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A transoceanic journey: *Melanochlamys diomedea*'s first report in the North Atlantic

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Abstract

Egg masses from an unknown mollusc have been found in South-West Iceland since 2020, but it was not until September 2023 that the adult organism was collected. Morphological analysis of both adults and egg masses pointed towards the identification of the species as *Melanochlamys diomedea*. This was further confirmed through DNA analyses using COI, H3, and 16S rRNA markers, which established the presence of a new non-indigenous species in the North Atlantic. Members of the genus *Melanochlamys* have predominantly been found in the Indo-Pacific basin and the Pacific Ocean, with only one species known to exist across the Madeira Islands, Canary Islands, and Cape Verde in the Atlantic. The known distribution range of *M. diomedea* extends from Alaska to California on the Pacific side of North America, where it typically inhabits sandy-muddy areas of the littoral in the tidal zone and below. It is not known how the species arrived in Iceland. However, maritime transport through either ballast water or biofouling is being considered as the most likely mode of dispersal.

Introduction

The family Aglajidae belongs to the order Cephalaspidea. Molluscs of this order are commonly known as bubble shells (Abbott, 1974) and are characterized by the presence of a headshield (Malaquias *et al.*, 2008). Identification of Aglajidae species is challenging due to their lack of radula, along with inconsistent shell morphology, external features, and colouration, leading to taxonomical conflicts (Camacho-Garcia *et al.*, 2014; Cooke *et al.*, 2014; Ortea *et al.*, 2014). In 2014, Camacho-Garcia *et al.* genetically resolved the taxonomic status of the genus *Melanochlamys* within the Aglajidae family. The genus exhibits several distinct morphological features, including small size, internal shells that are completely and strongly calcified, taking up a notable proportion of the posterior shield. Other characteristics include continuous tubular albumen and membrane glands, short and blunt caudal lobes at their apices, a headshield rounded at the front and truncated at the back, and eyes that are not externally visible and situated deep within the body cavity (Gosliner, 1978).

The genus *Melanochlamys* is represented by 17 accepted species (WoRMS, 2023, accessed 25 September 2023). Most of them are found in the Indo-Pacific basin and the Pacific Ocean (Cooke *et al.*, 2014) and up until now only one species, *Melanochlamys maderensis* (Watson, 1897), had been found in the Atlantic Ocean, in the Madeira Island, Canary Islands, and Cape Verde (Ortea and Moro, 1998; Cooke *et al.*, 2014).

Melanochlamys diomedea was first described by Bergh, 1894 as *Doridium diomedeum*, and later moved to the genus *Aglaja* (Renier, 1807). It had been identified as *Aglaja nana* in San Francisco Bay (Steinberg and Jones, 1960) and *Aglaja diomedea* (Marcus, 1961) in California. The species *A. nana* was moved to the genus *Melanochlamys* by Rudman, 1972. In 1978, Gosliner stated that *Melanochlamys nana* (Steinberg and Jones, 1960) was a junior synonym of *M. diomedea* (Bergh, 1894). Finally, in 2014, the study by Camacho-Garcia *et al.* on the Aglajidae phylogeny conclusively confirmed that *M. diomedea* belonged to the *Melanochlamys* genus.

The first reports of *M. diomedea* specimen were from two Alaskan Islands of the Aleutian archipelago, St. Paul in the Bering Sea and the Shumagin Island in the North Pacific. The current known native geographic range of the species is along the Pacific coast of North America, from Southern California to Alaska (Steinberg and Jones, 1960; Marcus, 1961; Cooke *et al.*, 2014) (Figure 1). One specimen is reported from the Gulf of Mexico (from 1951, Collections Smithsonian's, 2024, accessed online 10 January 2024) but Cooke *et al.* (2014) consider *M. maderensis* as the only Atlantic species, further confirmation is needed for the identification of the Gulf of Mexico specimen.

In September 2023, the presence of *M. diomedea* (Bergh, 1894) in Iceland was confirmed, being the second record of a species belonging to the genus *Melanochlamys* in the Atlantic.

Unlike other members of the genus, the penis of *M. diomedea* is complex, with an elongated prostate, a seminal vesicle that runs the length of the retractor muscle, and a muscular papilla with a cuticular apex (Rudman, 1972). The species has a slim and elongated body, with the cephalic shield taking up approximately one-third of its length. The shield is almost rectangular in shape but tends to be rounded at the front. The shell is completely calcified,

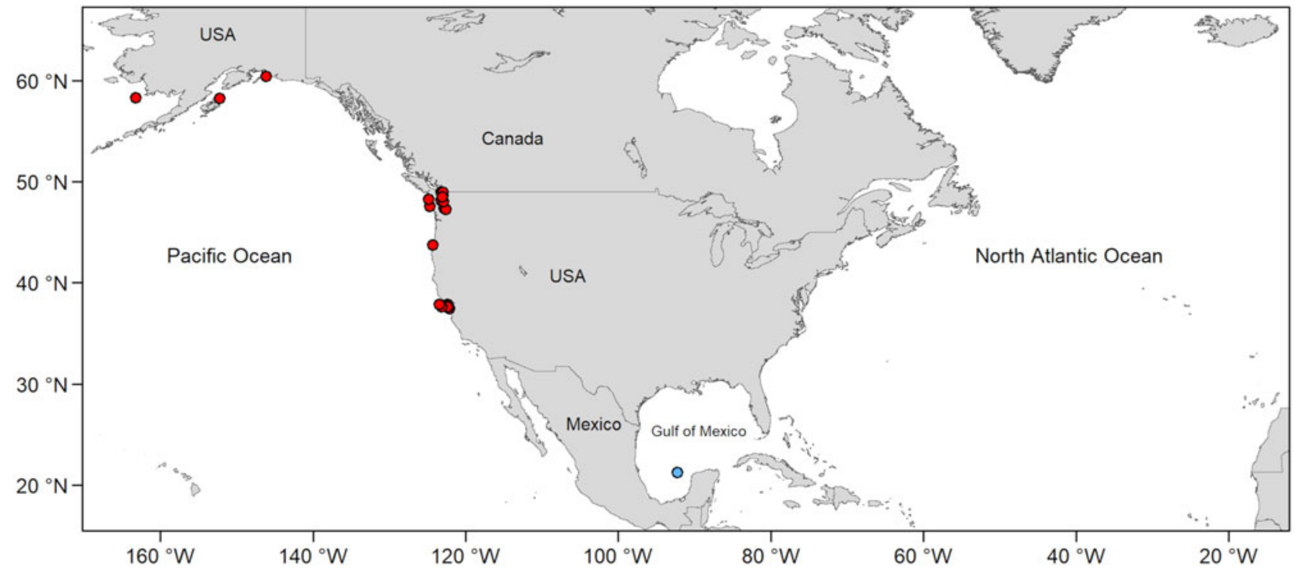

Figure 1. Map of the known distribution of *Melanochlamys diomedea* based on OBIS data (OBIS mapper, 2024, accessed online 10 January 2024). Confirmed species collection sites (red) occur only along the Pacific coast. A single unconfirmed sighting (blue) is reported in the Gulf of Mexico.

relatively thick, and slightly thinner at the front. The spiral of the shell is small and not free, with a large process that extends forwards and downwards. There is also a deep hollow in front of the spiral. The visceral region is elongated and terminates in two short and wide tails of equal size. The parapodia, except for the very front and back, are short and extend along almost the entire length of the animal (Bergh, 1894; Cooke *et al.*, 2014). The egg mass is a gelatinous sac with a spheroidal shape, measuring 1–2 cm. It is laid on the sediment and anchored to it by a strand of gel extending from the top of the mass (Hurst, 1967; Woods and DeSilets, 1997). A typical *M. diomedea* egg mass contains about 25,000–50,000 encapsulated embryos (1–4 eggs per capsule) that are arranged in a spiral pattern within the gelatinous matrix (Hurst, 1967; Woods and DeSilets, 1997). The gelatinous matrix protects the eggs from salinity fluctuations (Woods and DeSilets, 1997). Once attached to the substrate, the egg masses remain there for a period of 7–10 days until the embryos hatch, subsequently spending more than a month in the plankton as feeding larvae (Strathmann, 1987).

The first comprehensive overview of the Cephalaspidea order in Iceland was conducted by Lemche (1938). The work documented the presence of 11 species in Iceland, categorized under Cylichnidae (2), Diaphanidae (2), Laonidae (3), Aglajidae (1), Scaphandridae (2), and Retusidae (1). One additional species from Cylichnidae is mentioned, but its validity is currently being assessed by the World Register of Marines Species

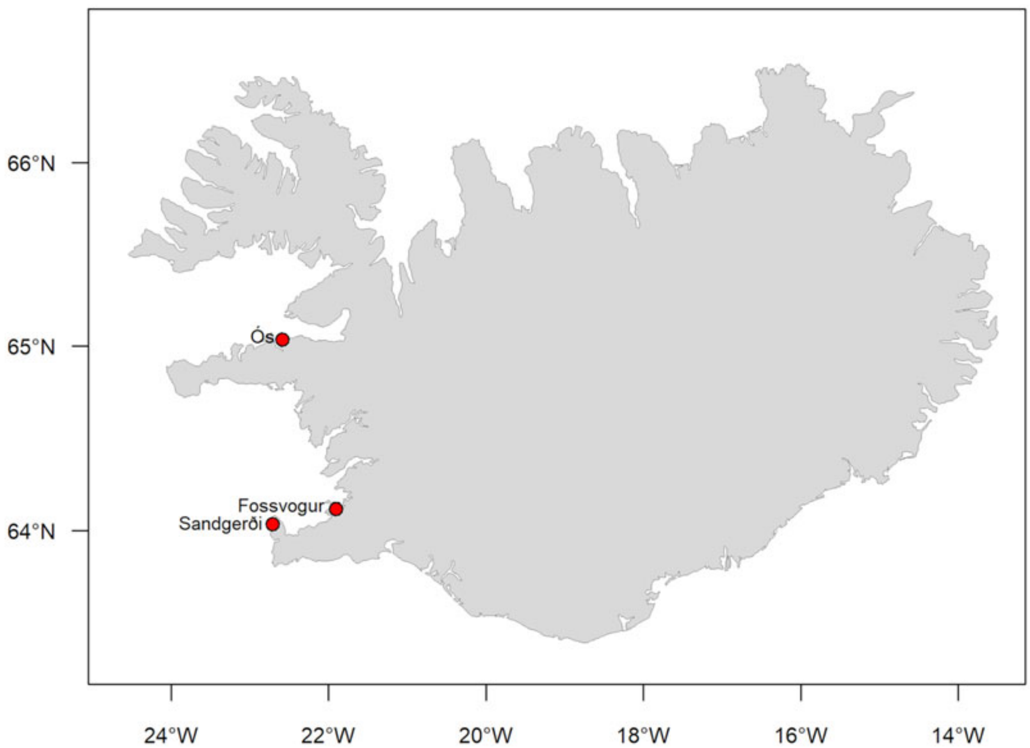

Figure 2. Map of Icelandic locations where adult specimens and egg masses of *Melanochlamys diomedea* were found.

(WoRMS). Decades later, Warén (1989) expanded the list by adding two more species from Cylichnidae. To the authors' knowledge, this study is only the second documented record of a member of the Aglajidae family in Iceland. Furthermore, this paper reports the first-ever verified occurrence of *M. diomedea* in the North Atlantic Ocean.

Materials and methods

In June 2020, abundant egg masses of an unknown mollusc were discovered in Reykjavík (Fossvogur), SW-Iceland (64.119607°N, 21.904163°W). Eggs were subsequently found in Sandgerði, SW-Iceland (64.035624°N, 22.710856°W) in April 2021 and June 2023; samples were taken on both occasions and preserved in 96% ethanol (EtOH). Egg masses were also found in June and December 2022 and June 2023 at Ós, in the inner part of Breiðafjörður, W-Iceland (65.03785°N, 22.58558°W) (Figure 2). In August 2023, a search for the mollusc was conducted at low tide at Ós, in the area where attached egg masses were found. Fifteen adult specimens were kept alive in seawater to photograph in a studio, while three specimens were preserved in 96% EtOH. Tissue from the three adult snails collected at Ós and an egg mass collected in June 2023 in Sandgerði were used for the DNA analysis.

DNA isolation was performed with a Congen SureFood Prep Basic kit according to the manufacturer's recommendations, with an additional overnight Proteinase K digestion step for the egg mass at Matís, Icelandic Food and Biotech R&D. Three markers were amplified from each extraction; two mitochondrial, cytochrome c oxidase I (COI) (Folmer *et al.*, 1994) and 16S ribosomal RNA (16S) (Palumbi *et al.*, 1991), and one nuclear, histone H3 (H3) (Colgan *et al.*, 1998), as reported in Galindo *et al.* (2016) (Table 1). Sanger sequences were generated on an ABI3730XL by Eurofins Genomics, Cologne, Germany. Sequences were trimmed in Geneious v. 6R and aligned with the built-in ClustalW algorithm (gap open cost 15, gap extend cost 6.66). Comparative marker sequences from *Melanochlamys* individuals, which had sequences for all three markers (COI, 16S, and H3), were retrieved from NCBI GenBank, as well as sequences from an *Aplysia punctata* individual for use as an outgroup (online Table S1). Maximum likelihood phylogenetic analysis, including nucleotide substitution model selection, was performed with the Linux version of IQtree (1.6.12). A consensus tree for a combined partitioned dataset was produced with 1000 bootstrap resampling. Additionally, separate consensus gene trees for the three individual markers were similarly generated using all available *Melanochlamys* sequences on NCBI GenBank (i.e. with sequences omitted from the combined analysis). Visualization of the resulting Newick file was done with FigTree v.1.4.4.

Table 1. Primers as described in Galindo *et al.* (2016)

Gene	Primers	Sequence 5′–3′	Source
COI	LCO1	GGTCAACAAATCATAAAGATATTG	Folmer *et al.* (1994)
	HCO1	TAAACTTCAGGGTGACCAAAAAATCA	
16S	16SA	CGCCTGTTTATCAAAAACAT	Palumbi *et al.* (1991)
	16SB	CCGGTCTGAACTCAGATCATGT	
H3	H3R1	ATATCCTTRGGCATRATRGTGAC	Colgan *et al.* (1998)
	H3F1	ATGGCTCGTACCAAGCAGACVGC	

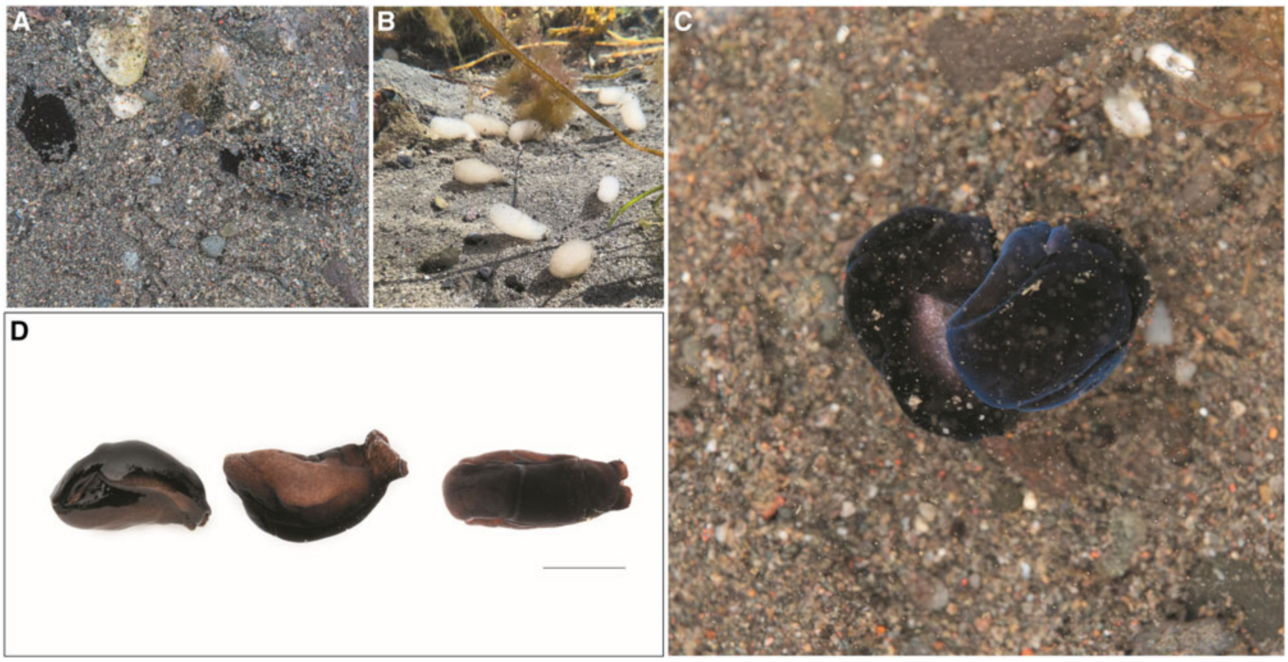

Figure 3. (A) Half-buried specimen of *Melanochlamys diomedea*. (B) Egg masses lying on the sediment. (C) Lateral, ventral, and dorsal view (from left to right) of *M. diomedea*. (D) Specimens of *M. diomedea* exhibiting a blue iridescence. Scale bar: C, 1 cm.

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Figure 4. *Melanochlamys diomedea* shell from Ós specimen. Scale bar: 1 cm.

Results

Specimens of *M. diomedea* were found half buried in the sandy-muddy sediments (Figure 3A), just as described by Gosliner (1978). In Ós, they were located at the periphery of vegetation patches with some in proximity to the egg masses. Egg masses were found lying at the surface of the sediment in a zone relatively free of vegetation (Figure 3B). A recent re-examination of photos taken from 2020, in Reykjavík, when the first egg masses were sampled showed specimens laying eggs that were not noted at the time.

The molluscs measured approximately 1.5–2 cm in length and 1 cm in width. They exhibited a dark purple-blue colour, with the foot area being slightly lighter and showing a mottled pattern (Figure 3C). Live animals also exhibit a blue iridescence, particularly visible on the edge of the parapodial extension of the foot (Figure 3D).

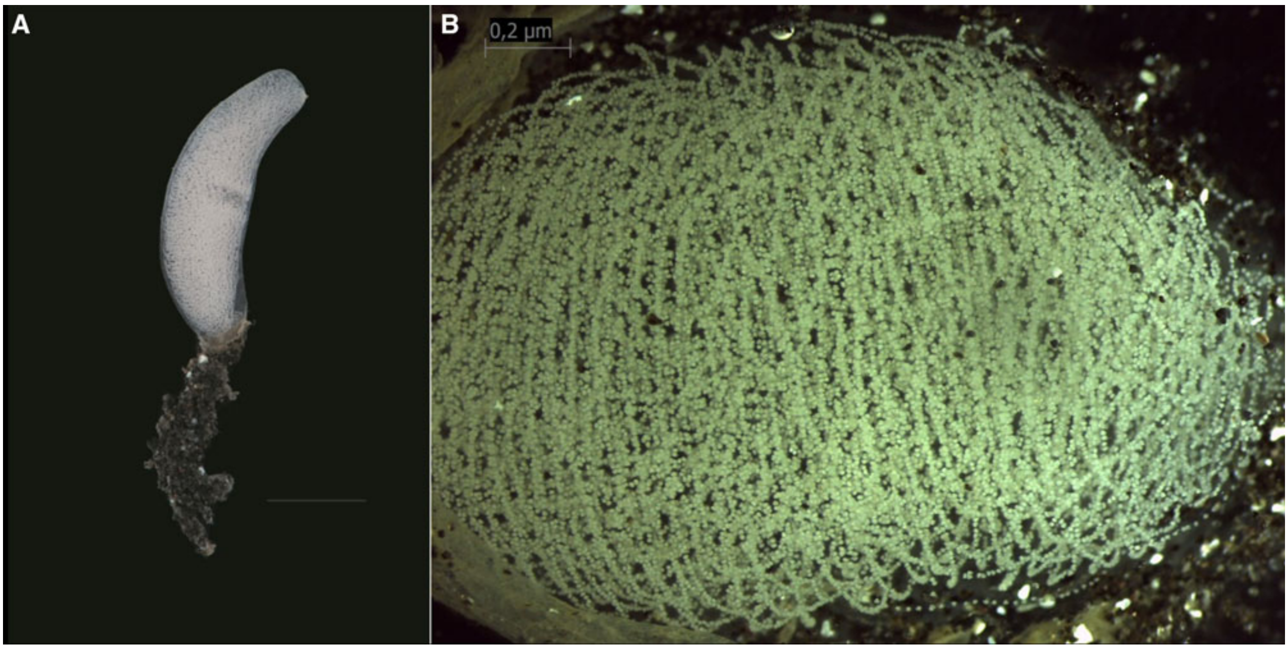

Figure 5. (A) Egg mass and strand of *Melanochlamys diomedea*. (B) Detailed stereoscopic photograph highlighting the intricate spiral pattern of the encapsulated embryos found within the gelatinous matrix. Scale bar: A, 1 cm.

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External anatomy and colour are not valid characters to distinguish species within Aglajidae, so shell morphology was used as the primary criterion (Ortea *et al.*, 2014) to narrow down our identification. Observed shell features corresponded to the genus *Melanochlamys* (Bergh, 1894) (Figure 4).

The shape of the collected egg mass and the egg disposition inside were consistent with that described for *M. diomedea* egg masses (Figure 5A, B) (Castro and Podolsky, 2012).

For the DNA sequence analysis, the same nucleotide substitution model was selected based on BIC scores for COI and 16S (Kimura 3-parameter model with unequal empirically determined base frequencies and discrete four-category gamma distributed rates). On the other hand, the H3 marker was assigned a Kimura 2-parameter model with equal base frequencies and discrete four-category gamma distributed rates. Using the combined dataset partitioned by all three genetic markers, a consensus phylogenetic tree (Figure 6) was constructed. In this tree, the Icelandic samples were grouped with 100% bootstrap support together with a *M. diomedea* voucher specimen collected from the San Juan Islands in Washington State, USA, preserved at the California State Polytechnic University, Pomona, Invertebrate Collection (CPIC00700).

Since only one *M. diomedea* individual in GenBank had sequences for all three employed markers, individual gene trees for each marker were generated to incorporate additional *M. diomedea* sequences for comparison purposes. Phylogenetic placements of the three adult snail specimens and the egg mass consistently grouped them with other *M. diomedea* individuals, as shown in the separate genetic marker analysis (Figure 7). The consensus gene trees for COI and 16S demonstrated a

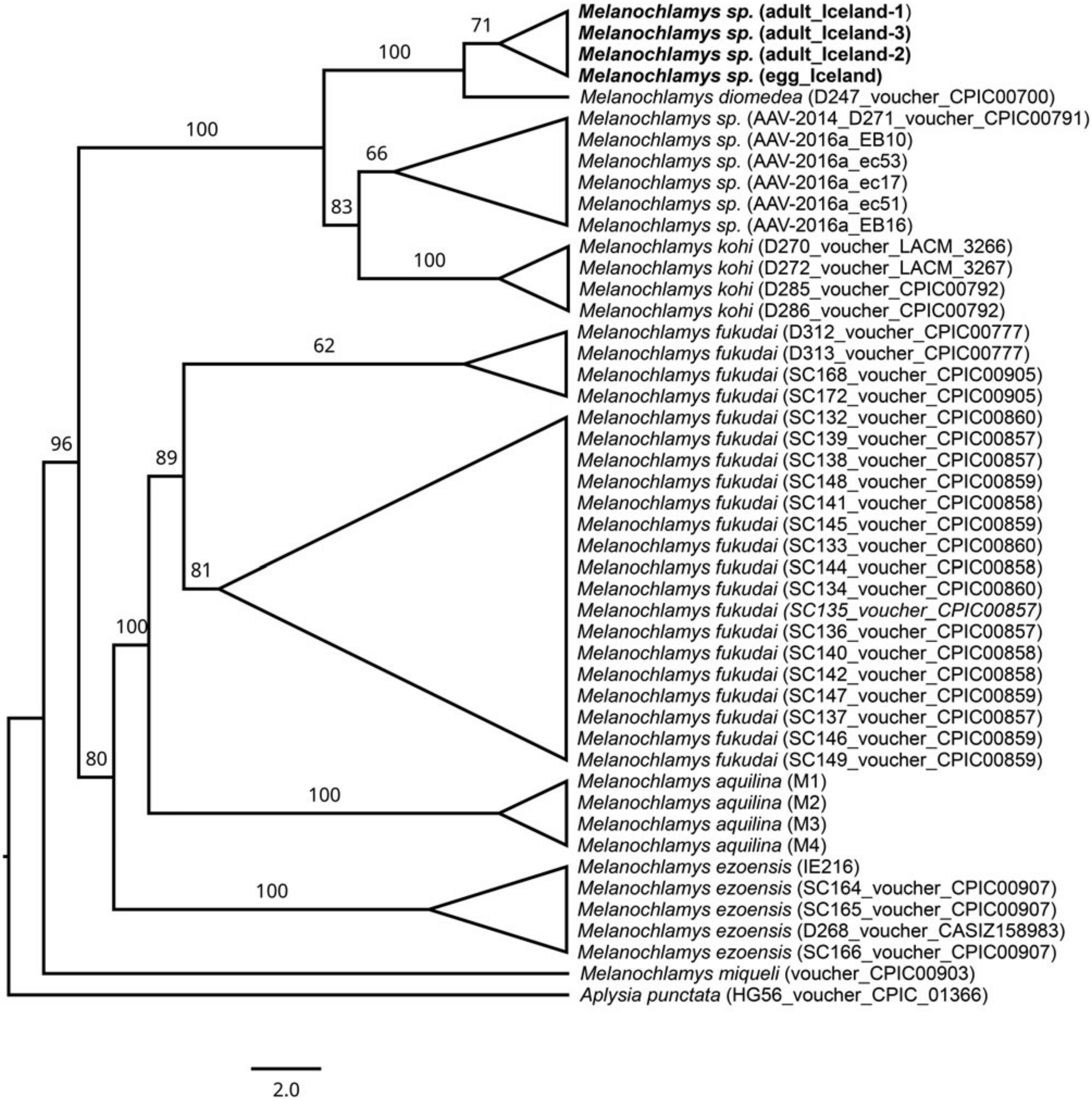

Figure 6. Maximum likelihood consensus tree calculated using two mitochondrial markers (COI, 16S) and one nuclear marker (H3), generated by IQtree and drawn with FigTree. Bootstrap node support percentages from 1000 reiterations are indicated on each branch. Specimens collected from Iceland are drawn at the top of the tree and are listed in bold. The *Melanochlamys diomedea* voucher specimen that clustered 100% of the time with the Icelandic sequences (CPIC00700) was collected from the San Juan Islands in Washington State, USA.

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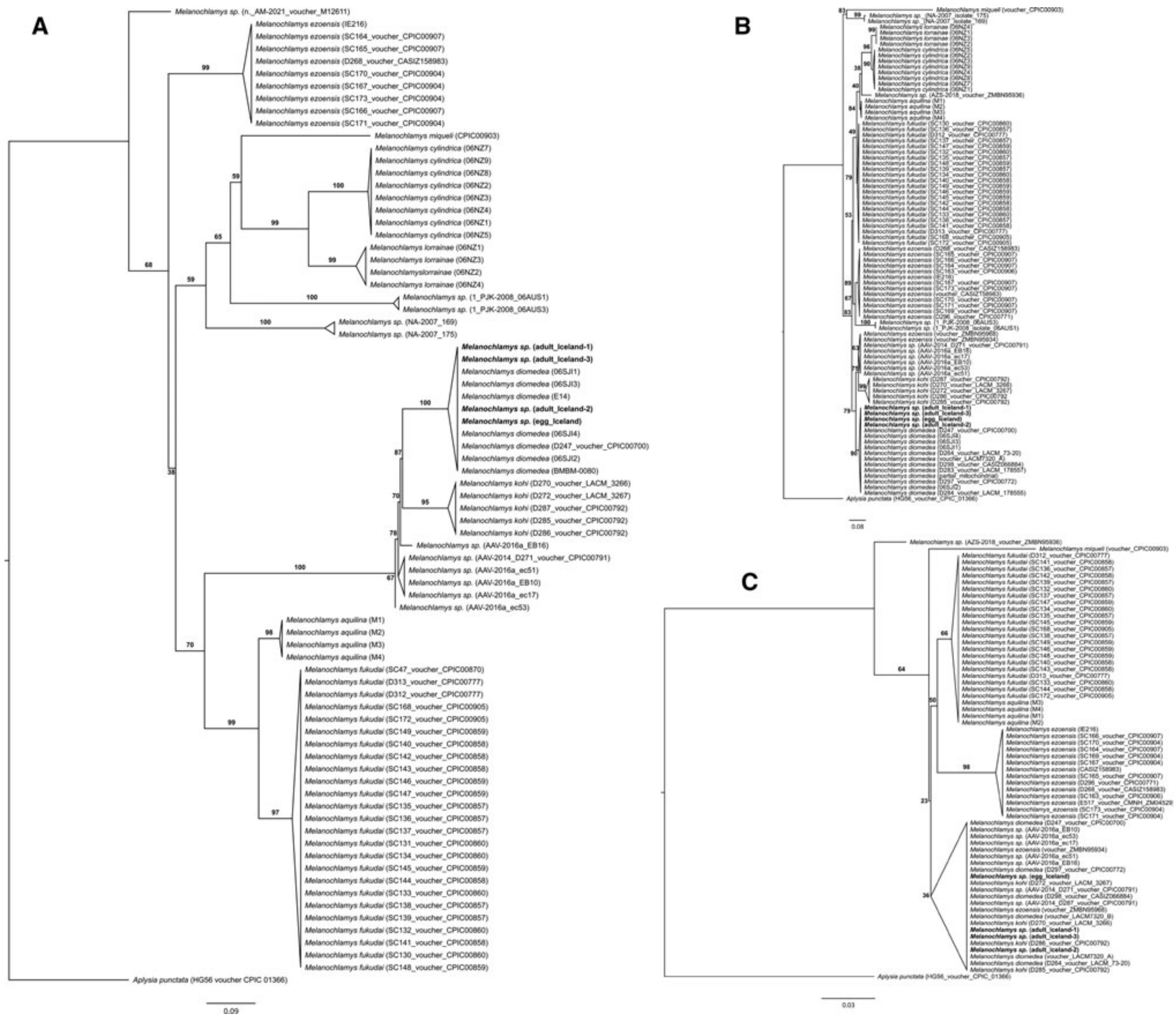

Figure 7. Maximum likelihood consensus trees calculated for each marker sequence individually: (A) cytochrome c oxidase I (COI); (B) 16S ribosomal RNA (16S); (C) histone H3 (H3). Each tree was generated using IQtree and drawn with FigTree. Bootstrap node support percentages from 1000 reiterations are indicated on each branch. Specimens collected from Iceland are listed in bold.

monophyletic grouping of the four Icelandic samples with all other *M. diomedea* sequences, with bootstrap support of 100 and 90%, respectively. However, the H3 marker analysis alone was unable to determine the relationship between *M. diomedea*, *M. kohi* (Cooke *et al.*, 2014), and a proposed cryptic species of *Melanochlamys* collected in Vladimir Bay, Russia (Breslau *et al.*, 2016).

Discussion

Melanochlamys diomedea (Bergh, 1894) is an intertidal and subtidal species occurring in muddy sand bays (Strathmann, 1987), where it is known to feed on nematodes, polychaetes, and crustaceans (Behrens and Hermosillo, 2005; Zamora-Silva and Malaquias, 2018). The species exhibits great phenotypic plasticity in colouration, shell, and external morphology. The colour is mostly commonly dark brown-black with some white spots; however, individuals within a single population can range in colour from solid black to mottled patterns and even pure white (Gosliner, 1978; Cooke *et al.*, 2014). The inconsistent shell and external morphology within the genus pose challenges for accurate taxonomic classification to the species level, highlighting the importance of DNA sequencing for positive identification (Cooke *et al.*, 2014).

The species is widespread on the Pacific coast of North America from California to Alaska (Cooke *et al.*, 2014). The species was also reported as a new addition to the Russian fauna in the Sea of Japan to south Kurile Island (Chaban and Martynov, 1998) but it was later confirmed as *Melanochlamys enzoensis* (Baba, 1957) (Cooke *et al.*, 2014; Zamora-Silva and Malaquias, 2018). One specimen was reported in 1951 in the Gulf of Mexico; however, this was never confirmed as a true recording of *M. diomedea*, with available sample, and no further records of the species have ever been reported from Gulf of Mexico. It is therefore likely that the specimen (Collections Smithsonian and OBIS mapper accessed online 10 January 2024) may well have been misidentified. So far *M. diomedea* (Bergh, 1894) has only been confirmed on the Pacific coast of North America, based on molecular analysis (Cooke *et al.*, 2014; Zhang *et al.*, 2020). This is the first confirmed occurrence in the Atlantic Ocean (both North and South) as well as a new addition to the Icelandic fauna.

It is not known how *M. diomedea* arrived in Iceland; however, in recent decades, the majority of introduced species appear to have been transported via ships, either as part of biofouling or through ballast water (Gíslason *et al.*, 2013, 2014; Gunnarsson *et al.*, 2015, 2023; Ramos-Esplá *et al.*, 2020; Micael *et al.*, 2021, 2023). This mode of transportation aligns with the location of arrival, which is southwest Iceland, an area characterized by significant maritime traffic (Gíslason *et al.*, 2013; Gunnarsson *et al.*, 2015, 2023).

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S002531542400047X.

Data availability statement. Specimens used in this study for morphological identification are preserved at the Marine and Freshwater Research Institute, Hafnarfjörður, Iceland. All DNA sequences generated have been submitted to the NCBI GenBank, with accession numbers listed in online Table S1.

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Competing interest. None.

Ethical standards. All sampling and data acquisition for this project were performed in accordance with local ordinances (no collection permits were required) and standards for ethical research.

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