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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

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Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis of the 5S ribosomal DNA region has been applied to the establishment of DNA-based molecular markers for the identification of five razor clam species: *Ensis arcuatus*, *E. siliqua*, *E. directus*, *E. macha*, and *Solen marginatus*. PCR amplifications were carried out using a pair of universal primers from the coding region of 5S rDNA. *S. marginatus* was simply distinguished by the different size of the amplicons obtained. Species-specific restriction endonuclease patterns were found with the enzymes H*ae* III for *E. arcuatus*, *E. siliqua*, and *E. directus*, and A*cs* I for *E. macha*, and when two enzymes were combined, the four species were also identified. Thus, this work provides a simple, reliable, and rapid protocol for the accurate identification of *Ensis* and *Solen* species in fresh and canned products, which is very useful for traceability and to enforce labeling regulations.

KEYWORDS: Species identification; razor clam; *Ensis* spp; *Solen marginatus*; 5S rDNA marker; PCR-RFLP; traceability; seafood.

INTRODUCTION

The Solenoidea superfamily contains the Pharidae family, which includes the *Ensis* genus among others, and the Solenidae family, which includes the *Solen* genus. They are commonly named razor clams and assemble numerous species that belong to soft bottom marine fauna, inhabiting the fine sand, silt, or mud biotopes. They are adapted for life in these habitats because of their capacity for rapid vertical burrowing into sand, and the characteristic shape of their shell, which is elongated, laterally compressed, narrowed, and with almost semicylindrical valves, plays a major role in this adaptation process. Their peculiar morphology and way of life attracted the attention of naturalists and malacologists, who focused their first studies in the analysis of morphologic characters (*1*, *2*) and later on the anatomical, reproductive, physiological, pathological, and ecological features (*3*−*8*); however, genetic studies are nonexistent.

The economic valuation for razor clams has experienced a considerable development during the last 5 years, making them a highly valuable species. In 2004, the import value of the razor market within Europe reached a quote of 550 million euros. The key countries within this market are Spain, Italy, France, Portugal, and The Netherlands, with Spain being the most significant with imports of nearly 43% of the total for the European Union (data obtained from the Eurostat information database, http://epp.eurostat.ec.europa.eu/). In terms of the razor species, there are two genuses that are commercialized in Europe, the *Ensis* and *Solen* genuses, with *Ensis arcuatus*, *E. siliqua*, and *E. directus* being the main commercialized ones. *E. arcuatus*, *E. siliqua*, and *S. marginatus* are native species in Europe, distributed along the European Atlantic coasts, while *E. directus* is a species introduced into Europe in 1978 by means of larvae in the ballast water of ships. It was initially detected in the German coast, reaching France and the English Channel in 1991 (*9*). During the last 2 years, there has been an increasing reliance on imports from outside, mainly, Argentina and Chile, which contribute another razor species (*E. macha*) to European markets (data obtained from the Eurostat information database, http://epp.eurostat.ec.europa.eu/). This razor clam is distributed throughout the coasts of Southern Argentina and Chile, representing a valuable fishery resource (*10*).

Razor clams, mainly *E. siliqua* and *E. arcuatus*, are the favored species in the canning sector and in the food industry in Europe, reaching the highest commercial value. Because of the elevated similarity in form and size of the shell between those and other razor clam species (*E. directus* and *E. macha*), it is very difficult to clearly differentiate them on the basis of their morphology. Under European legislation (Regulation No. 104/2000 of Dec. 17, 1999) for the common organization of the markets in fishery and aquaculture products, these products must be correctly labeled with the commercial name of the species, the production method, and the capture zone before retail distribution (*11*). Therefore, it is necessary to develop analytical methods for species identification in order to detect and avoid commercial fraud as well as the unintentional substitution of different razor clam species and enforce labeling regulations.

Most genetic approaches developed to overcome this problem are focused on the identification of targeted species and are based on the use of conserved mitochondrial or nuclear DNA

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primers for polymerase chain reaction (PCR) amplification, followed by sequencing (*12*, *13*), by restriction fragment length polymorphism studies (PCR−RFLPs) (*14*−18), by PCR-SSCP analysis (*19*), and by use of multiplex PCR (*20*, *21*). The aim of this work was to develop an appropriate and reliable methodology of identification of five razor clams species by means of PCR amplification of 5S rDNA and also by an analysis of restriction fragment length polymorphism of amplified fragments.

MATERIALS AND METHODS

Sample Collection. Razor clams samples of five closely related razor clams, *Ensis arcuatus*, *E. siliqua*, *E. directus*, and *E. macha* belonging to the *Ensis* genus and *Solen marginatus* belonging to the *Solen* genus, were studied. The samples of *E. arcuatus*, *E. siliqua*, and *S. marginatus* were collected from local fishermen in Barra de Cangas (Pontevedra, Spain). *Ensis directus* and *E. macha* specimens were purchased from local fish suppliers. All specimens were morphologically identified by bivalve taxonomists at the Centre of Marine Investigations (CIMA), and then a piece of foot was dissected and immediately stored in 95% ethanol until DNA preparation (less than 5 days).

DNA Extraction. Total genomic DNA was prepared from foot tissue (20 mg) preserved in 95% ethanol according to a method developed in our laboratory. Samples were immersed in 400 μL of lysis buffer (1 M Tris, 0.2 M EDTA, and 2.5% *N*-Laurylsarcosine) containing 10 μL of Proteinase K (2 mg/mL) (Sigma), mixed by vortex, and incubated at 60 °C overnight. For canning tissues, an initial step was performed before incubating in lysis buffer: samples were immersed in distilled water for 15 min. A 150 μL volume of Precipitation solution (3 M ammonium acetate pH 6.0) was added to each sample and vortexed vigorously, and then samples were incubated on ice for 15 min. Following centrifugation at 10,000*g* for 5 min, the supernatant was collected, and a 600 μL volume of isopropyl alcohol was added, mixed by tube inversion, and centrifuged again at 17,500*g* for 5 min. The pellet was washed twice with 70% ethanol (−20 °C), dried in a vacuum pump, and resuspended in 100 μL of TE (10 mM Tris and 1 mM EDTA).

PCR Amplification. PCR amplification of the 5S rDNA was performed in 25 μL of PCR Supermix buffer (Life Technologies) containing 25 ng of template DNA, 0.5 U of *Taq* DNA polymerase, and 0.2 mM of each primer. The primers used were 5SF: 5′-CGTCCGATCACCGAAGTTAA-3′ and 5SR: 5′-ACCGGTGTTTT-CAACGTGAT-3′ (Martínez-Lage, personal comunication). PCR amplification was carried out in a Bio Rad Gene Cycler, and the amplificaton profile consisted of one initial denaturation cycle of 3 min at 94 °C, followed by 35 amplification cycles of 45 s at 94 °C, 45 s at 50 °C, 1 min at 72 °C, and a final extension step at 72 °C for 10 min. PCR products (5 μL) were migrated on a 2.0% agarose gel electrophoresis. The gels were stained by immersion in 0.5 μg/mL ethidium bromide for 30 min, visualized on a transilluminator, and the image recorded with a UVP (Ultra-Violet Products, Gel Documentation System ImageStore 5000, v7.12) video camera.

DNA Sequencing. DNA from selected electrophoretic bands was excised under UV light and isolated using the Concert Gel Extraction Systems kit (Life Technologies), according to the manufacturer's instructions. Cloning was achieved by employing the pGEM-T Easy Vector System II (Promega, Madison, Wis.). After growing the recombinant colonies, plasmids were purified by means of a QuiaPrep Spin Miniprep Kit (Quiagen), following the manufacturer's instructions. DNA was sequenced in both directions, using an AutoRead ALFexpress Sequencing Kit (Amersham Biosciences), in an ALFexpress DNA sequencer.

PCR−RFLP Analysis. The PCR amplification product of the 5S rDNA was digested with *Hae* III and *Acs* I restriction endonucleases. The reaction was carried out in 15−20 μL volume, containing 70−80 ng of DNA (3−5 μL of PCR product), 5 U of enzyme, and 2 μL of the recommended buffer for each enzyme. The digestion mixture was incubated at 37 °C in an oven overnight. The reaction was stopped by adding 2 μL of loading buffer. The digestion products were loaded onto a 2% agarose gel electrophoresis, stained with ethidium bromide, and visualized with an ultraviolet light transilluminator.

RESULTS AND DISCUSSION

Nowadays, there is a growing requirement to develop techniques that allow consumers to know what they purchase, guarantying the correct identification and traceability of seafood products in order to ensure the composition and safety of commercial marine products (*21*). Recently developed molecular biological techniques and in particular the polymerase chain reaction have been shown very appropriate for this purpose. The characteristics of the 5S rDNA, which forms a multigene family of tandem arrays, with a highly conserved region of 120 bp that facilitates the primer design for its amplification and that contains a non-transcribed spacer (NTS) that evolves more rapidly, makes it attractive as a tool in the search of species-specific DNA molecular markers. The use of the 5S rDNA was reported to be useful for the discrimination of fish species with commercial value: salmons (*22*, *23*), perches (*24*), and sole (*21*), and also molluscs, such as oysters (*20*) and cockles (*25*).

In the present study, a simple and reliable PCR−RFLP analysis using the 5S rDNA region was developed for accurate discrimination of closely related species belonging to the *Ensis* and *Solen* genuses. The whole units of 5S rDNA of the five species were amplified by means of PCR using primers corresponding to two sequences that bound in the coding region (**Figure 1**). Sizes of the amplified products, estimated in 10 individuals of each species, were about 420 bp for the *Ensis* spp. and 530 bp for *Solen marginatus* (**Figure 2**). Although sizes of PCR products varied between *S. marginatus* and the species belonging to the *Ensis* genus allowing us to differentiate them, the *Ensis* spp. showed an NTS of similar size; thus, *E. arcuatus*, *E. siliqua*, *E. macha*, and *E. directus* could be misidentified. In order to characterize the different amplification fragments of the *Ensis* genus species, we purified, cloned, and sequenced PCR products from two individuals of each species. The exact lengths of the PCR products were determined to be 420 bp for *E. arcuatus*, 422 bp for *E. siliqua*, 443 bp for *E. directus*, and 434 bp for *E. macha*. When the sequences were aligned, a low similarity between the spacer regions of the four species was found. These nucleotide differences between the NTS sequences of the species provided the basis for selecting diagnostic restriction enzymes. However, when the sequences of *E. arcuatus* and *E. siliqua* were compared, the degree of similarity obtained was very high. The appropriate restriction enzyme patterns identifying the PCR products obtained were predicted by the WEBcutter program (http://rna.lundberg.gu.se/cutter2/). The comparison of the restriction maps obtained allowed the identification of, at least, two restriction endonucleases (H*ae* III and A*cs* I) that could differentiate the four razor clam species. The target sites for the two enzymes are gg/cc (H*ae* III) and r/aatty (A*cs* I). The restriction profiles generated after H*ae* III digestion allowed the direct identification of *E. arcuatus* and *E. directus* (type A and type C, respectively) (**Table 1** and **Figure 3**), meanwhile *E. siliqua* and *E. macha* remained misidentified (both type B). After the identification of the first two species with H*ae* III, A*cs* I enzyme allowed the direct discrimination of *E. siliqua* (type A) and *E. macha* (type B) (**Figure 4**), and also *E. arcuatus* and *E. siliqua* when combined with the pattern of the H*ae* III enzyme. It should be noted that the 205 and 214 bp restriction fragments generated after H*ae* III digestion in *E. directus* were visualized as a single band because they cannot be discriminated in 2% agarose gels and also that these gels do not allow the clear resolution of the smallest fragments (<30 bp), although they are unnecessary for

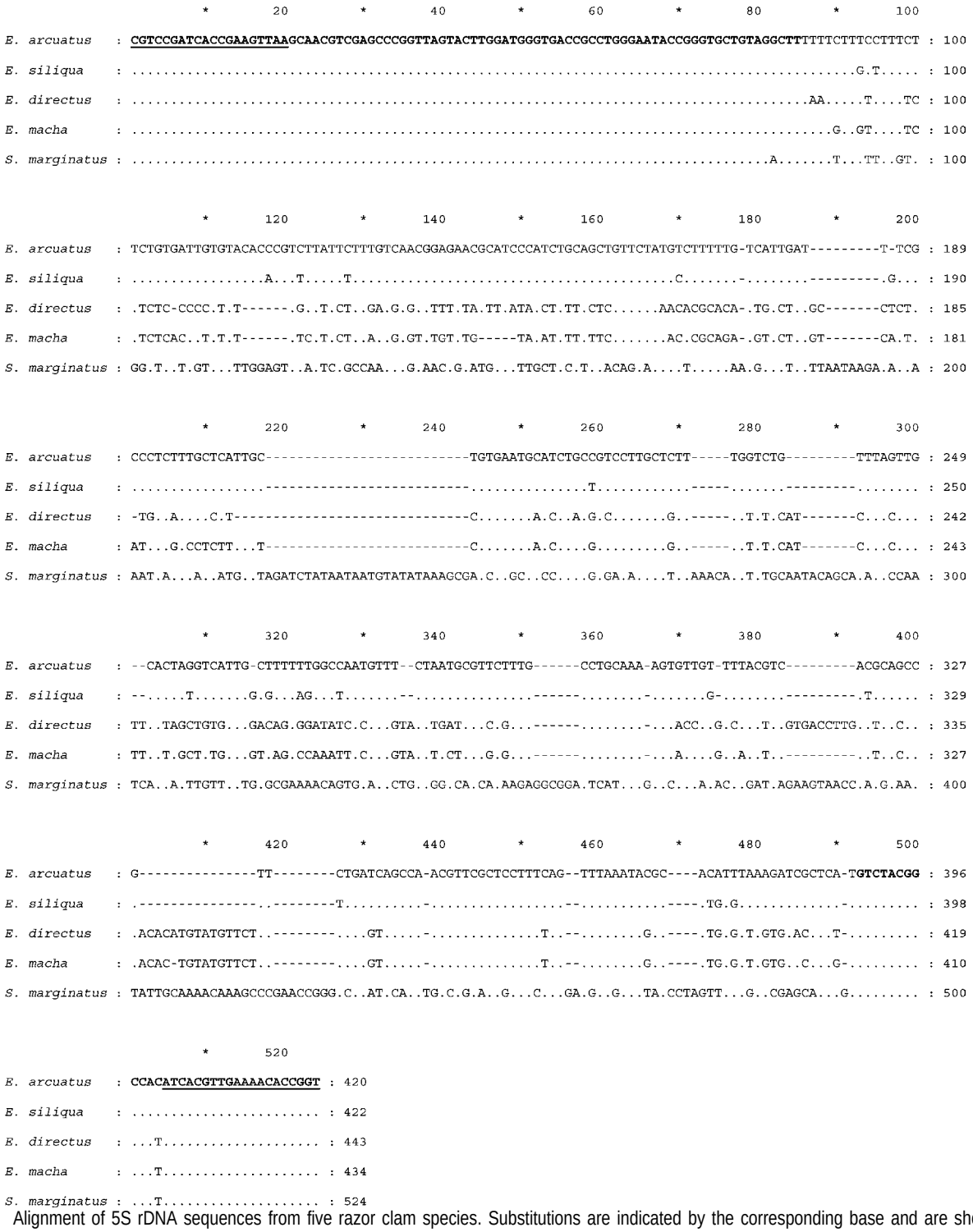

Figure 1. Alignment of 5S rDNA sequences from five razor clam species. Substitutions are indicated by the corresponding base and are shown with respect to the first sequence. The dots indicate identical base with respect to the first sequence. The gaps are represented by dashes. The sequences corresponding to 5SR and 5SF primers used to amplify this region are underlined. The coding region is shown in bold.

appropriate species differentiation. An extra fragment of approximately 430 bp was visualized in some 5S rDNA amplicons of *E. arcuatus*, *E. directus*, and *E. macha* digested with H*ae* III. A possible explanation can lie in an incomplete digestion at the recognition site. The cause for this incomplete reaction is unknown, but it is not due to the presence of mixed species in the PCR product because H*ae* III endonuclease did not yield such a mixed digestion profile. Similar results were reported by Ram et al. (*26*) for the restriction analysis of a 120 bp fragment of the cytochrome *b* in canned tuna species. Further nucleotide sequencing was performed in both strands in 10 individuals belonging to four *Ensis* spp. in order to verify that intraspecific polymorphism does not exist at the H*ae* II and A*cs* I target sites. Therefore, amplification of 5S rDNA region allowed the identification between *S. marginatus* and *Ensis* spp., and species-specific PCR−RFLP electrophoretic patterns made it possible to identify *E. arcuatus*, *E. siliqua*, *E. directus*, and *E. macha*, demonstrating that the NTS region is a functional nuclear marker for the identification of related bivalve species belonging even to the same genus.

As has been cited before, our aim was to find an appropriate and reliable method for the identification of the described

Table 1. Restriction Fragments (in Bp) Obtained after Digestion of 5S rDNA Products from *E. arcuatus*, *E. siliqua*, *E. directus*, and *E. macha* with H*ae* III and A*cs* I Enzymes[a]

		Hae III		*Acs* I		
razor clam species	5S rDNA length	fragment sizes	type	fragment sizes	type	pattern
E. arcuatus	420	24, 125, 271	A	x	A	AA
E. siliqua	422	24, 398	B	x	A	BA
E. directus	443	24, 205, 214	C	x	A	CA
E. macha	434	24, 410	B	24, 140, 270	B	BB

[a] The fragment sizes shown are exact, as determined by sequencing. x, not cleavage fragments.

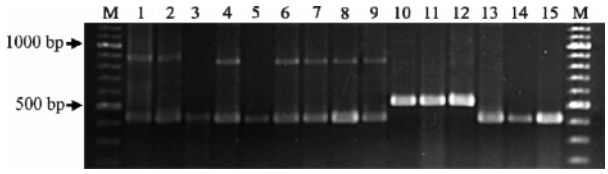

Figure 2. PCR products of the 5S rDNA repetition unit in *E. directus* (lanes 1–3), *E. siliqua* (lanes 4–6), *E. arcuatus* (lanes 7–9), *S. marginatus* (lanes 10–12), and *E. macha* (lanes 13–15). M = 100 bp molecular weight marker.

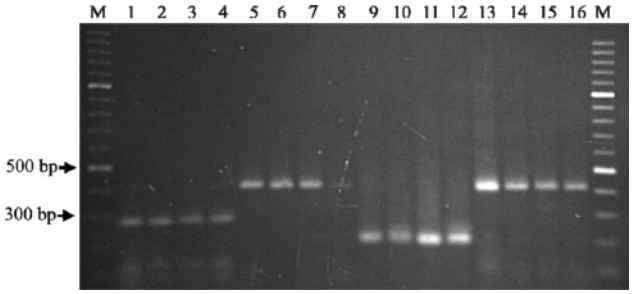

Figure 3. Electrophoretic analysis (2% agarose gel) showing the restriction profiles of the 5S rDNA PCR products digested with H*ae* III from the razor clam species *E. arcuatus* (lanes 1–4), *E. siliqua* (lanes 5–8), *E. directus* (lanes 9–12), and *E. macha* (lanes 13–16). M = 100 bp molecular weight marker.

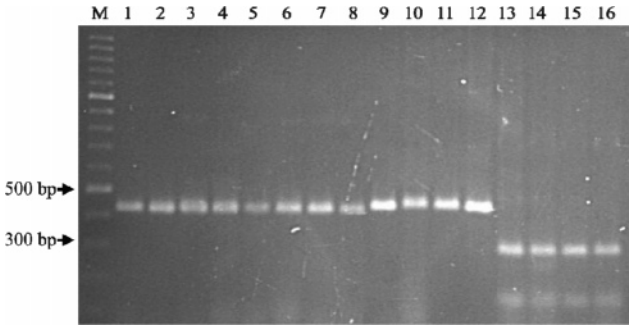

Figure 4. Electrophoretic analysis (2% agarose) showing the restriction profiles of the 5S rDNA PCR products digested with A*cs* I from the razor clam species *E. arcuatus* (lanes 1–4), *E. siliqua* (lanes 5–8), *E. directus* (lanes 9–12), and *E. macha* (lanes 13–16). M = 100 bp molecular weight marker.

species. The above-mentioned analyses were carried out on individuals preserved in alcohol after being collected. Nevertheless, a great variety of razor clam cans exist in the market where the morphologic identification of the samples cannot be accomplished. Therefore, we decided to test whether our method is easily applicable to manufactured and canned products. In order to do this, we extracted the DNA, according to the method

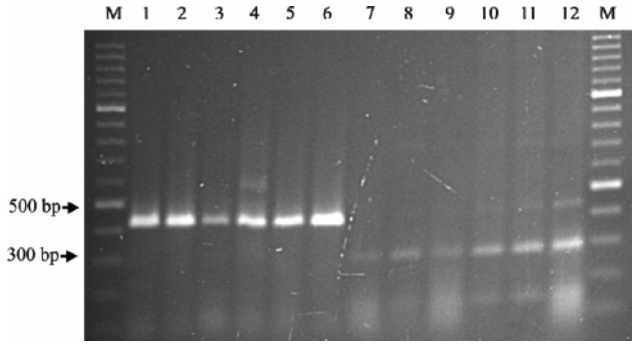

Figure 5. Electrophoretic analysis (2% agarose) showing the restriction profiles of the 5S rDNA PCR products digested with A*cs* I from the razor clam canned products: *Ensis* spp from Galicia (lanes 1–6) and *E. macha* (lanes 7–12). M = 100 bp molecular weight marker.

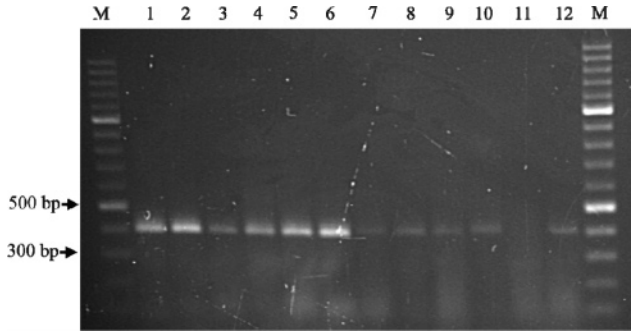

Figure 6. Electrophoretic analysis (2% agarose) showing the restriction profiles of the 5S rDNA PCR products digested with H*ae* III from the razor clam canned products: *Ensis* spp from Galicia (lanes 1–6) and *E. macha* (lanes 7–12). M = 100 bp molecular weight marker.

developed in our laboratory, from 50 individuals belonging to four commercial razor clam cans (12−15 specimens per can), two labeled as *E. macha*, and the other ones labeled as *Ensis* spp. of Galician origin (*E. arcuatus* or *E. siliqua*). In most cases, extracted DNA length was 1 kb, but in some occasions, the efficiency of the extraction was lower, although its size always reached 500 bp. First, we carried out a PCR amplification using the primers 5SR and 5SF. After that, we proceeded to the digestion with the A*cs* I enzyme, and the electrophoretic pattern observed (**Figure 5**) showed that samples labeled as *E. macha* were cleaved in two fragments, as previous ones preserved in ethanol, and the other samples, corresponding to Galician origin, were not digested, demonstrating that these could belong to *E. siliqua* or to *E. arcuatus*. A second step was performed in order to identify them; amplicons were digested with H*ae* III, and the restriction profile generated (**Figure 6**) evidenced that all samples corresponded to *E. siliqua*.

In order to make a methodological validation, we tested our identification method in fresh or frozen samples (50 individuals per species ranging in size from 10 to 15 cm) and in manufactured products (10 commercial cans) purchased in the market. Genetic identification of both types of products was correct since all analyzed samples were always identified as expected. Our methodological approximation allowed us to verify the species identification and the correct labeling of the analyzed products.

The results obtained in this work show that the developed technique a simple, robust, reliable, and inexpensive protocol involving PCR amplification of the 5S rDNA region and RFLP analysis with H*ae* III and A*cs* I restriction endonucleases can be routinely applied to differentiate razor clam species. Because of the speed, approximately a working day, and the fact that

this approach does not require conventional sequencing of PCR products followed by detailed comparison of individual sequences, it can be routinely performed in food inspection laboratories, especially when large numbers of samples have to be analyzed.

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LITERATURE CITED

(1) Deshayes, G. H. Historie Naturelle des Mollusque. In *Exploration Scientifique de l'Álgérie*, 2nd ed.; Science Physiques, Zoologie: Paris, France, 1834; Vol. 18, pp 40−42.

(2) Jeffreys, G. 1865. *British Conchology, or an Account of the Mollusca which Now Inhabit the British Isles and the Surrounding Seas*, 2nd ed.; Plymouth Marine Faune, Eds.; Marine Biological Association: London, UK, 1931; Vol. III.

(3) Gaspar, M. B.; Monteiro, C. C. Reproductive cycles of the razor clam *Ensis siliqua* and the clam *Venus striatula* of Vilamorura, southern Portugal. *J. Mar. Biol. Assoc. U. K.* **1998**, *78*, 1247−1258.

(4) Darriba, S.; Iglesias, D.; Harshbarger, J. C.; López, C. Germinoma in razor clam Ensis arcuatus (Jeffreys, 1865) in Galicia (NW Spain). *J. Invertebr. Pathol.* **2006**, *93*, 140−142.

(5) Darriba, S.; San Juan, F.; Guerra, A. Energy storage and utilization in relation to the reproductive cycle in the razor clam *Ensis arcuatus* (Jeffreys, 1865). *ICES J. Mar. Sci.* **2005**, *62*, 886−896.

(6) Darriba, S.; San Juan, F.; Guerra, A. Gametogenic cycle of *Ensis siliqua* (Linnaeus, 1758) in the Ría de Corcubión, Northwestern Spain. *J. Molluscan Stud.* **2005**, *71*, 47−51.

(7) Darriba, S.; San Juan, F.; Guerra, A. Reproductive cycle of the razor clam *Ensis arcuatus* (Jeffreys, 1865) in northwest Spain and its relation to environmental conditions. *J. Exp. Mar. Biol. Ecol.* **2004**, *311*, 101−115.

(8) López, C.; Darriba, S. Presence of *Martelia* sp. (Paramyxea) in the razor clam *Solen marginatus* (Pennántt, 1777) in Galicia (NW Spain). *J. Invertebr. Pathol.* **2006**, *92*, 109−111.

(9) Luczak, C.; Dewarumez, J. M. Note on the identification of *Ensis directus* (Conrad, 1843). *Cah. Biol. Mar.* **1992**, *33*, 515−518.

(10) Baron, P. J.; Real, L. E.; Ciocco, N. F.; Re, M. E. Morphometry, growth and reproduction of an Atlantic population of the razor clam *Ensis macha (Molina, 1782). Sci. Mar.* **2004**, 68, 211−217.

(11) Council Regulation (EC) No 104/2000 of 17 December 1999 on the common organization of the markets in fishery and aquaculture products. *Off. J. Eur. Commun.* **2000**, *L17*, 22−52.

(12) Rodríguez, M. A.; García, T.; González, I.; Asensio, L.; Mayoral, B.; López-Calleja, I.; Hernández, P. E.; Martín, R. Identification of goose, mule duck, chicken, turkey, and swine in foie gras by species-specific polymerase chain reaction. *J. Agric. Food Chem.* **2003**, *51*, 1524−1529.

(13) Infante, C.; Crespo, A.; Zausti, E.; Ponce, M.; Pérez, L.; Funes, V.; Catanese, G.; Manchado, M. PCR-based methodology for the authentication of the Atlantic mackerel *Scomber scombrus* in commercial canned products. *Food Res. Int.* **2006**, *39*, 1023−1028.

(14) Quinteiro, J.; Vidal, R.; Izquierdo, M.; Sotelo, C G.; Chapela, M. J.; Pérez-Martín, R. I.; Rehbein, H.; Hold, G. L.; Russel, V. J.; Pryde, S. E.; Rosa, C.; Santos, A. T.; Rey-Méndez, N. Identification of hake species (*Merluccius* genus) using sequencing and PCR-RFLP analysis of mitocondrial DNA control region sequences. *J. Agric. Food Chem.* **2001**, *49*, 5108−5114.

(15) Fernández, A.; García, T.; Asensio, L.; Rodríguez, M. A.; González, I.; Hernández, P. E.; Martín, R. PCR-RFLP Analysis of the internal transcribed spacer (ITS) region for identification of 3 clam species. *Food Chem. Toxicol.* **2001**, *66*, 657−661.

(16) López-Piñón, M. J.; Insua, A.; Méndez, J. Identification of four scallop species using PCR and restriction analysis of the ribosomal DNA internal transcribed spacer region. *Mar. Biotechnol.* **2002**, *4*, 495−502.

(17) Santaclara, F. J.; Espiñeira, M.; Cabado, G.; Aldasoro, A.; González-Lavin, N.; Vieites, J. M. Development of a method for the genetic identification of mussel species belonging to *Mytilus*, *Perna*, *Aulacomya* and other genera. *J. Agric. Food Chem.* **2006**, *54*, 8461−8470.

(18) Aranishi, F. Rapid PCR-RFLP method for discrimination of imported and domestic mackerel. *Mar. Biotechnol.* **2005**, *7*, 571−575.

(19) Fernández, A.; García, T.; Asensio, L.; Rodríguez, M. A.; González, I.; Lobo, E.; Hernández, P E.; Martín, R. Genetic differentiation between the clam species *Ruditapes decussatus* (grooved carpet shell) and *Venerupis pullastra* (pullet carpet shell) by PCR-SSCP analysis. *J. Sci. Food Agric.* **2002**, *82*, 881−885.

(20) Cross, I.; Rebordinos, L. Species identification of *Crassostrea* and *Ostrea* species by polymerase chain reaction amplification of the 5S rRNA gene. *J. AOC Int.* **2006**, *89*, 145−148.

(21) Céspedes, A.; García, T.; Carrera, E.; González, I.; Fernández, A.; Hernández, P. E.; Martín, R. Indentification of sole (*Solea solea*) and Greenland halibut (*Reinharius hippglossoides*) by PCR amplification of the 5S rDNA gene. *J. Agric. Food Chem.* **1999**, *47*, 1046−1050.

(22) Pendás, A. M.; Morán, P.; Martínez, J. L.; García-Vázquez, E. Applications of 5S-rDNA in Atlantic salmon, Brown trout, and in Atlantic salmon x Brown trout hybrid identification. *Mol. Ecol.* **1995**, *4*, 275−276.

(23) Carrera, E.; García, T.; Céspedes, A.; González, I.; Fernández, A.; Hernández, P E.; Martín, E. PCR-restriction fragment length polymorphism of the p53 gene as a genetic marker for indentification of smoked Atlantic salmon (*Salmo salar*) and rainbow trout (*Onchorhyncus mykiss*). *J. AOC Int.* **2000**, *83*, 341−346.

(24) Asensio, L.; González, I.; Fernández, A.; Céspedes, A.; Rodríguez, M. A.; Hernández, P. E.; García, T.; Martín, R. Identification on nile perch (Lates nitolicus), grouper (*Epinephelus guaza*), and wreck fish (*Polyprion americanus*) fillets by PCR amplification of the 5S rDNA gene. *J. AOC Int.* **2001**, *84*, 777−781.

(25) Freire, R.; Insua, A.; Méndez, J. *Cerastoderma glaucum* 5S ribosomal DNA: characterization of the repeat unit, divergence with respect to *Cerastoderma edule*, and PCR-RFLPs for the identification of both cockles. *Genome* **2005**, *48*, 427−442.

(26) Ram, J. L.; Ram, M. L.; Baidoun, F. F. Authentication of canned tuna and bonito by sequence restriction site analysis of polymerase chain reaction products of mitochondrial DNA. *J. Agric. Food Chem.* **1996**, *44*, 2460−2467.

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