The Ruddy Turnstone, *Arenaria interpres interpres*, a New Definitive Host for *Gynaecotyla squatarolae* (Digenea: Microphallidae)

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Abstract : The ruddy turnstone, *Arenaria interpres interpres*, a migratory Korean bird, was proved to be a natural definitive host for *Gynaecotyla squatarolae* (Digenea: Microphallidae). The ruddy turnstone was found dead at the seashore of Okgueup, Gunsan-si, Jeollabuk-do. The intestinal tract was examined, and 98 unknown flukes were recovered. The worms were $600 \times 284 \ \mu m$ in size, and had 2 ventral suckers. The seminal vesicle was large, the genital atrium was prominent, and the average egg size was $20 \times 12.5 \ \mu m$. Based on these results, the worms were identified as *G. squatarolae*. This is the first report on the ruddy turnstone as a natural definitive host of *G. squatarolae* in the Republic of Korea.

Key words: Gynaecotyla squatarolae, Intestinal fluke, Ruddy turnstone, Arenaria interpres interpres, Definitive host

Gynaecotyla squatarolae (Digenea: Microphallidae) was first found in the small intestine of birds in Japan [1], and then from experimentally infected rats, rabbits, chickens, and quail [2]. The second intermediate host of *G. squatarolae* was proved to be the brackish water crab, *Macrophthamus dilatatus* and *M. japonicus* in Japan [2,3]. In the Republic of Korea, the metacercariae of *G. squatarolae* were discovered from the shore crab, *M. dilatatus* in Taean-eup, Chungcheongnam-do, and their adult flukes were recovered from experimentally infected rats [4]. Considering the fact that the shore crabs are locally consumed in the coastal area, the possibility of human infections cannot be overlooked.

Many human-infecting trematodes in Korea originated as parasites in birds. For example, the paleartic oystercatchers, *Haematopus ostralegus osculans*, have been reported as the natural final host for *Gymnophalloides seoi* [5], and the family Microphallidae Travassos, 1920, occur primarily as intestinal parasites of birds [6]. An example is *Gynaecotyla riggini* of which the natural final host is a ruddy turnstone, *Arenaria interpres morinella*, from Florida, USA [7]. Also, a herring gull was found infected with *Gynaecotyla adunca* [8]. Hence, it is reasonable to expect that birds play a role as the natural final host of *G. squatarolae*. In this study, a ruddy turnstone was opportunistically collected from a western coastal area of Korea, from which adult worms

of G. sqautarolae were recovered.

The ruddy turnstone, *Arenaria interpres interpres*, a migratory Korean bird, was found dead by a bird researcher at the seashore of Okgu-eup, Gunsan-si (= city), Jeollabuk-do, in April 2006. The intestinal tract was removed and stored at -70°C immediately, and transferred to our laboratory after 17 days. The sample was thawed at room temperature, opened longitudinally in saline, and examined for the presence of parasites under a stereomicroscope. Collected worms were fixed in 10% neutral formalin, washed with distilled water, and stained with Semichon's acetocarmine.

The number of recovered parasites was 103 flukes. A taxonomic analysis revealed 2 different types of parasites; 98 unknown flukes and 5 *Acanthoparyphyium* sp. Among the 98 unknown flukes, 26 (26.6%) were mature. Identification of the mature worms was based on the following characteristics. Esophagus is long, and ceca are widely divergent. Cirrus pouch is large, between large ventral sucker and cecal arch, containing a seminal vesicle (Fig. 1). Large ventral sucker is located under the center of cirrus pouch, and small ventral sucker is right to large one. Genital atrium is close to small ventral sucker in the right side. Below them the right testis is located, with the left one on the opposite side. Ovary is located above the left testis. Uterus is filled with numerous eggs, which are brownish in color, thick-shelled, and $20 \times 12.5~\mu m$ in average size. Two groups of vitellaria are scattered in posterior part of the worm,

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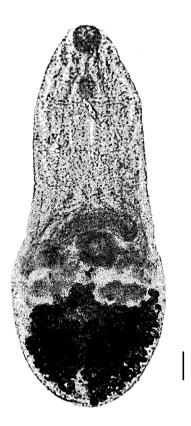


Fig. 1. An adult worm recovered from the ruddy turnstone, *Arenaria* interpres interpres (Bar = $50 \mu m$).

not beyond the level of testes (Fig. 2). Based on these results, these worms were identified as *G. squatarolae*.

Measurements were done only on mature worms. The size of worms from experimental rats was $601 \pm 53 \times 284 \pm 20 \ \mu m$. Oral sucker $49 \pm 2 \times 51 \pm 3$, ventral sucker $62 \pm 6 \times 64 \pm 5$. Esophagus 130 ± 17 . Cirrus sac $137 \pm 9 \times 36 \pm 4$, right testis $34 \pm 10 \times 72 \pm 12$, left testis $37 \pm 5 \times 65 \pm 10$. Ovary $40 \pm 10 \times 48 \pm 13$, seminal vesicle $115 \pm 12 \times 29 \pm 4$. In comparison with the worms reported by Seo et al. [4], the worms from the ruddy stone were significantly smaller in length and width (P < 0.05). The size of suckers, genital atrium, and seminal vesicle showed little difference between them, but testis and ovary were significantly larger in worms from rats than in those from the ruddy turnstone (P < 0.05), up to $57 \ \mu m$ in the case of right testis.

In relation to morphological characteristics, the genus *Gynae-cotyla* has 2 ventral suckers [7,9] and the genital atrium is equipped with separated male and female genital organs [8]. Additionally, the vitelline follicles did not exceed the level of each testis [7,10]. Based on this information, we concluded that the recovered worms were a species of *Gynaecotyla*. In terms of species

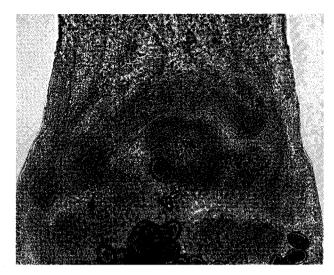


Fig. 2. Magnification of Fig. 1. LVS: large ventral sucker, SVS: small ventral sucker, GA: genital atrium, T: testes, O: ovary, C: cirrus, SV: seminal vesicle. VD: vitelline duct. (× 400).

identification, the number of vitelline follicles, the location of ovary, and the comparative size of 2 ventral suckers are important [7]. Our specimen had more than 10 vitelline follicles on each side, and had an ovary in the dextral position, compatible with the criteria of *G. squatarolae*. Although the size difference between 2 suckers was more than 0.02 mm in these specimens, it was similar to *G. squatarolae* described by Seo et al. [4]. However, the size of eggs showed somewhat difference with that of Seo et al. [4]. The reported egg size was $21 \times 17 \ \mu \text{m}$ in Seo et al. [4], while the eggs from this study were measured $20 \times 12.5 \ \mu \text{m}$, on average. Of course, the eggs of Seo et al. [4] were recovered from the stool of rats, whereas in this study we measured the intrauterine eggs. Considering that the eggs of *G. nassicola* were measured at $20 \times 10 \ \mu \text{m}$ [11], the egg size of *G. squatarolae* were close to that of the present study.

The second intermediate host of *G. squatarolae* was the shore crab, *M. dilatatus*. The term 'turnstone' refers to the fact that the bird turns over the pebble searching for food [12]. Hence, the ruddy turnstone can feed on the shore crabs while discharging the eggs of *G. squatarolae*. Since the ruddy turnstone was discovered at Okgu-eup, Jeollabuk-do, and the site has been known as the visiting place of migratory birds, it is reasonable to expect that the crabs of Okgu-eup may be infected with the metacercariae of *G. squatarolae*. Investigations on the shore crabs in this area must be conducted. Furthermore, other on-route visiting locations of migratory birds, such as the Youngsan lake, in Jeollanam-do, have to be also examined.

Many worms of G. squatarolae were identified as immature

in the ruddy turnstone. Even in mature worms, the length and width of the worm, as well as the size of testes and ovary were smaller than those of the worms isolated from infected rats reported by Seo et al. [4]. Considering that Gynaecotyla species originated as parasites of birds, it was unexpected that the worms from the ruddy turnstone were smaller was unexpected. It may be possible that the ruddy stone died on the morrow of feeding on shore crabs, and the excysted metacercariae did not have sufficient time to grow. It is possible that the ruddy turnstone may not be a highly suitable host for G. squatarolae. Studies on migratory birds should shed more light on this issue.

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