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Grooved razor clam (*Solen marginatus*) in the mid-Atlantic Azores: unravelling ecology, phylogeny, and population biology of a new population

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Abstract

The first insights on habitat and phylogenetic origin of a newly found population of *Solen marginatus* are provided in the mid-North Atlantic Azores archipelago, in the bay of Praia da Vitória, Terceira Island. Distribution is confined to the northern portion and most sheltered part of the bay down to 14.2 m depth. Densities with an average of 12.69 individuals/m^2 were found at 8.4 m depth, using 4 × 20 sqm visual transects. Sizes of shell length between 10 and 12 cm comprised 60% of collected specimens (n = 118), ranging between 8.79 and 15.4 cm and averaging 11.28 cm. Considering shell length, the high densities and dispersion area, a settlement period above 20 years is estimated. Greater genetic affinity was found in the Ria de Aveiro (North of Portugal) and the Asturias populations (North of Spain). The source origin remains undetermined, with intentional or non-intentional anthropogenic introduction, as well as natural dispersion remaining possible, although more unlikely. Due to the commercial value of this species, a new clam fishery is likely to develop in the area, requiring further studies and immediate conservation measures.

Introduction

Razor clams are ecologically and economically important marine bivalves (Saeedi and Costello, 2019*a*), inhabiting sand and muddy bottoms within the lower intertidal and subtidal areas. Razor clams have been part of the pre-glaciation malacofauna of the mid-North Atlantic nine-island Azores archipelago (36–40°N, 24–31°W). *Ensis minor* (Chenu, 1843) was present on Pleistocene deposits in Santa Maria Island (Ávila *et al.*, 2002), although, possibly due to a severe drop in sea surface temperature (Ávila *et al.*, 2008), disappeared from the archipelago with other littoral bivalves living in fine sand. *Solen marginatus* Pulteney, 1799 was referenced for the shores of São Miguel by Drouet (1861: 47). *Solen vagina* Linnaeus, 1758 was also indicated to the Azores (0 to −18 m) by Jeffreys (1881: 929), but it has been considered a dubious record (Ávila *et al.*, 2000). After the submission of this manuscript, Álvaro *et al.* (2024) reported the presence of *S. marginatus* from beach observations at one site in Praia da Vitória, Terceira Island (Figure 1). Based on 11 individuals and opportunistic observations down to 2.5 m, the species was validated through molecular tools, advocating the existence of a new population (Álvaro *et al.*, 2024), however, no phylogenetic analysis or information on species density and dispersion were provided.

As part of the ecological studies undertaken onboard 'OCEANUS II' around the Archipelago of the Azores, *S. marginatus* was identified on the 17 August 2023 in several locations in the bay of Praia da Vitória, Terceira Island. The species was verified by classic and molecular taxonomy using cytochrome oxidase I (COI) mitochondrial gene to improve the accuracy of species-level identification and allow to perform intraspecies diversity analysis in an attempt to understand level genetic divergence and population source (Hebert *et al.*, 2003; Fernández-Tajes and Méndez, 2007; Cunha *et al.*, 2008; Leray and Knowlton, 2015). In support of conservation measures, the species' habitat range and population biology were further addressed for the bay of Praia da Vitória, by analysing dispersion and densities through visual surveys and size ranges, providing the first ecological insights on habitat and phylogeny of the new population of grooved razor clam in the Azores.

Material and methods

Razor clams were observed and collected on several locations in the north part of the bay of Praia da Vitória, Terceira Island on the 17 August 2023. Samples were kept in 96% ethanol for molecular studies and −20°C for subsequent analysis at the Atlantic Naturalist Collection in

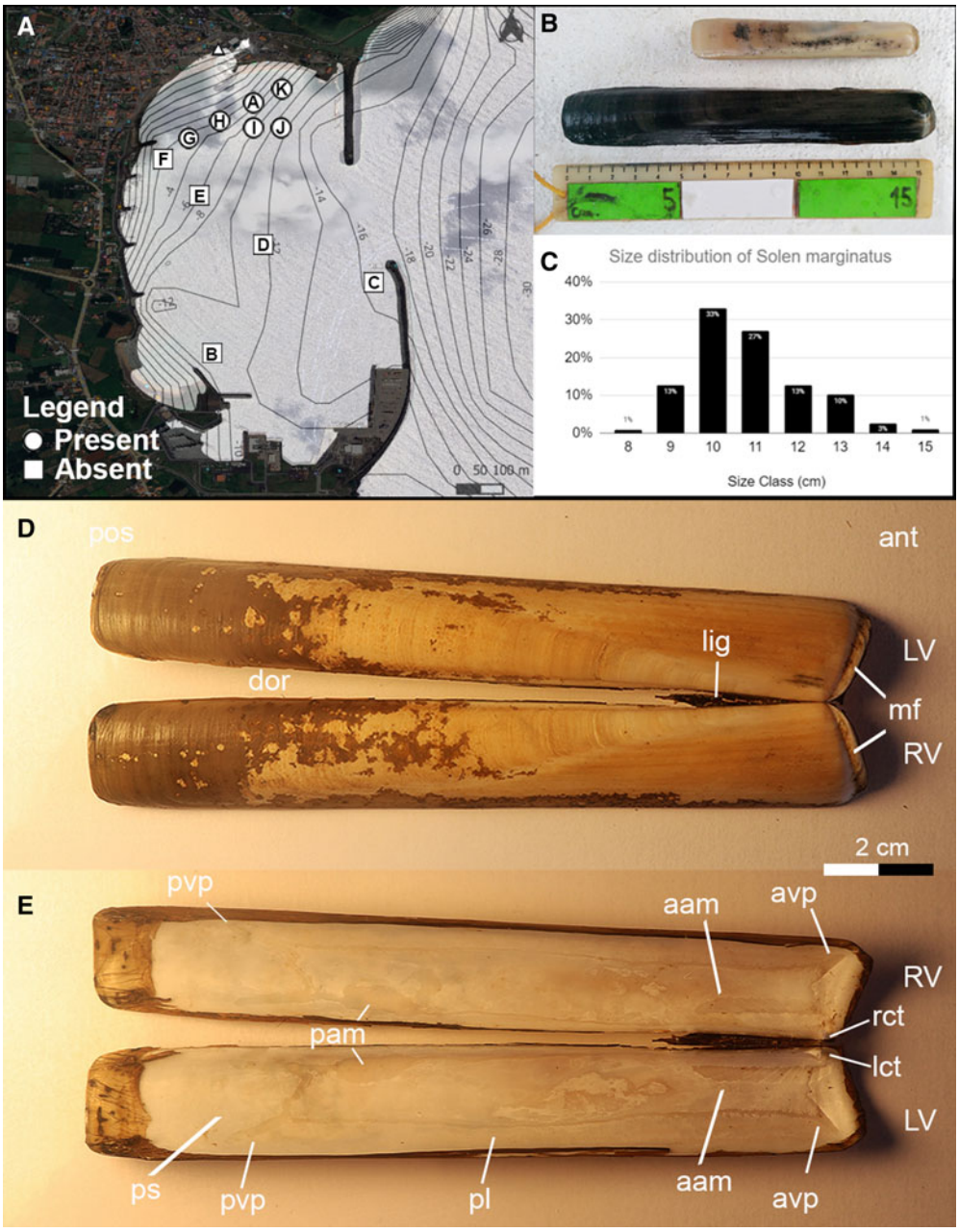

Figure 1. Observations of *Solen marginatus* in Praia da Vitória bay, Terceira Island, Azores; (a) Circles indicate presence, (A, G–K), squares indicate absence, (B–F) with letters representing different sampling sites of *S. marginatus* in August 2023, triangle indicates the single sighting location of Álvaro *et al.* (2024); (b) largest and a smaller specimen collected at Site A; (c) size distribution at Site A ($n = 118$); (d) dorsal and (e) ventral view of shells: aam, anterior adductor muscle; avp, antero-ventral mantle projection; ant, anterior; dor, dorsal; lig, ligament; LV, left valve; mf, marginal furrow, pam, posterior adductor muscle; pl, pallial line; pos, posterior; pvp, postero-ventral mantle projection; ps, pallial sinus; rct, right cardinal tooth; lct, left cardinal tooth; RV, right valve.

Horta, Faial Island, Azores. Identification was conducted by morphology-based taxonomy and molecular analysis. For the molecular identification 20 razor clam individuals from Praia da Vitória, Terceira Island, together with an additional sample of five specimens obtained in Faro (south Portugal) were used to establish a control group. A fragment of the foot was dissected and preserved in 96% ethanol until DNA processing. DNA extractions were conducted on 10–20 mg of foot tissue using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The COI amplification of the 'Folmer' barcode region at the 5′ start of the cytochrome c oxidase 1 gene (CO1), was done with LCO 1490 (5′-GGTCAACAAATCATAAAGATATTG-3′) and HCO 2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) universal primers (Folmer *et al.*, 1994). Amplifications consisted of 25 µl mix containing 1 µl (10–100 ng) of genomic DNA, 5 mM GoTaq buffer (5×), 0.2 mM dNTP (Promega, Madison, WI, USA), 1.5 mM $MgCl_2$, 0.2 mM of each primer, and 1 U of GoTaq DNA polymerase (Promega). The PCR cycling profile for COI amplification included an initial denaturation step at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 42°C for 30 s, extension at 72°C for 40 s, and a final extension at 72°C for 5 min. The PCR products were purified through ethanol/sodium acetate precipitation Field (Green and Sambrook, 2016) and subsequently sequenced using the corresponding PCR primers. Sequencing was performed on an Applied Biosystems 3130xl Genetic Analyser, utilising Sanger technology and the BigDye® Terminator v3.1 kit. The generated COI gene sequences were assembled and trimmed using the Geneious Prime (version 2020.0.3, Biomatters, New Zealand, https://www.geneious.com). The assembled sequences were BLASTn-searched in the National Centre for Biotechnology (NCBI) and compared with closely related sequences. Sequences

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were aligned using MUSCLE in Geneious Prime. Overall mean diversity was computed with MEGA v 11.0.13. The maximum likelihood (ML) analysis was conducted in RAxMLGUI v 2.0.10 (Tamura *et al.*, 2021) with 100 bootstrap replicates and K81uf + gamma model. As an outgroup, we used a COI sequence from *Ensis ensis* (ACCN: HF970367).

To address habitat range, a visual assessment was conducted on the seafloor at 10 locations throughout the bay of Praia da Vitória (Figure 1). The presence of *S. marginatus* was verified using specific bioturbation burrows with an eight or keyhole shape. With a low diversity of sand bivalves, the identification was straightforward, however, caution was taken by excluding old or unclear lebensspurren (e.g. Pereyra *et al.*, 2023). Quadrats of 0.5 × 0.5 m were used three times in each location to assess densities per m^2 at the various locations throughout the bay density was estimated in more detail using visual line transects conducted by two scuba divers at Site A, within the area of occurrence of the species at 8.6 m depth (Site A). Four non-overlapping random line transects of 20 square metres were conducted at this site, totalling 80 sqm.

Population biology was assessed through size distribution by collecting 118 specimens at site A with a 'salting method' using a saline solution and measuring shell length from posterior to the anterior furthest distance using a vernier calliper (0.1 cm). Size distribution was evaluated with a Kolmogorov–Smirnov test ($P < 0.05$).

Results

Morphological analysis

The species was identified from its equivalve shell, elongated and rather broad shape–shell ratio 1:6.1, with razor-like dorsal and ventral margins straight and parallel (Figure 1; Fischer *et al.*, 1987; Carpenter and De Angelis, 2016). The shells are anterior obliquely truncated and longer at the ventral edge. There is only one cardinal tooth on each valve (with no lateral teeth). Anterior adductor muscle scar is nearly as long as the ligament. The distinct marginal furrow bordering the anterior margin of valves is very conspicuous and distinct from other species (Figure 1d; Dautzenberg, 1897; Luczak and Dewarumez, 1992). The outer colour of the shell ranges from yellowish to pale brownish growth zones.

Phylogenetic analysis

The COI gene sequence generated from the *Solen* samples from Azores and Faro shared 100% similarities with *S. marginatus* from Ria de Aveiro, Portugal (ACCN: MK779736) and Asturias, Spain (ACCN: KJ818881). Furthermore, the Azorean samples shared 99% similarity with samples from Asturias, Spain (ACCN: KJ818881, KJ818886, KJ818884, KJ818877, KJ818890, KJ818889, KJ818882, KJ818879) and Ria de Aveiro, Portugal (ACCN: MK779734). Within the *S. marginatus* the lowest shared similarity (90%) was with a sample from Italy (ACCN: MN630857). The overall mean diversity of the *S. marginatus* clade (including *S. marginatus* and the Azorean samples) was 1%. Phylogenetic relationships of the samples from Azores with other closely related *Solen* species, as inferred by the COI gene sequences using ML analysis, showed a formation of a distinct *S. marginatus* clade (Figure 2).

Habitat range and density

The habitat range of *Solen marginatus* in Praia da Vitória, Terceira Island from the preliminary visual census is presented in Figure 1a. The species was present on the sites encircled (average densities given from the quadrats) as follows: A (8,6 m depth; 12.4 ind/m^2), I (9.6 m; 9.3 ind/m^2), K (8.9 m; 10.7 ind/m^2); with some of the sites of occurrence hosting densities below 1 ind/m^2: G (7.1 m depth), H (7.5 m), and J (14.5 m). No burrows were observed on locations B (7.2 m depth), C (20 m), D (14.2 m), E (10 m), and F (4.6 m).

The distribution seems confined to the northern part of the bay (Figure 1). Presence was noted northward of a theoretical line between the green lighthouse (right peer as you enter the bay), and the peer south of Praia Grande (next to location F, Figure 1). Next to Prainha they were mainly observed on depths of 8 m and deeper (Figure 1, sample location K), although generally spread, with greater incidence at the bottom of the slope. At this site, only at specific locations *S. marginatus* was found up to 2.5 m depths. Densities estimated by visual transects at Site A were 12.69 individuals/m^2 (STDEV = 2.6; $n = 1015$ per 80 sqm). Shell length of *S. marginatus* at Site A averaged 11.3 cm (STDEV = 1.3), ranging between 8.79 and 15.4 cm. Size distribution is presented in Figure 1c. Kolmogorov–Smirnov test revealed a non-normal distribution ($D = 0.345$), with a leptokurtic positive skewness (excess kurtosis = 1.2585) around size classes between 10 and 11.99 cm length, consisting of 60% of the population. The preliminary weight measurements averaged 19.4 g (Stdev = 6.9) per specimen.

Discussion

Solen marginatus is widespread along the European continent, east of the Azores, from the Baltic Sea to the Mediterranean, and from the Black Sea along the African coast to down to Senegal (Saeedi and Costello, 2019*b*). The mid-North Atlantic Azorean malacofauna has affinities with the Mediterranean, Portugal, and Madeira Island (Ávila *et al.*, 2000), so the occurrence of this population is not unexpected. It is noticeable however, that such a dense aggregation of a species with high commercial value remained unreported until present. *Solen marginatus* has not been reported on other islands since the original report by Drouet (1861), and was not included in the recent check-list for the Azores (Ávila *et al.*, 1998; Borges *et al.*, 2010). Our findings on size distribution and preliminary habitat range of the community demonstrate that this population is well established and reaching high densities in the northern area of Praia da Vitória Bay, with no individuals observed in the south of the Bay. The settling location is one of the less hydrodynamic areas of the bay, including the recreational dock and larger sandy beaches. Considering the densities revealed and the historical presence of the solenoids in the region, the grooved razor clam may expand to other locations in the archipelago.

The dominance of reproductive sizes also indicates a well-established population, as *S. marginatus* reaches maturity between the first and the second year (ca. 4.7 cm length; Maia, 2006, in Ria de Aveiro, Portugal). Having 60% of the population with a medium age of 4 years old shows potential for reproduction and growth. While settlement occurred more than 10 years ago, judging from the existence of individuals above 14 cm length (Maia, 2006), a settlement period of over 20–25 years is expected, as it is very unlikely that only 10 years of recruitment as proposed by Álvaro *et al.* (2024) would result in such high densities, as here reported over a considerably large area. Genetically it is a recent settlement as supported by the shallow phylogenetic divergence with other populations. The origin of this population remains undetermined, with greater genetic affinity to the Ria de Aveiro (North of Portugal) and the Asturias (North of Spain) populations. Our findings on the source populations, agree with the hypothesis of Álvaro *et al.* (2024), that suggested an

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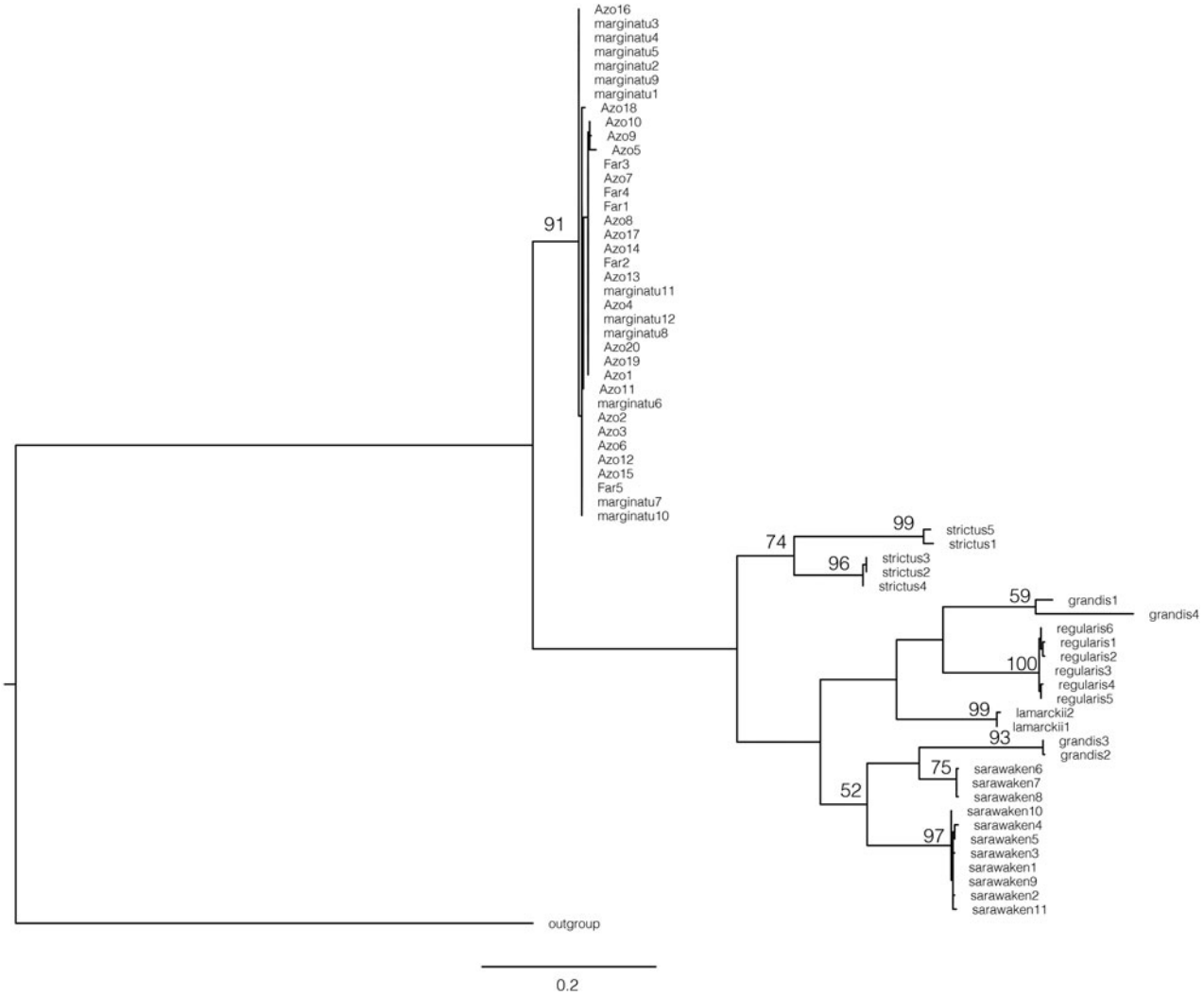

Figure 2. Phylogenetic relationships of the samples from the Azores with other closely related *Solen* species, as inferred by the COI gene sequences using ML analysis. Numbers above branches are >50% bootstrap values.

anthropogenic origin, likely from Portugal or Spain, due to the 8–9 day settlement period of the razor clam larvae (Da Costa *et al.*, 2012). Intentional or non-intentional anthropogenic sources remain possible, as well as natural dispersion. The regular arrival of boats, coupled with the availability of live specimens for purchase in local marketplaces for use as human consumption or fish bait, will more likely function as introduction vectors once the distance to the Northeast Atlantic coastal areas (at least 1800 km) makes natural dispersion more complex.

Future works should cover the entire sand bank habitats on the island as well as other potential habitats in the archipelago. The use of different genetic markers may prove useful in the search for genetic divergence and understanding the population origin. Future population studies should comprise reproductive histological analysis, toxicological and finer distribution assessments if fisheries exploitation is to be considered. These would support establishing no-take areas, closure dates, minimum sizes, or total allowable catches. Future extractive techniques may include dredging, which places this newly described and confined population and the overall habitat under imminent threat requiring urgent conservation.

Data. Data is kept at Atlantic Naturalist collection in Horta, Faial and can be made available under request.

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Competing interests. All authors have no conflict of interest.

Ethical Standards. The present survey complied with all ethical standards.

References

Álvaro N, Sinigaglia L, Madeira P, Hipólito A, Melo CS, Arruda S, Fernandes JF, Baptista L and Ávila SP (2024) The razor clam *Solen marginatus* Pulteney, 1799: a new anthropogenic marine introduction in the Azores Archipelago. *Regional Studies in Marine Science* **70**, 103387.

https://doi.org/10.1017/S0025315424000572 Published online by Cambridge University Press

Ávila SP, Amen RG, Azevedo J, Cachão M and García-Talavera F (2002) Checklist of the Pleistocene marine molluscs of Praínha and Lagoínhas (Santa Maria Island, Azores). *Açoreana. Revista de Estudos Açoreanos* **9**, 343–370.

Ávila SP, Azevedo J, Gonçalves JM, Fontes J and Cardigos F (1998) Checklist of the shallow-water marine molluscs of the Azores: 1-Pico, Faial, Flores and Corvo. *Açoreana. Revista de Estudos Açoreanos* **8**, 487–523.

Ávila SP, Azevedo J, Gonçalves JM, Fontes J and Cardigos F (2000) Checklist of the shallow-water marine molluscs of the Azores: 2-São Miguel Island. *Arquipélago. Life and Marine Sciences*. **Supplement 2**, 99–131.

Ávila SP, Madeira P, Mendes N, Rebelo A, Medeiros A, Gomes C, Garcia-Talavera F, Silva F, Silva C, Cachão M, Hillaire-Marcel C and de Frias Martins AM (2008) Mass extinctions in the Azores during the last glaciation: fact or myth? *Journal of Biogeography* **35**, 1123–1129.

Borges P, Bried J, Costa A, Cunha R, Gabriela R, Gonçalves V, Martins A, Melo I, Parente M, Raposeiro P, Rodrigues P, Santos R, Silva L, Vieira P, Vieira V, Mendonça E and Boieiro M (2010) *Listagem dos organismos terrestres e marinhos dos Açores*. Cascais, Portugal: Princípia.

Carpenter KE and De Angelis N (2016) *The Living Marine Resources of the Eastern Central Atlantic. Volume 2 Bivalves, Gastropods, Hagfishes, Sharks, Batoid Fishes and Chimaeras*. Rome: Food & Agriculture Organization.

Cunha R, Tenorio M, Afonso C, Castilho R and Zardoya R (2008) Replaying the tape: recurring biogeographical patterns in Cape Verde Conus after 12 million years. *Molecular Ecology* **17**, 885–901.

Da Costa F, Nóvoa S, Ojea J and Martínez-Patiño D (2012) Effects of algal diets and starvation on growth, survival and fatty acid composition of *Solen marginatus* (Bivalvia: Solenidae) larvae. *Scientia Marina* **76**, 527–537.

Dautzenberg P (1897) *Atlas de poche des coquilles des Côtes de France: Manche, Atlantique, Méditerranée*, Vol. **6**. Paris: P. Klincksieck, p. 54.

Drouët H (1861) *Éléments de la faune Açoréenne*. Paris: J.B. Baiilliére & Fils, Libraires de l'Academie de Médecine, 245pp.

Fernández-Tajes J and Méndez J (2007) Identification of the razor clam species *Ensis arcuatus*, *E. Siliqua*, *E. Directus*, *E. Macha*, and *Solen marginatus* using PCR-RFLP analysis of the 5S rDNA region. *Journal of Agricultural and Food Chemistry* **55**, 7278–7282.

Fischer W, Bauchot ML and Schneider M (1987) *Fiches FAO d'identification des espèces pour les besoins de la pêche. Rév. 1. Méditerranée et mer Noire. Zone de pêche 37. Vol. I. Végétaux et invertébrés. Publication préparée par la FAO et la Commission des Communautés européennes (Projet GCP/INT/422/EEC) financées conjointement par ses deux organisations*. Rome: FAO, 60pp.

Folmer OM, Black W, Hoeh R, Lutz R and Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology & Biotechnology* **3**, 294–299.

Green MR and Sambrook J (2016) Precipitation of DNA with ethanol. *Cold Spring Harbor protocols* **2016**. doi: 10.1101/pdb.prot093377

Hebert PDN, Cywinska A, Ball SL and deWaard JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **270**, 313–321.

Jeffreys JG (1881) On the Mollusca procured during the "Lightning and Porcupine" expeditions 1868-1870. Part IV. *Proceedings of the Zoological Society of London*, 922–952.

Leray M and Knowlton N (2015) DNA barcoding and metabarcoding of standardized samples reveal patterns of marine benthic diversity. *Proceedings of the National Academy of Sciences* **112**, 2076–2081.

Luczak C and Dewarumez JM (1992) Note on the identification of *Ensis directus* (Conrad, 1843). *Cahiers de biologie marine* **33**, 515–518.

Maia FMSR (2006) *Estudo do ciclo reprodutor e do crescimento de Solen marginatus e Venerupis pullastra na Ria de Aveiro* (Tese de Mestrado). Universidade de Aveiro.

Pereyra CA, Bel Haouz W and Lagnaoui A (2023) New bivalve burrows from the mid-Holocene of northeastern Buenos Aires Province (Argentina): ichnotaxonomy and ethology. *Palaeoworld* **32**, 174–187.

Saeedi H and Costello MJ (2019*a*) The biology, ecology, and societal importance of razor clams. *Reference Module in Earth Systems and Environmental Sciences*, 494–498.

Saeedi H and Costello MJ (2019*b*) A world dataset on the geographic distributions of Solenidae razor clams (Mollusca: Bivalvia). *Biodiversity Data Journal* **7**, e31375.

Tamura K, Stecher G and Kumar S (2021) MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution* **38**, 3022–3027.

https://doi.org/10.1017/S0025315424000572 Published online by Cambridge University Press