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PCR-SSCP and Isoelectric Focusing as Screening Methods for Identification of (Sub-) Tropical Fish Species

PCR-SSCP und isoelektrische Fokussierung als Testverfahren zur Identifizierung von (sub-) tropischen Fischarten

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Abstract

To ensure the authentication of fishery products lacking biological characters, rapid species identification methods are required.

Two DNA- and protein-based methods, PCR-SSCP (polymerase chain reaction - single strand conformation polymorphism) of a 464 bp segment of the cytochrome b – gene and isoelectric focusing (IEF) of water-soluble proteins from fish fillets, were applied to identify fillets of (sub-) tropical fish species available on the European market. Among the samples analysed were two taxonomically identified species from the family Sciaenidae and one from Sphyraenidae.

By comparison of DNA- and protein patterns of different samples, information about intra-species variability of patterns, and homogeneity of batches (e.g. fillet blocks or bags) can be obtained. PCR-SSCP and IEF may be useful for pre-checking of a large number of samples by food control laboratories.

Keywords: fish species identification, PCR, SSCP, IEF, Sciaenidae, Sphyraenidae

Zusammenfassung

Zur Sicherstellung der Authentizität von Fischerei-Erzeugnissen ohne biologische Merkmale sind schnelle Verfahren zur Speziesidentifizierung hilfreich. Zwei Methoden der DNA- bzw. Protein-Analyse wurden eingesetzt, um Filets (sub-) tropischer Fischarten, die auf dem europäischen Markt angeboten werden, zu identifizieren. Bei diesen Methoden handelt es sich um die PCR-SSCP (Polymerase-Kettenreaktion – Einzelstrang-Konformationspolymorphismus) – Analyse der PCR-Produkte und die IEF (isoelektrische Fokussierung) der wasserlöslichen Fischmuskelproteine. Unter den untersuchten Proben waren zwei taxonomisch bestimmte Arten aus der Familie Sciaenidae und eine Spezies aus der Familie Sphyraenidae.

Durch Vergleich der DNA- bzw. Proteinmuster lassen sich Informationen über die intra-spezifische Variabilität solcher Muster und die Einheitlichkeit von Partien (beispielsweise Filetblöcke oder Filetbeutel) gewinnen. PCR-SSCP und IEF können in Laboratorien der Lebensmittelüberwachung als Vortest gerade bei hohen Probenzahlen sinnvoll eingesetzt werden.

Stichwörter: Fischarten-Identifizierung, PCR, SSCP, IEF, Sciaenidae, Sphyraenidae

Introduction

The global trade of fish and seafood is changing. Permanently new marine fish species from tropical and subtropical countries are no longer found only on their local markets, but are increasingly exported into the European Union and the USA. In Germany and other countries official lists of fish names to be used by industry and trade have been published, but labelling regulations are not unequivocal in every case.

Quite often more than one fish species can be sold under the same trade name; this can be the generic name of species e.g. *Lutjanus sp*. or a common name like snapper. In recent years, numerous cases of mislabelling of fish have been reported; among other reasons, insufficient supply and large differences of prices of fish are responsible for the development in unfair trade (Jacquet and Pauly, 2008).

Nowadays, the mostly applied method for fish species identification is sequencing of amplicons obtained by PCR of mitochondrial genes; PCR with specific primers and probes, SSCP and RFLP are used less frequently (Rasmussen and Morrissey, 2008). However, when it comes to the analysis of large sample numbers, SSCP may be a useful technique for pre-checking or even identification of fishery products. Also IEF of sarcoplasmic proteins, which has been widely replaced by DNA-based methods, is still in use for rapid comparison and identification of raw fish fillet (Schiefenhövel and Rehbein, 2013).

Aims of the present study were (i) the application of rapid low cost methods of protein- and DNA-electrophoresis, IEF and SSCP, for controlling incoming goods (fish industry) or compliance with labelling (food control), and (ii) submitting DNA sequences of tropical and subtropical food fish to GenBank.

This work describes results obtained for some commercially important tropical and subtropical fish species belonging to the families Sciaenidae, Sphyraenidae, Lutjanidae and other species like *Lepidocybium flavobrunneum*, *Coryphaena hippurus* and *Aluterus monoceros*. Previously, similar reports have been published for tilapia (*Oreochromis spp.*), barramundi (*Lates calcarifer*) and Sparidae species (Schiefenhövel and Rehbein, 2010; 2013).

Sciaenidae Fish species of the family Sciaenidae are common in the Atlantic, Pacific and Indian oceans (Fishbase, 2013). They are demersal carnivores, feeding on crustaceans, other benthic invertebrates and fish (Whitehead et al., 1986). Due to the fact, that they can produce sounds with muscles attached to the swim bladder, they are also called croakers or drums (Smith and Heemstra, 2003). There are 270 species in 70 genera in this family, among them there are species which are of high commercial importance. For South America it is most likely the Whitemouth croaker (*Micropogonias furnieri*) with a total annual production of 94,000 tons. In Asia, China is the main producer of the Yellow croakers (*Larimichthys polyactis*) with almost 400,000 tons and the African state Angola had a production with 17,000 tons of the species Southern meagre (*Argyrosomus hololepidotus*) in 2011 (FAO statistics, 2013). *Argyrosomus regius (meagre)* is mainly distributed in the Mediterranean and Atlantic coastal areas of Europe and Africa. In those areas it is a highly esteemed food fish with a total production volume of 5,000 tons in 2011 (FAO statistics, 2013).

Umbrina canariensis also called Canary drum is frequently found in the Bay of Biscay southward along the western African Atlantic coast and in the western Mediterranean area and only of local commercial importance (Whitehead et al., 1986).

Sphyraenidae Species of the family Sphyraenidae, genus Sphyraena (barracudas), are worldwide distributed in tropical and subtropical oceans. They are commercially fished especially in the Asian waters with about 80,000 tons in 2011, highest production was in Thailand (12,700 t) and India (12,400 t) (FAO statistics, 2013). Apart from the major fished species *Sphyraena barracuda* (Great barracuda), there are other species of the family Sphyraenidae of commercial interest: *Sphyraena obtusata* (Obtuse barracuda), *Sphyraena jello* (Pickhandle barracuda), *Sphyraena putnamae* (Sawtooth barracuda) and in Europe, fished by Italy and Spain, *Sphyraena sphyraena* (European barracuda) (FAO statistics, 2013).

Sphyraena putnamae is mainly distributed in the tropical and subtropical Indo-West Pacific, preferring bays and turbid waters (Carpenter and Niem, 2001a). It is described as valuable species for local fish markets (Mohammadizadeh et al., 2010). Recently, *S. putnamae* repeatedly appeared also on the European market. However, especially the larger Sphyraenidae species like the great barracuda, *Sphyraena barracuda*, should be avoided eating, as fillets could contain ciguatoxin (Carpenter and Niem, 2001a). Ciguatoxin, produced by dinoflagellates like *Gambierdiscus toxicus*, can accumulate in the fillet of tropical herbivore or carnivore fish species. Consumption of tropical top predators like Sphyraenidae species are at high risk causing ciguatera poisoning. There are reports were consumers of barracuda flesh or eggs showed typical symptoms of nausea, vomiting, diarrhea and neurologic abnormalities for a toxication by ciguatera (Hung et al., 2005).

Lutjanidae. Lutjanidae, also named snappers, are found worldwide in all tropical and subtropical oceans, where many of the adult snappers are mainly living demersal. They are mostly marine species, but some Indo-Pacific members and juveniles of several species enter estuaries and fresh water areas for living or just feeding. Almost all species feed on crustacean and fish; others are plankton-feeders (Smith and Heemstra, 2003; Carpenter and Niem, 2001b). Currently, about 103 species in 17 genera are recognized (Fishbase, 2013), whereas the genera Lutjanus includes the most species, in total 65 (Allen, 1985). Many Lutjanus species are slow-growing and have solitary life, and some

stocks are depleted due to overfishing (Huang et al., 1995).

For both commercial and game fishing, many snapper species are of high value, but they are also a major food source for the local population in their distributive range (Allen, 1985). In some fish species in certain areas accumulation of ciguatoxin is reported, for example in the species *Lutjanus bohar* (Two-spot red snapper) and *Lutjanus argentimaculatus* (Mangrove red snapper) (Carpenter and Niem, 2001b; Allen, 1985). With a total production of almost 120,000 tons, Indonesia reported the highest rate for *Lutjanus spp.* in 2011 (FAO statistics, 2013).

Misidentification of members of the genus Lutjanus often occurs due to morphological similarity and their natural ability to hybridize (Guo et al., 2007). Different countries are using Red snapper as a market name for different Lutjanus species, e.g. for *Lutjanus malabaricus* (in Germany) or *Lutjanus campechanus* (in U.S.A.). Mislabelling of Red snapper through other snapper species has occurred (Huang et al., 1995), and Red snapper and other snapper products are replaced by less favoured and cheaper species (Logan et al., 2008), e.g. fish belonging to the family Scorpaenidae (Huang et al., 1995).

Lepidocybium flavobrunneum, Coryphaena hippurus, Aluterus monoceros

The escolar *Lepidocybium flavobrunneum*, belonging to the family Gempylidae (snake mackerels), is distributed in temperate, tropical and subtropical waters (Fishbase, 2013). (Carpenter and Niem, 2001b; Smith and Heemstra, 1986). Escolar is sometimes also sold under the name "butterfish" or "oilfish" (Karl and Rehbein, 2004). These names probably derive from a high content of wax ester-rich fats (14-25 % of wet weight) in the flesh (Nichols et al., 2001). The high wax ester content (over 90 % of total lipids) arise from the diet, as the fish cannot metabolize the wax ester. Consumption of the flesh can lead to diarrhoea (Nichols et al., 2001; Smith and Heemstra, 1986).

Coryphaena hippurus, also called common dolphinfish or mahi mahi, is found offshore in the Atlantic, Indian and Pacific ocean, from tropical to subtropical waters. It is the only species of the family Coryphaenidae besides the species *Coryphaena equiselis* (Fishbase, 2013). *Coryphaena hippurus* is a very important game fish due to its length up to 2 m and high fighting ability. In some countries, like Mexico, it is even reserved by law for that branch of industry (Rocha-Olivares and Chávez-Gonzáles, 2008). Commercially fished with long lines or trolling as well as by artisanal and game fishing it reached a global production of 90,800 tons in 2011 (FAO species, 2013). *Coryphaena hippurus* is supposed to be a good candidate for aquaculture due to favourable properties of this species like fast growth, high fecundity and food conversion ratio (Fishbase, 2013). It can be easily adapted to culture condition (Lee, 1997; Kraul, 2007) and additionally has a high market price (Fishbase, 2013). Controlled feeding in aquaculture could probably solve the problem of ciguatera poisoning which is reported for that species in some areas where large algae blooms occurs (Fishbase, 2013).

The Unicorn leatherjacket, *Aluterus monoceros*, is favoured for its delicious and less bony fillet (Wu et al., 2008). This species belongs to the Filefishes (Monacanthidae) and inhabits tropical and subtropical oceans (Smith and Heemstra, 1986). China was the main producer of filefish species in 2011 with more than 200,000 tons (FAO statistics, 20013). For European countries it is less important, but also appears from time to time on the market. Wu et al. (2008) and Hsieh et al. (2010) reported that filefish species, and as a cheap substitute also sometimes nontoxic puffer fish species, are usually taken for production of dry dressed fish fillets, favourite in Taiwan, Japan and mainland China, but here the danger of confusion cannot be excluded, that also, with or without purpose, toxic puffer fish species could be used instead.

Material and Methods

Type and origin of fish samples

Fish samples were collected at research cruises of the MRI to the Bay of Biscay or purchased at retail shops or from wholesale traders in Northern Germany. Gutted fish was obtained deep frozen or fresh; fillets were received deep frozen with or without skin. Species from the families Sciaenidae, Sphyraenidae and Lutjanidae as well as several products of the species *Lepidocybium flavobrunneum*, *Coryphaena hippurus* and *Aluterus monoceros* were analysed. In Tab. 1a authenticated samples are listed, they were identified by visual inspection of the whole animal (Carpenter and Niem, 2001a, 2001b, 2002; Smith and Heemstra, 2003; Whitehead et al., 1986) and/or by comparing the sequence of a 464 bp cytochrome b gene sequence with sequences from GenBank using the BLAST tool. In Tab.1b analysed, but not taxonomically identified commercial samples are listed.

Analytical methods

DNA-based methods. A 464 bp segment of the mitochondrial cytochrome b gene was chosen for identification of fish species. Extraction of DNA, conditions of PCR, SSCP analysis, DNA sequencing and application of BLAST were performed as described recently (Schiefenhövel and Rehbein, 2013).

Table 1a: List of authentic samples.
Tabelle 1a: Liste der authentischen Proben.

Code	Origin	Product	Latin name	English name	Authentication
1	wholesale trader	Whole fish	*Argyrosomus regius* A	Meagre	BLAST: 100% id to DQ197924.1
2	wholesale trader, caught in Middle East Atlantic	Whole fish	*Argyrosomus regius* B	Meagre	vi*; BLAST: 100% id to DQ197924.1
3	wholesale trader, caught in Middle East Atlantic	Whole fish	*Argyrosomus regius* C	Meagre	vi
4	Research vessel, Bay of Biscay, wild catch	Whole fish	*Umbrina canariensis* A	Canary drum	vi; BLAST: 99% id to EF392638.1
5	Research vessel, Bay of Biscay, wild catch	Whole fish	*Umbrina canariensis* B	Canary drum	vi
6	Wholesale trader, wild catch, India	Whole fish	*Sphyraena putnamae*** A	Sawtooth barracuda	vi; BLAST: 90% id to Z70778.1 (*Sphyraena barracuda*)
7	Wholesale trader, wild catch, India	Whole fish	*Sphyraena putnamae*** B	Sawtooth barracuda	vi
8	Wholesale trader, wild catch, India	Whole fish	*Sphyraena putnamae*** C	Sawtooth barracuda	vi
9	Wholesale trader, caught in Pacific Ocean	Whole fish	*Lepidocybium flavobrunneum*	Escolar	BLAST: 100% id to AM265576.1
10	Wholesale trader, caught in Pacific Ocean	Whole fish	*Coryphaena hippurus*	Common dolphinfish	BLAST: 100% id to EF439196.1
11	Wholesale trader, caught in Pacific Ocean	Fllet	*Aluterus monoceros*	Unicorn leatherjacket	BLAST: 100% id to EU216740.1

(*vi = visual inspection of a whole animal; **visual identified species: not in Genbank)

Sequences of the cytochrome b gene of some authentic fish samples were submitted to GenBank: *Argyrosomus regius* (Accession-No. HM352755), *Umbrina canariensis* (Acc.-No. HM352761), *Sphyraena putnamae* (Acc.-No. HM352756).

Protein-based methods. Water-soluble proteins were extracted from white muscle of fish, and conditions of IEF were the same as described by Schiefenhövel and Rehbein (2013). Protein bands were visualized with Coomassie staining. The different pI values of fish proteins were calculated against a standard protein mixture (Broad pI Kit 3.5-9.3, GE Healthcare; Serva IEF Marker 3.5-10.7, SERVA) by means of the computer program Quantity One (version 4.6.8, Bio-Rad).

Results and Discussion

DNA analysis. A 464 bp cytochrome b gene segment was amplified by PCR and yielded an amplicon for all species studied. By sequencing the amplicons and application of BLAST, a number of samples could be authenticated (see Table 1: sample 1 and 2: A. *regius*, sample 4: *U. canariensis*, sample 9: *L. flavobrunneum*, sample 10: *C. hippurus*, sample 11 and 20c: *A. monoceros*. Together with the fish identified by biological characters, these samples served as references for SSCP analysis.

In SSCP analysis three to four strong bands and some weaker bands of single-stranded DNA (ssDNA) were received for every species (Figure 1 and 2). In general, the SSCP method showed that examined fish species had different patterns of ssDNA. Intra-species variability was only observed in case of *Aluterus monoceros* (sample 20c).

Sciaenidae. The three samples (A-C) of the species *Agyrosomus regius* A, B and C, gave the same pattern of ssDNA bands (Figure 1).

Sphyraenidae. Although the SSCP analysis displayed very slight differences in the position of DNA bands between *Sphyraena putnamae* products A, B and C

Table 1b: List of other commercial samples (species not identified) from the German market.
Tabelle 1b: Liste nicht authentifizierter Handelserzeugnisse des deutschen Marktes.

Code	Origin	Product	Labelling (Latin name)	English name	Result of BLAST
12	Wholesale trader	Whole fish	*Sphyraena sp.*	Barracuda	BLAST: 90% id to Z70778.1 (*Sphyraena barracuda*)
13	Wholesale trader	Whole fish	*Sphyraena sp.*	Barracuda	BLAST: 90% id to AY115984.1 (*Sphyraena barracuda*)
14	Wholesale trader	Fillet	*Sphyraena sp.*	Barracuda	n.d.
15a	Wholesale trader, caught in West Central Pacific	Fillet	*Lutjanus malabaricus*	Malabar blood snapper	BLAST: 99% id to AP004431.1 (*Ostichthys japonicus*)
15b	Wholesale trader, caught in West Central Pacific	Fillet	*Lutjanus malabaricus*	Malabar blood snapper	n.d.
15c	Wholesale trader, caught in West Central Pacific	Fillet	*Lutjanus malabaricus*	Malabar blood snapper	n.d.
15d	Wholesale trader, caught in West Central Pacific	Fillet	*Lutjanus malabaricus*	Malabar blood snapper	n.d.
16a	Wholesale trader, caught in Pacific Ocean	Fillet	*Lutjanus sp.*	Snapper	BLAST: 89% id to FJ416614.1 (*Lutjanus kasmira*)
16b	Wholesale trader, caught in Pacific Ocean	Fillet	*Lutjanus sp.*	Snapper	n.d.
16c	Wholesale trader, caught in Pacific Ocean	Fillet	*Lutjanus sp.*	Snapper	n.d.
16d	Wholesale trader, caught in Pacific Ocean	Fillet	*Lutjanus sp.*	Snapper	n.d.
16e	Wholesale trader, caught in Pacific Ocean	Fillet	*Lutjanus sp.*	Snapper	n.d.
16f	Wholesale trader, caught in Pacific Ocean	Fillet	*Lutjanus sp.*	Snapper	n.d.
17a	Wholesale trader, caught in Pacific Ocean	Fillet	*Lepidocybium flavobrunneum*	Escolar	n.d.
17b	Wholesale trader, caught in Pacific Ocean	Fillet	*Lepidocybium flavobrunneum*	Escolar	n.d.
17c	Wholesale trader, caught in Pacific Ocean	Fillet	*Lepidocybium flavobrunneum*	Escolar	n.d.
17d	Wholesale trader, caught in Pacific Ocean	Fillet	*Lepidocybium flavobrunneum*	Escolar	n.d.
18a	Wholesale trader, caught in Pacific Ocean	Fillet	*Coryphaena hippurus*	Common dolphinfish	n.d.
18b	Wholesale trader, caught in Pacific Ocean	Fillet	*Coryphaena hippurus*	Common dolphinfish	n.d.
18c	Wholesale trader, c aught in Pacific Ocean	Fillet	*Coryphaena hippurus*	Common dolphinfish	n.d.
18d	Wholesale trader, caught in Pacific Ocean	Fillet	*Coryphaena hippurus*	Common dolphinfish	n.d.
18e	Wholesale trader, caught in Pacific Ocean	Fillet	*Coryphaena hippurus*	Common dolphinfish	n.d.
19	Wholesale trader	Whole fish	*Coryphaena sp.*		n.d.
20a	Wholesale trader, caught in Pacific Ocean	Fillet	*Aluterus monoceros*	Unicorn leatherjacket	n.d.
20b	Wholesale trader, caught in Pacific Ocean	Fillet	*Aluterus monoceros*	Unicorn leatherjacket	n.d.
20c	Wholesale trader, caught in Pacific Ocean	Fillet	*Aluterus monoceros*	Unicorn leatherjacket	BLAST: 99% id to EU216740.1

(n.d. = not determined)

Table 2: List of pI-values of samples shown in IEF analysis
Tabelle 2: Liste der pI-Werte für Proben, die mittels IEF untersucht wurden.

code	Species (Latin)	pI-values: < 6	6 - 5	> 5	pI-values (heated and dialysed)
1	*Argyrosomus regius* A	7.48 6.57	5.68	4.27	4.14
2	*Argyrosomus regius* B	7.54 6.55 6.38	5.61 5.53 5.45	4.28	5.48 4.20
3	*Argyrosomus regius* C	7.52 6.56 6.37	5.62 5.53 5.45	4.28	4.19
4	*Umbrina canariensis* A	6.94 6.55 6.40	5.59 5.51 5.28	4.45 3.88 3.80	4.45 3.93 3.82 3.74
5	*Umbrina canariensis* B	6.95 6.55 6.39	5.58 5.52 5.28	4.46 3.88 3.80	4.46 3.93 3.82 3.73
12	Sample 12	7.46 6.84 6.48 6.43 6.39 6.32 6.22		4.84 4.50 4.37	4.91 4.51 4.34
13	Sample 13	7.45 6.84 6.39 6.33 6.22		4.49 4.40 4.37	4.51 4.36 4.34
14	Sample 14	7.41 6.83 6.48 6.44 6.40 6.33 6.23		4.48 4.39	4.52 4.37
6	*Sphyraena putnamae* A	7.43 6.85 6.49 6.44 6.39 6.33 6.22		4.84 4.49 4.40	4.89 4.51 4.36
7	*Sphyraena putnamae* B	7.45 6.85 6.48 6.43 6.39 6.33 6.23		4.86 4.50	4.91 4.52
8	*Sphyraena putnamae* C	7.43 6.85 6.49 6.44 6.40 6.34 6.23		4.47 4.38 4.36	4.51 4.37 4.34
15a	Sample 15a	7.04	5.83 5.74 5.47	4.71 4.11 3.75	
16a	Sample 16a	7.11 6.81	5.97 5.84 5.78 5.42	4.65 4.38	
9	*Lepidocybium flavobrunneum*	7.22 6.83 6.77 6.57 6.42 6.26			
10	*Coryphaena hippurus*	7.22 7.06 6.69 6.64	5.87 5.44 5.38 5.25		
19	Sample 37	7.22 7.11 6.67 6.62	5.40 5.36 5.23		
11	*Aluterus monoceros*	6.82 6.34 6.17 5.98		5.01 4.79	
20c	Sample 20c	6.81 6.34 6.17 6.00		5.01 4.80	

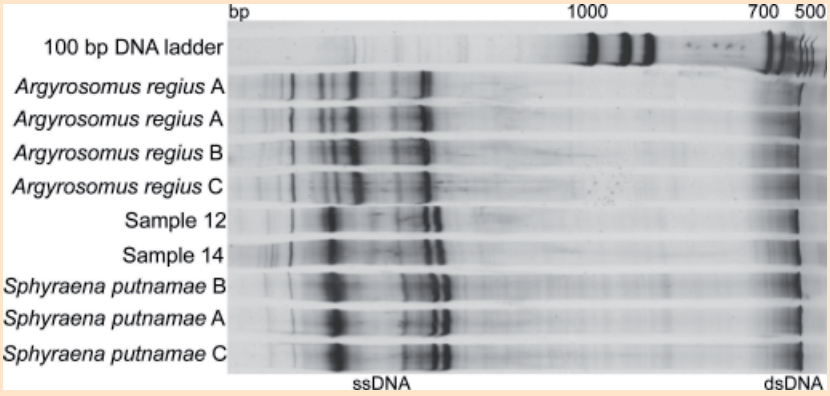

Figure 1: PCR-SSCP of different products of Argyrosomus regius, Sphyraena putnamae and not identified commercial fish samples. CleanGel 10%, anode is on the right side.
Abbildung 1: PCR-SSCP verschiedener Erzeugnisse aus *Argyrosomus regius*, *Sphyraena putnamae* und nicht identifizierter Handelsprodukte. CleanGel 10%, die Anode befindet sich rechts.

and samples 12 and 14, labelled as Sphyraena sp. (Figure 1), the sequences of *S. putnamae* A and sample 12 were identical (data not shown). Possbibly the small shift in bands positions was caused by inhomogenities in the electrophoresis gel.

Lutjanidae, *Lepidocybium flavobrunneum*, *Coryphaena hippurus* and *Aluterus monoceros*. Several commercial products of snapper consisting of four to six fillets each, labelled as *Lutjanus malabaricus* (sample 15a-d) or *Lutjanus sp.* (sample 16a-f) ex-

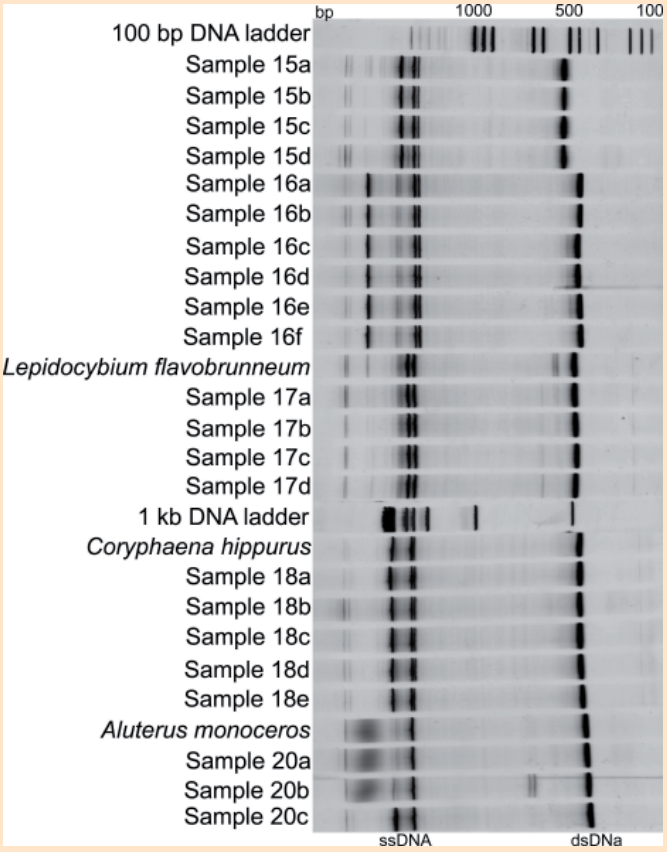

Figure 2: PCR-SSCP of different products of Lepidocybium flavobrunneum, Coryphaena hippurus, Aluterus monoceros and not identified commercial fish samples. CleanGel DNA-HP 10%, anode is on the right side.
Abbildung 2: PCR-SSCP verschiedener Erzeugnisse aus *Lepidocybium flavobrunneum Coryphaena hippurus, Aluterus monoceros* und nicht identifizierter Handelsprodukte. CleanGel DNA-HP 10%, die Anode befindet sich rechts.

pressed identical patterns within each batch (Figure 2), but a different pattern of ssDNA between the two products of snapper. According to the result of sequencing and BLAST, the sample 15a, labelled as *L. malabaricus*, was not a snapper but consisted of the species *Ostichthys japonicus* with high probability. *O. japonicus* (Japanese soldierfish) is living in the Indo-West Pacific south off Japan; the genus *Ostichthys comprises* more than 20 species (FishBase, 2013), which are not related to the Lutjanidae.

It is also very questionable whether the fillets declared to be *Lutjanus* sp. (sample 16a-f) were snappers at all, because of the low score of identity to *Lutjanus kasmira* obtained by BLAST (Table 2, sample16a).

L. flavobrunneum (sample 17a-d), *C. hippurus* (sample 18a-d) and *A. monoceros* (sample 20a-c), were analysed by SSCP and compared to authenticated samples of *L. flavobrunneum* (sample 9), *C. hippurus* (sample 10) and *A. monoceros* (sample 11) (Figure 2).

In case of products from *L. flavobrunneun* and *C. hippurus* correct labelling was confirmed by SSCP analysis of all commercial samples of those species by comparing the ssDNA patterns of samples and references.

The ssDNA patterns of *A. monoceros* was the same as for sample 20a and b, but different to the pattern of 20c; close inspection of the pattern obtained for *A. monoceros* and samples 20a and b showed a diffuse band in the cathodic part of the gel, which might have been focussed in case of sample 20c.

Protein analysis. The protein patterns of different samples and references obtained by IEF are shown in Figure 3 and 4. They proved to be different between species, and with the exception of *Sphyraena putnamae*, invariable within species. The pI-values of different species are listed in Table 2.

Furthermore, IEF analysis presented the same protein pattern for *A. monoceros* reference and sample 20c (Fig. 4). In general, the protein pattern of *A. monoceros*, *C. hippurus* and *L. flavobrunneum* were devoid of strong acidic protein bands (Figure 4).

The two different batches of fillet samples of Lutjanus displayed different protein profiles, but as long as there

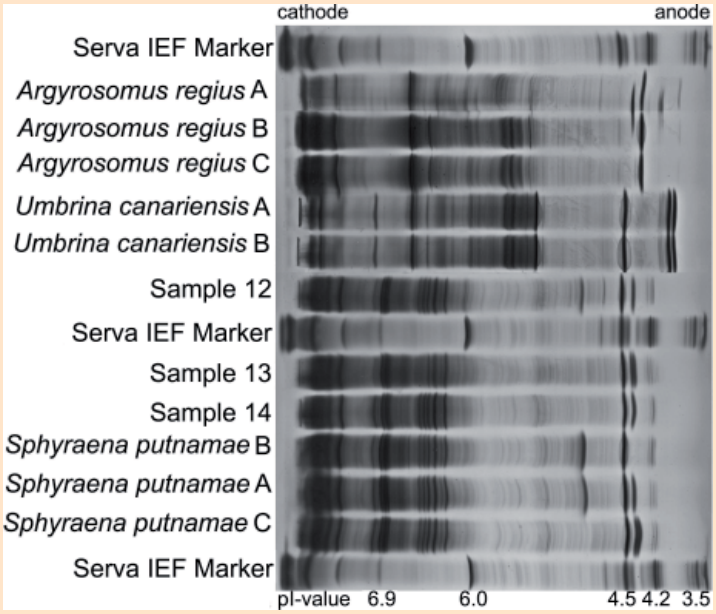

Figure 3: IEF analysis of different products of croakers and barracudas. Servalyt-Precote 3-10, anode is on the right side.
Abbildung 3: IEF verschiedener Erzeugnisse aus Trommlern und Barrakudas. Servalyt-Precote 3-10, die Anode befindet sich rechts.

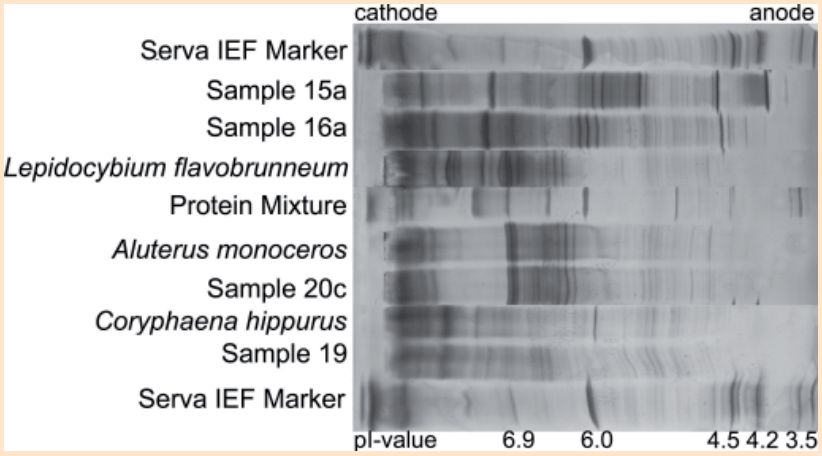

Figure 4: IEF analysis of different commercial fish products. Servalyt-Precote 3-10, anode is on the right side.
Abbildung 4: IEF verschiedener Fischerei-Erzeugnisse aus dem Handel. Servalyt-Precote 3-10, die Anode befindet sich rechts.

is no reference available, either on DNA (sequence deposited in GenBank) or on protein basis, samples 16a-f (labelled as *Lutjanus sp.*) remain unidentified. Huang et al. (1995) have reported about substitutions of snapper species by Pacific coast rockfish from the family Scorpaenidae; about 58 % of mislabelled Red snapper products have occurred.

4 Conclusions

To identify species in seafood products, IEF of sarcoplasmic proteins and SSCP of PCR-products, are successful and rapid differentiation and identification methods. Genetically related fish species can be differentiated from each other through their unique protein and SSCP profiles, and DNA sequences. But both methods could possess intra-species variability in SSCP and protein patterns. To be aware of intra-specific variants in species, a significant number of authentic specimens from different stocks and origin have to be tested (Mackie et al., 1999). Additionally, results of both methods should be confirmed by DNA sequencing.

Although most of the species actually did not show heterogeneity in protein patterns, in some species, e.g. *Sphyraena putnamae*, minor variations of protein bands appeared, probably due to different regional catch areas (Altinelataman et al., 2009).

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