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▣ BRIEF COMMUNICATION

Spirometra decipiens (Cestoda: Diphyllobothriidae) Collected in A Heavily Infected Stray Cat from the Republic of Korea

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Abstract: Morphological and molecular characteristics of spirometrid tapeworms, *Spirometra decipiens*, were studied, which were recovered from a heavily infected stray cat road-killed in Eumseong-gun, Chungcheongbuk-do (Province), the Republic of Korea (=Korea). A total of 134 scolices and many broken immature and mature proglottids of *Spirometra* tapeworms were collected from the small intestine of the cat. Morphological observations were based on 116 specimens. The scolex was 22.8-32.6 mm (27.4 mm in average) in length and small spoon-shape with 2 distinct bothria. The uterus was coiled 3-4 times, the end of the uterus was ball-shaped, and the vaginal aperture shaped as a crescent moon was closer to the cirrus aperture than to the uterine aperture. PCR amplification and direct sequencing of the *cox1* target fragment (377 bp in length and corresponding to positions 769-1,146 bp of the *cox1* gene) were performed using total genomic DNA extracted from 134 specimens. The *cox1* sequences (377 bp) of the specimens showed 99.0% similarity to the reference sequence of *S. decipiens* and 89.3% similarity to the reference sequence of *S. erinaceieuropaei*. In the present study, we report a stray cat heavily infected with *S. decipiens* identified by mitochondrial *cox1* sequence analysis and morphological examinations of the adult worms.

Key words: *Spirometra decipiens*, scolex, molecular detection, *cox1*, cat

The stray or feral cat is a powerful predator in the ecosystem of wild life in Korea and has a wide range of food stuffs originating from mammalian, avian, reptilian, and amphibian animals. The free-roaming stray cats act as the potential source of environmental contamination and transmit the pathogenic parasites to humans even to domesticated cats as the reservoir host of zoonotic helminths. Among intestinal helminths, *Spirometra* species of cats are the most important in terms of public health. *Spirometra* species in cats have been reported sporadically by many authors in the Republic of Korea (=Korea). Kang (1967) examined 41 cats in Gyeongsangnam-do (Province) with helminth infections such as *Clonorchis sinensis, Paragonimus* sp. *Taenia taeniaeformis, Spirometra* sp., and *Toxocara cati* [1]. Huh et al. (1993) reported 7 helminth species, *T. cati, Anisakis simplex* larvae, *C. sinensis, Pharyngostomum cordatum, Spirometra erinaceieuropaei* (formerly *S. erinacei*), and *T. taeniaeformis*, from 41 cats in Seoul [2]. Yang et al. (1995) detected 4 helminth species, *T. cati, Diphyllobothrium latum, S. erinacei*, and *T. taeniaeformis*, from 133 cats in Jeollanam-do (Province) [3]. Sohn and Chai [4] reported more than 29 helminth species from feral cats purchased from a market in Busan. In addition, Chai et al. [5] reported 4 nematode species, 23 trematode species, and 5 cestode species in cats. Thus, intestinal parasite infections in stray or feral cats have been reported involving more than 29 species of helminths, including 23 trematodes, 5 cestodes and 4 nematodes in Korea [4,5].

With respect to cestodes of cats, a total of 4 species, comprising *S. erinaceieuropaei*, a diphyllobothriid tapeworm, *T. taeniaeformis*, and *Hymenolepis diminuta*, have been commonly found in Korea [1-5]. *S. erinaceieuropaei* has been recognized as the only *Spirometra* species in Korea before the report of Jeon et al. [6]. *Spirometra* species inducing human sparganosis are being identified by morphological and genetic analyses, and the complete mitochondrial genomes of *S. erinaceieuropaei* and *S. decipiens* have been characterized and recorded in Korea [6,7]. The genetic distance of *S. decipiens* vs *S. erinaceieuropaei* and *S.*

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decipiens vs *S. proliferum* was estimated on the basis of partial *cox1* sequences, respectively [8]. Presently, 2 *Spirometra* species were reported in Korea, therefore, identification of *Spirometra* species is required. In this study, we identified a *Spirometra* species from a stray cat by molecular analysis using cytochrome *c* oxidase (*cox1*) in addition to morphological observations of mature proglottids.

A road-killed stray cat (*Felis catus*) was found in the Eumseong-gun area in Chungcheongbuk-do in November 2011. The small intestine was isolated and longitudinally opened with a pair of scissors in 0.85% saline and rinsed in PBS until the supernatant remained clear. The sediment containing the intestinal content was carefully examined under a stereomicroscope. Two types of worms were detected in the small intestines during the postmortem examination. The collected worms were fixed with 10% neutral buffered formalin under a glass slide with pressure for microscopic observations after carmine staining. Immature and mature proglottids of the tapeworms were pressed and fixed in alcohol-formalin-acetic acid (AFA) for carmine staining. Mature proglottids were observed with respect to the vaginal opening, uterus, uterine pore, cirrus, genital pore, testes, and vitellaria, based on the morphological data of Faust et al. [9]. The scolices and proglottids were preserved at -70˚C until use for molecular identification.

A single scolex was chopped into small pieces, and total genomic DNA was extracted using a DNeasy tissue kit (Qiagen, Valencia, California, USA). PCR and DNA sequencing for differential diagnosis were performed according to established protocols [9,10]. Pairwise genetic distances on the partial mitochondrial *cox1* sequences (377 bp) were analyzed using the neighbor-joining method with Kimura's 2-substitution model. The partial mitochondrial *cox1* sequences (377 bp) of 4 taxa of Diphyllobothriidae comprised *S. decipiens* (GenBank no. KJ599679), *S. erinaceieuropaei* (GenBank no. KJ599680), *Diphyllobothrium nihonkaiense* (GenBank no. EF420138), and *Diphyllobothrium latum* (GenBank no. DQ985706). The parasite materials (PRB000720) used in the present study were provided by the Parasite Resource Bank of Korea, National Research Resource Center, Korea.

A total of 134 scolices and mature proglottids of *Spirometra* species were found in the cat examined. Morphological observations were based on 116 scolices and mature proglottids. The scolex was 2.74±0.43 mm×0.73±0.11 mm in average size and small spoon-shape with 2 distinct bothria (Fig. 1A).

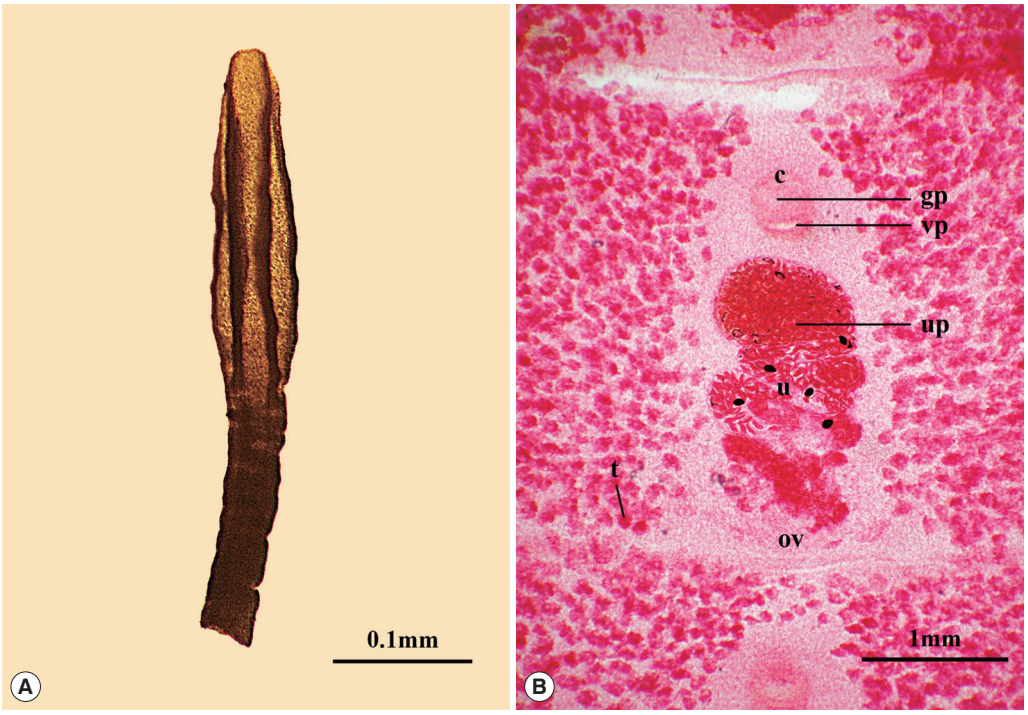

Fig. 1. *Spirometra decipiens* collected from a road killed stray cat in Chungbuk, Korea. (A) Scolex. (B) Whole-mounted specimen of proglottids showing the uterus and cirrus pouch (×40). Abbreviations; cirrus (c), uterus (u), genital pore (gp), vaginal pore (vp), uterine pore (up), ovaries (ov) and testes (t) (carmine stain).

Mature proglottids measured 4.2±0.03 mm in width and 1.70±0.01 mm in length (Fig. 1B). The uterus was coiled 3-4 times, the end of the uterus was ball-shaped, and the vaginal aperture shaped as a crescent moon was closer to the cirrus aperture than to the uterus aperture (Fig. 1B). The uterine ball measured 457.7±4.8 μm in diameter, and uterine pores were on the midline behind the anterior margin of the uterine ball. The cirrus pouch measured 260.3±2.6 μm in diameter. The genital pore was situated ventrally on the midline in the anterior 1/5 segment. The testes measured 71.4±0.7 μm maximum diameter (Fig. 1B) (Table 1).

PCR amplification and direct sequencing of the *cox1* target fragment (377 bp in length and corresponding to positions 769-1,146 bp of the *cox1* gene) were performed using total genomic DNA extracted from 134 specimens. The partial *cox1* sequences (377 bp) of the scolices (n=134) and proglottids (n=41) showed 99.0% similarity to the *S. decipiens* reference sequence (GenBank no. KJ599679) and 89.3% similarity to the *S. erinaceieuropaei* reference sequence (GenBank no. KJ599680). The difference in nucleotide sequence of the partial *cox1* gene between the specimens and *S. decipiens* was 0.1%, while that with *S. erinaceieuropaei* was 9.7%. The pairwise genetic distance between the specimens (scolices and proglottids) and *S. decipiens* was 0.0026, while that with *S. erinaceieuropaei* was 0.0079±0.0237 (Table 2).

In the present study, all scolices and proglottids were identified as *S. decipiens* using nucleotide sequence analysis of the mitochondrial *cox1* gene. Pairwise genetic distances between *S. decipiens* and *S. erinaceieuropaei* isolated from the cat examined were calculated as being the same as those of previous studies of snakes (0.0079±0.0237) [8]. The major distinguishing morphologic characteristics of *Spirometra* species are the number of uterine coils. The mature proglottids in this study were identical with those of *S. decipiens* by the morphological characteristics of reproductive organs, especially 3-4 spiral rotations of the uterus.

The taxonomy of the genus *Spirometra* has been controversial because of its species classification. Approximately 38 species of the genus *Spirometra* have been reported from intestinal parasites of canines and felines [9,11-13]. All species in the genus *Spirometra* were considered synonymous with *S. erinaceieuropaei* found in Asian countries, including China, Japan, and Korea [11-13]. The mitochondrial DNA sequences of the genus *Spirometra* have been used as identification and genetic variation of species to resolve uncertain taxonomic status. The nucleotide sequence variation of *S. erinaceieuropaei* was reported by Okamoto et al. (<2.6%) [15] and Liu et al. (0.0-3.1%) [16]. The complete mitochondrial genomes of genus *Spirometra* have been recently published for *S. erinaceieuropaei* and *S. decipiens* based on morphological analyses of *Spirometra* tapeworms by Eom et al. [7]. Thirty-seven human sparganosis were reported from Africa, including 34 from South Sudan and 3 from Ethiopia by Eberhard et al. [17]. The partial *cox1* nucleotide divergence of Asian isolate identified as *S. decipiens* dif-

Table 1. Morphological features of spirometrid tapeworms recovered from a road killed cat

Organs	Size
Scolex (n=116)	2.74±0.43 mm (diameter) 0.73±0.11 mm (length)
Mature proglottids (n=41)	4.19±0.02 mm (width) 1.70±0.01 mm (length)
Uterus	3-4 loops
Uterine ball	457.6±4.77 μm (diameter)
Cirrus pouch	260.3±2.62 μm (diameter)
Genital pore	115.7±1.55 μm (diameter)
Testes	71.4±0.71 μm (diameter)

Table 2. Pairwise genetic distance of the mitochondrial *cox1* gene of *Spirometra* specimens isolated from a cat in comparison with *Spirometra* spp., *Diphyllobothrium nihonkaiense*, and *Diphyllobothrium latum*

Species	Scolices	Proglottids	*Spirometra decipiens*	*Spirometra erinaceieuropaei*	*Diphyllobothrium nihonkaiense*	*Diphyllobothrium latum*
Scoleces (n=134)						
Proglottids (n=41)	0.00265±0.00000					
Spirometra decipiens	0.00265±0.00000	0.00265±0.00000				
Spirometra erinaceieuropaei	0.00796±0.02369	0.00796±0.02369	0.01031±0.02251			
Diphyllobothrium nihonkaiense	0.15467±0.00218	0.15467±0.00218	0.15201±0.00218	0.14834±0.00284		
Diphyllobothrium latum	0.16490±0.00099	0.16490±0.00099	0.16490±0.00099	0.15429±0.00099	0.07206±0.00099	

Genetic distance (mean±SD) calculated by the neighbor-joining method using Kimura's 2-parameter model based on 377 bp partial *cox1* sequences.

fered from South America isolate (17.8-19.2%), but it was closely related to the Asian isolates of *S. erinaceieuropaei* (0.3-4.0%) [18]. The South American isolates of *Spirometra* species were revealed as neither *S. erinaceieuropaei* nor *S. decipiens* by mitochondrial *cox1* sequence analyzed. Those cases differed from Asian isolates of *S. erinaceieuropaei* and *S. decipiens* as well as those of African countries by analysis of mitochondrial DNA sequence data. Above studies provoked epidemiological questions concerning what is the geographical distribution of *Spirometra* spp., including *S. erinaceieuropaei* and *S. decipiens*? In addition, why is a high level of nucleotide variation found among Brazilian, Asian, and African isolates? The taxonomic status of *Spirometra* species has been unsettled. Further studies are needed investigations using a number of global samples to clarify the taxonomy of the genus *Spirometra*.

The final hosts of *Spirometra* spp. are known to be carnivorous animals such as cats and dogs; however, we only found *S. decipiens* in the absence of other *Spirometra* species, including *S. erinaceieuropaei* in natural infections in cats in those studies. A previous study identified *S. decipiens* plerocercoids in terrestrial snakes from Korea and China, without any exceptions [9]. In another report concerning human sparganosis cases, 35 and 15 instances of *S. erinaceieuropaei* and *S. decipiens*, respectively, were identified, revealing approximately twice as many cases of *S. erinaceieuropaei* compared to *S. decipiens* [6]. These findings indicated not only the presence of epidemiological discrepancies between the prevalence of *Spirometra* spp. in humans and intermediates or final hosts, but also that the infection route of *S. erinaceieuropaei* for humans remained unclear.

Since it is very difficult to identify the species of eggs of *Spirometra* using morphology alone, DNA-based analysis is considered necessary to taxonomically classify the genus *Spirometra*. Many studies had documented *Spirometra* spp. in surveys undertaken among cats before *S. decipiens* had been reported in Korea [1-5]. Those previous studies may need to be reexamined using molecular techniques in an effort to better understand the epidemiological status of *Spirometra* spp. in Korea. Although many studies have examined the prevalence of intestinal parasites in stray cats, none of the recent reports have indicated a heavy infection of *Spirometra* species in Korea as detailed in our present study. Moreover, all worms were identified as *S. decipiens* rather than *S. erinaceieuropaei* by morphological and molecular analyses.

In conclusion, we report a stray cat heavily infected with *S. decipiens* identified by mitochondrial *cox1* sequence analysis and morphological examinations of the adult worms. These findings suggested that stray cats play an important role of a reservoir host for *S. decipiens* inducing human sparganosis in Korea.

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CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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