



DNA barcoding reveals a high incidence of fish species misrepresentation and substitution on the South African market

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ARTICLE INFO

Article history:

Received 11 April 2011

Accepted 8 November 2011

Keywords:

DNA barcoding

Fish species

Identification

Mislabelling

Species authentication

ABSTRACT

The mislabelling of fishery products has emerged as a serious problem on global markets, raising the need for the development of analytical tools for species authentication. DNA barcoding, based on the sequencing of a standardised region of the cytochrome c oxidase I (COI) gene, has received considerable attention as an accurate and broadly applicable tool for animal species identifications. The aim of this study was to investigate the utility of DNA barcoding for the identification of a variety of commercial fish in South Africa and, in so doing, to estimate the prevalence of species substitution and fraud prevailing on this market. A *ca.* 650 base pair (bp) region of the COI gene was sequenced from 248 fish samples collected from seafood wholesalers and retail outlets in South Africa, following which species identifications were made in the Barcode of Life Database (BOLD) and in GenBank. DNA barcoding was able to provide unambiguous species-level identifications for 235 of 248 (95%) samples analysed. Overall, 10 of 108 (9%) samples from wholesalers and 43 of 140 (31%) from retailers were identified as different species to the ones indicated at the point of sale. Although some cases of mislabelling were potentially unintentional due to misapplied market nomenclature, a far greater proportion represented serious and seemingly deliberate acts of fraud for the sake of increased profits. This study has highlighted that the existing legislation pertaining to seafood marketing in South Africa is inadequate or poorly enforced and requires urgent revision. In the light of the results presented here, DNA barcoding appears to hold great potential for fish authentication monitoring by both regulatory bodies and industry, the utilisation of which could enhance transparency and fair trade on the domestic fisheries market.

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1. Introduction

The world's marine fish stocks, which were considered just over a century ago to be 'inexhaustible' (Huxley, 2007), now face extreme fishing pressure as the insatiable human appetite for seafood continually outpaces supply (Delgado, Wada, Rosegrant, Meijer, & Ahmed, 2003). Current data indicate that widespread overfishing has fully exploited, over-exploited or depleted up to 75% of global fish stocks (FAO, 2009) and has had deleterious effects on aquatic ecosystems (Pauly, Watson, & Alder, 2005; Worm, Barbier, & Beaumont, 2006). In a pertinent four-year study on 10 large marine ecosystems around the world, Worm, Hilborn, Baum, et al. (2009) reported that 63% of the assessed fish stocks were below desired levels and still require rebuilding, in spite of the numerous restrictions (annual harvest quotas, rights allocations, fishing gear modifications and seasonal or area closures) that have been imposed to promote more sustainable fisheries

management (Beddington, Agnew, & Clark, 2007; Brunner, Jones, Friel, & Bartley, 2009).

During the last two decades, there has been a growing realisation that the incorporation of consumer behaviour into marine conservation strategies will be required if the trends in fisheries declines are to be reversed (Kaiser & Edward-Jones, 2006). This realisation has led to a number of sustainable seafood awareness campaigns being initiated in many parts of the world, including the United Kingdom (UK), United States (US), Australia and Canada. The Southern African Sustainable Seafood Initiative (SASSI) was established in 2004 with similar aims of educating the local population on marine conservation issues and shifting consumer choices towards more sustainable seafood species. Typically, such organisations compile seafood lists that rank species according to sustainability criteria (e.g. 'best choice' or 'avoid'), the details of which are publicly disseminated via wallet cards, electronic databases and mobile phone applications (Roheim & Sutinen, 2006). A fundamental requirement for the success of all consumer awareness campaigns, as well as for fisheries management in general, is the accurate naming and labelling of fish products at the point of sale. Unfortunately, with escalating demand and globalisation of seafood trade, the current market climate in many countries

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is highly conducive to fraud and mislabelling of fish products (Jacquet & Pauly, 2007).

The mislabelling of fish species can manifest in several forms, as well as at any stage in the fisheries supply chain (Logan, Alter, Haupt, Tomalty, & Palumbi, 2008). A portion of the mislabelling that occurs is likely unintentional as fish species identities may be easily mistaken. Confusion may also arise due to the fact that different fish species can be referred to by a common vernacular name, or by different vernacular names in different regions (Buck, 2009). Of greater concern, however, is that some unscrupulous traders may deliberately use mislabelling as a means to launder illegally-caught fish into legitimate markets, or simply to defraud consumers for the purpose of accruing greater profits (Ogden, 2008). Since the flesh of many fish species is similar in appearance, taste and texture, it becomes relatively easy for species of high commercial value to be substituted, either partially or entirely, with species of lower value. The lack of traceability in the fisheries supply chain also provides a considerable opportunity for mislabelling. Fish products often change hands several times on route from the fishing vessels to the consumer's plate, making it difficult to identify the link in the supply chain where the fraud or substitution occurred (Thompson, Sylvia, & Morrissey, 2005).

Whether accidental or deliberate, fish mislabelling is not only a form of economic deception, but it also undermines the efforts of seafood awareness campaigns and can further erode already threatened fisheries (Jacquet & Pauly, 2007). For instance, 77% of the fish labelled as 'red snapper' in the US have been found to be substituted with less expensive and/or overexploited species (Marko, Lee, Rice, et al., 2004). In South Africa, shortfin mako shark has been sold as 'ocean fillets' or 'sokomoro' to increase its appeal (Atkins, 2010), even though it is listed as 'vulnerable' by the International Union for Conservation of Nature (IUCN, 2010). Furthermore, just as ichthyologic name-swapping can prevent consumers from making choices in favour of conservation, it also infringes on their right to safeguard their own health. Certain fish species can cause fatal allergic reactions (Triantafyllidis, Karaïskou, Perez, et al., 2010), while others contain potent toxins or high levels of contaminants. Reports have emerged on the mislabelling of pufferfish and oilfish as 'monkfish' and 'cod', respectively, where both cases have caused serious illness (Cohen, Deeds, Wong, et al., 2009; Lam, 2007).

Government regulations in many countries, including South Africa, require the full disclosure of food product content and stipulate that food labelling must not be misleading (DoH (Department of Health, South Africa), 2010; Martinez, James, & Loréal, 2005; NRCS, 2003). Nevertheless, such provisions have done little to deter mislabelling as they are often poorly enforced, or because the penalties for non-compliance are small in comparison to the profits resulting from fraudulent fish trading (Buck, 2009). There is now mounting evidence that molecular species identification methods, particularly those based on DNA analysis, can serve as critical tools for industry self-regulation, governmental monitoring and prosecution of illegal activities (Ogden, 2008). In particular, DNA barcoding – the sequencing of an approximately 650 base pair (bp) region of the cytochrome c oxidase I (COI) gene – has gained widespread support in the scientific literature as a rapid, cost effective and standardised method for the identification of a diverse range of animal lineages, including fish species (Hebert, Cywinska, Ball, & deWaard, 2003a; Hebert, Ratnasingham, & deWaard, 2003b; Ward, Zemlak, Innes, Last, & Hebert, 2005). This mitochondrial DNA (mtDNA) locus has been validated as a diagnostic marker for forensic identification applications (Dawnay, Ogden, McEwing, Carvalho, & Thorpe, 2007). In addition, COI barcoding is under consideration by the United States Food and Drug Administration (FDA) for uptake into their current regulatory framework and to serve as a replacement for the technique of protein isoelectric focusing for fish species identification (Handy, Deeds, Ivanova, et al., 2011; Ward, Hanner, & Herbert, 2009; Yancy, Zemlak, Mason, et al., 2008). Adoption of the COI gene for DNA barcoding purposes by the Consortium for the Barcode of

Life (CBOL) has led to the initiation of a number of international collaborative research efforts, including the Fish Barcode of Life Initiative (FISH-BOL), which aims to barcode all fish species of the world (Steinke & Hanner, 2011; Swartz, Mwale, & Hanner, 2008; Ward et al., 2009).

DNA barcoding has been utilised to evaluate the incidence of fish species substitutions in North America (Wong & Hanner, 2008), Europe (Miller & Mariani, 2010) and Italy (Barbuto, Galimberti, Ferri, et al., 2010; Filonzi, Chiesa, Vaghi, & Nonnis Marzano, 2010). However, to date, there have been no published reports on the use of this method to estimate the prevalence of such substitutions in South Africa. Considering that South Africa plays a leading role on the African continent in terms of both fish production and trade (INFOSA, 2007), such an evaluation is imperative to determine the incidence of mislabelling that could perpetuate locally or in exported commodities. The aim of this study was to investigate the utility of DNA barcoding for the identification of a large variety of fish products commercially traded at the wholesale and retail levels in South Africa, and in so doing, to assess the extent of misrepresentation and substitution occurring on this market.

2. Materials and methods

2.1. Sample collection

Fish samples were collected over a two-year period (2008–2010) in four provinces of South Africa, namely the Western Cape (WC), Eastern Cape (EC), KwaZulu-Natal (KZN) and Gauteng (GP). The former three provinces are the major coastal fishing provinces in South Africa and were included as these were expected to have access to a large variety of locally-caught fish species. Gauteng (GP) was included in order to evaluate the commercial fish trading practices in an inland province, principally because it is the most populated province in South Africa with the highest per capita income (Schlemmer, 1998). A total of 257 samples were collected, of which 108 (42%) were obtained from the wholesaler/distributor level, while 149 (58%) were obtained from retail outlets, which included both supermarkets and fish markets. Supermarkets were defined as those stores that sold fish and various other grocery items, while fish markets were defined as those stores selling primarily seafood commodities. All samples collected from wholesalers/distributors were purchased frozen, but these included both whole and processed specimens. Fresh, frozen, whole and processed fish samples were acquired from the retail outlets. All samples were stored in a laboratory freezer (−20 °C) following collection.

2.2. DNA extraction

Tissue was excised from the lateral muscle of each fish specimen with a sterile scalpel and forceps. Total genomic DNA was extracted from ca. 500 mg of the muscle tissue using the SureFood® PREP Allergen Kit (r-Biopharm, supplied by AEC-Amersham, Cape Town, South Africa), following the manufacturer's instructions. The concentrations and purities of the extracted DNA were assessed in a spectrophotometer (Beckman Coulter DU530, Beckman Instruments, Fullerton, USA) at 260 and 280 nm. DNA extracts were stored at −20 °C prior to further analysis.

2.3. Polymerase chain reaction (PCR)

A 652 base pair (bp) fragment from the 5' region of the COI gene was PCR amplified using the M13-tailed primer cocktail (C_FishF1t1/C_FishR1t1) previously described for the DNA barcoding of fish species (Ivanova, Zemlak, Hanner, & Hebert, 2007). The 25 µl PCR reaction mixtures contained 2.5 µl (1 ×) reaction buffer (MgCl₂ free) (Super-Therm, supplied by Southern Cross Biotechnologies, Cape Town, South Africa), 2.5 µl (2.5 mM) MgCl₂ (25 mM, Super-Therm), 0.25 µl (100 nM) of each primer (10 µM stocks), 0.125 µl (0.625 U) *Taq* DNA polymerase

(5 U/μl, Super-Therm), 0.5 μl (0.2 mM) of mixed dNTPs (10 mM, AB gene, supplied by Southern Cross Biotechnologies) and 2 μl (ca. 2 μg) of DNA template. PCR amplifications were performed in a Mastercycler Personal (Eppendorf, Germany) utilising the following thermal cycling parameters: initial denaturation at 94 °C for 2 min, 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 52 °C for 40 s and chain elongation at 72 °C for 60 s, followed by final extension at 72 °C for 10 min. The success of amplification was assessed by electrophoresis (90 V, 45 min) of the PCR products in 1.5% (m/v) agarose (Sigma-Aldrich, Gauteng, South Africa) gels, with subsequent visualisation under an ultraviolet light (Vilber Lourmat, Marne La Vallée, France).

In the few cases where COI barcoding did not deliver unequivocal species resolutions for certain specimens, a ca. 450 bp fragment of the faster-evolving mtDNA control region (d-loop) was amplified for confirmatory purposes according to Alvarado Bremer (1994), since this region contains hypervariable sequences which are reportedly useful for analysing the nucleotide variability in both populations and closely-related species (Chow, Okamoto, Uozumi, Takeuchi, & Takeyama, 1997; Donaldson & Wilson, 1999; Faber & Stepien, 1997; Jie, Fenghua, & Houcai, 2011). The reaction mixtures for the control region PCR (25 μl final volume) comprised 2.5 μl (1×) reaction buffer (MgCl₂ free) (Super-Therm), 2.0 μl (2.0 mM) MgCl₂ (25 mM, Super-Therm), 1.0 μl (400 nM) of each primer (10 μM stocks), 0.10 μl (0.50 U) *Taq* DNA polymerase (5 U/μl, Super-Therm), 2.0 μl (0.8 mM) of mixed dNTPs (10 mM, AB gene) and 1 μl (ca. 1 μg of DNA template). PCR cycling conditions included an initial denaturation step at 94 °C for 5 min, 35 cycles of 94 °C for 45 s, 54 °C for 45 s, and 72 °C for 60 s, followed by a final extension step of 72 °C for 10 min.

2.4. Sequencing and sequence analysis

PCR amplification products were purified with the NucleoFast 96 PCR Clean-up Kit (Macherey-Nagel, supplied by Separations, Gauteng, South Africa) following the instructions of the manufacturer. Sequencing of the purified PCR products was performed using BigDye chemistry and analysis on an ABI 3100 Genetic Analyser (Applied Biosystems, Foster City, USA). The primers for M13-tailed PCR products described by Messing (1983) were used for the sequencing of the COI amplicons, while the PCR amplification primers were used as sequencing primers for the control region amplicons. Sequences were aligned and manually edited using BioEdit sequence alignment editor, version 7.0.9.0 (Hall, 1999). The generated COI and control region sequences were identified in GenBank (www.ncbi.nlm.nih.gov) using the BLASTn search tool. Identification results for the COI sequences were cross-referenced within BOLD (www.barcodinglife.org) (Ratnasingham & Hebert, 2007). As a general rule, a top match with a sequence similarity of at least 98% was used as a criterion to designate potential species identifications (Barbuto et al., 2010). Species identifications made through GenBank and BOLD were compared to the market names and species names (when available) under which the queried samples had been sold. Since the COI and control region sequences generated in this study were not derived from voucher samples or expertly-identified fish specimens, these sequences were not submitted to either GenBank or BOLD.

2.5. Evaluation of species authenticity and mislabelling

For the evaluation of the accuracy of fish species labelling, a multiple-step protocol was employed to ensure that all samples were consistently evaluated in a rigid and literal manner. Since both GenBank and BOLD databases rely on FishBase (<http://www.fishbase.org>) as a taxonomic authority for valid fish species names (Froese & Pauly, 2011; Wong & Hanner, 2008), top species matches (highest percentages of similarity) for each specimen were compared with the species and corresponding common names within the FishBase database. In cases where inconsistencies were found between the market names of

the queried samples and the currently accepted fish names in FishBase, species and common names were cross-checked in van der Elst (1997), Smith, Smith, and Heemstra (2003) and the SASSI database (www.wwfasss.co.za). The three latter sources were used in the absence of authoritative lists in South Africa containing acceptable market names for seafood species sold on the local market (such as those available in the US Food and Drug Administration (FDA) list, www.fda.gov).

3. Results and discussion

The DNA extracted from 248 of the 257 (96%) collected market samples was successfully amplified with the COI primer cocktail. From these, the resulting PCR products were sequenced to obtain full length DNA barcodes averaging 650 bp in length. No insertions, deletions or stop codons were observed in any of the COI sequences, consistent with all amplified sequences being functional mitochondrial COI sequences. Additionally, the fact that all COI sequences exceeded 600 bp in length suggests that nuclear DNA sequences originating from mtDNA (NUMTs) were not sequenced (vertebrate NUMTs are generally smaller than 600 bp) (Zhang & Hewitt, 1996).

Nine samples, constituting canned products labelled as 'tuna chunks', 'pink salmon' and 'mackerel', did not amplify with the COI cocktail and were therefore not included in further analyses. This amplification failure was not anticipated to have been due to incompatible PCR primers, as barcode sequences for all of the nominal species comprising these samples have been successfully amplified in the past and are available in BOLD and GenBank (Rasmussen, Morrissey, & Hebert, 2009; Ward et al., 2005). Rather, it is well established that extensive processing conditions, such as the thermal treatments used in canning operations, can lead to the degradation of DNA into fragments smaller than 200 bp (Pardo & Pérez-Villareal, 2004; Quinteiro, Sotelo, Rehbein, et al., 1998; Ram, Ram, & Baidoun, 1996). Consequently, poor DNA quality in these nine samples is the most likely explanation for the failure in PCR amplification. Universal primers targeting shorter fragments of the COI gene (described as 'mini barcodes') have been reported for the identification of specimens whose DNA is expected to be degraded (Hajibabaei, Smith, Janzen, et al., 2006; Meusnier, Singer, Landry, et al., 2008). Such methods may be more suitable for the authentication of highly processed fish samples, such as those that failed to amplify in this study using the conventional COI primer cocktail for fish DNA barcoding.

For all 248 generated COI sequences, maximum species identities in the range of 98–100% were obtained in GenBank and/or BOLD. For two specimens identified in BOLD as *Coryphaena equiselis* and *Etelis coruscans* (Table 1), corresponding COI sequences were not available in GenBank to permit species identifications. Additionally, in the large majority of cases where sequence similarities below 100% were attained, BOLD yielded superior species resolutions in comparison to those achieved in GenBank. Although barcode data from BOLD and GenBank are continuously exchanged, a possible reason for this finding is that the BOLD database currently contains a wider representation of COI sequences with which unknown specimens can be compared (Wong & Hanner, 2008). As of March 2011, over one million barcode sequences were contained within BOLD, while a search for available COI sequences in GenBank returned about half this number. DNA barcoding permitted explicit species resolution for 235 of the 248 (95%) sequenced samples. Only *Helicolenus dactylopterus* and two species of the genus *Thunnus* showed overlapping COI barcodes with congeneric species and could not be unequivocally differentiated on this basis. The non-coding mtDNA control region was recognized as the most promising marker for the confirmatory identification of these closely-related fish species since it evolves more rapidly than the coding regions of the mtDNA (such as the COI and cytochrome *b* genes) and it is reported to be the most variable segment in the mitochondrial genome (Brown, Bechenbach, & Smith, 1993; Chauhan & Rajiv, 2010; Jie et al., 2011; Zhao, Wang, Li, & Cai,

Table 1

Identification results based on cytochrome c oxidase I (COI) and control region (CR) sequencing for 108 fish samples from fish wholesalers and distributors in South Africa, where N indicates the number of fish samples that were correctly labelled or potentially mislabelled. Cases of suspected mislabelling are indicated in bold typescript, while GenBank Accession numbers in italics indicate sequences derived from fish specimens caught in South African waters.

Fish marketed as: (expected species)	N	Process state	Gene target	BOLD		GenBank		
				Species identification	Similarity (%)	Species identification	Similarity (%)	Accession Number
Angelfish (<i>Brama brama</i>)	3	Filleted	COI	<i>Brama brama</i>	100	<i>Brama brama</i>	100	EF609300
Alaskan salmon (<i>Oncorhynchus keta</i>)	2	Filleted	COI	<i>Oncorhynchus keta</i>	100	<i>Oncorhynchus keta</i>	100	HQ611085
Atlantic salmon (<i>Salmo salar</i>)	5	Filleted	COI	<i>Salmo salar</i>	100	<i>Salmo salar</i>	100	AF133701
Barramundi (<i>Lates calcarifer</i>)	5	Filleted	COI	<i>Seriola brama</i> (common warehou)	100	<i>Seriola brama</i> (common warehou)	100	AY899437
Black butterfish (<i>Schedophilus velaini</i>) ^b	2	Filleted	COI	<i>Hyperoglyphe moselii</i> ^a	100	<i>Hyperoglyphe moselii</i> ^a	100	DQ107610
Black ruff (<i>Centrolophus niger</i>)	2	Filleted	COI	<i>Schedophilus velaini</i>	100	<i>Schedophilus velaini</i>	100	HQ611131
	1	Filleted	COI	<i>Centrolophus niger</i>	100	<i>Centrolophus niger</i>	100	AB205434
Bluenose (<i>Hyperoglyphe antarctica</i>)	3	Filleted	COI	<i>Hyperoglyphe antarctica</i>	100	<i>Hyperoglyphe antarctica</i>	100	DQ107615
				<i>Hyperoglyphe moselii</i>^a	100	<i>Hyperoglyphe moselii</i>^a	100	DQ107610
				<i>Schedophilus velaini</i> (violet warehou)	100	<i>Schedophilus velaini</i> (violet warehou)	100	HQ611131
Blue warehou (<i>Seriola brama</i>)	1	Filleted	COI	<i>Seriola brama</i>	100	<i>Seriola brama</i>	100	AB205439
Butterfish (<i>Ruvettus pretiosus</i>)	4	Filleted	COI	<i>Ruvettus pretiosus</i>	100	<i>Ruvettus pretiosus</i>	100	EU752173
Lepidocybium flavobrunneum	1	Filleted	COI	<i>Lepidocybium flavobrunneum</i>	100	<i>Lepidocybium flavobrunneum</i>	100	FJ605745
Cape dory (<i>Zeus capensis</i>)	2	Whole	COI	<i>Zeus capensis</i>	100	<i>Zeus capensis</i>	100	HM007762
Cape gurnard (<i>Chelidonichthys capensis</i>)	2	Whole	COI	<i>Chelidonichthys capensis</i>	100	<i>Chelidonichthys capensis</i>	100	HM007756
Cape salmon (<i>Atractoscion aequidens</i>)	3	Filleted	COI	<i>Atractoscion aequidens</i>	100	<i>Atractoscion aequidens</i>	100	GU946593
Cardinal (<i>Epigonus telescopus</i>)	3	Filleted	COI	<i>Epigonus telescopus</i>	100	<i>Epigonus telescopus</i>	100	HM007701
							99	EF609350
Deep-water Cape hake (<i>Merluccius paradoxus</i>)	3	Whole	COI	<i>Merluccius paradoxus</i>	100	<i>Merluccius paradoxus</i>	100	GU324176
Dorado (<i>Coryphaena hippurus</i>)	5	Filleted	COI	<i>Coryphaena hippurus</i>	100	<i>Coryphaena hippurus</i>	100	HM007683
							100	DQ885089
	1	Whole	COI	<i>Coryphaena equiselis</i> (Pompano dolphinfish)	99.7	No COI sequences for <i>C. equiselis</i> are available in GenBank		HM007705
East coast sole (<i>Austroglossus pectoralis</i>)	3	Dressed	COI	<i>Austroglossus pectoralis</i>	100	<i>Austroglossus pectoralis</i>	99	EU513717
							99	HM007679
Flame snapper (<i>Etelis coruscans</i>)	1	Filleted	COI	<i>Etelis coruscans</i>	99.7	No COI sequences for <i>E. coruscans</i> are available in GenBank		
Gastora (<i>Gasterochisma melampus</i>)	3	Filleted	COI	<i>Gasterochisma melampus</i>	100	<i>Gasterochisma melampus</i>	100	DQ107691
							100	HM007708
Japanese amberjack (<i>Seriola quinqueradiata</i>)	1	Filleted	COI	<i>Seriola quinqueradiata</i>	100	<i>Seriola quinqueradiata</i>	100	HQ641665
Kippers (<i>Clupea harengus</i>)	3	Filleted	COI	<i>Clupea harengus</i>	100	<i>Clupea harengus</i>	99–100	GU324181
Kingklip (<i>Genypterus capensis</i>)	12	Filleted	COI	<i>Genypterus capensis</i>	100	<i>Genypterus capensis</i>	99–100	HM007746
Marlin (<i>Makaira</i> spp.)	1	Filleted	COI	<i>Makaira nigricans</i>	100	<i>Makaira nigricans</i>	99	GQ202124
				<i>Makaira mazara</i>	100	<i>Makaira mazara</i>	99	AB470304
	1	Filleted	COI	<i>Tetrapturus angustirostris</i> (shortbill spearfish)	99.7	<i>Tetrapturus angustirostris</i> (shortbill spearfish)	99	AB470303
Monk (<i>Lophius vomerinus</i>)	2	Filleted	COI	<i>Lophius vomerinus</i>	100	<i>Lophius vomerinus</i>	99	EU683994
							100	HM007765
New Zealand ling (<i>Genypterus blacodes</i>)	2	Filleted	COI	<i>Genypterus blacodes</i>	100	<i>Genypterus blacodes</i>	99	HQ611135
							100	EU074430
Pilchard (<i>Sardinops sagax</i>)	2	Whole	COI	<i>Sardinops sagax</i>	100	<i>Sardinops sagax</i>	99	FJ165127
							100	HQ611132
Shallow-water Cape hake (<i>Merluccius capensis</i>)	4	Whole	COI	<i>Merluccius capensis</i>	100	<i>Merluccius capensis</i>	100	GQ988405
							100	HM007690
Silver kob/Kabeljou (<i>Argyrosomus inodorus</i>)	2	Filleted	COI	<i>Argyrosomus inodorus</i>	100	<i>Argyrosomus inodorus</i>	100	HM007711
Snoek/barracouta (<i>Thyrsites atun</i>)	3	Filleted	COI	<i>Thyrsites atun</i>	100	<i>Thyrsites atun</i>	100	HQ611106
							99	EU263813
	3	Filleted	COI	<i>Thyrsites atun</i>	100	<i>Thyrsites atun</i>	100	HQ641670
Spanish mackerel (<i>Scomberomorus commerson</i>)	2	Filleted	COI	<i>Scomberomorus commerson</i>	100	<i>Scomberomorus commerson</i>	100	DQ885055
							100	HM007790
West coast sole (<i>Austroglossus microlepis</i>)	8	Dressed	COI	<i>Austroglossus microlepis</i>	100	<i>Austroglossus microlepis</i>	100	GU946575
Yellowtail (<i>Seriola lalandi</i>)	3	Filleted	COI	<i>Seriola lalandi</i>	100	<i>Seriola lalandi</i>	100	HM007727
							99	EF609460
Yellowfin tuna (<i>Thunnus albacares</i>)	3	Filleted	COI	<i>Thunnus albacares</i>	100	<i>Thunnus albacares</i>	100	EF609629
				<i>Thunnus atlanticus</i>	99.85	<i>Thunnus atlanticus</i>	99	DQ107588
				<i>Thunnus obesus</i>	99.85	<i>Thunnus obesus</i>	99	DQ107642
				<i>Thunnus tonggol</i>	99.69	<i>Thunnus tonggol</i>	99	DQ107634
			CR	–	–	<i>Thunnus albacares</i>	99	AF301200
	1	Filleted	COI	<i>Thunnus obesus</i>	100	<i>Thunnus obesus</i>	100	GU451754
				<i>Thunnus atlanticus</i>	100	<i>Thunnus albacares</i>	99	GU256528
				<i>Thunnus albacares</i>	99.8	<i>Thunnus thynnus</i>	99	GU451772

(continued on next page)

Table 1 (continued)

Fish marketed as: (expected species)	N	Process state	Gene target	BOLD		GenBank		
				Species identification	Similarity (%)	Species identification	Similarity (%)	Accession Number
				<i>Thunnus thynnus</i>	99.5	<i>Thunnus tonggol</i>	99	DQ107634
				<i>Thunnus tonggol</i>	99.5			
			CR	–	–	<i>Thunnus obesus</i>	99	DQ126626

^a *Hyperoglyphe moselii* and *Schedophilus velainii* refer to the same species, although *S. velainii* is the currently accepted name.

2006). In addition, since control region sequences have been frequently employed for intra- and interspecific genetic studies of fishes (Aboim, Menezes, Schlitt, & Rogers, 2005; Alvarado Bremer, Naseri, & Ely, 1997; Carlsson, McDowell, Díaz-Jaimes, et al., 2004; Cawthorn, Steinman, & Witthuhn, 2011a; Lee, Conroy, Hunting Howell, & Kocher, 1995; Smith, Struthers, Paulin, McVeagh, & Daley, 2009; Viñas & Tudela, 2009), it was anticipated prior to analyses that reference sequences for this region would be readily available in DNA sequence repositories to allow identification of species not discriminated by COI sequencing.

Overall, 53 of the 248 (ca. 21%) fish samples analysed from all outlets were genetically identified as different species to the ones indicated and could consequently be considered mislabelled (Tables 1 and 2). The frequency of fish species mislabelling observed here is comparable to those results obtained from similar studies in Europe (Miller & Mariani, 2010), Italy (Barbuto et al., 2010; Filonzi et al., 2010) and North America (Logan et al., 2008; Marko et al., 2004; Wong & Hanner, 2008), emphasising that this problem is pervasive on a worldwide scale. A surprising finding emerging from this study was the large discrepancy in the incidence of mislabelling manifesting at the wholesale and retail levels in South Africa. Mislabelling was found to be considerably more pronounced in retail outlets than in wholesale outlets and both sets of results are subsequently discussed in turn.

3.1. Mislabelling at the wholesale level

Discrepancies were found between the identified species and the declared market names for 10 of the 108 (ca. 9%) frozen fish samples obtained from seafood wholesalers/distributors in South Africa (Table 1). Although the currently available literature suggests that most species mislabelling transpires following procurement from the fishermen for the sake of increased profits (Jacquet & Pauly, 2008), it is, nevertheless, quite conceivable that some of this mislabelling may have occurred prior to fish receipt by the respective wholesalers or distributors. As suggested by Logan et al. (2008), a factor potentially contributing to this problem is the loss of clear identification characteristics of fish specimens between the time of capture and the time that these appear for sale. The results obtained in this study indeed indicated that the majority of species substitutions at the wholesale level involved fish products that had been processed to some degree, meaning that the distinguishing morphological characteristics had been damaged or removed. Nine of the 94 (ca. 10%) filleted or processed samples were mislabelled, compared to just one of the 14 (ca. 7%) whole samples.

Some of the substitutions detected at the wholesaler/distributor level appeared to be more blatant than others. One case of potentially unintentional substitution involved a sample labelled as 'dorado' (*Coryphaena hippurus*), which exhibited 100% sequence similarity in BOLD with Pompano dolphinfish (*C. equiselis*) (Table 1). Both of these congeneric species occur worldwide in tropical or sub-tropical waters and it has been reported that *C. equiselis* is frequently misidentified as juvenile or female *C. hippurus* (Froese & Pauly, 2011). Somewhat less likely to be unintentional was the misrepresentation of confamilial species, such as the substitution of marlin with shortbill spearfish (*Tetrapturus angustirostris*). In addition, three samples

labelled as 'bluenose' showed a 100% sequence similarity with another member of the Centrolophidae family, namely *Schedophilus velainii* (violet warehou/black butterfish) (Table 1). Although marlin and shortbill spearfish may be targeted by the same fisheries, as may also be the case for bluenose and violet warehou, the morphological characteristics of these species should allow for relatively simple discrimination when the specimens are in their whole forms.

A major case of apparently deliberate fraud detected at the wholesaler/distributor level concerned the substitution of five samples marketed as the highly-valued 'Australian barramundi', expected to be *Lates calcarifer*, but identified by COI sequencing as the lower-valued common warehou (*Seriola lalandi*) (Table 1). Although all five samples tested were derived from the same distributor, these were representative of different batches, suggesting that the species substitution was not an isolated incident. A further four samples of the same product derived from retail outlets in all four provinces surveyed also showed 100% sequence similarity with *S. lalandi* in both BOLD and GenBank (Table 2).

3.2. Mislabelling at the retail level

Of the 140 retail samples that were sequenced, 43 (ca. 31%) of these were found to be misnamed or mislabelled (Table 2). As seen at the wholesale level, mislabelling at the retail level appeared to be particularly problematic with processed fish products. Thirty-three of 96 (ca. 34%) filleted or processed samples were deemed to be mislabelled, compared to 10 of 44 (ca. 23%) samples sold in their whole state. The overall frequency of fish mislabelling, as well as the frequency of mislabelling per province surveyed, is presented in Table 3. The highest incidence of fish mislabelling at the retail level was found in the coastal province of KZN, where 19 of the 34 (ca. 56%) samples analysed by DNA sequencing were misrepresented. Fish species substitution was the second most frequent in GP, where 8 of the 28 (29%) collected samples appeared to be mislabelled. A lack of vendor familiarity with marine fish nomenclature in an inland province may have been a factor leading to this high incidence of mislabelling in GP. However, it should also be noted that a corresponding lack of knowledge at the consumer level in an inland province could make it reasonably simple for dishonest retailers to mislabel their fish products for the sake of financial gain. The prevalence of retail-level mislabelling in the WC and EC was lower than that observed in KZN and GP, with 13 of 51 (25%) and 4 of 27 (15%) of the collected samples in the respective provinces being identified as other species.

Fourteen of the 43 (ca. 33%) cases of mislabelling discovered were seemingly subtle in nature, representing substitutions with congeneric species. One such instance of inconspicuous mislabelling involved a sample marketed as 'deep water hake', for which a 'product of South Africa' declaration was also included on the packaging (Table 2). According to the recently published labelling regulations in South Africa (DoH, 2010), a 'product of South Africa' label would imply that the main ingredient of the foodstuff was derived from South Africa. 'Deep water hake' caught in South African waters is expected to be *Merluccius paradoxus*, however, COI sequencing revealed a 100% sequence similarity between the enclosed fish and the imported species *Merluccius productus* (North Pacific hake). Thus, this case not only involved misrepresentation at the species

Table 2

Identification results based on cytochrome c oxidase I (COI) and control region (CR) sequencing for 148 fish samples from retail outlets (supermarkets and fish markets), where N indicates the number of fish samples that were correctly labelled or potentially mislabelled. Cases of suspected mislabelling are indicated in bold typescript, while GenBank Accession numbers in italics indicate sequences derived from fish specimens caught in South African waters.

Fish marketed as: (expected species)	N	Process state	Gene target	BOLD		GenBank		
				Species identification	Similarity (%)	Species identification	Similarity (%)	Accession number
Angelfish (<i>Brama brama</i>)	2	Filleted	COI	<i>Brama brama</i>	100	<i>Brama brama</i>	100	EF609300
	1	Filleted	COI	<i>Taractichthys longipinnis</i> (big-scale pomfret)	99.5	<i>Taractichthys longipinnis</i> (big-scale pomfret)	99	EF609476
Alaskan salmon (<i>Oncorhynchus keta</i>)	1	Filleted	COI	<i>Oncorhynchus keta</i>	100	<i>Oncorhynchus keta</i>	99	EU525057
Barracuda (<i>Sphyraena</i> spp.)	1	Filleted	COI	<i>Acanthocybium solandri</i> (wahoo)	100	<i>Acanthocybium solandri</i> (wahoo)	99	DQ107693
	1	Filleted	COI	<i>Thyrsites atun</i> (snoek)	100	<i>Thyrsites atun</i> (snoek)	98	EU263814
Barramundi (<i>Lates calcarifer</i>)	4	Filleted	COI	<i>Seriolella brama</i> (common warehou)	100	<i>Seriolella brama</i> (common warehou)	100	EF609461
								HM007731
Black ruff (<i>Centrolophus niger</i>)	2	Filleted	COI	<i>Centrolophus niger</i>	100	<i>Centrolophus niger</i>	100	AB205434
Cape dory (<i>Zeus</i> spp.)	2	Whole	COI	<i>Zeus capensis</i>	100	<i>Zeus capensis</i>	100	HM007762
Cape gurnard (<i>Chelidonichthys capensis</i>)	1	Whole	COI	<i>Chelidonichthys capensis</i>	100	<i>Chelidonichthys capensis</i>	100	HM007756
Cape monk (<i>Lophius vomerinus</i>)	2	Filleted	COI	<i>Lophius vomerinus</i>	100	<i>Lophius vomerinus</i>	99	EU683994
							100	HM007765
Cape salmon (<i>Atractoscion aequidens</i>)	5	Filleted	COI	<i>Atractoscion aequidens</i>	100	<i>Atractoscion aequidens</i>	100	GU946593
	1	Filleted	COI	<i>Tetrapturus angustirostris</i> (shortbill spearfish)	100	<i>Tetrapturus angustirostris</i> (shortbill spearfish)	100	DQ107621
								HQ611111
Cardinal (<i>Epigonus telescopus</i>)	1	Filleted	COI	<i>Epigonus telescopus</i>	99	<i>Epigonus telescopus</i>	98	EF609350
							99	HM007701
Cod fish (<i>Gadus morhua</i>)	1	Whole	COI	<i>Brama brama</i> (Atlantic pomfret/angelfish)	99.7	<i>Brama brama</i> (Atlantic pomfret/angelfish)	99	EF609300
								HQ611085
Couta (<i>Scomberomorus commerson</i>)	1	Filleted	COI	<i>Scomberomorus commerson</i>	100	<i>Scomberomorus commerson</i>	100	DQ885055
								HM007790
Deep water hake (product of South Africa) (<i>Merluccius paradoxus</i>)	1	Filleted	COI	<i>Merluccius paradoxus</i>	100	<i>Merluccius paradoxus</i>	100	GU324176
	1	Filleted	COI	<i>Merluccius productus</i> (North Pacific hake)	100	<i>Merluccius productus</i> (North Pacific hake)	100	HM007683
								FJ164854
Dorado (<i>Coryphaena hippurus</i>)	3	Filleted	COI	<i>Coryphaena hippurus</i>	99–100	<i>Coryphaena hippurus</i>	99–100	GU225592
								HM007704
East coast sole (<i>Austroglossus pectoralis</i>)	4	Dressed	COI	<i>Austroglossus pectoralis</i>	100	<i>Austroglossus pectoralis</i>	100	HM007679
							99	EU513717
Englishman (<i>Chrysoblephus anglicus</i>)	1	Whole	COI	<i>Chrysoblephus anglicus</i>	100	<i>Chrysoblephus anglicus</i>	100	HQ611099
Hake (<i>Merluccius</i> spp.)	3	Filleted	COI	<i>Merluccius paradoxus</i>	100	<i>Merluccius paradoxus</i>	100	HM007683
	2	Filleted	COI	<i>Merluccius capensis</i>	100	<i>Merluccius capensis</i>	100	HM007690
	1	Whole	COI	<i>Helicolenus dactylopterus</i>	100	<i>Helicolenus dactylopterus</i>	100	HM007747
				<i>Helicolenus barathri</i>	99.5	<i>Helicolenus hilgendorfi</i>	99	AP002948
				<i>Helicolenus percoides</i>	99.5	<i>Helicolenus barathri</i>	99	EF609370
				<i>Helicolenus hilgendorfi</i>	99.3			
			CR	–	–	<i>Helicolenus dactylopterus</i> (blackbelly rosefish/jacopever)	99	AY563096
Hottentot seabream (<i>Pachymetopon blochii</i>)	3	Whole	COI	<i>Pachymetopon blochii</i>	100	<i>Pachymetopon blochii</i>	100	HM007693
Jacopever/Jacs (<i>Helicolenus dactylopterus</i>)	3	Whole	COI	<i>Helicolenus dactylopterus</i>	100	<i>Helicolenus dactylopterus</i>	100	HM007747
				<i>Helicolenus barathri</i>	99.5	<i>Helicolenus hilgendorfi</i>	99	AP002948
				<i>Helicolenus percoides</i>	99.5	<i>Helicolenus barathri</i>	99	EF609370
				<i>Helicolenus hilgendorfi</i>	99.3			
			CR	–	–	<i>Helicolenus dactylopterus</i>	99	AY563096
Kabeljou (<i>Argyrosomus</i> spp.)	4	Filleted	COI	<i>Argyrosomus inodorus</i>	100	<i>Argyrosomus inodorus</i>	100	HM007711
Kahawai (<i>Arripis trutta</i>)	1	Filleted	COI	<i>Arripis trutta</i>	99.9	<i>Arripis trutta</i>	99	AB205452
King fish (<i>Carangoides/Caranx</i> spp.)	1	Filleted	COI	<i>Genypterus capensis</i> (kingklip)	99.8	<i>Genypterus capensis</i> (kingklip)	99	HM007746
Kingklip (<i>Genypterus capensis</i>)	9	Filleted	COI	<i>Genypterus capensis</i>	99–100	<i>Genypterus capensis</i>	99–100	HM007746
	3	Filleted	COI	<i>Genypterus blacodes</i> (pink cusk eel/ling)	100	<i>Genypterus blacodes</i> (pink cusk eel/ling)	99	EU074430
							100	HQ611135
Line fish (?)	1	Filleted	COI	<i>Atractoscion aequidens</i> (geelbek/Cape salmon)	99.8	<i>Atractoscion aequidens</i> (geelbek/Cape salmon)	99	GU946593
Longfin tuna (<i>Thunnus alalunga</i>)	2	Filleted	COI	<i>Thunnus alalunga</i>	100	<i>Thunnus orientalis</i>	100	DQ107659
				<i>Thunnus orientalis</i>	99.8	<i>Thunnus alalunga</i>	100	DQ107631
			CR	–	–	<i>Thunnus alalunga</i>	99	AF390331
Maasbanker (<i>Trachurus capensis</i>)	2	Whole	COI	<i>Trachurus capensis</i>	100	<i>Trachurus capensis</i>	100	HM007775
Mackerel (<i>Scomber japonicus</i>)	2	Whole	COI	<i>Scomber japonicus</i>	100	<i>Scomber japonicus</i>	100	HQ611117
							98	EF433290
Mackerel (<i>Scomber scombrus</i>)	2	Canned	COI	Failed to amplify with COI cocktail				
Musselcracker (<i>Sparodon durbanensis/Cymatoceps nasutus</i>)	2	Filleted	COI	<i>Pseudopentaceros richardsoni</i> (pelagic armourhead)	100	<i>Pseudopentaceros richardsoni</i> (pelagic armourhead)	99	DQ107734
	1	Filleted	COI	<i>Hyperoglyphe antarctica</i> (bluenose warehou)	100	<i>Hyperoglyphe antarctica</i> (bluenose warehou)	100	DQ107615
Norwegian salmon (<i>Salmo salar</i>)	3	Filleted	COI	<i>Salmo salar</i>	100	<i>Salmo salar</i>	100	AF133701
	1	Filleted	COI		100		100	DQ288270

(continued on next page)

Table 2 (continued)

Fish marketed as: (expected species)	N	Process state	Gene target	BOLD		GenBank		
				Species identification	Similarity (%)	Species identification	Similarity (%)	Accession number
				<i>Oncorhynchus mykiss</i> (rainbow trout)		<i>Oncorhynchus mykiss</i> (rainbow trout)		
Panga (<i>Pterogymnus laniarius</i>)	2	Whole	COI	<i>Pterogymnus laniarius</i>	100	<i>Pterogymnus laniarius</i>	100	HQ592212
Pignose grunter (<i>Lithognathus lithognathus</i>)	1	Filleted	COI	<i>Seriola lalandi</i> (yellowtail amberjack)	99.6	<i>Seriola lalandi</i> (yellowtail amberjack)	99	EF609460
Pilchard (<i>Sardinops sagax</i>)	2	Whole	COI	<i>Sardinops sagax</i>	100	<i>Sardinops sagax</i>	100	HM007727
							100	FJ165127
							100	HQ611132
Pink salmon (<i>Oncorhynchus gorbuscha</i>)	2	Canned	COI	Failed to amplify with COI cocktail				
'Red fish' (?)	1	Whole	COI	<i>Pterogymnus laniarius</i> (panga seabream)	100	<i>Pterogymnus laniarius</i> (panga seabream)	100	HQ592212
	1	Whole	COI	<i>Chrysoblephus puniceus</i> (slinger seabream)	100	<i>Chrysoblephus puniceus</i> (slinger seabream)	100	HQ611088
Red snapper (<i>Lutjanus</i> spp.)	3	Whole	COI	<i>Lutjanus Argentimaculatus</i> (river snapper)	99.9	<i>Lutjanus Argentimaculatus</i> (river snapper)	99	DQ885026
	1	Whole	COI	<i>Pterogymnus laniarius</i> (panga seabream)	100	<i>Pterogymnus laniarius</i> (panga seabream)	100	HM007781
	1	Filleted	COI	<i>Chrysoblephus laticeps</i> (Roman seabream)	100	<i>Chrysoblephus laticeps</i> (Roman seabream)	100	HM007750
Red stumpnose (<i>Chrysoblephus gibbiceps</i>)	1	Filleted	COI	<i>Chrysoblephus puniceus</i> (slinger seabream)	100	<i>Chrysoblephus puniceus</i> (slinger seabream)	100	HQ611087
Ribbon snoek (<i>Lepidopus caudatus</i>)	2	Filleted	COI	<i>Lepidopus caudatus</i>	100	<i>Lepidopus caudatus</i>	99	EU869824
							99	HM007784
Roman/red roman (<i>Chrysoblephus laticeps</i>)	2	Whole	COI	<i>Chrysoblephus laticeps</i>	100	<i>Chrysoblephus laticeps</i>	100	HM007750
	1	Whole	COI	<i>Cheimerius nufar</i> (santer seabream)	100	<i>Cheimerius nufar</i> (santer seabream)	100	HQ611102
	1	Whole	COI	<i>Chrysoblephus puniceus</i> (slinger seabream)	100	<i>Chrysoblephus puniceus</i> (slinger seabream)	100	HQ611087
Sailfish (<i>Istiophorus</i> spp.)	1	Filleted	COI	<i>Tetrapturus angustirostris</i> (shortbill spearfish)	99.9	<i>Tetrapturus angustirostris</i> (shortbill spearfish)	99	HM071007
							99	HQ611111
Santer/soldier (<i>Cheimerius nufar</i>)	4	Whole	COI	<i>Cheimerius nufar</i>	99–100	<i>Cheimerius nufar</i>	99–100	HQ611102
Silver (<i>Argyrozona argyrozona</i>)	3	Whole	COI	<i>Argyrozona argyrozona</i>	100	<i>Argyrozona argyrozona</i>	100	GU946638
Skipjack tuna (<i>Katsuwonus pelamis</i>)	2	Filleted	COI	<i>Katsuwonus pelamis</i>	100	<i>Katsuwonus pelamis</i>	100	EU014261
							100	HQ611090
Slinger (<i>Chrysoblephus puniceus</i>)	2	Whole	COI	<i>Chrysoblephus puniceus</i>	100	<i>Chrysoblephus puniceus</i>	100	HQ611087
Snoek (<i>Thyrsites atun</i>)	2	Filleted	COI	<i>Thyrsites atun</i>	100	<i>Thyrsites atun</i>	100	EU263813
							100	HQ641670
	1	Filleted	COI	<i>Scomberomorus plurilineatus</i> (Kanadi kingfish)	100	–	–	–
Swordfish (<i>Xiphias gladius</i>)	1	Filleted	COI	<i>Xiphias gladius</i>	100	<i>Xiphias gladius</i>	100	AB470301
							100	HM007787
	1	Filleted	COI	<i>Ruvettus pretiosus</i> (oilfish)	99.9	<i>Ruvettus pretiosus</i> (oilfish)	99	EU752173
							99	HM007721
Tomato rockcod (<i>Cephalopholis sonnerati</i>)	1	Whole	COI	<i>Cephalopholis sonnerati</i>	99.8	<i>Cephalopholis sonnerati</i>	99	HQ611096
Tuna chunks in brine (Not specified)	5	Canned	COI	Failed to amplify with COI cocktail				
Wahoo (<i>Acanthocybium solandri</i>)	1	Filleted	COI	<i>Scomberomorus commerson</i> (Spanish/king mackerel)	100	<i>Scomberomorus commerson</i> (Spanish/king mackerel)	100	DQ885055
							100	HM007790
White steenbras (<i>Lithognathus lithognathus</i>)	1	Filleted	COI	<i>Taractichthys longipinnis</i> (bigscale pomfret)	99.5	<i>Taractichthys longipinnis</i> (bigscale pomfret)	99	EF609476
	1	Filleted	COI	<i>Ruvettus pretiosus</i> (oilfish)	99.9	<i>Ruvettus pretiosus</i> (oilfish)	99	EU752173
White stumpnose (<i>Rhabdosargus globiceps</i>)	3	Whole	COI	<i>Rhabdosargus globiceps</i>	100	<i>Rhabdosargus globiceps</i>	100	HM007759
Yellowbelly rockcod (<i>Epinephelus marginatus</i>)	1	Whole	COI	<i>Epinephelus marginatus</i>	100	<i>Epinephelus marginatus</i>	100	HQ611093
Yellowfin tuna (<i>Thunnus albacares</i>)	3	Filleted	COI	<i>Thunnus albacares</i>	100	<i>Thunnus albacares</i>	100	EF609629
				<i>Thunnus atlanticus</i>	99.9	<i>Thunnus atlanticus</i>	99	DQ107588
				<i>Thunnus obesus</i>	99.9	<i>Thunnus obesus</i>	99	DQ107642
				<i>Thunnus tonggol</i>	99.7	<i>Thunnus tonggol</i>	99	DQ107634
			CR	–	–	<i>Thunnus albacares</i>	99	AF301200
							99	HQ853215
	1	Filleted	COI	<i>Katsuwonus pelamis</i> (skipjack tuna)	100	<i>Katsuwonus pelamis</i> (skipjack tuna)	100	GU225630
Yellowtail (<i>Seriola lalandi</i>)	4	Filleted	COI	<i>Seriola lalandi</i>	100	<i>Seriola lalandi</i>	99	EF609460
							100	HM007727
	5	Filleted	COI	<i>Seriola quinqueradiata</i> (Japanese amberjack)	100	<i>Seriola quinqueradiata</i> (Japanese amberjack)	100	HQ641665

level, but also at the country-of-origin level. Similarly, the common name 'yellowtail' is expected to refer to *Seriola lalandi* (yellowtail amberjack), a fish regularly consumed on the South African market. Nonetheless, 5 of 9 retail samples labelled as 'yellowtail' showed a 100% sequence similarity with *Seriola quinqueradiata* (Japanese amberjack) (Table 2), a species most likely imported from Asian

countries. This finding appears to indicate that retailers in South Africa may label this closely-related species as 'yellowtail', rather than take the risk of consumers rejecting the foreign counterpart due to unfamiliarity with its common name. Obviously, such practices are facilitated by the fact that there is no legal definition of which species constitutes a 'yellowtail' in South Africa. In addition, samples labelled

Table 3

The percentages of fish samples obtained from retail outlets that were found to be correctly and incorrectly labelled in all four surveyed provinces.

Province surveyed	No. of fish samples analysed	Fish samples correctly labelled (%)	Fish samples mislabelled (%)
WC	51	75	25
EC	27	85	15
KZN	34	44	56
GP	28	71	29
All provinces	140	69	31

as 'Roman seabream' and 'red stumpnose', expected to be the species *Chrysoblephus laticeps* and *Chrysoblephus gibbiceps*, respectively, were both found to be slinger seabream (*Chrysoblephus puniceus*) (Table 2). These cases of species substitution may hold economic implications, as the former members of the Sparidae family (particularly red stumpnose) normally command a higher price than the latter.

In South Africa, it has long been recognised that the popular and highly-valued kingklip (*Genypterus capensis*) is prone to market substitution (Smith & Smith, 1966). Kingklip stocks were heavily exploited in the 1980s by an experimental longline fishery in this country and these have not since recovered to their former abundance (McLean & Glazewski, 2009). Today, kingklip is managed as a bycatch in the offshore demersal trawl and demersal longline fisheries in South Africa, with a total allowable catch (TAC) of only about 3000 tons per annum (compared to the TAC of ca. 150 000 ton per annum for hake) (DEAT, 2005; SASSI, 2010). In spite of this stringent limit, kingklip remains one of the most commonly marketed fish species on the domestic market (Cawthorn et al., 2011b). While it is suspected that the congeneric counterpart from Australia or New Zealand known as ling/pink cusk eel (*Genypterus blacodes*) may serve as a substitute for kingklip (SASSI, 2010; von der Heyden, Barendse, Seebregts, & Matthee, 2010), this suspicion has not been definitively confirmed to date through DNA analysis. Although *G. capensis* and *G. blacodes* have been observed to be genetically closely-related, indistinguishable for example by 16S ribosomal RNA (rRNA) gene sequencing (von der Heyden et al., 2010), it was shown in this study that the two species possess unique COI barcodes and can be readily discriminated on this basis. Indeed, 3 of the 12 (25%) retail samples marketed as 'kingklip' exhibited 100% sequence similarity with *G. blacodes* (Table 2), in spite of a number of COI sequences for *G. capensis* being available in the BOLD and GenBank databases. This form of mislabelling may not only defraud consumers financially, but it also likely creates a skewed impression of the abundance of kingklip stocks in South Africa, contradicting the messages being sent by organisations such as SASSI on the conservation status of the species.

Other cases of convoluted labelling observed at the retail level involved the use of generic or group names to describe certain fish samples. One sample labelled as 'linefish' was found to be geelbek/Cape salmon (*Atractoscion aequidens*) (Table 2), a species that has been heavily exploited by overfishing in the past (Hutton, Griffiths, Sumaila, & Pitcher, 2001). Although *A. aequidens* is indeed a species caught in the traditional South African linefishery, there are approximately 150 other linefish species caught in this country (van Schalkwyk, 2007). These species may not only differ in commercial value, but they can also have markedly different conservation statuses. In addition, retail samples labelled by the generic term 'red fish' provided 100% sequence similarities with two endemic species of the Sparidae family, namely panga seabream (*Pterogymnus laniarius*) and slinger seabream (*C. puniceus*) (Table 2). Panga is among the most abundant of seabreams occurring in South African waters (van der Elst, 1997), while stocks of slinger have been heavily exploited as a result of extensive fishing pressure (Chopelet, Helyar, Mann, & Mariani, 2009). Therefore, this data emphasises that the ambiguities arising from the use of vernacular or generic names to group multiple species holds important

ramifications for sustainable seafood campaigns, with the potential to prevent even the most knowledgeable of consumers from making informed purchases for the benefit of conservation.

Twenty-eight of the 42 (67%) incidents of retail-level mislabelling involved species which were from entirely different genera to the ones declared. Some of these cases appeared to have no clear explanation, with no apparent association with any form of financial or marketing incentive. Examples of such cases, most of which were perpetuated in KZN, included samples labelled as 'wahoo' which were identified as king mackerel (*Scomberomorus commerson*) and, conversely, 'barracuda' that turned out to be wahoo (*Acanthocybium solandri*) (Table 2). Samples of 'Cape salmon' and 'sailfish' were found to be substituted with shortbill spearfish (*T. angustirostris*), while big-scale pomfret (*Taractichthys longipinnis*) was misdescribed as 'angel-fish'. Additionally, one sample denoted as 'codfish' on the label showed 99% sequence similarity with angelfish (*Brama brama*). When questioning the vendor on the origin of the 'cod' sample, it was indicated that the fish was caught in the local offshore trawl. Ironically, while cod (*Gadus* spp.) is heavily exploited in many parts of the world, it is not caught anywhere close to South Africa (Froese & Pauly, 2011). Apart from the possibility that the incorrect names were used to increase the market appeal of these sample, it appeared more likely in some instances that the misrepresentation was simply a result of ignorance on the part of the retailers. Confused nomenclature could also have been a reason for the mislabelling of kingklip as 'kingfish', where the retailer may not have recognised that these refer to different species (Table 2). The marketing of kingfish, nevertheless, presents an obstacle to consumer education and conservation strategies. All 53 species of kingfish are designated specially-protected and are illegal to sell in South Africa. However, these legal provisions would likely go unnoticed by the public if these species are touted on the market. In KZN, *Scomberomorus plurilineatus* is normally referred to as 'Natal snoek', but by shortening the name to just 'snoek', the vendors may not have comprehended that they were referring to a different species (*Thyrsites atun*). Furthermore, snoek (*T. atun*) may often be sold under the name 'barracouta', particularly when the species is imported. The one sample sold as 'barracuda' (expected to be *Sphyræna* spp.) which was found to be 100% similar to *T. atun* (Table 2), may have potentially represented an instance where the mere misspelling of the market name altered the species being referred to. Even in the case that the aforementioned mislabelling incidents were unintentional, these results highlight the need for improved and more uniform fish naming in South Africa as a means of promoting fair trade, conservation efforts and consumer rights.

On the other hand, a number of cases of potentially deliberate mislabelling were uncovered, where financial incentives could have been a driving factor for the substitution of highly-valued fish species with lower-valued ones. One sample denoted as the highly-priced 'Norwegian salmon' on the product label showed 100% sequence similarity with the cheaper member of the Salmonidae family, rainbow trout (*Oncorhynchus mykiss*) (Table 2). Another sample labelled as 'halfmoon rockcod' turned out to be the totally unrelated and lower-valued jacobever (*H. dactylopterus*). In addition, COI sequencing showed that a sample marketed as 'yellowfin tuna' was actually more genetically similar to the less-valuable skipjack tuna (*Katsuwonus pelamis*). Probably the most prominent case of economic fraud at the retail level concerned the misrepresentation of five fish samples sold as 'red snapper' (Table 2). According to the US FDA list of acceptable market names for fish (FDA, 2010), the term 'red snapper' is the legally designated common name for *Lutjanus campechanus*, a highly-prized but severely overexploited species found in the southern Atlantic and Gulf of Mexico (Froese & Pauly, 2011; Jacquet & Pauly, 2008). However, the results of this study showed that a variety of alternative species may masquerade under this title to increase their market appeal, a finding consistent with numerous reports of 'snapper' mislabelling from other parts of the world (Hsieh, Woodward, & Blanco, 1995; Logan et al., 2008; Marko et al., 2004; Wong &

Hanner, 2008). COI sequencing revealed that that two samples marketed as 'red snapper' exhibited 100% sequence similarity with other red-coloured species, namely panga seabram (*P. laniarius*) and Roman seabram (*C. laticeps*) (Table 2). In addition, the discovery of three samples of river snapper (*Lutjanus argentimaculatus*) incorrectly labelled as 'red snapper' has serious conservation and legal ramifications. Since river snapper is an estuarine-dependant species with a high vulnerability to overexploitation, it has been designated as a recreational species only and is prohibited to sell in South Africa. However, when river snapper is concealed under the name 'red snapper', consumers are unable to avoid purchasing an illegal, locally-protected species.

Also of concern from a legality and conservation perspective was the blatant marketing of a number of samples in KZN retail outlets as 'musselcracker' and 'white steenbras'/'pignose grunter'. The term 'musselcracker' may refer to either one of the severely exploited black musselcracker (*Cymatoceps nasutus*) or white musselcracker (*Sparodon durbanensis*), with the latter being a recreational species prohibited for sale in South Africa. On the other hand, the market names 'white steenbras' and 'pignose grunter' both refer to the illegal-to-sell species *Lithognathus lithognathus*, the stocks of which are considered collapsed due to extensive overfishing (Bennett, 1993; SASSI, 2010). In spite of the use of these terms, two samples denoted as 'musselcracker' showed a 100% sequence similarity in BOLD with *Pseudopentaceros richardsoni* (pelagic armourhead), a species of limited commercial value in South Africa. A further sample sold as 'musselcracker' in the WC exhibited a 100% sequence similarity with bluenose warehou (*Hyperoglyphe antarctica*). This probably represented a case of intentional substitution as the latter is normally imported (von der Heyden et al., 2010), while the former species are endemic to South Africa (SASSI, 2010). Similarly, 'white steenbras'/'pignose grunter' was found to be misrepresented by a number of different species, including big-scale pomfret (*T. longipinnis*) and yellowtail (*S. lalandi*). At a first glance, the substitution of over-exploited fish species with more sustainable ones may not be considered unfavourable from a conservation viewpoint. Nonetheless, this remains a serious form of deception and exemplifies a common problem faced by seafood awareness campaigns in their endeavours to educate consumers and provide tools for informed purchasing decisions. Similar to the repercussions discussed in terms of the mislabelling of kingklip, the selling of protected or illegal species (even if they are not what they are indicated to be) conceals the true status of the stocks. Consumers would probably be led to believe that the heavily exploited musselcrackers and white steenbras are actually plentiful if they are perceived to be continually available in the marketplace.

Lastly, cases of species mislabelling warranting concern from a health perspective involved the substitution of samples labelled as 'swordfish' and 'white steenbras' with oilfish (*Ruvettus pretiosus*). It is well established that oilfish contain high levels of indigestible wax esters, the purgative effects of which have been implicated in numerous cases of oily diarrhoea (keriorrhea) worldwide (Ling, Cheung, Cheng, et al., 2008). Although the sale and import of oilfish have been banned in Italy, Japan and South Korea (Ling, Nichols, & But, 2009), oilfish is still sold in many countries due to its frequent bycatch in swordfish and tuna fisheries (Tserpes, Tatamanidis, & Peristeraki, 2006). The sale of oilfish is not prohibited in South Africa and no guidelines exist for the marketing of this fish. The fact remains that even if individuals wished to avoid the consumption of a species which could knowingly cause illness, they are denied the right to do so when it is labelled as 'swordfish' or 'steenbras'.

3.3. Regulatory aspects and mislabelling

Although many potential reasons for mislabelling have been presented here, these all ultimately stem from a lack of control on the fisheries market in South Africa. This lack of control can inevitably

be traced back to the prevailing government legislation, where the policies relating to the marketing of fishery products are clearly inadequate and/or poorly enforced. The existing regulations in South Africa relating to the labelling of packaged foods have been published by the Department of Health (DoH), while those pertaining to the labelling of local and imported frozen fish products fall under the domain of the National Regulator for Compulsory Specifications (NRCS). Both of these sets of regulations specify that product labelling should not be misleading to the consumer (DoH (Department of Health, South Africa), 2010; NRCS, 2003). In addition, the NRCS regulations expressly stipulate that a 'true description (of the) variety of fish' must be stated on the packaging of frozen fish products. In spite of these requirements, neither of these regulations include any rules or guidelines on the acceptable market names that should be used for different imported and domestic fish sold in South Africa, nor do they require the declaration of the Latin names for the species being marketed. Such weaknesses in the legal provisions evidently provide seafood suppliers with a substantial amount of leeway in terms of the names they use to sell their products, providing ample opportunities for mislabelling and fraud to present itself on the local market.

Confusion in fish nomenclature has been one of the driving factors for the compilation of lists of 'acceptable market names' for seafood products by many countries, such as Canada's Food Inspection Agency Fish List, the FDA's seafood list in the US and those lists compiled by member states of the European Union (EU) (BIM, 2001; CFIA, 2010; FDA, 2010; FSA, 2010). The establishment of such lists in South Africa could provide some relief to the problems of mislabelling observed in this study, however, it is unlikely that these alone will completely eliminate the problem. A number of studies have shown that seafood mislabelling persists in both the US and EU in spite of their possession of uniform seafood naming lists, where poor enforcement, irregular updating and a lack of harmonisation between trading partners have been cited as factors impeding the usefulness of such endeavours (Logan et al., 2008; Marko et al., 2004; Miller & Mariani, 2010; Wong & Hanner, 2008). The ambiguities existing in market nomenclature in today's global economy plainly signal the need for the additional declaration of the Latin names of fish species on product labels (Gerson, Cudmore, Mandrak, et al., 2008). This practice has been made mandatory in the EU (EC, 2000; 2001) and, in the context of the results obtained here, is one that could markedly improve transparency and advance uniformity in the labelling of fish species in South Africa. In addition, the implementation of traceability policies for fishery products in this country, as have been implemented under EU law (EC, 2002), could promote industry responsibility, assisting in the curtailing of fraud and the prevention of the leakage of illegal, unreported, and unregulated (IUU) fishing products into the market (FAO, 2001; Miller & Mariani, 2010).

Exacerbating the complexities faced in this country due to the inadequacy of regulations are those problems associated with the monitoring and enforcement of the existing ones. At present, regulatory authorities inspect only a small portion of the fish products traded on the local marketplace to verify that the species are correctly identified. It is questionable as to whether the authentication methods presently utilised by the regulators, which rely on sensory evaluation to detect fish substitutions (Trutter, 2010, NRCS, South Africa, personal communication), are sufficiently accurate for the purposes intended. The use of more sophisticated and validated species authentication techniques, such as the COI barcoding method used in this study, would certainly enhance the reliability of regulatory monitoring in South Africa, whilst providing superior evidence to warrant the prosecution of illegal activities.

Lastly, since regulatory bodies cannot be expected to inspect or test every fish product that appears on the market, the whole fisheries supply chain will undoubtedly need to take more responsibility in ensuring that the labelling of the fish products they sell is sufficiently informative and truthful. The latter will become increasingly important

in the light of the recently published Consumer Protection Act (DTI, 2009) in South Africa, the regulations of which came into effect in April 2011. This Act aims to protect consumers from exploitation of any kind. Since the entire supply chain will be held responsible for any incident or complaint relating to their products, the individual links of the chain will be required to prove their credibility in order to avoid considerable penalties.

4. Conclusions

Consumers should be able to expect that the information presented on fish products is correct, especially at a time when they are increasingly being encouraged to take responsibly for both their own health and the health of the environment. This study represents the first comprehensive report on the use of DNA barcoding to investigate the prevalence of fish species substitution and fraud on the South African market. The results presented here have highlighted that the mislabelling of fish species is a reality in this country, being particularly pronounced at the retail level and in terms of processed fish products. The lax application of generic group names or the use of more exotic names to increase the market appeal of fisheries products is not only potentially impacting consumers financially, but it may also be jeopardising their health. Of paralleled concern is that the prevailing frequency of species substitutions is likely to be contradicting the messages on conservation being sent by organisations such as SASSI, while undoubtedly hampering the ability of proactive consumers to make sustainable seafood choices.

Clearly, such findings raise considerable concern on the functioning of the fisheries supply chain in South Africa and should compel authorities to identify targets for improving labelling policies, applicable to both domestic and imported products. Modifications to the existing legislation should, at the very least, include the requirement for the declaration on product labels of a designated 'acceptable market name' and Latin name of the fish species being traded. Obviously, such regulations will be of little value if they are not properly enforced. Government will consequently also need to address questions on whether the current regulatory monitoring activities are adequate and if the penalties imposed for non-compliance are sufficient to dissuade dishonest practices. Against this backdrop, DNA barcoding had been confirmed as an extremely powerful and widely applicable technique for fish identification purposes. The utilisation of such a method could offer a superior level of precision to fish authentication monitoring by both regulators and the industry, whilst also providing an important tool to justify the issuing of penalties and the prosecution of unlawful practices. If greater transparency can be achieved on the market through regulator and industry co-operation, then public confidence might be restored in the seafood supply chain in South Africa and full efforts may be refocused on the conservations of the ocean's fish stocks, which are clearly not as 'inexhaustible' as once thought.

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