

Intestinal Flukes Recovered from a Herring Gull, *Larus argentatus*, in the Republic of Korea

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Abstract: Trematode specimens were collected from the intestine of a herring gull, *Larus argentatus*, which was found in a critical condition on the shore of a small island (Yubu-do, Seocheon-gun, Chungcheongnam-do) located at the western coast of the Korean peninsula. Total 11 specimens of intestinal flukes, including 3 *Cryptocotyle lingua* (Heterophyidae), 1 *Himasthla alincia* (Echinostomatidae), 5 *Cardiocephaloides medioconiger* (Strigeidae), and 2 *Diplostomum spathaceum* (Diplostomidae), were recovered. *C. lingua* was morphologically characterized by the presence of a large ventrogenital apparatus and 2 obliquely tandem testes. *H. alincia* had an elongated body and a head collar equipped with 31 collar spines. *C. medioconiger* had a bisegmented body and a voluminous copulatory bursa containing the seminal vesicle and ejaculatory duct. *D. spathaceum* also had a bisegmented body and its vitellaria extended up to the anterior border of the tribocytic organ. It is of note that *C. lingua* is potentially zoonotic that can occur in birds and humans. Three of them, i.e., *C. lingua*, *C. medioconiger*, and *D. spathaceum*, are new trematode fauna in Korea. Studies on trematode fauna of migratory birds should be continued in Korea.

Key words: *Cryptocotyle lingua*, *Himasthla alincia*, *Cardiocephaloides medioconiger*, *Diplostomum spathaceum*, intestinal fluke

Various species of intestinal flukes infecting avian or mammalian hosts are known to be able to infect also humans [1]. They are taxonomically diverse and can be classified into the family Heterophyidae (heterophyids), Echinostomatidae (echinostomes), and miscellaneous families, including Diplostomidae (diplostomes) and Strigeidae (strigeids) [1]. In the Republic of Korea (= Korea), trematode fauna in birds and mammals has not been extensively studied, and studies on this topic are urgently needed.

The herring gull, *Larus argentatus*, is one of the commonly observed migratory birds along the seashores of Korea during winter season. They are typically found in harbors, on rocky shores, or at river mouths and known to breed in North-East Siberia spanning Chuckchi peninsula and Taymyr peninsula. During the survey of parasites in migratory birds, we discovered adult or juvenile specimens of intestinal flukes, including *Cryptocotyle lingua* (Creplin, 1825) Fiscoeder, 1903, *Himasthla*

alincia Diez, 1909, *Cardiocephaloides medioconiger* (Dubois et Perez-Vigueras, 1949) Baer, 1969, and *Diplostomum spathaceum* (Rudolphi, 1819) Olsson, 1876, from the small intestine of a herring gull. In this paper, we described the morphology of each species with discussion on their possibility for human infections.

In November 2010, a herring gull in a critical condition was found on the shore of a small island (Yubu-do, Seocheon, Chungcheongnam-do Province) near the western coast of the Korean peninsula during the routine surveillance of parasitic infections in migratory birds. This island was chosen as one of the eco-tour zones along with several nearby areas since a lot of migratory birds drop by on their way to the breeding or wintering grounds. The herring gull was immediately transferred to our laboratory, and the small intestine was separated. Then, the intestinal segment was opened longitudinally in saline, and the intestinal contents were examined for the presence of parasites. Some trematode specimens were observed, and they were isolated, fixed in 10% neutral formalin, and stained with Semichon's acetocarmine. Then, their species was identified under light microscopy.

Total 11 adult or juvenile trematode specimens, including 3 *C. lingua* (Heterophyidae), 1 *H. alincia* (Echinostomatidae), 5

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C. medioconiger (Strigeidae), and 2 *D. spathaceum* (Diplostomidae), were recovered. Their measurements (Tables 1-4) and morphological characteristics were as follows.

Specimens (n=3) of *C. lingua* (Heterophyidae) were small, leaf-like or tongue-shaped, 1.53-2.03 (av. 1.81) mm in length and 0.58-0.63 (0.59) mm in width (Table 1; Fig. 1A). The size of uterine eggs (n=12) was 40-44 (42) μ m long and 16-19 (18) μ m wide. They were morphologically characterized by

having a small ventral sucker imbedded into the large and complex ventrogenital apparatus, 2 obliquely tandem testes, and extensive distribution of vitellaria from the posterior extremity up to far anterior level of the ventrogenital apparatus.

Only 1 adult specimen of *H. alincia* (Echinostomatidae) was obtained, which was slender and elongated, 8.75 mm long and 0.93 mm wide (Table 2; Fig. 1B). The size of uterine eggs (n=10) was 98-110 (104) μ m long and 65-70 (68) μ m wide. It

Table 1. Measurements (μ m) of *Cryptocotyle lingua* (adults) in comparison with a previous report

Item	Our specimens (n=3)	Ransom (1920) [4]
Body (length \times width)	1,530-2,030 \times 580-630	550-2,000 \times 200-900
Oral sucker (diameter)	50-90	66-110
Prepharynx (length)	30	Smaller than pharynx
Pharynx (length \times width)	65-80 \times 40-55	40-80 \times 30-48
Esophagus (length)	60-75	About 50
Ventral sucker (diameter)	70-90	55-85
Genital apparatus (diameter)	110-115	120-250
Left testis (length \times width)	300-340 \times 175-225	120-250 \times 70-130
Right testis (length \times width)	275-300 \times 175-250	120-250 \times 70-130
Ovary (length \times width)	75-200 \times 75-125	140-180 \times 70-120
Egg (length \times width)	40-44 \times 16-19	40-50 \times 18-25

Table 2. Measurements (μ m) of *Himastha alincia* (adult) in comparison with a previous report

Item	Our specimen (n=1)	Han et al. (2009) [15]
Body (length \times width)	8,750 \times 930	5,400-10,000 \times 340-630
Head collar (width)	300	300-360
No. of collar spines	31	31
No. of corner spines	4	4
Oral sucker (length \times width)	153 \times 198	80-125 \times 75-113
Pharynx (length \times width)	124 \times 94	103-133 \times 80-120
Esophagus (length)	248	138-200
Ventral sucker (diameter)	290 \times 327	240-300 \times 200-610
Anterior testis (length \times width)	650 \times 480	290-820 \times 180-340
Posterior testis (length \times width)	750 \times 550	280-850 \times 180-350
Ovary (length \times width)	200 \times 200	63-170 \times 100-168
Egg (length \times width)	98-110 \times 65-70	108-113 \times 65-80

Table 3. Measurements (μ m) of *Cardiocephaloides medioconiger* (adult) in comparison with a previous report

Item	Our specimen (n=1)	Dubois (1970) [23]
Body (length \times width)	4,750 \times 1,100	9,000 \times 1,360-1,400
Forebody (length \times width)	1,250 \times 650	630-1,500 \times 450-1,360
Hindbody (length \times width)	3,500 \times 1,100	2,130-7,500 \times 500-1,400
Oral sucker (length \times width)	175 \times 90	81-179 \times 75-136
Pharynx (length \times width)	100 \times 200	66-183 \times 66-192
Ventral sucker (diameter)	150 \times 125	104-157 \times 75-138
Anterior testis (length \times width)	250 \times 600	240-560 \times 410-707
Posterior testis (length \times width)	300 \times 650	285-570 \times 500-750
Ovary (length \times width)	150 \times 250	150-278 \times 217-300
Egg (length \times width)	110-120 \times 70-78	96-131 \times 63-78

characteristically had a prominent head collar equipped with a crown of 31 collar spines (including 4 end group spines on each side). Two tandem testes were located near the terminal portion of the body, and vitellaria were distributed from the posterior extremity up to the posterior level of the cirrus sac.

One adult and 4 juvenile specimens of *C. medioconiger* (Strigeidae) were recovered. They were bisegmented, with a pyriform or cordiform forebody (1.25 mm long and 0.65 mm wide) and a cylindrical hindbody (3.50 mm long and 1.10 mm wide), 4.75 mm in total length (Table 3; Fig. 1C). The size of uterine eggs (n = 10) was 110-118 (115) μm long and 70-78 (74) μm wide. Their forebody was longer than wide, from which freely emerged the lobes of the tribocytic organ. It characteristically had a well-developed voluminous copulatory bursa containing seminal vesicle and ejaculatory duct in the

posterior part of the hindbody.

Two adult specimens of *D. spathaceum* (Diplostomide) were obtained. They were distinctively bisegmented, with a spoon-shaped forebody (0.68-0.85 mm long and 0.65-0.70 mm wide) equipped with prominent pseudosuckers, the anterior end of which was more or less trilobate, and a long cylindrical hindbody (1.08-1.10 mm long and 0.50-0.52 mm wide), 1.75-2.00 mm in total length (Table 4; Fig. 1D). The size of uterine eggs (n = 13) was 108-125 (114) μm long and 65-85 (73) μm wide. The size of the ventral sucker was almost equal to that of the oral sucker. They had a sucker-like tribocytic organ, a ventral sucker near or close to the tribocytic organ, vitellaria distribution up to the anterior border of the tribocytic organ, and an ovary far from the junction of the fore- and hindbody.

Table 4. Measurements (μm) of *Diplostomum spathaceum* (adults) in comparison with a previous report

Item	Our specimens (n=2)	Dubois (1970) [23]
Total body (length \times width)	1,750-2,000 \times 650-700	Up to 4,450
Forebody (length \times width)	680-850 \times 650-700	600-1,800 \times 270-960
Hindbody (length \times width)	1,080-1,100 \times 500-520	520-3,220 \times 210-750
Oral sucker (length \times width)	60-65 \times 75-100	40-100 \times 46-104
Pharynx (length \times width)	50-52 \times 55-65	39-91 \times 25-75
Ventral sucker (diameter)	75-85 \times 80-100	48-110 \times 48-140
Tribocytic organ (length \times width)	175-275 \times 125-160	125-450 \times 90-390
Anterior testis (length \times width)	325-350 \times 140-150	95-460 \times 130-560
Posterior testis (length \times width)	300-325 \times 175-225	90-485 \times 190-650
Ovary (length \times width)	100-135 \times 75-100	50-205 \times 70-235
Egg (length \times width)	108-125 \times 65-85	84-115 \times 52-76

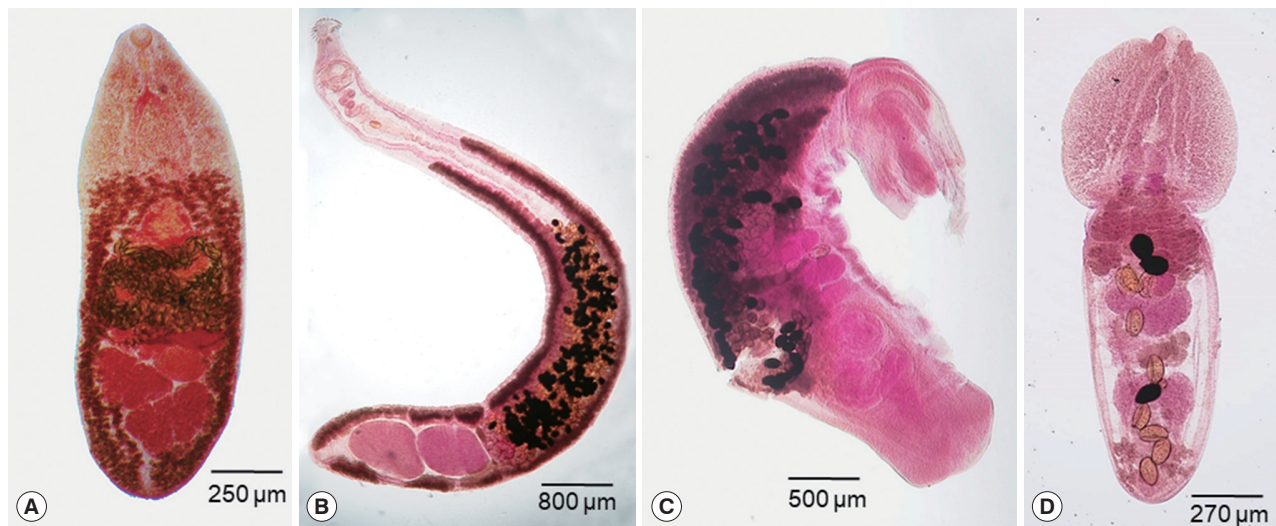


Fig. 1. Intestinal trematodes collected from a herring gull. (A) *Cryptocotyle lingua*. (B) *Himasthla alincia*. (C) *Cardiocephaloides medioconiger*. (D) *Diplostomum spathaceum*.

Foreshore soil and environment along the western coast of the Korean peninsula have provided adequate conditions for migratory birds to drop by, and comprehensive surveys on parasites among these birds, including intestinal flukes, are required. In addition, it should be noted that some of the trematode species infected in migratory birds, for example, *C. lingua* is known to have a potential to infect humans also [1].

C. lingua was originally described in Europe in 1825 from the intestine of birds by Creplin under the name *Distoma lingua* [2]. In 1899, Looss created *Tocotrema* as a new genus and designated this species as *Tocotrema lingua* [3]. Subsequently, in 1903, this fluke was renamed as *Cryptocotyle lingua* by Fischöder [4]. In 1915, Linton [5] found this fluke in USA but described it under the name *Tocotrema lingua*. However, in 1920, Ransom [4] synonymized the genus *Tocotrema* with *Cryptocotyle*, and in 1930, Stunkard [2] finally accepted *C. lingua*. It should be noted that human infection with this fluke was reported once in Greenland [6], and it is listed among the intestinal flukes potentially infecting humans [1]. The source of infection is various species of fish, including mullets [1]. In Korea, *Cryptocotyle concava* and *Cryptocotyle* sp. were reported several times from the small intestines of ducks [7] and feral cats [8-10]. However, no reports have been made on *C. lingua*, and this is a new fauna record in Korea. Major morphological differences between *C. lingua* and *C. concava* include the body shape (slightly elongated vs ovoid, respectively), ventrogenital apparatus or ventrogenital sac (large vs small), and testes (obliquely tandem vs almost side by side) [9].

H. alincia was originally described from the intestine of an avian species in Brazil [11] and is now well known to infect birds like gulls, herons, and oystercatchers in North and South Americas [12,13]. In Korea, the metacercariae of *H. alincia* were discovered in 2003 from a brackish water bivalve species (*Mactra veneriformis*) along the western coast [14]. Several other molluscan species were also found to harbor the metacercariae, and adult flukes were recovered from experimental chicks [15]. However, no natural infections of birds with *H. alincia* had been documented. Therefore, this is the first report of a natural infection of an avian host infected with *H. alincia* in Korea. This echinostome has never been reported from humans worldwide; however, it has a potential to infect humans considering that a related species, *Himasthla muehlensi*, was reported from a human infection [1,16]. It is also of note in Korea that the metacercariae of *Himasthla kusasigi*, another related species, were detected in a marine bivalve species, *Meretrix lu-*

soria [17], and its adult flukes were recovered from dunlin, *Calidris alpina sakhalina* [18]. The major differential points between *H. alincia* and *H. kusasigi* were the distribution of vitellaria, from the posterior extremity up to the posterior level of the cirrus sac (*H. alincia*) vs more anterior distribution beyond the cirrus sac (*H. kusasigi*). *H. alincia* also differs from a related species *Himasthla limnodromi* in the presence of small spines on the cirrus and larger body and egg sizes [14].

The genus *Cardiocephaloides* was created by Sudarikov in 1959 using *C. brandesii* as the type [19,20]. Prior to this, a related genus *Cardiocephalus* was erected by Szidat [21] in 1928-1929 and assigned *C. longicollis* (type), *C. musculosus*, *C. hillii*, and *C. brandesii* (n. sp.) in this genus. Another species, *C. medioconiger*, was described from birds (*L. argentatus*, *Larus atricilla*, and *Larus delawarensis*) in Pennsylvania, USA under the name *Strigea bursigera* [22]. Later, this species was assigned to *Cardiocephalus* with a new name *C. medioconiger* by Dubois and Pérez-Vigueras in 1949 [19,23]. However, it was found that the name *Cardiocephalus* was preoccupied as a taxon for an amphibian species by Broilli in 1904 [23]. Thus, Baer [24], Dubois [23], and Dubois and Macko [25] were of opinion that *C. medioconiger* should be assigned under the genus *Cardiocephaloides* but not *Cardiocephalus*. This opinion was accepted by Niewiadomska [26] in 2002 and the genus *Cardiocephalus* was synonymized with *Cardiocephaloides*. In our study, 5 specimens of *C. medioconiger* were recovered. Among them, 4 were immature, and only 1 was adult fluke having fully developed sexual organs. In Korea, this is the first report on the presence of this fluke. *C. medioconiger* is distinguished from other species of *Cardiocephaloides* in that it has a shorter and smaller body but has an enormous copulatory bursa containing the seminal vesicle and ejaculatory duct [23].

D. spathaceum was originally described from birds in Europe by Rudolphi in 1819 under the name *Distoma spathaceum* [20]. Later, in 1850, it was renamed as *Hemistomum spathaceum* by Diesing [27]. However, the name was changed into *Diplostomum spathaceum* by Olsson in 1876 [20]. Dubois [23] divided *D. spathaceum* into 4 subspecies (*D. spathaceum spathaceum*, *D. spathaceum huronense*, *D. spathaceum indistinctum*, and *D. spathaceum murrayense*). However, Yamaguti [20] elevated 3 of them (*D. spathaceum*, *D. huronense*, and *D. murrayense*) to distinct species and retained only *D. spathaceum indistinctum* as a subspecies. After Olsson, more than 50 species had been described in *Diplostomum* [20]. However, only 25 species were considered valid in a taxonomic revision of the genus by Shi-

gin in 1993 [28]. The most common species in Europe and Asia were 6 in number, including *D. spathaceum* (Rudolphi, 1819), *D. pseudospathaceum* Niewiadomska, 1984, *D. paracaudum* (Iles, 1959), *D. mergi* Dubois, 1932, *D. parviventosum* Dubois, 1932, and *D. baeri* Dubois, 1937 [29]. Adult flukes of these 6 species can be morphologically discriminated depending on 8 kinds of characters, including the extent of body division, forebody shape, form of holdfast organ, hindbody/forebody ratio, acetabulum/oral sucker ratio, acetabulum to the hold fast organ distance, vitellaria distribution, and ovary position [29]. Both of our 2 specimens were exactly matched with the characteristics of *D. spathaceum*. This is the first report on the presence of *D. spathaceum* in an avian species in Korea. The second intermediate host of *D. spathaceum* is known to be freshwater, brackish water, or marine fish, and the metacercariae parasitize particularly in their eyes [20]. The existence of the metacercariae of *Diplostomum* sp. were documented several times in freshwater fish in Korea [30-32]. However, the species of these *Diplostomum* metacercariae in fish should be determined in the near future through animal experimental infection.

In conclusion, this study reported 4 species of intestinal flukes parasitic in a herring gull found in Korea, which included *C. lingua*, *H. alincia*, *C. medioconiger*, and *D. spathaceum*. Three of them (*C. lingua*, *C. medioconiger*, and *D. spathaceum*) are new trematode fauna in Korea. Considering the adequate conditions of Korean peninsula for migratory birds to drop by, further extensive studies on the infection status of birds with trematode parasites should be performed.

CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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