Infections of Two *Isthmiophora* Species (Digenea: Echinostomatidae) in Wild Mammals from Republic of Korea with Their Morphological Descriptions

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Abstract: *Isthmiophora hortensis* (Digenea: Echinostomatidae) is a dominant echinostome in animal reservoir hosts and humans in the Republic of Korea (Korea). We intended to investigate the infection status with this echinostome species in the several species of wild animals and describe the morphological characteristics in the faunistic view point. A total of 175 animal carcasses belonging to 3 families, i.e., Canidae, Felidae and Mustelidae, were collected from the southern regions of Korea from March 2010 to July 2017. *Isthmiophora* spp. worms were recovered from the small intestines of each animal under a stereomicroscope after washing of intestinal contents. *Isthmiophora hortensis* was recovered from 4 species of wild carnivores, i.e., *Nyctereutes procyonoides* (3/107: 2.8%), *Mustela sibirica* (11/31: 35.5%), *Meles lucurus* (2/3: 33.3%) and *Martes flavigula* (1/2: 50%). The other 3 carnivores comprising stray dogs, cat and leopard cat were negative for *I. hortensis* infection (0/2, 0/10 and 0/12, respectively). Specimens obtained from the *Lutra lutra* (6/8: 75%) were identified as a distinct species, *I. inermis*, by morphological comparison. *Isthmiophora inermis* has thinner body, elongate testes and different anterior limits of vitelline fields. Detailed morphological descriptions and comparisons with the morphological characteristics are provided. Conclusively, it was confirmed for the first time that 3 species of mustelid mammals, i.e., *M. sibirica, M. lucurus,* and *M. flavigula*, are to be the new definitive hosts of *I. hortensis* in Korea. Additionally, *I. inermis* is to be newly added in the Korean echinostome fauna.

Key words: *Isthmiophora hortensis*, *Isthmiophora inermis*, mustelidae, wild carnivore

INTRODUCTION

Isthmiophora hortensis (Asada, 1926) (Digenea: Echinostomatidae) is an echinostome trematode species characterized by the possession a collar with 27 spines and has medical and veterinary importance. Human infection with *I. hortensis* has been reported frequently in East Asian countries such as China, Japan and the Republic of Korea (= Korea) [1].

Due to its public health importance, the life cycle of the species has been studied extensively. Freshwater snails of the family Lymnaeidae Rafinesque, 1815 are known to serve as first intermediate hosts [2-4], while freshwater fishes and frogs have been reported as second intermediate hosts [3,5-10]. Addition-

ally, a wide range of small mammals are known as respective definitive hosts, such as the rat (reported in Japan by Asada, 1926) [21], black-striped field mouse *Apodemus agrarius* (Pallas), Manchurian reed vole *Microtus fortis pelliceus* Thomas, domestic mouse *Mus musculus* L., brown rat *Rattus norvegicus* (Berkenhout), black rat *R. rattus* L., Daurian pika *Ochotona dauurica* (Pallas), domestc dog *Canis lupus familiaris* L., domestic cat *Felis catus* L., Japanese weasel *Mustela itatsi* Temminck (= *Lutreola itatsi itatsi*) and raccoon *Procyon lotor* L. [12-25]. Recently, Sohn et al. reported two additional species, namely, the raccoon dog *Nyctereutes procyonoides* (Gray) and wild boar *Sus scrofa* L., to the list of known definitive hosts of *I. hortensis* [23].

Ishtmiophora inermis (Fuhrmann, 1904) is one of 6 species belonging to the genus *Isthmiophora*. This species is morphologically very similar to *I. hortensis*, but has thinner body, elongate testes and different anterior limits of vitelline fields [26]. Moreover, this species has a somewhat different host range than *I. hortensis*. Unlike the hosts of *I. hortensis* as we mentioned above, this species has been found mainly in otters i.e.,

Lutra sp., *L. lutra* and *Lontra canadensis* (Schreber, 1777) [18,27-32]. Eurasian otter has been found in Korea as well, but the distribution of *I. inermis* is still unknown until present study.

Considering the life cycle of *Isthmiophora* flukes and their known hosts, wild mammals and especially carnivorous or omnivorous animals such as mustelids seem to be suitable hosts for *I. hortensis*. However, their infection status in Korea is not well known. Therefore, the present study aimed to investigate the infection status of *I. hortensis* for wild mammals in Korea and to determine the range of its definitive hosts. The present study provides reports on yellow-throated marten *Martes flavigula* (Boddaert), Aisan badger *Meles lucurus* (Hodgson) and Siberian weasel *M. sibirica* Pallas as new host records of *I. hortensis*, and presents a detailed overview of its infection status in Korea. In addition, we include detailed morphological characteristics of *I. inermis*, found from the intestines of Eurasian otter *Lutra lutra*, as a new member of the Korean parasite fauna.

MATERIALS AND METHODS

Collecting specimens

Carcasses of wild carnivores were collected from March 2010 to July 2017. A total of 175 wild carnivores belonging to three families comprising Canidae, Felidae and Mustelidae were collected and examined. Collection, identification and necropsy of the host animals were conducted with the support of several wild animal rescue centers or wildlife related institutes, namely, the Wildlife Center of Chungbuk (WCC), Cheongju-si, Chungcheongbuk-do, Korea, the Chungnam Wild Animal Rescue Center (CNWARC), Yesan-gun, Chungcheongnam-do,

Korea, the Korean National Park Service Species Restoration Technology Institute (KNPSSRTI), Gurye-gun, Jeollanam-do, Korea and the Conservation Genome Resource Bank for Korean Wildlife (CGRB), Seoul, Korea, as well as small-scale college laboratories. All of the examined hosts were collected from the Korean peninsula.

Examined hosts included stray dogs *C. lupus familiaris* L. (n=2), Korean raccoon dog *N. procyonoides koreensis* (Gray) (n=107), stray cats *F. cactus* L. (n=10), Amur leopard cats *Prionailurus bengalensis euptilurus* (Elliot) (n=12), Siberian weasels *M. sibirica* (n=31), Eurasian otters *L. lutra* L. (n=8), Asian badgers *M. lucurus* (n=3) and yellow-throated martens *M. flavigula* (n=2). Most of the animals were dead during rescue/care in the wildlife rescue center, or found on the road as a carcass.

The visceral organs were extracted from each dead body, and the lumen was exposed by a longitudinal incision. *Isthmiophora* spp. were recovered from the visceral organs using the filtration method [36]. Parasite numbers were then recorded and recovered specimens were stored in fixative agents (70% ethanol or 10% neutral buffered formalin) for further research. Detailed information concerning infection status and locality of positive cases is provided in Table 1.

Morphological identification

Identification of species was based on the key to the species of *Isthmiophora* provided by Kostadinova and Gibson [26]. Two species, *I. hortensis* and *I. intermis*, were distinguished using morphological observations. Based on the morphological condition of specimens and the representativeness among the morphological differences, 12 *I. hortensis* and 12 *I. inermis* were

Table 1. Prevalence of *Isthmiophora* spp. in wild mammals from the Republic of Korea

Host species	No. of animals examined	No. (%) of animals positive	No. of worms recovered	Survey areas[†]
Canidae				
Canis lupus familiaris (stray dog)	22	0 (0)	-	-
Nyctereutes procyonoides koreensis	107	3 (2.8)	1–5 (3±2)	Cheongju (3/107)
Felidae				
Felis cactus (stray cat)	10	0 (0)	-	-
Prionailurus bengalensis euptilurus	12	0 (0)	-	-
Mustelidae				
Mustela sibirica	31	11 (35.5)	2–150 (54±56)	Cheongju (7/10), Taean (1/1), Hongseong (1/2), Cheongyang (2/2), Sejong (1/1)
Meles lucurus	3	2 (33.3)	20–24 (22±3)	Ulju (1/1), Yesan (1/1)
Martes flavigula	2	1 (50)	5	Boryeong (1/1)
Lutra lutra	8	6 (75)	98–410 (268±134)	Yesan (1/1), Cheongju (2/2), Gurye (1/1), Chungju (1/1)

[†]Positive cases only.

chosen for morphological observation. Specimens of *I. hortensis* (host code no. B417) was obtained from a *M. sibirica* collected from Cheongju (city) in 2011 and *I. inermis* (host code no. B1330) was obtained from a *L. lutra* collected from Cheongju in 2015. They were stained with Semichon's acetocarmine and observed under light microscopy on a mounted slide in Permount (Fisher Scientific) [33]. The voucher specimens were deposited at the National Institute of Biological Resources (NIBR), Incheon Metropolitan City, Korea and the Parasite Resource

Bank (PRB), Cheongju-si, Chungcheongbuk-do, Korea.

Abbreviations used in the present study are as follows: BL: body length; BW: maximum body width; CL: collar length; CW: collar width; NCS: number of collar spines; OSL: oral sucker length; OSW: oral sucker width; PL: prepharynx length; PHL: pharynx length; PHW: pharynx width; ESL: esophagus length; CSL: cirrus sac length; CSW: cirrus sac width; SVL: seminal vesicle length; SVW: seminal vesicle width; VSL: ventral sucker length; VSW: ventral sucker width; OVL: ovary length;

Table 2. Morphometric comparisons of *Isthmiophora hortensis* (Asada, 1926) and *I. inermis* (Fuhrmann, 1904) in the present study

Species	*I. hortensis* (Asada, 1926)			*I. inermis* (Fuhrmann, 1904)		
Hosts	*Mustela sibirica* Pallas (n = 12)			*Lutra lutra* L. (n = 12)		
	Min.	Max.	Average	Min.	Max.	Average
BL	5,707	8,585	6,913	6,273	9,446	7,360
BW	861	1,107	961	615	984	820
CL	206	265	231	216	372	273
CW	343	412	372	304	451	392
DSpL	42	57	52	66	83	74
DSpW	9	14	12	14	20	16
LSpL	46	56	52	47	70	58
LSpW	7	14	10	8	16	12
ASpL	50	64	57	66	83	73
ASpW	14	17	14	19	15	17
OSL	167	216	189	137	216	180
OSW	186	274	219	147	245	205
PL	20	88	46	0	88	49
PHL	167	206	180	108	186	159
PHW	142	201	172	118	186	150
OL	235	451	315	294	588	452
CSL	294	608	444	333	539	453
CSW	157	265	209	196	353	262
VSL	539	706	629	412	608	538
VSW	529	715	612	421	617	525
OVL	176	284	231	98	176	140
OVW	176	274	234	98	186	143
MEL	196	343	259	176	392	262
MEW	304	490	368	157	480	304
ATL	402	735	592	402	666	532
ATW	470	637	534	176	372	305
PTL	588	843	695	470	715	616
PTW	392	539	485	206	431	299
EL	115	125	119	101	126	117
EW	65	74	71	61	77	72
FO	812	1,132	968	763	1,279	1,050
U	320	1,328	806	369	812	570
T	2,312	3,862	2,911	3,173	4,895	3,682
BW/BL (%)	11	17	14	9	12	11
FO/BL (%)	12	16	14	11	17	14
U/BL (%)	5	15	12	6	10	8
T/BL (%)	36	47	42	47	55	50

OVW: ovary width; MEL: Mehlis' gland length; MEW: Mehlis' gland width; ATL: anterior testis length; ATW: anterior testis width; PTL: posterior testis length; PTW: posterior testis width; EL: egg length; EW: egg width; FO: forebody length (distance from the anterior end to the anterior margin of the ventral sucker); U: uterine field length (distance from the anterior margin of the ovary to the posterior margin of the ventral sucker); and T: post-testicular field length. The size and proportions of standard structures relative to body length were calculated as described by Kostadinova [34]. Measurements are shown in Table 2.

RESULTS

Among the 175 hosts examined, 23 animals were positive for *Isthmiophora* flukes (13%). *Isthmiophora* flukes were found in all mustelid hosts and included Siberian weasel (11/31, 35.5%), Asian badger (2/3, 33.3%), Eurasian otter (6/8, 75%) and yellow-throated marten (1/2, 50%). Furthermore, only 3 of the 107 raccoon dogs (2.8%) were infected with *Isthmiophora* flukes. Dogs, cats, and leopard cats were negative for these flukes. However, the flukes that were found in these animals are thought to be *I. hortensis*. Moreover, morphological examination revealed
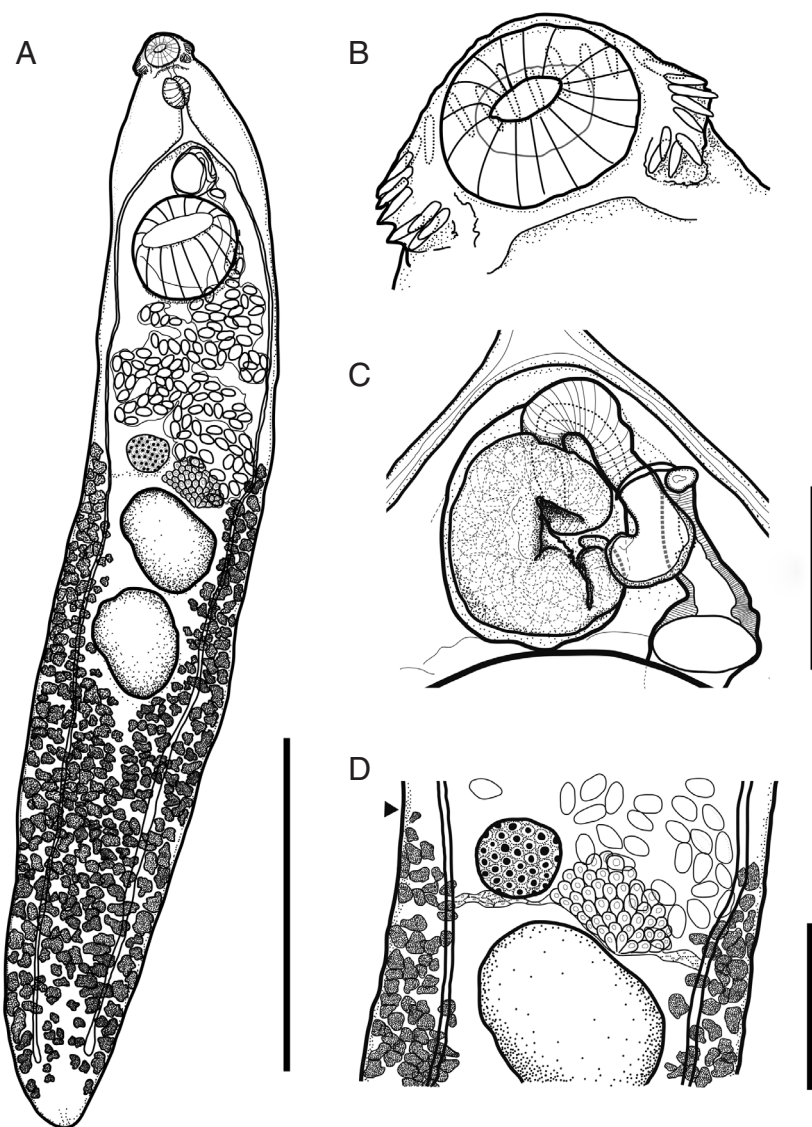
Fig. 1. Descriptions of *Isthmiophora hortensis* (Asada, 1926) recovered from a Siberian weasel *Mustela sibirica* Pallas. (A) Entire worm, (B) Head collar. (C) Terminal genitalia, (D) Body part showing the anterior distribution of vitelline follicles (black arrowhead) Scale bars: (A) 2 mm. (B-C) 200 μm. (D) 500 μm.

that all the *Isthmiophora* flukes found in Eurasian otters were *I. inermis*. Detailed information concerning infection status and locality of positive cases is provided in Table 1.

Descriptions in the present study: Family Echinostomatidae Looss, 1899, Genus *Isthmiophora* Luhe, 1909, *Isthmiophora hortensis* (Asada, 1926) Kostadinova and Gibson, 2002

Host: *Martes flavigula, Meles lucurus, Mustela sibirica, Nyctereutes procyonoides*

Localities: Chungcheongbuk-do (Cheongju-si), Chungcheong-nam-do (Boryeong-si, Cheongyang-gun, Hongseong-gun, Se-

jong-si, Taean-gun and Yesan-gun) and Ulsan (Ulju-gun); Republic of Korea.

Description (Fig. 1, n = 12): Body thin, foliated, dorso-ventrally flattened. Maximum body width at the level of ovary. Forebody short to long (FO/BL = 12-19%). Tegumental spine present, densely distributed in forebody but less dense in hind body. Collar reniform, small. Ventral lappets poorly developed. Collar spines 27 in number, angle spines aligned in double row (2 ventral and 2 dorsal), lateral spines in single row, and dorsal spines in double row. Oral sucker subterminal, subspherical, muscular. Prepharynx present. Pharynx muscu-
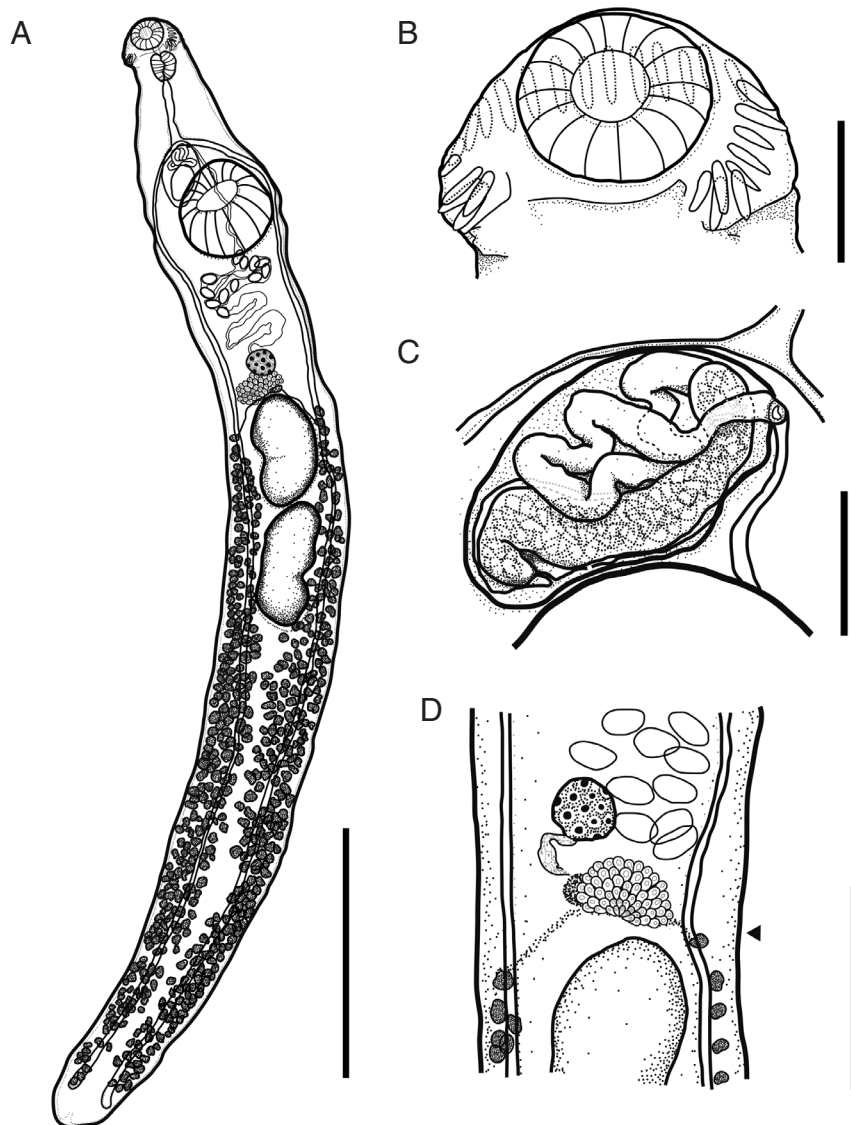
Fig. 2. Descriptions of *Isthmiophora inermis* (Fuhrmann, 1904) recovered from a Eurasian otter *Lutra lutra* L. (A) Entire worm, (B) Head collar. (C) Terminal genitalia, (D) Body part showing the anterior distribution of vitelline follicles (black arrowhead) Scale bars: (A) 2 mm. (B, C) 200 μm. (D) 500 μm.

lar, elongate-oval. Oesophagus short. Intestinal bifurcation in front of ventral sucker. Caeca blind, narrow, overlapped by vitelline follicles, almost reaching posterior end. Ventral sucker subspherical, well-developed, muscular.

Testes 2, tandem, large, oval to irregular shaped, located in equatorial region of body. Post testicular region long (T/BL = 36-47%). Cirrus sac oval, muscular, located antero-dorsal to ventral sucker, included elongated seminal vesicle with curved pars prostatica. Cirrus tubular, armed with spines. Genital pore located posterior to intestinal bifurcation.

Ovary spherical to oval, dextral to median, close to ventral sucker (U/BL = 5-15%). Mehlis' gland large, sub-median, contiguous but slightly behind ovary. Uterus intercaecal, short. Eggs oval, yellowish. Vitelline follicles small, distributed from the level of ovary to posterior extremity, occupy each side of the body and overlapped at post testicular field. Excretory vesicle Y-shaped.

Isthmiophora inermis (Fuhrmann, 1904) Kostadinova and Gibson, 2002

Host: *Lutra lutra*

Localities: Chungcheongbuk-do (Cheongju-si and Chungju-si), Chungcheongnam-do (Yesan-gun), and Jeollanam-do (Gurye-gun); Republic of Korea

Descriptions (Fig. 2, n = 12): Body thin, elongated and cylindrical (BW/BL = 10-12%). Maximum body width at level of anterior testis or ventral sucker. Forebody short (FO/BL = 11-17%). Tegumental spine obscure.

Collar weakly developed, armed with 27 spines. Ventral lappets poorly developed. Oral sucker subterminal, subspherical, muscular. Prepharynx present. Pharynx muscular, elongate-oval. Oesophagus relatively short, bifurcated slightly at anterior region of the ventral sucker. Ventral sucker large, subspherical, muscular, well-developed.

Testes 2, tandem, elongated, oval shaped. Post testicular region very long (47-55%). Cirrus sac located between intestinal bifurcation and ventral sucker, oval, muscular, includes elongated, curved seminal vesicle with convoluted and long pars prostatica. Cirrus tubular. Genital pore located posterior to intestinal bifurcation.

Ovary spherical, entire, dextral, located somewhat in spaces anterior to anterior testis. Mehlis' gland well-developed, median. Uterus short (U/BL = 6-10%), filled with eggs. Metraterm muscular. Vitelline follicles well-developed, covering region starting from posterior end to near anterior border of anterior testis.

DISCUSSION

Morphological observations of stained slide specimens representing the two groups of *Isthmiophora* flukes indicated that the characteristics of specimens matched well with the diagnostic characteristics of the genus *Isthmiophora* [26,34]. All specimens had 27 collar spines and exhibited the characteristics regarded as the major attributes of the genus *Isthmiophora* as noted by Kostadinova and Gibson [26]. Observations revealed a slightly greater anterior position of the testes (T/BL = 36-55%), armed cirrus, small head collar with not well-developed ventral lappets, longer oral spines than aboral, short forebody (FO/BL = 11-17%) and uterus (U/BL = 5-14%), and large eggs.

According to the differential keys of *Isthmiophora* suggested by Kostadinova and Gibson [26], our specimens were identified as comprising two *Isthmiophora* species, *I. hortensis* and *I. inermis*. All specimens collected from animal hosts investigated in this study, with the exception of Eurasian otters, had morphological characteristics identical to those of *I. hortensis*, which was the only species reported previously in Korea.

I. hortensis is regarded as very similar morphologically to *I. melis*, and the only difference between the two species is the position of cirrus sac (located between intestinal bifurcation and ventral sucker or antero-dorsal to the ventral sucker). Recently, Sohn et al. investigated body indices of *I. hortensis* obtained from 4 species of terrestrial mammals, namely, *A. agrarius*, *F. cactus*, *N. procyonoides* and *S. scrofa*, and compared results with those of experimentally infected albino rats (metacercariae were obtained from the Korean dark sleeper, *Odontobutis platycephala* Iwata & Jeon) in Korea [23]. They also noted that *I. hortensis* has a high similarity with *I. melis* and that it is very difficult to distinguish the two species, which can only be accomplished using a morphological aspect such as body indices. Our results also indicate that body indices are very similar to those of *I. melis*; however, our observations indicate that the position of the cirrus sac is similar to that reported by Kostadinova and Gibson [26].

I. inermis can be distinguishable from *I. hortensis* by its slender body, rather elongated testes and anterior limits of the vitelline fields at the level of the anterior testis [26]. These features can also be checked in our specimens collected from *L. lutra*. Additionally, morphometric comparisons supported the species identifications (Table 2). Observations showed that specimens of both species did not differ much since measure-

ments of size overlapped for most body parts. However, the measurements support the distinction of species based on differences in the ratios of body parts. In comparison with *I. hortensis*, *I. inermis* had a slender body, BW/BL: 9-12% (11%) and longer post-testicular fields, T/BL: 47-55% (50%). The BW and T ratio relative to the BL of *I. hortensis* were 11-19% (15%) and 36-47% (41%), respectively (Table 2).

The specimens collected from *Lutra lutra* were all identified as *I. inermis*, which had never been reported before as a member of the Korean parasite fauna. *Isthmiophora inermis* was primarily described as *Euparyphium inerme* in a Lutra sp. from Java, Indonesia [27], but Kostadinova and Gibson [26] considered this species as a member of the genus *Isthmiophora* and transferred it from the genus *Euparyphium* to *Isthmiophora*. As in the present study, *I. inermis* has been mainly detected from otters (*Lutra* sp., and *L. lutra*) in Eurasian countries such as China, Indonesia (Java), the Russian Far East and Vietnam [18,27-31]. Additionally, several suspicious findings of this species were recorded from North America [32]. The only known case of a non-mustelid host involved dogs in India [35]. Our results suggest that the species could be found along with the distribution of its main host *L. lutra*, and Korea is a new location for its distribution.

The generic conception of the genus *Isthmiophora* as re-defined by Kostadinova and Gibson [26] provides insights into the life history and host range of species identified in this study by comparison with other members of the genus. Generally, they have been known to use mammals as definitive hosts [26,34]. Among the known host species, mustelids represent a large portion of their host diversity. With the exception of two species having 29 collar spines, *I. lukjanovi* Chertkova, 1971 [36] and *I. citellicola* Kadenatsii in Skrjabin & Bashkirova, 1956 [37], which were only reported from *Gazella subgutturosa* and *Citellus pygmaeus*, the other 4 species are known to use at least one species of mustelid. The type species *I. melis* was reported from over 10 species of mustelids [29], and otters are known to act as definitive hosts of *I. inermis* [27-32]. A North American species, *I. beaveri*, was reported from a mustelid host, *Neovison vison* (Schreber) (=*Mustela vison*), and also from experimentally infected ferrets [26,38,39]. Several authors have noted the importance of mustelids as hosts of *Isthmiophora* flukes [13,40,41].

Isthmiophora hortensis has been known to use mammals as its definitive host. It is our understanding that the host list includes 11 animals belong to 6 families as follows: Canidae (*Canis lupus* and *N. procyonoides*), Felidae (*F. catus*), Ochotonidae (*Ochotona dauurica*), Muridae (*A. agrarius, R. norvegicus, R. rattus, fortisM. pellceus, M. musculus*), Mustelidae (*M. itatsi*), Procyonidae (*P. lotor*) and Suidae (*S. scrofa*) in Korea, China, Japan, Vietnam and the Russian Far East [12,14-25,29]. However, only one species of mustelid, the Japanese weasel *M. itatsi*, has been reported as a host of *I. hortensis* [13].

As with other *Isthmiophora* species that have 27 collar spines, *I. hortensis* was also found to use mustelids as its definitive hosts. In the present study, we found *I. hortensis* from 3 species of mustelids, i. e., *M. sibirica, M. lucurus* and *M. flavigula*. Furthermore, their infection rates seem relatively higher than those for other host species reported previously in Korea. Seo et al. [14] examined a total of 624 rodents comprising 5 species (*A. agrarius, R. norvegicus, R. rattus, M. musculus* and *M. fortis*) and a species of shrew, *Crocidura lasiura* Dobson (=*C. russula*) from the northern region of the Republic of Korea. They found *I. hortensis* from 15 rodents and the maximum number of infections was 134 from *R. rattus*. An additional survey was conducted inside a campus located in Seoul by Seo et al. [15]. They found only 4 positive rats among 325 rats (*R. norvegicus*). Follow-up studies of rodent species were then conducted by several authors, but the infection rates were not very high and measured 16/170 (rats) by Seo et al. [16], 1/34 (*R. rattus*) by Ahn and Ryang [42], and 6/236 (*A. agrarius*) by Sohn et al. [23]. The number of infections from the 3 studies was estimated as 1 to 65. Cho et al. [17] found *I. hortensis* from another host (dog) with an infection rate of 3.9% (4/102). Analysis of cats showed similar results.

Two surveys were conducted for helminth parasites of stray cats in Korea. *I. hortensis* was found in 5 of 438 (1.1%) cats in Busan, and 6 of 400 (1.5%) cats near 5 major river basins. The numbers of infection were 1-485 and 1-59, respectively [21,25]. Although the number of animals examined in the present study was not large, the infection rate and number of infections in mustelid hosts were relatively high (Table 1). On the other hand, our investigation of the infection status of the raccoon dog revealed results similar to those of a previously reported case of a dog (2.8 vs. 3.9%, respectively) and a smaller infection number (1-5 vs. 2-32). These results indicate that mustelid hosts act as predominant hosts of *I. hortensis* in wild carnivores within Korea.

The existence of additional species of *Isthmiophora* in Korea may prompt a revision of previously believed information on *I. hortensis*. The classical identifications of echinostome meta-

cercariae depend only on the number of collar spines [9]. This resulted in little impetus previously to consider distinguishing 2 *Isthmiophora* species that had the same number of collar spines (27-28) using the classical morphological identification. If fishes can mediate the metacercariae of *I. inermis*, then previously reported data may include the misidentification of *I. inermis*. Indeed, a consideration of the habitat and eating habits of *L. lutra* suggests strongly that freshwater fishes are the main intermediate host of *I. inermis* rather than other intermediate hosts. *Lutra lutra* has been known to use freshwater fishes as its main prey. Furthermore, Choe et al. [43,44] recorded cases of fluke species in Korea, *Pegosomum bubulcum* [43] and *Chaunocephalus ferox* [44], which had 27 collar spines and were thought to use freshwater fishes as intermediate hosts. Recently, Sohn and Na [45] found unidentified echinostomatid metacercariae that are morphologically very similar with those of *I. hortensis* metacercariae from freshwater fishes caught from Junam-jeosuji (reservoir) and Woopo-neup (swamp) in Gyeongsangnam-do, Korea. In this situation, clarification of the intermediate host of *I. inermis* is considered essential before metacercariae investigation. In addition, certainty is needed regarding differential diagnosis between metacercariae that have 27 collar spines, and such investigations should involve molecular analysis. Human infections of *I. inermis* are also an issue that can't be overlooked, and their potential for human infection needs to be investigated.

In conclusion, the results of the present study revealed the infection status of *I. hortensis* in various wild mammals and the existence of an additional *Isthmiophora* species, *I. inermis*, in Eurasian otters, in Korea. We provided their detailed morphometric comparisons, and showed the morphological differences between two species. However, our study is limited that the identification is based only morphological observations. DNA analysis should be conducted in near future for better understanding about the differences between *Isthmiophora* species and also the taxonomic aspects. As with other *Isthmiophora* species that have 27 collar spines, *Isthmiophora hortensis* appeared to have a significant relationship with mustelid hosts, and we added three more species to the host list of *Isthmiophora hortensis*. We also showed the relatively high prevalence of *I. hortensis* infection in mustelid hosts. Considering the present results and ecology of mustelid hosts, they seem to play an important role as dominant hosts in the life cycle of *I. hortensis* in the Korean ecosystem.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest with this article.

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