New Geographical Records of Three Gill Monogeneans (Platyhelminthes) Parasitic on *Pseudorasbora parva* (Cypriniformes: Cyprinidae) in Japan

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Three species of monogenean, *Dactylogyrus squameus* Gussev, 1955, *Ancyrocephalus pseudorasborae* Achmerow, 1952, and *Bivaginogyrus obscurus* (Gussev, 1955), were collected from the gills of the cyprinid *Pseudorasbora parva* (Temminck and Schlegel, 1846) in Japan. *Dactylogyrus squameus* and *A. pseudorasborae* were collected in Nara, Tottori, and Ibaraki prefectures, and in Ibaraki and Okayama prefectures, respectively, and are redescribed as new country records from Japan. *Bivaginogyrus obscurus* has been reported from only Ibaraki and Nagano prefectures, and its discovery in Nara and Okayama prefectures as represents as new locality records. Although *D. squameus* and *B. obscurus* are known as alien parasites in Europe, all three monogeneans found in this study are considered to be native to Japan.

**Key Words:** *Dactylogyrus squameus*, *Ancyrocephalus pseudorasborae*, *Bivaginogyrus obscurus*, Monogenea, *Pseudorasbora parva*, new geographical records, redescription, fish parasite.

**Introduction**

*Pseudorasbora parva* (Temminck and Schlegel, 1846) (Cypriniformes: Cyprinidae) is natively distributed in Far-East Asia, from south-eastern Russia and regions west of the Kantō District of Honshū, Japan, in the north to northern Vietnam in the south (Hosoya 2013). The fish has spread into Europe as an invasive species along with two dactylogyrid monogeneans, *Dactylogyrus squameus* Gussev, 1955 and *Bivaginogyrus obscurus* (Gussev, 1955) (Ondráčková et al. 2004; Galli et al. 2007; Gozlan et al. 2010; Anonymous 2012). The monogenean fauna of this fish is poorly known in Japan, where only *B. obscurus* has been reported from Ibaraki Prefecture (Nitta and Nagasawa 2014). In this paper, we examined *Pseudorasbora parva* collected from natural habitats in Japan and report *Dactylogyrus squameus* and *Ancyrocephalus pseudorasborae* Achmerow, 1952 as new country records and *Bivaginogyrus obscurus* as new prefectural records.

**Materials and Methods**

Seventeen specimens of *Pseudorasbora parva* (standard length 25.5–52.7 mm) were collected by a hand net or angling from four sites in Japan: two specimens from the Tomio River (34°41′50″N, 135°43′52″E), a tributary of the Hino River system at Tomio-gawa-nishi, Nara city, Nara Prefecture on 22 July 2012; four specimens from the Kitakata River (35°21′26″N, 133°19′00″E), a tributary of the Hino River system at Kitakata, Nanbu town, Saitahaku County, Tottori Prefecture on 6 August 2013; ten specimens from Lake Kasumigaura (36°04′05″N, 140°15′23″E) at Okijuku-machi, Tsuchiura city, Ibaraki Prefecture (eight and two specimens on 13 and 16 June 2014, respectively); and one specimen from the Senō River (34°36′16″N, 133°51′52″E), a tributary of the Kurashiki River system, at Uchio, Minami Ward, Okayama city, Okayama Prefecture on 16 March 2015. The fish were brought alive to the laboratory at Hiroshima University, Higashi-Hiroshima city, Hiroshima Prefecture, and examined for gill monogeneans under an Olympus BX51 compound microscope. Monogeneans were collected from the gills by using small needles and forceps, fixed in 70% ethanol, AFA (acetic acid-formalin-alcohol), or APG (ammonium picrate glycerin; in Lim 1991) under coverslip pressure and stained with Heidenhain’s iron hematoxylin or alum carmine, but some specimens fixed in APG were not stained. All specimens were dehydrated in 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 compound microscope. The hook measurements are presented in Fig. 1. The male copulatory organs and the sclerotized vaginae were measured on images taken by digital camera on an Olympus DP20 microscope at a magnification of ×1,000 using ImageJ software (version 1.48i). Measurements, in micrometers, are expressed as the mean±standard deviation followed in parentheses by the range of structure measurements and the number (n) of specimens examined. The numbering of hook pairs follows Llewellyn (1963). Fish identification was based on Hosoya (2013). Prevalence and intensity of infection are those defined by Bush et al. (1997). Specimens are deposited in the...
Platyhelminthes collection of the National Museum of Nature and Science (NSMT-Pl), Tsukuba city, Ibaraki Prefecture, Japan.

Results

Three species of monogeneans were collected from Pseudorasbora parva. Dactylogyrus squameus and Ancyrocephala pseudorasbora were collected in Nara, Tottori, and Ibaraki prefectures and Ibaraki and Okayama prefectures, respectively. Bivaginogyrus obscurus was found with D. squameus in Nara Prefecture and with A. pseudorasbora in Okayama Prefecture. Furthermore, these three monogenean species concurrently infected four host individuals in Lake Kasumigaura, Ibaraki Prefecture.

Genus Dactylogyrus Diesing, 1850

[Japanese name: Yubigata-mushi-zoku]

Dactylogyrus squameus Gussev, 1955

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


Neodactylogyrus squameus: Yamaguti 1963: 43, fig. 703.

Material examined. Ten specimens were used for the description: eight specimens fixed in APG (NSMT-Pl 6176, 6177); one specimen stained in Heidenhain’s iron hematoxylin (NSMT-Pl 6179 together with two specimens of B. obscurus); and one specimen (NSMT-Pl 6178) stained in alum carmine; from Ibaraki, Nara, and Tottori prefectures, respectively.

Description. Body length including haptor 526±133.0 (241–683, n=9), width at mid-body 76±15.3 (57–102, n=9). Three pairs of head organs. Two pairs of eye-spots. Alimentary system consisting of subshperical pharynx, length 31±5.0 (20–37, n=9), width 28±5.1 (20–35, n=9), followed by esophaguss and bifurcate intestine with branches confluent just anterior to tests. Testis pyriform, dorsal to ovary. Vas deferens arising from anterior region of testis, looping around left intestine towards ventral side of body, distended as seminal vesicle before entering base of copulatory organ. Two prostatic reservoirs both saccate. Copulatory organ (Fig. 2L) a curved tube, organ length 22±1.2 (20–24, n=8), tube length 22±1.7 (19–24, n=8). Ovary in mid-body. Oviduct arising from anterior side of ovary, continuing as ootype surrounded by Mehlis’ gland and uterus. Vagina un sclerotized, running ventrally from right side of seminal receptacle located to right of ootype to vaginal opening on right body surface. Vitellaria approximately co-extensive with intestine.

Haptor length 72±14.1 (43–94, n=9), width 106±30 (62–138, n=9). Dorsal anchor (Fig. 2B) of total length 33±2.4 (28–35, n=10), length to notch 28±1.9 (25–30, n=10), outer root length 5±0.5 (4–5, n=10), inner root length 8±1.2 (7–10, n=10), point length 10±0.7 (9–11, n=10). Dorsal bar (Fig. 2C) bow-shaped, of total length 22±1.4 (21–25, n=10), total width 8±0.7 (7–9, n=10), median width 5±0.6 (4–6, n=10). Ventral bar (Fig. 2D) thin, slightly curved, of total length 24±2.6 (21–28, n=7), total width 2±1.0 (1–4, n=7), median width 1.1±0.4 (1–2, n=7). Marginal hooks in 7 pairs; length: pair I (Fig. 2E) 17±1.0 (15–18, n=9); pair II (Fig. 2F) 19±1.1 (18–21, n=9); pair III (Fig. 2G) 22±1.0 (20–23, n=9); pair IV (Fig. 2H) 25±1.9 (22–27, n=9); pair V (Fig. 2I) 22±1.0 (21–24, n=9); pair VI (Fig. 2J) 21±1.0 (20–23, n=9); pair VII (Fig. 2K) 19±1.0 (17–20, n=9). Pair of needles (Fig. 2M) of length 9±0.9 (8–10, n=9), located near tips of second hooks.

Host. Pseudorasbora parva (Cypriniformes: Cyprinidae).

Site of infection. Gill.

Prevalence and intensity range (mean). 100% (2/2) and 1–2 (1.5) in the Tomio River, Nara Prefecture; 25% (1/4) and 1 in the Kitakata River, Tottori Prefecture; 40% (4/10) and 1–3 in Lake Kasumigaura, Ibaraki Prefecture.

Remarks. Dactylogyrus squameus was originally described by Gussev (1955) from the gills of Pseudorasbora parva from the Amur River and Lake Chanka, Far-East Russia, and subsequently reported from the same host in...
Gill monogeneans of *Pseudorasbora parva*

Fig. 2. *Dactylogyrus squameus* Gussev, 1955, NSMT-Pl 6179 for A, NSMT-Pl 6176 for B–M. A, whole mount (ventral view); B, dorsal anchors; C, dorsal bar; D, ventral bar; E, marginal hook of pair I; F, marginal hook of pair II; G, marginal hook of pair III; H, marginal hook of pair IV; I, marginal hook of pair V; J, marginal hook of pair VI; K, marginal hook of pair VII; L, male copulatory organs (from two specimens, left: ventral view, right: dorsal view); M, needle. Scale bars: A, 50 µm; B–M, 10 µm. Abbreviations: ap, accessory piece; co, copulatory organ; dan, dorsal anchor; e, eye-spots; h, haptor; ho, head organ; in, intestine; mg, Mehlis' gland; mh, marginal hook; o, oötype; od, oviduct; ov, ovary; ph, pharynx; pr, prostatic reservoir; sr, seminal reservoir; sv, seminal vesicle; t, testis; va, vagina; vd, vas deferens; vl, vitellaria; vo, vaginal opening.
Heilongjiang, Liaoning, Hubei, and Yunnan, China (Ji et al. 1982; Chen 1984; Wu and Wang 1991; Liang 2000). The species was transferred to the genus *Neodactylogyrus* by Yamaguti (1963), although this genus had been synony-

ized with *Dactylogyrus* by Mizelle and Donahue (1944). The specimens examined in this study almost conform to the descriptions and illustrations of *D. squameus* by Gussev (1955, 1985), Bykhovskaya-Pavlovskaya et al. (1962), Wu

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Fig. 3. *Ancyrocephalus pseudorasborae* Achmerow, 1952, NSMT-Pl 6180. A, whole mount (dorsal view); B, dorsal anchors; C, ventral anchors; D, dorsal bar; **G**, marginal hook; G, male copulatory organ; H, vagina. Scale bars: A, 50 µm; B–H, 10 µm. Abbreviations: ap, accessory piece; co, copulatory organ; dan, dorsal anchor; e, eye-spots; h, haptor; ho, head organ; in, intestine; mg, Mehlis' gland; mh, marginal hook; o, oötype; ov, ovary; pg, prostatic gland; ph, pharynx; pr, prostatic reservoir; sr, seminal reservoir; sv, seminal vesicle; t, testis; va, vagina; van, ventral anchor; vd, vas deferens; vl, vitellaria; vo, vaginal opening.
and Wang (1991), Liang (2000), Ondračkova et al. (2004), and Gussev et al. (2010). The dorsal anchors of our specimens are slightly smaller than those authors' measurements, but this may be due to intraspecific variation because the anchor's shape and the male copulatory organs of our specimens agree with the cited descriptions. The present collection in Japan represents a new country record for Dactylogyulus squameus. This monogenean established populations infecting *Pseudorasbora parva* in Kazakhstan, Tajikistan, the Kyrgyz Republic, Ukraine, the Czech Republic, Slovak Republic, and Italy after both species were introduced from Far-East Asia into these countries (Gvozdev and Agapova 1977; Gussev 1985; Ondračkova et al. 2004; Galli et al. 2007). Ondračkova et al. (2004) listed the monogenean as occurring in "Uzbekistan" by reference to Gussev (1985), but this was most probably a mistranslation of "Ukraine".

**Japanese name.** The new Japanese name is a combination of "motsugo" and "yubigata-mushi", which are the Japanese names of *P. parva* and the genus *Dactylogyulus* (as "yubigata-mushi-zoku"), respectively.

**Genus Ancyrocephalus** Creplin, 1839

[New Japanese name: Yotsume-ikari-zoku]

**Ancyrocephalus pseudorasborae** Achmerow, 1952

[New Japanese name: Mitsugo-yotsume-ikari]

(Fig. 3)


**Material examined.** Twenty specimens were used for the description. Soft anatomy was observed in 10 specimens (NSMT-Pl 6180) from Ibaraki Prefecture stained in Heidenhain's iron hematoxylin. Sclerotized structures were measured in eight and two specimens (NSMT-Pl 6180 and 6182) fixed in APG, from Ibaraki and Okayama prefectures, respectively.

**Description.** Body (Fig. 3A) elongate, length 400±43.6 (335–483, n=10) including haptor, width at mid-body 71±11.7 (55–94, n=10). Three pairs of head organs. Two pairs of eye-spots. Pharynx subspherical, length 26±2.0 (23–30, n=10), width 23±2.1 (19–26, n=10); esophagus present; intestine bifurcate with branches confluent just posterior to testis. Testis pyriform, dorsal to ovary. Vas deferens leaving from anterior region of testis, passing along dorsal to loop of sinusial intestine to ventral side, ascending to anterior part of copulatory organ, looping downwards and becoming distended to form seminal vesicle, narrowing to enter initial part of copulatory organ. Single prostatic reservoir elongate; prostatic gland occupying dorsal region of anterior trunk, with its duct leading into the anterior part of prostatic reservoir. Copulatory organ (Fig. 3G) a slender curved tube, organ length 28±1.6 (25–30, n=10), tube length 50±4.0 (41–55, n=10); accessory piece curved in same direction as copulatory organ, trifurcate at tip, length 20±1.4 (18–22, n=10). Ovary ovate, in mid-body. Oviduct arising from anterior side of ovary, continuing as oötype surrounded by Mehlis' gland and uterus. Seminal receptacle dorsal to oviduct. Vagina (Fig. 3H) sclerotized, a slightly curved tube, length 17±3.1 (13–21, n=10), arising from anterior part of seminal receptacle and opening at midlength on right ventral body surface. Vitellaria approximately co-extensive with intestine.

Haptor length 55±5.7 (44–61, n=10), width 74±11.2 (59–99, n=10). Dorsal anchor (Fig 3B) more slender than ventral anchor, of total length 24±0.6 (23–25, n=10), length to notch 20±0.8 (19–21, n=10), outer root length 1±0.7 (0–2, n=10), inner root length 7±0.7 (6–8, n=10), point length 4±0.5 (4–5, n=10). Ventral anchor (Fig. 3C) of total length 20±1.2 (19–22, n=10), length to notch 18±0.8 (16–19, n=10), outer root length 1±0.5 (1–2, n=10), inner root length 8±1.1 (7–10, n=10), point length 4±0.7 (3–5, n=10). Dorsal bar (Fig 3D) rod-shaped, of total length 18±0.7 (17–19, n=10), total width 4±0.4 (3–4, n=10), median width 3±0.4 (2–3, n=10). Ventral bar (Fig 3E) V-shaped with posteriorly directed enlargement at each end, of total length 25±0.8 (23–26, n=10), total width 5±0.6 (4–6, n=10), median width 3±0.5 (2–4, n=10). Seven pairs of marginal hooks (Fig 3F), all of approximately same length, 12±0.5 (11–12, n=10).

**Host.** *Pseudorasbora parva* (Cypriniformes: Cyprinidae).

**Site of infection.** Gills.

**Prevalence and intensity range (mean).** 90% (9/10) with no data for intensity in Lake Kasumigaura, Ibaraki Prefecture; seven worms infected one *P. parva* in the Senō River, Okayama Prefecture.

**Remarks.** *Ancyrocephalus pseudorasborae* was originally described by Achmerow (1952) from the gills of *Pseudorasbora parva* in Lake Bolon, Far-East Russia. It was subsequently reported from the same host in Heilongjiang, Liaoning, Shandong, Shanghai, Jiangxi, Hubei, Zhejiang, Liaoning, Shandong, Shanghai, Jiangxi, Hubei, Zhejiang, Fujian, and Yunnan, China (Anonymous 1973; Ji et al. 1982; Chen 1984; Wu 1991, 2000) and the Amur River, Russia (Gussev 1955, 1985; Bykhovskaya-Pavlovskaya et al. 1962; Gussev et al. 2010). The measurements of sclerotized structures examined in this study are slightly smaller than those reported by Gussev (1955, 1985), Bykhovskaya-Pavlovskaya et al. (1962), Anonymous (1973), Wu (1991, 2000), and Gussev et al. (2010), but the ranges of the measurements overlap, and all the descriptions of the male copulatory organ are in general agreement. The present collection represents the first record of *Ancyrocephalus pseudorasborae* in Japan.

**Japanese names.** The new Japanese generic name, “yotsume-ikari” ("zoku" means a genus), refers to the opisthobothrium with four anchors possessed by *Ancyrocephalus* spp. and means a grapnel anchor in Japanese. Part of the new Japanese name of the species is "motsugo", which is the Japanese name of the host.
Genus *Bivaginogyrus* Gussev and Gerasev, 1985
[New Japanese name: Futa-ana-mushi-zoku]

*Bivaginogyrus obscurus* (Gussev, 1955)
[New Japanese name: Motsugo-futa-ana-mushi]

Host. *Pseudorasbora parva* (Cypriniformes: Cyprinidae).
Site of infection. Gills.

Material examined. Two and three specimens collected from the Tomio River, Nara Prefecture (NSMT-Pl 6179), and the Senō River, Okayama Prefecture (NSMT-Pl 6182), respectively.

Prevalence and intensity. 50% (1/2) and 2 in the Tomio River, Nara Prefecture; four worms infected one *P. parva* in the Senō River, Okayama Prefecture.

Remarks. In Japan this monogenean was first described from *Pseudorasbora parva* and *P. pumila* Miyadi, 1930 in Lake Kasumigaura, Ibaraki Prefecture and in ponds of Shinonoi, Nagano Prefecture, respectively by Nitta and Nagasawa (2014). The specimens from Nara and Okayama prefectures correspond well with those from Lake Kasumigaura and ponds of Shinonoi. The present collections constitute new prefectural records and extend the geographical distribution of the species from Ibaraki and Nagano prefectures westward to Nara and Okayama prefectures.

Japanese names. The new Japanese generic name is a combination of “Futa-ana” and “mushi” which mean two holes (i.e., vaginae) and worms, respectively (“zoku” means a genus). Part of the new Japanese name of the species is “motsugo”, which is the Japanese name of the host.

Discussion

Two of the three monogeneans found in this study, *Dactylogyrus squameus* and *Ancyrocephalus pseudorasborae*, were collected from *Pseudorasbora parva* in the native habitats of this fish in Japan. Thus, as with *Bivaginogyrus obscurus* (Nitta and Nagasawa 2014), they are considered to be native to Japan. Recently, *P. parva* has been recorded in Hokkaidō, the Tōhoku District, and Okinawa Prefecture as a domestic alien freshwater fish (Matsuzawa and Senou 2008), and it has also been suggested that some individuals of the species were introduced from the Eurasian continent into several rivers in Japan (Watanabe et al. 2000; Yoshigou 2013). Two dactylogyrids, *D. squameus* and *B. obscurus*, have spread into Europe with *P. parva* (Ondráčková et al. 2004; Galli et al. 2007; Gozlán et al. 2010; Anonymous 2012), and they might have become established outside of their original range on this fish in Japan as well. Many species of freshwater fish are currently known as domestic alien species in Japan (Matsuzawa and Senou 2008), but no information is available about their monogeneans. It is necessary to clarify the monogenean fauna of such domestic alien freshwater fishes.

At present, five species of *Pseudorasbora* Bleeker, 1860 are recognized as valid, and three of them, *P. parva*, *P. pumila*, and *P. pugnax* Kawase and Hosoya, 2015, are distributed in Japan (Kawase and Hosoya 2015). The latter two species are endemic to Japan and have been designated as critical endangered species by the Ministry of the Environment of Japan (Sugiyama 2015; Kawamura 2015). To date, *Bivaginogyrus obscurus* has been reported from *P. pumila* (Nitta and Nagasawa 2014), but nothing is known about the monogenean fauna of *P. pugnax*. More study is needed on the monogeneans of these fishes because host-specific parasites of endangered hosts are sometimes threatened with co-extinction (Windsor 1990; Stork 1993; Baruš et al. 1997).

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References


Davydov, O. N., Kurovskaya, L. Y., Temnikhanov, Y. D., and Nebrochek, S. I. 2012. Parasites of some invasive fishes of the fresh...


Yoshigou, H. 2013. The inland water fishes of the Ohsaki-kamijima Island, Hiroshima Prefecture, Japan, with notes of the invasions factors of *Pseudorasbora*. Miscellaneous Reports of the Hiwa Museum for Natural History 54: 1–45. [In Japanese with English abstract]