

A new species and a new record of *Meghimatium* Slugs (Pulmonata: Philomycidae) in Korea

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Abstract: Until now, five species (*Meghimatium bilineatum*, *M. fruhstorferi*, *Limax flavus*, *L. marginatus*, *Deroceras reticulatum*) in three genera of families Philomycidae and Limacidae have been reported in Korea. Philomycidae is a family of air-breathing land snails. *Meghimatium hongdoensis* sp. nov. is described based on its middle size (60–80 mm in body length), its body coloration (dark-red yellow), no dorsal with streaks, its genitalia, and 16rDNA sequence analysis. Specimens of this new species were collected from Hongdo island, Sinan-gun, Jeollanam-do, Korea. This species is only known from the type locality at Hongdo to date. It could be found in high-humidity sites. *Meghimatium uniforme* (Laidlaw 1937) was the first one reported in Korea. It was also collected from Gageodo island, Sinan-gun, Jeollanam-do, Korea. In this study, morphological characteristics including their radula and genital structures of these two species were described. Preliminary results of mitochondrial 16S rDNA sequencing and phylogenetic analyses indicated that these species belonged to the Korean clade.

Keywords: Philomycidae, *Meghimatium hongdoensis* sp. nov., *M. uniforme*, new record, morphology, molecular analyses

INTRODUCTION

Slug, or land slug, is a common name for any apparently shell-less terrestrial gastropod molluscs. Especially, the genus *Meghimatium* is a common land slug in tropical and subtropical areas, including East Asia. These are known as considered a pest of great importance in numerous crops, horticultural facilities, plant nurseries, and garden. The word slug is also often used as part of the common name of any gastropod molluscs that has no shell, a very reduced shell, or only a small internal shell. The mantle is a fleshy lobe that secretes the shell in snails. It is vestigial in most slugs but useful for identification. The keel (carina) is a backbone-like ridge that runs the length of the back in some slug species. Philomycidae slug is mantle covering

nearly the entire back of the animal. This family of primitive aulacopod slugs is related to the slug family Arionidae, and to the Punctidae, the latter a family with well-developed external shells. Philomycids are found all over Korea. Coloration of live *Meghimatium* species is diverse and attractive (Schilthuizen and Liew 2008). Early authors have described numerous species of the genus in Asia (Cockerell 1890; Simroth 1902). These descriptions were based on external and internal characteristics, especially the coloration of preserved slugs, leaving the examination of genitalia out of consideration (Collinge 1901, 1903; Tsai *et al.* 2011). It was Hoffmann (1924) who critically revised and synonymized many of the described species. Five species (*Meghimatium bilineata*, *M. fruhstorferi*, *Limax flavus*, *L. marginatus*, and *Deroceras reticulatum*), three gen-

era of the families Philomycidae and Limacidae until now were reported of Korea. Morphological studies of land mollusks evolutionary relationships have been hampered by the long evolutionary history of the group, by relatively rapid radiations, and by convergences, and this has led to a number of competing classifications (Tillier 1988). The mitochondrial genome (mitogenome) can provide information for phylogenetic analyses and evolutionary biology (Wade *et al.* 2006; Tsai and Wu 2008). Although there are still many questions to be answered, recent molecular studies of the land snails and slugs (Emberton *et al.* 1990; Tillier *et al.* 1996; Thollesson 1999; Wade *et al.* 2001; Grande *et al.* 2004; Wade *et al.* 2006) have begun to provide remarkable new insights into stylommatophoran evolutionary relationships. This paper described a new species and a new record collected from Hongdo and Gageodo island, Korea. All type material has been lodged with the Laboratory of Environmental Medical Biology (LEMB), Gangneung, Gangwon-do (LEMB201700010-201700013, LEMB201700020-201700023).

MATERIALS AND METHODS

1. Specimen collections and morphology-based identifications

Slugs are collected with hands and some of the slugs were transferred to the laboratory for their morphological determination and dissection. The soft body were removed for anatomy. The genital structures were dissected with tweezers, fixed in 70% alcohol for 10–15 minutes, and examined under a Nikon XMZ 800N stereo microscope (Nikon, Japan). The radula removed from the proboscis was kept in a boiling tube containing alkaline solution. For radula extraction, live specimens were initially anaesthetized using 7.5% magnesium chloride to relax and loosen the body muscles attached to the shell and facilitate easy removal of the organism. Subsequently, an incision was made on the dorsal surface of the head to expose the radular sac located within the mantle cavity. The radular sac was transferred to 19% KOH solution for digestion of radular tissue. Subsequently, the radula was washed with distilled water and dehydrated using alcohol. Dehydration was done by immersing the radula in increasing concentration of alcohol (50, 70, 90, and 100%) (Rios *et al.* 2019). Then the dehydrated radulae were brought to the next step of coating making them suitable for FESEM observation (Field Emission Scanning Electron Microscope, FEI, Inspect F,

Brno, Czech Republic). Initially, the radulae mounted on brass stubs with a double-sided tape were coated with gold and placed into the specimen chamber of the SEM. Subsequently, photographs were taken at various magnifications depending on the size of the radula. Radula length and rachidian width were measured from the photographs. The terminology used in the description of radula follows Kool (1987). Voucher specimens are deposited in the Department of Environmental Medical Biology, Catholic Kwandong University, Gangwon-do, Republic of Korea.

2. Molecular analysis

DNA was extracted from the tissue pieces of five specimens to each species, using the QIAamp Tissue Kit (Qiagen Inc., Valencia, CA). Cell lysis was achieved overnight in a 56°C constant temperature shaker with proteinase K and Qiagen lysis buffer. Polymerase chain reaction (PCR) was used to amplify a 443 bp fragment of the mitochondrial 16S rDNA gene (Mc Donnell *et al.* 2011). The aligned 16S sequences were then compared with those for the different species of *Meghimatium striatum* (accession no.: FJ896801, Taiwan), *M. pictum* (FJ896783, Taiwan), *M. burchi* (FJ896795, Taiwan), *M. fruhstorferi* (FJ896776, Taiwan) and *M. uniforme* (FJ896800, Taiwan). Neighbor-Joining (NJ) trees based on Kimura-2-parameter distances, 1000 bootstrap replicates and using *Limax flavus* (FJ896815, Taiwan) and *Arion alpinus* (AY947347, Belgium) as outgroups were used to confirm the association of the sequences with haplogroups. The NJ tree reconstruction and analysis were carried out with MEGA v. 4 software (Kumar *et al.* 2008).

SYSTEMATIC ACCOUNTS

Class Gastropoda Cuvier, 1795
Order Stylommatophora A. Schmidt, 1855
Family Philomycidae Gray, 1847
Genus *Meghimatium* van Hasselt, 1823

Meghimatium hongdoensis n. sp.

홍민달팽이 (신칭) (Figs. 1, 2)

Material examined. South Korea: Jeollanam-do, Shinan-gun, Heuksan-myeon, Hongdo, 34°41'05.32"E, 125°11'43.09"E.

Measurements.

Holotype: 82 mm L, 9 mm W.

(23 vii 2017, G.M. Park, LEMB201700010)

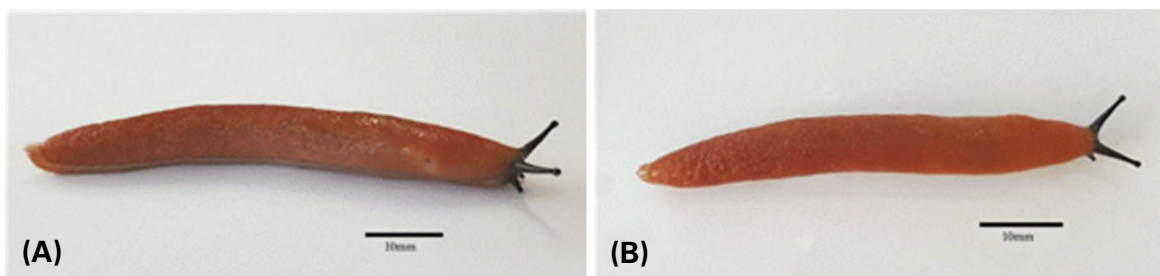


Fig. 1. *Meghimatium hongdoensis* n. sp. (A) Habitus, lateral view; (B) Habitus, dorsal view. Scale bars = 10 mm.

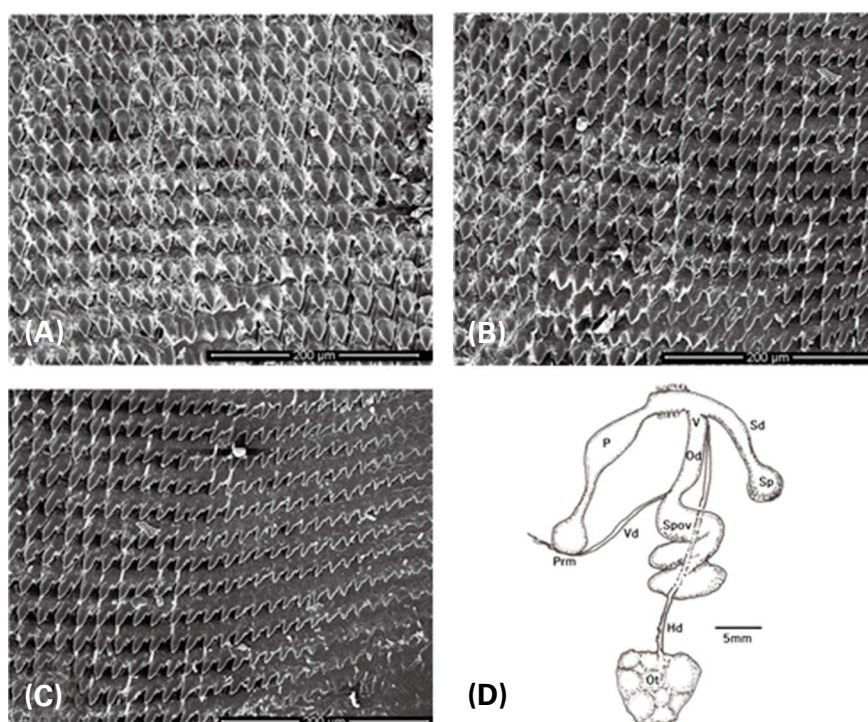


Fig. 2. *Meghimatium hongdoensis* n. sp. radula showing central tooth (11th tooth from the left side) and lateral teeth (A and B), lateral and marginal teeth (B), right side of lateral and marginal teeth (C), and genitalia (D). Hd, hermaphroditic duct; Od, oviduct; Ot, ovotestis; P, penis; Prm, penial retractor muscle; Sd, spermathecal duct; Sp, spermatheca; Spov, spermoviduct; V, vagina; Vd, vas deferens.

Paratype 1: 75 mm L, 6 mm W.

(23 vii 2017, G.M. Park, LEMB201700011)

Paratype 2: 60 mm L, 6 mm W.

(24 vii 2017, G.M. Park, LEMB201700012)

Paratype 3: 80 mm L, 8 mm W.

(24 vii 2017, G.M. Park, LEMB201700013)

Diagnosis. Body length 60–80 mm, width 8 mm; Body coloration: dark red yellow, no dorsal with streaks (Fig. 1). Lateral sides and dorsal side generally same color; genital pore

located at 1/7 body length from anterior end. Sole white. Radula: 12 mm long and 2.8 mm wide. Radula with about 120 transverse rows; each transverse row with a central tooth and about 34 lateral and marginal teeth. Central tooth with a pointed cusp and a slit on both sides of it; lateral teeth with an elongated triangular cusp, and a slit on outer side of cusp which becomes more distinct and appears as a separate denticle; outwardly marginal teeth with cusps and denticles becoming smaller and eventually disappearing

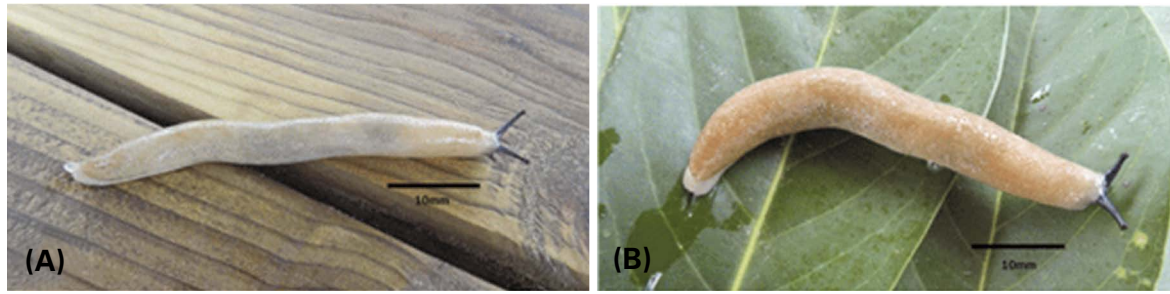


Fig. 3. *Meghimatium uniforme* Laidlaw, 1937. (A) Habitus, lateral view; (B) Habitus, dorsal view. Scale bars = 10 mm.

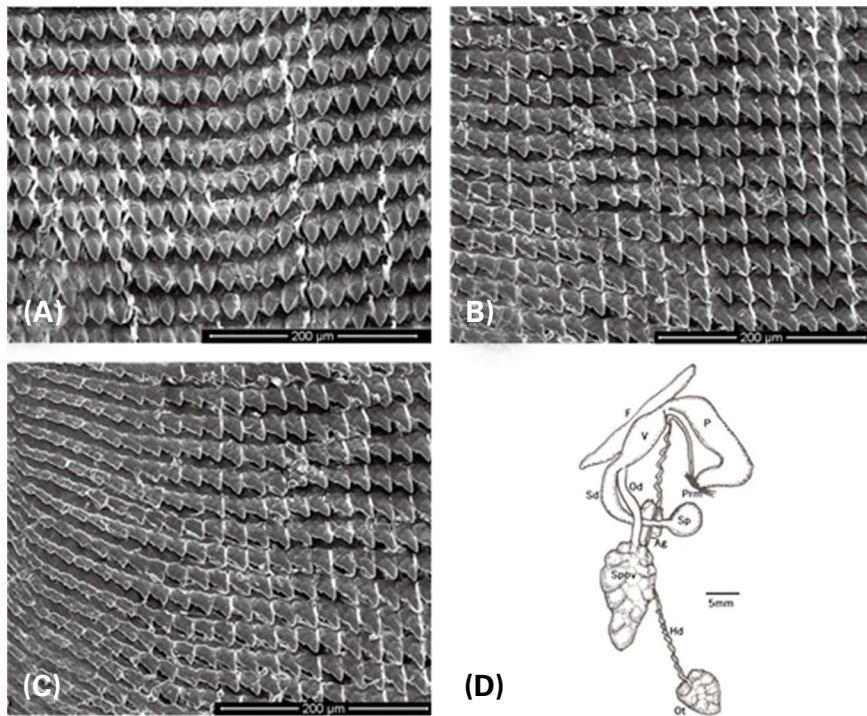


Fig. 4. *Meghimatium uniforme* radula showing central tooth (11th tooth from the left side) and lateral teeth (A), lateral and marginal teeth (B), the right side of lateral and marginal teeth (C), and genitalia (D). Ag, Albumin gland; F, foot; Hd, hermaphroditic duct; Od, oviduct; Ot, ovotestis; P, penis; Prm, penial retractor muscle; Sd, spermathecal duct; Sp, spermatheca; Spov, spermooviduct; V, vagina.

(Fig. 2A–C). Genitalia (Fig. 2D): Ovotestis (Ot) of moderate size, about 3 mm in diameter and 2 mm in height; hermaphroditic duct (Hd) of moderate length (about 15 mm in length) and convoluted; spermooviduct (Spov) long (about 7 mm in length) and thick; free oviduct (Od) about 5 mm long; spermatheca (Sp) oval-shaped; penis about 22 mm long; spermathecal duct thick and short (about 6 mm long); vagina of moderate size (about 2.5 mm long).

Remarks. Coloration of live *Meghimatium* species is diverse. The color of alcohol-preserved specimens, however, fades and changes within a short period of time. Based on

its size the slug can well be distinguished from the middle other *Meghimatium* spp. External morphological characteristics of *M. hongdoensis* have middle size, dark-red in body coloration and no dorsal with streaks. In case of *Meghimatium bilineatum* (Benson 1842), three rows of black bands extend from head to tail, one row at each side of the body, and one row at the center of the back along the body axis. On the other hands, *M. fruhstorferi* have a black spot in the center extends from the head to the tail, and cloud-shaped pattern appear on both sides, and body color changes to black as they mature. The radular morphology of *M. hong-*

doensis sp. nov. is similar to those of the other three Korean *Meghimatium* species. Genitalia of *M. hongdoensis* sp. nov. differ from those of the other three Korean *Meghimatium* in the proximal end of sperm duct expanding then narrowing before entering the penis. Looking at the morphological differences of radula reported so far within the genus *Meghimatium*, *M. pictum* had 138 transverse rows and *M. burchi* 140 rows, whereas this species showed a differences of 120 rows (Tsai and Wu 2008; Gomes *et al.* 2011).

Habitat. Humid soil on the ground, crack in the stone or rocks.

***Meghimatium uniforme* Laidlaw, 1937**

가거민달팽이 (신칭) (Figs. 3, 4)

Material examined. South Korea: Jeollanam-do, Shinnan-gun, Heuksan-myeon, Gageodo, 34°03'29.42"E, 125°07'15.59"E.

Measurements.

Sample 1: 51 mm L, 10 mm W.

(3 ix 2017, G.M. Park, LEMB201700020)

Sample 2: 53 mm L, 10 mm W.

(3 ix 2017, G.M. Park, LEMB201700021)

Sample 3: 45 mm L, 10 mm W.

(4 ix 2017, G.M. Park, LEMB201700022)

Sample 4: 55 mm L, 10 mm W.

(4 ix 2017, G.M. Park, LEMB201700023)

Sample 5: 43 mm L, 8 mm W.

(VQUMIV000008645, deposited in National Institute of Biological Resources (NIBR))

Diagnosis. Body length 30–50 mm, width 4 mm (Fig. 3); Moderately slender, with uniform coloration that can

range from pale grey to yellow close to white. Radula: 10 mm long and 2.4 mm wide. Number of rows of teeth 114 + nascent. Formula 34-1-34. Outer marginal rudimentary. Central tooth with a pointed cusp, and a slit on outer side of cusp which becomes more distinct and appears as a separate denticle; outwardly marginal teeth with cusps and denticles becoming smaller and eventually disappearing (Fig. 4A–C). Upper tentacles black. Penis extremely short (9 mm long). Genitalia (Fig. 4D): Ovotestis (Ot) is about 2.5 mm in diameter and 1.3 mm in height; hermaphroditic duct (Hd) about 18 mm in length; spermooviduct (Spov) is oval shape and long about 17 mm in length; oviduct (Od) about 8 mm long; spermatheca (Sp) oval-shaped; Penis (P) vermiform and long, slightly folded and penial retractor muscle (Prm) is attached; vagina (V) slightly swollen.

Remarks. Laidlaw (1937) original descriptions on *Meghimatium uniforme* - "The specimens average about 20 mm. in length, and are of a uniform gray-brown color, entirely without markings". In the case of *M. uniforme* found this time, differed from other congeneric species by body color and radula form reported in Korea so far. The type locality of *M. uniforme* is Mount Kinabalu, North Borneo, Malaysia. This species has an average mature body length of 20 mm and has a uniform grayish-brown ground color this no markings on its dorsal body. When comparing the body size of the type species, the Korean specimen is generally much larger (45–55 mm) than the type specimens. Tsai *et al.* (2005) compared 4 species of the genus *Meghimatium* from Taiwan. According to their sizes, there were the large-sized *M. fruhstorferi* and *M. rugosum*, and the medium-sized *M. bilineatum* and *M. pictum*.

Habitat. Humid soil on the ground, crack in the stone.

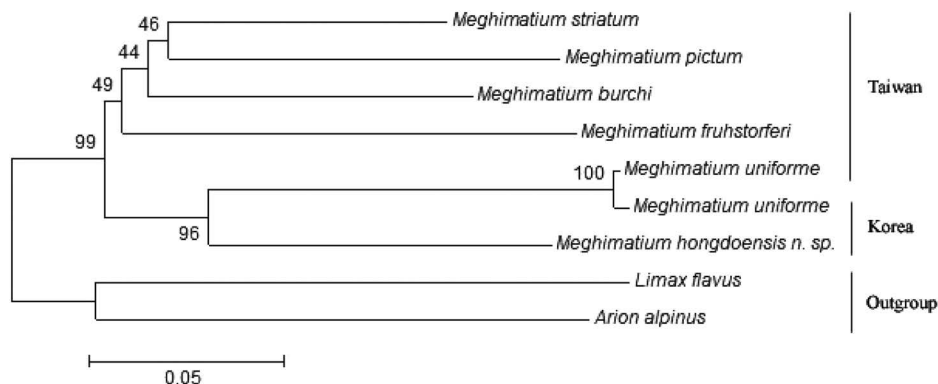


Fig. 5. Assignment of partial 16S gene sequences (443 bp) of Korean specimens of *M. uniforme* and *M. hongdoensis* with other *Meghimatium* species from GenBank. A neighbor-joining tree was constructed based on Kimura-2-parameter distances with 1000 bootstrap replicates using *Limax flavus* and *Arion alpinus* as outgroups.

DISCUSSION

Genetic divergence in geographically isolated populations is a prerequisite for allopatric speciation, generally considered one of the most common modes of speciation in animals (Barraclough and Vogler 2000; Coyne and Orr 2004). Such divergence may involve either adaptation to differences in the environment, or environmentally neutral divergence, or a combination of processes (Schilthuizen *et al.* 2006). Shell morphology alone could not reveal clearly the differences between these species, hence a combination of shell, radula and genital structures known to be important in the classification molluscs were investigated for their significance in the identification of the invasive snail species. This study was undertaken to reveal the differences in the morphology, radula and genital structures of the two species and provide notes on characters that can be important for their identification and to contribute to the available information on the terrestrial molluscan species that is still poorly investigated.

From the point of view of body size, *M. hongdoensis* sp. nov. is the small size than *M. fruhstorferi* and *M. bilineatum* (Kwon *et al.* 2001). This species has an average mature body length of 20 mm and has a uniform grayish-brown ground color with no markings on its dorsal body. The Korean *M. uniforme* is larger body lengths than of type locality species and light-brown body color. The radular morphology of *M. hongdoensis* sp. nov. is similar to the other three Korean species, namely *M. uniforme*, *M. fruhstorferi* and *M. bilineatum*. A comparison of these two *Meghimatium* species showed that the proximal ends of the vas deferens (VD) are all expanded.

To elucidate the phylogeny of these species, we sequenced an approximately 443-bp fragment of the mitochondrial 16S gene from five individuals per species, and performed maximum parsimony analysis. This showed that all species are monophyletic (Fig. 5). *Meghimatium hongdoensis* sp. nov. belongs to the monophyletic Korean ingroup. Phylogenetic reconstruction presents two clades on *Meghimatium*: clade I composed of four species in the Taiwan species, and clade II containing the Korea species. In the future, it is necessary to collect more slugs in this country and find new species by comparing them.

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