

## Discrimination of Anemonefish Species by PCR-RFLP Analysis of Mitochondrial Gene Fragments

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### Abstract

A means of discriminating among species of clown anemonefishes, based on restriction enzyme analysis of partial mitochondrial DNA sequences, was investigated. Mitochondrial 16S rRNA and cytochrome *b* genes from 6 species (7 strains) of anemonefish (*Premnas biculeatus*, *Amphiprion polymnus*, *A. sandaracinos*, *A. perideraion*, *A. ocellaris*, *A. ocellaris* var. and *A. percula*) were PCR-amplified. A 623-bp portion of 16S rRNA gene was obtained from different fishes using the same pair of primers. Further investigation of this 16S rRNA fragment, by restriction endonuclease digestion with *BfuCI* and *RsaI*, was not able to distinguish all fishes studied, but did yield 3 different digestion patterns. The first was specific to *P. biculeatus*, the sole member of the genus *Premnas*, while the remaining two separated the *Amphiprion* species into 2 groups: 1) *A. polymnus*, *A. sandaracinos* and *A. perideraion*, and 2) *A. ocellaris*, *A. ocellaris* var. and *A. percula*. In contrast to this, restriction endonuclease digestion of a 786-bp fragment of the cytochrome *b* gene with *HinfI* and *RsaI*, was able to differentiate different 7 anemonefishes. This utility marker is valuable for unambiguous species/strain identification of juvenile anemonefishes.

**Keywords:** anemonefish, species identification, 16S rRNA, cytochrome *b*, PCR-RFLP

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### 1. Introduction

Anemonefishes of the genus *Amphiprion* and *Premnas* (Perciformes: Pomacentridae: Amphiprioninae) are one of the most attractive marine ornamental fishes. Approximately 28 species have been recorded in the warm waters of Indian and Pacific Oceans, including Australia's Great Barrier Reef (Robertson, 1998). Typical classification and species identification of anemonefishes are based on morphological characteristics, such as tooth, shape, scalation of head and body proportions (Allen and Fautin, 1992). However, to distinguish anemonefishes in the field, colour pattern is the most important feature. Identification of newly hatched fish larvae to species level is often difficult, due to poorly defined morphological characteristics and great differing juvenile morphology in comparison to the adults (Fautin and Allen, 1997). In such cases molecular based taxonomy, particularly methods employing analysis of polymerase chains reaction (PCR) amplified DNA fragments, can provide an accurate alternative means of identification of individuals to genus, species or even strain level. This approach has been widely applied in the study of teleost fishes, because of the relative simplicity, specificity and sensitivity of the technique. Often sufficient diagnostic information can be obtained from analysis of PCR amplicons digested with re-

striction enzymes, generating potentially discriminatory restriction fragment length polymorphism (PCR-RFLP) markers. PCR-RFLP analysis is faster, more cost effective and more accessible than the alternative of sequencing each PCR amplicon.

Mitochondrial DNA (mtDNA) genetic markers have been widely used as a tool to distinguish within and among species (e.g. Patarnello *et al.*, 1994; Chirstian *et al.*, 2000; Klossa *et al.*, 2002; Moyses and de Almeida, 2002; Aranishi *et al.*, 2005, Hsieh *et al.*, 2007; and references therein). MtDNA sequences are almost exclusively maternally inherited (Gyllensten *et al.*, 1985) and the rate of evolution of the mtDNA genome is considered to be approximately ten times greater than that of the nuclear genome (Brown *et al.*, 1979). In this study the use of PCR-RFLP analysis of two mitochondrial gene fragments to distinguish among six species (two genera) of anemonefish is investigated.

### 2. Materials and methods

#### 2.1 Fish samples

Four different anemonefish species (5 strains) comprising *P. biculeatus*, *A. sandaracinos*, *A. ocellaris*, *A. ocellaris* var., and *A. percula* were gifted from Percula Farm, Chonburi, Thailand. These





