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Differentiation of *Hortonia ovalifolia* and *Hortonia floribunda* by DNA barcoding

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Hortonia is an endemic genus which belongs to the family Monimiaceae. In Sri Lanka, Monimiaceae is represented only by the genus *Hortonia* which, according to the Flora of Ceylon, consists of three species. A recent phytochemical investigation, found identical chemical compounds among the three species which has raised the question of species limits of *Hortonia*. This led us to investigate the genus *Hortonia* using short orthologous DNA sequences known as DNA barcodes to re-evaluate its species limits. DNA barcoding is recognized as a powerful framework for identifying organisms and discriminating among closely related species.

Leaf samples of *H. ovalifolia and H. floribunda* were collected and kept at -20^{0}C until use. Genomic DNA was extracted using a recommended procedure**.** Purity of the extracted DNA was confirmed by spectrophotometry. DNA from both species was subjected to polymerase chain reaction (PCR) using specific primers for nuclear internal transcribed spacer (ITS) and *trnH-psbA* spacer regions. PCR amplified ITS and *trnH-psbA* regions were purified using gene clean kit, phosphorylated and ligated to a pBlueScript (pBS) vector previously digested with *EcoR* V. The ligated vector was transformed to *E.coli* strain JM109 and the cells were grown at 37^{0}C in LB medium containing ampicillin for 12-15 hours. Cells were harvested followed by plasmid purification. Purified plasmids containing the DNA of interest were subjected to sequencing. Sequence homology of ITS and *trnH-psbA* regions from both species were compared using MacVector software.

DNA sequence comparison of ITS and *trnH-psbA* regions of *H. ovalifolia* and *H. floribunda* show a 2.49% and 1.5 % sequence divergence, respectively. ITS is the most commonly sequenced locus used in plant phylogenetic investigations at the species level and *trnH-psbA* spacer is the most variable plastid region in angiosperms. These high sequence divergence values clearly indicate that *H. ovalifolia* and *H. floribunda* are two different species. Therefore the present study strongly supports the recognition that there are two distinct species of *Hortonia.* DNA sequencing studies on *H. angustifolia* are in progress to completely re - evaluate the species limits of the genus *Hortonia.*

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