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A molecular analysis of the interrelationships of tetraodontiform fishes (Acanthomorpha: Tetraodontiformes)

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Abstract

Tetraodontiform fishes (e.g., triggerfishes, boxfishes, pufferfishes, and giant ocean sunfishes) have long been recognized as a monophyletic group. Morphological analyses have resulted in conflicting hypotheses of relationships among the tetraodontiform families. Molecular data from the single-copy nuclear gene RAG1 and from two mitochondrial ribosomal genes, 12S and 16S, were used to test these morphology-based hypotheses. Total evidence (RAG1 + 12S + 16S), RAG1-only, and mitochondrial-only analyses were performed using both maximum parsimony and Bayesian criteria. Total evidence and RAG1-only analyses recover a monophyletic Tetraodontiformes. However, the relationships recovered within the order differ, and none completely conform to previous hypotheses. Analysis of mitochondrial data alone fails to recover a monophyletic Tetraodontiformes and therefore does not support any of the morphology-based topologies. The RAG1 data appear to give the best estimate of tetraodontiform phylogeny, resulting in many strongly supported nodes and showing a high degree of congruence between both parsimony and Bayesian analyses. All analyses recover every tetraodontiform family for which more than one representative is included as a strongly supported monophyletic group. Balistidae and Monacanthidae are recovered as sister groups with robust support in every analysis, and all analyses except the Bayesian analyses of the mitochondrial data alone recover a strongly supported sister-group relationship between Tetraodontidae and Diodontidae. Many of the intrafamilial relationships recovered from the molecular data presented here corroborate previous morphological hypotheses.

Keywords: RAG1; 12S; 16S; Phylogenetics; *Takifugu*; *Tetraodon*

1. Introduction

Order Tetraodontiformes comprises approximately 350 species of fishes displaying an incredible range of morphological diversity (Santini and Tyler, 2003). The group consists primarily of tropical and subtropical marine reef-dwellers but also includes species that are entirely pelagic, some that are benthic slope-dwellers, and a small number of brackish and freshwater species (Leis, 1984; Nelson, 1994; Tyler, 1980). The number of families recognized within the order varies between eight (e.g., Winterbottom, 1974) and 10 (e.g., Tyler, 1980); specific families recognized by various authors are discussed below.

Because of its considerable diversity, the order (as well as many of its subdivisions) has been historically difficult to define, even though the tetraodontiforms were already recognized as a group of related fishes by the 17th century by workers such as Willughby (1686) and Artedi (1738) (Tyler, 1980). Tyler (1980) provides a comprehensive historical review of work on the order prior to the publication of his own monograph.

Recent researchers have used a variety of morphological methods to investigate the order's monophyly and

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the relationships between groups within the order (Lauder and Leim, 1983; Leis, 1984; Rosen, 1984; Santini and Tyler, 2003; Tyler, 1980; Winterbottom, 1974). Although all have agreed that the group is monophyletic, each provides a different hypothesis of the interrelationships of the tetraodontiform subgroups. This is not surprising given that the authors of these previous studies used different methods of phylogeny reconstruction, different outgroups for comparison, and different suites of morphological characters.

Explicitly phylogenetic analysis of Tetraodontiformes began with Winterbottom's (1974) myological analysis, which included 75 muscle characters for 46 extant species and employed Hennig's (1966) phylogenetic methods (i.e., only synapomorphies were used to infer phylogeny). Characters were weighted to de-emphasize characters thought to result from parallel evolution, reduction, and serial homology relative to those characters that involved shifts in the origin and insertion of existing muscles and the development of new muscles. The transformation series were polarized by inspecting various groups of perciform fishes.

Based on his analysis, Winterbottom (1974) divided the order into two suborders (Fig. 1A). Suborder Triacanthoidei comprised two families, Triacanthodidae and Triacanthidae. Suborder Tetraodontoidei comprised six families in two superfamilies. Superfamily Balistoidea contained two families, Balistidae and Ostraciidae. Monacanthidae, which is traditionally considered a separate family, was recognized as a monophyletic subfamily of Balistidae. Superfamily Tetraodontoidea contained the remaining four families; Triodontidae was basal, and Molidae was sister to a clade containing Diodontidae and Tetraodontidae. Two subfamilies were recognized within Tetraodontidae, Tetraodontinae and Canthigasterinae.

Tyler (1980) based his study primarily on osteology and a limited number (10) of soft anatomical features. In addition to examining the relationships within Tetraodontiformes, Tyler (1980) aimed to define the order anatomically and to interpret the phylogenetic relationships of both fossil and Recent tetraodontiform fishes. He examined 167 Recent and 25 fossil species of tetraodontiform fishes as well as 39 Recent and 10 fossil species of non-tetraodontiform fishes. He presented a comparative diagnosis of each suborder, infraorder, and family, along with a discussion of infraordinal relationships. The various categories within the order, as well as the order itself, were presented within an evolutionary taxonomic (contra phylogenetic) framework. Tyler (1980) pointed out that the order's defining characteristics consist largely of the loss of bones present in perciform fishes.

In contrast to Winterbottom's (1974) phylogeny and classification, Tyler (1980) recognized Suborder Balistoidei containing the families Triacanthodidae, Triacanthidae, Balistidae, Monacanthidae, Aracanidae, and Ostraciidae, along with a monophyletic suborder Tetraodontoidei containing the families Triodontidae, Tetraodontidae, Diodontidae, and Molidae (Fig. 1B). Although his analysis led him to conclude that the triacanthids are more closely related to the balistoids and ostracoids (Ostraciidae + Aracanidae), Tyler (1980) placed them within Superfamily Triacanthoidea. In addition, he differed from Winterbottom (1974) and followed a more traditional classification by recognizing Monacanthinae, Ostraciinae, and Aracaninae as families rather than subfamilies. Like Winterbottom (1974), Tyler (1980) placed Triodontidae basally within a clade that also contained Tetraodontidae, Diodontidae, and Molidae.

Lauder and Leim (1983) addressed the phylogeny of the tetraodontiform fishes within their larger review and summary of the interrelationships of the actinopterygian fishes. They employed Hennig's (1966) methods in their analysis using a wide variety of morphological characters. They presented a phylogeny of the tetraodontiform fishes based upon Tyler (1980) and Winterbottom (1974). Lauder and Leim (1983) concluded that the order is monophyletic on the basis of the following synapomorphies: entire branchiostegal region covered by a thick layer of skin; greatly restricted gill opening does not extend far below the base of pectoral fin; suborbital bones, parietals, nasals, sensory canals in skull bones, and anal fin spines absent.

Lauder and Leim (1983) recognized Triacanthidae and Triacanthodidae as a clade, sister to a monophyletic lineage they refer to as Sclerodermi, which contained Balistidae, Ostraciidae, Triodontidae, Tetraodontidae, Diodontidae, and Molidae (Fig. 1C). Like Winterbottom (1974), they recognized the monacanthids as a subgroup of Balistidae and the aracanines as a subgroup of Ostraciidae. They also hypothesized a sister-group relationship between Balistidae and Ostraciidae. Within the clade containing Triodontidae, Tetraodontidae, Diodontidae, and Molidae, they hypothesized a sister-group relationship between the tetraodontids and the diodontids, but they placed this clade in an unresolved trichotomy with the triodontids and molids.

Arai (1983) utilized 17 osteological characters to analyze the order using Wagner's groundplan/divergence method (Wagner, 1961, 1969, 1980). He used both symplesiomorphic and synapomorphic characters to infer relationships, allowed non-dichotomous branching, and allowed an extant species to represent the ancestor of another extant species. Arai (1983) recognized six families of tetraodontiform fishes (Fig. 1D). He recognized Triacanthodidae as the "stem family" or ancestor of five lineages corresponding to Triacanthidae, Balistidae (including Monacanthidae), Ostraciidae, Triodontidae, and Tetraodontidae (including Diodontidae). He was unable to place a monophyletic lineage corresponding to