

## Discovery of *Endocotyle incana* and *Spelotrema pseudogonotyla* (Digenea: Microphallidae) from Scolopacid Migratory Birds in Korea

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**Abstract:** Migratory birds have been suspected as playing a central role in transmission of various trematodes, but few surveys have been undertaken in the Republic of Korea. In the present study, we describe new fauna of microphallid flukes in Korea. Two species of microphallids were found in the intestines of 2 migratory bird species, namely the red necked stints (*Calidris ruficollis*) and the terek sandpiper (*Xenus cinereus*), in a coastal area of Gunsan-si, Jeollabuk-do. The microphallids recovered from the latter were 451 µm in length and 217 µm in width, and the eggs were very small, 13 µm by 8 µm. They had the characteristic extracecal vitellaria intruding into the median posttesticular area, belonging to *Endocotyle incana*. Another microphallids were discovered from both migratory birds, 417 µm in length and 249 µm in width. The cirrus was projecting into the genital atrium in form of male papilla, and bounded by a muscular flap, termed pseudogonotyl. They were identified as *Spelotrema pseudogonotyla* in consideration of the distinctive male papilla and pseudogonotyl. Besides these flukes, *Maritrema obstipum* and *Gynaecotyla squatarolae* also were recovered. This paper is in fact the first report on the presence of *Spelotrema* and *Endocotyle* in Korea.

**Key words:** *Endocotyle incana*, *Spelotrema pseudogonotyla*, red necked stint, terek sandpiper

The prevalence of parasitic diseases in Korea has been dramatically reduced over the past 30 years, though the public health importance of intestinal trematodes is remained due to high prevalences in coastal areas [1]. A total of 21 species, transmitted typically through consumption of raw sea foods, have been reported as the human infecting intestinal flukes in Korea [1,2]. Migratory birds have been suspected of playing a central role in that transmission as their reservoir hosts, but few surveys on the avian trematode fauna have been undertaken. The paleartic oystercatcher, *Haematopus ostralegus osculans*, has been reported to be a natural final host of *Gymnophalloides seoi* [3], and the ruddy turnstone, *Arenaria interpres interpres* is the host of *Gynaecotyla squatarolae* [4]. Recently, adult worms of *Maritrema obstipum* were found in the intestines of certain migratory birds [5]. Considering the number and diversity of

migratory birds in Korea; however, additional investigation of their parasites certainly is required. In the present study, we made the first Korean discovery of adult worms of *Endocotyle incana* and *Spelotrema pseudogonotyla* in the intestines of migratory birds. We herein report our findings with morphological descriptions.

Five red necked stints (*Calidris ruficollis*), and 4 terek sandpipers (*Xenus cinereus*) were found in dead on the coastal area of Gunsan-si, Jeollabuk-do in October 2009, and were immediately transferred to the laboratory for further study. Their intestinal tracts were separated, fixed in 3% formalin, and examined under a stereomicroscope. The intestinal trematodes recovered from each bird were counted and preserved in 10% formalin, and subsequently stained with Semichon's acetocarmine for species identification.

From the red necked stints, a total of 174 flukes were recovered. Among them, 1 fluke was easily identified as the adult worm of *M. obstipum* by the presence of the cirrus sac, the ring-like distribution of the vitellaria, and the posteromedially curved ejaculatory duct. The others belonged to the same species, *E. incana*, and the infection rate was 80% (4/5). The num-

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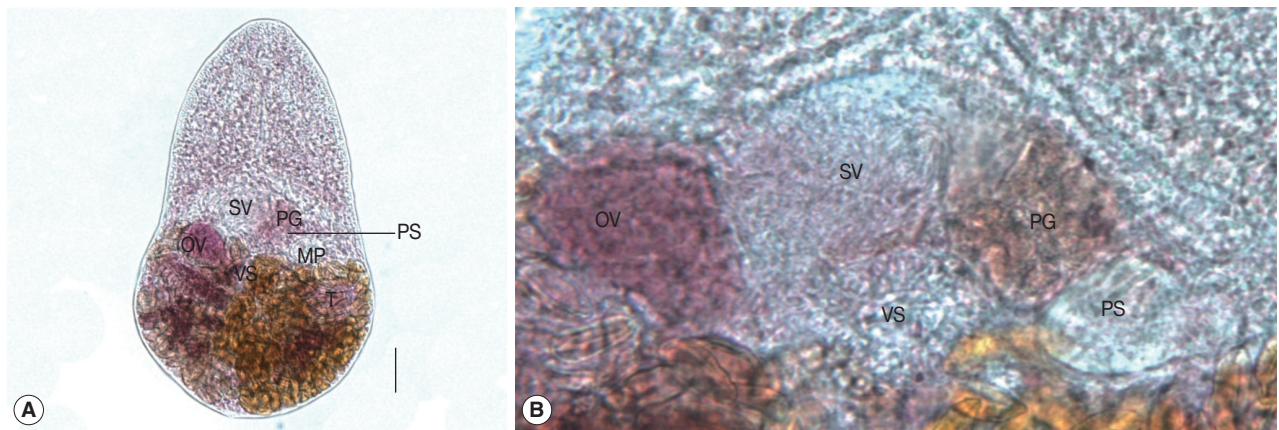
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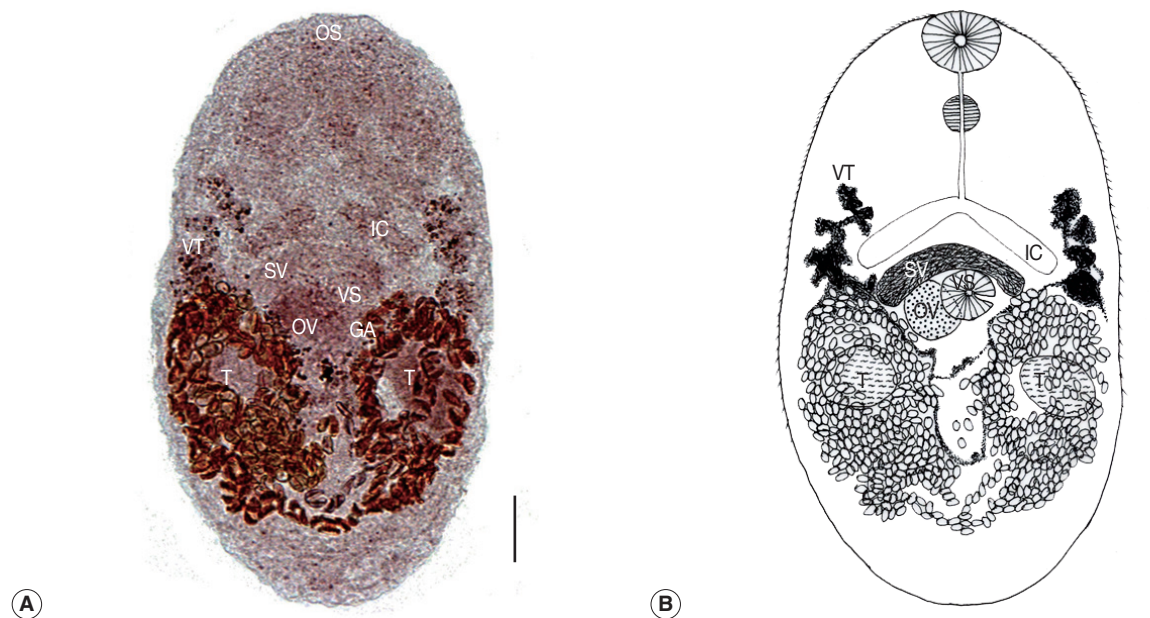
ber of specimens per host was 162, 3, 3, 5, and 0 (mean 34.6), and the morphological characteristics were as follows (Fig. 1).

Body pyriform, 417 (405-440)  $\mu\text{m}$  long and 249 (230-263)  $\mu\text{m}$  wide. Cuticle is armed with numerous spines, but less conspicuous from the level of ventral sucker. Oral sucker terminal, 39 (30-43)  $\mu\text{m}$  long and 49 (45-53)  $\mu\text{m}$  wide. Prepharynx conspicuous, esophagus long. Ceca short, terminates at equatorial level. Ventral sucker distinctly smaller, 24 (18-38)  $\mu\text{m}$  long and 28 (23-33)  $\mu\text{m}$  wide, located at posterior third. Testes

postacetabular, right one 35 (30-38)  $\mu\text{m}$  by 60 (50-63) wide, left one 41 (25-65)  $\mu\text{m}$  by 53 (30-63)  $\mu\text{m}$ . Seminal vesicle in intercecal space, anterior to ventral sucker. Prostatic glands well developed. Cirrus pouch absent, and cirrus projecting into genital atrium in form of muscular papilla, termed male papilla. Male papilla bounded by muscular flap, termed pseudogonotyl. Ovary dextral to ventral sucker, lying close against right cecum, 41 (25-53)  $\mu\text{m}$  long and 53 (30-63)  $\mu\text{m}$  wide. Vitellaria forms 2 clusters, right one obscuring right testis. Uterus



**Fig. 1.** (A) An adult of *Spelotrema pseudogonotyli* recovered from a red-necked stint, *Calidris ruficollis*. Bar = 100  $\mu\text{m}$ . SV, seminal vesicle; VS, ventral sucker; PG, prostatic gland; PS, pseudogonotyla; OV, ovary; T, testis. (B) Magnification of the midbody of another worm. SV, seminal vesicle; VS, ventral sucker; PG, prostatic gland; PS, pseudogonotyla; OV, ovary.



**Fig. 2.** (A) An adult of *Endocotyle incana* recovered from a terek sandpiper, *Xenus cinereus*. Bar = 50  $\mu\text{m}$ . OS, oral sucker; IC, intestinal ceca; SV, seminal vesicle; VS, ventral sucker; GA, genital atrium; OV, ovary; T, testis; VT, vitellarium. (B) A line drawing of (A), the adult worm of *E. incana*. Bar = 50  $\mu\text{m}$ . IC, intestinal ceca; SV, seminal vesicle; VS, ventral sucker; OV, ovary; T, testis; VT, vitellarium.

voluminous, extending anterior to testes, even up to tip of cecum. Size of intrauterine eggs 20 by 13 (11-13)  $\mu\text{m}$ . Considering these morphological characteristics, especially the presence of pseudogonotyl, the present specimen was identified as *S. pseudogonotyla*.

In the intestines of the terek sandpipers, a total of 80 flukes were recovered. They were tentatively divided into 4 groups; 5 *G. squatarolae*, 2 *S. pseudogonotyla*, 71 unidentified microphallids, and 2 unknown flukes. Unidentified microphallids were found in only 1 out of 4 terek sandpipers, and had the following morphological characteristics (Fig. 2). Body linguiform, 451 (410-490)  $\mu\text{m}$  in length and 217 (161-272)  $\mu\text{m}$  in width. Oral sucker terminal, 37 in length by 53 in width. Prepharynx distinct, 24 (22-25)  $\mu\text{m}$  long; pharynx comparatively long, 27  $\mu\text{m}$  long by 25  $\mu\text{m}$ . Esophagus short to medium in length. Ceca wide, divergent in front of ventral sucker. Ventral sucker well developed, at about middle of body, 25  $\mu\text{m}$  in length by 29  $\mu\text{m}$  (27-30) in width. Genital atrium adjacent to ventral sucker, sinistral and posterior to it. Seminal vesicle anterior to ventral sucker. Testes symmetrical, posterior to ventral sucker, right one 39 (37-42)  $\mu\text{m} \times 38$  (37-40)  $\mu\text{m}$ , left one 37.1  $\times$  37.1  $\mu\text{m}$ . Ovary slightly to right of median line, immediately behind ventral sucker. Uterus mainly postcecal, not reaching posterior extremity. Eggs very small, 13 (12-15)  $\mu\text{m}$  by 8 (7-8)  $\mu\text{m}$ . Vitellaria composed of numerous, small follicles forming 2 sinusoidal ribbons, initially prececal and marginal in forebody, and then passing into space between ventral sucker and testis. Considering these morphological characteristics, it was concluded that the microphallids belonged to *E. incana*.

Prior to the present study, only 2 species of microphallids had been discovered in migratory birds in Korea; *G. squatarolae* from the ruddy turnstone, and *M. obstipum* from the sanderling, Kentish plover, Mongolian plover, and red-necked stint [4,5]. In the present study, *M. obstipum* was also recovered from red necked stints and *G. squatarolae* from terek sandpipers, respectively, proving that terek sandpipers also are the definitive hosts for *G. squatarolae*. In addition, *S. pseudogonotyla* and *E. incana* were found, for the first time, in the intestines of migratory birds in Korea [6,7]. These represent the 3rd and 4th such microphallids recovered in migratory birds in Korea. Certainly, the close scrutiny on the parasitic infections in migratory birds will be needed, as the study of bird parasites in littoral areas can provide key insights into the trematode distribution.

The present *S. pseudogonotyla* specimens fell within the 405-440  $\mu\text{m}$  length range, which is smaller than *S. nicolli* [8]. The

body width increased without interruption from the anterior towards the broadly rounded posterior end, differentiating them from *Spelotrema simile* [8]. Whereas in *Spelotrema pygmaeum* the uterus does not extend anterior to the testes, the present specimens showed intrauterine eggs located anteriorly to the testes [8]. They were identified definitively as *S. pseudogonotyla* on the basis of the location of the acetabulum and the presence of pseudogonotyl [9]. *S. pseudogonotyla* originally was discovered in the intestines of domestic ducks in Hong Kong [9], but the present results indicated that the migratory birds were also the natural definitive host of this parasite, transmitting them to other localities. The presence of metacercariae of *Spelotrema nicolli* in the tissues of the green crab, *Carcinus maenas*, indicates that migratory birds are infected with *S. pseudogonotyla* through consumption of crustaceans in the western coastal areas of Korea [8]. And notably, the first description of *Spelotrema brevicaca* was reported in a human autopsy case, 11 additional cases having been found in the Philippines [6,7]. Considering the raw-seafood-eating habit of Koreans, human infection with *Spelotrema* sp., as seen in the case of *G. squatarolae* [2], is possible.

As for the genus *Endocotyle*, only 3 species, *E. incana*, *E. africanus* and *E. bushi*, have been reported thus far. In *E. incana*, the type species, its extracecal vitellaria is characteristically intruding into the median posttesticular area, as in the present specimen [10]. Adult worms of *E. bushi* have been found in willets (*Catotrophorus semipalmatus*) in Florida and Texas, given the disposition of their posterior uterine loops, the extension of the vitellarium, and their geographical distribution, among other indicators, they are thought to represent a new species [5,11]. Indeed, *E. bushi* was found to be longer (550  $\mu\text{m}$ ) than *E. incana* (400  $\mu\text{m}$ ) and to possess large intrauterine eggs (22-24  $\mu\text{m}$ ) [5], differentiating it from the present specimens. Moreover, *E. africanus*, identified as a new species in Kenya, is 404  $\mu\text{m}$  long by 326  $\mu\text{m}$  wide, with intrauterine eggs of 15.3  $\mu\text{m}$  length, clearly differentiating it from the present flukes [12]. Therefore, the present specimens were deemed to be adult worms of *E. incana*.

In October 2010, an investigation of the terek sandpiper was conducted, though on only 1 individual [5]. However, only 4 adults of *G. squatarolae* were recovered, and there were no cases of *E. incana* [5]. In the current study, adult worms of *E. incana* were recovered from only 1 terek sandpiper out of 4 (25%), reflecting the low infection rate of this fluke. By contrast, in the study of October 2010, 19 unknown worms from red-necked

stints were newly identified as adults of *S. pseudogonotyla* [5]. This is an illustration of why investigations should be conducted on a frequent basis.

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