A Real-Time PCR method for the authentication of common cuttlefish (*Sepia officinalis*) in food products

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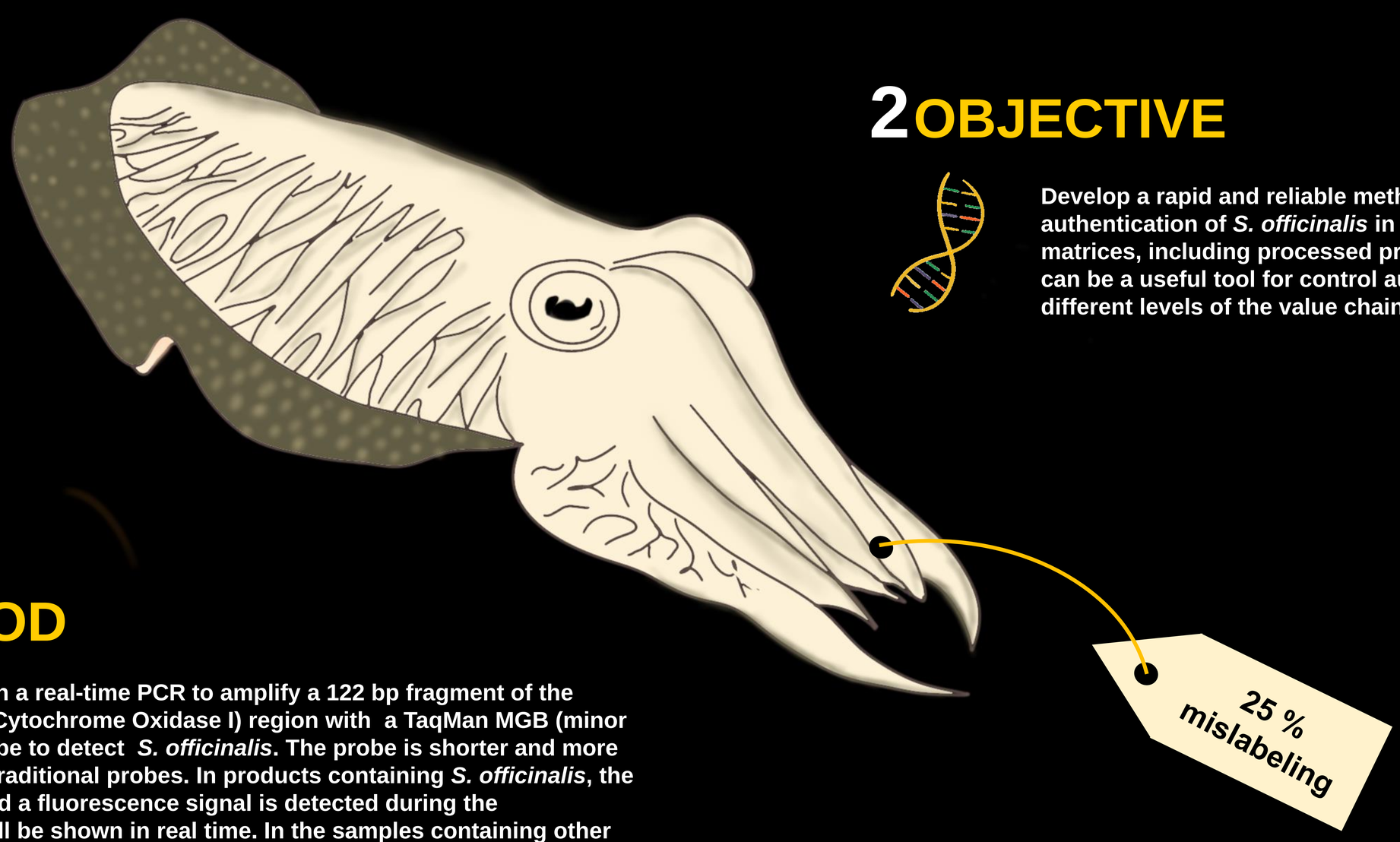
1 BACKGROUND

The common cuttlefish (*Sepia officinalis*) is a cephalopod highly appreciated by consumers around the world. It is traded with different presentations as fresh, frozen, canned, etc. European regulations regarding the labeling of fishery products establish the obligatory nature to show commercial and/or scientific name depending on the type of product. Processed (e.g., peeled, cut, cooked and canned) makes the visual differentiation almost impossible. Illicit substitutions of one species for another may constitute economic fraud and/or misbranding violations due to incorrect mislabeling.



2 OBJECTIVE

Develop a rapid and reliable method for the authentication of *S. officinalis* in different food matrices, including processed products, that can be a useful tool for control authorities at different levels of the value chain.

3 METHOD

The method relies on a real-time PCR to amplify a 122 bp fragment of the mitochondrial COI (Cytochrome Oxidase I) region with a TaqMan MGB (minor groove binding) probe to detect *S. officinalis*. The probe is shorter and more specific than other traditional probes. In products containing *S. officinalis*, the probe hybridates and a fluorescence signal is detected during the amplification and will be shown in real time. In the samples containing other species, there is no signal or it appears weakly in the last cycles.

43 reference samples of *S. officinalis* and 16 commercial samples (frozen, canned, cooked and thawed and grilled) were tested with this new method.

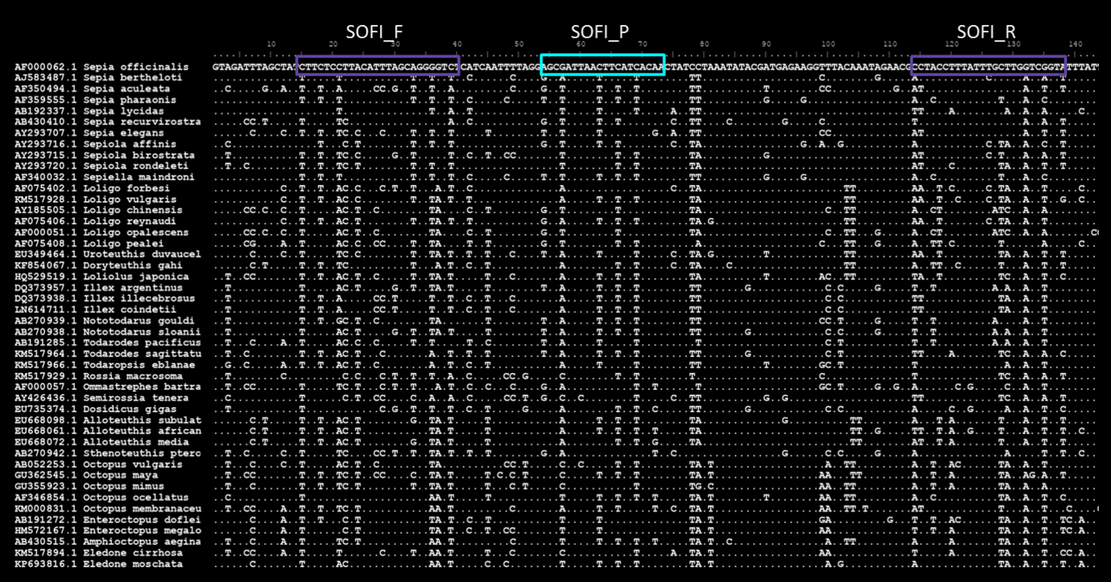


Figure 1. Multiple sequence alignment of the mitochondrial COI (Cytochrome Oxidase I) fragment, showing the position of the primers (SOFI_F and SOFI_R) and probe (SOFI_P) designed.

4 MAIN RESULTS

The MGB TaqMan real-time PCR system was able to differentiate between products containing *S. officinalis* and products containing other species of the Sepiidae family, with statistical significance ($p < 0.001$), based on Ct values. The type of processing did not affect the differentiation not even in highly processed samples such as canned.

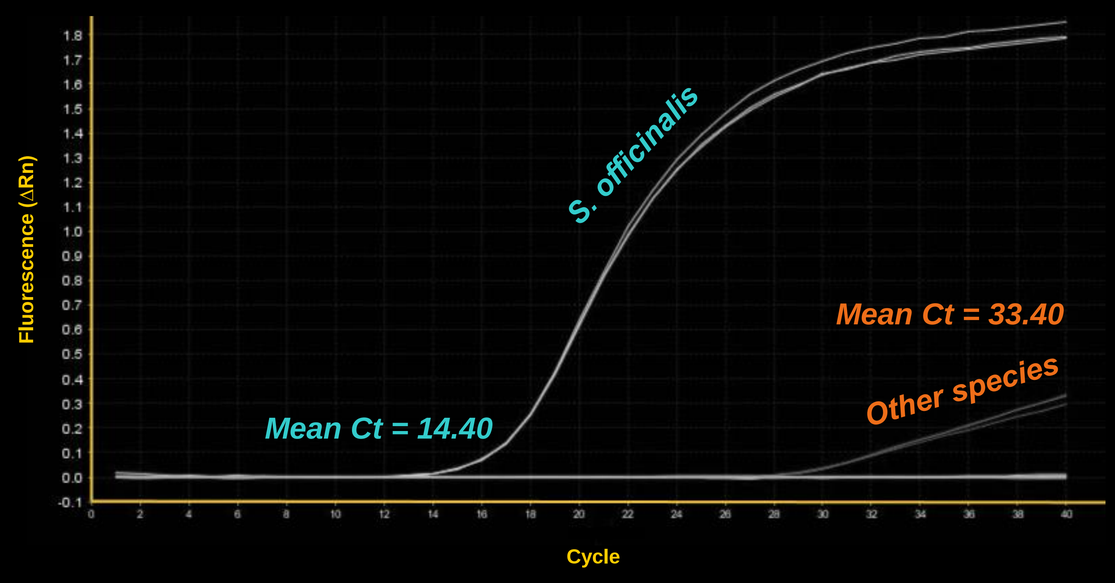


Figure 2. Specificity test: amplification pattern of reference samples of *Sepia officinalis* and the rest of the species

5 CONCLUSIONS

- **The developed method is able to differentiate *S. officinalis* from other species of the genus Sepia and other cephalopod species in food samples, including those highly processed. It is a rapid (the complete analysis from the tissue sample can be carried out in 3 - 4 h), reliable and non-expensive (less than 5 € per sample) method.**
- **The level of mislabeling in Sepia products found (25%) highlights the need for a more efficient control of the authenticity of these products.**

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