Redescription of *Dactylogyrus petruschewskyi* Gussev, 1955 (Monogenea: Dactylogyridae), a Newly Recorded Alien Monogenean from an Alien Cyprinid, *Megalobrama amblycephala* Yih, 1955 (Cypriniformes: Cyprinidae), in Ibaraki Prefecture, Central Japan

Masato Nitta^{1,4}, Takanori Ishikawa², and Tomiji Hagiwara³

¹ Graduate School of Science, Kobe University, 1-1 Rokkodai, Nada, Kobe 657-8501, Japan
E-mail: licht.bsn.mono@gmail.com

² College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-0880, Japan

³ Global Environmental Forum, 8th Floor Kuramae Intelligent Bldg., 3-17-3 Kuramae, Taito, Tokyo 111-0051, Japan

⁴ Corresponding author

(Received 27 April 2020; Accepted 4 September 2020)

Dactylogyrus petruschewskyi Gussev, 1955 (Monogenea: Dactylogyridae) was found infecting the gills of *Megalobrama amblycephala* Yih, 1955 (Cypriniformes: Cyprinidae: Cultrinae) that was sampled from the Ono River flowing into Lake Kasumigaura in Ibaraki Prefecture, central Japan. This is the first record of this monogenean from Japan, and this parasite was most likely introduced and established along with *M. amblycephala* from China into Japan. The 28S rDNA sequence of *D. petruschewskyi* is available in the database but not based on a published study. We analyzed molecular data based on newly collected specimens, and the obtained sequence corresponded to the available sequence.

Key Words: Monogenea, fish parasite, alien species, 28S rDNA sequence, Lake Kasumigaura, Tone River system.

Introduction

Megalobrama amblycephala Yih, 1955, a cyprinid fish belonging to the subfamily Cultrinae, is natively distributed throughout lakes in the middle reaches of the Yangtze River, China (Wu *et al.* 1979). Recently, this cyprinid was collected from Lake Kasumigaura, Ibaraki Prefecture, in central Japan, as an alien fish (Hagiwara 2017). Over 30 species of alien freshwater fishes have been reported from the Tone River system, which includes Lake Kasumigaura (*e.g.*, Hagiwara and Kumagai 2007; Yanai *et al.* 2008; Sekine 2009; Nemoto *et al.* 2011; Arayama *et al.* 2012; Hagiwara 2017), and some alien parasites have been co-introduced and have established with them (Nitta and Nagasawa 2015, 2020). In this study, we examined *M. amblycephala* collected from the Ono River, which flows into Lake Kasumigaura, and collected some specimens of a dactylogyrid monogenean, *Dactylogyrus petruschewskyi* Gussev, 1955. This dactylogyrid was originally described from *Parabramis pekinensis* (Basilevsky, 1855) (Cyprinidae: Cultrinae) in Lake Hanka at Astrakhanka, Primorsky Krai, Far East Russia (Gussev 1955), then reported from China (see Long 2000). However, to date, no morphological character other than the sclerotized parts of this species has been described. This study presents the first record of this monogenean as an alien species in Japan, along with its redescription and molecular information.

The 28S rDNA sequence of *D. petruschewskyi* is available

in the database (AY548927) but not based on a published study. Nevertheless, this data has been used for molecular phylogenetic studies (*e.g.*, Šimková *et al.* 2006). Since the phylogenetic relationships of species are important information that is referred to when constructing a taxonomy, we covered all studies that performed phylogenetic estimation based on this unpublished sequence data. Furthermore, based on the newly obtained sequence in this study, we confirmed the correctness of this registered sequence.

Materials and Methods

Two *M. amblycephala* specimens were collected by set-nets from the Ono River (35°58'12.1"N, 140°21'09.0"E) flowing into Lake Kasumigaura (the Tone River system), at Horinouchi, Inashiki City, Ibaraki Prefecture, central Japan on 10 March and 28 September 2019. The first specimen (standard length, SL: 36.3 mm) was fixed in 99% ethanol in the field, and the second one (SL: 338.0 mm) was kept in a freezer before the examination for gill parasites. Gills were removed from each fish, and monogeneans were collected using needles by observing under a dissecting microscope. Two monogenean specimens collected from a host fixed in ethanol were stained in alum carmine. Bodies of two other specimens were cut from their haptors using needles and preserved in 99% ethanol for molecular analysis. The rest of the haptors and the other specimens were flattened under

coverslips and fixed in modified picrate glycerin (Nitta and Nagasawa 2018). All such specimens used for morphological analysis were dehydrated using a series of graded ethanol, cleared in xylene, and mounted in Canada balsam.

Drawings were made with the aid of a drawing tube fitted on an Olympus BX60 light microscope. Sclerotized parts were measured following the methods reported by Nitta and Nagasawa (2016). All measurements are in micrometers and given as the range followed by the mean and the number (n) of specimens examined in parentheses. The scientific names of fishes used in this paper follow Froese and Pauly (2019). Prevalence and intensity of infection are as defined by Bush *et al.* (1997). Voucher specimens of dactylogyrids were deposited in the Platyhelminthes collection of the National Museum of Nature and Science (NSMT-PI 6451, 6452), Tsukuba City, Ibaraki Prefecture, Japan.

DNA was extracted from bodies using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, 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 (Mollaret *et al.* 1997). 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, 54°C for 30 s, 72°C for 30 s, and 10 min at 72°C for a final extension. Amplified PCR products were purified using the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany) and were sequenced using a 3130xl Genetic Analyzer (Applied Biosystems, Foster, USA) with the PCR primers. Sequence data and electropherograms were inspected and edited manually using MEGA7 (Kumar *et al.* 2016), then the partial 28S rDNA (796 bp) sequence obtained was submitted to the DNA Data Bank of Japan Centre (DDBJ; accession number LC538183) and was compared with the available sequences for dactylogyrid species in GenBank using BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>) software on 11 April 2020.

***Dactylogyrus petruschewskyi* Gussev, 1955**

[New Japanese Name: Dantōbō-yubigata-mushi]
(Figs 1, 2)

Dactylogyrus petruschewskyi Gussev, 1955: 242–243, fig. 24–4; Bykhovskaya-Pavlovskaya *et al.* 1962: 334, fig. 663; Strelkov 1971: 72; Anonymous 1973: 132–134, figs 111–114; Chen 1981: 151; Long and Tao 1981: 546; Gussev 1985: 161, fig. 223; Wu *et al.* 1991: 18, 111–112, fig. 105; Li and Zhang, 1992: 90; Jin *et al.* 1993a: 326, 327, fig. 95; Jin *et al.* 1993c: 411; Gibson *et al.* 1996: 25; Wang *et al.* 1997: 91, 116, 117; Wu *et al.* 2000: 24; Long 2000: 196–197, fig. 145; Ding and Liao 2004: 629, 630, 631; Ding and Liao 2005: 246; Šimková *et al.* 2006: 44, 47; Gussev *et al.* 2010: 231, fig. 278; Singh and Chaudhary 2010: 123, 124, 125, 126; Wang *et al.* 2012: 45; Rajvanshi and

Agrawal 2013: 580, 582; Tan 2013: 71, 133, 159, 160, 161, 192; Mendoza-Palmero *et al.* 2015: 4, 6; Nitta and Nagasawa 2016: 485, 486; Mendoza-Palmero *et al.* 2017: 154, 159; Nitta and Nagasawa 2017: 2; Šimková *et al.* 2017: 5, 7; Chiary and Singh 2019: 143; Daghigh Roohi *et al.* 2020: 1056, 1057; Nitta and Nagasawa 2020: 64.

Neodactylogyrus petruschewskyi: Yamaguti 1963: 41, fig. 677.

Description. Body elongate, total length of body including haptor 278–291 (285, n=2), maximum width 69–97 (83, n=2) in mid-body. Cephalic region with well-developed medial and bilateral cephalic lobes. Three pairs of head organs. Head gland cells on both sides of body at level of posterior pharynx. Two pairs of eye-spots. Pharynx subspherical, 20–24 (22, n=2) long, 21–26 (24, n=2) wide; esophagus short; intestinal ceca bifurcate with caeca confluent posterior to testis.

Haptor 57–61 (59, n=2) long, 67–88 (78, n=2) wide. A pair of dorsal hamulus, 35–45 (41, n=10) long; shaft slightly expanded before bent and tapering to point; length to notch 26–33 (31, n=9) long; outer root slightly tapering distally, 3–5 (4, n=9) long; inner root elongate trapezoid, 13–16 (15, n=9); point 12–15 (14, n=9) long. Dorsal bar broadly W-shaped, total length 24–30 (28, n=9), total width 7–9 (8, n=9), median width 4–5 (4, n=9). Ventral bar T-shaped, total length 20–22 (21, n=8), total width 11–14 (12, n=8); base of each side of the vertical deeply concave (n=6, Fig. 2: VB), or concavity becoming circular holes (n=2, Fig. 2: VBc). Marginal hooks in 7 pairs; hook length: pair I 23–27 (25, n=8); pair II 28–37 (32, n=8); pair III 30–41 (36, n=8); pair IV 28–35 (32, n=8); pair V 25–29 (27, n=8), pair VI 24–31 (28, n=8); pair VII 27–35 (32, n=8). Pair of needles 8–9 (8, n=7) long, located near the tip of fifth marginal hooks.

Gonads intercecal. Testis pyriform, posterodorsal to germarium, 32–45 (39, n=2) long, 22–23 (23, n=2) wide. Vas deferens arising from anterior margin of testis, extending from intercaecal portion, looping around left intestinal ceca onto ventral, extending seminal vesicle lying left to base of male copulatory organ, entering base of penis. Two prostatic reservoirs saccate, rounded, posterior to male copulatory organ. Male copulatory organ consisting of penis and accessory piece, 29–33 (31, n=7) long. Penis slightly curved tube, 25–29 (26, n=7) long; its base touching base of rod of accessory piece. Sclerotized accessory piece, 25–28 (26, n=7) long, consisting of half-cylindrical shaped plate with hook-like projection on sinistral tip, and rod connected to postero-dextral side of the plate; hook-like projection bending inward; plate supporting the tip of the penis dorsally; rod slightly bent (n=6), sometimes divided into two parts (n=1, Fig. 2: MCot).

Germarium ovate in mid-body, 47–50 (49, n=2) long, 27–30 (29, n=2) wide. Oviduct arising from anterior margin of germarium, continuing to oötype. Mehlis' gland connecting base of oötype. Vagina unsclerotized, opening on left lateral side, mid-length of body, leading to seminal receptacle. Seminal receptacle lying left of oviduct, connecting at junction of oviduct and oötype by short, narrow duct.

Vitellaria approximately co-extensive with intestinal ceca. Egg not collected.

Materials examined. 11 specimens (NSMT-PI 6451,

Fig. 1. *Dactylogyrus petruschewskyi* Gussev, 1955. NSMT-PI 6451. Whole mount (ventral view). Abbreviations: DH, dorsal hamulus; E, eye spot; G, germarium; HG, head gland; HO, head organ; Hp, haptor; In, intestine; MCO, male copulatory organ; MG, Mehli's gland; MH, marginal hook; O, oötype; Od, oviduct; Ph, pharynx; PR, prosthetic reservoir; SR, seminal receptacle; SV, seminal vesicle; T, testis; V, vitellaria; Va, vagina; VD, vas deferens.

6452)

Locality. Ono River flowing into Lake Kasumigaura (35°58'14.5"N, 140°21'11.4"E), Inashiki City, Ibaraki Prefecture.

Host. *Megalobrama amblycephala* Yih, 1955 (Cypriniformes: Cyprinidae: Cultrinae).

Site on host. Gill filaments.

Prevalence and intensity. 100% and 2 and 9 worms were collected from two fish.

Representative DNA sequences. The partial 28S rDNA (796bp) sequences from the two specimens (NSMT-PI 6452) were identical and submitted to the DDBJ (accession number LC538183).

Etymology. The new Japanese name, "dantōbō" refers to the host, *M. amblycephala*, in Japanese, and "yubigatamushi" means the genus *Dactylogyrus*.

Remarks. *Dactylogyrus petruschewskyi* was originally described after being taken from the gills of *Parabramis pekinensis* in Lake Hanka at Astrakhanka, Primorsky Krai, Far East Russia (Gussev 1955). The species was transferred to the genus *Neodactylogyrus* Price, 1938 by Yamaguti (1963), although this genus had been synonymized with *Dactylogyrus* Diesing, 1850 by Mizelle and Donahue (1944). As a native parasite, the monogenean was then reported with the same host species in Hubei, Zhejiang, Guangxi, and in Hunan, China (Anonymous 1973; Wu *et al.* 1991; Li and Zhang 1992; Jin *et al.* 1993c). It was also reportedly obtained from *Megalobrama terminalis* (Richardson, 1846) in Hubei, Zhejiang, and in Guangxi (Anonymous 1973; Wu *et al.* 1991; Li and Zhang 1992); from *M. amblycephala* in Hunan (Wang *et al.* 2012); from *Sinibrama wui* (Rendahl, 1933) (Cyprinidae) as *S. wui polylepis* Yih and Wu, 1964 and *S. wui typus* Yih and Wu, 1964 in Guizhou (Long and Tao 1981); and from *Sinibrama macrops* (Günther, 1868) in Zhejiang and Guangxi, China (Wu *et al.* 1991; Li and Zhang 1992). In addition, Gussev *et al.* (2010) regarded *M. terminalis*, listed as the parasite host by Gussev (1985), to be *Megalobrama mantschuricus* (Basilevsky, 1855) as *M. skolkovii* Dybowski, 1872.

The measurements and morphology of sclerotized parts

Fig. 2. *Dactylogyrus petruschewskyi* Gussev, 1955. NSMT-PI 6452. Sclerotized structures. Abbreviations: ap, accessory piece; DB, dorsal bar; DH, dorsal hamulus; I, marginal hook of pair I; II, marginal hook of pair II; III, marginal hook of pair III; IV, marginal hook of pair IV; MCOd, male copulatory organ, normal type (dorsal view); MCOt, male copulatory organ, two-rod type (Anonymous 1973) (dorsal view); MCOv, male copulatory organ, normal type (ventral view); N, needle; p, penis; VB, ventral bar; VBc, ventral bar with circular holes; V, marginal hook of pair V; VI, marginal hook of pair VI; VII, marginal hook of pair VII.

of the specimens collected in the present study were almost identical to descriptions of *D. petruschewskyi* by Gussev (1955, 1985), Anonymous (1973), Wu *et al.* (1991), Long (2000), and Gussev *et al.* (2010). There are two types of accessory piece rod: the normal type consisting of a single rod (Gussev 1955, 1985; Gussev *et al.* 2010) and the two-rod type (Anonymous 1973). Both types were found in this study (Fig. 2); thus, these forms are judged to demonstrate intraspecific variation.

As found through a BLAST search, the newly obtained partial 28S rDNA sequence (LC538183) was identical to *D. petruschewskyi* (AY548927). Although this accession sequence has been used for molecular phylogenetic studies on dactylogyrids (Ding and Liao 2004, 2005; Šimková *et al.* 2006, 2017; Singh and Chaudhary 2010; Rajvanshi and Agrawal 2013; Tan 2013; Mendoza-Palmero *et al.* 2015, 2017; Nitta and Nagasawa 2016, 2017, 2020; Chiary and Singh 2019; Daghigh Roohi *et al.* 2020), the sequence data were not based on a published record of specimens morphologically identified. The present results support the correctness of the identification.

This dactylogyrid has been recorded in *Megalobrama amblycephala* from Datong Lake, Yangtze River basin, China, (Wang *et al.* 2012) but the specimens have not been described morphologically nor obtained molecular information. According to morphological and molecular information, we identified the dactylogyrid taken from *M. amblycephala* as *D. petruschewskyi* for the first time in Japan. Other than *D. petruschewskyi*, only *D. strelkowi* Gussev, 1955 has been recorded from *M. amblycephala* (Jin *et al.* 1993b) and resembles *D. petruschewskyi* in the T-shaped ventral bar and the shape of the dorsal hamulus (Gussev 1955; Long 2000; Gussev *et al.* 2010). However, *D. petruschewskyi* differs from *D. strelkowi* which has the longer and thicker penis (Gussev 1955; Long 2000; Gussev *et al.* 2010).

The internal structure of *D. petruschewskyi* has not been described. The results of our redescription show a common morphological feature between this species and *D. bicorniculus* Nitta and Nagasawa, 2016, which is the only closely related species whose internal morphology has been described (Nitta and Nagasawa 2016): both the prostatic reservoirs are round and small; the vagina is not sclerotized and leads parallel to the seminal receptacle; the vas deferens and the seminal vesicle do not extend above the male copulatory organ; and the testis is pyriform and located posterodorsal to the germarium. Even in recent years, the internal morphological descriptions of *Dactylogyrus* species are often neglected. As it consists of several genetically distinct groups (*e.g.*, Šimková *et al.* 2017), the internal morphology of *Dactylogyrus* will be important for future taxonomic organization of this genus, which consists of over 1,000 species (Gibson *et al.* 1996; WoRMS 2020).

All recorded hosts of *D. petruschewskyi* are not native to Japan (Hosoya 2015; Froese and Pauly 2019), and the dactylogyrid has not been collected in any native fish examined from the Tone River system to date (Nitta and Ishikawa, unpublished data); therefore, this species was determined to be an alien parasite that was introduced with *M. amblycephala*

from China.

Dactylogyrus strelkowi has also been obtained from *M. amblycephala* (Jin *et al.* 1993b). Although we examined only two fish specimens in the present study, no invasion of *D. strelkowi* was found. In Datong Lake, the native habitat of *M. amblycephala*, *D. strelkowi* prevalence on this cyprinid was low, at 33% (Jin *et al.* 1993b), and it has not been rediscovered in the survey by Wang *et al.* (2012). In contrast, all *M. amblycephala* examined in the area were infected by *D. petruschewskyi* (Wang *et al.* 2012). Based on this difference in parasitism, we conclude that only *D. petruschewskyi* may have been co-introduced into Japan.

Acknowledgments

We thank Yasushi Morooka, a fisherman of Lake Kasumigaura, for his help with fish sampling. We are also grateful to Takuya Sato, Kobe University, for providing laboratory facilities. We would like to thank three anonymous reviewers for valuable comments to improve the manuscript. This study was partially supported by JSPS KAKENHI grant (no. 18J00466 to MN).

References

- Anonymous. 1973. *Dactylogyrus petruschewskyi* Gussev, 1955. Pp. 132–133. In: Institute of Hydrobiology of Hubei Province (Ed.) *Húběi Shěng Yú bìng Bìngyuán Qū xī Tú Zhì* [An Illustrative Guide to the Fish Diseases and Causative Pathogenic Fauna of Hubei Province]. Science Press, Beijing. [In Chinese]
- Arayama, K., Matsuzaki, S. S., Mashiko, K., Hagiwara, T., Morosawa, T., Kanou, K., and Watanabe, K. 2012. Records and possible population establishment of the non-indigenous bagrid catfish *Pseudobagrus fulvidraco* in Lake Kasumigaura, Ibaraki Prefecture. *Japanese Journal of Ichthyology* 59: 141–146. [In Japanese with English abstract]
- Bush, A. O., Lafferty, K. D., Lotz, J. M., and Shostak, A. W. 1997. Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology* 83: 575–583.
- Bykhovskaya-Pavlovskaya, I. E., Gussev, A.V., Dubinina, M. N., Izyumova, N. A., Smirnova, T. S., Sokolovskaya, I. L., Shtein, G. A., Shul'man, S. S., and Epshtein, V. M. 1962. *Key to Parasites of Freshwater Fishes of the U.S.S.R.* Translated from Russian, Israel Program for Scientific Translation, Jerusalem, 890 pp.
- Chen, Z. 1981. Classification for Monogenea parasitized on freshwater fishes on Northern China. *Journal of Shandong University (Natural Science)* 1981: 113–123. [In Chinese with English abstract]
- Chiary, H. R. and Singh, H. S. 2019. Comparative studies of Indian (Assam), Chinese, UK's and Iranian *Dactylogyrus lamellatus* Achmerow, 1952 for structural conservedness using phylogenetic and molecular analysis inferred from 28rDNA. *International Journal of Life Sciences Research* 7: 141–147.
- Daghigh Roohi, J., Dalimi, A., Pourkazemi, M., Ghasemi, M., and Shamsi, S. 2020. Morphometric and molecular characterization of *Dactylogyrus lamellatus* isolated from farmed grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844), in Guilan province, Iran. *Iranian Journal of Fisheries Sciences* 19: 1051–1061.

- Ding, X.-J. and Liao, X.-H. 2004. The taxonomic status of *Dactylogyrus inversus* (Monogenea, Dactylogyridae) inferred from DNA sequences. *Acta Zootaxonomica Sinica* 29: 628–632. [In Chinese with English abstract]
- Ding, X.-J. and Liao, X.-H. 2005. Phylogenetic position of the monogeneans *Pseudodactylogyrus*, *Heteronchocleidus* and *Trianchoratus* inferred from the 5' terminal sequences of 28S rDNA. *Acta Zootaxonomica Sinica* 30: 244–251. [In Chinese with English abstract]
- Froese, R. and Pauly, D. (Eds) 2019. FishBase, version (12/2019). Available at <http://www.fishbase.org> (17 June 2020).
- Gibson, D. I., Timofeeva, T. A., and Gerashev, P. I. 1996. A catalogue of the nominal species of the monogenean genus *Dactylogyrus* Diesing, 1850 and their host genera. *Systematic Parasitology* 35: 3–48.
- Gushev, A. V. 1955. [Monogenetic trematodes of fishes of the Amur River system]. *Trudy Zoologicheskii Instituta, Akademiya Nauk SSSR* 19: 171–398. [In Russian]
- Gushev, A. V. 1985. Monogenea. Pp. 10–268. In: Bauer, O. N. (Ed.) *Opredelitel' Parazitov Presnovodnykh Ryb Fauny SSSR, Tom 2* [Key to the Parasites of the Freshwater Fish Fauna of the USSR, Vol. 2]. Nauka, Leningrad. [In Russian]
- Gushev, A. V., Gerashev, P. I., and Pugachev, O. N. 2010. Order Dactylogyridae. Pp. 15–337. In: Pugachev, O. N., Gerashev, P. I., Gushev, A. V., Ergens, R., & Khotenowsky, I. (Eds) *Guide to Monogeneoidea of Freshwater Fish of Palaearctic and Amur Regions*. Ledizioni Ledipublishing, Milano.
- Hagiwara, T. and Kumagai, M. (Ed.) 2007. *Heisei Chōsa, Shin Kasumigaura no Sakana-tachi*. [Heisei Research, Fishes of Kasumigaura New Edition]. Kasumigaura Citizen's Association, Tsuchiura, 158 pp. [In Japanese]
- Hagiwara, T. 2017. Records of the non-indigenous Wuchang bream *Megalobrama amblycephala* (Cyprinidae) in Lake Kasumigaura, Ibaraki Prefecture, Japan. *Izunuma Uchinuma Wetland Researches* 11: 75–81. [In Japanese with English abstract]
- Hosoya, K. 2015. *Freshwater Fishes of Japan*. Yama-kei Publishers, Tokyo, 559 pp. [In Japanese]
- Jin, X., Dai, Z., Liu, X., Zeng, G., Zhang, B., He, S., and Xiang, J. 1993a. Investigation and studies of fish parasites and pathogen flora in Hunan Province. *Journal of Hunan Agricultural College* 19: 297–389. [In Chinese with English abstract]
- Jin, X., Dai, Z., Zeng, G., Liu, X., He, S., and Xiang, J. 1993b. [Studies on the pathogen fauna of fish parasites in Datong Lake]. *Journal of Hunan Agricultural College* 19: 400–407. [In Chinese]
- Jin, X., Liu, X., and Zeng, G. 1993c. Studies on the pathogen fauna of fish parasites in Dongjiang Reservoir. *Journal of Hunan Agricultural College* 19: 408–414. [In Chinese with English abstract]
- Kumar, S., Stecher, G., and Tamura, K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874.
- Li, G. and Zhang, J. 1992. Monogenea of South China fresh water fishes XI—monogenetic list of freshwater fishes of Guangxi. *Journal of Guangxi Normal University* 10: 90–94. [In Chinese with English abstract]
- Long, S. 2000. *Dactylogyrus petruschewskyi* Gushev, 1955; *Dactylogyrus petruschewskyi* Gushev, 1955. Pp. 195–197. In: Wu, B., Long, S., Wang, W., Ma, C., Jiang, N., Chen, Z., Liu, J., Liang, R., Yao, W., and Zhao, Y. (Eds) *Fauna Sinica, Platyhelminthes Monogenea*. Science Press, Beijing. [In Chinese]
- Long, S. and Tao, S. 1981. Two new species of *Dactylogyrus* (Monogenea, Trematoda) from *Sinbrama wui typus* (Rend.) and *S. w. polylepis* Yih et Woo. *Oceanologia et Limnologia Sinica* 12: 545–548. [In Chinese with English abstract]
- Mendoza-Palmero, C. A., Blasco-Costa, I., and Scholz, T. 2015. Molecular phylogeny of Neotropical monogeneans (Platyhelminthes: Monogenea) from catfishes (Siluriformes). *Parasites & Vectors* 8: 164.
- Mendoza-Palmero, C. A., Blasco-Costa, I., Hernández-Mena, D. and Pérez-Ponce de León, G. 2017. *Parasciadicleithrum octofasciatum* n. gen., n. sp. (Monogeneoidea: Dactylogyridae), parasite of *Rocio octofasciata* (Regan) (Cichlidae: Perciformes) from Mexico characterised by morphological and molecular evidence. *Parasitology International* 66: 152–162.
- Mizelle, J. D. and Donahue, M. A. 1944. Studies on monogenetic trematodes XI. Dactylogyridae from Algonquin Park fishes. *American Midland Naturalist* 17: 600–624.
- Mollaret, I., Jamieson, B. G., Adlard, R. D., Hugall, A., Lecointre, G., Chombard, C., and Justine, J. L. 1997. Phylogenetic analysis of the Monogenea and their relationships with Digenea and Eucestoda inferred from 28S rDNA sequences. *Molecular and Biochemical Parasitology* 90: 433–438.
- Nemoto, T., Sugiura, M., and Nakamura, M. 2011. [Distribution and habitat of fishes in rivers flowing into lakes Kasumigaura and Kitaura]. *Bulletin of Ibaraki Prefectural Freshwater Fisheries Experimental Station* 44: 35–44. [In Japanese]
- Nitta, M. and Nagasawa, K. 2015. An alien monogenean, *Ligictaluridus pricei* (Platyhelminthes: Ancyrocephalidae), parasitic on the channel catfish *Ictalurus punctatus* (Actinopterygii: Siluriformes: Ictaluridae) in Japan. *Species Diversity* 20: 95–102.
- Nitta, M. and Nagasawa, K. 2016. A new species of *Dactylogyrus* (Monogenea: Dactylogyridae) parasitic on an endangered freshwater fish, *Rhodeus atremius atremius*, endemic to Japan. *Parasitology International* 65: 483–487.
- Nitta, M. and Nagasawa, K. 2017. *Dactylogyrus oryzias* n. sp. (Monogenea: Dactylogyridae) from *Oryzias latipes* (Beloniformes: Adrianichthyidae) in Japan. *Species Diversity* 22: 1–5.
- Nitta, M. and Nagasawa, K. 2018. *Gyrodactylus medaka* n. sp. (Monogenea: Gyrodactylidae) parasitic on wild and laboratory-reared medaka *Oryzias latipes* (Beloniformes: Adrianichthyidae) in Japan. *Parasitology International* 67: 651–658.
- Nitta, M. and Nagasawa, K. 2020. Alien gill parasites of the silver carp *Hypophthalmichthys molitrix* (Cypriniformes: Cyprinidae) in Tochigi Prefecture, central Japan. *Species Diversity* 25: 61–73.
- Rajvanshi, S. and Agrawal, N. 2013. One known and an unknown species of the genus *Thaparocleidus* Jain, 1952, infecting *Sperata aor* (Hamilton, 1822): comparison with species from China, on molecular basis. *Bioinformation* 9: 577–582.
- Sekine, K. 2009. *Watarase Yūsui-chi no Sakana Zukan* [Freshwater Fishes of the Watarase Retarding Basin]. Watarase Yūsui-chi Akurimēshon Shinkō Zaidan, Tochigi, 64 pp. [In Japanese]
- Šimková, A., Benovics, M., Rahmouni, I., and Vukić, J. 2017. Host-specific *Dactylogyrus* parasites revealing new insights on the historical biogeography of Northwest African and Iberian cyprinid fish. *Parasites & Vectors* 10: 589.
- Šimková, A., Matějsová, I., and Cunningham, C. O. 2006. A molecular phylogeny of the Dactylogyridae sensu Kritsky & Boeger (1989) (Monogenea) based on the D1-D3 domains of large subunit rDNA. *Parasitology* 133: 43–53.
- Singh, H. S. and Chaudhary, A. 2010. Genetic characterization of *Dactylogyrus longicirrus* (Tripathi, 1959) Gushev, 1976 by nuclear 28S segment of ribosomal DNA with a morphological redescription. *Scientia Parasitologica* 11: 119–127.
- Strelkov, J. A. 1971. Monogenetic trematodes from the fishes of the river Amur's basin. *Parazitologicheskii Sbornik* 25: 41–76. [In Russian with English title]
- Tan, W. B. 2013. *Morphological and Molecular Characterisation of Monogeneans*. PhD thesis, University of Malaya, Malaya, 248 pp.
- Wang, W., Li, L., Yu, Y., Feng, W., Xia, C., Wang, G., Liu, J., Yao, W., and Feng, S. 1997. Parasite fauna of fishes in Wuling Mountains area.

- Pp. 73–146. In: Song, D. (Ed.) *Invertebrates of Wuling Mountains Area, Southwestern China*. Science Press, Beijing. [In Chinese with English abstract]
- Wang, W.-b., Zou, W., Tang, L., Luo, Y.-s. Liu, L.-g., and Yang, P.-h. 2012. A sampling survey on the infection of enclosure culture fishes by *Dactylogyrus* in Datong Lake. *Journal of Hunan University of Arts and Science (Natural Science Edition)* 24: 43–45. [In Chinese with English abstract]
- WoRMS. 2020. *Dactylogyrus* Diesing, 1850. Available at <http://www.marinespecies.org/aphia.php?p=taxdetails&id=119282> (17 June 2020).
- Wu, B., Sun, X., and Song, C. 1991. *Fauna of Zhejiang. Trematoda*. Zhejiang Science and Technology Publishing House, Hangzhou, 8+14+416 pp., 4 pls. [In Chinese with English abstract]
- Wu, B., Wang, W., and Jiang, N. 2000. [General introduction]. Pp. 1–52. In: Wu, B., Long, S., Wang, W., Ma, C., Jiang, N., Chen, Z., Liu, J., Liang, R., Yao, W., and Zhao, Y. (Eds) *Fauna Sinica, Platyhelminthes Monogenea*. Science Press, Beijing. [In Chinese]
- Wu, X.-w., Yang, G.-r., Yue, P.-q., and Huang, H.-j. 1979. *Zhong guo Jing ji Dong wu Zhi, Dan shui Yu lei, Di er ban*. [Economic Fauna Sinica of China, Freshwater Fishes, Second Edition]. Science Press, Beijing, vi+153 pp., 38 pls. [In Chinese]
- Yamaguti, S. 1963. *Systema Helminthum IV. Monogenea and Aspidocotylea*. Interscience Publishers, New York, v+699 pp.
- Yanai, T., Arayama, K., and Tominaga, A. 2008. The introduction process in alien fishes found from Lake Kasumigaura and Kitaura. *Bulletin of Ibaraki Prefectural Freshwater Fisheries Experimental Station* 41: 47–54. [In Japanese with English title]