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Rapid Communication

Mollusc on the move; First record of the Newfoundland's razor clam, *Ensis terranovensis* Vierna & Martínez-Lage, 2012 (Mollusca; Pharidae) outside its native range

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Abstract

In recent years the number of non-indigenous marine species has been increasing in Icelandic waters. In May 2019, a razor shell (*Ensis* sp.) was found for the first time in Iceland. Since then, living and empty razor shells have been discovered at several locations in Southwest Iceland. Upon morphological examination, the specimens were thought to belong to either *Ensis leei* or *Ensis terranovensis*, both native to the east coast of North America. Molecular analysis, using COI and 16S rRNA markers, showed that the Icelandic specimens belong to the latter species. Native populations of *Ensis terranovensis* have, until now, only been reported in Newfoundland, Canada. This represents the first record of *E. terranovensis* outside its native range. The Newfoundland's razor shell has most likely arrived in Iceland as larvae discharged with ballast water. Based on the sizes of specimens found in Iceland, the species is likely to have arrived several years prior to this first record. Presumably it has already established viable spawning populations that are likely to spread along the coast.

Key words: Iceland, introduced species, DNA barcoding, COI, 16S rRNA

Introduction

In recent decades, anthropogenic interference has caused a significant increase in the translocation of marine organisms beyond their natural distribution range worldwide (Bax et al. 2003; Streftaris et al. 2005), often facilitated by suitable environmental conditions created by climate change at the site of new settlement. The most important vectors for long distance translocation of sessile marine organisms are thought to be transport in ballast water, fouling on ship hulls, or unintentional introduction of "hitchhikers" during intentional transport of aquaculture animals (Bax et al. 2003; Galil et al. 2014; Brenner et al. 2014; Grosholz et al. 2015; Geburzi and McCarthy 2018; Boudouresque et al. 2020). Such transport has helped

marine species surpass greater, natural distribution barriers, e.g. vast and deep oceans and large continents.

In Iceland, most of the known marine non-indigenous species (NIS) have first been observed in the southwestern part of the country, where maritime transport is most intense and sea temperatures are relatively higher than in other parts of the country (Gunnarsson et al. 2015; Ramos-Esplá et al. 2020; Micael et al. 2021, 2022). It is thought that most of the marine NIS in Iceland arrived from Europe although this can be difficult to verify. Some NIS of northwestern Atlantic origin are found in Iceland, but those species may have arrived through secondary spread from Europe as most of them had already established in the eastern Atlantic before they were found in Iceland (Gunnarsson et al. 2015; Ramos-Esplá et al. 2020; Micael et al. 2021). An exception is the Atlantic rock crab (*Cancer irroratus* Say, 1817) that was first found in Iceland in 2006 (Gíslason et al. 2014). *Cancer irroratus* is native to the east coast of North America and most probably arrived from there as this was the first record of the species outside its native range (Gíslason et al. 2013, 2014).

Here we report the occurrence of an introduced marine mollusc, a razor shell (*Ensis* sp.), recently discovered in Southwest Iceland. The present find represents the first record of the recently described, *Ensis terranovensis* Vierna and Martínez-Lage 2012, outside its native distribution range, which is in Newfoundland, Canada.

Materials and methods

Sampling

In May 2019, empty razor shells (*Ensis* sp.) were found on the shore, in a sandy estuary area by the farm Naustanes, at the head of Kollafjörður, Southwest (SW) Iceland (Figure 1). Since then, several living as well as empty *Ensis* shells have been found at a number of similar sandy estuarine areas in Kollafjörður, Hvalfjörður and Borgarfjörður, SW Iceland (Figure 1). Specimens were collected by hand in the intertidal zone as empty shells were found lying on the sand flats or living shells were protruding up from the surface sediment. Shells that were not broken were measured for maximum length and width to the nearest 1 mm. The form of the shells was noted, and the inside of shells was inspected for scars left by muscles and mantel. For morphological species determination we used the keys and descriptions provided by Cosel (2009) and Vierna et al. (2012).

DNA extraction, PCR and sequencing

DNA was extracted from approximately 0.2 g of foot tissue of three *Ensis* sp. individuals, one collected at each of three intertidal sand flats in SW Iceland: Naustanes in Kollafjörður (64.20°N; 21.71°W, collected in April 2022), Helguvík in Hvalfjörður (64.39°N; 21.43°W, collected in March 2021)

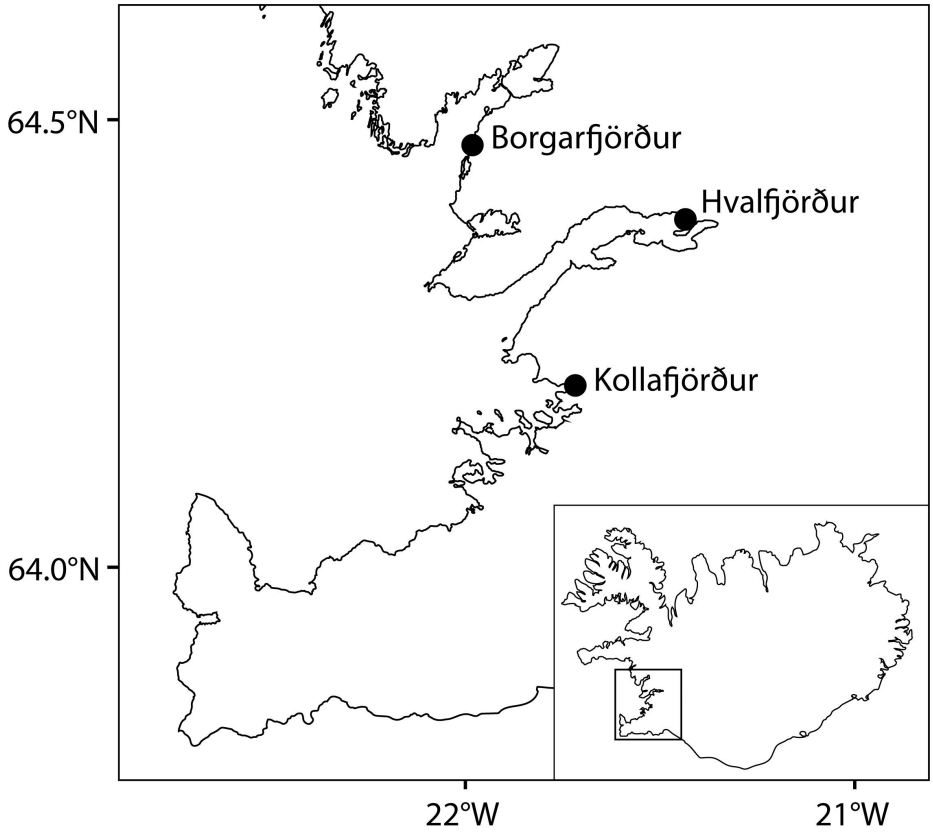

Figure 1. Locations in southwestern Iceland (black dots) where *Ensis* sp. was sampled for DNA analysis.

and Höfn in Borgarfjörður (64.47°N; 21.98°W, collected in February 2021) (Figure 1), using sbeadex livestock kit (LGC Biosearch Technologies) following the manufacturer's protocol. Concentrations of extracted DNA were assessed using NanoDrop 2000 (Thermo Scientific) spectrophotometer. Two mitochondrial genes COI and 16S rRNA were amplified using the primer pairs COIF-ALT (5'-ACAAATCAYAARGAYATYGG-3') / COIR-ALT (5'-TTCAGGRTGNCCRAARAAYCA-3') and 16Sar-ALT (5'-GCCTGTTTATCAAAAACATSG-3') / 16Sbr-ALT (5'-CCGGTCTGAACTCAGATCATGT-3') respectively (Mikkelsen et al. 2006). The PCR reaction was performed in a mixture consisting of: 2 µL DNA, 2 µL of 10X Standard Buffer, 2 µL of 10 mM DTP, 0.12 µL of forward and reverse primers at 100 µM concentration each, 13.6 µL of ddH_2O and 0.15 µL Taq (Taq DNA Polymerase with Standard Taq Buffer, NEB cat. M0273L). PCR amplifications were done in a MiniAmp™ Thermal Cycler (Applied Biosystems). We used identical PCR conditions for both regions: An initial denaturation step at 94°C for 4 min, followed by 35 cycles of denaturation at 95°C for 50 s, annealing at 48°C for 40 s and an extension at 68°C for 1 min, and then a final extension at 68°C for 7 min. PCR success was assessed by gel electrophoresis on 1% Agarose gel.

Sequencing was performed in an ABI 3730 DNA Analyzer (Applied Biosystems) using standard Sanger sequencing method (Sanger and Coulson 1975). Electropherograms were processed using phred v. 0.071220.c (Green

and Ewing 2002) and phrap v. 1.090518 (Green 2009). Finally, Consed v.29.0 was used for quality trimming and alignment of the forward and reverse sequences (Gordon and Green 2013). After visual inspections of the electropherograms and quality trimming, COI sequences of 542 bp and 16S rRNA sequences of 427 bp were obtained. The DNA sequences generated and analysed in this study are available from GenBank sequence database (Supplementary material Table S1).

Phylogenetic analysis

COI and 16S rRNA sequences from samples collected in the present study, and of 14 additional *Ensis* species from the Atlantic obtained from GenBank and BOLD systems, were used in the analysis. Only individuals that had both markers sequenced were selected. Information on GenBank accession numbers of the sequences is shown in Table S1. Sequences were cut to equal lengths and the marker pair from each individual was combined before constructing the maximum likelihood phylogenetic tree.

Phylogenetic analyses were performed with maximum likelihood and Bayesian methods using *Phaxas pellucidus* (Pennant, 1777) as outgroup. Maximum likelihood (ML) analyses were done using MEGA-X (Kumar et al. 2018). Support for ML analysis was assessed both with approximate likelihood ratio test and bootstrap values that were created with 1000 resampling replicates for both COI and 16S rRNA data. Bayesian analysis was conducted with MrBayes v3.2.6 (Ronquist et al. 2012) using a general time reversible model with invariable sites and gamma distribution (GTR+I+Γ) to describe the nucleotide sequence evolution, which was assessed as a good candidate by JModelTest2 with the Akaike Information Criterion (Guindon and Gascuel 2003; Darriba et al. 2012). This model was used in Bayesian analysis for both COI and 16S rRNA. Markov chain Monte Carlo analysis was run for 1,000,000 generations with sampling every 1000 generations. Potential scale reduction factor scores of < 0.01 were used to determine the number of generations run. The sump and sumt functions of "MrBayes" were used to calculate Bayesian posterior probability values. The first 25% of the samples from the cold chain were discarded as burn-in.

Results

At all localities where *Ensis* shells were encountered in SW Iceland both living individuals and empty shells were found. Living specimens of *Ensis* sp. had not previously been found in Iceland. At the localities where *Ensis* sp. was found, the most common infaunal molluscs were *Mya arenaria* Linnaeus 1758, *Cerastoderma edulis* (Linnaeus, 1758) and *Arctica islandica* (Linnaeus, 1767). *Mytilus edulis* Linnaeus, 1758 was a common epibenthic mollusc.

Figure 2. Live specimen of *Ensis terranovensis* from the shore by the farm Höfn in Borgarfjörður, southwestern Iceland, sampled in February 2021. The shell has its foot protruding out of the shell. Scale bar: 2 cm.

Intact *Ensis* sp. shells measured from 1.5 cm up to 18.0 cm long and were about 6 times longer than wide. The form of the shells resembled those described for both *Ensis leei* M. Huber, 2015 and *E. terranovensis* Vierna & Martínez-Lage, 2012, species that can be difficult to distinguish morphologically (cf. Cosel 2009; Vierna et al. 2012). The Icelandic shells had an average length-width ratio of about 6 as in the two mentioned species (Figure 2; see Cosel 2009; Vierna et al. 2012). The main difference between the two species is the ratio of the distance between the posterior abductor scar and pallial sinus and the shell width (Vierna et al. 2012). Scars left by muscles and mantel on the inside were difficult to discern but seemed to fit the description of *E. terranovensis*. Additionally, the shells of *E. terranovensis* from Newfoundland have thicker and stronger valves than *E. leei* (Vierna et al. 2012).

DNA gene sequences of the mitochondrial COI and 16S rRNA of the *Ensis* sp. found in Iceland most closely resembled sequences from *E. terranovensis* from Canada when blasted against sequences of the respective markers in GenBank and BOLD System. As both markers distinguished the Atlantic *Ensis* species quite well at the species level and resulted in a very similar phylogenetic tree, a Bayesian maximum likelihood tree was constructed using concatenated sequences of the two markers (Figure 3).

The phylogenetic tree constructed based on COI and 16S rRNA sequences showed monophyly encompassing two distinct clades of species (Figure 3). The *Ensis* species native to the Atlantic coast of Europe, i.e. *E. magnus* Schumacher, 1817, *E. ensis* (Linnaeus, 1758), *E. minor* (Chenu, 1843), and *E. siliqua* (Linnaeus, 1758), formed a distinct cluster along with *E. goerensis* (Clessin, 1888) from Senegal, clearly separated from the other

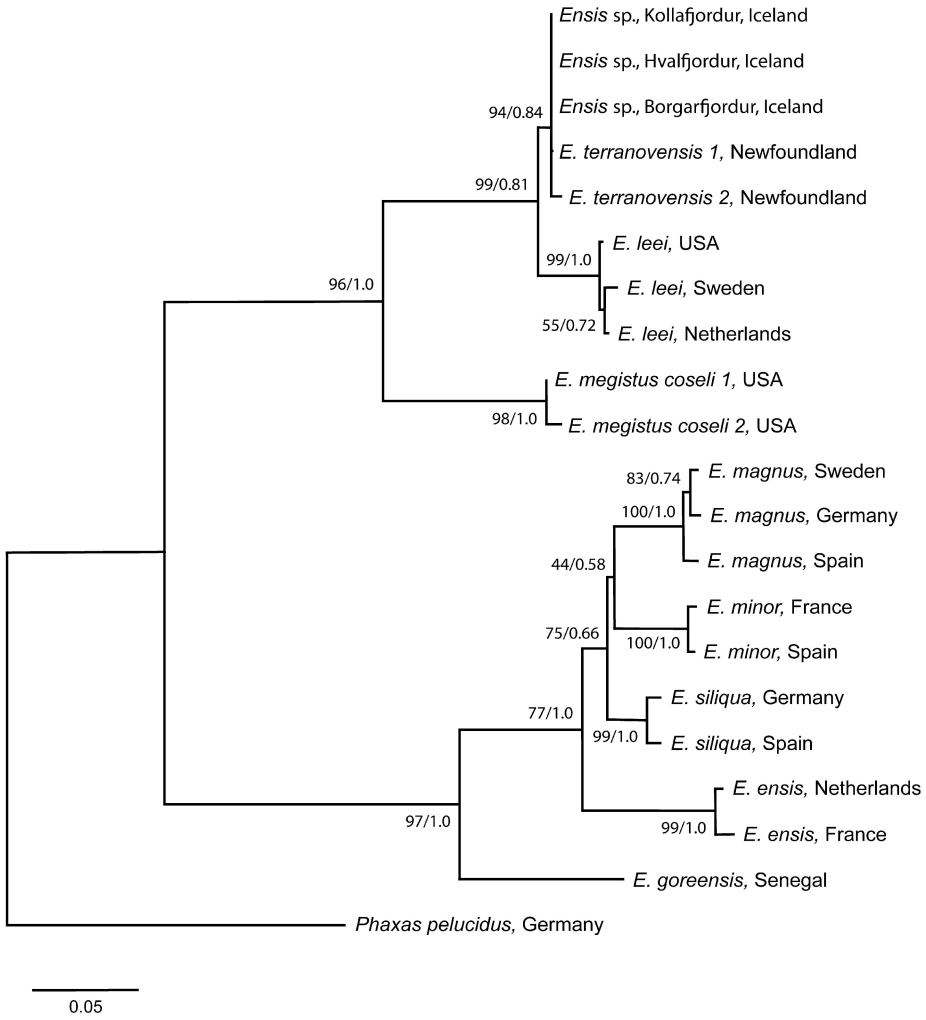

Figure 3. Maximum likelihood (ML) tree for Atlantic species of *Ensis* spp. drawn using concatenated COI and 16S rRNA sequences. Values at nodes: ML bootstrap /Bayesian posterior probability. Scale bar: substitutions per site. For details on the specimens, from which the sequences were obtained, see supplementary data in Table S1.

cluster comprising *Ensis* species from the western Atlantic, i.e. *E. terranovensis*, *E. leei* and *E. megistus coseli* Vierna, 2014. The specimens collected in Iceland formed an integral part of the *E. terranovensis* branch on the phylogenetic tree. Considering the morphological characteristics along with the molecular evidence we considered the species newly found in Iceland to be the recently described *E. terranovensis*.

Discussion

The finding of the razor shell specimens in Kollafjörður, Southwest (SW) Iceland in May 2019 was the first record of *E. terranovensis* outside its native range in Newfoundland, Canada. *Ensis terranovensis* is, so far, only found at a few localities in SW Iceland in addition to the three sites examined here. Due to the wide range in shell lengths, we suspect that it has been actively reproducing in Iceland for several years. Therefore, given favourable environmental conditions, the species may spread rapidly and extend its distribution in Iceland as more colonies are established.

In 1957 two empty razor shells, 18.5 cm long, were found in the upper littoral zone in Southeast Iceland (Óskarsson 1969). By the fact that the periostracum was still covering parts of the shells, they were thought to have died relatively shortly before they were found. Upon examination they were determined to be *E. magnus* (as *E. arcuatus* (Jeffreys, 1865), Óskarsson 1969). Since this discovery, *Ensis* sp. had not been recorded, either dead or alive, in Iceland until after May 2019 when *E. terranovensis* was discovered in SW Iceland.

There are presently 13 accepted species of *Ensis* spp., of which eight are found in the Atlantic. Five of them are native to the eastern Atlantic and three to the western Atlantic (MolluscaBase 2022). Molecular data clearly shows that *Ensis* specimens collected in SW Iceland belong to the species *E. terranovensis*, native to Canada. When the genetic relationship between *Ensis* species in the Atlantic is compared, the species originating from the east coast of North America form a distinctive clade clearly separated from those originating from the coast of Europe (Figure 3). This is in accordance with results obtained by Vierna et al. (2012, 2014). *Ensis terranovensis* was first described in 2012 from specimens collected in Long Pond, Conception Bay, Newfoundland (Vierna et al. 2012).

In late 1970s the jackknife shell *Ensis leei*, native to the east coast of North America, was introduced to Europe presumably via ballast water into the Elbe estuary in Germany (Cosel et al. 1982). It has since spread northwards along the coast to Denmark, Sweden and Norway and southwards to the Netherlands, Belgium, France, UK and Galicia in Spain (Gollasch et al. 2015). It has become a dominant species in the benthic communities in several estuaries in the North Sea, English Channel and Bay of Biscay (Gollasch et al. 2015; Ollivier and Arthur 2018).

Ensis leei and *E. terranovensis* both occur on the coast of Newfoundland and are occasionally found living together (Philip Sargent DFO, NL, Canada, *pers. comm*. 2021), and *E. leei* has been reported as far north as Labrador in Canada. If, as we suspect, *E. terranovensis* has been brought to Iceland by ballast water (see below), it is not unlikely that *E. leei* will appear in Iceland either from the east coast of North America or from Europe. Considering that *E. terranovensis* has established viable populations in southwestern Iceland, local environmental conditions seem to be favourable for the species and should be equally favourable for the growth of *E. leei*.

It seems most likely that the Newfoundland razor shells were introduced to Iceland as larvae discharged with ballast water into the coastal waters in SW Iceland, where shipping traffic is most intense and sea temperatures are relatively high compared to the rest of the Icelandic coast. As far as records show, there has been regular traffic of cargo ships travelling between Newfoundland and Faxaflói harbours in SW Iceland over the last decades (information from Icelandic Coast Guard records on ship traffic in Icelandic Waters from 2012 to 2022; Magnússon 1998).

In 2010 a ballast water management regulation was adopted to prevent the introduction of NIS in Icelandic waters. The general rule is that ballast water must not be discharged within Iceland's pollution jurisdiction which coincides with the 200 mile Exclusive Economic Zone. Discharging ballast water within Iceland's pollution jurisdiction is, however, authorised after being exchanged just before entering the jurisdiction or treated to secure a minimum number of organisms before discharge (Iceland Ballast Water Regulation 2010). It is possible that *E. terranovensis* was introduced before the Ballast Water Regulation was enforced but it may also have been introduced after 2010. Studies have shown that exchange of ballast water at sea does diminish but not eliminate the risk of species introductions (Darling et al. 2018). Maritime traffic has increased in the past years (cf. Borch et al. 2016; Carney et al. 2017) and that may have partly counterbalanced the effectiveness of the ballast water regulation, allowing for the introduction of *E. terranovensis* to Iceland.

Studies of age and growth of *E. terranovensis* are not available, as far as we know. Empty shells of *E. terranovensis* found in Iceland were up to 18 cm long. The closely related species *E. leei* normally reaches 12 to 15 cm in five years after which the growth slows down (Beukema and Dekker 1995; Kenchington et al. 1998; Cardoso et al. 2013). Comparing this size and growth with the maximum size of *E. terranovensis* found in Iceland, it seems clear that they must have been introduced to Iceland several years before they were first discovered, in May 2019. Further studies on the distribution, growth rate, age structure, reproduction and larval development of *E. terranovensis* populations are needed to establish possible time of arrival and the likely scenario for its future spreading in Iceland.

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Authors' contribution

All authors contributed to the study conception, sampling design, methodology and preparation. SS and DG were responsible for the DNA extraction and sequencing. KG, SS and DG did the phylogenetic analysis. KG wrote the first draft of the manuscript and all authors revised, edited and approved the final manuscript.

Data availability

The DNA sequences generated during the current study are publicly available from GenBank and coordinates for the specimens are included in the supplementary table S1. The new species records have been included in the open access AquaNIS databank (Gíslason 2023).

References

Bax N, Williamson A, Aguero M, Gonzalez E, Geeves W (2003) Marine invasive alien species: A threat to global biodiversity. *Marine Policy* 27: 313–323, https://doi.org/10.1016/S0308-597X(03)00041-1

Beukema JJ, Dekker R (1995) Dynamics and growth of a recent invader into European coastal waters: The American razor clam, *Ensis directus*. *Journal of the Marine Biological Association of the United Kingdom* 75: 351–362, https://doi.org/10.1017/S0025315400018221

Borch OJ, Andreassen N, Marchenko N, Ingimundarson V, Gunnarsdottir H, Ludin L, Petrov S, Jakobsen U, Dali B (2016) Maritime activity in the high north - Current and estimated level up to 2025. MARPART project report 1, Utredning nr. 7. Bodø: North University, 130 pp

Boudouresque C-F, Blanfuné A, Pergent G, Pergent-Martini C, Perret-Boudouresque M, Thibaut T (2020) Impacts of marine and lagoon aquaculture on macrophytes in Mediterranean benthic ecosystems. *Frontiers in Marine Science* 7: 218, https://doi.org/10.3389/fmars.2020.00218

Brenner M, Fraser D, Van Nieuwenhove K, O'Beirn F, Buck BH, Mazurié J, Thorarinsdottir GG, Dolmer P, Sanchez-Mata A, Strand O, Flimlin G, Miossec L, Kamermans P (2014) Bivalve aquaculture transfers in Atlantic Europe. Part B: Environmental impacts of transfer activities. *Ocean Coastal Management* 89: 139–146, https://doi.org/10.1016/j.ocecoaman.2013.10.009

Cardoso JFMF, Nieuwland G, Witbaard R, van der Veer HW, Machado JP (2013) Growth increment periodicity in the shell of the razor clam *Ensis directus* using stable isotopes as a method to validate age. *Biogeosciences* 10: 4741–4750, https://doi.org/10.5194/bg-10-4741-2013

Carney KJ, Minton MS, Holzer KK, Miller AW, McCann LD, Ruiz GM (2017) Evaluating the combined effects of ballast water management and trade dynamics on transfers of marine organisms by ships. *PLoS ONE* 12: e0172468, https://doi.org/10.1371/journal.pone.0172468

Cosel R von (2009) The razor shells of the eastern Atlantic, part 2. Pharidae II: the genus *Ensis* Schumacher, 1817 (Bivalvia, Solenoidea). *Basteria* 73: 9–56

Cosel R von, Dorjes J, Muhlenhardt-Siegel U (1982) Die amerikanische Schwertmuschel *Ensis directus* (Conrad) in der Deutschen Bucht. I. Zoogegraphie und Taxonomie im Vergleich mit den einheimischen Schwertmuschel-Arten. *Senckenbergiana maritima* 14: 147–173

Darling JA, Martinson J, Gong Y, Okum S, Pilgrim E, Pagenkopp Lohan KM, Carney KJ, Ruiz GM (2018) Ballast water exchange and invasion risk posed by intracoastal vessel traffic: An evaluation using high throughput sequencing. *Environmental Science and Technology* 52: 9926−9936, https://doi.org/10.1021/acs.est.8b02108

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772, https://doi.org/10.1038/nmeth.2109

Galil BS, Marchini A, Occhipinti-Ambrogi A, Minchin D, Narščius A, Ojaveer H, Olenin S (2014) International arrivals: widespread bioinvasions in European Seas. *Ethology Ecology & Evolution* 26: 152–171, https://doi.org/10.1080/03949370.2014.897651

Geburzi JC, McCarthy ML (2018) How do they do it? - Understanding the success of marine invasive species. In: Jungblut S, Liebich V, Bode M (eds), YOUMARES 8 - Oceans across boundaries: Learning from each other. Springer, Cham, pp 109–124, https://doi.org/10.1007/978-3-319-93284-2_8

Gíslason ÓS, Svavarsson J, Halldórsson HP, Pálsson S (2013) Nuclear mitochondrial DNA (NUMT) in the Atlantic rock crab *Cancer irroratus* Say, 1817 (Decapoda, Cancridae). *Crustaceana* 86: 537–552, https://doi.org/10.1163/15685403-00003191

Gíslason ÓS, Halldórsson HP, Pálsson MF, Pálsson S, Davíðsdóttir B, Svavarsson J (2014) Invasion of the Atlantic rock crab (*Cancer irroratus*) at high latitudes. *Biological Invasions* 6: 1865–1877, https://doi.org/10.1007/s10530-013-0632-7

Gollasch S, Kerckhof F, Craeymeersch J, Goulletquer P, Jensen K, Jelmert A, Minchin D (2015) Alien species alert: *Ensis directus*. Current status of invasions by the marine bivalve *Ensis directus*. ICES Cooperative Research Report No. 323, 32 pp, https://doi.org/10.17895/ices.pub.5491

Gordon D, Green P (2013) Consed: A graphical editor for next-generation sequencing. *Bioinformatics* 29: 2936–2937, https://doi.org/10.1093/bioinformatics/btt515

Grosholz ED, Crafton RE, Fontana RE, Pasari JR, Williams SL, Zabin CJ (2015) Aquaculture as a vector for marine invasions in California. *Biological Invasions* 17: 1471–1484, https://doi.org/10.1007/s10530-014-0808-9

Guindon S, Gascuel O (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology* 52: 696–704, https://doi.org/10.1080/10635150390235520

Gunnarsson K, Thórarinsdóttir GG, Gíslason ÓS (2015) Framandi sjávarlífverur við Ísland. *Náttúrufræðingurinn* 82(1–2): 4–14 [in Icelandic with English summary]

Kenchington E, Duggan R, Riddell T (1998) Early life history characteristics of the razor clam (*Ensis directus*) and the moonsnails (*Euspira* spp.) with applications to fisheries and aquaculture. Canadian Technical Report of Fisheries and Aquatic Science No. 2223, vii + 32 pp

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35: 1547–1549, https://doi.org/10.1093/molbev/msy096

Magnússon G (1998) Eimskip frá upphafi til nútíma, Vol 1. Hf. Eimskipafélag Íslands, Reykjavík, 424 pp [in Icelandic]

Micael J, Rodrigues P, Gíslason S (2021) Native vs. non-indigenous macroalgae in Iceland: state of knowledge. *Regional Studies in Marine Science* 47: 101944, https://doi.org/10.1016/j.rsma.2021.101944

Micael J, Denisenko NV, Gíslason S, Guðmundsson G, Kukliński P, Rodrigues P (2022) Diversity of Bryozoa in Iceland. *Polar Biology* 45: 1391–1402, https://doi.org/10.1007/s00300-022-03078-5

Mikkelsen PM, Bieler R, Kappner I. Rawlings TA (2006) Phylogeny of Veneroidea (Mollusca: Bivalvia) based on morphology and molecules. *Zoological Journal of the Linnean Society* 148: 439–521, https://doi.org/10.1111/j.1096-3642.2006.00262.x

Ollivier E, Arthur P (2018) Evaluation des gisements de couteaux de Cayeux-sur-Mer au Touquet en 2018. Rapport du GEMEL no 18-018, 19 pp

Óskarsson I (1969) Hefur nýr borgari bæzt í hóp íslenskra lindýra? *Náttúrufræðingurinn* 38: 199–201 [in Icelandic with English summary]

Ramos-Esplá AA, Micael J, Halldórsson HP, Gíslason S (2020) Iceland: a laboratory for non-indigenous ascidians. *BioInvasions Records* 9: 450–460, https://doi.org/10.3391/bir.2020.9.3.01

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542, https://doi.org/10.1093/sysbio/sys029

Sanger F, Coulson AR (1975) A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *Journal of Molecular Biology* 94: 441–448, https://doi.org/10.1016/0022-2836(75)90213-2

Streftaris N, Zenetos A, Papathanassiou E (2005) Globalisation in marine ecosystems: The story of non-indigenous marine species across European seas. *Oceanography and Marine Biology: Annual Review* 43: 419–453, https://doi.org/10.1201/9781420037449-10

Vierna J, Jensen KT, Gonzalez-Tizon AM, Martınez-Lage A (2012) Population genetic analysis of *Ensis directus* unveils high genetic variation in the introduced range and reveals a new species from the NW Atlantic. *Marine Biology* 159: 2209–2227, https://doi.org/10.1007/s00227-012-2006-6

Vierna J, Cuperus J, Marınez-Lage A, Jansen JM, Perina A, Van Pelt H, Gonzalez-Tizon AM (2014) Species delimitation and DNA barcoding of Atlantic *Ensis* (Bivalvia, Pharidae). *Zoologica Scripta* 43: 161–171, https://doi.org/10.1111/zsc.12038

Web sites, online databases and software

Gíslason S (2023) *Ensis terranovensis*. In: AquaNIS. Editorial Board. Information system on Aquatic Non-Indigenous and Cryptogenic Species. www.corpi.ku.lt/databases/aquanis. Version 2.36+ (accessed 29 April 2023)

Green P (2009) Phrap, version 1.090518. http://phrap.org

Green P, Ewing B (2002) Phred, version 0.071220.c. http://phrap.org

Iceland Ballast Water Regulation (2010) https://island.is/reglugerdir/nr/0515-2010 (accessed 19 June 2022)

MolluscaBase (2022) MolluscaBase. *Ensis* Schumacher, 1817. https://www.marinespecies.org/aphia.php?p=taxdetails&id=138333 (accessed 15 December 2022)

Supplementary material

The following supplementary material is available for this article:

Table S1. GenBank accession number, tentative species.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2023/Supplements/BIR_2023_Gunnarsson_etal_SupplementaryMaterial.xlsx