

Discovery of *Maritrema jebuensis* n. sp. (Digenea: Microphallidae) from the Asian Shore Crab, *Hemigrapsus sanguineus*, in Korea

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Abstract: *Maritrema* spp. (Digenea: Microphallidae) are parasites of birds, but have not been found in the Republic of Korea. In this study, metacercariae of *Maritrema* sp. were discovered in the Asian shore crab, *Hemigrapsus sanguineus*, caught in the mud-flats of Jebu-do, Hwasung-gun, Gyeonggi-do, and the adult flukes were confirmed by experimental infection into mice. Based on the symmetric ribbon-like vitellarium, adult flukes of *Maritrema* sp. were identified, but did not belong to previously described species in terms of the following morphologic characteristics: ceca reaching to the lateral wall at the anterior border of the ovary; ventral sucker larger than oral sucker; a prominent metraterm; and vitellarium forming a complete ring. Hence, we named this microphallid *M. jebuensis* n. sp. after the island where the second intermediate hosts were collected. From this study, it has been shown that *Maritrema* sp. is distributed in Korea and transmitted by the Asian shore crab, *H. sanguineus*.

Key words: *Maritrema jebuensis*, intestinal fluke, Asian shore crab, Jebu-do

Species of the genus *Maritrema* Nicoll, 1907 (Microphallidae) occur in widely separated parts of the world, a finding which is consistent with birds serving as the definitive hosts of *Maritrema* spp. [1]. *Maritrema* spp. are found in the intestines of birds, causing enteritis and ulceration [2,3]. The characteristic organ of *Maritrema* spp. is the vitellarium, which appears as a symmetric ribbon reaching close to the margins of hind body, surrounding the uterine coils and testes [4]. The life cycle involves gastropods as first intermediate hosts, and crustaceans as second intermediate hosts [5].

Until now, 4 species belonging to Microphallidae have been described in Korea (*Microphalloides japonicus* from *Helice tridens tridens*, *Levinseniella* spp. from *Macrophthalmus japonicus*, and

Gynaecotyla squatarolae and *Microphallus koreana* from *Macrophthalmus dilatatus*) [6-9]. *Maritrema* species have not been previously reported in Korea, but the metacercariae were found for the first time during a survey on shore crabs of the western Korea coastal areas. In this study, the metacercariae were shown to be those of *Maritrema* spp., and identified as a new species by experimental infection in mice. Although the suitable host of *Maritrema* was known to be the bird, but mice were used in this study due to unavailability of chicks. For the adult worms recovered from mice, we assigned the name *Maritrema jebuensis* n. sp. *Maritrema* is the fifth microphallid having been found in Korea.

In October 2009, the Asian shore crabs, *Hemigrapsus sanguineus*, were caught in the mud-flats of Jebu-do (island), Seosin-myeon, Hwaseong-si, Gyeonggi-do during low tides. The crabs were mainly found beneath stones, and well-differentiated from *Macrophthalmus* spp. by morphology (Fig. 1A). After transportation to the laboratory, 40 of 50 crabs were ground in a mortar with a pestle, and filtered through a series of nets ranging from 60-500 μ m in size. Then, the crab homogenates were washed

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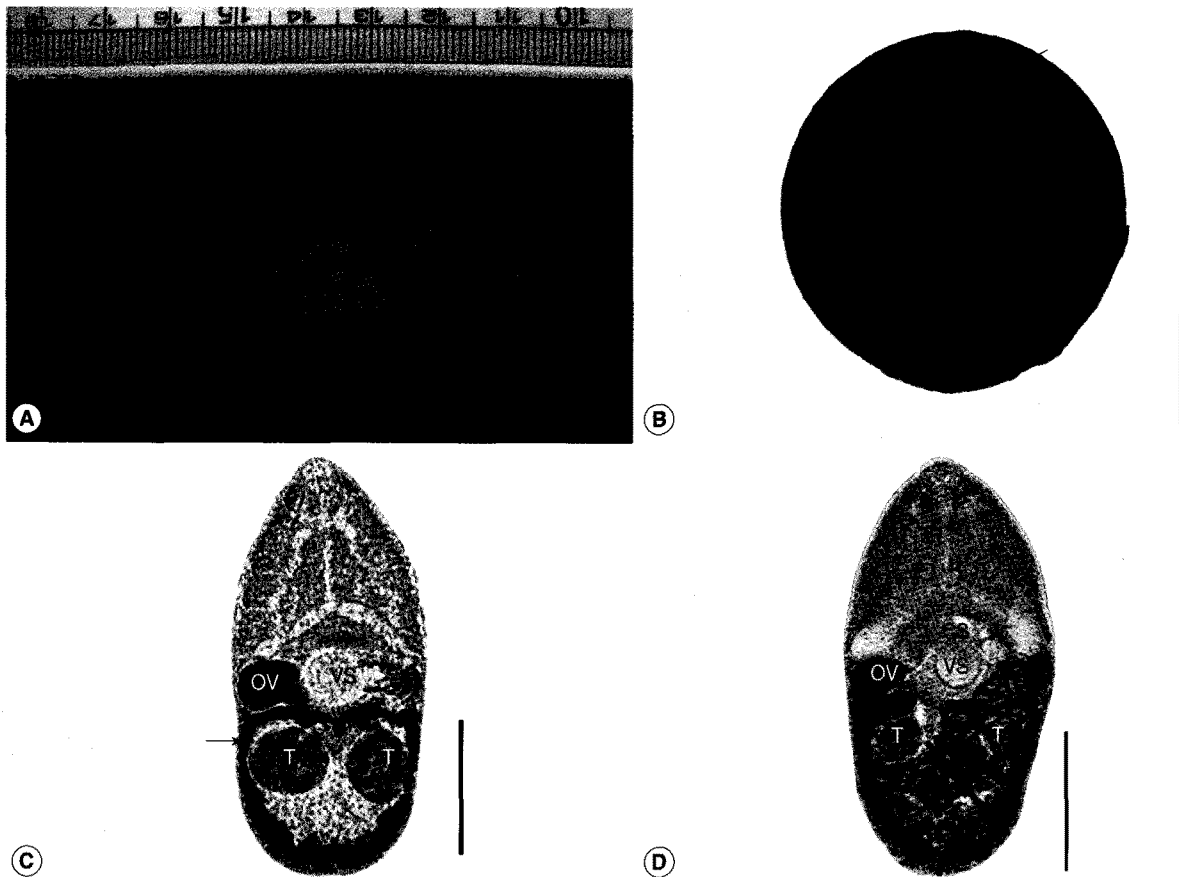


Fig. 1. (A) Asian shore crab, *Hemigrapsus sanguineus*, caught in the mud-flats of Jebu-do, Hwasung-gun, Gyeonggi-do, Korea. (B) A metacercaria of *Maritrema* n. sp. recovered from the Asian shore crab. The oral sucker (arrow) and the vitellarium (arrowhead) are indicated. Bar = 30 μ m. (C) A 1-day old worm of *Maritrema* n. sp. recovered from a mouse. Note that the vitellarine follicles appeared as a ring (arrow) in posterior part of the body. VS, ventral sucker; OV, ovary; T, testis. Bar = 20 μ m. (D) A 5-day old worm of *Maritrema* n. sp. The hindbody was filled with the intrauterine eggs, surrounded by the vitellarium. VS, ventral sucker; CS, cirrus sac; OV, ovary; T, testis. Bar = 20 μ m.

several times with PBS, and the metacercariae of *Maritrema* sp. were collected from the sediments using a stereomicroscope. Some of the metacercariae were fixed in 10% neutral formalin, and were used for morphological investigation after acetocarmine staining. The rests were used for experimental infection.

Then, specific pathogen-free (SPF) ICR mice, 5-week-old, were purchased from Koatech Co. Ltd. (Pyeongtaek, Korea) and maintained under SPF conditions at the Animal Facility of Dankook University. To obtain adult worms, 20 mice were orally infected each with 100 metacercariae, and 4 mice were sacrificed on days 1, 3, 5, 7, and 9 post-infection (PI). The small intestines were removed, and the microphallid worms were recovered from the intestinal contents under a stereomicroscope. The collected flukes were counted and fixed in 10% formalin. After staining with Semichon's acetocarmine, the flukes were observed under a light microscope for species identification.

Measurements were done on all of the recovered worms (in μ m).

The mean number of *Maritrema* n. sp. metacercariae per Asian shore crabs (*H. sanguineus*) was 30.5 (range 0-102). The metacercariae were mainly found in the muscles, especially near the legs. The metacercaria of *Maritrema* n. sp. was round and 300 \times 300 μ m in size. The wall was thick and composed of 2 layers. In outer layer, numerous striations were observed, and a ring-shaped vitellarium inside it (Fig. 1B). In addition to *Maritrema* n. sp., the metacercariae of *Gynaecotyla squatarolae* were also recovered (mean 32.6; range 0-125).

The average recovery rate of *Maritrema* n. sp. adult worms was 4.3% on day 1 PI, 1.3% on day 3 PI, 3.0% on day 5 PI, 1.0% on day 7 PI, and 0.0% on day 9 PI. The 1-day-old worm already had the ovary and testes (Fig. 1C), and the intrauterine eggs appeared on day 3 PI (Fig. 1D). The length of adult worms was

the highest on day 3 PI, 681 μm (614-762), continuously decreased to 530 μm (515-545) on day 7 PI. The size of intrauterine eggs was 22 \times 11 μm . The morphological characteristics of 3-day worms were as follows: Body linguiform, 681 μm long by 250 (228-287) μm wide at ventral sucker level. Oral sucker subterminal, 57 (53-64) μm \times 56 (53-61) μm . Anterior border of ventral sucker is located at midline of the body, 79 (66-86) μm \times 79 (77-86) μm . Sucker ratio (oral sucker to ventral sucker) was 0.72. Prepharynx 51 (26-88) μm long, pharynx 31 (24-33) μm long, and esophagus 147 (132-156) μm long. Ceca reached middle of ventral sucker. Both testes were symmetrical and same in size, 61 \times 61 μm . Cirrus sac intercecal, arcuate with thin wall, 103 (92-112) μm long by 29 (26-31) μm wide. The right end of cirrus sac reached the left border of ovary, not the right margin of body, and the left end reached at level of genital pore. Seminal vesicle and prostatic cells inside the cirrus sac, the latter surrounding curled ejaculatory duct. Invaginated cirrus slightly developed. Genital pore conspicuous, sinistrolateral to ventral sucker. Ovary dextral at level of ventral sucker, unilobed, 57 (46-70) μm \times 76 (66-86) μm . Uterus occupied the majority of hindbody, from anterior border of testis to the end of body. Metraterm thick walled, sinistral to genital pore. Vitellarium formed a complete ring, reaching close to margins of hindbody, surrounding uterine coils and testes.

Taxonomic summary

Experimental host: mouse

Locality: Jebudo (island), Seosin-myeon, Hwaseong-si, Gyeonggi-do, Republic of Korea

2nd intermediate host: Asian Shore Crabs, *Hemigrapsus sanguineus*

Site of infection: small intestine

Etymology: The specific name is derived from the name of the island where the second intermediate hosts were collected.

Holotype: Adult fluke is deposited in Department of Parasitology, College of Medicine, Dankook University, Cheonan-si, Chungnam 330-714, Republic of Korea.

In this study, the presence of *Maritrema* sp. has been demonstrated for the first time in Korea, and the Asian shore crab, *H. sanguineus*, has been shown to be the second intermediate host. Although the metacercariae of *M. novaezealandensis* were found in *Macrophthalmus* crabs [10], the survey on *M. japonicus* and *M. dilatatus* revealed no metacercariae of *Maritrema* spp. in Korea [11]. Since the tidal currents help to mix the cercariae popula-

tion from a large number of snails, the presumed 1st intermediate hosts, it was suggested that the crabs may commonly be infected by numerous kinds of cercariae [10].

The basis for identification of the present specimen as a new species was as follows: Above all, the vitelline follicles formed a complete ring in the present specimen. *M. pacifica*, *M. orensensis*, *M. neomi*, *M. patulus*, *M. magdalenense*, *M. borneoense*, *M. minuta*, *M. macropharynga*, and *M. jilinensis* were excluded due to the incomplete ring of vitellaria [12-15]. Ceca reached the lateral wall at the anterior border of the ovary in the specimen, but at the level of vitellaria in *M. arenaria* and in *M. paracadiæ* [13,16] and the testicular level in *M. bonaerensis* [17]. Postero-medial curving is present in *M. obstipum*, and ovary posterior to ventral sucker in *M. novaezealandensis* and *M. madryensis* [12,18,19]. Ventral sucker is larger than oral sucker in the present specimen, differentiating *M. pyrenaica* and *M. feluie* [15,20]. Ovary is trilobed in *M. spinosulum* and *M. eroliae* [14]. Ovary is more median in *M. ovata*, *M. gratiosum*, and *M. acadiaæ* [16]. The prominent metraterm is the characteristic feature shared by *M. bonaerensis* and *M. orensensis* [14,17]. Hence, it was concluded that the present specimen did not belong to the pre-existing *Maritrema* spp., and we suggested this new species of *Maritrema* as *M. jebuensis*.

However, the adults of *Maritrema* spp. are very similar in morphology, rendering separation of the species identification difficult. This difficulty is further complicated by the variation in structures generally used as taxonomic criteria [1]. For example, the disposition of the vitellaria, uterus, and sucker sizes showed considerable variation in *Maritrema obstipum* [18]. Genetic analysis is a powerful method to differentiate species, but few molecular studies have been performed on *Maritrema* spp., including *M. novaezealandensis* [10]. More information should be accumulated for molecular differentiation of *Maritrema* spp. Interestingly, the metacercariae of *Maritrema* n. sp. were mainly found in the muscles of crabs. This finding contrasts with those of *Gynaecotyla squatarolae*, being located in the hepatopancreas [8]. In addition, it was reported that the metacercariae of *M. novaezealandensis* were found encysted in the body cavity of the crustacean host [12]. These findings might help the differentiation of microphallid metacercariae during the research.

In case of *M. obstipum*, the infection in ducklings lasted 28 days, 24 days in chicks, 5 days in mice, and 2 days in hamsters [18]. It seems to be reasonable since *Maritrema* spp. utilize shorebirds as definitive hosts. In this study, the infection lasted only 7 days along with the low worm recovery rate due to the usage

of mice. Compared to *Microphallus koreana*, the adult worms of which were recovered from only chicks [9], the host specificity of *Maritrema* n. sp. might be low. From this study, the presence of *Maritrema* sp., named as *M. jebuensis*, was demonstrated in Korea for the first time. Because *Maritrema* is a parasite of birds, further study on birds is needed.

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