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A new record of the nudibranch *Halgerda batangas* from Vietnam confirmed by DNA barcoding

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Abstract

Although nudibranchs are common and attractive animals, our understanding of these marine gastropods in Vietnam remains limited. Prior research has suggested that combining morphological examination with molecular analysis results in more accurate identification of nudibranchs. However, previous studies in Vietnam have typically relied solely on morphological methods for nudibranch identification. In this study, the nudibranch species *Halgerda batangas* was recorded in Vietnam for the first time based on both morphological and molecular approaches. *Halgerda batangas* was characterized by a network pattern consisting of orange lines, relatively low dorsal tubercles with red-orange caps and white basal rings, and an orange line along the foot margin. Molecular analysis corroborated the morphological findings. These results suggest that integrating morphological and molecular methods is an effective approach for identifying nudibranchs.

Introduction

Nudibranchs are globally distributed marine gastropod molluscs, renowned for their vivid colours and striking forms, and considered among the most beautiful animals in the oceans. Approximately, 3000 valid species of nudibranchs have been recorded, and this number continues to rise as new species are described (Wägele and Klussmann-Kolb, 2005). Despite their diversity, our understanding of nudibranchs in Vietnam remains limited. Few studies have focused on the nudibranch community in Vietnam, with studies on this group rarely published. For example, Martynov and Korshunova (2012) documented approximately 120 nudibranch species from Vietnam, including the new species *Janolus savinkini*. Subsequently, Tuyen (2013) listed 55 nudibranch species from coral reefs in central Vietnam. Previous studies on nudibranchs in Vietnam have primarily relied on morphological examination method for species identification, often neglecting molecular methods. This limitation has made it challenging to identify species with similar appearances. *Halgerda* is a diverse nudibranch genus found in the Indo-Pacific (Tibirić *et al.*, 2018). In a recent investigation, Donohoo *et al.* (2023) described 14 new species of the genus. Nevertheless, information on the species composition of this genus in Vietnam remains sparse. To date, only *H. tessellata*, *H. wasinensis* and *H. willeyi* have been recorded in the country (Martynov and Korshunova, 2012; Tuyen, 2013). Continued research is essential to understand their diversity and distribution in Vietnamese waters.

DNA-based identification has gained prominence worldwide as an effective tool for identifying species (Hebert *et al.*, 2003). DNA-based methods offer unprecedented accuracy owing to their inherently high resolution. Moreover, using DNA barcoding is crucial for accurately estimating biodiversity, and it represents a pivotal technique in identifying nudibranchs. This approach has facilitated the detection of new records and the description of new species (Do *et al.*, 2020; Pola *et al.*, 2023). Given its effectiveness in identifying nudibranchs, the present study employs DNA barcoding alongside morphological examination to provide the first record of *Halgerda batangas* collected from the Con Dao Islands, Vietnam.

Materials and methods

Nudibranch specimens were collected from two coral reefs at depths of 4 and 7 m in waters off the Con Dao Islands, Vietnam via scuba diving. Detailed information on the specimens is presented in Table 1. Collected specimens were preserved in 10% formaldehyde for morphological examination, which were prepared, along with species descriptions, following the guidelines of Carlson and Hoff (2000). The radula used for scanning electron microscope examination was prepared following Do *et al.* (2020). The photographs of the radula were captured using the Hitachi TM4000Plus II with backscattered electron mode. Additionally, foot tissue samples were stored in 95% ethanol for DNA extraction. Total DNA was extracted from each sample



Table 1. Nudibranch specimens collected and examined in this study

Species name	Collection site	Coordinates	Storage	Specimen catalogue	GenBank accession numbers	
					COI	16S
<i>Halgerda batangas</i>	The Con Dao Islands, Vietnam	8°38'42"N 106°37'85"E	Institute of Marine Environment and Resources, Vietnam	IMER-NU0001	PP331423	PQ067230
<i>Halgerda batangas</i>	The Con Dao Islands, Vietnam	8°42'39"N 106°33'06"E	Institute of Marine Environment and Resources, Vietnam	IMER-NU0002	PP331424	PQ067231

using the G-spin™ Total DNA Extraction Mini Kit (iNtRON Biotechnology, South Korea). Polymerase chain reaction (PCR) analysis was performed using the primers LCO1490/HCO2198 (Folmer *et al.*, 1994) for a partial sequence of cytochrome *c* subunit 1 gene (COI) and the primers 16Sar-L/16Sbr-H (Palumbi, 1996) for a partial sequence of 16S ribosomal RNA gene (16S). The PCR reaction mixture consisted of 10 µl of 2X PCR Master Mix Solution (i-Taq) (iNtRON Biotechnology, South Korea), 1 µl of each primer (10 pmoles µl⁻¹), 100 ng of DNA and distilled water to a final volume of 20 µl. The amplification conditions were as follows: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 45 s, annealing temperature at 45°C (COI) and 48°C (16S) for 45 s, extension at 72°C for 1 min and final elongation at 72°C for 5 min. PCR products were verified via electrophoresis and sequences were obtained using the 3500xL Genetic Analyzer (Thermo Fisher Scientific, USA).

Consensus sequences were generated from forward and reverse sequences using Geneious Prime 2023 (Kearse *et al.*, 2012). Sequences of congeneric species were retrieved from GenBank to estimate genetic distances (Table S1). Sequence distances were calculated using MEGA X software via the K2P method (Kumar *et al.*, 2018). Assemble Species by Automatic Partitioning (ASAP) was employed to delineate putative species (Puillandre *et al.*, 2021).

For phylogenetic analysis, species without COI or 16S sequences were excluded. The optimal model for phylogenetic tree reconstruction was determined using PartitionFinder 2 (Lanfear *et al.*, 2017). Phylogenetic trees were constructed using the Bayesian inference (BI) method in MrBayes ver. 3.2.7a (Ronquist *et al.*, 2012). The BI analysis consisted of two runs with four chains for 10 million generations, a sampling interval of 100 generations, and a burn-in of 25%.

Results

Morphological identification

Halgerda batangas Carlson and Hoff, 2000 (Fig. 1)

Halgerda batangas Carlson and Hoff, 2000: 157–159, figs. 6–11; Gosliner *et al.*, 2018: 111.

Halgerda malesso Debelius, 1996: 257 (Non *H. malesso* Carlson & Goff, 1993).

Type locality: Mactan Island, Cebu, Philippines.

Distribution: Western and Central Pacific (Gosliner *et al.*, 2018).

Material examined: Two *Halgerda batangas* specimens were collected from the Con Dao Islands, Vietnam, on 29 May and 02 September 2023 (COI GenBank numbers: PP331423 and PP331424; 16S GenBank numbers: PQ067230 and PQ067231).

Description: The two specimens measured 52 and 45 mm in length, respectively. The living animal (Figure 1) was translucent white and ovate. Its dorsal surface featured a network of fine, solid, red-orange lines. Both ridges and tubercles were present on the mantle. Smaller tubercles were located next to larger

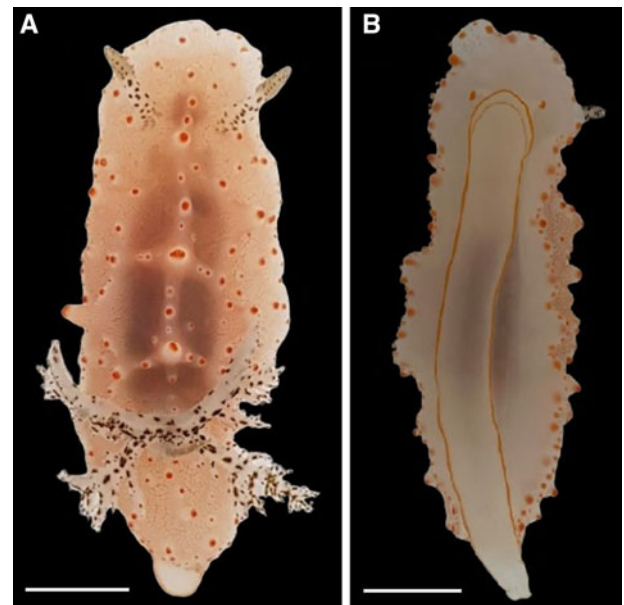


Figure 1. Photograph of *Halgerda batangas* collected in the Con Dao Islands, Vietnam. (A) Dorsal view; (B) ventral view. Scale bars: 10 mm.

tubercles or they occurred along the ridges. The rounded orange-capped tubercles were surrounded by white bands. The size of the tubercles decreased toward the mantle margin. Rhinophores and gills were translucent white, with scattered small dark brown spots. The foot margin was delineated by an orange band. The gill had four primary branchial leaf rachises, with dark brown spots scattered across it.

The radula (Figure 2) of the dissected 52-mm specimen (IMER-NU0001) had a formula of 65 × 53.0.53. The innermost lateral teeth were small with broad bases and shorter hooks compared to the mid-lateral teeth (Figure 2A). The size of the lateral teeth gradually increased toward the mid-lateral teeth. The mid-lateral teeth (Figure 2B) were hamate with long, pointed hooks. The outermost three to four teeth were reduced (Figure 2C).

Remark

Two other species in the genus *Halgerda*, namely *H. malesso* and *H. terramtuentis*, are similar to *H. batangas* in having dorsal patterns of lines. *H. batangas* is characterized by relatively low tubercles with orange-red caps and white basal rings, along with an orange marginal band along the foot. In contrast, *H. terramtuentis* has a golden linear pattern, relatively low, white-capped tubercles on the dorsum, and a golden submarginal band along the mantle (Carlson and Hoff, 2000). Conversely, *H. malesso* features orange lines on the dorsal surface, which may merge into orange markings in depressed areas of the dorsum, with relatively high tubercles (Carlson and Hoff, 2000).

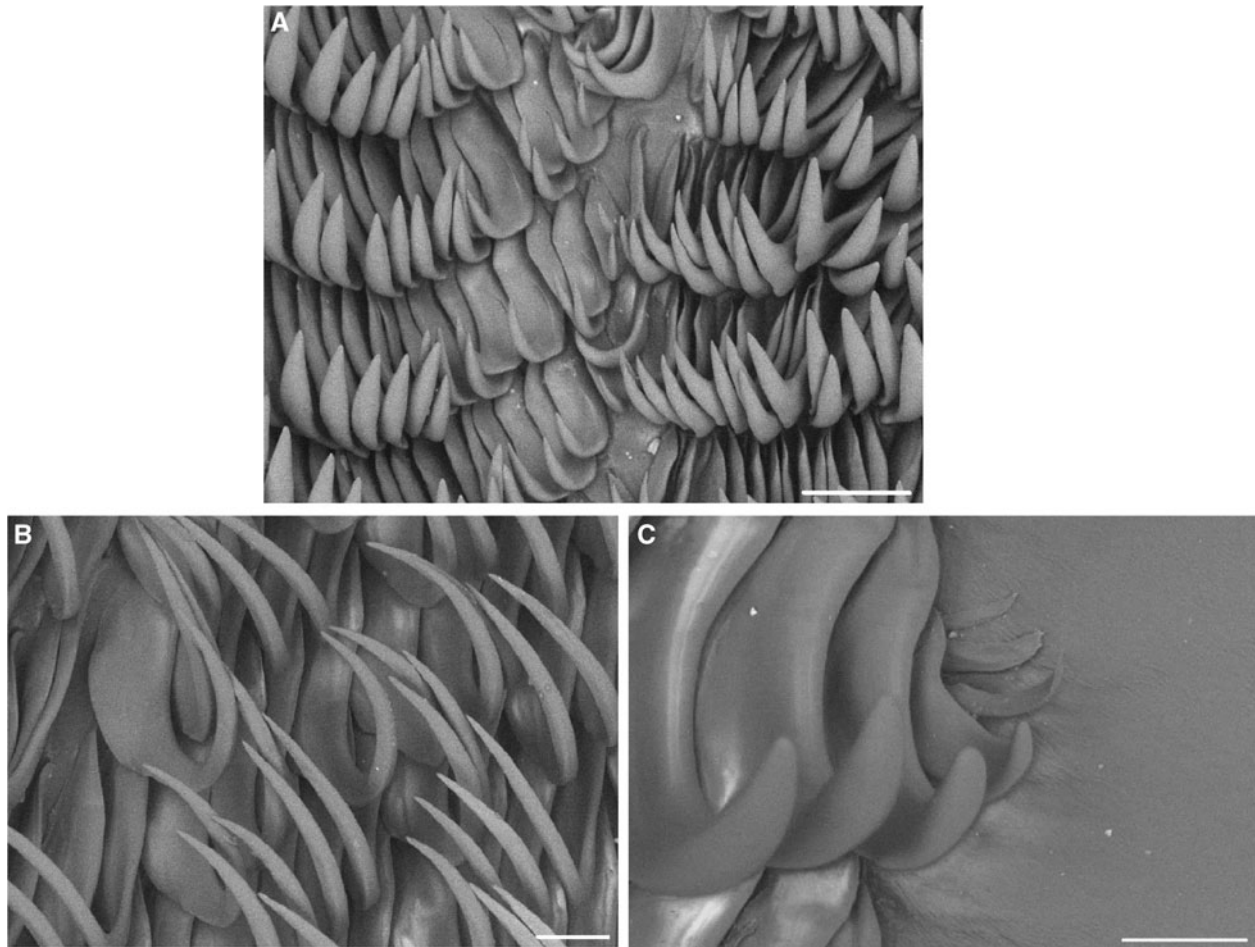


Figure 2. Radula of *Halgerda batangas*. (A) Inner lateral teeth; (B) mid-lateral teeth; (C) outer lateral teeth. Scale bars: 50 μm .

Analysis of COI and 16S sequences

The two COI sequences generated in this present study were 100% identical, resulting in 0% distance between the two sequences of *Halgerda batangas*. Intraspecific distances were 0–0.17%, whereas the minimum interspecific distance between *H. batangas* and other congeneric species was 1%, observed with *H. malesso*. ASAP analysis for species delimitation identified ten partitions, with *H. batangas* retrieved as a distinct taxonomic unit among the high number of subsets.

The 16S sequences showed 0% intraspecific distances, indicating that all examined 16S sequences were identical. However, the interspecific distances for 16S among *H. batangas*, *H. malesso* and *H. diaphana* were also 0%, indicating no difference in 16S across these species. ASAP analysis revealed 10 partitions, grouping *H. batangas* with *H. malesso* and *H. diaphana* owing to their sequence identities.

The phylogenetic tree for *Halgerda* constructed using BI (Figure 3) revealed that the specimens from this study formed a cluster with sequences obtained from GenBank. Specifically, *H. batangas* exhibited close relationships with congeneric species, including *H. terramtuentis*, *H. malesso* and *H. diaphana*. This suggests that the nudibranch samples collected in the present study belong to *H. batangas*.

Discussion

Coral reefs are among the most biologically diverse marine ecosystems on Earth, hosting a wide range of reef organisms, including nudibranchs. However, nudibranchs from coral reefs in

Vietnam remain under investigation. Most previous studies have compiled species lists, with only a few providing descriptions and illustrations. Continued research on nudibranchs is essential for understanding marine biodiversity in the country. In the present study, *Halgerda batangas* was recorded and described from the coral reefs of the Con Dao Islands, Vietnam. *H. batangas* belongs to a group in *Halgerda* known for its primarily white and orange markings. Originally described from the waters of the Philippines (Carlson and Hoff, 2000), *H. batangas* from Vietnam exhibits characteristics consistent with those original descriptions, including a network of fine, solid, red-orange lines on the mantle (Carlson and Hoff, 2000). It also features a white band around the mantle edge, with low, rounded, orange-capped tubercles. The mantle tubercles vary from small, rounded bumps to large, prominent structures. Previous studies in Vietnam have recorded three species of this genus: *H. tessellata*, *H. wasinensis* and *H. willeyi* (Martynov and Korshunova, 2012; Tuyen, 2013). Among these, *H. tessellata* is characterized by a yellow reticulated dorsum with small nodes (Martynov and Korshunova, 2012; Tibirićá *et al.*, 2018) as well as white spots forming a band around the mantle (Tibirićá *et al.*, 2018). In contrast, *H. wasinensis* typically displays irregular dark blotches and a network of orange lines on the dorsum (Tibirićá *et al.*, 2018). Finally, *H. willeyi* has a complex dorsal pattern with yellow/orange ridging and black/dark brown radiating lines on the mantle (Tibirićá *et al.*, 2018; Donohoo *et al.*, 2023). Meanwhile, *H. batangas* can be distinguished by its relatively low tubercles with orange-red caps and white basal rings, a reticulate network of orange lines on the dorsum, and an orange marginal band along the foot margin.

Competing interest. None.

Data. The nucleotide sequences generated during the current study were deposited in GenBank under accession numbers present in [Table 1](#).

References

- Carlson CH and Hoff PJ** (2000) Three new Pacific species of *Halgerda* (Opisthobranchia: Nudibranchia: Doridoidea). *The Veliger* **43**, 154–163.
- Debelius H** (1996) *Nudibranchs and Sea Snails: Indo-Pacific Field Guide*. Frankfurt: IKAN-Unterwasserarchiv.
- Do TD, Jung DW, Kil HJ and Kim CB** (2020) A report of a new species and new record of *Cadlina* (Nudibranchia, Cadlinidae) from South Korea. *Zookeys* **996**, 1–18.
- Donohoo SA, Villalobos SG, Hallas JM and Gosliner TM** (2023) Hyperdiversity of the genus *Halgerda* Bergh, 1880 (Nudibranchia: Discodorididae) with descriptions of fourteen new species. *Marine Biodiversity* **53**, 42.
- Fahey SJ** (2003) Phylogeny of *Halgerda* (Mollusca: Gastropoda) based on combined analysis of mitochondrial CO1 and morphology. *Invertebrate Systematics* **17**, 617–624.
- Folmer O, Black M, Hoeh W, Lutz R and 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.
- Gosliner TM, Valdés Á and Behrens DW** (2018) *Nudibranch & Sea Slug Identification: Indo-Pacific*, 2nd Edn. Jacksonville: New World Publications.
- Hebert PDN, Ratnasingham S and deWaard JR** (2003) Barcoding animal life: cytochrome c oxidase subunit I divergences among closely related species. *Proceedings of the Royal Society B: Biological Sciences* **270**, S96–S99.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheun M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P and Drummond A** (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647–1649.
- Kumar S, Stecher G, Li M, Knyaz C and Tamura K** (2018) MEGA x: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35**, 1547–1549.
- Lanfear R, Frandsen PB, Wright AM, Senfeld T and Calcott B** (2017) Partitionfinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* **34**, 772–773.
- Martynov AV and Korshunova TA** (2012) Opisthobranch molluscs of Vietnam (Gastropoda: Opisthobranchia). In Britayev TA and Pavlov DS (eds), *Benthic Fauna of the Bay of Nhatrang, Southern Vietnam*, vol. 2. Moscow: KMK Scientific Press Ltd, pp. 142–257.
- Palumbi SR** (1996) Nucleic acids II: the polymerase chain reaction. In Hillis DM, Moritz C and Mable BK (eds), *Molecular Systematics*, 2nd Edn. Sunderland, MA: Sinauer Associates, pp. 205–247.
- Pola M, Miguel-González M and Paz-Sedano S** (2023) New contributions to the subfamily Polycerinae (Nudibranchia, Polyceridae): description of three new species and one new genus. *Journal of the Marine Biological Association of the United Kingdom* **103**, e76.
- Puillandre N, Brouillet S and Achaz G** (2021) ASAP: assemble species by automatic partitioning. *Molecular Ecology Resources* **21**, 609–620.
- Ronquist F, Teslenko M, Mark PVD, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA and Huelsenbeck JP** (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**, 539–542.
- Tibiriçá Y, Pola M and Cervera JL** (2018) Systematics of the genus *Halgerda* Bergh, 1880 (Heterobranchia: Nudibranchia) of Mozambique with descriptions of six new species. *Invertebrate Systematics* **32**, 1388–1421.
- Tuyen HT** (2013) Composition of nudibranchs (Order Nudibranchia) in coral reefs in Central Vietnam. In Long BH, Tuan VS and Lam NN (eds) *Proceeding of the international conference on 'Bien Dong 2012'*, Vol. 1, Institute of Oceanography, Nha Trang, 12–14 September 2012. Ha Noi: Publishing House for Science and Technology, pp. 169–178.
- Wägele H and Klussmann-Kolb A** (2005) Opisthobranchia (Mollusca, Gastropoda) more than just slimy slugs. Shell reduction and its implications on defence and foraging. *Frontier in Zoology* **2**, 1–18.