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Oral presentation

## Emerging Risks in the European Seafood Chain: Molecular Identification of Toxic *Lagocephalus* spp. and Evaluation of the Public DNA Libraries Reliability in Supporting Species Identification<sup>#</sup>

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## Abstract:

Pufferfish may cause human intoxications due to Tetrodotoxin accumulation and their sale is banned in EU. In the last decades, some pufferfish (e. g. Lagocpehalus spp.) spread in the Mediterranean Sea through Lessepsian migration, representing an emerging risk within the seafood chain. Their illegal presence in mislabelled products is also reported. The identification of Lagocephalus spp. is hindered by morphological similarities among co-generic species and DNA amplification, sequencing and comparison to public DNA libraries is often required. This process can be affected by the presence of wrongly deposited sequences in the libraries. This study first aimed at finding a suitable molecular target for identifying all Lagocephalus spp. in commercial products. Moreover, the reliability of two the public libraries in supporting the discrimination of L. spadiceus and L. guentheri was assessed. All cytochrome oxidase subunit I (COI) and cytochrome b (cytb) genes sequences of Lagocephalus spp. were retrieved from official libraries and used to create phylogenetic trees depicting inter-species relationships. Given its higher inter-species variability, cytb was selected as target and 17 cytb sequences from 6 Lagocephalus spp. reference samples were produced. Then, a primer pair amplifying a 130bp cytb polymorphic fragment from all the Lagocephalus spp. was designed for identifying 16 mislabelled commercial products with degraded DNA. Also, 20 Mediterranean specimens of L. guentheri were collected; both COI and cytb sequences were ex-novo produced and phylogenetically analysed with the sequences from L. spadiceus and L. guentheri previously retrieved from official libraries. First, the presence of L. spadiceus in the commercial products was confirmed. Moreover, a scarce DNA libraries accuracy was observed since most sequences deposited as L. spadiceus of Mediterranean origin were instead misidentified L. guentheri, causing a significant underestimation of this species in the Mediterranean Sea. Overall, this study may provide a reliable tool to protect European consumers from the emerging risk associated to toxic pufferfish.

Keywords: Pufferfish, Lagocephalus spp., toxic species, mislabelling, Mediterranean environment

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