## Surveys of *Gynaecotyla squatarolae* and *Microphallus koreana* (Digenea: Microphallidae) Metacercariae in Two Species of Estuarine Crabs in Western Coastal Areas, Korea

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**Abstract:** To figure out the geographical distribution of *Gynaecotyla squatarolae* and *Microphallus koreana* metacercariae in Korea, shore crabs of southwestern coastal areas were examined. Eight coastal areas in Inchon-si (A), Gyeonggi-do (B), Chungcheongnam-do (C, D, and E), Jeollabuk-do (F), and Jeollanam-do (G and H) were selected, and 2 kinds of crabs, *Macrophthalmus dilatatus* and/or *Macrophthalmus japonicus*, were caught. After transportation to the laboratory, 15 crabs per each group were grouped and ground in a mortar and pestle, and examined for microphallid metacercariae. In *M. dilatatus, G. squatarolae* metacercariae were recovered from 3 (C, E, and H) out of 6 regions, but *M. koreana* metacercariae were not recovered. In the case of *M. japonicus, G. squatarolae* metacercariae were recovered from 5 regions (A, B, D, F, and H). These results indicate that the life cycle of *G. squatarolae* is maintained in the western coastal areas using *M. dilatatus* and *M. japonicus* as intermediate hosts, while that of *M. koreana* is maintained only using *M. japonicus*.

Key words: Gynaecotyla squatarolae, Microphallus koreana, shore crab, Macrophalmus japonicus, Macrophthalmus dilatatus, epidemiological survey

*Gynaecotyla squatarolae* (Digenea: Microphallidae) was originally discovered in the small intestine of birds [1], and the second intermediate host is known to be the shore crab, *Macrophathalmus japonicus* and *Macrophthalmus dilatatus* in Japan [2]. In the Republic of Korea, the metacercariae of *G. squatarolae* were discovered from the shore crab, *M. dilatatus* in Taean-eup, Chungcheongnam-do [3], but the investigation on *M. japonicus* has not been performed yet. In addition, the ruddy turnstone, *Arenaria interpres interpres*, a migratory Korean bird, was proved to be a natural definitive host for *G. squatarolae* [4]. Considering that the ruddy turnstone was found at the seashore of Gunsan-si, Jeollabuk-do, a survey on the western coastal areas was needed.

*Microphallus koreana* is also a kind of microphallid, described as a new species in Korea [5]. As in *G. squatarolae*, the crabs, *M. dilatatus*, are known to play a role in maintaining the life cycle of *M. koreana*, but the prevalence and infection density along the coastal areas has not been studied yet. The present study was performed to understand the geographical distribution of *G. squatarolae* and *M. koreana* by examining 2 species of estuarine crabs, M. dilatatus and M. japonicus, according to the localities.

A total of 8 areas along the western coasts of Korea were selected: Ganghwa-eup, Incheon-si (A), Jebu-ri Seosin-myeon Hwaseong-si, Gyeonggi-do (B), Mageum-ri Geunheung-myeon Taean-gun, Chungcheongnam-do (C), a market of Seosan-si Dongnam-dong, Chungcheongnam-do (D), Namdang-ri Seobu-myeon Hongseong-gun, Chungcheongnam-do (E), Sangdeung-ri Gyehwa-myeon, Buan-gun, Jeollabuk-do (F), Hasa-ri, Baeksu-eup Yeonggwang-gun, Jeollanam-do (G), Sinjang-ri, Aphae-myeon, Sinan-gun, Jeollanam-do (H). These areas were indicated in Fig. 1.

The crabs, *M. japonicus* and *M. dilatatus*, were collected at the areas described above by using a scoop. Along with the collection by scoop, the crabs of Seosan-si, Chungcheongnam-do, were bought in the Seosan market. After transportation to the laboratory, the hepatopancreas of each crab was separated, ground in a mortar with pestle, and filtered through a series of nets. Then, the presence of microphallid metacercariae was investigated under a stereomicroscopy. If metacercariae were present, the number of metacercariae per crab was counted. Each group was consisted of 15 crabs.

In M. dilatatus crabs, G. squatarolae metacercariae were recov-

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Fig. 1. Map showing the areas (A-H) where the crabs were collected and examined for *Gynaecotyla squatarolae* and *Microphallus koreana* metacercariae.

ered from C, E, and H out of 6 areas, and the highest density was recorded in H (79.1) (Table 1). All the crabs were infected with *G. squatarolae* in H, whereas the infection rate was 86.7% in C. The infection density was the highest in H, followed by E (44.3) (Table 1). However, metacercariae of *Microphallus koreana* were not recovered from *M. dilatatus* in the surveyed areas. A lot of metacercariae of unknown trematodes were recovered, but their identification is still underway.

In the case of *M. japonicus*, all the surveyed areas except A had *G. squatarolae* metacercariae (Table 2). Crabs from D had the highest prevalence (87.7%), whereas the number of metacercariae was the highest in crabs from H, followed by those from D. In the remaining areas positive for *G. squatarolae*, the prevalence ranged from 20% to 80%, and the number of metacercariae was less than 5.0 per crab (Table 2). Five out of 7 areas

Table 1. Number of metacercariae recovered from *M. dilatatus* 

	G. squatarolae	M. koreana	Unknown
Ganghwa-eup (A)	0	0	0
Jebu-ri (B)	0	0	160.7 (5-898)
Mageum-ri (C)	5.3 (1-16)	0	145.6 (45-325)
Namdang-ri (E)	44.3 (2-355)	0	25.0 (5-96)
Sangdeung-ri (F)	0	0	0
Sinjang-ri (H)	79.1 (6-510)	0	241.9 (61-721)

<sup>a</sup>The number of examined crabs were 15 per each site.

were positive for *M. koreana* metacercariae, and the prevalence was from 6.7% in A to 80% in D. The infection density was less than 10 per crab, and the highest number was recorded in F, followed by D. Undetermined metacercariae were also recovered from *M. japonicus*.

Our results suggested that G. squatarolae and M. koreana are distributed widely along the southwestern coastal areas of Korea. The crabs of Sinjang-ri, Shinan-gun, Jeollanam-do, was proved to be heavily infected with G. squatarolae. This Sinjang-ri area, Shinan-gun, is known to be the endemic area of Gymnophalloides seoi, a human infecting gymnophallid transmitted by oysters [6], and an avian host, the Paleartic oystercatcher, was proved to be a natural definitive host [7]. Microphallids have also been known as parasites of birds; for example, Microphallus sabanensis was collected from the wild birds of Venezuela [8], and Maritrema novaezealandensis was found from the red-billed gulls of New Zealand [9]. In addition, adult worms of G. squatarolae were collected from the intestine of the ruddy turnstone, a migratory bird of Korea [4]. Since the natural definitive host of M. koreana is not discovered yet, special attention should be paid to shore birds for the study of microphallids.

According to our previous researches [3,5], both *G. squatarolae* and *M. koreana* metacercariae were recovered from *M. dilatatus* purchased at a market of Seosan-si, Chungcheongnam-do, and the infection density was 4.3 per crab for *G. squatarolae* and 4.7 per crab for *M. koreana*. However, in the present study, *M. koreana* metacercariae were found only in *M. japonicus*, but not in *M. dilatatus*. Environmental changes, such as the oil outflow accident in Taean-gun, Chungcheongnam-do in 2007, might have changed the geographical distribution of larval trematodes. However, it may be reasonable to regard that *M. japonicus* is a more important intermediate host for *M. koreana* than *M. dilatatus* nowadays in Korea. More studies on microphallids are needed to elucidate this point.

Table 2. Number of metacercariae recovered from M. japonicus

Area surveyed	G. squatarolae	M. koreana	Unknown
Ganghwa-eup (A)	0	0.1 (2)	0
Jebu-ri (B)	4.8 (1-13)	3.0 (1-24)	1.1 (1-4)
A market, Seosan-si (D)	11.5 (1-36)	7.5 (1-53)	1.5 (1-9)
Namdang-ri (E)	2.7 (1-23)	0	6.8 (1-72)
Sangdeung-ri (F)	7.8 (2-20)	0.9 (2-9)	0.2 (1-2)
Hasa-ri (G)	1.8 (1-27)	0	1.4 (2-19)
Sinjang-ri (H)	17.6 (4-76)	1.8 (1-9)	75.7 (3-216)

<sup>a</sup>The number of examined crabs were 15 per each site.

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## REFERENCES

- 1. Yamaguti S. Studies on the helminth fauna of Japan. Part 25. Trematodes of birds, IV. Jap J Zool 1939; 8: 167-168.
- 2. Yamaguti S. Studies on the helminth fauna of Japan. Part 3. Avian trematodes, II. Jap J Zool 1934; 5: 546-550.
- Seo M, Guk SM, Lee SH, Chai JY. *Gynaecotyla squatarolae* (Digenea: Microphallidae) from rats experimentally infected with metacercariae from the shore crab, *Macrophthalmus dilatatus*. Korean J Parasitol 2007; 45: 199-204.
- 4. Seo M, Guk SM, Chai JY. The ruddy turnstone, *Arenaria interpres interpres*, a new definitive host for *Gynaecotyla squatarolae* (Digenea: Microphallidae). Korean J Parasitol 2008; 46: 41-43.

- Guk SM, Chai JY, Sohn WM, Kim YM, Sim S, Seo M. Microphallus koreana n. sp. (Trematoda: Microphallidae) transmitted by a marine crab, Macrophthalmus dilatatus. Korean J Parasitol 2008; 46: 165-169.
- 6. Chai JY, Lee GC, Park YK, Han ET, Seo M, Kim J, Guk SM, Shin EH, Choi MH, Lee SH. Persistent endemicity of *Gymnophalloides seoi* infection in a southwestern coastal village of Korea with special reference to its egg laying capacity in the human host. Korean J Parasitol 2000; 38: 51-57.
- 7. Ryang YS, Yoo JC, Lee SH, Chai JY. The Palearctic oystercatcher *Haematopus ostralegus*, a natural definitive host for *Gymnophalloides seoi*. J Parasitol 2000; 86: 418-419.
- Diaz MT, Bashirullah AK, Hernandez LE. A new species of *Microphallus* (Trematoda: Microphallidae) from Venezuela. Rev Biol Trop 2004; 52: 363-369.
- Martorelli SR, Fredensborg BL, Mouritsen KN, Poulin R. Description and proposed life cycle of *Maritrema novaezealandensis* n. sp. (Microphallidae) parasitic in red-billed gulls, *Larus novaehollandiae scopulinus*, from Otago Harbor, South Island, New Zealand. J Parasitol 2004; 90: 272-277.