

Distinguishing Two Morphologically Similar *Rhinacanthus* Species Using Chloroplast Barcoding Regions

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Rhinacanthus flavovirens (Maha Anitta) belonging to the family Acanthaceae, is an endemic species to Sri Lanka. *R. flavovirens* is distributed in North central, Central, Southern and Western provinces in the country. *R. flavovirens* and *R. nasutus* possess a high level of morphological similarity, creating confusions in differentiating between the two species. DNA barcoding is a widely used molecular method for herbal plant identification and authentication, especially when morphological features are not sufficient. Chloroplast barcoding regions *matK*, *rbcl* and *trnH-psbA* have been used for this study. DNA was extracted and quantified using a Nanodrop spectrophotometer before amplification. The amplified fragment lengths for *R. nasutus* were 762 bp (*matK*), 369 bp (*rbcl*), and 333 bp (*trnH-psbA*), while those for *R. flavovirens* were 769 bp (*matK*), 370 bp (*rbcl*), and 364 bp (*trnH-psbA*). The Sanger sequencing showed that, among these three markers *trnH-psbA* showed the highest nucleotide variation (68.46%) whereas *matK* showed the lowest (1.3%). Pairwise genetic distance analysis showed that *R. nasutus* and *R. flavovirens* have closer genetic relatedness for *matK* region (0.0133) and higher divergence in *trnH-psbA* region (1.83). The MEME suite program identified a higher frequency of insertion/deletion events in *trnH-psbA* compared to *matK* and *rbcl*. The result of this study showed that DNA barcoding, particularly using variable region *trnH-psbA*, can effectively distinguish *R. flavovirens* from *R. nasutus*. Future studies incorporating whole chloroplast genome sequencing may provide enhanced resolution for species identification, authentication and phylogenetic analysis.

Keywords: DNA barcoding, *Rhinacanthus flavovirens*, *matK*, *rbcl*, *trnH-psbA*

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