Molecular differentiation of the species of two squid families (Loliginidae and Ommastrephidae) based on a PCR study of the 5S rDNA gene

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A B S T R A C T

The present study aimed to demonstrate the effectiveness of the 5s rDNA gene for the identification of commercially-valuable species of cephalopod belonging to the families Loliginidae and Ommastrephidae. Our results demonstrate distinct banding patterns in each of the six species sampled (Loligo surinamensis; Loligo sompuresis; Loliguncula brevis; Sepioteuthis sepioida; Omithoteuthis antillarum; illex argentinus), as well as diagnostic traits at the genus and probably family levels. The results emphasize the efficiency of the 5s rDNA marker as a low-cost and rapid forensic technique, which not only permits the identification of species, but also differentiation of members of the Loliginidae and Ommastrephidae.

1. Introduction

Cephalopods represent an important fishery resource, corresponding to 3.6% of the total marine tonnage landed worldwide in 2001. Squid — including both coastal and oceanic species — make up around 70% of the cephalopod catch (FAO, 2000). Much of the catch is sold in processed form, which involves removal of the intestines and skin, the separation of the head, arms, tentacles and fins, and in the case of squid, the slicing of the body into rings (calamari). This processing obviously hampers the identification of the species, or even the genus, in some cases (Chapela, Sotelo, & Pérez-Martin, 2003).

This practice also facilitates the substitution of more expensive species (such as those of the family Loliginidae) by cheaper ones, generally members of the family Ommastrephidae (Chapela et al. 2002). Even considering only the ommastrephids, many species are marketed as higher-quality, more expensive ones, especially in Europe, the principal market. Some of the morphological traits used for the identification of cephalopod species are influenced by sexual maturity, age and gender, while others, such as the hectocotylus, are found only in mature males (Roper & Mangold, 1998).

Recent advances in molecular techniques have greatly enhanced the identification of mollusk species, in particular the cephalopods (Brierly & Thorpe, 1994; Colombo et al., 2002; Santaclara, Espiñeira & Vieites, 2007; Leite, Haimovici, Molina & Warnke, 2008; Warnke, Soller, Blohm & Saint-Paul, 2000). The majority of studies have involved the identification of processed fishery products, based on comparative analyses of fragment length of mitochondrial DNA or nuclear genes, in particular 5S rDNA (Carrera et al., 1999; Céspedes et al., 1999; Cocolin, D’Agoro, Manzano, Lanari & Comi, 2001; Pinhal et al., 2008; Quinteiro et al., 1998; Russel et al., 2000; Wolf, Rentsch & Hübner, 1999).

The 5S ribosomal DNA is a small molecule forming part of the large subunit of the ribosome. In eukaryotic species it is typically encoded by hundreds to thousands of gene copies located in large arrays of tandem repeats (5S rDNA) at one or more chromosomal sites (Long & Dawid, 1980). The repetitions of the 5S rDNA gene consist of highly-conserved coding sequences of 120 base pairs, which are separated from one another by variable non-transcribed spacers, or NTSs (Long & Dawid, 1980; Wasko, Martins, Wright & Galetti-Jr., 2001). This region is considered to be especially appropriate for PCR-based genetic studies, due to a number of considerations, which include: the gene is highly conserved, even in distantly-related species, allowing the isolations of its repetitions in different species using PCR analysis; given their relatively small size and tandem arrangement, the repetitions may be isolated from DNA of reduced quality (Martins & Wasko, 2004); The 5S rDNA gene copies are organized in tandem array and dispersed all over genome been distributed in different chromosomes (Martins & Wasko, 2004).

Species identification using 5S rDNA is based on the amplification of fragments containing the gene together with non-transcribed...
spacers (NTSS). The variation found in the fragments amplified is due to the presence of these NTSS (Martins & Wasko, 2004), which evolve relatively rapidly, with substitution rates comparable to those of pseudogenes or nonfunctional sequences (Li, Luo, & Wu, 1985; Pasolini, Costagliola, Rocco & Tinti, 2006).

Other authors have argued that the interspecific variation in the length of NTSS may also be caused by the presence of insertions and/or deletions, mini repetitive sequences, or even pseudogenes themselves (Sadjak, Reed & Phillips, 1998).

The usefulness of the 5S rDNA gene as a species-specific marker has been assessed for a number of different groups of fishes (Asensio et al., 2001; Insiridou, Minos, Katsares, Karaikou & Tsiora, 2007; Karaikou, Triantafyllidis & Triantaphyllid, 2003; Pinhal et al., 2008). This marker has not yet been evaluated in the cephalopods, however, which not only present highly-conserved characteristics in some species, but are also often marketed as a processed product, which hinders species identification. Given this, the present study aimed to demonstrate, for the first time, the efficiency of 5S rDNA for the identification of the cephalopod species of two commercially-important families (Loliginidae and Ommastrephidae).

2. Materials and methods

2.1. Collection of samples and DNA extraction

Tissue samples were collected from six squid species. (Table 1). Five species were collected locally in northern Brazil, where they were caught as by-catch by trawlers fishing. The sixth species, Illex argentinus, was obtained from the local supermarket as a whole frozen specimen and some samples were collected by trawlers fishing in RS state. The samples were preserved in 100% ethanol and stored in a −4 °C freezer until analysis. For extraction, the tissue was first washed, and then centrifuged twice in 600 μl of distilled water. The DNA was then extracted using Sambrook and Russell. (2001) standard phenol/chloroform protocol.

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Common Name</th>
<th>Origin</th>
<th>Number of samples analyzed in the present study</th>
</tr>
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<tbody>
<tr>
<td>Loliginidae</td>
<td>Loligo samponensis</td>
<td>São Paulo squid</td>
<td>Rio Grande, Rio Grande do Sul</td>
<td>25</td>
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<td></td>
<td>Brakoniecki, 1984</td>
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<tr>
<td></td>
<td>Loligo surinamensis</td>
<td>Surinam squid</td>
<td>Bragança, Pará</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Voss, 1974</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Loliguncula brevis</td>
<td>Western Atlantic brief squid</td>
<td>Caravelas, Bahia</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Blainville, 1823</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sepioteuthis septioidea</td>
<td>Caribbean reef squid</td>
<td>Barra Grande, Bahia</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Blainville, 1823</td>
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<tr>
<td>Ommastrephidae</td>
<td>Ornithoteuthis antillarum</td>
<td>Atlantic bird squid</td>
<td>Bragança, Pará</td>
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</tr>
<tr>
<td></td>
<td>Adan, 1957</td>
<td></td>
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</tr>
</tbody>
</table>

2.2. Polymerase chain reaction (PCR)

The repetitions of the 5S rDNA gene were amplified using the following primers: S5A (5'-TACGCCGATCCTCGCCAT-3') and S5B (5'-CAGGCTGTATGCGCGTAAAC-3') (Péndas, Mórán, Freije, & García-Vazquez, 1994). Each PCR reaction consisted of a mixture of 0.5 μl of each primer (S5A = 5 pmol/μl, S5B = 5 pmol/μl), 1 μl of MgCl2 (50 mM), 4 μl of the dNTP mixture (1.25 mM), 2.5 μl of the 10x buffer (Invitrogen, Carlsbad-CA USA – Tris–HCl and KCl, pH 7.8), 0.2 μl of Taq polymerase (5 U/μl: Invitrogen), approximately 100 ng of total DNA, and purified water to complete the final volume of 25 μl. The amplification protocol was 4 min at 95 °C for denaturation; 35 cycles of 20 s at 95 °C, followed for 1 min at 55 °C for annealing, 30 s at 72 °C for extension, and a final extension at 72 °C for 7 min.

2.3. Electrophoresis

The PCR products were stained with 0.5 μl ethidium bromide and electrophoresed in 1% agarose gel (Agarose D-1 Low EEO, CONDA Laboratories, Madrid-Spain), using the following conditions: V = 50, mA = 110, and W = 200 for 60 min. The gel was subsequently verified and photodocumented in a Vilber Lourmat UV transilluminator coupled to a Vilber Lourmat digital camera with a 51 mm lens (Vilber Lourmat, Marne-la-Vallée-France). A 1 kb Plus DNA ladder (Life Technologies, Inc. Carlsbad-CA USA) was used to estimate the width of each band observed during electrophoresis.

3. Results and discussion

The present study confirmed the use of the 5S rDNA gene for identification of species. The repetition pattern of the 5S rDNA gene provided the basis for the identification of six species of squid, using only a single gel run. Similar levels of precision have been recorded in studies which have identified subspecies of mice, species of fish, and plants (Céspedes et al., 1999; Linder, Moore & Jackson, 2000; Suzuki, Morigaki & Sakurai, 1994). The amplification of the repetitive 5S rDNA sequences generated distinct patterns among species, but no intraspecific variation was found in the present study (data not shown) even when the same species was collected from different localities as in the case of Illex argentinus. This pattern has also been observed in a number of other studies that used the same marker (Martins & Gatti-Jr., 2001; Martins & Wasko, 2004; Martins et al., 2002; Karaikou et al., 2003; Pinhal, Araki, Gadig, & Martins, 2009).

In the species analyzed here, fragments varied in size from 200 to 1000 base pairs (bps) (Fig. 1). Loligo surinamensis presented five bands, one with 1000 bps, one with more than 650 bps, one with approximately 450 bps, a band with only 200 bps and finally a band with 150 bps. A similar configuration was observed in Loligo


