

# Phylogenetic analyses of *s.* *kinabaluensis*



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Based on the results obtained from both phylogenetic analyses, the putative parents of *S. kinabaluensis* are related to two different lineages. In one case, clones #1, #7, #8, #9, and #10 appear to be related to a lineage that includes *S. crenata* and *S. nitidus* (Clade 1). In the other case, clones #4 and #6 are closely related to *S. alabensis* (Clade 2) (Figure 12, Figure 13). From both the MP and ML, it shows that *S. kinabaluensis* is most closely related to *S. alabensis* where they are aligned together in both trees.

Even though the consensus tree has an inclusive low level of resolution and bootstrap support (listed above branches) (Figure 12, Figure 13), the results are in agreement with previous analyses based on ETS and ITS sequence distinction (Shafreena, 2012) suggested that the *S. alabensis* as one of the potential parents since the polymorphic sites observed are additive between *S. alabensis* and the potential parents (Shafreena, 2012). However, there is not enough support for this relationship. More data are needed to positively confirm the result.

From the phylogenetic analyses, both trees of MP and ML yielded similar results (56% MP, 60% ML). The results of lineage that related to the *S. crenata* and *S. nitidus* (Clade 1) are considered as not supported and unresolved due to the low bootstrap value. Therefore, the morphological differences between these two species with *S. kinabaluensis* were not further evaluated in this study. As for the other lineage, it shows that *S. alabensis* could be one of the potential parents of *S. kinabaluensis* although with low bootstrap support value (Clade 2). Therefore, the vegetative and reproductive parts of these two species were observed closely and compared

to find out if there is any similarity to support the result of the phylogenetic analysis.

As already noted, the distinguishing feature of *S. kinabaluensis* is that this plant is covered with white stellate hairs on the vegetative and reproductive parts. Based on morphological observation, vegetative parts of *S. alabensis* are glabrous. However, the reproductive parts especially the inflorescence, branches, peduncles and pedicels do have sparse hairs.

Further morphological analysis based on the reproductive parts of both species could not be further observed due to the flowering and fruiting stage while conducting this study. The specimens examined for *S. kinabaluensis* only had fruits, and as for *S. alabensis*, the specimens examined were just past flowering and there is no fruit seen. However, the leaf margin which is toothed (only towards the apex) is shared between these two species. Based on the morphological observation and study by Shafreena (2012), the corymb-like inflorescence is also shared by these two species. Therefore, these morphological similarities somehow do support the phylogenetic analyses.

A study by Mallet (2007) has mention that in a hybrid speciation, it means that hybridization has had a principal role in the origin of a new species. The derived species initially have exactly one genome from each parent, a 50% contribution from each, although, in older polyploids, recombination and gene conversion may ultimately lead to unequal contributions (Mallet, 2007). However, Arnold (1997) confines the opposite view as, in his opinion, hybrids are often more fit due, not only to the intermix of genotypes from the

parents, but also due to the fact that hybridization often creates novel genotypes that may be more fit under certain environmental conditions, particularly disturbed or novel environmental conditions. In this study, the potential parents of *S. kinabaluensis* still in a question. Therefore, the features and characteristics of *S. kinabaluensis* could not be fully determined and examined thoroughly. The features and characteristics of *S. kinabaluensis* might be inherited by the other parents or maybe it is because of the evolution undergoes by the plant itself for its own adaptation to the surrounding.

Other than that, the type and phase of elevation of *S. kinabaluensis* and *S. alabensis* are more or less to each other which are 1986-2254 m and 1500-1800 m above sea level, respectively. There is not much difference in distance of elevation between this two species and therefore, increase the probability of *S. alabensis* as one of the putative parents of *S. kinabaluensis*. However, it seems that the phase of elevation of *S. kinabaluensis* is higher compared to *S. alabensis*. Based on this elevation data, it can be correlates with the facts of hybrid speciation.

A study by Chase et al. (2010) has stated that once a hybrid species is developed, they faced ecological challenges. When they do become established, there are several potential reasons why they succeed: a) they exhibit transgressive traits (novel mixtures of those exhibited by their parents) that assist their existence in habitats in which neither of their parents can grow, conferring the isolation needed to avoid their reabsorption into the gene pool of one of their parents; or b) their intermediacy in some morphological traits or ecological preferences allows them to utilize a novel

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niche, again delivering a degree of genetic isolation from both of their parents (Chase *et al.* , 2010).

Often such hybrids can only exploit the ecological transition zone between those of their parents, which places them near one or both parents and which may be so inadequate in area that they cannot form viable populations and this intermediates are unlikely to form viable species in their own right (Chase *et al.* , 2010). In this case, the *S. kinabaluensis* populations grows on a higher elevation compared to *S. alabensis* , showing that this hybrid species forming a populations near to their parents but with slightly different ecological changes based on the lower temperature at higher elevation.

Several lines of molecular evidence support that *S. kinabaluensis* is of hybrid origin. First, 14 and 16 polymorphic sites for each of two accessions were discovered in the ETS and ITS region (Shafreena, 2012). Based on observation of polymorphic sites of *Schefflera* species showed that there are 14 species that have between 1-3 polymorphic sites except for *S. avenis* that has 5 polymorphic sites (Shafreena, 2012). Thus, the presence of 14-16 polymorphic sites of *S. kinabaluensis* showed an unusual condition (Shafreena, 2012).

Second, phylogenetic analyses showed that within-individual cloned ITS repeats were not monophyletic; instead they arose from two distinct lineages (Small & Wendel, 2000). From this study , *S. kinabaluensis* cloned ITS sequences are related to two different lineages. The clones #1, #7, #8, #9

and #10 appear to be related to a lineage that includes *S. crenata* and *S. nitidus*. Other clones #4 and #6 are closely related to *S. alabensis*.

Third, although shared nuclear ancestry could simply point out sister relationships with *S. alabensis*, *S. kinabaluensis* also allocates nuclear ancestry with a different lineage of species including *S. crenata* and *S. nitidus*. To conclude, this molecular phylogenetic information verifies the hypothesis of a hybrid origin for *S. kinabaluensis*.

Quite a lot of additional studies of natural hybridization are needed before the nature of the claimed hybrid origin can be comprehended (Barkman & Simpson, 2002). First, it would be of relevance to know if *S. kinabaluensis* is an allopolyploid or diploid hybrid. Second, artificial crosses should be executed to resolve if there are any obstructions to hybridization between the potential parental species (Barkman & Simpson, 2002).

For future studies, the limitations in this study should be overcome such as the limitations on the amount of clones produced from the species in order to get a more precise analysis. In addition, a complete morphological description which includes the vegetative and reproductive parts of both the target hybrid species and the putative parents should be done to support the phylogenetic analysis.