

Note on occurrence of the land slug family Rathouisiidae Heude, 1885 from South Korea and its DNA barcode

Kazuki Kimura^{1,2*}, Takahiro Hirano², Satoshi Chiba² & Jae-Hong Pak¹

¹Research Institute for Ulleung-do and Dok-do islands, Department of Biology, Kyungpook National University, 80 Daehak-ro, Buk-gu, Daegu, 41566, South Korea

²Graduate School of Life Sciences, Tohoku University, Kawauchi 41, Aoba-ku, Sendai, 980-0862, Japan

*Corresponding author, email: k.kimura.000@gmail.com

ABSTRACT Rathouisiidae Heude, 1885 is a family of terrestrial slugs. Although only several species has so far been described in this family, rathouisiids are estimated to be highly diversified. In the present study, we report the presence of the slug of Rathouisiidae in South Korea and its DNA barcode for the first time.

KEY WORDS Land slugs; DNA barcoding; Rathouisiidae; South Korea.

Received 10.07.2020; accepted 20.12.2020; published online 30.12.2020

INTRODUCTION

Rathouisiidae Heude, 1885 is a family of terrestrial slugs. Although available information on this family remains insufficient at present, there are three recognized genera: (1) *Rathousia* Heude, 1884; (2) *Atopos* Simroth, 1891; and (3) *Granulilimax* Minato, 1989 (Wu et al. 2006; Schilthuizen & Liew 2008; Kimura et al. 2020). Rathouisiid slugs are found in Australia, Papua New Guinea, Indonesia, Singapore, Malaysia, Philippines, Vietnam, Laos, Thailand, Myanmar, India, China, and Japan (Barker, 2001; Schilthuizen & Liew, 2008; Tan & Chan, 2009; Minato, 2015; Tripathy et al., 2018; Inkhavilay et al., 2019). In this family, only several species have so far been described (e.g., Wu et al., 2006; Minato, 2015). However, rathouisiids are estimated to be highly diversified (e.g., Moriguchi, 2010; Kimura in press).

While rathouisiid slugs are not recorded from the Yangtze River northward in China (i.e., from the river to the base of the Korean peninsula), they

are known from the western region of Japan (Wu et al., 2006; Minato, 2015). Western Japan is geologically close to the Korean peninsula and had a land bridge to the peninsula during the last glacial period. Indeed, a lot of land gastropod genera and species are common in Japan and South Korea (e.g., Kuroda & Miyanaga, 1943; Kimura et al., 2019; Kimura & Noseworthy, 2020). However, there is so far no record of Rathouisiidae in the Korean peninsula.

MATERIAL AND METHODS

Study species and data collection

On 27 June 2018, a single specimen of the family Rathouisiidae was collected at the Bisulsan Natural Recreation Forest (35°43'41.9"N 128°32'23.8"E), Gachangmyeon, Daegu, South Korea. It was examined under a light microscope (Olympus SZ40). The examined specimen was pre-

served in the personal collections of K. Kimura (Voucher No: MNKS509).

Total DNA was isolated from a foot piece of the individual using Nucleospin tissue (TaKaRa, Shiga Pref., Japan) according to the manufacturer's instructions. A fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified and sequenced. The condition used for the polymerase chain reaction (PCR) followed the protocol described by Kimura et al. (2020) and the primer set LCO1490 (5'-GGTCAACAATCA-TAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATC-3') was used (Folmer et al., 1994). The PCR product was purified using Exo-SAP-IT (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). Sequencing was performed using a BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) and electrophoresed using an ABI 3130xl sequencer (Applied Biosystems, Carlsbad, CA, USA). The resulting COI sequence has been deposited in the DDBJ/EMBL/GenBank database (Accession No: LC598438). In addition to this new sequence of the Korean rathouisiid slug, already existing data of COI gene of Rathouisiidae and its sister family Veronicellidae were obtained from GenBank to conduct a phylogenetic analysis (Table 1).

Phylogenetic analyses

These sequences were aligned with MUSCLE

v3.8 (Edgar 2004). The phylogenetic tree was obtained for a COI gene data set (581 sites) using the maximum likelihood (ML) method. Evolution model selection and generation of a ML tree were conducted with MEGA (Kumar et al., 2018). As a result of the model selection, the GTR+G+I model was selected. For the ML tree obtained, we assessed nodal support by performing bootstrap analyses with 1000 replications. The species of Veronicellidae was used as the outgroup.

RESULTS AND DISCUSSION

Systematics

Phylum MOLLUSCA Cuvier, 1797
 Classis GASTROPODA Cuvier, 1795
 Ordo SYSTELLOMMATOPHORA Pilsbry, 1948
 Familia RATHOUISIIDAE Heude, 1885

Rathouisiidae sp.

DESCRIPTIONS. Long and slender. Measurements: length 14.6 mm, width 2.1 mm (Fig. 1, Voucher No: MNKS509). Notum round, lacking a dorsal keel, pale brown, covered with circular granules. Upper tentacles short, black. Lower tentacles pale grey, with a two-lobed shape. Foot with a narrow sole. A small groove between the footsole and each hyponatum. Male genital opening situated at the base of the right lower tentacle. Female genital



Figure 1. The rathouisiid slug used in this study. Voucher No MNKS509. Scalebar: 1.0 mm.

FAMILY	SPECIES NAME	LOCALITY	VOUCHER #	GENBANK
Rathouisiidae	Rathouisiidae sp.	Gachangmyeon, Daegu, South Korea	MNKS509	*LC598438
Rathouisiidae	<i>Granulilimax fuscicornis</i>	Tokushima, Japan	KC4519	LC508386
Rathouisiidae	<i>Granulilimax</i> sp.	Kuchinoshima Isl., Kagoshima, Japan	HC2897	LC522963
Rathouisiidae	Rathouisiidae sp.	Okinawa, Japan	KC9179	LC508385
Rathouisiidae	Rathouisiidae sp.	Izena Isl., Okinawa, Japan	HC4738	LC522965
Rathouisiidae	Rathouisiidae sp.	Amamioshima Isl., Kagoshima, Japan	HC2305	LC522962
Rathouisiidae	Rathouisiidae sp.	Kikai Isl., Kagoshima, Japan	HC3981	LC522964
Rathouisiidae	<i>Atopos</i> sp.	Batang Padang, Perak, Malaysia	Rathoui03	LC522961
Rathouisiidae	<i>Atopos</i> sp.	Myanmar	FLMNH494081	MF983573
Rathouisiidae	<i>Atopos</i> sp.	Myanmar	FLMNH494022	MF983574
Rathouisiidae	<i>Atopos</i> sp.	Myanmar	FLMNH494195	MF983575
Veronicellidae	<i>Laevicaulis natalensis</i>	South Africa	NM-W1444	HQ660051
Veronicellidae	<i>Laevicaulis</i> sp.	South Africa	NM-W4061	HQ660052
Veronicellidae	<i>Laevicaulis alte</i>	-	-	MN022749
Veronicellidae	<i>Phyllocaulis tuberculosus</i>	Brazil	MCP 8857	HQ660053
Veronicellidae	<i>Phyllocaulis variegatus</i>	Brazil	CASIZ 180487	HQ660054
Veronicellidae	<i>Vaginulus taunaisii</i>	Brazil	MCP 8858	HQ660056
Veronicellidae	<i>Veronicella cubensis</i>	Hawaii	CASIZ 180489	HQ660057

Table 1. List of the species included in the molecular phylogenetic analysis. Asterisk indicates the sequence newly obtained in this study.

opening on the foot groove, situated somewhat behind the head. Anal pore on the foot groove, slightly anterior to the female genital opening. Pulmonary orifice on the foot groove, slightly posterior to the female genital opening.

DISTRIBUTION AND BIOLOGY. This Korean rathouisiid was so far only encountered in deciduous broad-leaved forests at the Bisulsan Natural Recreation Forest, Gachangmyeon, Daegu, South Korea. The slug individual was observed in the leaf litter layer.

MOLECULAR PHYLOGENY. The result of the phylogenetic analysis (Fig. 2) suggested that the Korean rathouisiid slug reported in this study

belongs to neither *Atopos* in Southeast Asia (clade C) nor *Granulilimax* in Japan (clade B). Moreover, it was supposed that the slug is, to some extent, genetically distinct from the clade containing the remaining Japanese species (clade A). The Korean rathouisiid may belong to the remaining already-recognized genus *Rathouisia*, whose COI barcode was not available. Additional specimens of Rathouisiidae are needed to examine this possibility.

REMARKS. The specimen examined here had an undeveloped male reproductive organ and was an immature individual. Therefore adult body size and shape of developed reproductive organs remain to be elucidated.

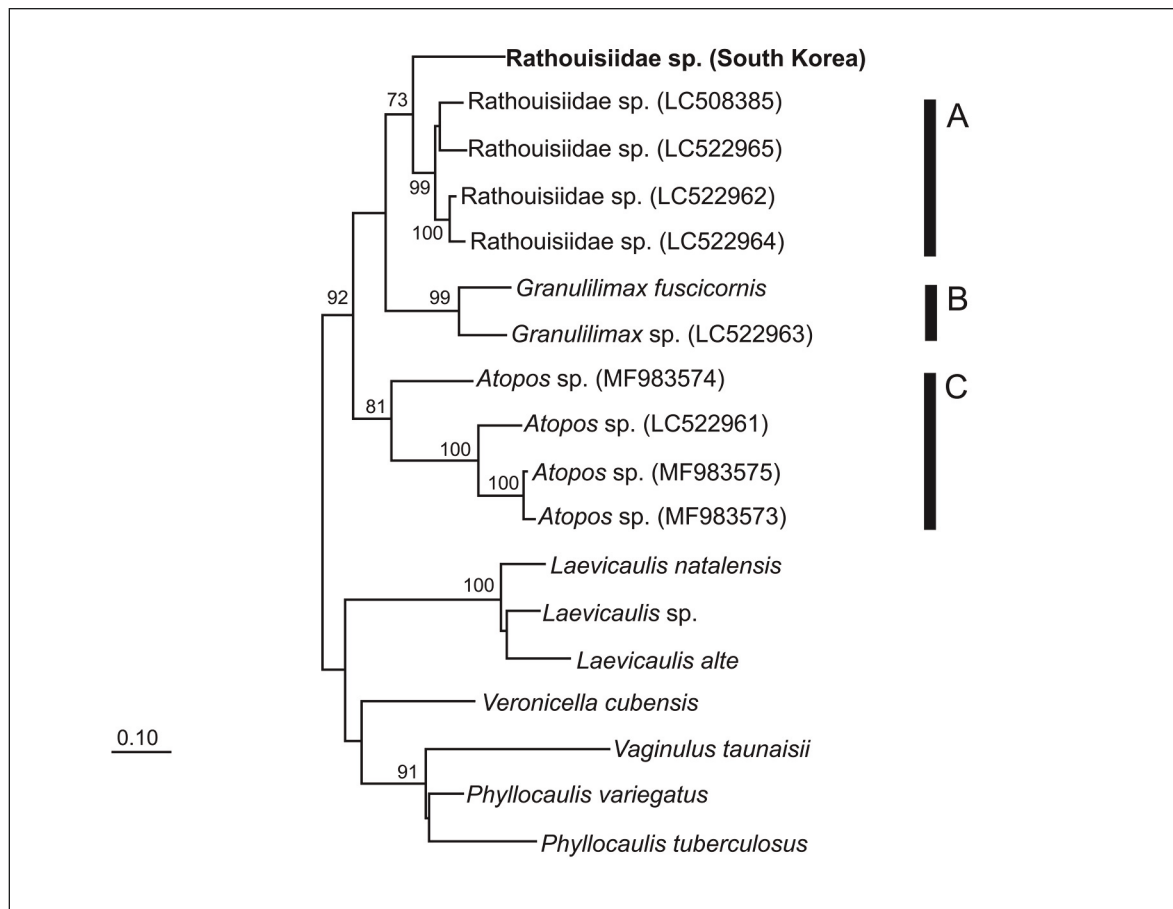


Figure 2. Maximum likelihood tree of the rathouisiid slugs based on 581 bp of the COI gene. Each OTU label represents a species name and the Korean specimen is in bold. Numbers on branches indicate maximum likelihood bootstrap values. Scale bar indicates 0.10 substitutions per site.

ACKNOWLEDGEMENTS

We express our sincere gratitude to Y. Hayase (Shizuoka, Japan) for important information on the study materials and W. Lee, J. Youn (Taegu, South Korea) and Y. Nakase (Kyoto, Japan) for considerable assistance in sample collection.

This research was supported by JSPS Grant-in-Aid for Scientific Research (No 17H04611) and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2016R1A6A1A05011910).

REFERENCES

- Barker G.M., 2001. Gastropods on land: phylogeny, diversity and adaptive morphology. In: Barker G.M. 2001. The biology of terrestrial molluscs. CABI Publishing, Wallingford, 1–146.
- Edgar R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32: 1792–1797.
- Folmer O., Black M., Hoeh W., Lutz R. & Vrijenhoek R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3: 294–299.
- Inkhavilay K., Sutcharit C., Bantaowong U., Chanabun R., Siriut W., Srisonchai R., Pholyotha A., Jiraprasit P. & Panha S., 2019. Annotated checklist of the terrestrial molluscs from Laos (Mollusca, Gastropoda). *ZooKeys*, 834: 1–166. <https://doi.org/10.3897/zookeys.834.28800>
- Kimura K., in press. DNA barcoding of rathouisiid slugs on the Ryukyu Islands, Japan. *Molluscan Diversity Journal*.

- Kimura K., Chiba S. & Pak J.-H., 2019. First record of the land gastropod genus *Otesiopsis* from South Korea (Helicarionoidea Bourguignat, 1877). Biodiversity Data Journal, 7: e46984. <https://doi.org/10.3897/BDJ.7.e46984>
- Kimura K. & Noseworthy R., 2020. First record of the little-known land gastropod genus *Nobuea* Kuroda et Miyanaga, 1943 (Gastropoda Diplommatinidae) from Jeju Island, South Korea. Biodiversity Journal, 11: 289–292. <https://doi.org/10.31396/Biodiv.Jour.2020.11.1.289.292>
- Kimura K., Sano I., Kameda Y., Saito T. & Chiba S., 2020. Phylogenetic Position of the Japanese Land Slug Genus *Granulilimax* Minato, 1989 Based on Preliminary Analyses of Mitochondrial and Nuclear Genes. American Malacological Bulletin, 37: 53–61. <https://doi.org/10.4003/006.037.0202>
- Kumar S., Stecher G., Li M., Knyaz C. & Tamura K., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Molecular Biology & Evolution, 35: 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Kuroda T. & Miyanaga M., 1943. Land shell fauna of Kyobun-to (Port Hamilton), Korean archipelago. Venus, 12: 119–129.
- Minato H., 2015. A memorandum of the studies of Japanese land snails (14), distribution of *Granulilimax fuscicornis* species complex (Systellommatophore: Rathouisiidae) and its bibliographic review. Kainakama, 49: 1–12.
- Moriguchi M., 2010. Quest for the Truth of Mysterious Goose-Skin Slugs. Kodama Publishing, Tokyo, 245 pp.
- Schilthuizen M. & Liew T.-S., 2008. The slugs and semislugs of Sabah, Malaysian Borneo (Gastropoda, Pulmonata: Veronicellidae, Rathouisiidae, Ariophantidae, Limacidae, Philomycidae). Basteria, 72: 287–306.
- Tan S.K. & Chan S.Y., 2009. New records of predatory slugs from Singapore with notes on their feeding behaviour. Nature in Singapore, 2: 1–7.
- Tripathy B., Sajan S.K. & Mukhopadhyay A., 2018. Mollusca. In: Zoological Survey of India 2018. Faunal Diversity of Indian Himalaya. Zoological Survey of India, Kolkata, 785–796.
- Wu M., Guo J., Wan F., Qin Q., Wu Q. & Wiktor A., 2006. A preliminary study on the biology of the predatory terrestrial mollusk *Rathouisia leonina*. The Veliger, 48: 61–74.

