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Optimization of Polymerase Chain Reaction (PCR) of Mitochondrial Cytochrome c Oxidase I (COI) Gene in Two Bornean Fanged Frogs

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ABSTRACT

Limnonectes kuhlii and *Limnonectes leporinus* are two of the Bornean fanged frogs (without advertisement call) which are widely distributed, thus thought to exhibit different evolutionary lineages and the existence of genetically cryptic species. Yet, the two species are still under study especially at the molecular level. Hence, cytochrome c oxidase I (COI) of mitochondrial gene was used to investigate suitable parameters for DNA amplification using the Polymerase Chain Reaction (PCR) method. Three PCR programmes (varied in the temperatures and period of each PCR step) were employed to identify the most efficient parameters in amplifying PCR products for both species. From the three programmes, Programme B (Initial denaturation: 96°C for 5 min; denaturation: 95°C for 45 sec; annealing: 48-53°C for 1 min 30 sec; extension: 72°C for 1 min 30 sec; final extension: 72°C for 10 min, 30 cycles) showed the highest percentage (53%) of optimal PCR products. The other two programmes showed non-specific products or "primer-dimers". The results also suggest that the annealing temperature of 52°C, 0.025-0.05 units/µl of 1.5mM *Taq* polymerase, 0.04 mM of dNTPs mix and optimal concentrations of magnesium in 50 µl of reaction mixture were sufficient enough to amplify high quality PCR products for both species. However, using Programme B, the re-amplification of the PCR products yielded "primer-dimer". In addition, a 'Hot-Start' PCR method was also applied and mostly yielded in an optimal PCR amplification. Nevertheless, further research on the second amplification of the two species should be conducted to determine the causes of the primer-dimer production.

Keywords: **Polymerase Chain Reaction (PCR) conditions, optimization, annealing temperature, Hot-Start PCR**

INTRODUCTION

Bornean fanged frogs, categorized under the sub-genus *Limnonectes* in the family of Ranidae (Dubois, 1992; Emerson and Ward, 1998; Frost *et al*., 2006) are divided into four species, namely *Limnonectes leporinus*, *L. ingeri*, *L. kuhlii*, and *L. ibanorum* (Inger, 1996; Emerson and Inger 1992; Dubois, 1992; Frost *et al*., 2006). Most of the *Limnonectes* species (except for *L. kuhlii*) are grouped in the *grunniens* (Emerson and Ward, 1998), and consist all the putative species which are difficult to identify due to the high similarity in their external morphology. Hence, there is confusion in taxonomically categorizing the species and its systematic relationship using the conventional methods (Emerson and Ward, 1998). With the revolution of the molecular techniques, the studies on the phylogenetic and taxonomy of the Bornean fanged frogs can be applied as an alternative method (Avise, 1994; Duellman and Trueb, 1994).

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