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A newly recorded species of *Assiminea grayana* J. Fleming, 1828 (Gastropoda: Assimineidae) from the Korean Peninsula

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ABSTRACT

The genus *Assiminea* (Gastropoda: Assimineidae) are amphibious, brackish snails that inhabit moist areas near marine or non-marine water bodies. Here, eleven specimens of *Assiminea grayana* are collected from river estuaries and the intertidal to supratidal zones of the western and southern seas of the Korean Peninsula. It is the first record of *Assiminea grayana* J. Fleming, 1828 in the Korean fauna. To verify the species identification, we examined its ecological characteristics, morphological features and DNA barcoding, which consistently supported the existance of *A. grayana* in Korea. This species has 6-7 slightly swollen whorls, impressive suture, and sharpened apex. The angle of the aperture acute was adapical. The blackish cephalic tentacles form short, rounded lobes each with eyes at the tip, and their gray snout is divided into two parts. Its *COI* sequence showed 99.7% similarity to those of individuals from the UK and Germany. The ML tree showed a close relationship of *A. grayana* from Korea and Europe, which formed a sister group with *A. hiradoensis* and *A. estuarina* exclusively inhabiting Northeastern Asia. It is likely to imply that *A. grayana* might be originated from Northestern Asia. Further studies integrating morphological and molecular data are essential for clarifying the taxonomic status and migration histories of *A. grayana* and related species.

Keywords: New record, *Assiminea grayana*, Molecular species identification, Molecular phylogeny, Species migration, the Korean Peninsula

INTRODUCTION

The family Assimineidae H. Adams and A. Adams, 1856 is considered one of the most derived families among truncatelloidean gastropods (Ponder, 1988). Assimineids are widely distributed in temperate and tropical regions (Marcus and Marcus, 1965; Abbott, 1974; van Aartsen, 2008). The type genus of the family Assimineidae, *Assiminea* J. Fleming, 1828 is known as one of the genera with the highest species diversity within the family. It is known that all species of the genus *Assiminea* are amphibious, residing in moist areas near the periphery of marine or permanent non-marine water bodies (Abbott, 1958). These snails feed on mud detritus and animal matter, contributing to the purification of the environment (Abbott, 1958). Until now, there have been reported 56 *Assiminea* species worldwide (Molluscabase, 2024). In the Korean Peninsula, only four *Assiminea* species have been recorded so far, which are exclusively found in Northeastern Asia: *A. estuarina*, *A. hiradoensis*, *A. japonica* and *A. lutea* (Kang, 1971; Kwon and Habe, 1979).

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The type species of the genus *Assiminea*, *Assiminea grayana* which inhabits brackish water areas (Fleming, 1828) has been recorded only in Northwestern Europe such as UK, Germany, France etc. Due to the continued decline of the habitat, this species has been listed for The IUCN Red List of Threatened Species as Least Concern (Killeen *et al.*, 2017). Many assimineid taxonomic groups have been challenged for exact species identification due to their indistinct morphological characteristics and insufficient morphological character descriptions in previous reports (e.g. Miranda, 2014; Das *et al.*, 2021). Thereby it is needed to conduct molecular species identification based on DNA barcode information like *COI* for reliable Assimineidae classification (e.g. Hwang and Kim, 1999; Hwang *et al.*, 2004; Baek *et al.*, 2013; Cha *et al.*, 2018; Baek *et al.*, 2020; Choi *et al.*, 2020; Choi *et al.*, 2021; Hong *et al.*, 2023). In this study, we first record *A. grayana* in the Korean fauna with its morphological diagnosis (shell, head-foot, radula, etc.), DNA barcoding, and molecular phylogenetic analyses.

MATERIALS AND METHODS

The eleven specimens of *A. grayana* were collected in the upper intertidal zone and estuary brackish area of the western and southern coasts of the Korean Peninsula. The collected samples were fixed in 95% ethanol, stored frozen at −20°C and deposited in the lab of UWH, Kyungpook National University (KNU), Daegu, South Korea (voucher nos. LEGOM030630-LEGOM030639) and Honam National Institute of Biological Resources (HNIBR), Mokpo, South Korea (HNIBRIV8507). The specimens were identified morphologically under a microscope (M205, Leica Camera AG, Germany). The dorsal, ventral, and lateral views of the shell were captured and combined from stacks of images with LAS v4.12. Morphological terminologies were followed by Hershler (1987). The mantle portion of the specimen was opened, and the radula was dissected and extracted from the organs. The separated radula was rinsed with distilled water, and the remaining tissues were gently removed with a fine brush. After cleaning, the sample was dried on the plate and coated with platinum ions. The shape of the radula was observed with Field Emission Scanning Electron Microscopy (FE-SEM) (SU8220, Hitachi Co., Japan).

For molecular species identification, DNA barcoding analyses based on the partial mitochondrial *COI* fragment (681 bp) were performed. 1 mm^3 of the foot muscle tissue was used to extract DNA from the specimen (Siheung, the western sea of the Korean Peninsula) following the protocol with a DNeasy Blood & Tissue kit (QIAGEN, California, USA). The mitochondrial *COI* fragment was amplified using the universal primer set (LCO1490 and HCO2198; Folmer *et al.*, 1994). The resultant *COI* nucleotide sequence from an individual of the species was registered under the NCBI GenBank accession no. PP470768. We conducted a BLAST search using the GenBank database to find sequence matches. The BLAST search with the parameters including the ‘Nucleotide’ collection database and the MegaBlast program resulted in a list of the 100 closest nucleotide sequences, from which we selected the top suggestion with the highest Bit-score or Max score.

The partial 46 *COI* sequences (= DNA barcoding region) of ten *Assiminea* species were retrieved from the NCBI GenBank database and aligned using BioEdit version 7.0 (Hall, 1999). The number of variable nucleotide sites and haplotypes were calculated in the *COI* sequences (579 bp) using DnaSP 6 software (Rozas, 2017). A unrooted phylogenetic tree of the genus *Assiminea* was conducted with the 32 haplotypes extracted from 46 *COI* sequences using the maximum likelihood (ML) method. A sequence evolutionary model selection performed within the IQ-tree webserver (http://iqtree.cibiv.univie.ac.at) identified the TIM3 + F + G4 substitution model as the best fit under the Bayesian information criterion. The ML tree was computed from 1000 ultrafast bootstrap replicates using the IQ-tree webserver. The GenBank accession numbers and sampling localities of 46 *COI* nucleotide sequences employed for the phylogenetic analyses are listed in Table 1.

Table 1. The summery of GenBank accession numbers, localities, and references of 32 *COI* haplotype sequences of ten *Assiminea* species employed in the present phylogenetic analyses.

No.	Species	GenBank Acc. No.	Locality	Reference
1	*Assiminea grayana*	PP470768	Siheung, KR	This study
2	*Assiminea grayana*	EF667310	Kent, UK	Hershler, 2008
		HQ623170	Niedersachsen, DE	Wilke, 2013
3	*Assiminea hiradoensis*	AB611807	Nagasaki, JP	Kameda, 2011
		OL877321* OL877322* OL877323*	KR	Unpublished
4	*Assiminea hiradoensis*	MT240258	CN	
5	*Assiminea estuarina*	PP389405		
6	*Assiminea cienegensis*	DQ533841	Coahuila, MX	Hershler, 2007
7	*Assiminea pecos*	DQ533842	Texas, US	
8	*Assiminea pecos*	DQ533843		
9	*Assiminea pecos*	DQ533844		
10	*Assiminea pecos*	DQ533845		
11	*Assiminea pecos*	DQ533847 DQ533848	New Mexico, US	
		DQ533846 DQ533850 DQ533852 DQ533853 DQ533854	Texas, US	
12	*Assiminea pecos*	DQ533849		
13	*Assiminea pecos*	DQ533851		
14	*Assiminea californica*	DQ533855		
15	*Assiminea californica*	DQ533856	California, US	
16	*Assiminea californica*	DQ533857		
17	*Assiminea californica*	DQ533858		
18	*Assiminea californica*	EF667296	Sonora, MX	Hershler, 2008
19	*Assiminea californica*	EF667297	California, US	
20	*Assiminea succinea*	DQ533859	Texas, US	Hershler, 2007
21	*Assiminea succinea*	DQ533860		
22	*Assiminea succinea*	DQ533861		
23	*Assiminea succinea*	DQ533862		
24	*Assiminea succinea*	EF667298	New York, US	Hershler, 2008
		OQ323272 OQ323344 OQ323415	Virginia, US	Unpublished
25	*Assiminea succinea*	OQ322741		
26	*Assiminea succinea*	OQ323138		
27	*Assiminea succinea*	DQ533863	California, US	Hershler, 2007
		EF667303		Hershler, 2008
28	*Assiminea infima*	EF667300		
29	*Assiminea infima*	EF667301		
30	*Assiminea infima*	EF667302		
31	*Assiminea andrewsiana*	HG973051	Christmas Island, AU	Unpublished
32	*Assiminea capensis*	KC439797	AU	Golding, 2014

*These species are suspected to be *A. hiradoensis* misidentified as *A. estuarina*.

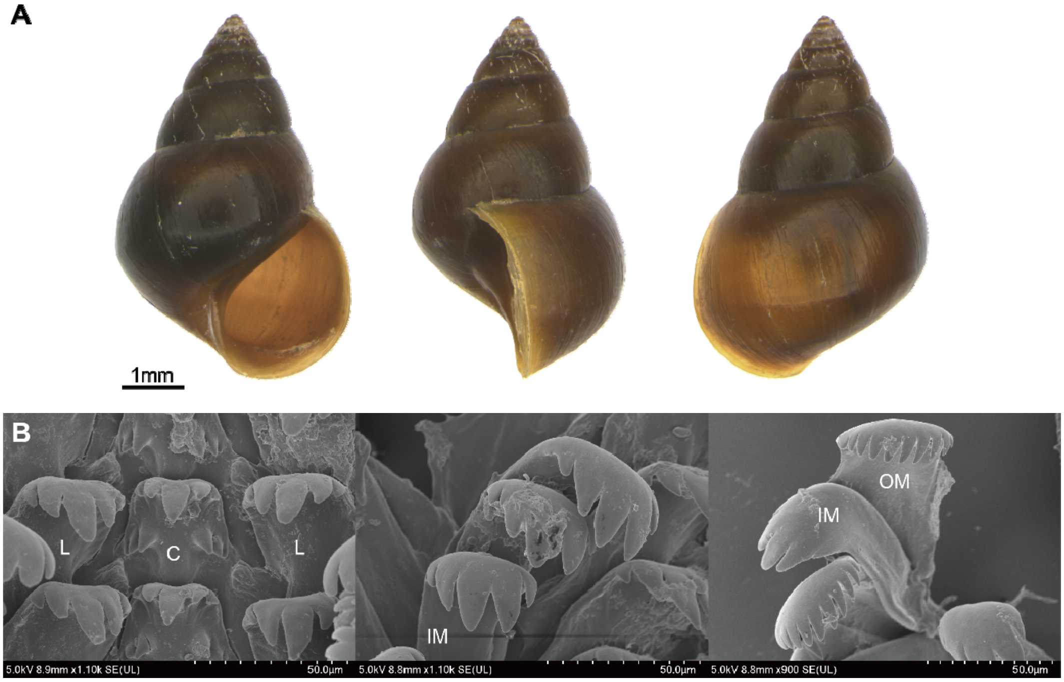

Fig. 1. Photographs of shell and radula of *Assiminea grayana*. **A.** Dissection microscopic photographs of dorsal, lateral, and ventral views of the shell in order; **B.** Scanning electron microscopic photographs of radula. L, lateral teeth; C, central teeth; IM, inner marginal teeth; OM, outer marginal teeth. A scale bar is depicted on each photograph.

SYSTEMATIC ACCOUNTS

Phylum Mollusca Linnaeus, 1758 연체동물문
Class Gastropoda Cuvier, 1795 복족강
Order Littorinomorpha A. N. Golikov and Starobogatov, 1975 총알고둥목
Family Assimineidae H. Adams and A. Adams, 1856 기수우렁이과
Genus *Assiminea* J. Fleming, 1828 기수우렁이속

***Assiminea grayana* J. Fleming, 1828 유럽기수우렁이 (신칭) (Fig. 1)**

Assiminea grayana J. Fleming, 1828: 275; *Assemania ostiorum* Bavay, 1920: 161.

Type locality. Greenwich marshes, Thames estuary, England.

Materials examined. Korea: 5 specimens, Janggok-dong, Siheung-si, Gyeonggi-do, 37°23'34.9"N, 126°46'49.2"E, 15 Mar 2023, Cho Rong Shin (HNIBRIV8507, LEGOM030630-0633); 3 specimens, Samdu-ri, Gunoe-myeon, Wando-gun, Jeollanam-do, 34°20'40.0"N, 126°38'44.0"E, 16 Jun 2023, Cho Rong Shin (LEGOM030634-0636) ; 3 specimens, Waryong-ri, Bukpyeong-myeon, Haenam-gun, Jeollanam-do, 34.25.29.0N, 126°39'04.0"E, 16 Jun 2023, Cho Rong Shin (LEGOM030637-0639).

Diagnosis.

Shell 5.26-7.98 mm in height; 3.34-4.73 mm in width; 2.12-3.52 mm in aperture height; 1.61-2.79 mm in aperture width. Solid, conical, chestnut or horn-brown, shiny, with minute lines of growth; nearly flat 6-7 whorls, last bend more convex, half or more of the height of the shell; spiral small, not sharp; impressive sutures; aperture slightly oblique, rounded-ovate, the right edge thin, left margin applied to the last bend, closing the umbilicus (Fig. 1A); clear, semicircular, thin, and translucent operculum.

Head-foot Anterior of body covered in black, gradated changing to ivory towards the end of foot; dark black snout broadly bilobed and terminated with grey or whitish lobes, flat and longer than wide; tentacle short, thickened, rounded at the tips.

Radula Typical radula formula; central teeth

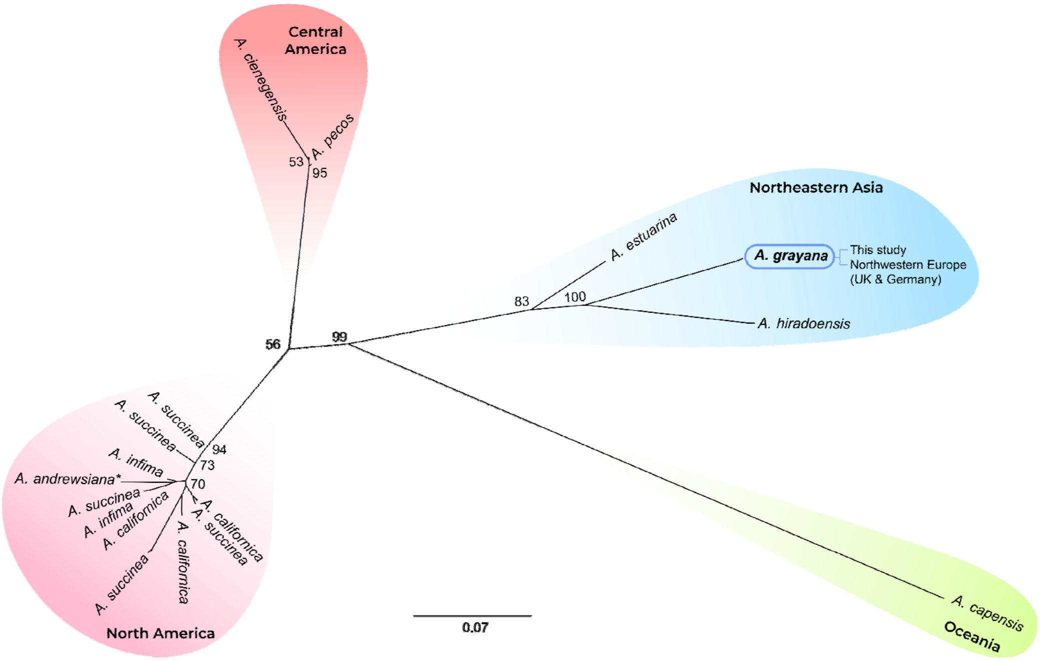

Fig. 2. An unrooted maximum likelihood tree of ten *Assiminea* species based on 32 *COI* haplotype sequences. The bootstrap values are shown for each node. The statistical significance of internal nodes was determined by performing bootstrap analysis based on 1000 resamplings of the data. The scale bar represents an evolutionary distance of 0.07 nucleotides per sequence position. Species marked with an asstarisk (*) is from Australia.

2-1-2/3-3, lateral teeth 2(1)-1-2, inner marginal teeth 4-5, outer marginal teeth 9-11 (Fig. 1B).

Distribution. Korea (new record), Northwestern Europe.

Habitat. Gathered in high density, under the halophytic plants such as reeds and gravel crevices, edge of the shallow waters of estuary salt marshes.

Remarks. We collected the samples of *A. grayana* from the estuary of a river near the western and southern coasts in the Korean Peninsula. The sample collection was conducted from the three sites, in which both *A. japonica* and *A. hiradoensis* were sympatrically found. Compared with the two co-inhabited *Assiminea* species, *A. grayana* can be easily distinguished by some distinct morphological differences in the shell such as the number of whorls and the shape of the apex.

To reaffirm the species identification of *A. grayana* found in Korea, molecular species identification was performed with mitochondrial *COI* nucleotide sequences. It showed that the resultant sequences had high similarity (99.7%; only two nucleotide sequence differences) with those of the species reported from the UK (GenBank accession no: EF667310) and Germany (HQ623170). Those of UK and Germany had an identical sequence. The unrooted ML tree revealed that *A. grayana* from Korea and Northwestern Europe are grouped together (Fig. 2). The results evidently showed that the specimens collected in the Korean Peninsula would be classified as *A. grayana*, supporting the morphological identification.

DISCUSSION

It is known that the shape of the radula is an important classification key for distinguishing Assimineidae subfamilies, and the number of cusps on the radula is one of the key characteristics of classifying assimineid species (Fukuda and Ponder, 2003). Until now, the radula of *A. grayana* has been described by Thiele (1927), Abbott (1958), Marchand

(1972), and Bandel (1984) with morphological descriptions, illustrations, or pictures. Integration of these results revealed that the central tooth features five cusps along the cutting edge and three cusps on each basal side. The lateral tooth exhibits five cusps, the inner marginal four to five, and the outer marginal eleven. The SEM photographs were taken from five individuals of *A. grayana* (data not shown), which confirmed that the shapes and the number of cusps of the radula in *A. grayana* are identical to the previous studies (Thiele, 1927; Abbott, 1958; Marchand, 1972; Bandel, 1984). Interestingly, it was revealed that the cusps at the side edges of the radula were sometimes absent or nearly absent even within the same individuals, particularly in the anterior part of the radula. It is likely to be interpreted as the result of being worn out along with aging. Moreover, we found that some cusps on the marginal teeth of radula are very small, making them easily overlooked. Because of these characteristics, careful observation is required in counting the number of radula cusps of *A. grayana*.

The ML tree reconstructed with 32 *COI* haplotypes of the ten *Assiminea* species showed that they were divided into the four different groups along geographical continents like Northeastern Asia, Central America, North America, and Oceania. *A. grayana* from Korea and Northwestern Europe were closely related to *A. estuarina* and *A. hiradoensis* which are distributed exclusively in Northeastern Asia. Although Central American and North American species are divided into distinct groups, their genetic distances were relatively close. A. capensis from Oceania exhibited significant genetic differences from the other groups.

Abbott (1958) suggested that *A. grayana* might be originated not from Europe but from Asia, with bases of no report of sister species in Europe and a limited distribution area restricted to Northwestern Europe. *A. violacea* (synonymized as *Euassiminea violacea*) that have similar morphological characteristics to those of *A. grayana* has been reported from the estuary of Yantz river, China (Heude, 1882). If *A. violacea* and *A. grayana* are an identical species, *A. grayana* found in Northwestern Europe may have been migrated from China between the 15th and 18th centuries by people (Abbott, 1958; Fukuda, 2019). According to the present phylogenetic tree (Fig. 2), *A. grayana* are grouped with *A. estuarina* and *A. hiradoensis* exclusively distributed only in Northeastern Asia. It may indicate that *A. grayana* origin can be not Europe but Northeastern Asia. Through further studies, it is needed to be examined species taxonomic status and migration histories of *A. grayana* and *A. violacea*. In addition, due to the difficulties of morphological identification of assimineids, it is necessary to conduct exploring more distinguishable morphological characteristics as well as reconfirming species identification with DNA barcoding and molecular systematic studies.

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