Alien Gill Parasites of the Silver Carp
_Hypophthalmichthys molitrix_ (Cypriniformes: Cyprinidae)
in Tochigi Prefecture, Central Japan

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Four species of alien parasites, _Dactylogyrus skrjabini_ Achmerow, 1954, _D. hypophthalmichthys_ Achmerow, 1952, _D. wuhuensis_ Lee, 1960 (Platyhelminthes: Monogenea: Dactylogyridae), and _Sinergasilus lieni_ Yin, 1949 (Crustacea: Copepoda: Ergasilidae), were collected from the gills of the alien cyprinid, the silver carp _Hypophthalmichthys molitrix_ (Valenciennes, 1844) in the Watarase River flowing into the Watarase Retarding Basin, Tochigi Prefecture, central Japan. These alien parasites represent new geographical records from Japan. The generic position of _D. skrjabini_ has been doubted because its dorsal anchor shape is similar to those of _Pellucidhaptor_ spp. (Dactylogyridae), but the species should be assigned to _Dactylogyrus_ based on the phylogenetic analysis of 28S rDNA made in this study. The scientific name "_Pseudergasilus polycolpus_ Markewitsch, 1939" is an incorrect subsequent spelling of _P. undulatus_ Markewitsch, 1940, and _S. lieni_ is the oldest available name that can be applied to the ergasilid found in this study.

Key Words: Gill parasite, _Dactylogyrus_, Monogenea, _Sinergasilus_, Copepoda, _Hypophthalmichthys molitrix_, alien species, new country record, Japan.

Introduction

The silver carp _Hypophthalmichthys molitrix_ (Valenciennes, 1844) (Cypriniformes: Cyprinidae) is a freshwater fish, being natively distributed in southern Asia, eastern China, and Far-East Russia (Kolar et al. 2005). This species has been widely introduced and become established in aquaculture facilities in about 30 countries and territories around the world (Kolar et al. 2005). It was introduced into Japan from China and Taiwan between 1878 and the 1940s and established only in the Tone River system, central Japan (Maruyama et al. 1987; Matsuzawa and Senou 2008).

The Watarase Retarding Basin, a floodplain wetland belonging to the Tone River system, holds many endangered plants and animals and has been registered as a Ramsar site since 2012 (Obata et al. 2012; Ogawa 2012). About 50 species of freshwater fishes, including 19 alien species, have been reported from the basin (Sekine 2009; Nitta et al. 2016), in which the silver carp is known to occur as one of those alien species (Nakamura 1949; Sekine 2009). This cyprinid has been introduced in different water bodies along with its parasites in many countries (e.g., Hoffman and Schubert 1984), and the Tone River system is most likely under the same situation. We examined the gills of silver carp collected from the Watarase River flowing into the Watarase Retarding Basin and the results of this examination are presented herein.

Materials and Methods

Four specimens of the silver carp (507–750 mm in standard length) were collected by a cast net from the Watarase River (36°12′09.0″N, 139°40′59.4″E), a tributary of the Tone River system, in Fujioka, Tochigi City, Tochigi Prefecture, Japan on 19 August 2015. The fish were kept in a freezer before examination for gill parasites. After they were thawed, all gills were removed from them: the gills from one fish were examined for parasites in fresh conditions, while those from the second and third-fourth fish were examined after fixation in 5% formalin and 99% ethanol, respectively. The fish identification was based on Hosoya (2013).

Monogeneans were picked up from the gills using small needles and flattened between a glass slide and a coverslip. For molecular analysis, some specimens identified as _D. skrjabini_ under an Olympus BX51 light microscope were preserved in 99% ethanol. For morphological study, other specimens of this and other monogenean species were fixed in acetic acid-formalin-alcohol or 70% ethanol and stained in alum carmine, or fixed in modified picrate glycerin (Nitta and Nagasawa 2018). Monogeneans except the specimens for molecular analysis were dehydrated through a graded ethanol series, cleared in xylene, and mounted in Canada
balsam. Measurements of sclerotized parts of monogeneans are presented in Fig. 1 (see Gussev et al. 2010). The penis and accessory piece lengths were measured from images taken by an Olympus DP20 microscope digital camera using ImageJ software (version 1.48i) attached on an Olympus BX51 light microscope. The numbering of marginal hook pairs follows Mizelle (1936).

Copepods were removed from the gills using small needles and forceps and fixed in 70 or 99% ethanol. Copepods were cleared and dissected in lactic acid. The whole body was examined using the wooden slide method (Humes and Gooding 1964). The removed appendages and parts of the body were dehydrated through a graded ethanol series, cleared in xylene, mounted in Canada balsam, and examined for morphological characters.

Drawings were made with the aid of a drawing tube fitted on an Olympus BX51 light microscope. Measurements, in micrometers, are expressed as the range. The monogenean and copepod specimens are deposited in the Platyhelminthes and Crustacea collections of the National Museum of Nature and Science (NSMT-Pl and NSMT-Cr), Tsukuba City, Ibaraki Prefecture, Japan, respectively.

DNA was extracted from two specimens of D. skrjabini using the DNeasy blood and tissue kit (Qiagen) in accordance with the manufacturer's instructions. The DNA was amplified 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′) to amplify partial 28S rDNA (Vân Le et al. 1993). A total of 25 µL PCR reaction consisted of 1 µL of DNA template, 10×Titanium Taq PCR Buffer (Clonetech), 0.2 mM of each dNTP, 1 µM of each primer, and 1×Titanium Taq DNA Polymerase (Clonetech). 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 product was purified using NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel) and sequenced with a 3130xl Genetic Analyzer (Applied Biosystems) with the same primers that generated the PCR product. The newly generated 28S rDNA sequence was aligned with sequences for 14 Dactylogyrus species and two Pseudodactylogyrus species collected in East Asia retrieved from the GenBank database (Fig. 3). Alignment was performed with ClustalW using the default parameters. Phylogenetic trees were constructed for maximum likelihood methods under the GTR+G+I model selected as the best-fit model using AICc, and with the neighbor-joining (NJ) method under the K2 model, with the phylogeny tested by 1,000 bootstrap repeats using MEGA7 (Kumar et al. 2016).

Results

Class Monogenea van Beneden, 1858
Subclass Monopisthocotylea Odhner, 1912
Order Dactylogyridae Bychowsky, 1937
Family Dactylogyridae Bychowsky, 1933
[Japanese name: Shi-sen-chū-ka]
Genus Dactylogyrus Diesing, 1850
[Japanese name: Yubigata-mushi-zoku]
Dactylogyrus skrjabini Achmerow, 1954
[New Japanese name: Dai-yubigata-mushi]
(Fig. 2)
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16; Zhang 2012: 123; Al-Jawda and Asmar 2015: 129; Mhaisen and Al-Rubaie 2016: 5, 7.

*Dactylogyrus scrjabini* [lapsus]: Bykhovskaya-Pavlovskaya *et al.* 1962: 254–255, fig. 603; Babayev 1964: 51; Gussev 1967: 56, 58, figs 1ge, 2ze; Osmanov 1971: 104; Yukhimenko 1972: 155, 156; Anonymous 1973a: 139, pl. 78, figs 157–158; Musselius 1973: 20–21, fig. 6be; Anonymous 1978: 50; Chen 1981: 115; Ji *et al.* 1982: 20; Molnár 1984: 154; Gussev 1985: 22, 122–123, figs 9–8, 157; Huang 1986: 16; Salih *et al.* 1988: 371, 378, 381–382, fig. 7; Gerasev 1989: 39–40, fig.1-1; Gerasev 1990: 367; Gerasev 1991: 224–226, fig. 5-18; Hoffmann 1999: 128; Urazbaev and

Fig. 2. *Dactylogyrus scrjabini* Achmerow, 1954. NSMT-Pl 6393. A, whole mount (ventral view); B, dorsal anchor; 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, needle; M, male copulatory organ. Scale bars: A, 500 µm; B–M, 20 µm. Abbreviations: ap, accessory piece; e, eye-spot; dan, dorsal anchor; g, germarium; h, haptor; ho, head organ; in, intestine; mg, Mehlis’ gland; mh, marginal hook; o, ootype; od, oviduct; p, penis; ph, pharynx; pr, prostatic reservoir; sv, seminal vesicle; t, testis; va, vagina; vd, vas deferens; vl, vitellaria; vo, vaginal opening.
Material examined. Five specimens stained in alum carmine and three fixed in modified picrate glycerin (NSMT-Pl 6393).

Description. Body elongate (Fig. 2A), 1208–2618 long including haptor and long peduncle, width at mid-body. Three pairs of head organs. Two pairs of eye-spots. Pharynx subspherical, 80–103 long, 82–104 wide; esophagus followed by bifurcated intestine with branches confluenting posterior to testis. Testis ovate to pyriform, posterodorsal to germarium, 128–298 long, 115–158 wide. Vas deferens arising from anterior end of testis, looping dorsoventrally around left intestine, forming seminal vesicle. Two saccate prostatic reservoirs. Male copulatory organ sclerotized, consisting of penis and accessory piece, length 104–128 (Fig. 2M). Penis slightly curved tube, length 68–84. Accessory piece rod-shaped, its widened tip holding distal end of penis, length 104–127. Germarium ovate, in mid-body, 60–229 long, 82–120 wide. Oviduct arising from anterior margin of germarium, continuing to oötype. Mehlis’ gland surrounds base of oötype. Vagina unsclerotized, opening on right lateral side, mid-length of body, leading to right side of oviduct. Vitellaria approximately co-extensive with intestine.

Haptor 185–250 long, 180–250 wide. Dorsal anchor (Fig. 2B), total length 71–76; length to notch 28–35; outer root well developed, length 38–45, inner root length 11–18, point length 9–11. Dorsal bar plate-shaped, total length 17–25, total width 11–23, median width (Fig. 2C). Ventral bar broadly V-shaped with notched edge, total length 14–20 (16, n=4), total width 7–10, median width 3–4 (Fig. 2D). Marginal hooks 7 pairs; hook length: pair I (Fig. 2E) 40–47; pair II (Fig. 2F) 30–34; pair III (Fig. 2G) 31–37; pair IV (Fig. 2H) 36–45; pair V (Fig. 2I) well developed, 60–64, pair VI (Fig. 2J) 37–43; pair VII (Fig. 2K) 39–48. Pair of needles (Fig. 2L) located near fifth hooks, length 10–13 (12, n=3).

Host. Silver carp Hypophthalmichthys molitrix (Cypriniformes: Cyprinidae)

Site of infection. Gill rakers.

Molecular analysis. The partial 28S rDNA (731 bp) sequences from the two specimens were identical and submitted to the DNA Data Bank of Japan Centre (DDBJ) (LC414156). Two species of Pseudodactylogyrus were used as the outgroup for the phylogenetic analysis, the tree agree with the part of analysis by Nitta and Nagasawa (2016), and Dactylogyrus skrjabini forms a sister group with D. hypophthalmichthys (Fig. 3).

Remarks. This species was originally described from the gills of H. molitrix in the Amur River Basin, Far-East Russia (Achmerow 1954). It was subsequently reported from the gills of the same host in the natural distribution range of the host: Lake Taihu, and Anhui, Hubei, Fujian, Beitan, Habae, and Burqin provinces in China (Long and Lee 1960; Lee 1963; Anonymous 1973a; Huang 1986; Zhao 2011). The dorsal anchor shape and well developed fifth marginal hook are characters to distinguish D. skrjabini from the other congeneric species, and the specimens examined in this study agree with the descriptions by Achmerow, (1954), Bykhovskaya-Pavlovskaya et al. (1962), Long (2000), and Gussev et al. (2010). The detailed internal anatomy of the species was firstly described herein and showed the common dactylogyrid form.

The present finding represents the first record of D. skrjabini from Japan. This monogenean is established along with H. molitrix in the European region of Russia (Musselius 1969, 1973; Osmanov 1971), Turkmenistan (Babayev 1964), Kazakhstan (Gvozdev and Agapova 1977), Hungary (Hoffman and Schubert 1984; Molnár 1984), Iraq (Salih et al. 1988; Ali et al. 1989; Mhaisen et al. 2012; Al-Jawda and Asmar 2015), and the Aral Sea (Urazbaev and Kurbanova 2006; Zonn et al. 2009).

Japanese name. The species is one of the biggest species in the genus, and the new Japanese name refers it: “dai” and “yubigata-mushi” mean large and the genus, respectively.

Dactylogyrus hypophthalmichthys Achmerow, 1952
[New Japanese name: Hakuren-yubigata-mushi] (Fig. 4)
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3a; Gussev 1955: 395; Long and Yu 1958: 10–11, fig. 3; Bykhovskaya-Pavlovskaya et al. 1962: 292, 294–295, fig. 683; Akmetov 1963: 462; Lee 1963: 76; Wu 1963: 112; Babayev 1964: 51; Agapova 1966: 134; Akmetov 1966: 258; Osmanov and Yusupov 1967: 212; Musselius 1969: 238, 240; Chentsov and Kirichenko 1970: 119; Osmanov 1971: 106; Kirichenko 1972: 115; Yukhimenko 1972: 154–156; Anonymous 1973a: 139, pl. 78, figs 155–156; Musselius 1973: 19–21, fig. 6a; Volovic 1973: 130–141, figs 1–3; Bauer and Hoffman 1976: 165; Belova 1977: 38–42; Gvozdev and Agapova 1977: 109; Musselius 1977: 146; Anonymous 1978: 41; Chang and Ji 1978: 355; Chen 1981: 115; Zhang 1981: 72; Ji et al. 1982: 13; Molnár 1984: 154; Gussev 1985: 166–168, fig. 241; Margaritov and VanThan 1985: 52–54, fig. 2; Huang 1986: 15; Mhaisen et al. 1988: 893; Salih et al. 1988: 371, 378, 380, fig. 6; Ali et al. 1989: 152–153; Jalali and Molnár 1990: 241; Ma and Li 1991: 3; Wu and Wang 1991: 18, 71–72, fig. 46; Li and Zhang 1992: 91; Zhang et al. 1992: 129; Jin et al. 1993: 327–328, fig. 99; Pojmanika 1995: 81; Gibson et al. 1996: 17; Blanc 1997: 497; Gelnar and Spakulová 1997: 189; Xia and Wang 1998: 19, 20; Hoffman 1999: 126; Long 2000: 92–93, fig. 40; Yao 2000: 25; Moravec 2001: 17; Xu et al. 2001: 715; Grigorovich et al. 2002: 1208; Yao and Nie 2004: 664–665; Johnson and Lunde 2005: 131; Šefrová and Laštůvka 2005: 157; Jalali and Barzegar 2006: 50–51, fig. 9; Molodozhnikova and Zhokhov 2006: 328, 334; Urazbaev and Kurbanova 2006: 537; Žy and Tě 2007: 121, 124, fig. 91; Wu et al. 2007: 653, 655, 657; Karabekova 2008: 331, 333; Zonn et al. 2009: 125; Gussev et al. 2010: 243–244, fig. 296; Singh and Chaudhary 2010: 123–126; Mhaisen et al. 2010: 96, 99–100; Tan et al. 2011: 133–134; Zhang et al. 2011: 30–34; Bozorgnia et al. 2012: 251; Davydov et al. 2012: 141; Mhaisen et al. 2012: 106, 116; Zhang 2012: 123; Paskornik and Masoumian 2012: 575; Fedorowych et al. 2013: 242–244; Gussev et al. 2013: 31; Zhatkanbayeva et al. 2013: 149; Zhou et al. 2014: 18, 20, figs 2L, J; Zaichenko 2015: 73, 77; Mhaisen and Al-Rubaie 2016b: 5, 7.

**Neodactylogyrus hypophthalmichthys**: Yamaguti, 1963a: 38, fig. 659.

**Material examined.** Eleven specimens fixed in modified picrate glycerin (NSMT-Pl 6394).

**Description.** Body elongate, 384–544 long including haptor, width at mid-body 73–117 (104, n = 5). Pharynx spherical, 14–24 long, 15–24 wide. Male copulatory organ (Fig. 4L) sclerotized, consisting of penis and accessory piece, length 32–34. Penis tapered, sigmoid tube, length 31–35. Accessory piece (Fig. 4L) rod-shaped, its tip holding middle of penis, length 28–32. Vagina un sclerotized.

Haptor 55–77 long, 88–125 wide. Dorsal anchor (Fig. 4A) total length 33–40; length to notch 29–34; outer root length 4–6, inner root length 11–18 (13, n = 6), point length 9–11 (10, n = 6). Dorsal bar (Fig. 4B) broadly V-shaped, total length 24–27, total width 4–9, median width 3–5. Ventral bar (Fig. 4C) T-shaped, total length, total width 8–12, median width 5–11. Marginal hooks 7 pairs; hook length: pair I (Fig. 4D) 27–31; pair II (Fig. 4E) 31–38; pair III (Fig. 4F) 28–35; pair IV (Fig. 4G) 26–33; pair V (Fig. 4H), 30–34, pair VI (Fig. 4I) 33–44; pair VII (Fig. 4J) 26–43. Pair of needles (Fig. 4K) located near second hooks, length 9–11.

**Host.** Silver carp *Hypophthalmichthyus molitrix* (Cyprinidae-formes: Cyprinidae)

**Site of infection.** Gill filaments.

**Remarks.** This species was originally described by Achmerow (1952) from the gills of *H. molitrix* in Lake Petro pavlovsk, Lakes Bolon and Udyl, Far-East Russia. Subsequently, Yamaguti (1963a) transferred the species to the genus *Neodactylogyrus* Price, 1938, but this genus had already been synonymized with *Dactylogyrus* by Mizelle and Donahue (1944). As a native parasite, *D. hypophthalmichthys* was reported from the same host in Jiangsu, Anhui, Hubei, Zhejiang, Wuzhou, Chongqing, Liaoning, Jiangxi, Fujian, and Heilongjiang provinces, and Shanghai, China (Long and Yu 1958; Lee 1963; Wu 1963; Anonymous 1973a; Chang and Ji 1978; Zhang 1981; Huang 1986; Ma and Li 1991; Wu and Wang 1991; Li and Zhang 1992; Xia and Wang 1998; Yao 2000; Wu et al. 2007; Zhou et al. 2014) and the Amur River, Russia (Gussev 1955; Chentsov and Kirichenko 1970). Yao (2000) reported this monogenean from the gills of the grass carp *Ctenopharyngodon idellus* (Valenciennes, 1844) (Cyprinidae) in Jiangxi Province, China. The life cycle and development of *D. hypophthalmichthys* were described by Volovic (1973).

The specimens examined in this study conform to the descriptions and illustrations of *D. hypophthalmichthys* by Achmerow (1952), Bykhovskaya-Pavlovskaya et al. (1962), Long (2000), and Gussev et al. (2010): the tip of accessory piece holding the middle of the sigmoid penis is one of the features in *D. hypophthalmichthys*.

The present collection represents the first record of *D. hy-
Dactylogyrus wuhuensis Lee, 1960

[New Japanese name: Buko-yubigata-mushi] (Fig. 5)

Dactylogyrus wuhuensis Lee, 1960: 33–36, figs A, B; Lee 1963: 76; Yukihmenko 1972: 155; Anonymous 1973a: 138, pl. 77, figs 153–154; Musselius 1973: 20, 22, fig. 6e; Gvozdev and Agapova 1977: 109; Anonymous 1978: 52–53; Chen 1981: 116; Ji et al. 1982: 22; Gussev 1985: 125–126, fig. 161; Huang 1986: 15; Wu and Wang 1991: 18, 102–103, fig. 90; Long 2000: 94–95, fig. 41; Xia et al. 2000: 152; Urzaizav and Kurbanova 2006: 537; Ký and Tê 2007: 136, 139, fig. 116; Gussev et al. 2010: 189–190, 192, fig. 217; Davydov et al. 2012: 141; Zhang 2012: 123.

Dactylogyrus chenshuchenae Gussev in Bykhovskaya-Pavlovskaya et al., 1962 described from the gill filaments of the same host in Hubei, Fujian, and Zhejiang provinces, China (Anonymous 1973a; Huang 1986; Wu and Wang 1991) and Far-East Russia (Yukihmenko 1972; Musselius 1973). Dactylogyrus wuhuensis Gussev in Bykhovskaya-Pavlovskaya et al., 1962 described from the gill filaments of the same host in the Liao River, Liaoning Province, China, has been synonymized with D. wuhuensis (Anonymous 1973a). The haptoral structures and the accessory piece of the specimen examined in this study conform to those of Lee (1960), Wu and Wang (1991), Long (2000), and Gussev et al. (2010).

The present collection represents the first record of D. wuhuensis from Japan. This monogenean has been reported as an alien species parasitic on Hypophthalmichthys molitrix from Turkmenistan (Babayev 1964; Osmanov 1971), the Amu Darya River (Osmanov and Yusupov 1967), the European region of Russia (Musselius 1969), Kazakhstan (Gvozdev and Agapova 1977), the Aral Sea (Urzaizav and Kurbanova 2006), and Vietnam (Ký and Tê 2007).

Japanese name. In the new Japanese name, “buko” means Wuhu, the type locality of D. wuhuensis, in Japanese, and “yubigata-mushi” means the genus Dactylogyrus.

Material examined. One specimen fixed in modified picrate glycerin (NSMT-Pl 6395).

Description. Male copulatory organ (Fig. 5L) sclerotized, consisting of penis and accessory piece, composition of male copulatory organ of our specimen damaged during preparation. Penis long, length 138. Accessory piece (Fig. 5L) rod-shaped with bifurcated base and widened tip, length 60. Vagina unsclerotized.

Dorsal anchor (Fig. 5A) total length 40; length to notch 35; outer root length 5, inner root length 9, point length 1. Dorsal bar (Fig. 5B) bow-shaped, total length 22, total width 6, median width 3. Ventral bar (Fig. 5C) broadly M-shaped, total length 35, total width 5, median width 3. Marginal hooks 7 pairs; hook length: pair I (Fig. 5D) 29; pair II (Fig. 5E) 30; pair III (Fig. 5F) 30; pair IV (Fig. 5G) 30; pair V (Fig. 5H), 27, pair VI (Fig. 5I) 29; pair VII (Fig. 5J) 32. Pair of needles (Fig. 5K) length 11.

Host. Silver carp Hypophthalmichthys molitrix (Cypriniformes: Cyprinidae)

Site of infection. Gill filaments.

Remarks. This species was originally described by Lee (1960) from the gills, oral cavity, and nasal cavity of H. molitrix in Wuhu, Anhui Province, China. Subsequently, it was reported from the same host in Hubei, Fujian, and Zhejiang provinces, China (Anonymous 1973a; Huang 1986; Wu and Wang 1991) and Far-East Russia (Yukihmenko 1972; Musselius 1973). Dactylogyrus chenshuchenae Gussev in Bykhovskaya-Pavlovskaya et al., 1962 described from the gill filaments of the same host in the Liao River, Liaoning Province, China, has been synonymized with D. wuhuensis (Anonymous 1973a). The haptoral structures and the accessory piece of the specimen examined in this study conform to those of Lee (1960), Wu and Wang (1991), Long (2000), and Gussev et al. (2010).

The present collection represents the first record of D. wuhuensis from Japan. This monogenean has been reported as an alien species parasitic on H. molitrix from Turkmenistan (Babayev 1964; Osmanov 1971), the Amu Darya River (Osmanov and Yusupov 1967), the European region of Russia (Musselius 1969), Kazakhstan (Gvozdev and Agapova 1977), the Aral Sea (Urzaizav and Kurbanova 2006), and Vietnam (Ký and Tê 2007).

Pseudergasilus polycolpus (not of Markewitsch, 1940): Markewitsch, 1946: 233. (in part, incorrect subsequent spelling for Pseudergasilus undulatus Markewitsch, 1940)
Sinergasius lieni Yin, 1949. NSMT-Cr 26718. A, whole mount (dorsal view); B, antennule; C, antenna; D, mandible, maxillule and maxilla; E, leg 1; F, leg 2; G, legs 3; H, leg 4; I, leg 5; J, urosome (ventral view). Scale bars: A, 500 µm; B–J, 50 µm.
Antenna (Fig. 6C) 4-segmented; first segment shorter than second segment and claw of the antenna (Bykhovskaya-Pavlovskaya et al. 1962). The antennule armature of S. lieni is obscure in the past descriptions (e.g., Yin 1949, 1956; Mirzoeva 1973), but the present description almost agrees with the figure of the redescription by Yin (1956: pl. 16, fig. 2) and redefined herein the armature formula.

The present collection represents the first record of S. lieni from Japan. This copepod is natively distributed in China and Far-East Russia with H. molitrix and H. nobilis (Markewitsch 1946; Yin 1956; Wang et al. 2014). It also infects H. harmandi Sauvage, 1884 (Cyprinidae) in Vietnam (Ký and Tê 2007). Sinergasilus lieni has been reported as an alien species from the European region of Russia (Musselius 1969; Mirzoeva 1972, 1973; Zhokhov and Molodzhnikova 2006), Hungary (Molnár and Székely 2004), and Macedonia (Dimovska and Stojanovski 2015) from H. molitrix and H. nobilis; and Serbia and Montenegro from H. nobilis (Cakic et al. 2004).

Japanese name. The new Japanese generic and specific name is a combination of "chūka" and "era-jirami", which mean China and a gill louse in Japanese, respectively ("zoku" means a genus).

Discussion

The dorsal anchor of Dactylogyrus skrjabini is similar to those of the species in the genus Pellucidhaptor Price and Mizelle, 1964 (Dactylogyroridae), and the generic position of D. skrjabini was doubted (Gerasiev 1990). Based on the phylogenetic analysis of 28S rDNA (Fig. 3), this species should be assigned to Dactylogyrus as in the past. Unfortunately, there are no available sequence data of Pellucidhaptor, and the residual problem is a future study subject. Dactylogyrus skrjabini and D. hypophthalmichthys form a sister group in the phylogenetic tree. This affinity can be supported by the observed similar composition of both male copulatory organs, i.e., the tip of rod-shaped accessory piece holds the penis. The infection sites of both species are different: D. skrjabini mainly infects the gill rakers, while D. hypophthalmichthys only the gill filaments. The speciation of these species might have been prompted by the differences in infection site on the same host species.

The ergasilid collected in this study is herein identified as Sinergasilus lieni, but our use of this scientific name needs some explanations. The species was first reported as “Pseudergasilus polycopus” Markewitsch, 1939” by Markewitsch et al. 2010: 1192, pl. 1; Delibaier et al. 2014: 93; Wang et al. 2014: 180; Dimovska and Stojanovski 2015: 33–37, figs 1–7.

Pseudergasilus undulatus (not of Markewitsch, 1940): Markewitsch 1956: 64–66 (in part); Markewitsch 1976: 98–100 (in part).

Material examined. Six whole and three dissected females (NSMT-Cr 26718).

Description of adult female. Body (Fig. 6A) elongate, cylindrical, length 1760–2066. Prososome length 1334–1775, width, 394–494, depth 181–340, 2.5–3.2 times as long as wide.

Body (Fig. 6A) elongate,

cylindrical, length 1760–2066. Prosome length 1334–1775, width, 394–494, depth 181–340, 2.5–3.2 times as long as wide.

Table 1. Armature formula of legs 1–4 of Sinergasilus polycolpus Yin, 1949, adult female, NSMT-Cr 26718. Arabic numbers = number of seta, Roman numbers = number of spines.

|       | Coxa | Basis | Exopod | Endopod |
|-------|------|-------|--------|---------|
| Leg 1 | 0-0  | 1-0   | I-0; 1-1; II-5 | 0-1; 0-1; II-4 |
| Leg 2 | 0-0  | 1-0   | I-0; 0-1; 1-6 | 0-1; 0-2; I-4 |
| Leg 3 | 0-0  | 1-0   | I-0; 0-1; 1-6 | 0-1; 0-2; I-4 |
| Leg 4 | 0-0  | 1-0   | I-0; 1-5 | 0-1; 0-2; I-3 |

Host. Silver carp Hypophthalmichthys molitrix (Cyprini-formes: Cyprinidae)

Site of infection. Gill filaments.

Remarks. The specimens examined in this study conform to the descriptions of S. lieni by Yin (1949, 1956) from the bighead carp Hypophthalmichthys nobilis (Richardson, 1845) and H. molitrix in China. This species is differentiated from two congeners, Sinergasilus major (Markewitsch, 1940) and S. undulatus, based on the ratio of the length of the second segment and claw of the antenna (Bykhovskaya-Pavlovskaya et al. 1962). The antennule armature of S. lieni is obscure in the past descriptions (e.g., Yin 1949, 1956; Mirzoeva 1973), but the present description almost agrees with the figure of the redescription by Yin (1956: pl. 16, fig. 2) and redefined herein the armature formula.

The present collection represents the first record of S. lieni from Japan. This copepod is natively distributed in China and Far-East Russia with H. molitrix and H. nobilis (Markewitsch 1946; Yin 1956; Wang et al. 2014). It also infects H. harmandi Sauvage, 1884 (Cyprinidae) in Vietnam (Ký and Tê 2007). Sinergasilus lieni has been reported as an alien species from the European region of Russia (Musselius 1969; Mirzoeva 1972, 1973; Zhokhov and Molodzhnikova 2006), Hungary (Molnár and Székely 2004), and Macedonia (Dimovska and Stojanovski 2015) from H. molitrix and H. nobilis; and Serbia and Montenegro from H. nobilis (Cakic et al. 2004).

Japanese name. The new Japanese generic and specific name is a combination of "chūka" and "era-jirami", which mean China and a gill louse in Japanese, respectively ("zoku" means a genus).

Discussion

The dorsal anchor of Dactylogyrus skrjabini is similar to those of the species in the genus Pellucidhaptor Price and Mizelle, 1964 (Dactylogyroridae), and the generic position of D. skrjabini was doubted (Gerasiev 1990). Based on the phylogenetic analysis of 28S rDNA (Fig. 3), this species should be assigned to Dactylogyrus as in the past. Unfortunately, there are no available sequence data of Pellucidhaptor, and the residual problem is a future study subject. Dactylogyrus skrjabini and D. hypophthalmichthys form a sister group in the phylogenetic tree. This affinity can be supported by the observed similar composition of both male copulatory organs, i.e., the tip of rod-shaped accessory piece holds the penis. The infection sites of both species are different: D. skrjabini mainly infects the gill rakers, while D. hypophthalmichthys only the gill filaments. The speciation of these species might have been prompted by the differences in infection site on the same host species.

The ergasilid collected in this study is herein identified as Sinergasilus lieni, but our use of this scientific name needs some explanations. The species was first reported as “Pseudergasilus polycopus” Markewitsch, 1939” by Markewitsch...
(1946) from the gills of crucian carp Carassius carassius (Linnaeus, 1758) from Lake Hanka and Hypophthalmichthys molitrix from the Amur River in Russia. However, the ergasilid was named as Pseudergasilus undulatus Markewitsch, 1940 in Markewitsch (1940a). Later, Markewitsch (1956: 64–68), the same author, stated that “The name P. polycolpus used for this species [= P. undulatus] in our [sic] article (Markewitsch, 1946) is due to oversight” (English translation in Markewitsch [1976]) and did not recognize P. polycolpus as a valid taxon. Further, there was no description of P. polycolpus in the Markewitsch’s paper (Markewitsch 1939, actually 1940a) and book (Markewitsch 1940b), and then Markewitsch (1946) did not propose a diagnosis of P. polycolpus nor indicate his purpose to establish a new taxon. Thus, “Pseudergasilus polycolpus Markewitsch, 1939” can be regarded as an incorrect subsequent spelling of “Pseudergasilus undulatus Markewitsch, 1939 (=1940)” (ICZN 1999: Article 33.3, 33.5). In China, Yin (1949) described S. polycolpus as the scientific name of the ergasilid. Based on the prevailing usage (ICZN 1999: Article 33.3.1), S. polycolpus can be regarded as a correct original spelling. However, this treatment does not replace “polycolpus” with “polycolpus” and “undulatus” and is considered inappropriate because P. undulatus (= S. undulatus) is still accepted as a valid taxon differed from S. polycolpus (=S. lieni) (e.g., Yin 1956; Anonymous 1973b; Bykhovskaya-Pavlovskaya et al. 1962; Gussev 1987). In addition, Markewitsch’s (1936) opinion to synonymize S. lieni with P. undulatus was rejected by Bykhovskaya-Pavlovskaya et al. (1962) and Gussev (1987), both of whom regarded S. lieni as a valid taxon. As stated above, “Pseudergasilus polycolpus Markewitsch, 1939” is an incorrect subsequent spelling of P. undulatus, and S. lieni is the oldest available scientific name of the ergasilid and, therefore, is herein used an accepted name for the ergasilid found in this study.

Nineteen species of alien fishes have been established in the Watarase Retarding Basin (Sekine 2009; Nitta et al. 2016). The parasites reported herein have a simple life cycle (Mirzoeva 1972, 1973; Volovic 1973), and other alien monogeneans and parasitic copepods may be co-established in the basin with the other alien fishes. It is necessary to clarify the alien and native parasite fauna of this area and the imperfections of alien parasites on domestic fishes.

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