. sp.

Type-host: *Rhinogobius similis* Gill (Perciformes: Gobiidae).
Type-locality: River Teima (26°33′29″N, 128°04′42″E) at Mihara, Nago City, Okinawa-jima Island, Okinawa Prefecture, Japan
Type-material: Holotype (NSMT-Pl 6397a) and 20 paratypes (NSMT-Pl 6397b, 6398–6400).
Site of infection: Gill filaments.
Prevalence and intensity: 80.0% (4/5) and 4–20 (mean 9.0 worms per infected fish).
Representative DNA sequences: DDBJ accession numbers LC494516 and LC494517 (28S rDNA),

and LC494519 and LC494520 (18S+ITS1+5.8S rDNA). The sequences were obtained from two paratypes (NSMT-Pl 6399, 6400)

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 *Gobioecetes longibasis* n. sp. is urn:lsid:-zoobank.org:act:3ADADFD1-94EB-4C7D-8C9C-5CCFDC892A77.

Etymology: The new scientific name is from Latin (*longus* = long + *basis* = base) and refers to the long internal process of the dorsal hamuli.

Description (Fig. 1)

[Based on 14 stained and 7 unstained specimens.] Total length of body including haptor 342–662 (502, n = 18), maximum width 124–165 (144, n = 18) (Fig. 1A). Three pairs of head organs. Head gland cells on both sides of body at posterior level of pharynx. Two pairs of eye-spots, sometimes dissociated. Pharynx subspherical, 42–62 × 30–63 (52 × 44) (n = 18); oesophagus short; intestine bifurcates into 2 caeca confluent posterior to testis.

Haptor 57–118 (76, n = 17) long, 73–187 (112, n = 17) wide. Dorsal hamuli (Fig. 1B) smaller than ventral hamuli, 42–48 (45, n = 19) long; hamulus base 30–36 (32, n = 19) long, ratio of hamulus length to base length 1.33–1.53 (1.40, n = 19); external process slightly tapering distally, 4–12 (9, n = 19) long, 4–7 (5, n = 19) wide; internal process straight, not tapering, sometimes with triangular tip, 16–25 (20, n = 19) long, 6–8 (7, n = 19) wide; point 8–14 (11, n = 19) long. Ventral hamuli (Fig. 1C) 46–52 (50, n = 19) long; hamulus base 30–35 (33, n = 19) long, ratio of hamulus length to base length 1.39–1.63 (1.51, n = 19); external process tapering distally, 7–13 (10, n = 19) long, 4–8 (6, n = 19) wide; internal process straight, with triangular tip, 22–30 (26, n = 19) long, 6–8 (7, n = 19) wide; point length 9–14 (11, n = 19). Dorsal bar absent. Ventral bar (Fig. 3D) straight or slightly bent, slightly thickened at both ends, 22–30 (28, n = 19) long, 3–10 (7, n = 19) wide. Marginal hooks 7 pairs (Fig. 1E), all of approximately same length, 12–15 (14, n = 18) long.

Testis pyriform, posterodorsal to germarium, 53–135 × 36–64 (83 × 51) (n = 13). Vas deferens leaving from anterior region of testis, looping around left intestinal caecum, distended as seminal vesicle before leading into base of penis. Seminal vesicle 34–50 × 11–28 (41 × 20) (n = 13). Prostatic reservoir single, pyriform, dorsal to seminal vesicle, 15–31 × 11–17 (24 × 14) (n = 9). Male copulatory organ (Fig. 1F) sclerotised, consisting of tubular penis and accessory piece. Penis long, coiled, tapering, 97–120 (109, n = 15) long, 1–2 (1, n = 17) wide, 6–9 (7, n = 17) wide at base, with unsclerotised and non-muscular bulb. Accessory piece, 20–29 (24, n = 17) long, rod-shaped with distal claw-like bifurcation.

Germarium ovate, in mid-body, intercaecal, 39–93 × 28–52 (77 × 38) (n = 13). Oviduct arising from anterior side of germarium, continuing as oötype. Mehlis' gland opening at base of oötype. Seminal receptacle dorsal to oviduct. Vagina opening on right lateral side of body; vaginal duct slightly curved, leading into anterior part of seminal receptacle. Vitellarium approximately coextensive with intestinal caeca. Eggs (Fig. 1G) ovate, 56–70 × 43–51 (65 × 47) (n = 5), with short filament.

Remarks

Gobioecetes longibasis n. sp. corresponds with the diagnostic morphological characters of the genus proposed by Ogawa & Itoh (2017) and is similar to the two other congeneric species, *G. rhinogobius* and *G. biwaensis* parasitising *Rhinogobius* spp. The new species differs from *G. rhinogobius* by having larger ventral hamuli (46–52 *vs* 37–40 µm) (see Ling, 1973a) and from *G. biwaensis* in the ratio of dorsal hamulus length to base length (1.33–1.53 *vs* 1.03–1.26) (see Ogawa & Itoh, 2017). The internal process of the dorsal hamuli in *G. longibasis* n. sp. is also slightly longer and slender than that in *G. biwaensis* (16–25 × 6–8 *vs* 11–17 × 8–12 µm) (Ogawa & Itoh, 2017).

Molecular data comparison

The pairwise sequence divergence between *Gobioecetes longibasis* n. sp. and *G. biwaensis* in the sequences of partial 18S rDNA+ITS1+partial 5.8S rDNA and the partial 28S rDNA were 2.4% and 1.5%, respectively (Tables 2, 3). No intraspecific variation

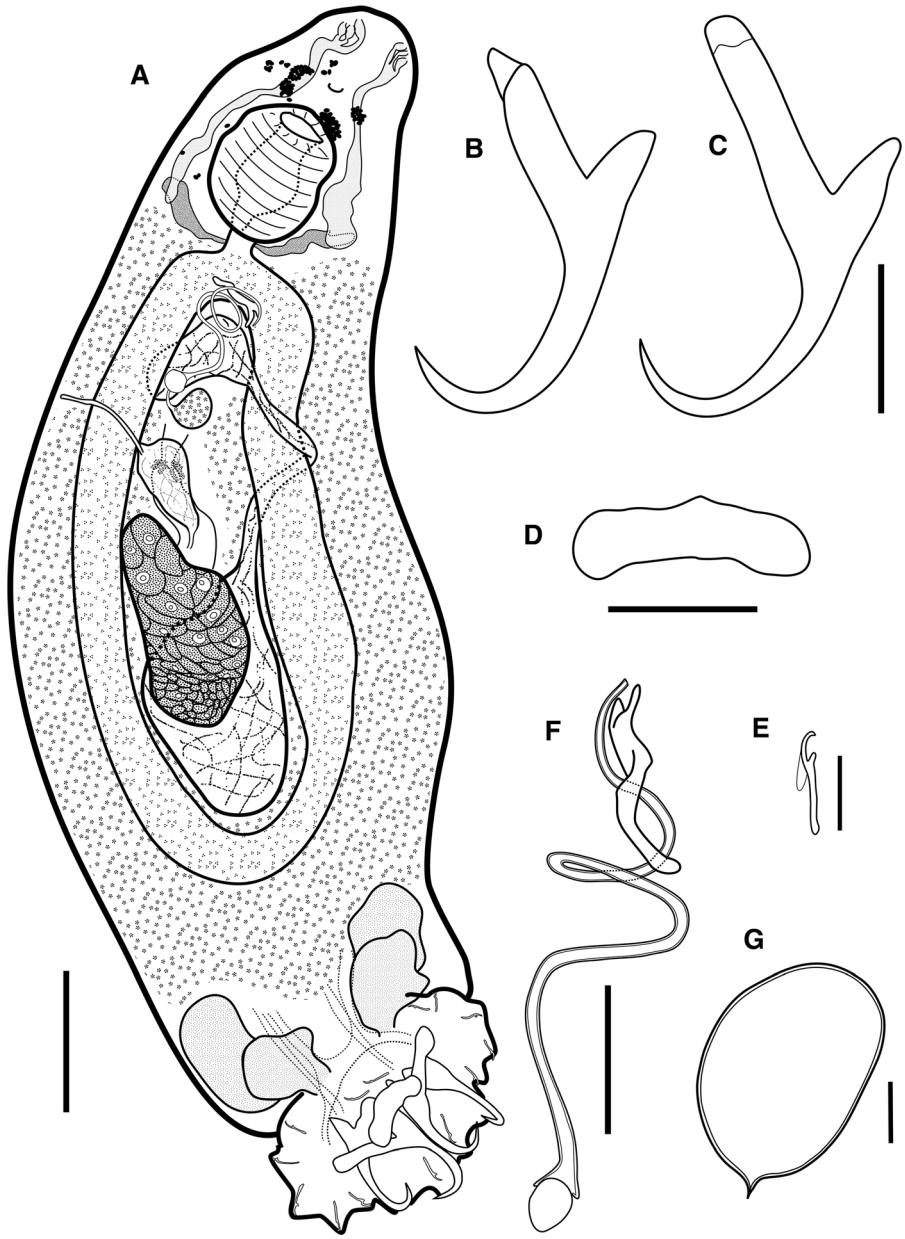

Fig. 1 *Gobioecetes longibasis* n. sp. ex *Rhinogobius similis* Gill in the River Teima, Okinawa-jima Island, Japan. A, Holotype (NSMT-Pl 6397a); B–F, Paratype (NSMT-Pl 6398); G, Paratype (NSMT-Pl 6397b). A, Whole body, ventral view; B, Dorsal hamulus; C, Ventral hamulus; D, Ventral bar; E, Marginal hook; F, Male copulatory organ; G, Egg. *Scale-bars*: A, 50 μm; B–E, 10 μm; F, 20 μm

in the sequence data was found for *G. longibasis* n. sp., but the genetic distance of partial 18S rDNA+ITS1+-partial 5.8S rDNA between *G. biwaensis* collected from *Rhinogobius* sp. OM and *R. biwaensis* was 0.2% (Table 1).

Discussion

To date, eleven species of monogeneans have been reported from freshwater fishes of Okinawa-jima Island (Nitta & Nagasawa, 2018b), and *G. longibasis* n. sp. is the 12th species. Since *G. longibasis* n. sp. has never been collected from the mainland of Japan

Table 2 The sequence divergence (in %) for the partial 18S rDNA+ITS1+partial 5.8S rDNA (1,018 bp) sequences between *Gobioecetes longibasis* n. sp. and *G. biwaensis*

Species	Host	GenBank ID	LC494516	LC494517	LC494515	LC176446
G. longibasis n. sp.	*R. similis*	LC494516	–			
G. longibasis n. sp.	*R. similis*	LC494517	0.0	–		
G. biwaensis	*Rhinogobius* sp. OM	LC494515	2.4	2.4	–	
G. biwaensis	*R. biwaensis*[a]	LC176446	2.4	2.4	0.2	–

[a]The host registered as *Rhinogobius* sp. BW was described as *Rhinogobius biwaensis* by Takahashi & Okazaki (2017)

Table 3 The sequence divergence (in %) for the partial 28S rDNA (753 bp) sequences between *Gobioecetes longibasis* n. sp. and *G. biwaensis*

Species	Host	GenBank ID	LC494519	LC494520	LC494518
G. longibasis n. sp.	*R. similis*	LC494519	–		
G. longibasis n. sp.	*R. similis*	LC494520	0.0	–	
G. biwaensis	*Rhinogobius* sp. OM	LC494518	1.5	1.5	–

(Ogawa & Itoh, 2017; Shimizu & Nagasawa, 2018; this study), the species is most likely to be endemic to Okinawa-jima Island or the Ryukyu Archipelago. About 100 species of fluvial and diadromous fishes are known from the island (Yoshigou, 2014), and many undescribed monogeneans may parasitise these fishes.

Gobioecetes rhinogobius and *G. longibasis* n. sp. are both parasitic on *R. similis* but occur in Hubei, China (Ling, 1973a) and on Okinawa-jima Island, Japan (this study), respectively. Diadromous *Rhinogobius* spp. are widely distributed in Japan, Korea, Taiwan, China and northern Vietnam (Suzuki et al., 2015), and their populations off Okinawa-jima Island and Honshu, the main island of Japan, are genetically related to each other (Yamasaki et al., 2015). However, no dactylogyrid was collected in this study from *R. similis* from the seven localities in the western and central Japan. The southern and central Ryukyu Archipelago including Okinawa-jima Island is affected by a strong current, the Kuroshio, and since the early Pleistocene, this region has been separated from the northern Ryukyu Archipelago by the Tokara Channel (see Matsuura & Senou, 2007; Osozawa et al., 2012). This study reveals that *G. longibasis* n. sp. is host-specific to *R. similis*, and, as has been suggested for some other freshwater and terrestrial animals (e.g. Shokita, 1996; Ota, 1998; Kaito & Toda, 2016), *G. longibasis* n. sp. or its ancestor might have colonised the Okinawa-jima Island with *R. similis* in the Pleistocene through a land bridge connected to the Eurasian continent.

Rhinogobius biwaensis is endemic to Lake Biwa and considered having one-year life-cycle (Takahashi & Okazaki, 2002, 2017; Fujita, 2015), whereas *Rhinogobius* sp. OM occurs in the coastal waters of the lake and inflowing rivers throughout the year and has two or three-year life-cycle (e.g. Kurumi & Suzuki, 1998; Takahashi & Okazaki, 2002; Takahashi & Ohara, 2006, Ishizaki et al., 2016). In the present study, the prevalence and intensity of *G. biwaensis* on *Rhinogobius* sp. OM were higher than those on *R. biwaensis*, and thus, this monogenean may use *Rhinogobius* sp. OM as a stable host.

Over 80 species of *Rhinogobius* are known to occur in East and Southeast Asia (see Suzuki et al., 2019). However, only four species of the genus have reported to host monogeneans (Ling 1973a, b; Ogawa & Itoh, 2017; Shimizu & Nagasawa, 2018; this study). The monogenean fauna of this fish group remains poorly understood.

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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.

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