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Enfoque multidisciplinario para la identificación de aletas de tiburones pelágicos con datos moleculares, morfométricos y análisis digitales

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ABSTRACT

Accurate species identification is one of the most important issues to conserve and manage shark fisheries. A multidisciplinary approach involving molecular (using variation at ITS2 sequences), morphometrical and image processing species identification was performed to evaluate their discriminating power with three pelagic shark species common to the coasts of Chile (*Prionace glauca* Linnaeus 1785, *Isurus oxyrinchus* Rafinesque 1810, and *Lamna nasus* (Bonnaterre, 1788). Species-specific DNA markers and multivariate analyses based on twenty morphometrical measurements were used to identify fresh and dry fin sets for each shark species. Additionally, coloring patterns and fin shape were jointly used to distinguish dry fin sets of shark species by using digital invariant correlation (relation target and problem image independent of their changes in position, scale and rotation). Our results showed that morphometrical analysis was the least accurate approach, whereas DNA-based identification and image processing approaches were 100% successful on the identification of shark species. Thus ITS2 sequences and morphological diagnostic characteristics such as the ones related to color patterns, allow the correct identification of shark species. Therefore, the implementation of molecular and/or image tools can be applied to confidently identify the main pelagic shark species involved in Chilean landing and fin trade.

Key words: Invariant digital correlation, molecular markers, multivariate analysis, pelagic sharks.

RESUMEN

La identificación correcta de las especies es uno de los más importantes temas para la conservación y el manejo de las pesquerías de tiburones. Un análisis multidisciplinario que involucra el procesamiento de datos moleculares, morfométricos e imágenes fue realizado para evaluar su capacidad de discriminación de tres especies de tiburones pelágicos comunes en las costas de Chile (*Prionace glauca* Linnaeus 1758*, Isurus oxyrinchus* Rafinesque 1810,and *Lamna nasus* Bonnaterre, 1788). Marcadores moleculares especie-específicos y análisis multivariados basados en 20 mediciones morfométricas, fueron usados para identificar grupos de aletas húmedas y secas de cada especie de tiburón. Adicionalmente, patrones de coloración y forma de la aleta fueron usados conjuntamente para distinguir grupos de aletas secas de las especies de tiburones utilizando correlación digital invariante (relación entre la imagen conocida y la imagen problema, independiente de sus cambios de posición, escala y rotación). Nuestros resultados muestran que el análisis morfométrico es el enfoque menos exacto, mientras que la identificación basada en el ADN y el procesamiento de imágenes fue 100% exitosa para reconocer las especies de tiburones en cuestión. Así, secuencias del ITS2 y caracteres diagnósticos en la morfología, tales como aquellos relacionados al patrón de coloración, permiten la identificación correcta de las especies de tiburones. Por lo tanto, la implementación de herramientas moleculares y/o de imagen permiten confidentemente identificar a las principales especies de tiburones pelágicos involucrados en el desembarque y comercio de aletas en Chile.

Palabras clave: Análisis multivariado, correlación digital invariante, marcadores moleculares, tiburones pelágicos.

INTRODUCTION

International marine fisheries communities have focused their attention in the worldwide increase of Chondrichthyans catches during the last decades. Landing reports have shown a sustained growth from approximately 270.000 t in 1950 to 830.000 t in 2000 (Stevens *et al.* 2000; Barker & Schluessel 2005). However, these statistics are probably twice of tones due to there are large unreported bycatches in several countries such as Japan, Taiwan, Brazil, Venezuela, Perú , and Chile (Stevens *et al.* 2000; Lamilla *et al.* 2005). In contrast, many countries report their Chondricthyans catches, landings and exports of their meat, fins, cartilage, liver and other products (Vannuccini 1999, Stevens *et al.* 2000). However, many of these data records have deficient species-specific information in their statistics. Also, there are difficulties to identify species or part of them such as fins and carcasses (Shivji *et al.* 2002). Under this scenario, is even more difficult to make effective assessment and management strategies based on species-level data.

Shark products are the most important group of Chondrichthyans which are traded to Asian market, especially the fins. These are identified and traded principally like a grouped generic category (Shivji *et al.* 2002; Clarke *et al.* 2006; Hernández *et al.* 2008). In this classification, it is possible to obtain a mix of closely related shark species, principally of the families Carcharhinidae, Lamnidae, Sphyrnidae, and Alopiidae (Shivji *et al.* 2002: Hernández *et al.* 2008). Difficulties in the accurate species identification are imposed in these shark species due to morphological similarities (Castro 1993, Shivji *et al.* 2002). In addition, shark finning (fin cutting while discarding the rest of the body into the sea) complicates even more the correct species identification due to removal of the sharks' main diagnostic characteristics (head and fins) (Vannuccini 1999, Shivji *et al.* 2002). In consequence, the accurate species identification is a complex and imperative issue.

In Chile, at least six pelagic shark species have been reported as bycatch in the swordfish fishery: *Prionace glauca,* Linnaeus, 1758, *Isurus oxyrinchus* Rafinesque 1810, *Lamna nasus* Bonnaterre 1788, *Alopias superciliosus,* Lowe, 1841 *A. vulpinus* Bonnaterre, 1788 and *Sphyrna zygaena* Linnaeus 1758*,* (SERNAPESCA 1996-1999; Acuña *et al.*, 2002; Lamilla *et al.*, 2005). According to Acuña *et al.* (2002), 72.2% of the bycatch associated with swordfish fisheries are Chondrichthyans species, with *P. glauca* as the predominant shark (58.5%). The remnant is composed of *I. oxyrinchus* (6.4%), *L. nasus* (3.5%) and *A. superciliosus* (0.5%). The trunks and fins of *I. oxyrinchus* and *L. nasus* are landed, while *P. glauca* trunks are usually discarded and only the fins are retained (Acuña *et al.*, 2002; Lamilla *et al.*, 2005; Hernández *et al.,* 2008). After the landing process, the fins are sold to fin-traders, who transport and process them in "driers". The fins are sun-dried for three to five days, and are subsequently packed and exported particularly to China and Hong Kong (Lamilla *et al.*, 2005; Hernández *et al.,* 2008).

Genetic approaches to identify shark species, especially threatened species or illegally hunted ones, are based on the species-specific Ribosomal Internal Transcriber Spacer 2 (ITS2), PCR markers (Pank *et al.*, 2001; Shivji *et al.*, 2002; Chapman *et al.*, 2003; Abercrombie *et al.*, 2005; Shivji *et al.*, 2005; Magnussen *et al.,* 2007; Hernández *et al.*, 2008). Multiplex PCR-assays based on the nuclear gene Internal Transcribed Spacer 2 (ITS2) and the Cytochrome *b* gene of the mitochondrial genome are powerful and robust markers for accurate discrimination between shark species using shark tissue samples. This approach is based on simultaneous use of a multiplex PCR that allows exact discrimination of commercially exploited shark species from different

geographic origins. However, given the financial and logistical restrictions for molecular analyses, it would be useful to define cheaper techniques (e.g. the image analysis and/or morphometry) that could be used instead of molecular analyses for species identification.

In addition to the problems related to identification of shark fins there are limited records on shark catches, landings and trade, and therefore the assessment of shark conservation and management programs is difficult (Shivji *et al.*, 2002). There is lack of knowledge about the shark fins' morphological characteristics required for species identification since the available data corresponds to preliminary studies based on qualitative criteria, such as shape and coloring patterns (Nakano & Kitamura, 1998; Anonymous, 1999; Vannuccini, 1999). In this sense, shark morphological measurements, including those for each fin type (e.g. pectoral, dorsal, and caudal) should be standardized (Compagno, 2002). However, to date, the usefulness of the fin shape to discriminate between shark species have not been demonstrated. Some studies have shown that digital image analysis is a technique that can provide reliable results for species identification based on invariant digital correlation, which is defined as relation between target and problem image independent of their changes in position, scale and rotation (Pech-Pacheco & Álvarez-Borrego, 1998; Álvarez-Borrego & Chávez-Sánchez, 2001; Álvarez-Borrego & Castro-Longoria, 2003).

In this research we used three different approaches to identify shark species from fins: (i) DNA-based markers, (ii) morphometrical measurements, and (iii) image fin analysis. The goal was to evaluate their possible usefulness in determining the species-specific status of sharks involved in the Chilean finning trade. The results are applicable to the management and conservation strategies of these species caught in Chile (Lamilla *et al.*, 2005; Hernández *et al.*, 2008).

MATERIAL AND METHODS

Collection of samples. This study was based on 192 sets of shark fins of *P. glauca, I. oxyrinchus* and *L. nasus*. Of these, 145 were collected from whole sharks captured during a fishery trip of commercial swordfish (*Xiphias gladius* Linnaeus, 1758) and shortfin mako (*Isurus oxyrinchus*) (sample size is detailed in Table 1). Forty-seven sets consisted of dry fins collected from a warehouse located in north-central Chile (Paico, 33° 40' S, 71° 02' W). All sets consisted in the right pectoral, first dorsal, and caudal fins, whereas for dry fins only the lower lobule of caudal fins was measured due to its high commercial value. Samples of 115 sets of fresh fins plus all sets of dry fins were used for the DNA- based identification. All sets (fresh and dry sets separated) were used to discriminate fin type between the shark species using the morphometric analysis approach. Finally, only the dry fin sets were used to discriminate fin types between species using image analysis due to cooperative trader just to get photos from dry fins. The fresh ones were dried and stored in a different place which we had not access (Table 1).

The fin sets belonged mainly to juvenile sharks because the bycatch of the Chilean swordfish fishery consists principally of this type of individuals, >50% of *Prionace glauca*, >90% of *I. oxyrinchus* and >80% of *Lamna nasus* (Acuña *et al.*, 2001; Acuña *et al.* 2002). Shark tissue samples of approximately 1 cm^3 were collected with a clean metal knife from each right pectoral fin from whole sharks and all kinds of dry fins (table 1). The tissue was kept on 95% ethanol at -20º C. Molecular identification of tissue samples were based on multiplex PCR-format of nuclear gene Internal Transcribed Spacer 2 (ITS2) region. All these procedures are detailed in Hernández *et al.* (2008).

Morphometric analysis. Morphometrical measurements were recorded for each species *P. glauca, I. oxyrinchus* and *L.*

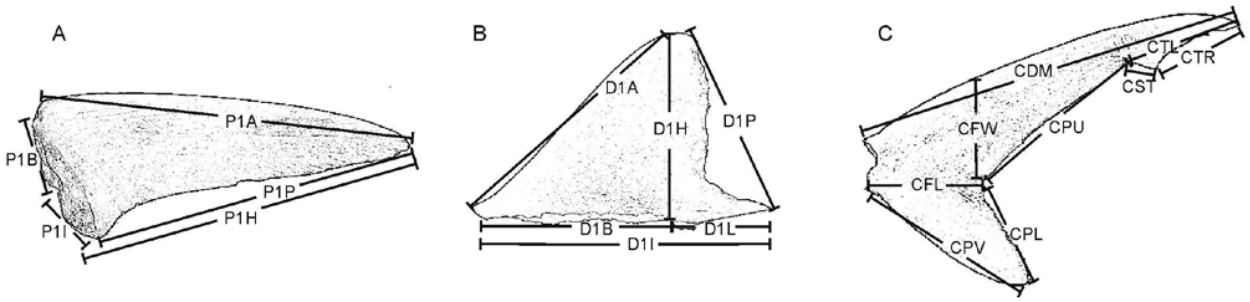

Figure 1. Diagrams of pectoral fin (A), first dorsal fin (B) and caudal fin (C) of sharks, showing the morphometrical measurements used for Principal Component Analyses and Discriminant-Function Analyses. P1A= Pectoral anterior margin; P1B = Pectoral base; P1P= Pectoral posterior margin; P1H = Pectoral height; and P1I = Pectoral inner margin; D1A= Dorsal anterior margin; D1P = Dorsal posterior margin; D1H = Dorsal height; D1B = Dorsal base; D1I = Dorsal inner margin; and D1L = Dorsal length; CDM = Dorsal caudal margin; CTR = Terminal caudal margin; CTL = Terminal caudal lobe; CST = Subterminal caudal margin; CPU = Upper postventral caudal margin; CFW = Caudal fork width; CPL = Lower postventral caudal margin; CPV = Preventral caudal margin; and CFL = Caudal fork length.

Table 1. Sample size of fresh and dry fins of the right pectoral, first dorsal and caudal, or lower lobule of caudal fins, of *Prionace glauca*, *Isurus oxyrinchus* and *Lamna nasus*. The values between parentheses, without parentheses and with asterisk correspond to the sample sizes used for the DNA-based species identification, morphometric analysis and image analysis respectively.

Data set	*Type of fins*	*P. glauca*	*I. oxyrinchus*	*L. nasus*	Total
Fresh fins	Pectoral	15(80)	15(28)	10(7)	45(115)
	First dorsal	15	15	9	39
	Caudal	15	15	10	45
Dry fins	Pectoral	16(16)	16(16)	15(15)	47(47)*
	First dorsal	16(16)	16(16)	15(15)	47(47)*
	Lower lobule caudal	16(16)	16(16)	15(15)	47(47)*

nasus following Compagno (2002) (figure 1): five measurements of the right pectoral fin, six of the first dorsal fin and nine of the fresh caudal fin, and only three measurements of dry ones. It because the shark fins traders only deal with the lower lobule of the caudal fins (for shark fin soup purposes). Fresh and dry fins were measured, and each data set was analyzed separately. Previous to multivariate analyses, correlations between body size and the respective fin measurements were performed to adjust for the effect of differences in body sizes among shark species. Then, we calculated the ratios between each fin measurement and the measured variable that is more correlated with body size. Thus, each morphometrical variable was divided by P1A for pectoral fins; by D1L for dorsal fins; and by CTL for caudal fins (see figure 1). In order to evaluate differences of each fin type for the studied species, and considering that the morphometrical measurements of the fins are highly correlated variables, Principal Component Analyses (PCA), based on correlation matrices of the previously log-transformed morphometric data, was performed. The importance of the original variables to explain the morphometrical patterns among shark species fins was obtained from the factor loadings in PCA. Then, the orthogonal variables (principal components or factors), which keep the original information, were used to perform Discriminant Function Analyses (DFA) (Legendre & Legendre, 1998; Vivanco,1999; Quinn & Keough, 2002). With DFA, the ability of correct classification of the different fins between shark species was assessed. All statistical analyses were performed using STATISTICA 6.0.

IMAGE ANALYSIS OF SHARK FINS.

Image analysis was based on digital invariant correlations using species-specific composite filters (Álvarez-Borrego & Fájer-Ávila, 2006), which include information of morphological variations of each target species (*P. glauca, I. oxyrinchus and L. nasus*). Color images of each of the studied fins (right pectoral, first dorsal and lower lobule of the caudal fins) of the three shark species considered were obtained using a high resolution digital camera (Nikon Coolpix 8400) and later transferred to the computer for its analysis. The size of the images $(f(x,y))$ was 500 x 500 pixels. Since fins have variable morphologies (e.g. according to size, finning process, etc.), this information needs to be included in the composite filters, where the composite filters are the fins to be recognized, according to the species to be studied, using the shape information.

The composite filter for each fin type per species was built using several images randomly chosen, that correspond to different random fixed views for each single shark fin, denoted as $f_1(x,y), f_2(x,y),..., f_n(x,y)$. An inverse Gaussian filter G(x,y), was applied to each image to only enhance the high frequencies. The filter is given by the equation:

$$G(x,y) = \left| \exp\left[\frac{(x-x_o)^2+(y-y_o)^2}{2\sigma^2} \right] - 1 \right|$$

where x_0 and y_0 are the center of the image to be analyzed. The term σ^2 gives the filter's width. After this process, a new image is recovered where it is possible to see the enhancement of the image's fine details. For each image, the modulus of the fast Fourier transform was calculated to assess position invariance, $|F_1(w_x,w_y)|, |F_2(w_x,w_y)|, ..., |F_n(w_x,w_y)|$, (in other words, the input image must not be necessarily in the center of the field of the image) where F is the variable defining the frequency of the image and w defines the coordinates in x and y directions. Then, all the modules are added to get a single module containing several random fixed views. This provides, in a single matrix, all the frequency information related to the morphology of the species to be recognized. In order to recognize shark fins independently of rotation, the Cartesian coordinates (w_x, w_y) were mapped to the polar coordinates defined by $F(r,\theta)$. In addition, a bilinear interpolation of the coordinate conversion data was introduced (for details see Pech-Pacheco et al., 2003). This was done to minimize sampling error, which affects the identification of the "unknown" species. Finally, the Fourier transform was applied only considering the phase information, and the resultant image represents the species-specific composite filter denoted by $S_{cpf}(U_\rho, V_\theta)$. In order to test the accuracy of the species-specific composite filters, we used sets of dry fins previously characterized using DNA based markers.

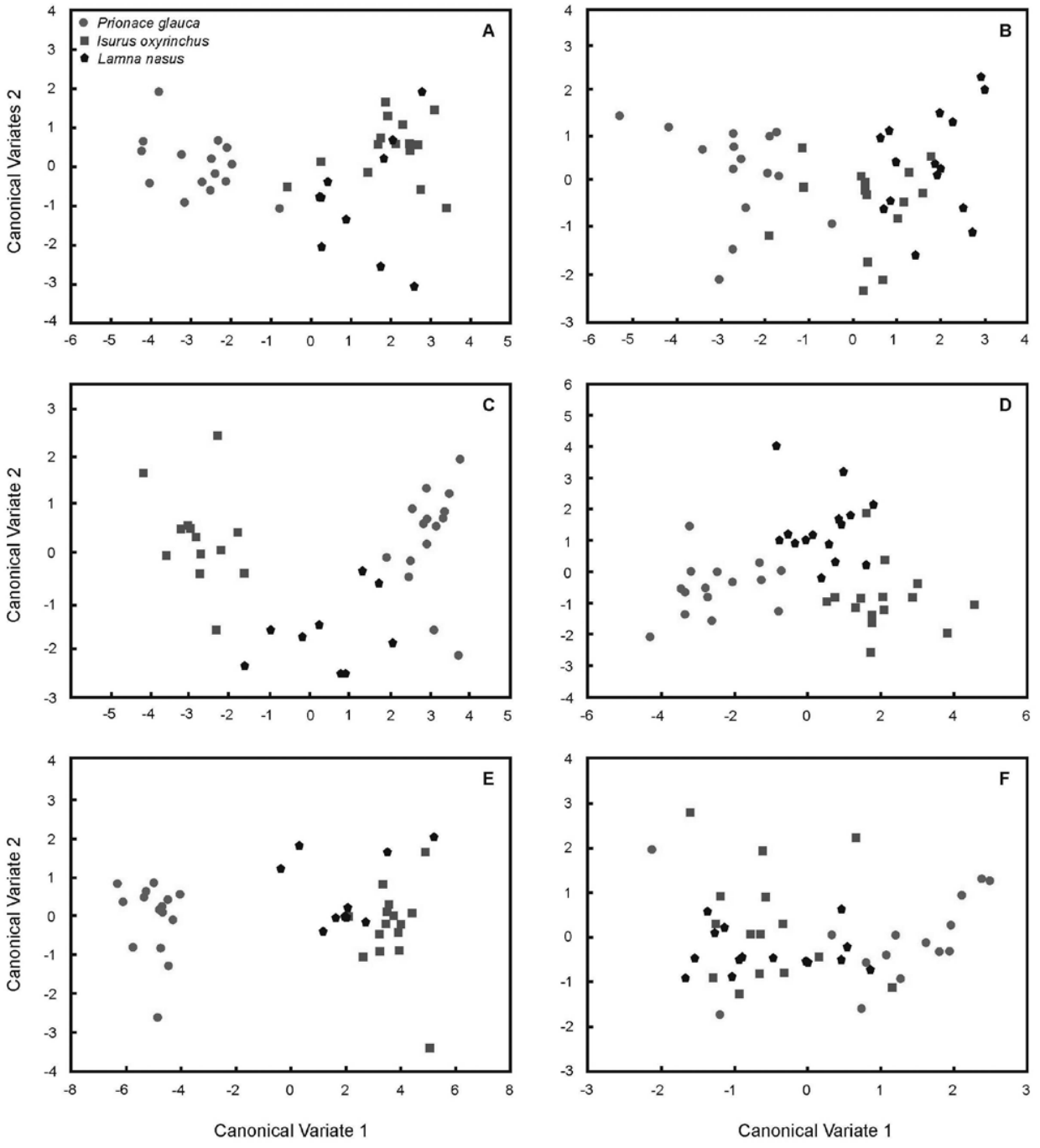

Figure 2. First and second canonical variates of morphometric data of the: (A) fresh pectoral fin, (B) dry pectoral fin, (C) fresh first dorsal fin, (D) dry first dorsal fin, (E) fresh caudal fin, and (F) dry caudal fin of *P. glauca*, *I. oxyrinchus* and *L. nasus*.

To discriminate between different shark species fins, images of each individual to be recognized were transformed as described above, except that in the first step a single image provides the input. Finally, the invariant digital correlation was made between the shark fins to be recognized and the individual species-specific composite filters. The digital correlations were performed by an algorithm specifically built by us using MATLAB software (The Mathworks, Inc.). Inside the invariant digital correlation box, the positive correlation (same shark species) is displayed by a high central unique correlation peak; in contrast,

Table 2. Factor loadings of morphometrical data of pectoral fins from PCA based on correlation matrix. Abbreviations of the ratios measurements are detailed in legend of figure 1.

	Fresh pectoral fins		Dry pectoral fins	
	Factor 1	Factor 2	Factor 1	Factor 2
P1B/P1A	0.800	-0.043	0.780	-0.405
P1P/P1A	-0.728	-0.274	-0.452	-0.718
P1H/P1A	-0.837	0.385	-0.700	-0.496
P1I/P1A	0.166	0.947	0.619	-0.573

Table 3. Factor loadings of morphometrical data of dorsal fins in the first two axes from PCA based on correlation matrix. Abbreviations of the ratios measurements are detailed in legend of figure 1.

	Fresh dorsal fins		Dry dorsal fins	
	Factor 1	Factor 2	Factor 1	Factor 2
D1A/D1L	0.647	0.568	0.879	0.277
D1P/D1L	0.805	0.126	0.870	0.284
D1B/D1L	0.889	-0.404	0.856	-0.500
D1I/D1L	-0.776	0.576	-0.839	0.524
D1H/D1L	0.887	0.380	0.811	0.465

Table 4. Factor loadings of morphometrical data of caudal fins (fresh ones) or lower lobule of caudal fins (dry ones) in the first two axes of PCA based on correlation matrix. Abbreviations of the ratios measurements are detailed in legend of figure 1.

	Fresh caudal fins		Dry caudal fins	
	Factor 1	Factor 2	Factor 1	Factor 2
CDM/CTL	-0.979	-0.016		
CTR/CTL	-0.673	-0.371		
CST/CTL	0.428	-0.864		
CPU/CTL	-0.968	-0.163		
CFW/CTL	-0.984	0.008		
CPL/CTL	-0.976	-0.011		
CPV/CTL	-0.985	0.052	0.940	0.338
CFL/CTL	-0.976	0.005	0.940	-0.338

the negative correlation does not show a significant correlation peak (different shark species).

RESULTS

Species-specific markers. The DNA-based identification analyses carried out on the pelagic shark species using species-specific primers shows that 100% of the samples identified from the fresh and dry sets of fins as (1) blue sharks were *P. glauca*, (2) shortfin makos were *I. oxyrinchus,* and (3) porbeagles were *L. nasus.*

Morphometric discrimination. The different PCAs carried out indicated that generally over 90% of the morphometrical variance in fins were explained by the first two PCA axes. The morphometric characters of the fresh pectoral fins used in the multivariate analyses were useful to distinguish clearly *P. glauca* fins from the ones of the other two shark species considered in the study (Wilks' Lambda= 0.126; $F_{(8,68)}$= 15.39; p <0.001). However, an overlap between the fins of *I. oxyrinchus* and *L. nasus* was observed (figure 2A). Similarly, dry pectoral fins of the three shark species showed significant differences in their form (Wilks' Lambda= 0.184; $F_{(8,78)}$= 12.97; p <0.001), with a little overlap between *I. oxyrinchus* and *L. nasus* (figure 2B). For fresh pectoral fins, in the first component (= Factor 1), the ratios pectoral base/pectoral anterior margin (P1B/P1A), pectoral height/pectoral anterior margin (P1H/P1A) and pectoral posterior margin/pectoral anterior margin (P1P/P1A) showed high loadings, but in Factor 2 the P1I/P1A ratio contributed most to the distinction between species, whereas ratios P1B/P1A, P1I/P1A and P1H/P1A explained better the differences among dry fins (figure 1; table 2).

The morphometry of the first dorsal fin (both fresh and dry sets) revealed significant differences among the studied shark species (Wilks' Lambda= 0.04; $F_{(10,54)}$= 19.608; p <0.001 for fresh fins; and Wilks' Lambda= 0.098; $F_{(10,76)}$= 16.590; p <0.001 for dry fins) (figure 2C and 2D). In the first component, all variables showed high loadings, while in Factor 2 the variable that most contributed to the differentiation between groups was the dorsal inner margin (D1I) for fresh and dry fins, respectively (figure 1; table 3).

In the case of fresh caudal fins, the morphometric proportions allowed to clearly distinguish the fins of the three species (Wilks' Lambda= 0.086; $F_{(16,60)}$= 36.503; p <0.001; figure 2E). However, the next three measurements recorded for the lower lobule of dry caudal fins: lower postventral caudal margin (CPL), preventral caudal margin (CPV), and caudal fork length (CFL), were insufficient to distinguish between lamnid species and showed high overlap between *I. oxyrinchus*, *L. nasus* and *P. glauca* (Wilks' Lambda= 0.569; $F_{(4,80)}$= 6.505; p <0.001; figure 2F). For fresh caudal fins, most morphometrical measurements were important in the differentiation between species (figure 1, table 4)

Between 87.5% and 80% of the fresh and dry fins were classified correctly (Tables 5). For fresh pectoral fins of lamnid species only 70% of the assignments were correct. For dorsal fins, the correct classification was high for the shark species, and the assignments were consistent for fresh and dry fins with 94.1% and 93.3%, respectively. Finally, the 100% of fresh caudal fins were correctly assigned to the respective shark species. In contrast, the correct classification of dry lower lobule of caudal

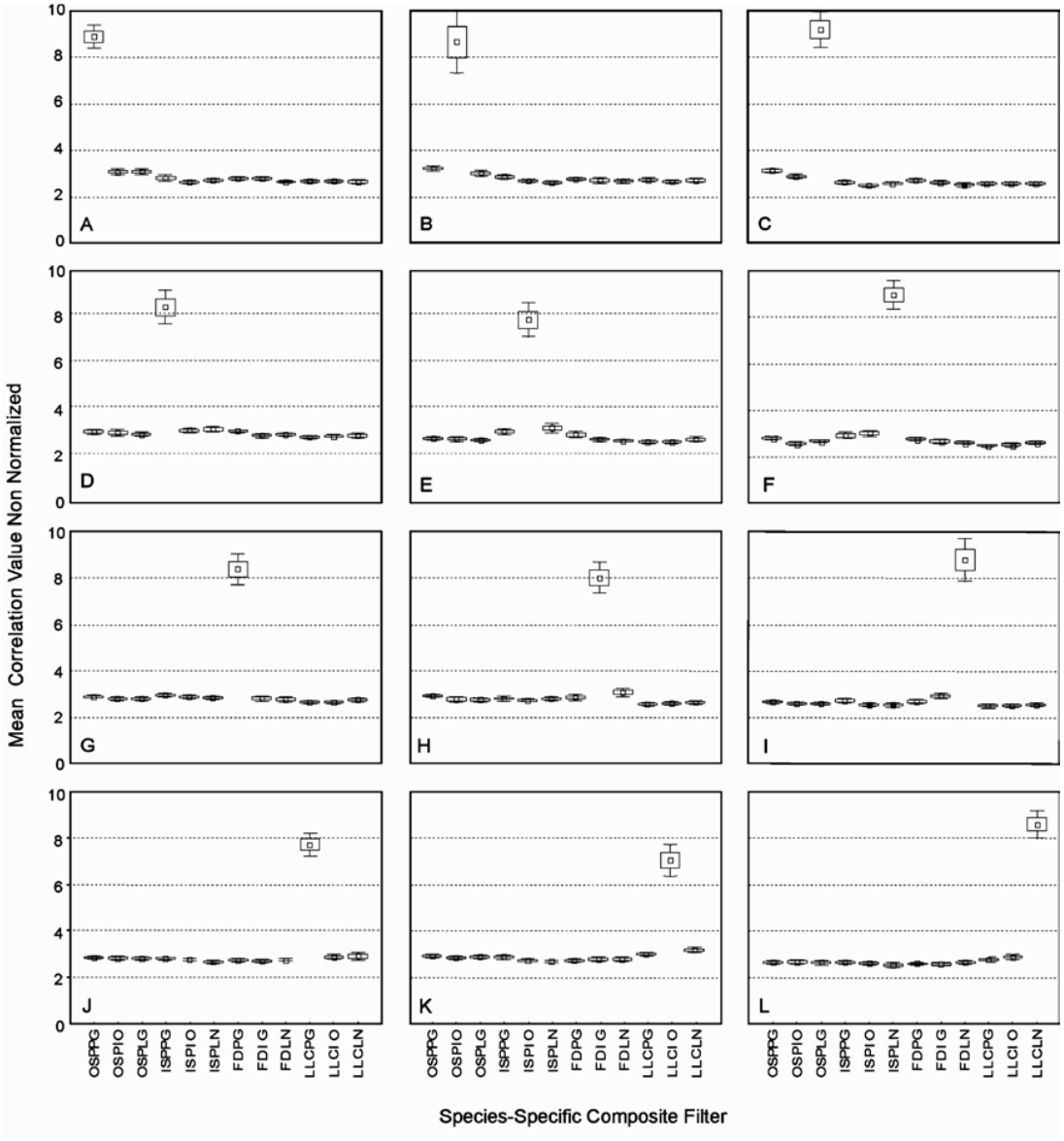

Figure 3 A-L. Mean correlation values of diffraction patterns of studied shark fins using the following species-specific composite filters: OSPPG, outer side pectoral *P. glauca*; OSPIO, outer side pectoral *I. oxyrinchus*; OSPLG, outer side pectoral *L. nasus*; ISPPG, inner side pectoral *P. glauca*; ISPIO, inner side pectoral *I. oxyrinchus*; ISPLN, inner side pectoral ***L. nasus***; FDPG, first dorsal *P. glauca*; FDIG, first dorsal *I. oxyrinchus*; FDLN, first dorsal *L. nasus*; LLCPG, lower lobule of caudal *P. glauca*; LLCIO, lower lobule of caudal *I. oxyrinchus*; LLCLN, lower lobule of caudal *L. nasus*. Boxes represent ±1 SE, whiskers ±2 SE.

fins was low for lamnids, only 50% and 53% for *L. nasus* and *I. oxyrinchus* respectively.

Digital imaging analyses. The numerical simulation performed on fin type revealed that all the composite filters built for *P. glauca*, *I. oxyrinchus* and *L. nasus* performed well (100%) when they were correlated with the modules of right pectoral (outer and inner side), first dorsal and lower lobule of caudal fin belonging to the same shark species (figure 3).

In all cases the identification of the shark species was successful. Thus, with this method is possible to identify with a 100 % of confidence level the different shark species.

DISCUSSION

The three methods used to identify fin-type for *P. glauca*, *I. oxyrinchus* and *L. nasus* (species-specific DNA-based markers, morphometric analyses, and digital invariant correlation) showed

Table 5. Percentages of correct assignment of species *(Prionace glauca*, *Isurus oxyrinchus* and *Lamna nasus*) by using DFA. The values between parentheses and without parentheses correspond to the fresh fins and dry ones respectively.

True Identity		Predicted identity		
Pectoral	*P. glauca*	*I. oxyrinchus*	*L. nasus*	Percentage correct
P. glauca	15(14)	0(1)	0(0)	100(93.3)
I. oxyrinchus	0(2)	13(10)	2(3)	86.6(66.6)
L. nasus	0(0)	3(3)	7(12)	70.0(80.0)
Total	15(16)	16(15)	9(15)	87.5(80.0)
Dorsal	*P. glauca*	*I. oxyrinchus*	*L. nasus*	Percentage correct
P. glauca	15(14)	0(0)	0(1)	100(93.3)
I. oxyrinchus	0(0)	13(14)	0(1)	100(93.3)
L. nasus	2(0)	0(1)	4(14)	66.6(93.3)
Total	17(14)	13(15)	4(16)	94.1(93.3)
Caudal	*P. glauca*	*I. oxyrinchus*	*L. nasus*	Total
P. glauca	15(13)	0(1)	0(1)	100(86.6)
I. oxyrinchus	0(2)	15(8)	0(5)	100(53.3)
L. nasus	0(4)	0(3)	10(7)	100(50.0)
Total	15(19)	16(12)	9(13)	100(63.3)

differential abilities as tools to discriminate between shark species. The fins' morphometry indicated that *P. glauca* was the species most clearly discriminated according to fin shape and size. The morphometrical record of right pectoral, first dorsal and caudal fin showed that the components of the multivariate analysis, in particular, the use of the first two canonical variates, explain 90% of the morphological variation. In other words, the variations in the morphological measurements of the fins of *P. glauca* allow their differentiation with 100% of reliability, respect to the other lamnid shark fins (*I. oxyrinchus* and *L. nasus*). However, the similarity in fin morphometrical characteristics hinders the accurate identification of *I. oxyrinchus* and *L. nasus*. This was confirmed by the classification matrix results for the fresh right pectoral and fresh first dorsal fins, where some specimens of *I. oxyrinchus* were classified as *L. nasus* and vice versa. Therefore, only the morphometrical proportion measurements (figure 1C), which were used in this study for the fresh caudal fins are 100% effective as a shark species recognition tool.

Image analysis is particularly helpful because it can be used in automated systems integrating a huge amount of information in digital format. So far, image processing has been widely used in biological research, including individual shark recognition (Arzoumanian *et al.*, 2005). The results obtained herein using an invariant digital correlation show that this technique is able to differentiate fins of different shark species. Whereby, both specific coloring patterns and shape were combined with morphological differences of the right pectoral (outer and inner side), first dorsal and lower lobule of caudal fins. The invariant correlation obtained clearly distinguishes the fins of the three studied species, *P. glauca*, *I. oxyrinchus* and *L. nasus*. The main reason of their discriminatory power was that the composite filter built for each species contains a combination of diagnostic morphological characters with specific coloring patterns. Apparently, the fins of *I. oxyrinchus* and *L. nasus* have few distinguishing characters, but the two species can be differentiated using a combination of different morphological characters. The main diagnostic characteristic to distinguish between these species is the coloring pattern of the inner side of the pectoral fin. In *I. oxyrinchus* it is white with a rounded black edge on the posterior margin, whereas *L. nasus* has a remarkable black coloring pattern and a barely rounded black front edge (Anonymous, 1999; Hernández *et al.*, 2008). Additionally to the diagnostic characteristic above described, the outer fin's side differs in the skin texture and in the presence/absence of loose hanging threads in the posterior edge. *Isurus oxyrinchus* exhibits a smooth skin texture without the presence of loose hanging threads in the posterior margin of fin, whereas *L. nasus* shows rough skin texture with the presence of loose hanging threads (Hernández *et al.*, 2008). Other characters, such as the coloring patterns in the underside region of keel, could also be useful for identification because *I. oxyrinchus* has a white coloring, while *L. nasus* has a completely black coloring. Finally, both lamnid species can also be distinguished by the first dorsal and caudal fins. *Lamna nasus* has a light free rear tip on the first dorsal fin and secondary keels in the caudal fin, while *I. oxyrinchus* does not (Compagno, 2002).

DNA-based markers have demonstrated their utility for effective forensic identification of shark individuals from market

places in order to determine the composition and proportion of species (Clarke *et al*., 2006). Proportion of shark species identified using the DNA-based markers agree with data of bycatch shark species associated to Chilean swordfish fishery and the shark species linked to pelagic shark fins traded in north-central Chile (Acuña *et al*., 2002, Hernández *et al.,* 2008). DNA-based species identification is also concordant with data known for the commercial shark fishery worldwide (Castro, 1993; Bonfil, 1994; Buencuerpo *et al*., 1998; Walker, 1998; Stevens *et al*., 2000). Approximately five families of pelagic shark species are caught, traded and exported from Chile (carcharinids, lamnids, aloppids, sphyrnids and possibly hexanchids). However, the quantitative estimate of shark fin landing and trading has been restricted to *P. glauca*, *I. oxyrhinchus* and *Lamna nasus* whereas the rest of shark species is unknown due to the general lack of species-specific records (Lamilla *et al*., 2005; Hernández *et al*., 2008).

Another advantage of the DNA-based identification is that it has high recognition power even when only fragmented body parts such as shark fins are available. The greatest advantage of this approach is that it could independently identify shark species, despite alterations or modifications due to handling or finning process.

Our study demonstrates that DNA-based and image analyses can be both useful to identify shark species. Morphological diagnostic patterns associated to coloring patterns, texture, and presence of anatomic structures (loose hanging threads and keels) allows the correct identification of shark species. However, the diagnostic characters should be prudently used since most of individuals studied here were juvenile sharks. In the absence of molecular tools, both image analyses and/or fresh caudal fin morphometry can be useful for monitoring landed shark fins. This information is crucial to orient the implementation of future shark conservation measures and management plans in Chile and other regions were the studied shark species are common.

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