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Microsatellite Analysis of Perch (*Perca fluviatilis*) and its Genetic Authentication of Geographical Localization

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Abstract: European perch (*Perca fluviatilis*) is an economically important freshwater species in Europe. In Switzerland, where the demand largely exceeds the production coming from Swiss lakes, nearly 90% of the requirements come from importation with the majority of perch originating from Estonia and Russia. The price of perch fillet varies considerably depending on the origin. Therefore traceability in the fish food sector plays an increasingly important role for consumer protection. Currently the traceability of perch can be assessed through chemical isotopic analysis. The 180/160 isotopic abundance ratio is used as geographical traceability marker, but several aspects affect the accuracy of the method, *i.e.* the distinct geographical area ratio differs only very slightly with overlapping standard deviation, the need for a large amount of fish material requires the mix of many fillets, the impossibility of analyzing processed matrix, the comparison of the ratio with the ratio of a sample of the presumed originating water makes the analyses more complicated. New application of DNA markers for the traceability of food products plays an increasingly important role for consumer protection. Microsatellites, which are short tandemly repetitive DNA sequences, are genetic markers of choice for traceability because of their abundance and high polymorphism. Moreover, fluorescent labelling and capillary electrophoresis separation increase efficiency and precision of genotyping microsatellites. The method can also be efficiently applied in processed food products where other methods have limited applications. In this study, we tested the efficiency of three polymorphic microsatellites and their combinations for their ability to correctly assign or exclude 195 reference perch to their origin population. Using the maximum likelihood and Bayesian methods computed by the software GeneClass2, the three loci microsatellite were optimized and allowed the correct assignation of all but two Swiss perch (60/62) into Swiss population. The markers also exclude 132/133 imported fish from the Swiss population with a match probability of more than 95%. The number of markers required for correct assignation differs from species to species, and depends on many factors such as genetic diversity and population structure. For perch populations, the results showed that only three polymorphic microsatellite markers are required to perform a reliable attribution or exclusion of a perch to the Swiss population with more than 98% correct assignations.

Keywords: European perch · Food authentication · Geographical origin · Microsatellite · Statistical analysis

1. Introduction

The European market for freshwater fish is considerable and according to FAO (Food and Agriculture Organization of the United Nations), world global fishery production from wild capture is set at about 90 million tonnes worldwide in 2013, with almost 28 million tonnes in Europe.[1] Fish belonging to the Percidae family are economically important in Europe with a particularly emphasis on the following commercial species: pike perch (*Sander lucioperca*) and European perch (*Perca fluviatilis*).[2]

Switzerland has a significant market for freshwater fish with European perch being one of the most popular species. Its economical importance is due to its delicate texture and mild flavor and European perch, which frequently appears on the menu in restaurants, is a typical dish in Swiss cuisine. Because of its popularity, the consumer demand largely exceeds the production coming from Swiss lakes. In 2003, 485 tonnes of perch were supplied from Swiss lakes, and the imported volume of perch is in the order of 3500 to 5000 tonnes,[1,3] representing more or less 90 percent of perch present on the Swiss market. Perch is imported in the form of frozen fillet mostly from Eastern Europe (primarily Estonia and Russia), and to a lesser extent from Northern Europe. The market price of perch varies considerably, doubling or even multiplying by three the price for Swiss perch in comparison to foreign fillets. Thus financial aspects lead to considerable fraud concerning geographical origin declaration. Besides, consumers are ever more concerned with the origin of fish, as several factors as pollution may contribute to growing risk of hazardous and toxic compound accumulation in sea products from the Baltic Sea and to product quality deterioration.[4–6]

Therefore traceability of the geographical origin of fish plays an increasingly important role for consumer protection. Traceability systems giving information on origin will enhance consumer confidence in food products and would represent a valuable tool for the authorities to carry out control and detect fraud.

Current analytical methods for traceability include spectroscopic and chromatographic techniques,[7,8] as well as high-resolution NMR.[9] Previous experience has shown that these methods do not always allow the differentiation of the geographical origin of distinct individuals from the same species, because analyzed markers do not have sufficient reliability and accuracy for geographical origin. Recently, isotope abundance ratio analysis

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has been shown to be particularly suited for this purpose.[10,11] But several aspects affect the precision of the method for individual geographical assignment. The need for a large amount of DNA material, as well as the comparison of the isotope ratio with a presumed originating environment sample makes the analysis difficult. Moreover, distinct geographical area ratios differ only very slightly with overlapping standard deviation leading to high error rates and decrease the accuracy of traceability systems.

New application of DNA markers for the traceability of food products can overcome these existing problems by tracking individuals through their DNA.[12–15] Microsatellites, which are short non-coding tandemly repetitive DNA sequences, are the markers of choice for traceability, because of their high abundance, high polymorphism and random distribution throughout the genomes of all eukaryotes.[16,17] The ease with which these can be characterized using PCR, fluorescent labelling and electrophoresis capillary separation increases efficiency and precision. Moreover, only small amounts of DNA are required and highly degraded DNA still allows robust analysis, making the analysis of processed foodstuffs possible.[18]

Numerous statistical tests have been developed for assignation or exclusion of populations as origins of individuals using multiple microsatellite loci.[18–21] They are based on likelihood estimation and Bayesian methods based on allelic frequency to assign or exclude an individual from reference populations.[21,22]

The objective of this study was to develop a precise DNA-based tracking system for genetic authentication of geographical localization of European perch present on the Swiss market. For this purpose, 195 fish fillets coming from seven distinct countries were collected representing the reference populations. To date, no microsatellite DNA markers have been used in such genetic traceability method in European perch. For this reason the markers used in this study were developed for walleye (*Stizostedion vitreum*)[23,24] and were first evaluated for their ability to be applied for European perch. Thus, five polymorphic microsatellite loci (Svi6, Svi17, Svi18, Svi26 and Svi29) were individually examined for their polymorphisms and power for individual geographical origin authentication. The efficiency of these microsatellite loci was evaluated for their ability to correctly assign or exclude each individual perch to the Swiss reference population. The assignments tests were performed using GeneClass 2.0 software, which is a program using multilocus genotypes to select or exclude populations as origins of individuals.[25] Assignment of an individual to a population was performed using two test types: assignment of an individual to the population from which it most probably originated with a score for each of the reference populations, and assignment of an individual by computing the statistic probability of a multilocus genotype in a reference population compared to the probability that an individual belongs to each reference population.

2. Material and Methods

2.1 *Fish Samples*

Perch (*Perca fluviatilis*) for the Swiss reference population were collected from fishermen at different localities around Lake Geneva. Additionally, perch from six other Swiss lakes were included in the study: Lake Neuchatel, Lake Zurich, Lake Sempachsee, Lake Bodensee, Lake Bielersee and Lake Walensee. Perch from foreign origin were obtained either from fishmongers or from supermarkets and originate from Estonia, Russia, Poland, Ireland, Sweden and Germany. In total, 62 fish samples corresponded to the Swiss reference population and 135 to the foreign perch population. All samples were collected as fish fillet.

2.2 *DNA Extraction*

For each specimen, genomic DNA was extracted from 300 mg of tissue using standard Wizard DNA Cleanup System (Promega),[26] as detailed below. Tissue was homogenized in 1 mL lysis buffer (pH 8.0) containing 10 mM Tris, 150 mM NaCl, 2 mM EDTA, 1% SDS. Directly before use, proteinase K to a final concentration of 0.8 mg/mL and guanidine hydrochlorate to a final concentration of 500 mM were added. The mixture was incubated in a water bath at 58°C for 2 hours, cooled to room temperature and centrifuged for 10 minutes in a microcentrifuge at maximum speed. The supernatant was incubated 10 minutes at room temperature with 3 µL of RNase A (10 mg/mL) and was loaded on a Wizard mini-column (Promega) containing 1 mL of Wizard Resin (Promega). The sample was passed through the column, washed with 2 mL 80% isopropanol and eluted with 60 µL of pre-warmed (70 °C) water after centrifuging 1 minute at maximum speed. DNA quantification was done by measuring absorbance at 260 nm using a Nanodrop spectrophotometer (ND-2000, Thermo Fischer Scientific).

2.3 *Species Determination*

2.3.1 *PCR Amplification*

Primers amplified a region of approximately 464 bp of the spanning the 3'-end of the tRNA-*Glu* and the 5'-end of the cytochrome b gene[27] (H15149ad: 5'-GCICCTCARAATGAYATTTGTCCTCA-3' and L14735: 5'-AAAAACCACCGTTGTTATTCAACTA-3'). PCR reactions were performed in 50 µL volume containing: 2.5 mM $MgCl_2$, 1× enhancer solution (PeqLab), 400 µM dNTPs (Thermo Fisher Scientific Biosciences GmbH), 800 nM of each primer, 1.25 units of Taq polymerase (PeqGold Hot Taq-DNA polymerase, PeqLab) and 500 ng of template DNA. PCR was carried out using a thermocycler (Biometra Tri-Professional Trio) as follows: activation step, 94 °C, 30 s; cycling conditions, 94 °C, 30 s, 50 °C, 105 s, 72 °C, 90 s, 33 cycles; final extension, 68 °C, 7 min. PCR products (5 µL) were electrophoretically separated on an agarose 2% gel, and visualized on a UV screen after staining with ethidium bromide.

2.3.2 *Restriction Fragment Length Polymorphism (RFLP)*

Restriction digestions were performed using the following enzymes: RsaI, AluI, HinfI and HaeIII (all from Thermo Fisher Scientific Biosciences GmbH). For each restriction enzyme, 10 µL of PCR amplicon was digested with 5 units of enzyme for 2 hours at 37 °C. Digest products were separated by electrophoresis on a 2% molecular screening (MS) agarose gel stained with ethidium bromide. The size of the DNA fragments were estimated by comparison with a 100 bp ladder (Thermo Fisher Scientific Biosciences GmbH) and compared with RFLP reference patterns of a *Perca fluviatilis* reference fish.

2.3.3 *Sequencing of PCR Amplicon*

PCR products were purified using Wizard SV Gel and PCR Clean-Up System (Promega). The PCR amplicons were sequenced on both DNA strands with the same primer pair used for PCR amplification. Sequencing was performed using the dye terminator cycle sequencing methodology. Sequencing reactions were prepared with the GenomeLab DTCS Quick Start Kit (Beckman Coulter, Inc.). Briefly, 50 fmol of purified PCR amplicons were added to 8 µL of Quick Start mix of the aforementioned kit and 4 pmol of the corresponding primer. Distilled water completed the volume to 20 µL. The sequencing reaction consisted of 30 cycles of 95 °C for 20 s and 55 °C for 3 min. The extension products were purified using an ethanol purification procedure for removal of the non-incorporated dye terminators. The pellet was dried, resuspended in Sample Loading Solution (SLS, Beckman Coulter, Inc.) and analyzed on the GenomeLab GeXP Genetic Analysis System (Beckman Coulter, Inc.).

2.4 *Microsatellite Analysis*

PCR amplification and fragment analysis were conducted for five dinucleotide microsatellite loci using primers developed for walleye (*Stizostedion vitreum*).[23,24] The loci studied and primers used are shown in Table 1. Forward primers were labelled on 5'-end using cyanine-based fluorescent dyes (WellRED oligos, sold by Sigma-Aldrich under license by Beckman Coulter, Inc. to be used CEQ Genetic Analysis Systems).

2.4.1 *PCR Amplification*

PCR reactions were performed in 25 µL volume containing: 1.5 mM (Svi18 and Svi29), 2.0 mM (Svi26) or 2.5 mM (Svi6 and Svi17) $MgCl_2$, 400 µM dNTPs (Thermo Fisher Scientific Biosciences GmbH), 400 nM of the labelled forward primer and of the reverse primer, 1.5 units of Taq polymerase (PeqGold Hot Taq-DNA polymerase, PeqLab) and 20 ng of template DNA. PCR were carried out using a thermocycler (Biometra Tri-Professional Trio) as follows: activation step, 94 °C, 30 s; cycling conditions, 94 °C, 30 s, 50 °C (Svi26) or 60 °C (Svi6, Svi17, Svi18, Svi29), 45 s, 72 °C, 45 s, 25 cycles.

2.4.2 *Fragment Analysis*

The amplified products were diluted in sample loading buffer (SLS) and a size standard ladder (Size standard 400 ladder, Beckman Coulter, Inc.) was added following the manufacturer's instructions. The products were separated on the GenomeLab GeXP Genetic Analysis System (Beckman Coulter, Inc.) following the manufacturer's procedure for fragment analysis. The microsatellite alleles for each locus were analyzed using the CEQ System software (Beckman Coulter, Inc.).

2.5 *Statistical Analysis*

Assignment or exclusion of individual fish to reference populations was performed using GeneClass 2 software.[19,25] which is a program using multilocus genotypes to select or exclude populations as origins of individuals. Assignment of individual fish to their most likely population of origin was performed using Bayesian criteria.[20,22] For each individual, the best-matching populations were sorted and a score is given for each population (scoring in percentage). Moreover, assignment or exclusion of individual fish to reference populations was also achieved by calculating statistic probabilities that an individual belongs to each reference population.[21] The scoring and the statistical parameters were considered to assign or exclude perch to their origin population. In order to assign an individual to the Swiss reference population, the criteria were: a) the scoring for the Swiss population was superior to 70% and b) the statistical probability was $p > 0.05$. On the contrary, to exclude an individual from Swiss reference population, a) the scoring was inferior to 5% and b) the probability was $p < 0.05$.

3. Results

3.1 *Fishes Composing the Reference Populations*

The reference samples consisted of fillet collected either from local fishermen, or from importing companies or supermarkets. Fishes from seven countries were indexed in the reference population. The countries were chosen as they represent the major perch importing countries on Swiss market, with more than 90% of total perch coming from Estonia, Russia and Poland.[1] Among the 197 reference fillets, 62 came from Swiss lakes, 61 from Russia, 40 from Estonia, 22 from Poland, 5 from Ireland, 5 from Sweden and 2 from Germany.

3.2 *Fish Identification: RFLP Patterns*

PCR amplification of the 3'-end of the tRNA-*Glu* and the 5'-end of the cytochrome b gene generated a single expected size amplicon of 464bp for all of the 197 fish samples.[26] PCR product digested using the four enzymes Tru1I, TaqI, RsaI and AluI were resolved using a 2% molecular screening (MS) agarose gel stained with ethidium bromide. For 195 fish fillets, we obtained RFLP profiles (Fig. 1, left profile) confirmed as European perch (*Perca fluviatilis*) profile by comparison with a reference profile. However, for 2 fillets originating from Poland, their RFLP profile (Fig. 1, right profile) did not match with European perch profile and the result of sequencing of the PCR amplicon revealed that the fishes corresponded to pike perch fish (*Sander lucioperca*). Therefore, these two fishes were not used for further investigations. Of the 197 fish fillet analyzed for species identification, 195 were used as reference population for European perch genetic authentication of geographical localization in further investigations.

3.3 *Microsatellite Marker Characteristics and Polymorphism*

Though the microsatellite loci were developed for walleye (*Stizostedion vitreum*),[23,24] all loci amplified correctly one or two alleles for most of the individual fillets, as illustrated in Fig. 2. Allele size for each microsatellite marker was recorded by the size of peaks produced after capillary electrophoresis. In the few cases where no fragments were amplified per one locus, it was coded as a missing allele in the respective individual. Altogether, we examined 5 loci for each of the 195 perch and we obtained fragment results for all loci for 181 perch, and for 14 perch (7.2%), further analyses were based only on 4 loci.

Allele number and proportion of heterozygotes were estimated using GeneClass 2.0 software. In total, 61 alleles were observed from the 5 loci. The number of alleles per locus, which is an indicator of genetic diversity, ranged from 4 (for Svi29) to 31 (for Svi17) across populations with a mean of 12.2 (Table 1), and from 1 to 21 within each populations (Table 2). For the Swiss population, the average number

Table 1. Microsatellite characteristics and primer sequences.

Locus	Number of alleles	% of most common allele	% of heterozyzotes	Primer sequences
Svi6	9	43.2	40.2	F: 5'-[D4]CAT ATT ATG TAG AGT GCA GAC CC-3' R: 5'-TGA GCT TCA CCT CAT ATT CC-3'
Svi17	31	31.9	62.5	F: 5'-[D4]GCG CAC TCT CGC ATA GGC CCT G-3' R: 5'-CGT TAA AGT CCT TGG AAA CC-3'
Svi18	12	33.4	53.4	F: 5'-[D4]GAT CTG TAA ACT CCA GCG TG-3' R: 5'-CTT AAG CTG CTC AGC ATC CAG G-3'
Svi26	5	86.9	22.2	F: 5'-[D4]CAG ACA AAC AGG TTG GAG AG-3' R: 5'-CTA CTT ATC TTC TGG CGG AC-3'
Svi29	4	97.8	2.1	F: 5'-[D4]GAT CCC CCT CCT CTC CCC TC-3' R: 5'-CTT TTT CTG CCC TGT CCC AAC-3'

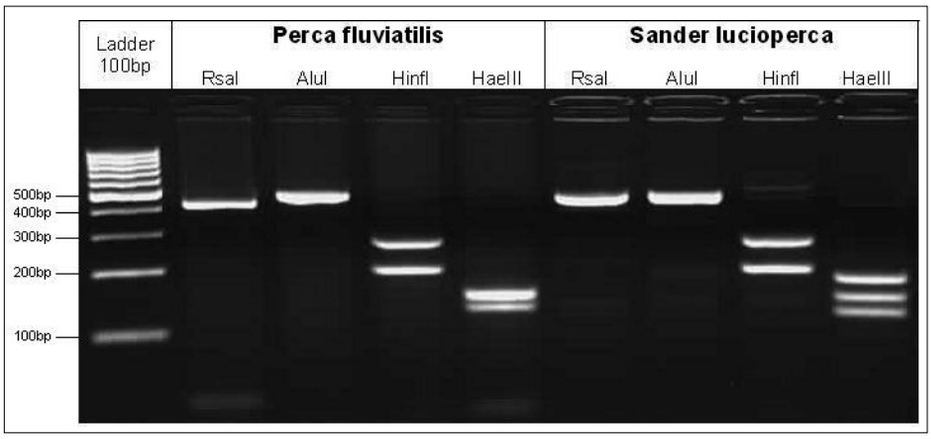

Fig. 1. RFLP profiles obtained for the 197 fish fillets. Restriction digestions were performed using RsaI, AluI, HinfI and HaeIII. Two distinct RFLP patterns were observed differing for the first and the fourth lanes. Left: RFLP profile obtained for the 195 European perch (*Perca fluviatilis*). Right: RFLP profile obtained for 2 pike perch (*Sander lucioperca*), confirmed by sequencing PCR amplicon. These two fishes were not used for further investigations.

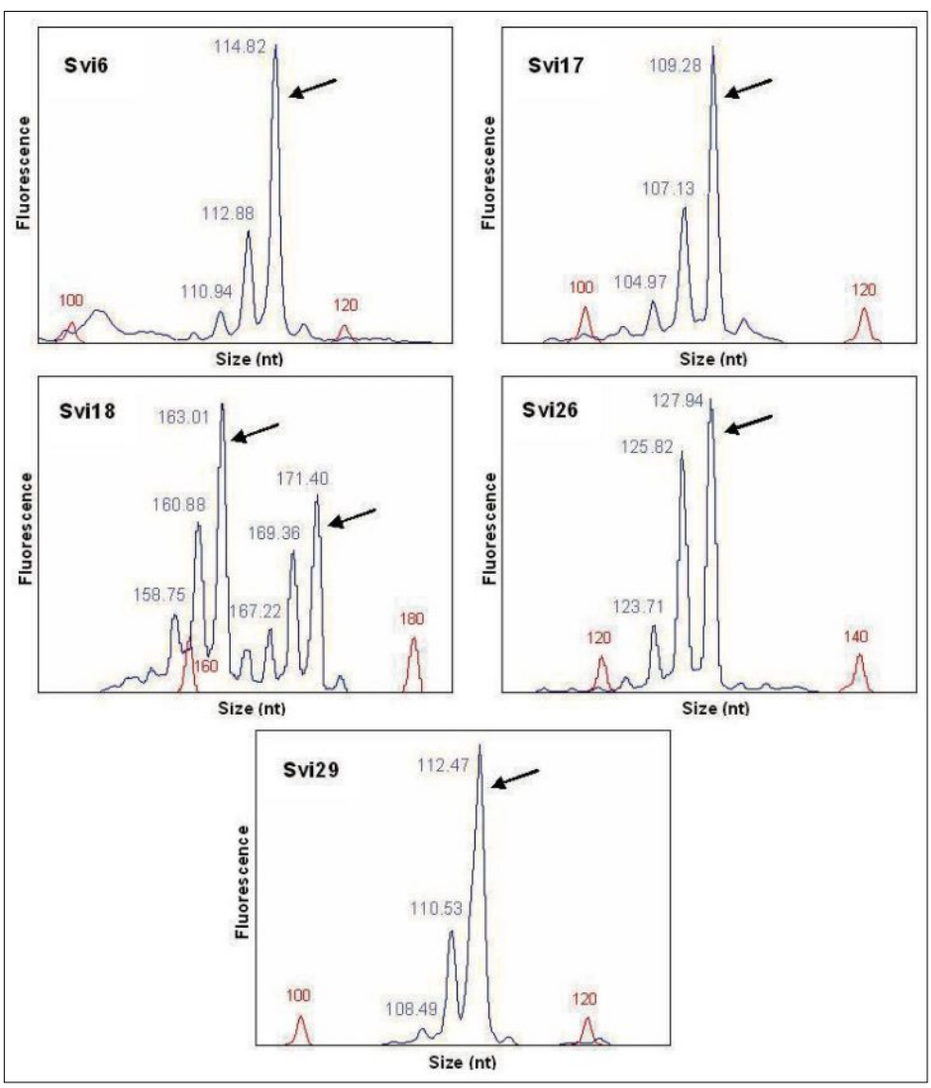

Fig. 2. Chromatogram examples of allelic determination for Svi6, Svi17, Svi18, Svi26 and Svi29. These chromatograms were all obtained for a Swiss fillet coming from Lake Geneva. Arrows indicated the allelic peaks, where minor peaks differing by two or four nucleotides are stutter peaks. We determined this fish was homozygote for Svi6, Svi17, Svi26 and Svi29, and heterozygote for Svi18.

of alleles per locus was approximately half those of Estonian, Russian and Polish populations. This low level of genetic diversity could perhaps be explained by intensive fishing and decrease of total perch population in Swiss lakes. Moreover, the small geographical area and natural barriers, as well as environmental stability could also be part of an explanation for the reduced genetic diversity.[28] For the three other populations (Irish, Swedish and German populations), the average number of alleles per locus was very low and might be explained by the reduced sample size of the three populations (respectively only 5, 5 and 2 individuals).

Heterozygosity of the whole population investigated was situated at 0.345, while the minimum of heterozygosity was observed for Irish (0.080) and Swiss (0.207) populations (Table 2). The values of heterozygosity for three loci (Svi6, Svi17 and Svi18) ranged from 0.402 (Svi6) to 0.625 (Svi17), which indicated the potential robustness of these loci as genetic markers. Heterozygosity level for the 2 other loci was very low with 0.222 and 0.021 for respectively Svi26 and Svi29. Moreover, the number of private allele found for these two loci represented each one less than 5% of private alleles found among the five loci for all populations (Table 3). Consequently, these two loci were only moderately polymorphic and they produced almost similar patterns for the seven reference populations. They were found to have the same predominant allele in each of the populations. Thus, due to their poor utility as genetic marker, these two microsatellite loci were not exploited for individual assignment or exclusion for perch origin determination.

3.4 *Statistical Criteria for Individual Assignment or Exclusion*

The accuracy with which individual perch could be correctly assigned or excluded from the Swiss reference population was assessed using individual assignment tests implemented in the GeneClass 2.0 software.[19,25] Assignment or exclusion of individual fish to reference populations was performed using two criteria: i) assignment performed through Bayesian statistical methods based on allelic frequency to determine the best-matching populations with a percentage scoring,[22] and ii) assignment or exclusion of individual fish to reference populations by calculating statistic probabilities that an individual belongs to each reference population.[21] To compute probability parameters, the Monte Carlo resampling method allowed the random generation of a large number of multilocus genotype individuals (10,000 in our study) to approximate the distribution of genotype likelihoods in reference population samples, and the comparison of a to-be-assigned individual to that distribution.[21,25]

In order to assign an individual to the Swiss reference population, the scoring for the Swiss population had to be superior to

Table 2. Number of observed alleles (A) and proportion of heterozygotes (Ho) in % for perch reference populations.

Reference population		Individual loci										Mean of all loci	
		Svi6		Svi17		Svi18		Svi26		Svi29			
	n	A	Ho	A	Ho	A	Ho	A	Ho	A	Ho	A	Ho
Switzerland	61	5	4.9	9	56.5	3	40.3	1	0	2	1.6	4.0	20.7
Russia	61	7	59.0	21	63.8	9	50.8	3	15.5	3	8.2	7.2	41.1
Estonia	40	6	55.0	14	52.8	8	72.5	5	20.0	3	5.0	8.6	39.5
Poland	19	5	52.6	14	94.7	7	80.0	3	50.0	1	0	6.0	55.5
Ireland	5	1	0	2	20.0	1	0	2	20.0	1	0	1.4	8.0
Sweden	5	4	60.0	7	100	5	80.0	1	0	1	0	3.6	48.0
Germany	2	2	50.0	2	50.0	3	50.0	2	50.0	1	0	2.0	40.0

Table 3. Number of private alleles (PA) for each of the reference population at each locus.

Private alleles (PA)	Svi6	Svi17	Svi18	Svi26	Svi29	For all loci
Switzerland	2	0	0	0	0	2
Russia	0	5	1	0	1	7
Estonia	0	3	1	1	0	5
Poland	0	3	1	0	0	4
Ireland	0	0	0	0	0	0
Sweden	0	3	0	0	0	3
Germany	0	0	0	0	0	0
Total	2	14	3	1	1	21

70% and the statistical probability $p > 0.05$. On the contrary, to exclude an individual from Swiss reference population, and consequently to assign the individual as foreigner, the scoring had to be inferior to 5% and the probability $p < 0.05$.

3.5 *Perch Genetic Authentication of Geographical Localization*

Using only three powerful microsatellite DNA markers, the Bayesian method revealed 99% of correct assignment or exclusion of Swiss or foreign fillets to the Swiss reference population, and the statistic probabilities criteria correctly assigned 98.5% of the perch population. Considered together, these two parameters correctly assigned or excluded 192 individual perch of a total of 195 (98.5%), regarding genetic authentication of geographical localization. A total of 60 perch among the 62 fillets originating from Switzerland were attributed to the Swiss reference population. However, for the two other fishes, one of the two parameters was not achieved. The statistical likelihood in order to be assigned in the Swiss population was inferior at 0.05 (respectively 0.0445 and 0.0086), consequently excluding these two fillets as originating from Swiss lakes, even though the scoring was superior to 70% for the Swiss population. However, they did not match the criteria for foreign assignment (scoring inferior to 5% and probability $p < 0.05$). The geographical origin result for these two fillets was set as undetermined. This case is probably not fraud, but it's rather explained by the uncertainty of the statistical method. In such a case during an official control, traceability of fish products would be checked by the fish supplier.

The third fish incorrectly assigned was a foreign fillet from Russia, for which scoring was superior to 90% for a Swiss origin assignation. Moreover, its probability of belonging to the Swiss population was also significant with $p = 0.2431$. Thus, declared geographical origin for this particular fish was considered as incorrect as attribution criteria demonstrated that it certainly originated from the Swiss lakes.

In conclusion, the results demonstrated a correct assignation or exclusion for more than 98% of individuals, which confirmed the robustness of microsatellites as genetic markers for individual assignment. Moreover, our results are completely in agreement with the percentages obtained for other traceability studies.[13,14]

4. Discussion

Recently, DNA microsatellite markers have been used more and more for the geographical traceability of food products.[12–15] Although these markers are accurate and precise, it is often difficult to identify correct peaks, because of PCR artifacts, like non-specific peaks in the vicinity of the main allele peak, or stutter bands and repeat slippage giving rise to extra amplification products that are one or more repeats larger or smaller than the authentic alleles.[29] However, most of the allelic profiles obtained in this study exhibited good peak resolutions and none of the stutter peaks impaired the recognition of the one or two alleles for each individual at each locus, allowing reliable interpretation of data.

In this study, we tested the power of five microsatellite loci in assigning individuals to their origin populations. While three markers exhibited a high degree of polymorphism and seemed powerful for genetic analysis, the two other loci were only poorly polymorphic and were subsequently not appropriate as genetic markers and therefore not used for further statistical analysis. Thus, using only three efficient markers, over 98% of a population of 195 perch individuals were correctly assigned or excluded from the Swiss reference population regarding their maximum likelihood scoring and statistical probabilities. These data are encouraging for routine future analysis of perch geographical authentication on Swiss market.

Recently, many microsatellites have been developed for fishes belonging to the Percidae family,[2,30–32] including *P. fluviatilis*. Characterizing new DNA markers for perch geographical origin authentication would most likely be advantageous and the use of data based on more than three microsatellite loci will probably improve the performance of assignments methods.

In conclusion, traceability is a critically important topic in ensuring food safety and improving consumer confidence in the food industry.[11,33] Geographical origin authentication through microsatellite DNA markers has already been successfully used in the livestock industry[34] and seafood industry.[18,35] but is in its early stages. In the future, methods for individual assignment

based on molecular markers will probably be standard tools in foodstuff traceability procedures and will find many applications for consumer protection.

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