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Monogeneans of *Cichlidogyrus* Paperna, 1960 (Dactylogyridae), gill parasites of tilapias, from Okinawa Prefecture, Japan

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Abstract. Three species of the monogenean genus *Cichlidogyrus* Paperna, 1960 (*C. sclerosus* Paperna & Thurston, 1969, *C. halli* (Price & Kirk, 1967) and *C. tilapiae* Paperna, 1960) are reported for the first time from Japan based on specimens from the gills of tilapias (Nile tilapia *Oreochromis niloticus niloticus* and Mozambique tilapia *O. mossambicus*) in Okinawa Prefecture. These host species are of African origin, and the monogeneans found are definitely alien parasites that were introduced along with their African tilapias to Okinawa Prefecture.

Key words: *Cichlidogyrus*, Monogenea, tilapia, alien fish parasites, new country records

Introduction

Tilapias (Perciformes: Cichlidae) were introduced to the main island of Japan through the importation of live fish from Thailand in 1954 and from the Middle East in 1962 (Yamaoka, 1989). Tilapias were also introduced to Okinawa, the southernmost prefecture of Japan, from Taiwan in 1954 and from the main island of Japan in the 1970's (Takehara *et al*., 1997). In Japan, tilapias were farmed soon after introduction, but, due to lack of popularity as food, their culture production has currently remained very low. On the other hand, tilapias have been established in natural waters of Japan (Yamaoka, 1989). They are Mozambique tilapia *Oreochromis mossambicus* (Peters, 1852), Nile tilapia *O. niloticus niloticus* (Linnaeus, 1758) and redbelly tilapia *Tilapia zillii* (Gervais, 1848). These tilapias are thought to have brought various parasites into Japan, but nothing is known about such parasites. The aim of the present study is to clarify the fauna of monogeneans infecting tilapias in Okinawa Prefecture, where tilapias occur abundantly in fresh and sometimes brackish waters.

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Materials and Methods

Three species of tilapias, Nile tilapia *O. n. niloticus*, Mozambique tilapia *O. mossambicus* and redbelly tilapia *T. zillii*, were collected using hook and line and cast nets in 14 localities on five islands (Okinawa-jima Island, Kume-jima Island, Miyako-jima Island, Ishigaki-jima Island, Minamidaito-jima Island), Okinawa Prefecture, southern Japan, from November 2009 to November 2011 (Table 1). Fish were fixed in 10% formalin in the field and transported to the laboratory for parasitological examination. Gills were removed and examined with an Olympus SZX7 stereoscopic microscope. Monogeneans were taken from the gills: some were transferred into a drop of ammonium picrate-glycerin for the sclerotized structures (Ergens, 1969), while, for the internal anatomy, others were flattened and fixed in 5% formalin on slide glasses with slight cover-slip

Table 1. Sampling localities and dates of tilapias in Okinawa Prefecture, southern Japan.

Locality no.	Locality	Latitude and longitude	Sampling date (No. of fish collected)	Sampling method
1	Haebaru Reservoir, Okinawa-jima Island	26°21'33"N,127°44'36"E	23 Apr. 2010 (6)15 Jul. 2010 (2), 14 Aug. 2010 (3), 15 Sep. 2010 (2), 18 Oct. 2010 (3), 18 Nov. 2010 (2), 19 Dec. 2010 (3), 17 Jan. 2011 (1)	cast net
2	Senbaru Reservoir, Okinawa-jima Island	26°14'57"N, 127°45'50"E	21 Apr. 2010 (1), 24 Jun. 2010 (8)	cast net
3	Ohshiro Reservoir, Okinawa-jima Island	26°9'36"N, 127°46'2"E	8 Jun. 2010 (10), 16 Nov. 2011 (16)	angling
4	Kinjo Reservoir, Okinawa-jima Island	26°12'42"N, 127°43'6"E	18 Jun. 2010 (21)	cast net
5	Nakahodo Reservoir, Okinawa-jima Island	26°10'15"N, 127°45'19"E	8 Jun. 2010 (10)	cast net
6	Hija River, Okinawa-jima Island	26°21'58"N, 127°45'31"E	21 Apr. 2010 (3), 16 Nov. 2011 (16)	angling
7	Okukubi River, Okinawa-jima Island	26°27'30"N, 127°56'18"E	25 Jun. 2010 (5)	cast net
8	Yamakura Reservoir, Kume-jima Island	26°21'25"N, 126°45'40"E	14 Nov. 2009 (9)	angling
9	Irrigation canal in Shimoji, Miyako-jima Island	24°45'18"N, 125°16'35"E	5 Oct. 2011 (26)	angling
10	Miyara River, Ishigaki-jima Island	24°25'10"N, 124°13'13"E	27 Nov. 2009 (5), 14 May 2010 (10)	angling
11	Nagura River, Ishigaki-jima Island	24°24'12"N, 124°9'13"E	28 Nov. 2009 (6), 15 May 2010 (14)	cast net
12	Irrigation canal in Uratabaru, Ishigaki-jima Island	24°23'23"N, 124°9'6"E	28 Nov. 2009 (3)	cast net
13	Hyotan Pond, Minamidaito-jima Island	25°50'4"N, 131°14'15"E	25 Sep. 2010 (16)	angling
14	Tansui Pond, Minamidaito-jima Island	25°50'52"N, 131°14'30"E	24 Sep. 2010 (12)	angling

pressure, stained in Heidenhain's iron hematoxylin, alum carmine or Semichon's acetic carmine, dehydrated in a graded ethanol series, cleared in xylene, and mounted in Canada balsam. Drawings were made with the aid of a drawing tube attached to an Olympus BX51 compound microscope. Average measurements (all in micrometers) are followed by the range in parentheses. Monogenean identification was made using the key given by Pariselle & Euzet (2009). The morphological terminology follows Douëllou (1993). Voucher specimens are deposited in the Platyhelminthes (Pl) collection at the National Museum of Nature and Science, Tsukuba (NSMT-Pl 5993–6003 for *Cichlidogyrus sclerosus* Paperna & Thurston, 1969; NSMT-Pl 6004–6006 for *C. halli* (Price & Kirk, 1967); NSMT-Pl 6007–6012 for *C. tilapiae* Paperna, 1960). Tilapias were identified based on Hatooka (2002), and their scientific and common names used herein are those recommended by Froese & Pauly (2012).

Results

Three species of the genus *Cichlidogyrus* (*C. sclerosus, C. halli and C. tilapiae*) were found on the gills of two species of tilapias (*O. n. niloticus* and *O. mossambicus*) (Table 2). No infection was found on *T. zillii*.

Cichlidogyrus sclerosus Paperna & Thurston, 1969

(Fig. 1)

Description (10 specimens measured): Body elongate, 451 (392–550) long and 177 (164–198) wide. Cephalic lobe with 4 pairs of head organs. One pair of eyes with lens. Mouth not observed. Pharynx spherical, 50 (43–61) in diameter; intestine bifurcated posterior to pharynx and becoming confluent posterior to testis. Haptor rounded, with 2 pairs of hamuli and 7 pairs of hooklets. Ventral and dorsal hamuli of similar size and shape, 31 (27–38) and 32 (29–41) long, respectively, with no distinct roots and strongly curved shaft. Hamulus filament present. Ventral bar V-shaped, 64 (46–70) in transverse length, with rounded extremities. Dorsal bar

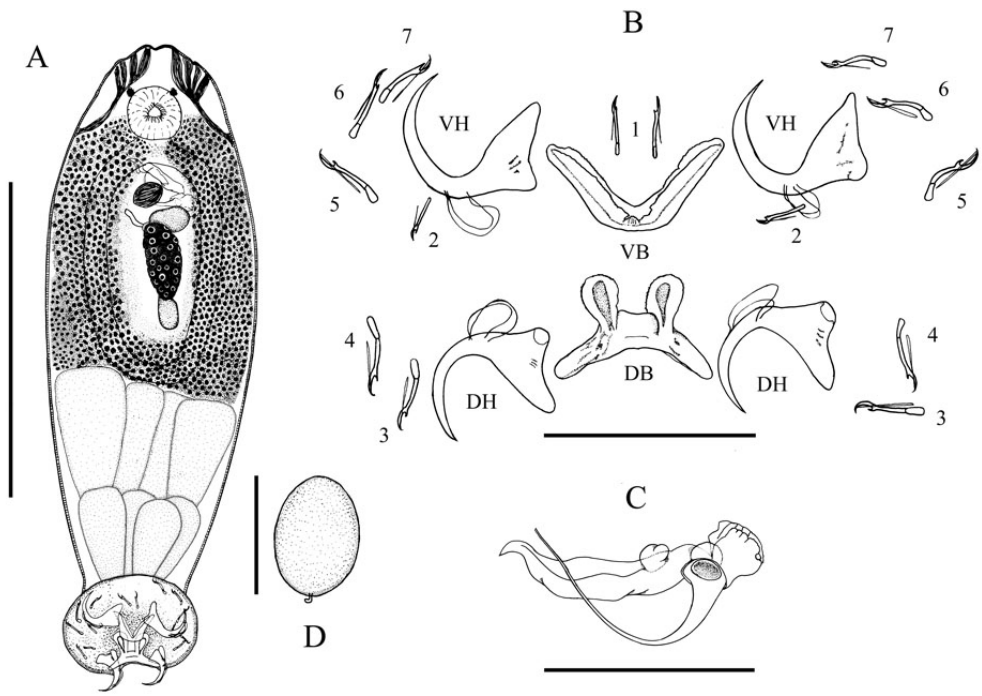

Fig. 1. *Cichlidogyrus sclerosus* Paperna & Thurston, 1969, ventral view. A, whole animal; B, haptoral armature; C, copulatory organ; D, egg. VH, ventral hamulus; DH, dorsal hamulus; VB, ventral bar; DB, dorsal bar. Scale bars: A, 250 μm; B-C, 50 μm; D, 100 μm.

massive and X-shaped, 38 (30–48) in transverse length; branches wide; appendages pyriform with rounded ends, 16 (13–20) long. Hooklets short; pair 2 shortest without base, other pairs with short base; hooklet lengths 15 (13–18), 12 (10–15), 15 (15–16), 17 (12–28), 17 (14–28), 16 (14–18) and 16 (13–18), respectively, for pairs 1–7.

Testis spindle-shaped and located dorsoposterior to ovary. Vas deferens not encircling the intestinal caecum. One prostatic reservoir situated posterior to copulatory organ. Copulatory organ very large and located posterior to intestinal bifurcation, with large serrated plate; copulatory tube thin and arched, 69 (61–76) long, with tapered end; accessory piece 47 (33–52) long, with finger-like extension. Ovary ovoid and located anteroventral to testis. Vagina sub-median. Seminal receptacle attached to vagina. Vitelline follicles well-developed and co-extensive with intestine. Mehlis' gland and uterine pore not observed. Eggs oval-shaped, 61 (56–66) long and 47 (45–48) wide, with appendix at terminal end.

Hosts: Oreochromis niloticus niloticus and *O. mossambicus* (Perciformes: Cichlidae).

Site of attachment: Gills.

Localities: Haebaru Reservoir, Ohshiro Reservoir, Kinjo Reservoir, Hija River, Okukubi River on Okinawa-jima Island; Yamakura Reservoir on Kume-jima Island; Miyara River, Nagura River, an irrigation canal in Uratabaru on Ishigaki-jima Island; Hyotan Pond, Tansui Pond on Minamidaito-jima Island (Table 2).

Remarks: The finding of *C. sclerosus* in this study represents the first record from Japan. This parasite was originally described by Paperna & Thurston (1969) based on specimens from the gills of *O. mossambicus* (as *Tilapia mossambica*), *O. n. niloticus* (as *T. nilotica*), *O. leucostictus* (as *T. leucosticta*), *Haplochromis* sp. and *T. zillii* in Uganda, Africa. The species so far has been reported from various cichlid fishes from Africa (Uganda, Zimba-

Table 2. Prevalence (P) and mean intensity (MI) of three species of *Cichlidogyrus* on tilapias (*Oreochromis niloticus niloticus, O. mossambicus* and *Tilapia zillii*) from Okinawa Prefecture, southern Japan.

Locality no.	Host	No. of fish examined	Body length (mm) mean (range)	*C. sclerosus*		*C. halli*		*C. tilapiae*	
				P	MI (range)	P	MI (range)	P	MI (range)
1*	*O. n. niloticus*	22	73 (48-230)	78	5.6 (1-15)	0	-	0	-
2	*O. n. niloticus*	5	115 (95-133)	0	-	0	-	0	-
	T. zillii	4	88 (73-107)	0	-	0	-	0	-
3	*O. n. niloticus*	26	114 (59-175)	92	14.5 (2-23)	0	-	0	-
4	*O. n. niloticus*	10	147 (115-182)	70	20.9 (3-39)	0	-	0	-
	T. zillii	11	109 (76-185)	0	-	0	-	0	-
5	*T. zillii*	10	90 (72-103)	0	-	0	-	0	-
6	*O. n. niloticus*	19	112 (66-185)	84	8.9 (1-77)	0	-	5	1.0 (1)
7	*O. n. niloticus*	5	140 (103-235)	60	2 (1-4)	0	-	0	-
8	*O. n. niloticus*	9	105 (60-140)	78	40.0 (11-167)	0	-	44	24.3 (1-58)
9	*O. n. niloticus*	26	79 (45-125)	0	-	0	-	0	-
10	*O. n. niloticus*	15	103 (73-142)	87	13.7 (9-66)	47	1.1 (1-2)	20	1.3 (1-2)
11	*O. n. niloticus*	20	154 (70-220)	95	32.8 (1-124)	75	2.3 (1-5)	25	1.8 (1-2)
12	*O. mossambicus*	3	163 (153-170)	100	10.3 (8-14)	33	1.0 (1)	0	-
13	*O. n. niloticus*	16	92 (70-125)	69	1.7 (1-4)	0	-	0	-
14	*O. n. niloticus*	12	92 (66-115)	75	4.8 (1-23)	0	-	0	-

* See Table 1 for detailed information on the sampling localities.

bwe, Egypt, South Africa), Middle East (Israel), Asia (Philippines, Hong Kong, Singapore, Thailand) and Americas (Colombia, Mexico, Cuba) (see Douëllou, 1993; Jiménez-García *et al*., 2001; Kohn *et al*., 2006; Mendora-Franco *et al*., 2006; Sanchez-Ramirez *et al*., 2007; Lerssutthichawal, 2008; Pariselle & Euzet, 2009; Le Roux & Avenant-Oldewage, 2010; Madanire-Moyo *et al*., 2011; Akoll *et al*., 2012).

Cichlidogyrus halli (Price & Kirk, 1967)

(Fig. 2)

Description (10 specimens measured): Body elongate, 546 (363–629) long and 215 (159–242) wide. Cephalic lobe with 4 pairs of head organs. One pair of eyes with lens. Mouth not observed. Pharynx spherical, 34 (31–38) in diameter; intestine bifurcated posterior to pharynx and confluent posterior to testis. Haptor ellipsoid, with 2 pairs of hamuli and 7 pairs of hooklets. Ventral hamuli robust, 49 (48–50) long, with massive base and short shaft and point; outer root broad with rounded end; inner root longer than outer. Dorsal hamuli smaller, 45 (41–48) long; outer root wide with rounded end; inner root narrower and much longer than outer root. Hamulus filament present. Ventral bar V-shaped, 107 (100–114) in transverse length, with membranous extensions. Dorsal bar large and massive, 60 (53–63) in transverse length; branches long; appendages wide apart, 23 (20–27) long. Hooklets long; pair 2 shortest without base, pair 1 with short base, other pairs with long base; hooklet lengths, 15 (13–18), 14 (13–15), 24 (23–25), 28 (25–31), 33 (30–35), 29 (25–30) and 26 (20–29), respectively, for pairs 1–7.

Testis ovoid and located dorsoposterior to ovary. Vas deferens not encircling the intestinal caecum. One prostatic reservoir situated posterior to copulatory organ. Copulatory organ very large and located posterior to intestinal bifurcation; copulatory tube S-shaped, 73 (60–80) long, with irregular base; ac-

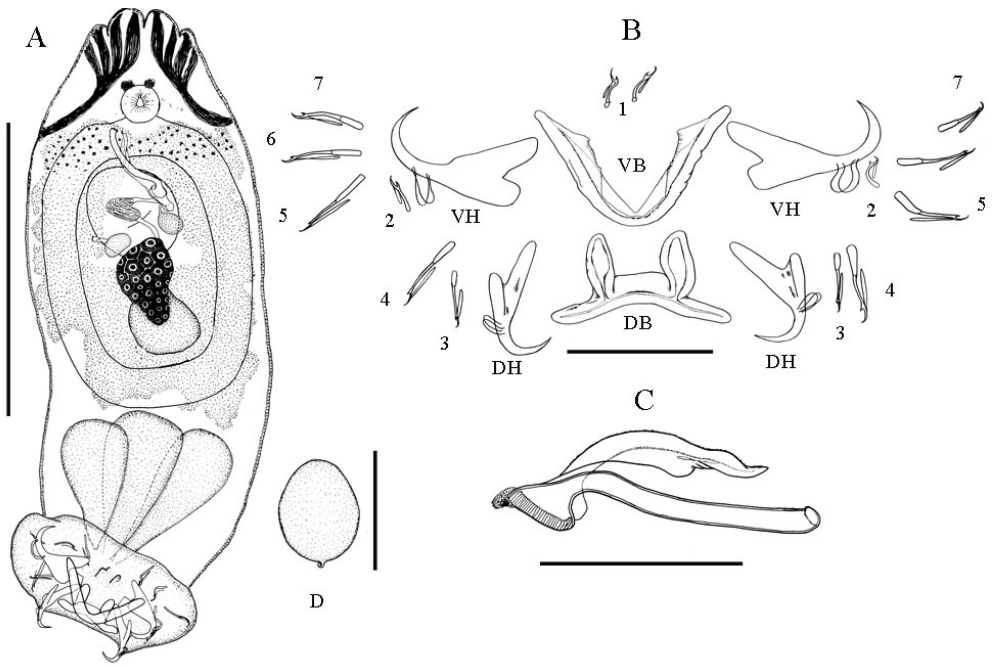

Fig. 2. *Cichlidogyrus halli* (Price & Kirk, 1967), ventral view. A, whole animal; B, haptoral armature; C, copulatory organ; D, egg. VH, ventral hamulus; DH, dorsal hamulus; VB, ventral bar; DB, dorsal bar. Scale bars: A, 250 µm; B-C, 50 µm; D, 100 µm.

cessory piece lancet-shaped and shorter, 60 (54–67) long, than copulatory tube. Ovary irregularly ovoid and located anteroventral to testis. Vagina sub-median. Seminal receptacle attached to vagina. Vitelline follicles well-developed and co-extensive with intestine. Mehlis' gland and uterine pore not observed. Eggs oval-shaped, 94 (84–98) long and 71 (57–79) wide, with appendix at terminal end.

Hosts: Oreochromis niloticus niloticus and *O. mossambicus* (Perciformes: Cichlidae).

Site of attachment: Gills.

Localities: Miyara River, Nagura River, an irrigation canal in Uratabaru on Ishigaki-jima Island (Table 2).

Remarks: Cichlidogyrus halli is reported herein for the first time in Japan. This species was originally described as *Cleidodiscus halli* by Price & Kirk (1967) from the gills of *O. shiranus shiranus* (as *T. s. shirana*) in Malawi, Africa. It has been recorded from various cichlid fishes from Africa (Malawi, Ghana, Uganda, Egypt, Benin, Guinea, Sierra Leone, Burkina Faso, South Africa, Ivory Coast, Senegal) (see Douëllou, 1993; Pariselle & Euzet, 1997; Pouyaud *et al*., 2006; Boungou *et al*., 2008; Pariselle & Euzet, 2009; Le Roux & Avenant-Oldewage, 2010; Mendlová *et al*., 2010; Madanire-Moyo *et al*., 2011). To the best of our knowledge, this paper constitutes the first record of *C. halli* from outside Africa.

Cichlidogyrus tilapiae **Paperna, 1960**

(Fig. 3)

Description (10 specimens measured): Body slender, tapering at posterior of body, 242 (214–280) long and 90 (83–100) wide. Cephalic lobe with 4 pairs of head organs. Two pairs of eyes with lens in the first pair. Mouth not observed. Pharynx spherical, 23 (21–24) in diameter; intestine bifurcated posterior to pharynx and becoming confluent posterior to testis. Haptor ellipsoid with 2 pairs of hamuli and 7 pairs of hooklets. Ventral hamuli similar to dorsal hamuli in shape. Ventral hamuli 30 (28–32) long, with broad base and shaft; outer root narrow; inner

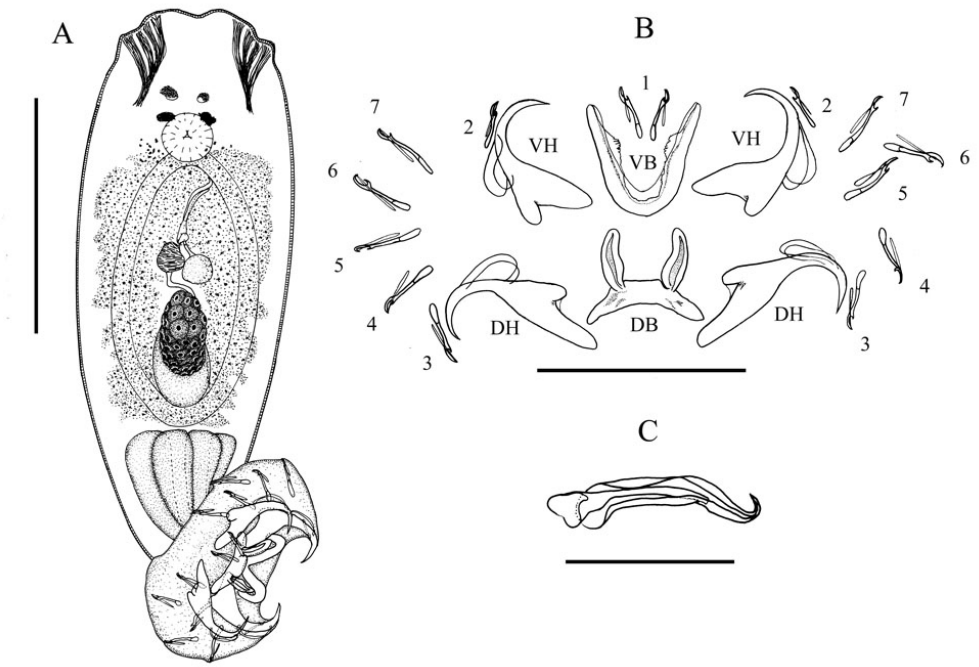

Fig. 3. *Cichlidogyrus tilapiae* Paperna, 1960, ventral view. A, whole animal; B, haptoral armature; C, copulatory organ. VH, ventral hamulus; DH, dorsal hamulus; VB, ventral bar; DB, dorsal bar. Scale bars: A, 100 μm; B, 50 μm; C, 30μm.

root broad. Dorsal hamuli slightly longer, 36 (35–38) long; outer root short; inner root elongate. Hamulus filament present. Ventral bar U-shaped, 54 (50–56) in transverse length, with indented membranous extensions. Dorsal bar slightly arched; branches pointed; appendages slender, 14 (13–15) long. Hooklets small; pair 2 shortest without base, other pairs with short base; hooklet lengths 13 (12–15), 10 (8–12), 14 (12–17), 14 (12–15), 16 (15–17), 16 (15–18) and 14 (13–15), respectively, for pairs 1–7.

Testis ovoid and located dorsoposterior to ovary. Vas deferens not encircling the intestinal caecum. One prostatic reservoir situated posterior to copulatory organ. Copulatory organ located one-third of total length from anterior extremity of body; copulatory tube straight and wider at base, 29 (28–30) long; accessory piece almost straight with sharp hook at terminal end, 33 (31–37) long. Ovary ovoid and located anteroventral to testis. Vagina not observed. Vitelline follicles well-developed and co-extensive with intestine. Mehlis' gland and uterine pore not observed.

Hosts:Oreochromis niloticus niloticus and *O. mossambicus* (Perciformes: Cichlidae).

Site of attachment: Gills.

Localities: Hija River on Okinawa-jima Island; Yamakura Reservoir on Kume-jima Island; Miyara River, Nagura River on Ishigaki-jima Island (Table 2).

Remarks: Our collection of *C. tilapiae* in this study constitutes the first record from Japan. This parasite was originally described by Paperna (1960) using specimens from the gills of *O. n. niloticus* (as *T. nilotica*), *Sarotherodon galilaeus galilaeus* (as *T. galilaea*), *Tristramella sacra* and *Tristramella simonis simonis* (as *Tilapia simonis*) in Israel. It has been reported from various cichlid fishes from Africa (Ghana, Uganda, Tanzania, Egypt, Zimbabwe, Ivory Coast, South Africa, Tanzania, Burkina Faso), Middle East (Israel), Asia (Philippines, Bangladesh, Thailand) and Americas (Colombia, Mexico, Cuba) (see Douëllou, 1993; Jiménez-García *et al*., 2001;

Pouyaud *et al*., 2006; Kohn *et al*., 2006; Mendora-Franco *et al*., 2006; Lerssutthichawal, 2008; Boungou *et al*., 2008; Pariselle & Euzet, 2009; Le Roux & Avenant-Oldewage, 2010; Madanire-Moyo *et al*., 2011; Akoll *et al*., 2012).

Discussion

The morphology and measurements of the specimens from the gills of tilapias from Okinawa Prefecture, Japan, correspond, more or less, to those of *C. sclerosus, C. halli* and *C. tilapiae* described by Paperna & Thurston (1969), Price & Kirk (1967) and Paperna (1960), respectively. These species were redescribed by Douëllou (1993). Duncan (1973) reported the detailed morphology of *C. sclerosus* from the Philippines. Also, Kritsky & Thatcher (1974) gave the measurements of *C. sclerosus* and *C. tilapiae* from Colombia, and Ergens (1981) provided those of *C. tilapiae* from Egypt. The body lengths of *C. sclerosus*, *C. halli* and *C. tilapiae* reported in this paper are smaller than those earlier recorded: for example, the mean body length of *C. sclerosus* is 451 × 177 in this study vs. 1,106 × 251 in Douëllou (1993). This difference is definitely caused by the fixation method used: in this study, the specimens were not collected from live or freshly-killed hosts but from the hosts fixed in 10% formalin in the field. Moreover, *C. tilapiae* was reported by Douëllou (1993: 167) to possess only two eyes, but this study has confirmed that the species has two pairs of eyes, as originally reported by Paperna (1960).

The two species of tilapias infected with *Cichlidogyrus* spp. in this study are not native to Okinawa Prefecture but are of African origin. These tilapias have been transplanted to many places around the world. In Okinawa Prefecture, *O. mossambicus* was introduced alive from Taiwan in 1954, whereas both *O. n. niloticus* and *T. zillii* were transported from the main island of Japan in the 1970's (Takehara *et al*., 1997), to which these two species were introduced from the Middle East in 1962 (Yamaoka, 1989). All these species of tilapias have established well in natural waters of Okinawa Prefecture (Takehara *et al*., 1997; Tachihara *et al*., 2002; Kochi, 2003; Shimadzu, 2011). On the other hand, the monogeneans of *Cichlidogyrus* reported in this paper are specific to cichlid fishes, and it is, therefore, reasonable to consider that these monogeneans are alien parasites that were introduced to Okinawa Prefecture along with their cichlid hosts.

There was a difference in geographical distribution of *C. sclerosus, C. halli* and *C. tilapiae* in Okinawa Prefecture (Table 2). *C. sclerosus* was most widely collected (11 of the 14 localities surveyed on Okinawa-jima Island, Kume-jima Island, Ishigaki-jima Island, and Minamidaito-jima Island), but both *C. halli* and *C. tilapiae* showed a restricted distribution (*C. tilapiae* in 4 localities on Okinawa-jima Island, Kume-jima Island and Ishigaki-jima Island; *C. halli* in 3 localities only on Ishigaki-jima Island). None of the fish from Miyako-jima Island was infected. Furthermore, no specimens of *T. zillii* examined were parasitized. These differences in the geographical distribution and occurrence of the parasites between fish species may be affected by differences in the history of introduction of tilapias into those islands.

Tilapias are also found in some rivers and lakes in the main islands of Japan, such as Kyushu and Honshu (Yamaoka, 1989). Since we have only limited information on the parasites of tilapias in Japan (Miyashita, 2006; Ogawa *et al*., 1995; Nagasawa & Uyeno, 2009), more work is needed on the parasites of tilapias from Japan.

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