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PCR Technique for Identification of Mussel Species

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Random amplified polymorphic DNA (RAPD) analysis has been applied to the identification of four mussels species: *Mytilus edulis*, *Mytilus chilensis*, *Mytilus galloprovincialis*, and *Perna canaliculus*. Amplifications of DNA from mussel were carried out using random primers. The most distinctive bands were then isolated, cloned, and sequenced to design specific primers. Finally, DNA from different mussels was amplified with these specific primers, and results allow genetic identification of *M. galloprovincialis* from the rest of the mussel species.

KEYWORDS: Mussels; *Mytilus*; RAPD; species identification

INTRODUCTION

Mussels, being one of the most harvested and commercialized species of bivalve molluscs, are widespread throughout the coasts of many countries of the world, showing an antitropical distribution pattern, so that closely related taxa inhabit mild climate zones of the northern and southern hemispheres (*1*). They do not show sexual dimorphism but exhibit a special characteristic revealed by the existence of two highly divergent genomes of mtDNA, one found in all individuals and transmitted by females (F-type) and the other found only in males (M-type) and transmitted to their male descendants. This special inheritance pattern is called "double uniparental inheritance" (*2*, *3*). Among the different species harvested are *Mytilus edulis*, *Mytilus galloprovincialis*, *Mytilus chilensis* (*Mytilus edulis chilensis*), and *Perna* sp. (mainly *P. canaliculus*). However, each of these species has different meat taste and texture, the most valuable characteristic for their commercialization. Because of this, the identification and authentication of commercial food species are very important for accurate labeling and assessment and to avoid unfair trading competition. To achieve this objective, laboratories need to have techniques and methodologies at their disposal to ascertain the species used in the food industry.

Mussels used for food are sold in two forms: (i) raw (with the shell) and (ii) frozen or canned (without the shell). Differentiation between *Mytilus* spp. and *Perna* sp. individuals with the shell is easy according to the color of the shell, which is black-violet in *Mytilus* and green in *Perna*, but is difficult if the individuals do not have the shell. However, identification of *Mytilus* spp. samples according to morphological criteria (for example, color of the shell, size, or height) is unreliable because of the great plasticity of these characteristics. To overcome this problem, allozyme markers (*4*, *5*), mitochondrial DNA analysis (*6*, *7*) or amplification of different nuclear DNA regions (*8*−*10*) have been applied to differentiate between species and populations of mussels. However, the results obtained show the existence of intrapopulation polymorphisms so, then, they cannot be used for identification at the individual level.

Different techniques for DNA analysis have been applied for food quality control in other animal species (*11*, *12*). PCR methods for species identification are based on mitochondrial DNA amplification (*13*−*16*), lysine sperm gene (*17*), α-actin gene (*18*), 5S rDNA genes (*19*), and anonymous DNA regions by random amplified polymorphic DNA analyses (RAPDs) (*20*).

RAPD using short primers (*21*) has been shown to be very useful in the analysis of genetic polymorphism; the method is a relatively fast, cheap, and simple technique that can be done without information about the genome of the organism being studied. However, reproducibility may suffer if the Polymerase Chain Reaction (PCR) conditions are not constant and stringent enough or if template DNA has a low quality. This technique has been used to identify only meat products (*20*, *22*) and fishes (*23*, *24*). In bivalves, this methodology has been applied in the genus *Donax* (*25*, *26*), in *Cerastoderma edule* and *C. lamarckii* (*27*), and in *Pecten maximus* (*28*). Depending on the number of priming sites, the RAPD technique may amplify from 0 to 30 products (*29*) among short inverted repetitions. These products can be scattered throughout the genome (*21*), and many of these loci could be neutral markers (*29*). The aim of this study is to analyze the suitability of RAPD products in order to search for a diagnostic pattern to be used in the identification of different mussel species or populations.

MATERIALS AND METHODS

Sample Collection and DNA Extraction. Mussels were collected from six harvested populations: Yerseke (Holland), Prince Edward Island (Canada), Puerto Aguirre (Chile), Ria de Arousa (northwest coast

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of Spain), Ebro Delta (east coast of Spain), and New Zealand. Samples from Yerseke, Ría de Arousa, and Ebro Delta were alive upon receipt; muscle, mantle, and foot were stored separately at −80 °C. Samples from Prince Edward Island, Puerto Aguirre, and New Zealand arrived frozen at −20 °C.

The population from Yerseke was previously characterized as *M. edulis* by Sanjuan et al. (*30*) and Quesada et al. (*31*), who also characterized, by allozyme markers, populations from Ria de Arousa and Ebro Delta as *M. galloprovincialis*. The population from Prince Edward Island was characterized as *M. edulis* according to the amplification of the internal transcribed spacer (ITS) and subsequent digestion with *Hha*I as described by Heath et al. (*8*). The mussels from Puerto Aguirre were determined to be *M. chilensis* (*M. edulis chilensis*) according to their geographical distribution (*1*, *4*). *P. canaliculus* was characterized in origin according to the color and morphology of the shell.

Prior to DNA extraction, tissues were washed in PBS (0.137 M NaCl, 2.68 mM KCl, 10.1 mM Na_2HPO_4, and 1.76 mM KH_2PO_4) and distilled water. Samples containing 25−30 mg of adduct muscles, mantle, or foot tissues were homogenized with 625 μL of lysis buffer (10 mM Tris, 100 mM EDTA, 400 mM NaCl, and 0.75% SDS) and 3 μL of proteinase K at 60 °C for 2 h. Later, 280 μL of saturated NaCl was added, and the tubes were vortexed for 4 min and then centrifuged (17500*g*, 10 min). Supernates were transferred to clean tubes, chloroform (500 μL) was added, and the tubes were vortexed for 2 min and centrifuged (17500*g*, 1 min). Supernates were transferred to clean tubes, 2-propanol at −20 °C (800 μL) was added, and the contents were mixed by inverting the tubes. DNA was precipitated at −80 °C for 30 min or at −20 °C overnight and recovered by centrifugation (17500*g*, 5 min). The pellet was washed twice with 70% ethanol (−20 °C), dried under vacuum, and dissolved in 100 μL of TE (10 mM Tris-HCl and 1 mM EDTA, pH 8). We observed that DNA of the foot produces the lowest quantity, but if the mussel is mature, the high-performance DNA is obtained from the gonad tissue.

PCR Amplifications. All amplification reactions were performed twice in 25 μL of reaction volume containing 25 ng of template DNA, 1.25 units of Taq polymerase (Bioline), 0.2 mM dNTPs, and 200 nM each of the primers tested. The reaction buffer contained 1.75 mM $MgCl_2$. The amplifications were performed in a Bio-Rad Gene Cycler as follows: initial denaturation of 3 min at 94 °C, then incubation for 10 cycles at 94 °C for 45 s, at 40 °C for 60 s, and at 72 °C for 75 s; then, 30 cycles at 94 °C for 45 s, at 50 °C for 60 s, and at 72 °C for 60 s; and finally the primer extension was carried out at 72 °C for 5 min.

Amplification of the *M. galloprovincialis* diagnostic band was performed as follows: initial denaturation at 94 °C for 2 min, followed by incubation for 35 cycles at 94 °C for 60 s, at 55 °C for 40 s, and at 72 °C for 40 s, and finally the primer extension was carried out at 72 °C for 3 min.

Amplified DNA was resolved by running 5 μL of PCR product on 1.5% agarose gels in Tris-acetate buffer (0.04 M Tris-acetate and 0.001 M EDTA, pH 8.0) at 3 V/cm and stained with ethidium bromide. Molecular weights were estimated using UVP specific densitometry software (Gelwld 2.51).

DNA Isolation and Sequencing. DNA from selected 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). After growing of recombinant colonies, plasmids were purified as in Sambrook et al. (*32*). DNA was sequenced in both directions, using an ALFexpress AutoRead Sequencing Kit (Amersham Pharmacia Biotech), in an ALFexpress DNA sequencer. Finally, we designed two primers (**Table 1**) to amplify this region.

RESULTS AND DISCUSSION

Twenty 10-mer random oligonucleotide primers (Advanced Biotechnologies) were screened to analyze banding patterns in 30 individuals from each population. Primers were selected attending to the reproducibility criteria in different assays with the same individuals and, on the other hand, to the high quality in banding pattern. The aim of this work is to find those that would allow the identification of these four mussel species. Primers ABA-02, ABA-05, ABA-07, and ABA-10 induced the best amplifications (**Table 1**).

Table 1. DNA Primers Used in This Study[a]

primer	sequence	reproducibility	quality	primer selected
ABA-01	5′-CAGGCCCTTC-3′	−	bad	−
ABA-02	5′-TGCCGAGCTG-3′	+	good	+
ABA-03	5′-AGTCAGCCAC-3′	+	bad	−
ABA-04	5′-AATCGGGCTG-3′	−	bad	−
ABA-05	5′-AGGGGTCTTG-3′	+	good	+
ABA-06	5′-GGTCCCTGAC-3′	−	bad	−
ABA-07	5-GAAACGGGTG-3′	+	good	+
ABA-08	5′-GTGACGTAGG-3′	+	bad	−
ABA-09	5′-GGGTAACGCC-3′	−	bad	−
ABA-10	5′-GTGATCGCAG-3′	+	good	+
ABA-11	5′-CAATCGCCGT-3′	−	bad	−
ABA-12	5′-TCGGCGATAG-3′	−	bad	−
ABA-13	5′-CAGCACCCAC-3′	−	bad	−
ABA-14	5′-TCTGTGCTGG-3′	−	bad	−
ABA-15	5′-TTCCGAACCC-3′	−	bad	−
ABA-16	5′-AGCCAGCGAA-3′	−	good	−
ABA-17	5′-GACCGCTTGT-3′	−	regular	−
ABA-18	5′-AGGTGACCGT-3′	−	bad	−
ABA-19	5′-CAAACGTCGG-3′	−	bad	−
ABA-20	5′-GTTGCGATCC-3′	−	bad	−
580-1F	5′-GAGCTGAGCGAGGAGA-3′			
580-1R	5′-ACCAGACTGCAACCTGA-3′			

[a] Only the primers that showed a good-quality banding pattern and high reproducibility in several amplifications of the same individuals were selected. Primers that produced highly smearing and/or inconsistent amplification products were not selected. Therefore, primer ABA-16 was not selected, despite its good quality. + indicates "yes", and − indicates "no".

Analysis of banding patterns revealed that primers ABA-02 and ABA-07 were the most discriminative. Primer ABA-02 can induce both 580 and 1050 bp fragments in the different individuals analyzed (**Figure 1A**). Each of the individuals of *M. galloprovincialis* populations presented the 580 bp band, whereas fewer than 40 and 50% of individuals from *M. edulis* and *M. chilensis* populations, respectively, showed this same band. More than 50% of *M. edulis* and *M. chilensis* individuals and fewer than 30% of those from *M. galloprovincialis* showed a 1050 bp fragment.

On the other hand, individuals from *P. canaliculus* never showed the 580 bp band when amplified with primer ABA-02, but 80% of them showed a characteristic band of 700 bp, whereas amplification with primer ABA-07 produced a pattern ranging from 700 to 800 bp (**Figure 1C**). Furthermore, primer ABA-07 amplified a 400 bp band in all of the individuals except in *P. canaliculus*. Primer ABA-05 generated a 975 bp fragment in >60% of individuals of each population, except for those from *P. canaliculus* (**Figure 1B**). Finally, primer ABA-10 yielded a fragment of 500 bp in 10−55% of mussels in each of the populations analyzed except for the Chilean population, in which this fragment was never amplified (**Figure 1D**).

Therefore, these results distinguish *P. canaliculus* from *Mytilus* mussel populations. Subsequently, we carried out the sequencing of the 580 bp band obtained from the Ria de Arousa population, because the different intensities of bands observed in *M. galloprovincialis* populations with regard to the other populations could be due to differences in this sequence. A sequence of 594 nucleotides with an adenine−thymine proportion of 63.24% was obtained from one individual from the Ria de Arousa population (Genbank Accession No. AJ315480).

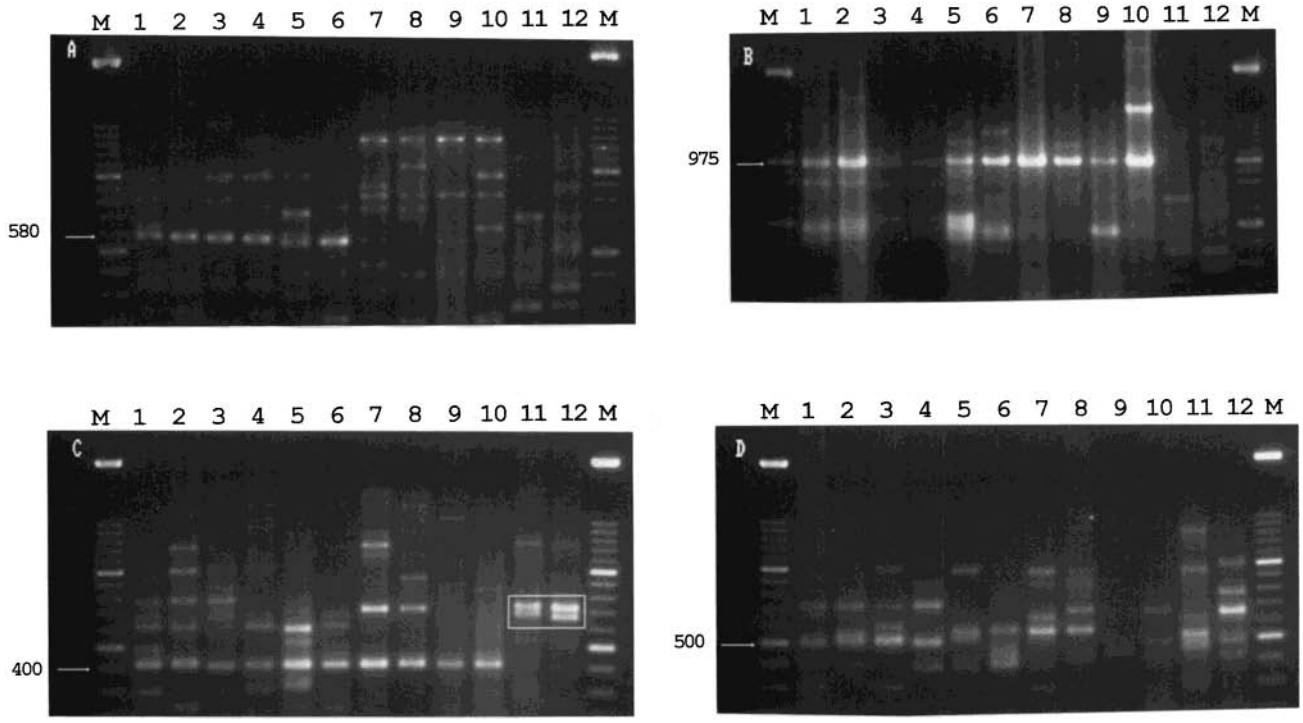

Figure 1. RAPD markers on 1.5% agarose gels: (lane M) 100 bp ladders; (lanes 1 and 2) *M. galloprovincialis* (Ria de Arousa); (lanes 3 and 4) *M. galloprovincialis* (Ebro Delta); (lanes 5 and 6) *M. edulis* (Yerseke); (lanes 7 and 8) *M. edulis* (Prince Edward Island); (lanes 9 and 10) *M. chilensis* (Puerto Aguirre); (lanes 11 and 12) *P. canaliculus* (New Zealand). Amplifications were performed using (A) primer ABA-02, (B) primer ABA-05, (C) primer ABA-07 (in the square, *P. canaliculus* pattern), and (D) primer ABA-10.

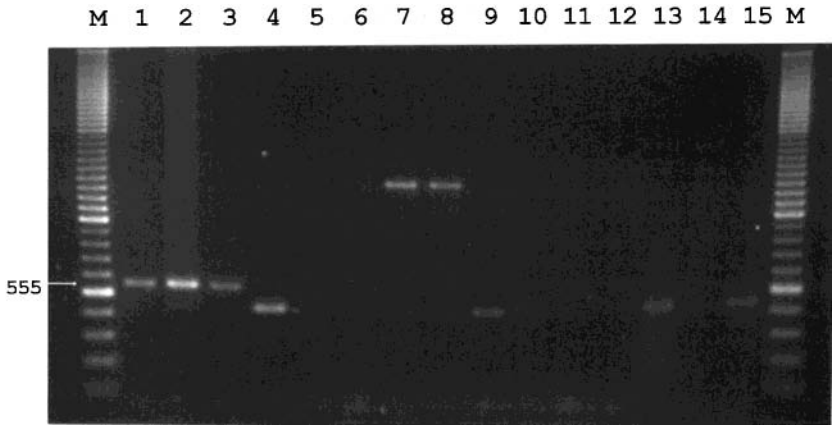

Figure 2. Species-specific PCR products on 1.5% agarose gel obtained from amplification with primers 580-1F and 580-1R in different individuals: (lane M) 100 bp ladders; (lanes 1–3) *M. galloprovincialis* (Ria de Arousa); (lanes 4–6) *M. edulis* (Yerseke); (lanes 7–9) *M. edulis* (Prince Edward Island); (lanes 10–12) *M. chilensis* (Puerto Aguirre); (lanes 13–15) *P. canaliculus* (New Zealand). Individuals from *M. galloprovincialis* show a specific 555 bp fragment. Alternatively, individuals outside *M. galloprovincialis* populations can show a 400–450 bp band or a 1300 bp band.

Sequence comparison with EMBL and Genbank nucleic acid databases revealed no significant homology with any recorded sequence. From these data, we designed two primers, 580-1F and 580-1R, of 16 and 17 bases, respectively (**Table 1**), to specifically amplify this fragment and try to differentiate between *M. galloprovincialis* and the rest of the populations. Amplification of the DNA from the different populations revealed that all *M. galloprovincialis* individuals (40 from Ria de Arousa and 40 from Ebro Delta) presented a 555 bp fragment (**Figure 2**), whereas only 17.85% of *M. edulis* from Prince Edward Island (5 of 28), 5.88% from Yerseke (2 of 34), and 9.09% from Puerto Aguirre (3 of 33) showed this band. This band was never observed in *P. canaliculus* individuals. ANOVA analysis of these five populations led us to observe a significant F value of 170.64 (df = 4/170). Tamhane post hoc contrast between Prince Edward Island, Puerto Aguirre, and Yerseke populations showed no significant differences. Subsequent ANOVA analysis between these populations showed an F value of 1.22 ($p = 0.301$; df = 2/92).

Molecular and biochemical methods for the identification of species exist for several purposes and use species-specific protein and DNA polymorphisms (*17*). Food quality control and authentication of contents have been used in fishes and other marine products (e.g., refs *13*, *17*, and *33*−*35*). Mollusc PCR-RFLP analysis of mitochondrial DNA was applied for identification of snail meat species (*14*) and nuclear PCR-RFLP for identification of abalone tissue (*17*) and clams (*18*). Although different methods, such as mitochondrial DNA analysis (*36*−*38*), RFLP of ribosomal 18S-5.8S-28S loci (*8*), or PCR of some gene fragments (*9*, *10*), have been used to identify mussel

species, none of these proved to be useful for distinguishing between the different harvested populations and commercially processed and packaged mussels.

The method presented in this work is simple, easy, and reproducible for identifying *M. galloprovincialis* from other mussel species. DNA amplification with 580-1F and 580-1R primers produced a 555 bp band, which allows clear identification of the *M. galloprovincialis* population. DNA of mussels not presenting the 555 bp band can be amplified with the ABA-07 primer, and then, if a banding pattern ranging from 700 to 800 bp appears, these mussels would be identified as *P. canaliculus*.

LITERATURE CITED

(1) Hilbish, T. J.; Mullinax, A.; Dolven, S. I.; Meyer, R. K.; Koehn, R. K.; Rawson, P. D. Origin of the antitropical distribution pattern in marine mussels (*Mytilus spp*.): routes and timing of transequatorial migration. *Mar. Biol.* **2000**, *136*, 69−77.

(2) Skibinski, D. O. F.; Gallagher, C.; Beynon, C. M. Mitochondrial DNA inheritance. *Nature* **1994**, *368*, 817−818.

(3) Zouros, E.; Ball, A. O.; Saavedra, C.; Freeman, K. R. Mitochondrial DNA inheritance. *Nature* **1994**, *368*, 818.

(4) McDonald, J. H.; Seed, R.; Koehn, R. K. Allozyme and morphometric characters of three species of *Mytilus* in the Northern and Southern hemispheres. *Mar. Biol.* **1991**, *111*, 323−335.

(5) Koehn, R. K. The genetics and taxonomy of species in the genus *Mytilus*. *Aquaculture* **1991**, *94*, 125−145.

(6) Geller, J. B.; Carlton, J. T.; Powers, D. A. Interspecific and intrapopulation variation in mitochondrial ribosomal DNA sequences of *Mytilus* spp. (Bivalvia: Mollusca). *Mol. Mar. Biol. Biotechnol*. **1993**, *2*, 44−50.

(7) Rawson, P. D.; Hilbish, T. J. Asymmetric introgression of mitochondrial DNA among European populations of blue mussels (*Mytilus* sp.). *Evolution* **1998**, *52*, 100−108.

(8) Heath, D. D.; Rawson, P. D.; Hilbish, T. J. PCR-based nuclear markers identify alien blue mussel (*Mytilus* spp.) genotypes on the west coast of Canada. *Can. J. Fish. Aquat. Sci.* **1995**, *52*, 2621−2627.

(9) Inoue, K.; Waite, H.; Matsuoka, M.; Odo, S.; Harayama, S. Interspecific variations in adhesive protein sequences of *Mytilus edulis, M. galloprovincialis* and *M. trossulus*. *Biol. Bull*. **1995**, *189*, 370−375.

(10) Rawson, P. D.; Joyner, K. L.; Meetze, K.; Hilbish, T. J. Evidence for intragenic recombination within a novel genetic marker that distinguishes mussels in the *Mytilus edulis* species complex. *Heredity* **1996**, *77*, 599−607.

(11) Leighton Jones, J. DNA probes: applications in the food industry. *Trends Food Sci. Technol.* **1991**, *2*, 28−32.

(12) Carrera, E.; García, T.; Céspedes, A.; González, I.; Fernández, A.; Hernández, P. E.; Martín, R. Salmon and trout by PCR-RFLP for identity authentification. *J. Food Sci.* **1999**, *64*, 410−413.

(13) Ram, J. L.; Ram, M. L.; Baidoun, F. F. Authentification of canned tuna and bonito by sequence and restriction site analysis of Polymerase Chain Reaction products of mitochondrial DNA. *J. Agric. Food Chem.* **1996**, *44*, 2460−2467.

(14) Borgo, R.; Souty-Grosset, C.; Bouchon, D.; Gomot, L. PCR-RFLP analysis of mitochondrial DNA for identification of snail meat species. *J. Food Sci.* **1996**, *61*, 1−4.

(15) Quintero, J.; Sotelo, C. G.; Rehbein, H.; Pryde, S. E.; Medina, I.; Pérez-Martín, R. I.; Rey-Méndez, M.; Meckie, I. M. Use of mtDNA direct Polymerase Chain Reaction (PCR) sequencing and PCR-restriction fragment length polymorphism methodologies in species identification of canned tuna. *J. Agric. Food Chem.* **1998**, *46*, 1662−1669.

(16) Russel, V. J.; Hold, G. L.; Pryde, S. E.; Rehbein, H.; Quinteiro, J.; Rey-Méndez, M.; Sotelo, C. G.; Pérez-Martín, R. I.; Santos, A. T.; Rosa, C. Use of restriction fragment length polymorphism to distinguish between salmon species. *J. Agric. Food Chem.* **2000**, *48*, 2184−2188.

(17) Sweijd, N. A.; Bowie, R. C. K.; Lopata, A. L. L.; Marinaki, A. M.; Harley, E. H.; Cook, P. A. A PCR technique for forensic, species-level identification of abalone tissue. *J. Shellfish Res.* **1998**, *17*, 889−895.

(18) Fernández, A.; García, T.; Asensio, L.; Rodríguez, M. A.; González, I.; Céspedes, A.; Hernández, P. E.; Martín, R. Identification of the clam species *Ruditapes decussates* (Grooved carpet shell), *Venerupis pullastra* (Pullet carpet shell), and *Ruditapes philippinarum* (Japanese carpet shell) by PCR-RFLP. *J. Agric. Food Chem.* **2000**, *48*, 3336−3341.

(19) Carrera, E.; García, T.; Céspedes, A.; González, I.; Fernández, A.; Asensio, L. M.; Hernández, P. E.; Martín, R. Differentiation of smoked *Salmo salar*, *Oncorhynchus mykiss* and *Brama raii* using the nuclear marker 5s rDNA. *Int. J. Food Sci. Technol.* **2000**, *35*, 401−406.

(20) Martínez, I.; Malmheden Yman, I. Species identification in meta products by RAPD analysis. *Food Res. Int.* **1998**, *31*, 459−466.

(21) Williams, J. G. K.; Kubelik, A. R.; Livak, K. J.; Rafalski, J. A.; Tingey, S. V. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*. **1990**, *18*, 6531−6535.

(22) Appa Rao, K. B.; Bhat, K. V.; Totey, S. M. Detection of species-specific genetic markers in farm animal trough random amplified polymorphic DNA (RAPD). *Genet. Anal. Biomol. E* **1996**, *13*, 135−138.

(23) Dinesh, K. R.; Lim, T. M.; Chan, W. K.; Phang, V. P. E. Genetic variation inferred from RAPD fingerprint in three species of tilapia. *Aquacult. Int.* **1996**, *4*, 19−30.

(24) Partis, L.; Wells, R. J. Ientification of fish species using random amplified polymorphic DNA. *Mol. Cell. Probes* **1996**, *10*, 435−441.

(25) Adamkewicz, S. L.; Harasewych, M. G. Use of random amplified polymorphic DNA (RAPD) markers to assess relationships among beach clams of the genus *Donax*. *Nautilus* **1994**, *2*, 51−60.

(26) Adamkewicz, S. L.; Harasewych, M. G. Systematics and biogeography of the genus *Donax* (Bivalvia: Donacidae) in eastern North America. *Am. Malacol. Bull.* **1996**, *13*, 97−103.

(27) André, C.; Lindegarth, M.; Jonsson, P. R.; Sundberg, P. Species identification of bivalve larvae using random amplified polymorphic DNA (RAPD): differentiation between *Cerastoderma edule* and *C. lamarcki*. *J. Mar. Biol. Assoc. U.K.* **1999**, *79*, 563−565.

(28) Heipel, D. A.; Bishop, J. D. D.; Brand, A. R.; Thorpe, J. P. Population genetic differentiation of the great scallop *Pecten maximus* in western Britain investigated by randomly amplified polymorphic DNA. *Mar. Ecol. Prog. Ser*. **1998**, *162*, 163−171.

(29) Grosberg, R. K.; Levitan, D. R.; Cameron, B. B. Characterization of genetic structure and genealogies using RAPD-PCR markers: a random primer for the novice and nervous. In *Molecular Zoology, Advances, Strategies and Protocols*; Ferraris, J. D., Palumbi, S. R., Eds.; Wiley-Liss: New York, 1996; pp 67−100.

(30) Sanjuan, A.; Zapata, C.; Alvarez, G. *Mytilus galloprovincialis* and *M. edulis* on the coasts of the Iberian Peninsula. *Mar. Ecol. Prog. Ser.* **1994**, *113*, 131−146.

(31) Quesada, H.; Zapata, C.; Alvarez, G. A multilocus discontinuity in the mussel *Mytilus galloprovincialis*: the interaction of ecological and life-history factors. *Mar. Ecol. Prog. Ser.* **1995**, *116*, 99−115.

(32) Sambrook, J.; Fritsch, E. T.; Maniatis, T. *Molecular Cloning. A Laboratory Manual*; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1989.

(33) Rehbein, H.; Kress, G.; Schmidt, T. Application of PCR-Sscp to species identification of fishery products. *J. Sci. Food Agric.* **1997**, *74*, 35−41.

(34) Mckay, S. J.; Smith, M. J.; Devlin, R. H. Polymerase chain reaction-based species identification of salmon and coastal trout in British Columbia. *Mol. Mar. Biol. Biotechnol.* **1997**, *6*, 131−140.

(35) Céspedes, A.; García, T.; Carrera, E.; González, I.; Sanz, B.; Hernández, P. E.; Martín, R. Identification of flatfish species using Polymerase Chain Reaction (PCR) amplification and restriction analysis of the cytochrome *b* gene. *J. Food Sci.* **1998**, *63*, 206−209.

(36) Skibinski, D. O. F. Mitochondrial DNA variation in *Mytilus edulis* and the Padstow mussel. *J. Exp. Mar. Biol. Ecol.* **1985**, *92*, 251−258.

(37) Carlton, J. T.; Geller, J. B. Ecological roulette: the global transport and invasion of nonindiogenous marine organs. *Science* **1993**, *261*, 78−82.

(38) Geller, J. B.; Carlton, J. T.; Powers, D. A. PCR-based detection of mtDNA haplotypes of native and invading mussels on the northeastern Pacific coast: latitudinal pattern of invasion. *Mar. Biol.* **1994**, *119*, 243−249.

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