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DOI: https://doi.org/10.3897/arphapreprints.e99672

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First reliable report of the *Nassarius sinarum* (Mollusca, Gastropoda) from Korea as an invasive species

Sungha Cho‡, Inho Yang‡, Jong Seong Khim§, Jinsoon Park‡

‡ Department of Convergence Study on the Ocean Science and Technology, Korea Maritime and Ocean University, Busan, South Korea

§ School of Earth and Environmental Sciences and Research Institute of Oceanography, Seoul National University, Seoul, South Korea

Corresponding author: Jinsoon Park (jpark@kmou.ac.kr)

Abstract

Background

Two live specimens of the mud snail, *Nassarius sinarum*, was collected from the Yeongsan River estuary in Jeollanam-do, Korea, during June 2022. The species was identified based on its morphology and DNA barcoding.

New information

Morphological descriptions and photographs of *N. sinarum* were used to verify its occurrence in Korean waters as an invasive species. The species is characteristic in its axial ribs, which weaken towards the outer lip. Molecular analysis of the mitochondrial DNA cytochrome oxidase subunit I gene showed that the specimen belonged to *N. sinarum* with 98.48–99.69% sequence similarity. The new Korean name of “No-lan-jul-job-ssal-mu-nui-go-dung; 노란줄좁쌀무늬고둥” was proposed for the species.

Keywords

DNA barcoding, SEM, COI, H3, Gastropoda, invasive species

Introduction

The family Nassariidae is a group of shelled gastropods that occur globally. The family has a wide habitat range, encompassing temperate, subtropical, tropical, and cold waters, from

the intertidal zone to deeper waters (1000 m depth) (Cernohorsky 1972). Most species of the family Nassariidae, commonly known as “nassa” mud snails, inhabit tidal flats. These species are carnivorous, carcass-eating scavengers (Okutani 2017). To date, the family Nassariidae includes more than 600 species belonging to at least 23 genera (MolluscaBase eds. 2022b). Twenty-four species from five genera in the family Nassariidae have been recorded in Korea (NIBR 2019). However, the biodiversity of this group remains poorly understood. Furthermore, the species Nassariidae inhabiting Korea have not been systematically investigated.

In June 2022, examination of specimens collected from the Yeongsan River estuary in Jeollanam-do revealed the presence of a previously unrecorded species in Korea, *Nassarius sinarum* (Philippi 1851). This species is endemic to China (Cernohorsky 1984). This species was previously observed once in Suncheon Bay (Korea), with the wrong spelling of a specific name, *N. (Zeuxis) sinarus*. It has never been added to the national species list of Korea (Hong et al. 2010, MolluscaBase eds. 2022a). Therefore, information on this species is lacking, including detailed morphological description and verification, detailed structural images and SEM images, and molecular verification.

Here, we collected samples of *Nassarius sinarum* at Yeongsan River estuary in Jeollanam-do, and verified the morphological characteristics of this species. Molecular analysis was also conducted. The genus *Nassarius* has no radular anatomical differences. It is difficult to identify sibling species due to the morphological instability of the shell (Wang et al. 2007, Yang and Zhang 2011). DNA barcoding is important for the accurate species-level identification of Gastropoda (Trivedi et al. 2016). We used the mitochondrial gene cytochrome c oxidase subunit I (COI) and the nuclear gene Histone H3 (H3) as gene markers. As such, this study formally reported and described *N. sinarum* to increase the taxonomic knowledge of *Nassariidae* found in Korean waters.

Materials and methods

Specimens were collected in June 2022 from the Yeongsan River estuary, which is situated on the west coast of Korea (Fig. 1). Samples were identified morphologically based on current taxonomic classification criteria. The samples were then frozen at –80 °C in a 50 mL conical tube filled with natural seawater. They were stored at this temperature until DNA extraction procedures could be performed.

The shells were observed using a stereo microscope S9D (Leica, Wetzlar, Germany) that was equipped with a camera K3C (Leica, Wetzlar, Germany) (Fig. 2). For scanning electron microscopy (SEM) observations, the shell was soaked in 10% sodium hydroxide (NaOH) solution, and was subsequently rinsed with distilled water. After being placed in an ultrasonic water bath, the shell was thoroughly dried, mounted on stubs, and gold sputtered. SEM analysis was performed on aluminum stubs with carbon tape using a Field Emission Scanning Electron Microscope (MIRA-3, Tescan, Brno, Czech Republic) (Fig. 2). We extracted genomic DNA from 2 mg muscle tissue using a HiGene Genomic DNA prep kit (Biofact, Daejeon, Korea). Samples were subjected to bidirectional sequencing, with two

DNA regions used for amplification: COI and H3. We then amplified the COI gene using forward and reverse primers LCO1490 (5'-TGTAAAACGACGGCCAGTGGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-CAGGAAACAGCTATGACTAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). Primer pairs H3aF (5'-ATGGCTCGTACCAAGCAGAC(ACG)GC-3') and H3aR (5'-ATATCCTT(AG)GGCAT(AG)AT(AG)GTGAC-3') (Colgan et al. 1998) were used for the H3 gene. We registered sample and sequence files with the National Center for Biotechnology Information (NCBI) (accession number: OP693482 for COI, OP719775 for H3), which store GenBank data for DNA barcode records. The 25 *Nassarius sinarum* COI gene sequences in the NCBI database were used for sequence comparison. We performed multiple sequence alignments using ClustalW (Thompson et al. 1994) in BioEdit ver. 7 (Hall 1999). Genetic distances were calculated, and a neighbor-joining (NJ) tree was generated with MEGA 11 (Tamura et al. 2021), based on the Kimura 2-parameter model (Kimura 1980), with 10,000 bootstrap replications (Felsenstein 1985) (Fig. 3).

Data resources

The data underpinning the analysis reported in this paper are deposited in the Mendeley Data Repository at http://dx.doi.org/10.17632/hkzgstr9wp.1.

Taxon treatment

Nassarius sinarum (Philippi, 1851)

- World Register of Marine Species (WoRMS) https://www.marinespecies.org/aphia.php?p=taxdetails&id=560319
- GenBank OP693482
- GenBank OP719775

Nomenclature

Buccinum sinarum Philippi, 1851 - Philippi (1851): 8–63; Cernohorsky (1984), pl. 31, Figs. 10, 11. pl. 32, Fig. 1.

Nassa (Niotha) sinarum Philippi, 1851 - Philippi (1851): 8–63; Okutani (2017), p. 912, ed. 2. 2 vols.

Nassarius (Tritonella) semiplicatus Adams, 1852 - Adams (1852): pl. 19–107; Cernohorsky (1984), pl. 31, Figs. 10, 11. pl. 32, Fig. 1; Liu (2008). p. 1267.

Nassarius (Zeuxis) semiplicatus Adams, 1852 - Adams (1852): pl. 19–107; Zhang and Yang (2010), 41–5, p. 791.

Nassarius (Zeuxis) sinarus Philippi, 1851 - Philippi (1851): 8–63; Cernohorsky (1984), pl. 31, Figs. 10, 11. pl. 32, Fig. 1; Lutaenko et al. (2013), p. 74, Fig. 50.

Nassarius semiplicatus Adams, 1852 - Adams (1852): pl. 19–107; Zhang and Yang (2010), 41–5, p. 791.

Zeuxis semiplicata Adams, 1852 - Adams (1852): 24–233, pl. 23, Fig. 164. pl. 19–107; Cernohorsky (1984), pl. 31, Figs. 10, 11. pl. 32, Fig. 1.

Nassarius sinarum Philippi, 1851 - Cernohorsky (1984), pl. 31, Figs. 10, 11. pl. 32, Fig. 1; Leung and Morton (2003), p.655; Zhang and Yang (2010), 41–5, p. 791.

Material

a. scientificName: Nassarius sinarum (Philippi, 1851); taxonConceptID: https://www.ebi.ac.uk/ena/browser/view/Taxon:1541843; taxonomicStatus: ACCEPTED; taxonID: ASV:c3f7e432967a463be282c759d18ccb39; kingdom: Animalia; phylum: Mollusca; class: Gastropoda; order: Neogastropoda; family: Nassariidae; taxonRank: SPECIES; genus: Nassarius; specificEpithet: sinarum; country: Korea, Republic of; countryCode: KR; locality: Jeollanam-do, Yeongsan River Estuary; decimalLatitude: 34.779999; decimalLongitude: 126.440002; geodeticDatum: WGS84; eventDate: 2022-06-16T00:00:00; year: 2022; month: 6; day: 16; individualCount: 1; occurrenceDetails: http://api.gbif.org/v1/occurrence/3969249286; occurrenceRemarks: Nassarius sinarum isolate NF5 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial.; recordedBy: Sungha Cho; occurrenceStatus: PRESENT; associatedSequences: https://www.ebi.ac.uk/ena/browser/api/embl/OP693482; identifiedBy: Sungha Cho; basisOfRecord: MATERIAL_SAMPLE; occurrenceID: B94FF589-E5C1-5B37-B5C7-43C3EFB146A4

Description

Shell up to 17.0 mm in length, elongate-ovate, light in weight, teleoconch of 5.5–6 convex and slightly inflated whorls, protoconch worn in examined specimens but clearly paucispiral; sculptured whorl, ribs tend to become obsolete on last dorsal half of body whorl; 12–13 spiral sculpture that is prominent from on the body whorl, followed by 4–5 weakly nodulose cords and 4–6 cords on the siphonal fasciole; sutural nodules distinct. Aperture narrow, outer lip thickened and narrowly variced, interior with 6–7 denticles, columella with a narrow callus and 5–10 round denticles, siphonal notch distinct, anal canal very prominent. Off-white in color, spire whorls ornamented with a posterior broader brown sutural band and a narrow band anterior to suture; body whorl with 3 moderately broad brown bands, anterior band usually weakly indicated. Aperture white, deep interior with brown bands. Operculum brown and simple at margins (Philippi 1851) (Fig. 2).

Distribution

Previously, *N. sinarum* was only recorded in China (Cernohorsky 1984); However, it was first observed 40 years ago in Japan, and was recorded as an invasive alien species (Mito and Uesugi 2004). In Korea, the species was first observed in 2010 in Suncheon Bay (Hong et al. 2010). 100 km from Suncheon Bay, the current study

reports its presence in the Yeongsan River estuary at Jeollanam-do, Korea (present study).

Type locality

Yangtze River, China (*B. sinarum*); Zhoushan, China (*N. semiplicata*).

Material examined

219, Daeburyeok-ro, Samho-eup, Yeongam-gun, Jeollanam-do (34°46'48"N, 126°26'31"E), Yeongsan River estuary at Jeollanam-do, Korea on 16 June 2022 (Fig. 1), 24.5°C, Dissolved Oxygen (DO) 5.69 mg/L, 33.24 Practical Salinity Unit (PSU), Potential of Hydrogen (pH) 8.37, 2 specimens, The collector is Sungha Cho.

Molecular identification

Genetic divergence shows that our specimens were identical to *N. sinarum* with 98.48–99.69% sequence similarity. These results were based on an analysis of the mitochondrial DNA COI sequence (660 bp). The identity of our specimens as *N. sinasum* was further verified by the Neighbor-Joining tree, in which *N. sinarum* was very closely clustered (Fig. 3Lee et al. 2010). Histone H3 genetic distance and gene phylogenetic tree of *N. sinarum* did not indicate an exact location because it is the first registration in NCBI.

Remarks

Species belonging to the genus *Nassarius* have morphologically developed spiral ribs and axial ribs, a columella fold located on the outer and inner lip, and a thick outer lip. *Nassarius sinarum* is characterized by axial ribs that weaken towards the outer lip (Cernohorsky 1984). When this species was first reported, this species was considered to be endemic to China (Cernohorsky 1984); however, it was recorded in Japan in 2004. It was assumed to be an exotic species introduced to Japan from mainland China (Mito and Uesugi 2004). The inflow of this species to Korean waters had been expected; however, detailed reports on the shell of living specimens were lacking (Lee et al. 2010). This study provides the first record of *N. sinarum* in Korean waters based on morphological analysis and DNA Barcoding. The new Korean name of “No-lan-jul-job-ssal-mu-nui-go-dung; 노란줄좁쌀무늬고둥” has been proposed for *N. sinarum*.

Acknowledgements

This work was supported by the project entitled “Development of Advanced Science and Technology for Marine Environmental Impact Assessment (20210427)”, which funded by the Ministry of Oceans and Fisheries, Korea.

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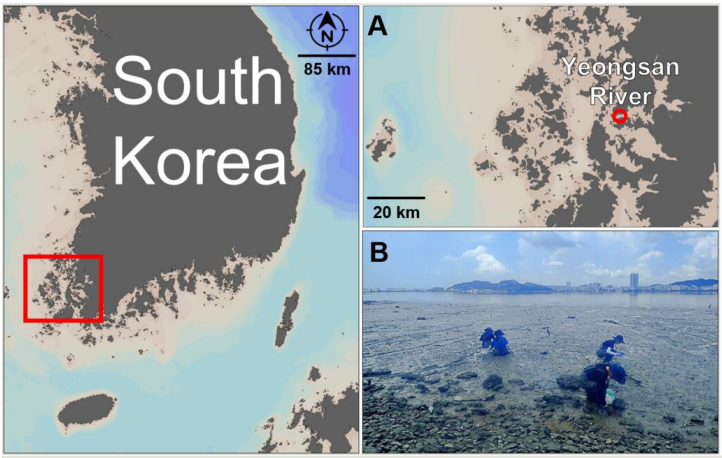

Figure 1.

Sample collection sites. **A** Fine-scale location of the collection site (34°46'48"N, 126°26'31"E) (Schlitzer R, Ocean Data View, http://odv.awi.de, 2015.); **B** Photograph of the sampling location at Yeongsan River. June 2022, Photograph by Sungha Cho.

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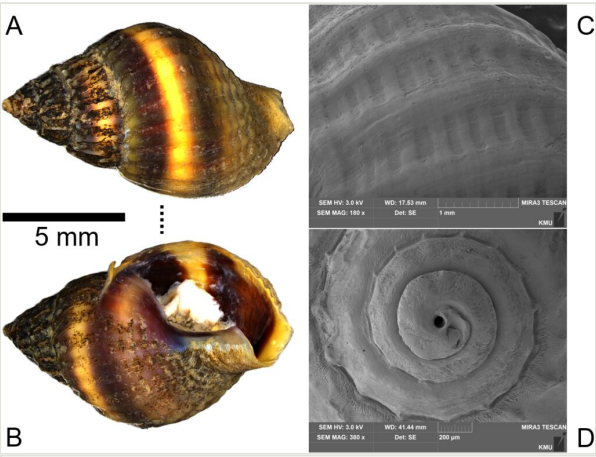

Figure 2.

Morphology of *Nassarius sinarum*. **A & B** Shell morphology; **C** SEM of the whorl sculpture of the body; **D** SEM of the protoconch. The specimen was collected from the Yeongsan River estuary in Jeollanam-do, Korea. Scale bar A, B = 5 mm; C = 1 mm; D = 200 µm. See Supplementary files for additional SEM images (Suppl. material 1) or original images and data (Suppl. material 2).

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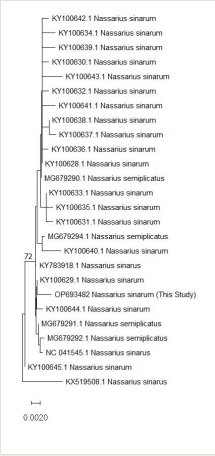

Figure 3.

Neighbor-joining tree of mitochondrial gene cytochrome c oxidase subunit I (COI) for 26 specimens of *Nassarius sinarum*. Number of branches (>70) corresponds to bootstrap probabilities in 10,000 bootstrap replications. Bar indicates genetic distance of 0.0020. Letter before the scientific name is the NCBI accession number. *Nassarius semiplicatus*, *N. sinarus*, is a synonymized name for *N. sinarum.*

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Supplementary materials

Suppl. material 1: SEM images of *Nassarius sinarum*

Authors: Sungha Cho, Inho Yang, Jong Seong Khim, Jinsoon Park
Data type: SEM images
Brief description: SEM images of Sculpture detail, Protoconch, Inside the spire (cross section), Columellar folds, and Posterior canal of *Nassarius sinarum* shell
Download file (557.42 kb)

Suppl. material 2: Original size images of *Nassarius sinarum*

Authors: Sungha Cho, Inho Yang, Jong Seong Khim, Jinsoon Park
Data type: Original size images
Brief description: Original images from SEM, live shell, dead shell, YSI
Download file (128.00 bytes)