Abstract

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Parvalbumin (*pvalb*), a low molecular weight calcium-binding protein, plays a crucial role in regulating Ca^{2+} switching in fast-twitch muscle fibres and has been identified as a major cause of fish-induced food allergies. The molecular evolution of *pvalb* genes in teleost fish and its cause, duplication of the whole genome, was investigated, revealing high diversity and complex gene repertoires, making detection and identification challenging. This study provides robust genomic evidence of the complex evolution of parvalbumin genes in teleost fish.

In addition to its role as a potent allergen, the *pvalb* gene, a nuclear gene, can serve as a valuable molecular marker. Keeping this in mind, a real-time PCR assay is developed to detect and quantify two European anglerfish species simultaneously, *Lophius piscatorius* and *Lophius budegassa*, which are susceptible to illegal species substitutions in the global seafood trade. The assay targets the intronic region of the *pvalb* gene, demonstrating high specificity, efficiency, and robustness, making it a potential forensic tool to prevent food fraud and ensure the accurate identification of fish species. Furthermore, a standardised quantitative PCR-based method is presented for the β-*pvalb* gene in *Lophius piscatorius,* utilising a plasmid DNA calibrator as an alternative to conventional genomic standards for absolute gene quantification. The plasmid calibrator exhibits superior PCR efficiency, linearity, and stability, offering a reliable approach to precise gene quantification.

A new universal primer assay is introduced for the identification of fish species in forensic applications, employing the nuclear gene marker pvalb instead of traditional mitochondrial gene-based markers. This alternative detection method shows promise, particularly for closely related fish species without external identification features. The assay successfully detects, identifies, and quantifies *pvalb*, making it useful in species detection and allergen quantification.

This Ph.D. thesis thoroughly explores the multifaceted role of *pvalb* in fish identification and its applications in forensic investigations. It provides comprehensive information on the evolutionary diversity of *pvalb* genes in fish, the development of sensitive detection and quantification methods for fish allergens, and the application of nuclear gene markers in the identification of forensic fish species. The research results have significant implications for addressing food fraud, species mislabeling, and illegal fish substitutions, benefiting the seafood industry and public health.

Keywords

Parvalbumin, Fish allergen, Molecular evolution, Real-time PCR, Forensic applications, Species identification, Food fraud, Teleost fish, Plasmid DNA calibrator, Nuclear gene marker.