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A description of new *Nerita* species (Gastropoda: Neritidae) from Korea and Thailand, with morphological comparison of the two blotched nerites

Bia Park[1], Seonghyeon Hong[1], Eun Hwa Choi[1,2], Ki Beom Kim[1,2] and Ui Wook Hwang[1,2,3,4]

[1]*Department of Biology Education, Teachers College and Institute for Phylogenomics and Evolution, Kyungpook National University, Daegu 41566, South Korea*
[2]*Phylomics Inc., Daegu 41910, South Korea*
[3]*Institute for Korean Herb-Bio Convergence Promotion, Kyungpook National University, Daegu 41566, South Korea*
[4]*School of Industrial Technology Advances, Kyungpook National University, Daegu 41566, South Korea*

ABSTRACT

The blotched nerite *Nerita albicilla* (Linnaeus, 1758) is widely distributed in intertidal areas of the Indo-Pacific. Interestingly, it is known that they inhabit in the southernmost region of Jeju Island, Korean Peninsula. Recently, it has been proposed that *N. albicilla* can be divided into two different species based on *COI* sequences. Herein, we describe the new species, *N. originalis* Hong, Park & Hwang, sp. nov., in Korea and Thailand. In addition to, we conduct the morphometric analysis between the two nerite species based on nine morphological characteristics of shells. The results show that there are no significant morphological differences of the shells between the two species. However, under SEM, we find a different morphological characteristic (lateral teeth on radula). *Nerita albicilla* has an inflection point close to a V-shape at the base in the lateral tooth, whereas *N. originalis* Hong, Park & Hwang, sp. nov. has a gentle inflection point. Through further study, we need to examine much more samples and more morphological characteristics to seek for significant morphological differences between the two species.

Keywords: lateral teeth, morphometric analysis, *Nerita albicilla*, *Nerita originalis*, SEM, species delimitation

Introduction

The blotched nerite *Nerita albicilla* (Linnaeus, 1758), which belongs to family Neritidae (Gastropoda, Cycloneritida), is widely distributed in intertidal areas of the Indo-Pacific, extending to the shores of East Africa in the west and reaching its eastern boundary at the Cook Islands (Crandall *et al*., 2008). Interestingly, it is known that they inhabit only at the southernmost part of Jeju Island in Korean Peninsula. Recently, Hong *et al*. (2023) reported that *N. albicilla* can be divided into two different species based on extensive analyses of *COI* haplotypes extracted from 697 individuals: *N. albicilla* mainly distributed in Palearctic, Australasia, Indo-Malay, and Oceania, and *N. originalis*, sp. nov. distributed mainly in Afrotropic area (Atlantic Ocean). They concluded species delimitation of *N. albicilla* and *N. originalis*, sp. nov. based on a variety of *COI* haplotype analyses such as population genetic analyses (i.e., TCS network, PCoA, Mantel test, and AMOVA test), DNA barcoding gap analyses, and phylogenetic tree reconstruction. Also, they discussed that *N. originalis*, sp. nov. from the Afrotropic region might be an origin of *N. albicilla*. However, they did not provide enough morphological comparison between the two species.

In this study, we describe *Nerita originalis*, sp. nov. in Korea and Thailand with morphological comparison

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Corresponding author: Ui Wook Hwang
Tel: +82 (53) 950-6344, e-mail: uwhwang@knu.ac.kr
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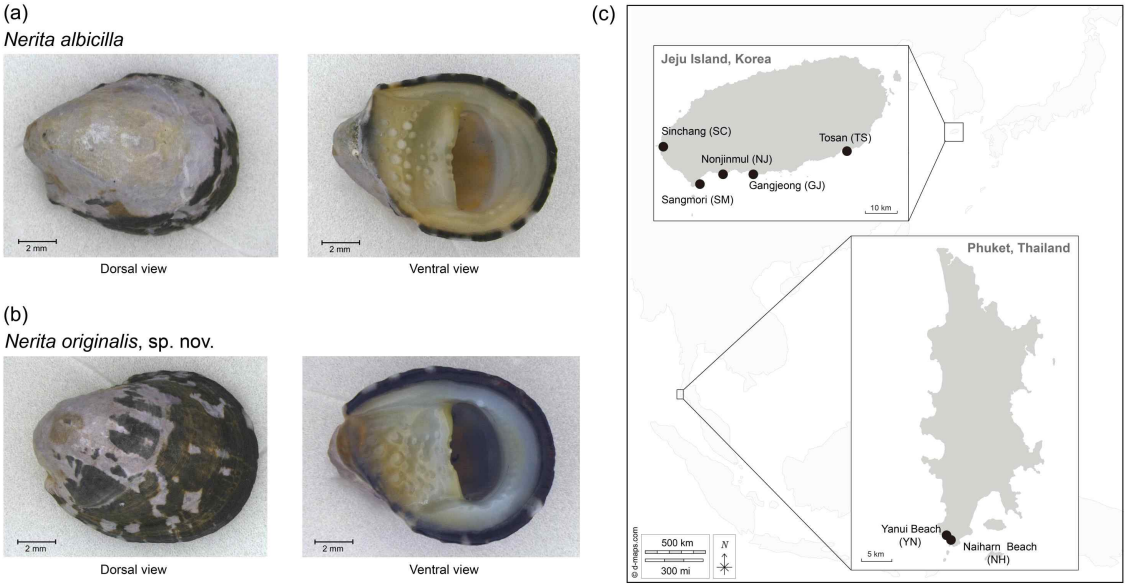

Fig. 1. Shell morphology and a map showing the collection sites of *Nerita albicilla* and *N. originalis*, sp. nov. inhabiting intertidal areas of Korea and Thailand (N = 75). (**a**) Shell of *N. albicilla*, (**b**) Shell of *N. originalis*, sp. nov. (holotype), (**c**) A map of collection sites. Black dots represent the sites where the samples are directly collected. The background map was adapted from the d-maps site (https://d-maps.com).

of the two blotched nerites. To examine their morphological differences, we collected 75 individuals of *N. albicilla* (N = 48) and *N. originalis*, sp. nov. (N = 27) and conducted the morphometric analysis based on shells. Although there are no significant morphological differences of the shells between the two species, we find a different morphological characteristic in lateral teeth of radula under SEM.

Materials and Methods

1. Sample collection

In total, 75 individuals of *Nerita albicilla* (N = 48) and *N. originalis*, sp. nov. (N = 27) were collected from five intertidal regions (GJ, NJ, SC, TS, and SM) of Jeju Island, Korean Peninsula in 2021 and from two intertidal regions (YN and NH) of Phuket, Thailand in 2022 (Table 1; Fig. 1c). The collected samples were immediately fixed with 100% alcohol. They were brought to the laboratory and stored at -20°C. With *COI* sequences previously reported by Hong *et al*. (2023), we did molecular species identification to distinguish *N. albicilla* from *N. originalis*, sp. nov. The identified individuals were subject to the subsequent analyses.

2. Description of new species

All samples examined in this study were deposited in the sample collection of Institute for Phylogenomics and Evolution, Kyungpook National University (KNU; Daegu, Korea) and Honam National Institute of Biological Resources (HNIBR; Mokpo, Korea). The 25 type specimens for *Nerita originalis*, sp. nov. are deposited under voucher nos. HNIBRIV7533 (holotype) in HNIBR and LEGOM030298-LEGOM030321 (paratypes) in KNU. We used the morphological terminology of Susintowati *et al*. (2018). *Nerita originalis*, sp. nov. was observed with a Leica M205 C stereo microscope (Leica Camera AG, Germany). The images of the new species were captured with a Leica MC190 HD camera mounted on a Leica M205 C stereo microscope (Leica Camera AG, Germany) and were produced with Leica Application Suite version 4.12.0 (Leica Camera AG, Germany). Final plates were prepared in Adobe Illustrator (Adobe Systems Incorporated, San Jose, USA). The following abbreviations are used throughout the text: SL, shell length; SW, shell width; H, shell height; AL, aperture length; AW, aperture width.

3. Morphometric analyses

Table 1. List of collection sites and the number of individuals of *Nerita albicilla* and *N. originalis*, sp. nov. from Korea and Thailand

Number	Species	Nations	Population names	Collection dates	Collection sites
1	*N. albicilla*	Korea	GJ02	2021.05.07	4968-7, Gangjeong-dong, Seogwipo-si, Jeju-do
2			GJ03		
3			GJ09		
4			GJ11		
5			GJ14		
6			GJ17		
7			GJ20		
8			GJ22		
9			GJ23		
10			GJ25		
11			NJ01	2021.05.07	253, Yeraehaean-ro, Seogwipo-si, Jeju-do
12			NJ03		
13			NJ04		
14			NJ07		
15			NJ09		
16			SC01	2021.05.07	1446-3, Sinchang-ri, Hangyeong-myeon, Jeju-si, Jeju-do
17			SC04		
18			SC07		
19			SC13		
20			SC14		
21			SC18		
22			SC19		
23			SC24		
24			SC27		
25			SC28		
26			SM01	2021.11.23	87-27, Sangmo-ri, Daejeong-eup, Seogwipo-si, Jeju-do
27			SM02		
28			SM04		
29			SM08		
30			SM13		
31			SM18		
32			SM22		
33			SM24		
34			SM27		
35			SM30		
36			TS01	2021.07.21	30-3, Tosan-ri, Pyoseon-myeon, Seogwipo-si, Jeju-do
37			TS05		
38			TS07		
39			TS09		
40			TS10		
41			TS12		
42			TS13		
43			TS19		
44			TS20		
45			TS22		
46		Thailand	YN06	2022.07.29	Yanui Beach, Phuket, Thailand
47			NH08	2022.07.31	Karon, Mueang Phuket District, Phuket, Thailand
48			NH31		

49	*N. originalis*, sp. nov.	Korea	GJ05	2021.05.07	4968-7, Gangjeong-dong, Seogwipo-si, Jeju-do
50			SM06	2021.11.23	87-27, Sangmo-ri, Daejeong-eup, Seogwipo-si, Jeju-do
51		Thailand	YN01	2022.07.29	Yanui Beach, Phuket, Thailand
52			YN03		
53			YN04		
54			YN07		
55			YN09		
56			YN10		
57			YN11		
58			NH01	2022.07.31	Karon, Mueang Phuket District, Phuket, Thailand
59			NH02		
60			NH05		
61			NH07		
62			NH11		
63			NH13		
64			NH14		
65			NH15		
66			NH16		
67			NH18		
68			NH20		
69			NH21		
70			NH23		
71			NH25		
72			NH26		
73			NH27		
74			NH28		
75			NH30		

Five morphological characters (SL, SW, H, AL, and AW) of shells from 48 *Nerita albicilla* and 27 *N. originalis*, sp. nov. were measured using a digital micrometer (SINCON, China). With five morphological characters, nine ratios were calculated: SW/SL, H/SL, H/SW, AL/SW, AL/SL, AL/H, AW/SW, AW/SL, and AW/H. To verify the distinctiveness of the two species, a principal component analysis (PCA) was conducted with the nine ratios using FactoRMine (Le *et al*., 2008). The histogram density was obtained for nine morphological characteristics, and Gaussian fitting was also performed for the histogram density using the maximum likelihood estimation algorithm.

4. Scanning electron microscopic analyses

To observe the radular morphology of *Nerita albicilla* and *N. originalis*, sp. nov., we obtained the pictures of SEM with a sample selected in each of *N. albicilla* (SM18) and *N. originalis,* sp. nov. (SM06). Subsequently, the snout part of specimen was dissected, and radula was extracted. The separated radula was immersed in 10% NaOCl to remove extra tissues. Detached radula was washed with distilled water and remove remain tissues with a soft brush. After cleaning process, the sample was dried and coated with gold ion particles to be observed with Field Emission Scanning Electron Microscopy (FE-SEM) (Hitachi; SU8220 & SU8230, Tokyo, Japan).

Results and Discussion

1. Systematic accounts

Class Gastropoda Cuvier, 1797 복족강
Order Cycloneritida Frýda, 1998 고리갈고둥목
Superfamily Neritoidea Rafinesque, 1815 갈고둥상과
Family Neritidae Rafinesque, 1815 갈고둥과
Genus *Nerita* Linnaeus, 1758 갈고둥속
***Nerita originalis* Hong, Park & Hwang, sp. nov.**
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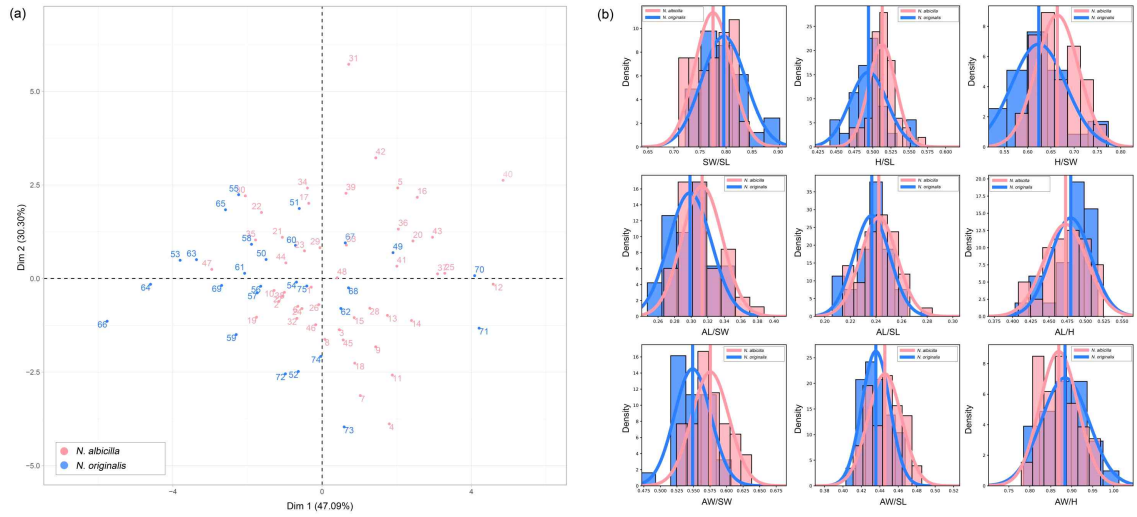

Fig. 2. The results of PCA and Gaussian fit analyses based on nine morphological characteristics of shells in *Nerita albicilla* and *N. originalis*, sp. nov. **(a)** The result of PCA, **(b)** A graph of Gaussian fit. Pink dots and bars represent *N. albicilla* and blue dots and bars represent *N. originalis*, sp. nov.

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Type specimens examined. [Holotype] SOUTH KOREA: 1 specimen, Jeju-do, Seogwipo-si, Daejeong-eup, Sangmo-ri 87-27, 23.XI.2021, S Hong, G Kim & B Park (SM06; HNIBRIV7533); [Paratypes] SOUTH KOREA: 1 specimen, Jeju-do, Seogwipo-si, Gangjeong-dong 4968-7, 7.V.2021, S Hong, G Kim & B Park (GJ05; LEGOM030298); THAILAND: 6 specimens, Phuket, Yanui Beach, 29.VII.2022, UW Hwang (YN03, YN04, YN07, YN09-YN11; LEGOM030299-LEGOM030304 in order); 17 specimens, Phuket, Mueang Phuket District, Karon, 31.VII.2022, UW Hwang (NH02, NH05, NH07, NH11, NH13-NH16, NH18, NH20, NH21, NH23, NH25-NH28, NH30; LEGOM030305-LEGOM030321 in order).

Description. SL 15.5-24.4 mm, SW 12.9-19.0 mm, H 7.1-12.1 mm, AL 3.3-6.1 mm, AW 6.8-11.0 mm. Shell white dorsally with varying amounts of black blotches; shell white to yellowish brown ventrally, with black blotches on the edge of the outer lip; operculum white to yellowish brown (Fig. 1b). Shell sculptured with narrowly rounded spiral ribs, its surface rough; spire low and flat. Inner lip with small teeth on median part; columellar deck with numerous columellar nodules. Outer lip thick and dentate with weak outer lips serrations. Aperture broad, its shape semicircular (Fig. 1b). Operculum semicircular, its outer side entirely covered with small granules. Lower margin of lateral teeth on radula gently descended in the form of a flat straight line, with a gentle inflection point at the base (Fig. 3c, d).

Distribution. Korea and Thailand (Fig. 1c); from the shores of East Africa in the west to its eastern boundary at the Cook Islands.

Habitat. This new species appears the intertidal rocky bottom or between rocks in the Indo-Pacific region.

Etymology. The species name is derived from the Latin word “originalis”, which means an English word “origin”. It indicates that *Nerita albicilla* may have originated from *N. originalis*, sp. nov.

Remarks. The two independent *Nerita* species are strongly supported by the results of population genetic analyses such as TCS network, PCoA, Mantel test, and AMOVA test, DNA barcoding gap analyses, and reconstructed phylogenetic tree (Hong *et al*., 2023). External morphology of *Nerita originalis*, sp. nov. is very similar to *N. albcilla*, but the two blotched nerites are distinguished by the shape of lateral teeth on radula.

2. Morphometric analyses

We conducted the morphometric analyses based on nine morphological characteristics of shells such as SW/SL, H/SL, H/SW, AL/SW, AL/SL, AL/H, AW/SW,

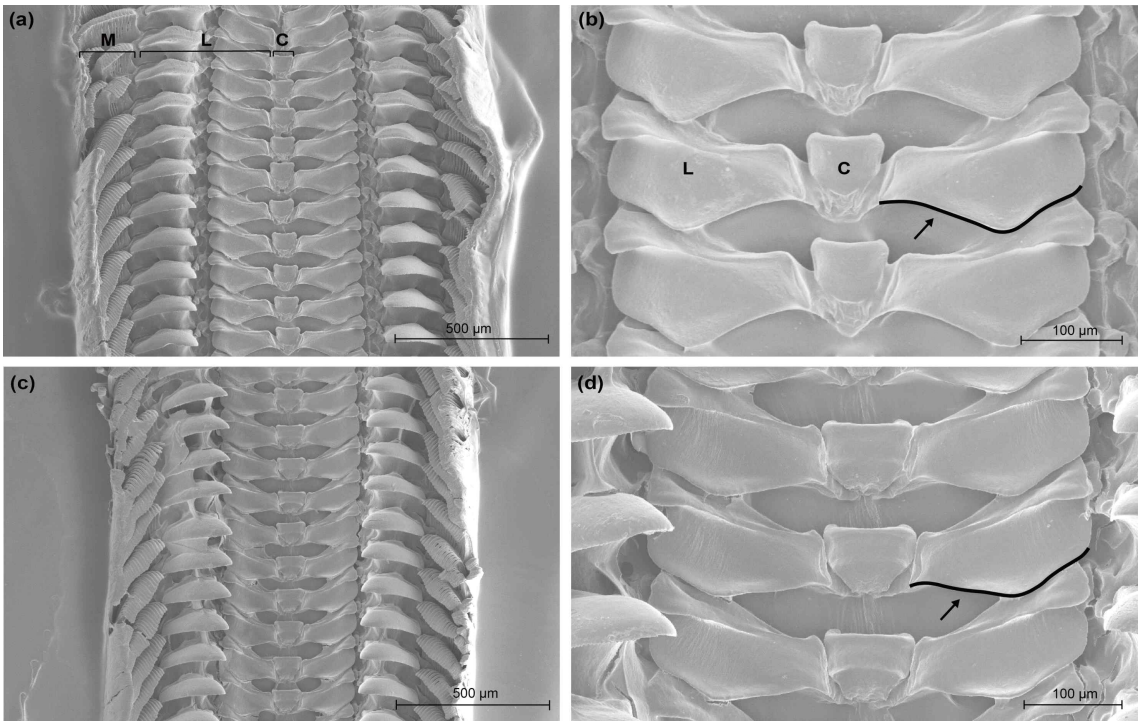

Fig. 3. The radular structures of *Nerita albicilla* (**a, b**) and *N. originalis*, sp. nov. (holotype; **c, d**) taken by the scanning electron microscope. (a, c) Radula, (b, d) Enlarged part of the radula. C: central teeth, L: lateral teeth, M: marginal teeth.

AW/SL, and AW/H. The analyses contained 75 individuals of *Nerita albicilla* (N = 48) and *N. originalis,* sp. nov. (N = 27), which are from Korea (N = 47) and Thailand (N = 28), as shown in Table 1. The morphometric analyses with nine morphological characteristics showed that the distribution patterns of *N. albicilla* and *N. originalis*, sp. nov. on the morphometrix (Fig. 2a) were not clearly divided into two apparent groups. However, there seems to be slightly different in major distribution patterns of the two species when we observed Fig. 2a by visual inspection, in spite that significant statistical reliability was not guaranteed (Fig. 2b). For examples, if we remove the three exceptional cases such as 49, 70, and 71, mainly *N. albicilla* individuals appeared in the far-right side. Whereas, if we remove the five cases such as 19, 22, 30, 35, and 47, mainly *N. originalis*, sp. nov. individuals appeared in the far-left side. Even though such by visual inspection with exclusion of exceptional cases cannot be statistically significant, if much more samples and more morphological characteristics are subjected to the analyses through further studies, morphological differences between the two might be robustly supported.

To find microstructural differences between the two, we fixed the radula and then took pictures under SEM. The SEM photos provided us a promising candidate of different morphological characteristics between the two: lateral teeth morphology (Fig. 3). In *Nerita originalis*, sp. nov., the lower part of the lateral tooth descends with a gentle slope in the form of a flat straight line and then has a gentle inflection point at the base (Fig. 3c, d). On the other hand, *N. albicilla* has an inflection point close to a V-shape at the base as the lower part of the lateral tooth goes down with a convex arch shape (Fig. 3a, b). If we examine the SEM photos of lateral teeth morphology from much more samples through further study, the utility of the lateral teeth difference might be proved or rejected. Moreover, it is necessary to do much more sample collection from other countries such as Japan, Vietnam, Indonesia, Australia, Philippine etc. as well

as Korea and Thailand.

Nerita albicilla was designated as one of the 100 Climate Change Biological Indicator Species (CBIS) by National Institute for Biological Resources (2010), Ministry of Environment, Korea. Based on the present study, we newly suggest that *N. originalis*, sp. nov. should be registered in the CBIS list. Continuous monitoring on distributional changes of *N. albicilla* and *N. originalis*, sp. nov. could play an important role in exploring and predicting the global warming effect in the Korean Peninsula.

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