

Comparative Morphology of Minute Intestinal Fluke Eggs That Can Occur in Human Stools in the Republic of Korea

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Abstract: The egg morphology of minute intestinal flukes (MIF) that can occur as human infections in the Republic of Korea, i.e., *Metagonimus yokogawai*, *M. miyatai*, *M. takahashii*, *Heterophyes nocens*, *Heterophyopsis continua*, *Stellantchasmus falcatus*, *Stictodora fuscata*, *Pygidiopsis summa*, and *Gymnophalloides seoi*, was studied in comparison with *Clonorchis sinensis*. The adult worms were obtained from residents of endemic areas, and their intrauterine eggs were studied and measured using light microscopy; the length, width, length-width ratio (LWR), and Faust-Meleney index (FMI). Several specimens were processed for scanning electron microscopy (SEM), and before gold-coating, the uterine portion of each fluke was etched with a sharp pin in order to expose the eggs. The MIF eggs were ovoid, pyriform, or elliptical with a size range of 21-35 × 12-21 μm. *S. fuscata* eggs revealed the highest FMI (largest in the area) and lowest LWR, whereas *P. summa* eggs showed the lowest FMI and medium LWR. SEM revealed that *G. seoi* and *S. fuscata* had remarkably clean shell surface lacking the muskmelon-like structure which is prominent in *C. sinensis* eggs. In *Metagonimus* spp., *H. continua*, *H. nocens*, and *S. falcatus* eggs, minute surface ridges were recognizable though less prominent compared with *C. sinensis*. On the surface of *P. summa* eggs, thread-like curly structures were characteristically seen. The results revealed that important differential keys for MIF eggs include the length, width, area (FMI), shape of the eggs, and the extent of the muskmelon-like structure or ridges on their shell surface and operculum.

Key words: *Metagonimus yokogawai*, *Metagonimus miyatai*, *Metagonimus takahashii*, *Heterophyes nocens*, *Heterophyopsis continua*, *Stellantchasmus falcatus*, *Stictodora fuscata*, *Pygidiopsis summa*, *Gymnophalloides seoi*, *Clonorchis sinensis*, egg, trematode, ultrastructure

INTRODUCTION

At least 70 species of foodborne intestinal trematodes are known to occur as human infections around the world [1]. In the Republic of Korea, about 20 species are known, which consist of species belonging to the family Heterophyidae (= heterophyids), Echinostomatidae, Gymnophallidae (= gymnophallids), Plagiorchiidae, and Neodiplostomidae [2]. Among them, the heterophyid and gymnophallid flukes are minute in their body size, so they are often called as minute intestinal flukes (MIF) [3]. Their eggs are also minute and morphologically close to one another, and it is practically difficult to differentiate the species when they are discharged in the human feces unless they are apparently smaller or larger than others

or extremely unique in shape.

The zoonotic MIF species that reproduce small-sized eggs so far known in the Republic of Korea include 11 species, namely, *Metagonimus yokogawai*, *Metagonimus miyatai*, *Metagonimus takahashii*, *Heterophyes nocens*, *Heterophyopsis continua*, *Stellantchasmus falcatus*, *Stictodora fuscata*, *Stictodora lari*, *Pygidiopsis summa*, *Gymnophalloides seoi*, and *Acanthotrema felis* [2,4]. The eggs of these flukes are 18-35 μm in length and 12-19 μm in width. In our previous study, the uterine eggs of *M. yokogawai*, *H. nocens*, *H. continua*, *S. falcatus*, and *P. summa* were taken out and examined in comparison with those of *C. sinensis* using light microscopy [5]. The study concluded that the 6 kinds of eggs could be morphologically differed if careful observation and measurement of sufficient numbers of eggs were done [5]. Nevertheless, specific diagnosis has been difficult in areas of no previously known endemicity as well as in areas with mixed infections with different species of heterophyids [6]. In such areas, recovery of adult flukes is needed to obtain a definite diagnosis [6]. However, to provide tentative diagnosis of MIF infections, additional information is needed on the comparative

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morphology of MIF eggs. In the present study, we examined the egg morphology of 9 MIF species by means of light microscopy and scanning electron microscopy (SEM).

MATERIALS AND METHODS

Collection of worms

The following 9 MIF species were studied; *M. yokogawai* (Katsurada, 1912), *M. takahashii* Suzuki, 1930, *M. miyatai* Saito et al., 1997, *P. summa* Onji and Nishio, 1916, *H. nocens* Onji and Nishio, 1915, *H. continua* (Onji and Nishio, 1916), *S. falcatus* Onji and Nishio, 1916, *S. fuscata* (Onji and Nishio, 1916), and *G. seoi* Lee, Chai and Hong, 1993. The adult specimens of these flukes were obtained from people who live in endemic areas of various localities (Shinan-gun, Gangjin-gun, Umseong-gun, Aphae-do, and Jeju-do in the Republic of Korea) after treatment with praziquantel and purging with MgSO₄. Adult specimens of *C. sinensis*, which were used for controls, were obtained from a human patient and also from an experimental rat infected with metacercariae from the freshwater fish.

Measurements of egg size

The worms obtained from stool samples were washed with PBS several times and fixed in 10% formalin. Some worms were stained with acetocarmine, and others were kept unstained. They were morphologically identified in both formalin-fixed and acetocarmine-stained specimens. Uterine eggs were examined and measured in formalin-fixed specimens under cover slip pressure using a light microscope, and pictures were taken. In each MIF species, the length and width of 10 uterine eggs were measured, and the mean \pm SD values were obtained. For better understanding of the egg dimensions, the length-width ratio (LWR=L/W) and the Faust-Meleney Index (FMI) ($=L$ [length] $\times W^2$ [width] of the eggs) [7], were also calculated.

Preparation for SEM

For SEM, the formalin-preserved worms were washed with 0.1 M phosphate buffer and post-fixed with 1% osmium tetroxide for 2 hr at 4°C. The fixed worms were dehydrated in graded series of ethanol, and several worms were pooled for each species. The prepared worms were dried using a critical point dryer (CO₂). They were mounted on stubs, and their body was dissected with a pin to etch the upper part of uterine branches and expose their eggs. Then, the surface of the worms and eggs was coated with gold. The surface ultrastructure of

the uterine eggs was observed with a ABT DS-130C Scanning Electron Microscope (Tokyo, Japan). The eggs were photographed with a fixed magnification for all different MIF species and *C. sinensis*.

RESULTS

Egg size

The length, width, and LWR of the eggs were variable by different MIF species (Table 1; Figs. 1-30) and seemed to be helpful for specific diagnosis. In addition, FMI appeared to be another useful index for distinguishing the MIF eggs under light microscopy, although there were some overlappings between different species (Table 1; Fig. 31).

Based on the measurements, the eggs of *S. fuscata* were the largest in length (33.6 \pm 1.4), width (20.8 \pm 0.7), and FMI (14,608 \pm 1,495), whereas the eggs of *P. summa* were the smallest of all in length (20.9 \pm 0.9), width (12.1 \pm 1.0), and FMI (3,046 \pm 417) (Table 1). With regard to the length, the second largest was *M. takahashii* (32.6 \pm 0.8), followed by *M. miyatai*, *M. yokogawai*, *S. falcatus*, *H. nocens*, *H. continua*, and *G. seoi* (Table 1). The length of *C. sinensis* eggs (28.5 \pm 1.3) was almost the same as that of *M. yokogawai* eggs (28.4 \pm 1.3). In the egg width, the second widest was *M. takahashii* (18.9 \pm 1.3), followed by *M. miyatai*, *M. yokogawai*, *H. continua*, *H. nocens*, *S. falcatus*, and *G. seoi* (Table 1). The width of *C. sinensis* eggs (15.7 \pm 1.0) was almost the same as that of *H. continua* eggs (15.5 \pm 1.2) and slightly smaller than that of *M. yokogawai* eggs (16.1 \pm 1.4). The LWR was the largest for *S. falcatus* (2.1 \pm 0.1) and the smallest for *S. fuscata* (1.6 \pm 0.1). In FMI values, the second largest was *M. taka-*

Table 1. Measurements of the eggs of minute intestinal flukes and *C. sinensis* that occur in the Republic of Korea

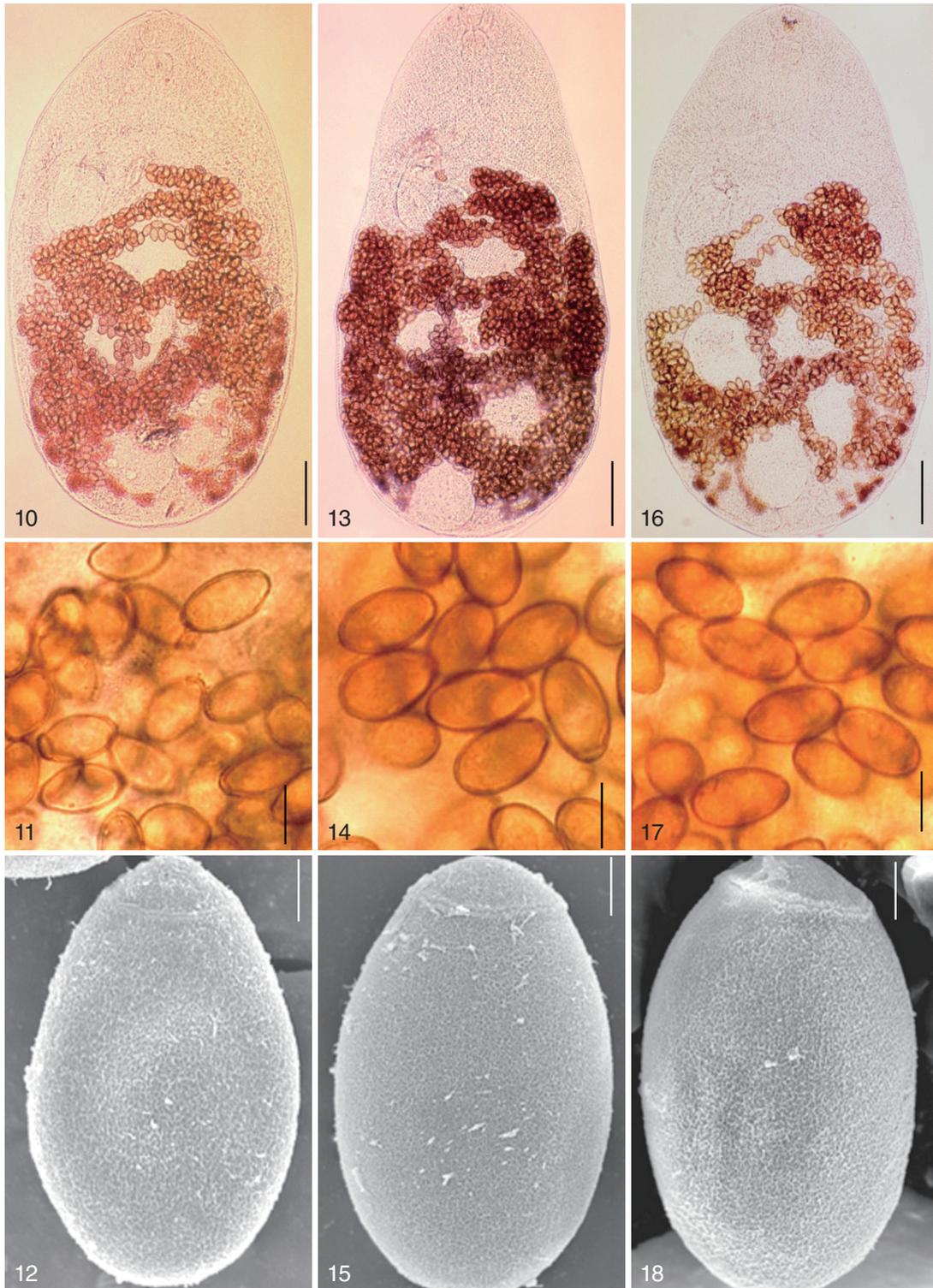
Fluke species	Length (μ m)	Width (μ m)	FMI ^a	LWR ^b
<i>Clonorchis sinensis</i>	28.5 \pm 1.3	15.7 \pm 1.0	6,872 \pm 593	1.8 \pm 0.2
<i>Pygidiopsis summa</i>	20.9 \pm 0.9	12.1 \pm 1.0	3,046 \pm 417	1.7 \pm 0.2
<i>Gymnophalloides seoi</i>	22.9 \pm 0.6	12.7 \pm 0.5	3,681 \pm 243	1.8 \pm 0.1
<i>Metagonimus yokogawai</i>	28.4 \pm 1.3	16.1 \pm 1.4	7,380 \pm 1,494	1.8 \pm 0.1
<i>Metagonimus miyatai</i>	30.8 \pm 0.9	17.8 \pm 0.8	9,702 \pm 1,042	1.7 \pm 0.1
<i>Metagonimus takahashii</i>	32.6 \pm 0.8	18.9 \pm 1.3	11,646 \pm 1,492	1.7 \pm 0.1
<i>Heterophyopsis continua</i>	24.0 \pm 1.0	15.5 \pm 1.2	5,747 \pm 757	1.6 \pm 0.2
<i>Heterophyes nocens</i>	26.0 \pm 1.6	14.1 \pm 1.2	5,151 \pm 975	1.9 \pm 0.2
<i>Stellantchasmus falcatus</i>	26.3 \pm 1.3	12.7 \pm 0.6	4,223 \pm 452	2.1 \pm 0.1
<i>Stictodora fuscata</i>	33.6 \pm 1.4	20.8 \pm 0.7	14,608 \pm 1,495	1.6 \pm 0.1

^aFMI (Faust-Meleney Index); length \times width².

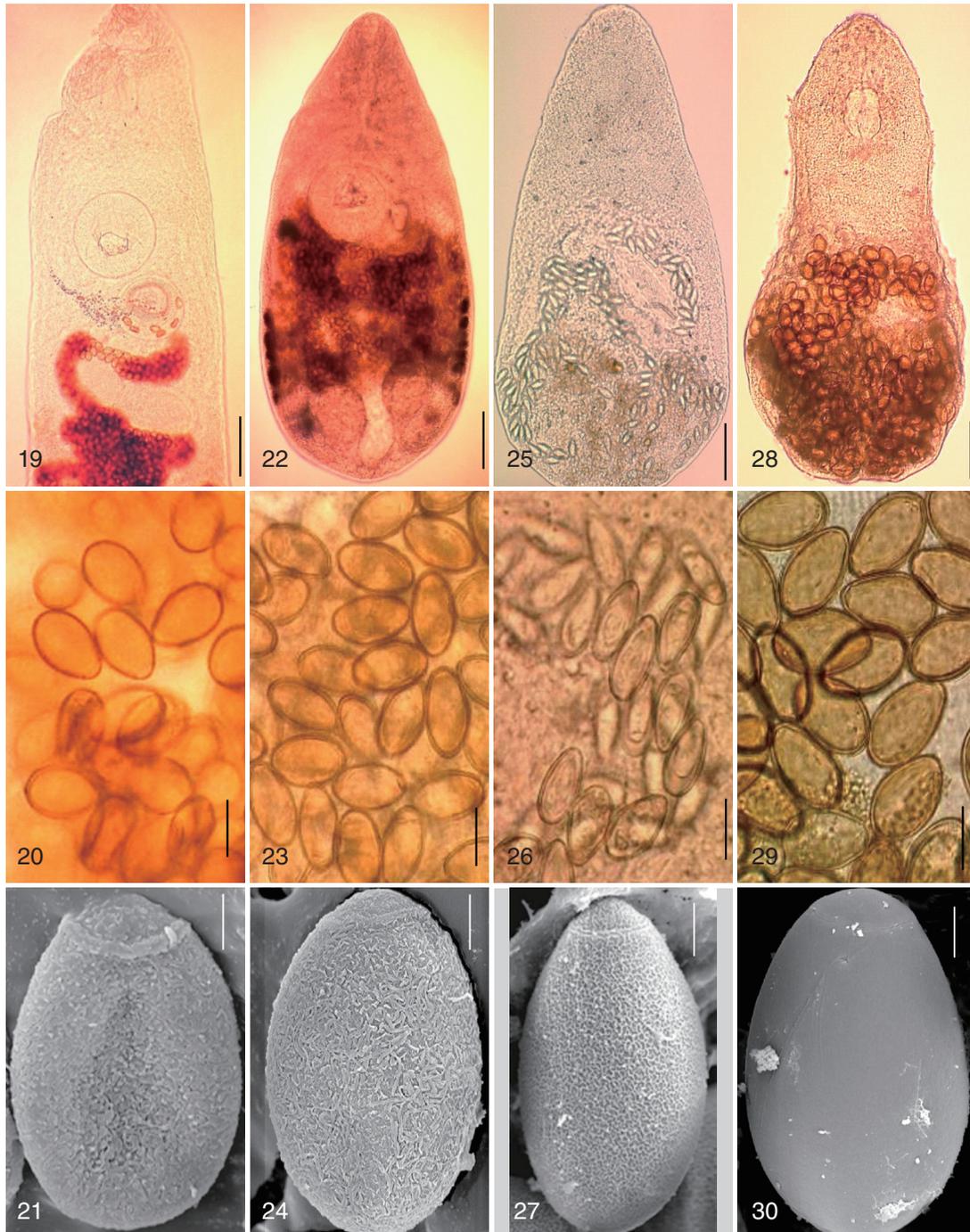
^bLWR (length-width ratio) = length: width.



Figs. 1-3. *Clonorchis sinensis*. 1. An adult worm obtained from an experimental rat. Bar=200 μ m. 2. Uterine eggs freed and seen under a light microscope. Bar=15 μ m. 3. SEM view of an egg showing the typical muskmelon-like structure on the surface. Bar=3 μ m. **Figs. 4-6.** *Pygidiopsis summa*. 4. An adult worm obtained from a naturally infected person on Aphae-do, Korea. Bar=100 μ m. 5. Uterine eggs. Bar=20 μ m. 6. SEM view of an egg showing the thread-like ridges on the surface. Bar=3 μ m. **Figs. 7-9.** *Gymnophalloides seoi*. 7. An adult worm obtained from a naturally infected person on Aphae-do, Korea. Bar=80 μ m. 8. Uterine eggs. Bar=20 μ m. 9. SEM view of an egg showing a big operculum and clean shell surface. Bar=3 μ m.



Figs. 10-12. *Metagonimus yokogawai*. 10. An adult worm obtained from a naturally infected person on Jeju Island, Korea. Bar = 150 μ m. 11. Uterine eggs. Bar = 20 μ m. 12. SEM view of an egg showing relatively clean surface only with multiple minute ridges. Bar = 4 μ m. **Figs. 13-15.** *Metagonimus miyatai*. 13. An adult worm obtained from a naturally infected person in Umseong-gun, Korea. Bar = 150 μ m. 14. Uterine eggs. Bar = 20 μ m. 15. SEM view of an egg showing relatively clean surface. Bar = 4 μ m. **Figs. 16-18.** *Metagonimus takahashii*. 16. An adult worm obtained from a naturally infected person in Umseong-gun, Korea. Bar = 150 μ m. 17. Uterine eggs. Bar = 20 μ m. 18. SEM view of an egg showing slightly rough surface. Bar = 4 μ m.



Figs. 19-21. *Heterophyopsis continua*. 19. Anterior part of an adult worm obtained from a naturally infected person on Aphae-do, Korea. Bar=150 μ m. 20. Uterine eggs. Bar=20 μ m. 21. SEM view of an egg showing rough surface with a prominent operculum and shoulder rims. Bar=4 μ m. **Figs. 22-24.** *Heterophyes nocens*. 22. An adult worm obtained from a naturally infected person in Gangjin-gun, Korea. Bar=150 μ m. 23. Uterine eggs. Bar=20 μ m. 24. SEM view of an egg showing rough surface with an inconspicuous operculum and shoulders. Bar=4 μ m. **Figs. 25-27.** *Stellantchasmus falcatus*. 25. An adult worm obtained from a naturally infected person in Shinan-gun, Korea. Bar=80 μ m. 26. Uterine eggs. Bar=20 μ m. 27. SEM view of an egg showing slightly rough surface and elongated shape. Bar=4 μ m. **Figs. 28-30.** *Stictodora fuscata*. 28. An adult worm obtained from a naturally infected person in Shinan-gun, Korea. Bar=120 μ m. 29. Uterine eggs. Bar=20 μ m. 30. SEM view of an egg showing clean surface and an inconspicuous operculum. Bar=4.5 μ m.

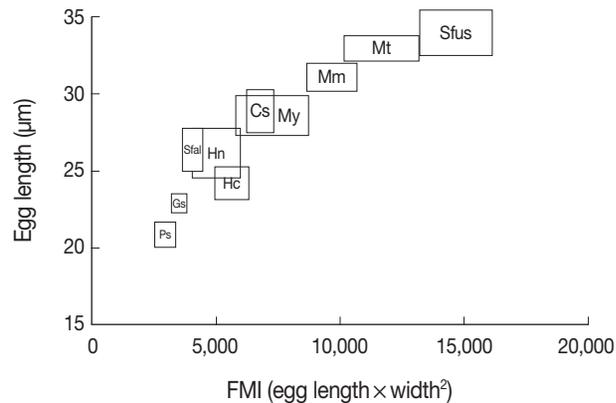


Fig. 31. A diagram showing the range (mean \pm SD) of the egg length and FMI of minute intestinal flukes and *Clonorchis sinensis* occurring in Korea. Ps, *Pygidiopsis summa*; Gs, *Gymnophalloides seoi*; My, *Metagonimus yokogawai*; Mm, *M. miyatai*; Mt, *M. takahashii*; Hc, *Heterophyopsis continua*; Hn, *Heterophyes nocens*; Sfal, *Stellantchasmus falcatus*; Sfus, *Stictodora fuscata*; Cs, *Clonorchis sinensis*.

hashii (11,646 \pm 1,492), followed by *M. miyatai*, *M. yokogawai*, *H. continua*, *H. nocens*, *S. falcatus*, and *G. seoi* (Table 1). The FMI of *C. sinensis* eggs (6,872 \pm 593) was greater than that of *H. continua* (5,747 \pm 757) and *H. nocens* (5,151 \pm 975) but smaller than that of *M. yokogawai* (7,380 \pm 1,494) (Table 1).

Egg shape

The adult flukes and their eggs were photographed under light microscopy and SEM (Figs. 1-30). In general, the eggs were ovoid, pyriform, or elliptical with a size range of 21-35 \times 12-21 μ m. *Metagonimus* spp., *H. nocens*, and *H. continua* eggs were ovoid, whereas *G. seoi* eggs were elliptical. *C. sinensis*, *P. summa*, *S. falcatus*, and *S. fuscata* eggs were pyriform. However, the morphology of some eggs was very similar to one another. *P. summa* eggs were highly similar to *C. sinensis* eggs except in their smaller size. Eggs of 3 *Metagonimus* species were indistinguishable except in their comparative sizes; largest for *M. takahashii*, followed by *M. miyatai* and then *M. yokogawai*. Eggs of *H. continua* were similar to eggs of *H. nocens* but the former was wider than the latter and stout. Eggs of *S. falcatus* were slender and long compared with those of *H. nocens* and *H. continua*. Eggs of *S. fuscata* were big and wide compared with those of *C. sinensis* and *Metagonimus* spp.

Surface morphology

The surface ultrastructure of the MIF eggs appeared to be a good marker for differential diagnosis from *C. sinensis* eggs.

Whereas the surface of *C. sinensis* eggs was covered with prominent, elevated ridges called muskmelon-like structures (Fig. 3), these structures were not observed in all MIF species eggs studied (Figs. 6, 9, 12, 15, 18, 21, 24, 27, 30). A similar surface morphology with *C. sinensis* was seen in *P. summa* eggs, in which thread-like curly structures were present (Fig. 9). In 3 *Metagonimus* spp., *H. continua*, *H. nocens*, and *S. falcatus* eggs, multiple surface ridges were recognized (Figs. 12, 15, 18, 21, 24, 27). However, the extent of the ridges was minute in *Metagonimus* spp., and relatively conspicuous in *H. continua*, *H. nocens*, and *S. falcatus*. By comparison, the egg shell surface was clean in *G. seoi* and *S. fuscata* eggs (Figs. 6, 30).

In some species, including *C. sinensis* (Fig. 3), *P. summa* (Fig. 9), *H. continua* (Fig. 21), and *H. nocens* (Fig. 24), the opercular junction was distinct and shoulder rims were prominent. However, shoulder rims were negligible in *G. seoi* (Fig. 6), *M. yokogawai* (Fig. 12), and *S. fuscata* (Fig. 30) eggs. The operculum was wide and big in *G. seoi* (Fig. 6), whereas it was narrow and small in *H. nocens* (Fig. 24) and *S. falcatus* (Fig. 27). The abopercular knob was present in some specimens of *C. sinensis*, *P. summa*, and *Metagonimus* spp., but absent in others (Figs. 3, 9, 15, 18).

DISCUSSION

MIF that include heterophyids and a gymnophallid represent significant health problems in various localities of the Republic of Korea [2,4,6]. The diagnosis is done by fecal examinations. However, morphological discrimination of the eggs in the feces using light microscopy is problematic [6]. The results of the present study revealed that not only the length, width, LWR, FMI, and shape of the eggs but also their surface ultrastructures as shown by SEM are distinctive markers that may be useful for discrimination of MIF eggs. The morphology of the shoulder rims, operculum, and abopercular knob was also helpful for the species differentiation.

The results of the present study were in good agreement with our previous study using light microscopy [5]. However, in the present study, eggs of *G. seoi*, *M. miyatai*, *M. takahashii*, and *S. fuscata* were newly added for study, and significant additional findings were obtained. The surface ultrastructures of MIF eggs occurring in the Republic of Korea were seldom studied by SEM, with the exception of a recent paleoparasitological report on *C. sinensis*, *M. yokogawai*, and *G. seoi* eggs [8]. In other countries, several reports were published on the SEM morphology of *C. sinensis*, *M. yokogawai*, *M. takahashii* (under the name *Metagoni-*

mus sp.), and *S. falcatus* eggs [9-11]. The results of the present study on these trematode species were well corresponded with those of the previous reports [8-11].

In our study, the eggshell surface of *P. summa*, *M. miyatai*, *H. nocens*, *H. continua*, and *S. fuscata* was studied for the first time using SEM. The surface ultrastructure of *P. summa* eggs were unique, having thread-like curly structures, which appeared to be useful for differential diagnosis of eggs. The eggshell surface of 3 *Metagonimus* spp. was characterized by the presence of minute ridges, although these ridges were more prominent in *H. nocens*, *H. continua*, and *S. falcatus* eggs. The present findings for *S. falcatus* were similar to those reported previously [11]. The surface ultrastructure of *S. fuscata* eggs was very smooth which was similar to *G. seoi* eggs [8]. In light microscopy, the eggshells of *S. fuscata* eggs were smooth and thick with an inconspicuous operculum [4]. In our study, *S. fuscata* eggs were much larger than those of *G. seoi* and had a small operculum.

In Thailand and Lao PDR, differentiation of *Opisthorchis viverrini* eggs from MIF eggs, including *Haplorchis taichui*, *Haplorchis pumilio*, *Haplorchis yokogawai*, *Prosthodendrium molenkampi*, *Phaneropsolus bonnei*, and *S. falcatus* was problematic, and studies on this subject were performed [11-14]. Light microscopic and SEM findings of these MIF eggs revealed unique features; for example, *Haplorchis* spp. eggs were characterized by the presence of filamentous mesh-like structures or thread-like ridges on the shell surface [11,12]. In *O. viverrini* eggs, the presence of muskmelon-like structures was characteristic and distinctly different from MIF eggs [11-13]. On the other hand, the presence of iodophilic bodies was characteristic in the eggs of *P. bonnei* and *P. molenkampi*, whereas such bodies were not seen in *O. viverrini* eggs [13,14]. Use of potassium permanganate solution (1% w/v) in fecal examinations was also useful for differentiation of *O. viverrini* eggs from *P. molenkampi* and *P. bonnei* eggs [15].

In the present study, the FMI appeared to be a useful index for MIF egg differentiation. Although there were some overlaps in the range, the mean values of FMI were quite specific for each different species of fluke. This was in agreement with a previous report in which FMI was applied to differentiate MIF, *O. viverrini*, and *C. sinensis* eggs [11].

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