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Molecular evidence for hybrid origin of *Melastoma intermedium*

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ABSTRACT

In this study, we sequenced two nuclear genes and one chloroplast spacer in *Melastoma intermedium*, a shrub species endemic to China, and its putative parental species, *Melastoma candidum* and *Melastoma dodecandrum*, to test the hybrid-origin hypothesis. Our results revealed that in one nuclear gene there were five fixed nucleotide substitutions between *M. candidum* and *M. dodecandrum*, and in the other nuclear gene, there were six. All but one individual of *M. intermedium* showed additivity in chromatograms at these sites of at least one gene. Haplotypes of *M. candidum* and *M. dodecandrum* at the two nuclear genes were well separated, and most haplotypes of *M. intermedium* were shared with those of *M. candidum* and *M. dodecandrum*. *M. candidum* and *M. dodecandrum* differed by three nucleotide substitutions in the chloroplast spacer, whereas individuals of *M. intermedium* had identical sequences to either *M. candidum* or *M. dodecandrum*. The molecular data clearly demonstrate that *M. intermedium* is of hybrid origin.

1. Introduction

The genus *Melastoma* L. (Melastomataceae) is centered in Southeast Asia and extends to India, southern China, Japan, northern Australia, and Oceania (Meyer, 2001). It was previously estimated that this genus comprises approximately 50–100 species (Chen, 1984; Wagner et al., 1999), but only 22 species are recognized in the latest taxonomic revision by Meyer (2001). According to the Chinese version of Flora of China (Chen, 1984), the nine Chinese *Melastoma* species are distributed in the south of the Yangtze River. However, the English version of Flora of China recognizes only five species, with *M. normale* D. Don, *M. affine* D. Don and *M. candidum* D. Don combined into one species, *M. malabathricum* L. (Chen and Renner, 2007). Two other species, *M. penicillatum* Naud. and *M. dendrisetosum* C. Chen, were described as endemic species to Hainan Island in the Chinese version (Chen, 1984). However, the former was considered to be misapplied to specimens of *M. sanguineum* Sims from Hainan, and the latter was not recognized in the English version (Chen and Renner, 2007). In this study, we will use the species names for *Melastoma* according to the Chinese version of Flora of China.

All of the *Melastoma* species in China have the same chromosome number of $2n = 24$ (Zhang et al., 2010). Among these species, *M. dodecandrum* Lour., *M. candidum* (*M. septemnervium* Lour. in Meyer's revision) and *M. intermedium* Dunn are often observed sympatrically in Guangdong, Guangxi and Fujian provinces. *M. dodecandrum*, a small shrublet, occurs in South China

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and North Vietnam (Chen, 1984). Usually less than 0.3 m high and with repent stems, it has the appearance of a herbaceous plant. *M. candidum* is another common species that is distributed in South China and Southeast Asia (Chen, 1984). Unlike *M. dodecandrum*, this species is an erect shrub, up to 1.5 m high. In addition, the trichomes on the branchlet, leaf, and hypanthium and the type of ovary all show striking differences between the two species (see details in Materials and Methods). *M. intermedium*, the only *Melastoma* species endemic to China, was initially found in Fujian and reported as a new species (Dunn, 1908). However, it was recently incorporated into *M. malabathricum* by Meyer (2001). This species is rare compared with *M. candidum* and *M. dodecandrum*. Interestingly, it has a species name "*intermedium*", implying its morphological intermediacy between other *Melastoma* species. In accordance with its species name, *M. intermedium* indeed has many morphological traits intermediate between those of *M. candidum* and *M. dodecandrum*. For example, *M. intermedium* has repent stems similar to those of *M. dodecandrum*, whereas it has a half-inferior ovary similar to that of *M. candidum*. Its height (0.3–0.6 m) and trichomes in branchlet, leaf and hypanthium are intermediate between these two species (see details in Materials and Methods). In habitat, these three species are very similar, occurring in open fields, grasslands and trailsides (Chen, 1984). Based on its uncommon occurrence, morphological intermediacy and overlapping distribution with *M. candidum* and *M. dodecandrum*, we propose that *M. intermedium* might represent an interspecific hybrid between *M. candidum* and *M. dodecandrum*.

Confirming the hybrid origins of questionable taxa is a prerequisite for taxonomic and evolutionary studies and for scientific conservation and management. In this study, we sequenced two low-copy nuclear genes and one chloroplast intergenic spacer in the three sympatric *Melastoma* species to test the hypothesis that *M. intermedium* has a hybrid origin. To this end, multiple individuals of each species were sampled from Shankou village, Zengcheng, Guangzhou, Guangdong, China, where they are sympatric.

2. Materials and methods

2.1. Plant materials

Our sampling site was located in Shankou village, Zengcheng, Guangzhou, Guangdong, China. On this site, three species of *Melastoma*, namely, *M. intermedium*, *M. candidum* and *M. dodecandrum*, are sympatric. Both *M. candidum* and *M. dodecandrum* are common species in this location, whereas *M. intermedium* is relatively rare. Plants were identified according to the diagnostic morphological characteristics described in the Chinese version of Flora of China (see Table 1). We sampled 11 individuals of *M. dodecandrum*, 12 individuals of *M. candidum*, and 13 individuals of *M. intermedium*. In addition, we sampled six individuals of *M. sanguineum* from Qinghushan, Zhaoqing, Guangdong, and used this species as an outgroup. Leaves from each individual were collected in plastic bags with silica gel before DNA extraction.

2.2. Sequencing of the two nuclear genes and the chloroplast trnL-trnF regions

Total genomic DNA was extracted from dried leaf tissues using the CTAB method (Doyle and Doyle, 1987). Two universal low-copy nuclear genes (*cam* and *gapC*) were used in this study (Strand et al., 1997). The two genes encode calmodulin (CaM) and cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPC). We first used universal primers for these two genes (Strand et al., 1997) to amplify these genes in one individual of each species. After these sequences had been obtained, we designed specific primers for these *Melastoma* species. These primers were MCamF: 5′ GTATTCGCGAGTTTCCTTTGAT 3′, MCamR: 5′ GGGGGTTCTCTCAATGCAC 3′, MgapCF: 5′ TGACATGCATTCGTCTTTAGTGC 3′ and MgapCR: 5′ TCAAGCTGTGAGACTGAGACCA 3′. The chloroplast *trn*L-*trn*F regions were amplified using the universal primers *trn*c and *trn*f (Taberlet et al., 1991). The PCR products were purified using a Pearl Gel Extraction Kit (Pearl Bio-tech, Guangzhou, China) and then directly sequenced on an ABI 3730 DNA automated sequencer with a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA). Intra-individual length polymorphisms of the nuclear genes could cause failure of direct sequencing from the polymorphic sites. In addition, many individuals, primarily from *M. intermedium*, had superimposed chromatograms at multiple sites of the nuclear genes, so the haplotypes could not be inferred. Under these circumstances, cloning sequencing was used to phase the haplotypes. Ligations were conducted using a pMD18-T&A cloning kit (Takara,

Table 1
Comparison of several morphological traits between *M. candidum*, *M. dodecandrum* and *M. intermedium*.

Organ or tissue	*M. dodecandrum*	*M. intermedium*	*M. candidum*
Stem	Repent	Repent	Erect
Branchlet	Strigose when young, later glabrous	Appressed strigose	Densely covered with appressed scales
Leaf	Sparsely strigose at margin only; 3–5 basal veins	Densely strigose; 3–5 basal veins	Densely strigose and puberulous; 7 basal veins
Hypanthium	Strigose	Densely strigose	Densely squamose-strigose
Ovary	Inferior	Half inferior	Half inferior