



Molecular phylogenetics of species of *Bulbophyllum* sect. *Trias* (Orchidaceae; Epidendroideae; Malaxidae) based on nrITS and plastid *rbcL* and *matK*

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Abstract

Phylogenetic relationships among species of *Bulbophyllum* sect. *Trias* and related taxa in *Bulbophyllum* has not been previously studied due to scarcity of appropriate samples. In this study, we aimed to assess the relationship of these taxa and investigate interspecific relationships of these orchids using DNA sequences from two plastid genes, *rbcL* and *matK*, and one nuclear region, nuclear ribosomal internal transcribed spacer, nrITS. Our results showed that all *Bulbophyllum* sect. *Trias* species were embedded among other groups of *Bulbophyllum*, supporting the already published transfer of *Trias* to synonymy of *Bulbophyllum*. All trees revealed that the Indochinese sect. *Trias* form a monophyletic group that could be divided into three groups coincident with their vegetative characters. This study further shows that nrITS sequences can be sufficient for inferring phylogenetic relationship among *Trias* species, although this marker and the combination of this plus the plastid genes are not able to distinguish the differences between some closely related species.

Introduction

Trias Lindley (1830: 60) is a genus of miniature epiphytic orchids consisting of at least 15 species (Dressler 1993, Cameron *et al.* 1999, Chase *et al.* 2003, van den Berg *et al.* 2005, Smidt *et al.* 2011). These epiphytic orchids are indigenous to tropical and subtropical rain forests in Southeast Asia, India, China and Australia (Liu *et al.* 2007), where they grow primarily on shady rocks along streams and in open woodlands (Liu *et al.* 2007, Schuiteman *et al.* 2008). The unique features of *Trias* are not just restricted to their floral morphology but are also found in their compact pseudobulbs and leaf shapes. Their characteristics were defined by Seidenfaden (1986) as having horn-like prolonged operculum with uniform broad triangular-ovate sepals and petals that are smaller than the sepals and mostly linear, rising vertically on each side of their column. Dressler (1993) included 15 orchid genera within this subtribe. However, recent evidence from molecular analyses has demonstrated that all former genera of Bulbophyllinae including *Trias* should be subsumed in *Bulbophyllum* Thouars (1822: t. 3; Pridgeon *et al.* 2014, Vermeulen *et al.* 2014). *Trias* was placed in *Bulbophyllum* in tribe Malaxideae, subtribe Bulbophyllinae (Chase *et al.* 2015).

The recent combination of molecular data with morphometric data has resulted in enhanced classification techniques over traditional approaches to taxonomy, with molecular analysis enabling a new classification of Orchidaceae (Chase *et al.* 2003, 2015). Sequences of both plastid DNA regions and nrITS have been used for phylogenetic analysis in Orchidaceae (Neubig *et al.* 2009, Li *et al.* 2011a, Batista *et al.* 2013, Xiang *et al.* 2013, Luo *et al.* 2014). Among the plastid DNA regions, the plastid *rbcL* and *matK* genes are the most commonly used regions as core barcodes (Hollingsworth *et al.* 2011). Plastid *rbcL* is the most commonly used gene and has been proven to aid in identification at a variety of taxonomic levels as it contains no length variation and has sufficient sequence divergence particularly at the genus level (Cameron *et al.* 1999). Similarly, the plastid gene *matK* has also been commonly used for assessing the phylogenetic relationships in Orchidaceae (Kores *et al.* 2000, Bateman *et al.* 2003, Ponsie *et al.* 2007, Hosseini *et al.* 2012, Batista *et al.* 2013) as it is one of the most rapidly evolving genes of the plastid genome (Hilu & Liang 1997, Hollingsworth *et al.* 2011). In addition to plastid genes, several studies have applied nuclear DNA regions, such as internal transcribed spacer of ribosomal DNA (nrITS), to phylogenetic analysis in order to identify relationship among species of orchids (Hollingsworth 2011). Many recent studies combined sequences of this region with plastid regions, such as *matK*, *rbcL*, *trnL-F* spacer, *trnL* intron, *psbA-trnH* spacer and *trnS-trnG* spacer (Kocyan *et al.* 2004, Smidt *et al.* 2011, Xiang *et al.* 2013) to improve resolution of the phylogenetic trees of various groups of orchids. ITS not only