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学位論文内容の要旨

博士の専攻分野の名称：博士（水産科学）　氏名　Alan Hertz Marin Guerrero

学位論文題名

Studies on Mitochondrial Genome for Phylogenetic Inference and Species Identification in Pectinidae

イタヤガイ科貝類の系統推定と種判別に向けたミトコンドリアゲノムに関する研究

The family Pectinidae (scallops) are species of high economic value and comprises 350 extant species within four commonly accepted groups: three subfamilies, Chlamydinae, Palliolinae, Pectininae and one tribe Aequipectini. Scallops display unique genetic characteristics that make them very interesting candidates for evolutionary studies. For instance, all available scallop mitogenomes possess dramatic mitochondrial gene rearrangement as well as the lack of the gene *atp8*. In spite of the abundant fossil record extending from the early Triassic (250 mya: Hautmann 2010), the phylogenetic relationship among members of this family is poorly understood and is still a matter of debate. Thus, in order to obtain further insights into the very complex taxonomy and evolutionary relationships among Pectinidae species, more studies on the mitochondrial genomes of scallop species are needed.

This dissertation has been divided in three chapters. Chapter one explores the characteristics of newly developed scallop mitochondrial genomes from three species: *Argopecten purpuratus*, *Pecten maximus* and *Pecten albicans*. The novel mitogenomes were used to infer the phylogenetic relationships among 9 scallop species with available mitochondrial genome. Phylogenetic relations were analyzed by using the nucleotide information from all mitochondrial protein-coding genes and gene order arrangement. By determining the complete protein-coding region of *P. maximus* and *P. albicans*, a novel mitochondrial gene order arrangement from the subfamily Pectininae was uncovered. As a result, the mitochondrial genomes of the three scallop species studied are typical of marine bivalves. They contained 12 protein-coding genes, lack of *atp8*, display a large level of gene order rearrangement, and their complete molecule size vary among species. All Pectinidae mitochondrial genes are encoded in the same strand. Information obtained from scallop gene order arrangement revealed that their gene order is clearly reflected in their phylogenetic

relationship supporting sister subfamilies relationships as well as relationships among members of the same subfamily. The putative "polyphyletic" status of the genus *Amusium* was discussed. My results based on phylogenetic analysis of the complete mitochondrial 16S gene supported the convergence evolution of shells and life habits theory (Mynhardt et al., 2014) between *Amusium pleuronectes* and *Amusium japonicum*. Thus, both *Amusium* species are not congeneric, a reclassification of the *Amusium* genus is suggested.

Chapter two describes the development of a DNA barcoding assay based on the 5' end of the mitochondrial 16S gene for scallop species identification. A partial region at the 5' end of the mitochondrial COI gene, known as the "Folmer region", has been proposed as the most suitable DNA barcoding marker. However, Folmer primers have failed to amplify PCR products in different organisms, including scallops. Searching for an alternative barcoding gene region, I analyzed the complete mitochondrial 16S rRNA gene in 15 scallop species. My results showed that the interspecific variation at the 5' end is twice as high as that of the 3' end. Based on that evidence, novel Pectinidae family-specific primer set was designed, aiming to amplify a partial region at the 5' end of the 16S rRNA gene, and tested its suitability as barcoding tool. A neighbor-joining analysis identified correctly 100% of the scallop specimens analyzed, with high bootstrap support. The new primers are well suited for DNA barcoding analysis and may contribute to scallop food industry as well as routine taxonomic surveys.

Chapter three was focused in the application of the mitochondrial 16S rRNA gene for the designing of scallop species-specific primers and multiplex PCR assay for scallop species identification. Two different multiplex PCR protocols were designed based in both extremes of the 16S gene. However, the low interspecific variability at the 3' end of this gene has limited its utility and only a few scallop species were assessed. The highly variable 5' end of the 16S gene allowed the development of a novel decaplex PCR assay that enabled a fast and accurate identification of 9 commercially important scallop species in a single PCR reaction. To enhance the utility of this assay, the PCR product amplified by the family-specific primer set that was utilized as positive control was also used for the identification of unknown (non-target) scallop species by DNA sequencing analysis. In its present form, this multiplex PCR method can be of great utility for different kinds of studies involving scallop species and for research institutes and governmental agencies that regulate seafood authentication around the world.