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Research

Phylogenetics of the plant genera *Dracaena* and *Pleomele* (Asparagaceae)

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Abstract

This research focuses on the biodiversity and the evolutionary history of the world-wide medicinal plant genus, *Dracaena*, and the plant genus *Pleomele.* The debate concerning the relationship between *Dracaena* and *Pleomele* has continued till date - some botanists continue to include *Pleomele* within *Dracaena* but others claimed to separate the two genera. *Dracaena is a genus* comprising of about 40-100 species world wide, mainly in tropics and subtropics, with the exception of America. *Pleomele* is a genus that has been circumscribed consisting of 10-50 species in Asia. Till date, its center of biodiversity is unknown. *Pleomele* is only classified well in Hawaii, but confused with *Dracaena* in the other parts of Asia. Phylogenetic relationship among the 33 taxa within the *Dracaena* and *Pleomele* were reconstructed. DNA sequences from the chloroplast DNA intergenic spacer, *trnL-trnF* and *trnH-psbA* were analyzed. A phylogeny was reconstructed using neighbor-joining, maximum parsimony in PAUP*, and likelihood criteria in RAxML, and Bayesian inference in MrBayes. The phylogeny with *Agave missionum* and *Agave attenuata* as outgroup taxa indicates that *Pleomele* is mixed with *Dracaena*. This study provides the first phylogenetic reconstruction with taxonomic sampling of the *Dracaena* and *Pleomele* to resolve their questionable placement. The relationships of the climate change adaptation, biogeography, and conservation with the two plant genera will be further discussed in this study. Some suggestions for the benefits of the biodiversity and natural resource conservation in Himalaya regions will be addressed. One significant contribution of this research will be in promoting molecular taxonomy to solve problems in systematics especially in cases when the classification is in debate.

Key-words: Phylogeny, chloroplast DNA, Asparagaceae, *Dracaena*, *Pleomele*.

Introduction

The two plant genera *Dracaena* Vand. and *Pleomele* Salisb. are important genera in the world not only because of their application in horticulture, medicine, agriculture, and worshiping in ceremonies by diverse cultures across different countries, but also in systematics, the two genera may provide the good evidence to give more stable classification for solving their unstable family placement from Liliaceae to current Asparagaceae (Brown 1914; Lee 1975; Wagner *et al*. 1990; Staples and Herbst 2005; Judd *et al*. 2007; APG 2009; Anonymous 2010). There are several problems about the two genera, such as unclear systematics, little research in their biogeography and evolution, conservational issues, and the using the species among different cultures. Therefore, the purpose of this study is to clarify their status of classification, to understand their evolution and biogeography, and to increase its application in conservation biology, horticulture and medicine.

Dracaena had been placed within in the family Liliaceae (Brown 1914). Among the characteristics that support this includes a superior ovary; leaves that are not twisted at base; bulbs present; fruits being fleshy, etc (Brown 1914; Bos 1980; Waterhouse 1987). However, this classification is no longer used because *Dracaena* species are woody and flowers have six stamens, unlike the typical herbaceous Liliaceae. Others

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have classified *Dracaena* in the family Agavaceae based on the features of flowers with 6 stamens, paniculate inflorescences, and plants with rosettes of fleshy fibrous leaves (Hutchinson 1973; Huang 1993; Staples and Herbst 2005). However, the ovary in *Dracaena* is superior, unlike other Agavaceae, and this classification is also no longer used.

Dracaena has been classified within the family Ruscaceae since 2003 (APGII 2003; Judd *et al*. 2007). Monophyly of *Dracaena* is supported by molecular analysis of 18S rDNA, *rbcL*, *atpB*, and *matK*, and morphologically by the presence of resin canals in their leaves and bark (APG 2003; Hilu *et al*. 2003; Judd 2003; Judd *et al*. 2007). Other key characters for Ruscaceae include superior ovary; fruits are fleshy and a berry; leaves are photosynthetic, and stems are cylindrical, green to brown, but not the major photosynthetic organ of the plant (Judd *et al*. 2007). However, currently, Ruscaceae is combined into the larger family Asparagaceae based on Angiosperm Phylogeny Group III system (APG 2009) because the research group's conclusion of uniting those confusing families into the same family when they do not show too much distinct from each other in the molecular data. Thus, *Dracaena* and *Pleomele* are replaced into the family Asparagaceae.

Furthermore, due to morphology though similar to the characteristics of Ruscaceae, several botanists claimed *Dracaena* within the family Dracaenaceae, the family of only one genus, *Dracaena* or two genera *Dracaena* and *Sansevieria* (Bos *et al.* 1992; Brummitt 1992; Watson and Dallwitz 1992; Kubitzi 1998; Marrero *et al*. 1998).

Dracaena was first described in 1768 by Vandelli (Brown 1914). The genus *Dracaena* comprises about 40-100 species world-wide, mainly in tropics and subtropics, with the exception of South America (Bos *et al.* 1992; Kubitzi 1998; Staples and Herbst 2005; Judd *et al*. 2007). Several species have been investigated for their medicinal and horticultural value (Lee 1975; Bos 1980, 1984; Bos *et al.* 1992; Chun 1994; Kubitzi 1998; Edward *et al*. 2001; Milburn, 1984; Staples and Herbst 2005). Africa is the center of diversity of *Dracaena* with some species distributed in Madagascar, Asia, Socotra, Mediterranean regions, Central America, Cuba, Macronesia, Northern Australia, and Pacific islands (Gwyne 1966; Kubitzi 1998; Marrero *et al*. 1998; Staples and Herbst 2005). Two extinct *Dracaena* species from the Neogene (23.03 ± 0.05 million years ago) have been identified based on the analysis of pollen (Van Campo and Sivak 1976). They are *Dracaena saportae*, recorded in Bohemia, Czech Republic, and *Dracaena guinetii*, recorded in Tunis, Tunisia Republic (Van Campo and Sivak 1976; Bonde 2005). The stomata of leaves are present and anomocytic (Kubitzi 1998).

Additionally, the huge uncertain species numbers of *Dracaena* are mainly due to be classified mixed with several other genera, such as *Sansevieria*, *Cordyline*, *Yucca*, and *Pleomele.* Similar situation is seen in *Pleomele*. Many juvenile and mature *Dracaena* often looked very different in morphology and the both stages can produce flowers and fruits. That makes the taxonomist to have difficult time to give really proper classification. Thus, clarifying the taxonomy and give proper genus based on phylogenetics becomes urgent.

The genus *Pleomele* was first described by Salisbury in 1796 (Brown 1914). Wagner recognized *Pleomele* in the family Agavaceae in 1990 and then in the family Ruscaceae in 2003 with no explanation (Wagner *et al*. 1990; Wagner and Herbst 2003). *Pleomele* has been circumscribed as a genus consisting of 40-50 species world-wide (Wagner *et al*. 1990; Wagner and Herbst 2003) and there are six endemic *Pleomele* species currently recognized in the Hawaiian Islands (Wagner *et al.* 1990; Wagner and Herbst 2003). St. John (1985) had classified nine *Pleomele* species in Hawaii and described their morphological features. However, Wagner *et al*. (1990) reclassified St. John's nine species into six and addressed morphology of all six *Pleomele* species endemic to Hawaiian flora (Wagner *et al*. 1990; Wagner and Herbst 2003).

Taxonomic ambiguity regarding the uncertain relationship of *Dracaena* and *Pleomele* has existed for a long time. Some species of *Pleomele* had been described as part of the larger genus, *Dracaena.* Brown (1914) separated *Pleomele* from *Dracaena* based on the difference of flowers. *Dracaena* has a very short perianth tube with tepals divided to the base of the flower and thickened staminal filaments near the middle. In contrast, the perianth tube of *Pleomele* has tepals connate for at least one-third of their length (Wagner *et al*. 1990). St. John (1985) and Wagner *et al*. (1990) agreed with this placement. However, in recent studies, *Pleomele* was used as a synonym of *Dracaena* based on similar morphological characteristics (Stevens 2001; Staples and Herbst 2005; Anonymous 2010). Stevens (2001) also combined *Pleomele* into *Dracaena*. Carlquist (1970) addressed *Pleomele* as an endemic Hawaiian genus. Wagner *et al*. (1990) stated *Pleomele* is a worldwide genus. However, those are only based on morphological classification. Because of the lack of phylogenetic evidence, the monophyletic status of *Pleomele* is not affirmed, although it has been regarded as monophyletic based on several morphological treatments (Brown 1914; Bos

1980; St. John 1985; Wagner *et al*. 1990; Kubitzi 1998; Staples and Herbst 2005). Therefore, the purpose of this study is to provide molecular phylogenetic evidence for the classification of *Dracaena* and *Pleomele* and resolve the systematic problems between *Pleomele* and *Dracaena* at the genetic level.

Materials and Methods

SAMPLE COLLECTION

Leaf tissues from species of *Dracaena* and *Pleomele* were collected from living material and the DNA extracted from fresh tissue as much as possible or from silica dried tissue when necessary. Two chloroplastic gene regions were used to examine these species. The *trnH-psbA* intergenic spacer (APG 2003; Shaw *et al*. 2005, 2007) was examined for 18 species with *Agave missionum* used for outgroup comparison. The *trnL-trnF* intergeic spacer (APG 2003; Shaw *et al*. 2005, 2007) was examined with 33 species, and both *A. missionum* and *A. attenuate* were used for outgroup comparison.

DNA EXTRACTION AND AMPLIFICATION

DNA was extracted from leaves using the CTAB as previously described (Morden *et al*. 1996; Randall and Morden 1999). DNA amplification by polymerase chain reaction (PCR), and template purifications was performed with Taq PCR Core Kit. Finally, PCR products were purified by EXOSAP method. DNA products were used for the following experiments. The *trnL-F* region was amplified by the primer pairs *trnL-tabE* (GGT TCA AGT CCC TCT ATC CC) and *trnF* (ATT TGA ACT GGT GAC ACG AG) (Taberlet *et al*. 1991) with the parameters 80°C for 5 min; 29 X (94°C for 1 min, 60°C for 1 min, 72°C for 2 min); 72°C for 5 min (Shaw *et al*. 2005). The *psbA-trnH* region was amplified by the primer pairs *psbA* (GGTATG CAT GAA CGT AAT GCT C) (Sang *et al*. 1997) and *trnH* (CGC GCA TGG TGG ATT CAC AAT CC) (Tate and Simpson 2003) with the parameters 80°C for 5 min; 35 X (94°C for 30 s, 57°C for 30 s, 72°C for 1 min); 72°C for 10 min (Shaw *et al*. 2005).

PHYLOGENETIC ANALYSIS

After DNA extraction and sequencing, sequences were aligned using Clustal X (Thompson *et al*. 1997), then edited and assembled using MEGA (Tamura *et al*. 2007). DNA sequences from the chloroplast genes were analyzed. The aligned in and manual adjustments were made in MEGA and in MacClade 4.0 (Maddison and Maddison 2000). Maximum parsimony analyses and maximum likelihood were performed in PAUP* (Swofford 2002) using the same heuristic search strategy. All characters were equally weighted, and gaps were treated as missing data. A Bayesian phylogenetic approach was used to generate a set of phylogenetic trees with estimated branch lengths that could then be converted to time in a rate analysis. MrBayes version 3.1 (Huelsenbeck and Ronquist 2001) were used to search tree parameter space. The general time reversible model (GTR+I+Ã) was selected for Bayesian analysis with intervals of 10,000 generations. Nonparametric bootstrap values (Felsenstein 1985), decay indices (Bremer 1988; Sorenson 1999), and Bayesian posterior probabilities were calculated for the phylogenetic reconstructions to estimate internal branch support.

Results

The length of the *trnL-F* sequences among the 33 taxa varied from 393 bases in *D. goldiena* to 438 bases in outgroup *Agave missionum* and *Agave attenuata*. The aligned *trnL-F* matrix is 621 bp long, and has 104 variable characters of which 155 are parsimony informative. The maximum likelihood search of the *trnL-F* of 33 taxa data set retained 550945 trees with Length (L)=621 (CI=0.564, and RI=0.568; both CI and RI were calculated including parsimony uninformative characters). Strict consensus tree obtained from 10 retained trees. Some nodes have no similar patterns on DI, PP, and BP. The node of *D. steudneri, D. multiflora, and D. umbraculifera* have very strong decay index 14 and Bayesian PP 100, but not strong in bootstrap percentages 70 (Fig. 1). The node of *D. serrulata* and *D. augustifolia* have very strong decay index 12 and good Bayesian PP 94, but not strong in bootstrap percentages 64 (Fig. 1). The node of *P. forbesii* and *P. fernaldii* has strong Bayesian PP 100, but has weak decay index 1 and bootstrap percentages 68 (Fig. 1). Some nodes have no similar patterns on DI, PP, and BP such as the node between *P. fernaldii* and the clade of *P. aurea, P forbesii, D. cemcina* has high PP 100 and low BP 68 (Fig. 2).

The length of the *psbA-trnH* sequences of 19 taxa varied from 542 bases in all of the ingroup taxa to 597 bases in outgroup *Agave missionum*. The maximum likelihood search of the *psbA-trnH* data set retained 2026821 trees with L=84

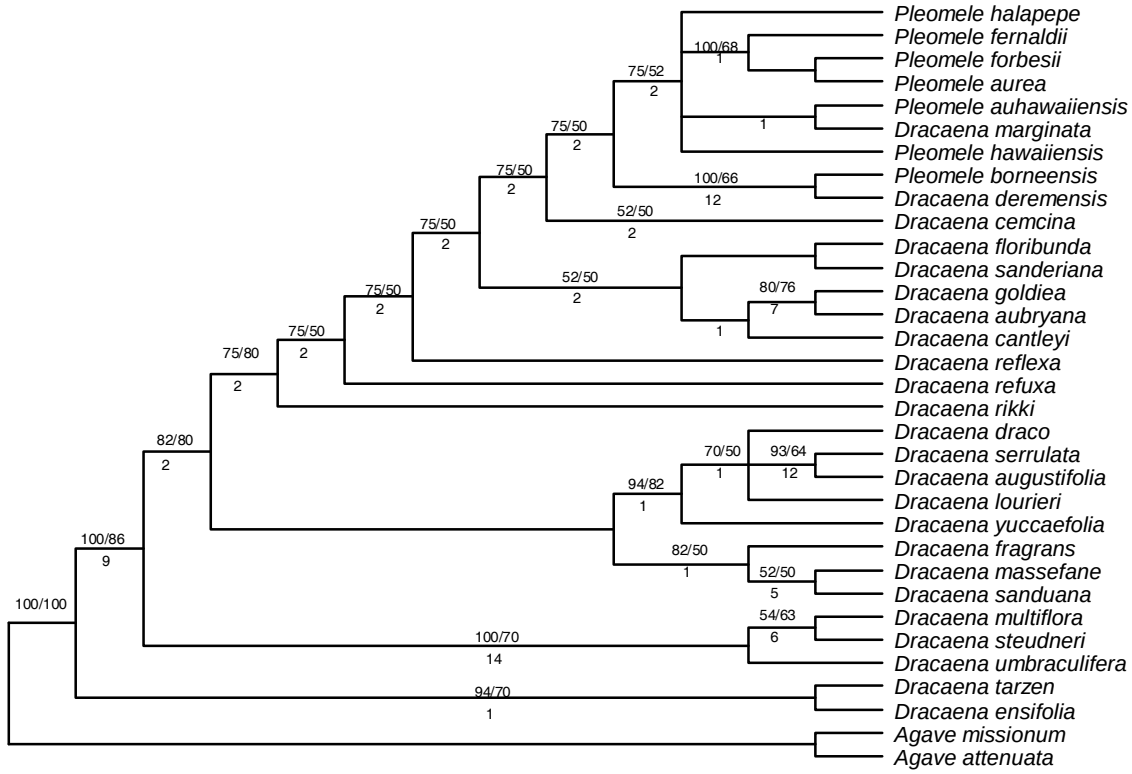

Figure 1. Strict consensus of the maximum parsimony tree with 33 taxa resolved using *trnL-trnF* sequence data. Posterior probability values/ Bootstrap percentages > 50% are above branches and decay indices are below branches.

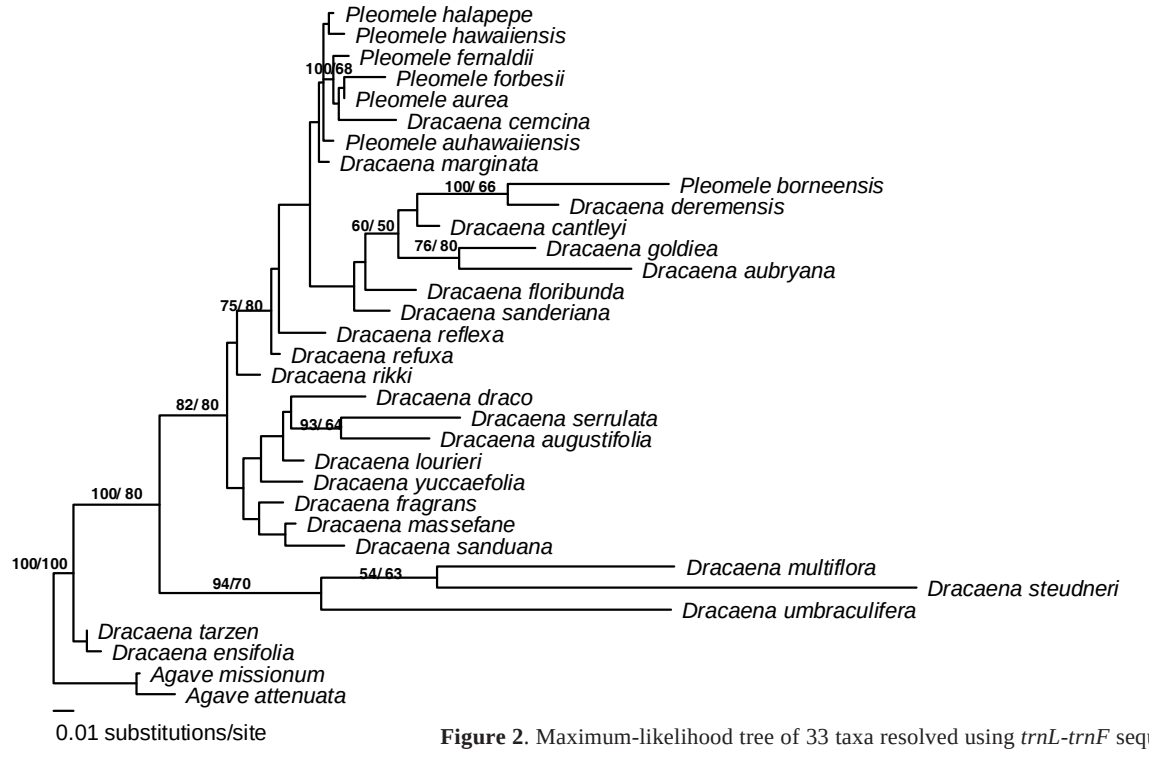

Figure 2. Maximum-likelihood tree of 33 taxa resolved using *trnL-trnF* sequence data. Posterior probability values/ Bootstrap percentages > 50% are above branches.

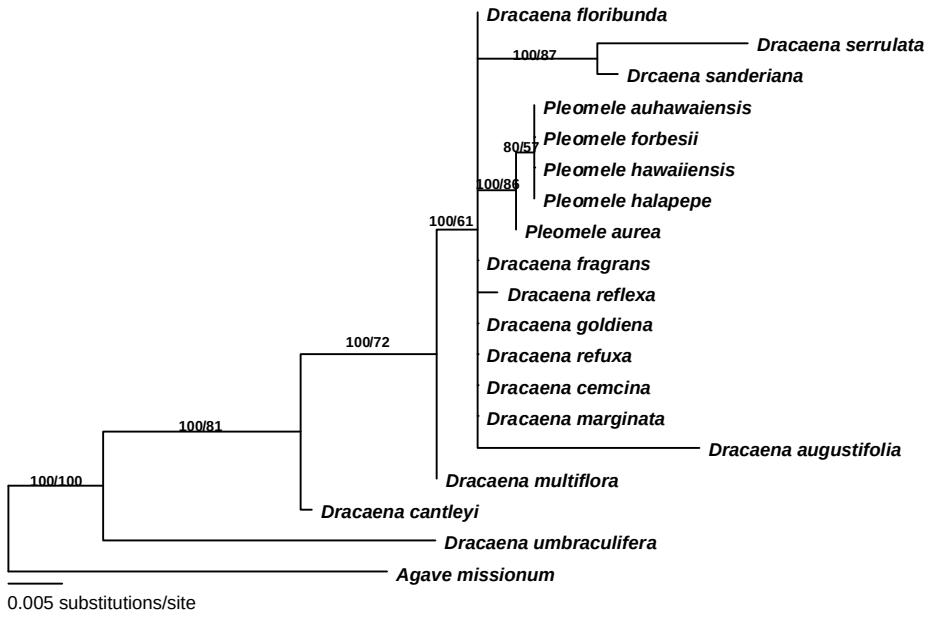

Figure 3. Maximum-likelihood tree of 19 taxa resolved using *psbA-trnH* sequence data. Posterior probability values/ Bootstrap percentages > 50% are above branches.

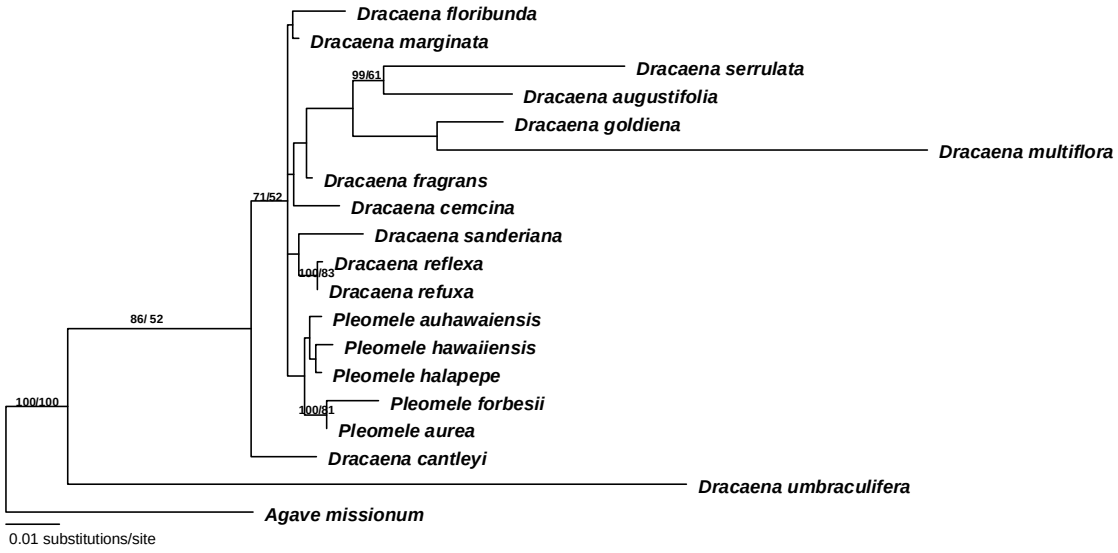

Figure 4. Maximum-likelihood tree of 19 taxa resolved using combined *psbA-trnH* and *trnL-trnF* sequence data. Posterior probability values/ Bootstrap percentages >50% are above branches.

(CI=0.905, and RI=0.818; both CI and RI were calculated including parsimony uninformative characters). Strict consensus tree obtained from 1359 retained trees. All of the nodes have similar patterns on DI, PP, and BP (data not shown). Some of the nodes have no similar patterns on DI, PP, and BP such as the node of *D. multiflora* and the clade of the most of *Pleomele* and *Dracaena* taxa has high PP 100 and low PB 61 (Fig. 3).

The length of the combined *psbA-trnH* and *trnL-F* sequences of 19 taxa varied from 935 bases in all of the ingroup

taxa to 1035 bases in outgroup *Agave missionum*. The maximum likelihood search of the combined data set retained 335021 trees with L=430 (CI=0.744, and RI=0.476; both CI and RI were calculated including parsimony uninformative characters). Strict consensus tree obtained from 153 retained trees. Some nodes have no similar patterns on DI, PP, and BP such as the node of *D. multiflora* and *D. goldiena* have low BP 50 and PP 50 but has high decay index 6 (data not shown). On the combined data set all of the nodes have similar patterns on DI, PP and BP (Fig. 4).

Analyses of all datasets supported a monophyletic clade containing both of the genera *Pleomele* and *Dracaena* (Fig. 1-4). The strict consensus trees also recovered a polyphyletic *Pleomele* with *Dracaena* on those data analyses (parsimony and Bayesian) (Fig. 1, 2). The *trnL-F* of 33 taxa data set in maximum parsimony tree shows that all of the Hawaiian *Pleomele* group together and are closely aligned with a *Dracaena* species, *D. marginata*, but the support for this relationship is not strong [Bayesian posterior probabilities (PP): 75, bootstrap percentages (BP): 52, decay index (DI): 2] (Fig. 2). Either of the separated data set of 19 taxa of *psbA-trnH* and *trnL-F* does not have good resolution. The *psbA-trnH* of 19 taxa data set in maximum parsimony tree shows that the polytomy of *Dracaena and Pleomele* with the exception of *D. cantleyi and D. umbraculifera* (Fig. 3). The two trees are not incompatible in their basic structure. Therefore a combined analysis using the 19 species that were sequenced in both analyses was undertaken (Fig. 4). The combined data set in maximum parsimony tree shows all of the Hawaiian *Pleomel*e nested together without *D. marginata* (PP: 100, BP: 86, DI: 2) (Fig 4). The placement of *D. cantleyi* and *D. multiflora* are different in the phylogenetic trees of *trnL-F* and *psbA-trnH*. *Dracaena multiflora* is on the more basal position based on *trnL-F* analysis and its support is good (PP: 100, BP: 80) based on *psbA-trnH* analysis. On the other hand, *D. cantleyi* is on the related basal position based on the *psbA-trnH* analysis and its support is strong (PP: 100, BP: 81) based on *trnL-F* analysis though it has the situation of a long branch attraction.

Discussion

This is the first time that *Pleomele* has been included in a phylogenetic analysis, and the results indicate that it is nested within *Dracaena*. The differentiation between *Dracaena* and *Pleomele* was uncertain from the time that Vandelli described the genus *Dracaena* (in 1768) and Salisbury named the genus *Pleomele* (in 1796). It remained confused until Brown (1914) separated them by more clear morphological characteristics based on the difference of flower structure. However, the debate between the relationship between *Dracaena* and *Pleomele* has never stopped. Bos (1992), Stevens (2001), and Staples and Herbst (2005) had recently placed the genus *Pleomele* into the genus *Dracaena* but without explanation. In contrast, Degener (1980), St. John (1985) and Wagner (1990) agreed that *Pleomele* should be separated from *Dracaena*. This study provides clear evidenced that *Pleomele* is not monophyletic and should be combined into *Dracaena* based on phylogenetic analysis.

The two *Dracaena* species, *D. tarzen* and *D. ensifolia*, are the basal clade with high support on the node, and for a sister group to the other *Dracaena* and *Pleomele* species. The second basal group with high support on the node is included the three species, *D. steudneri, D. multiflora, and D. umbraculifera.* It is formed sister group with the remaining *Dracaena* and *Pleomele* taxa. According to the species distribution of historical biogeography, we cannot have any further interpretation. Thus, the examination of morphological characteristics is needed to discover the patterns.

The combined data sets did not resolve the relationships among the species Hawaiian *Pleomele*. It is uncertain which species first colonized in the Hawaiian Islands or the direction of the radiation from island to island. Therefore, searching for faster evolving genetic markers is crucial.

In the analysis of *trnL-F*, the three species, *D. steudneri*, *D. multiflora* and *D. umbraculifera* may have the problem of long branch attraction or be truly evolved in a higher rate of base substitution at a faster rate. However, in the analysis of *psbA-trnH*, the situation does not exist perhaps because *psbA-trnH* is relatively slower evolving cpDNA marker compare to *trnL-F* for the genus *Dracaena* and *Pleomele*. Adding more non-terminal related taxa on the branches may break up the long branch or be evolving in a faster rate. Further examination is necessary.

On the data set of *trnL-F*, the CI value is not high enough and has related higher homoplasy. The data set of *psbA-trnH* and the combined data set had high CI values and thus their phylogenies can have more confidence to be trusted. However, the combined data set has low RI and RC value. The phylogeny of the data set of combined *trnL-F* and *psbA-trnH* has few synapomoprhy characters. Therefore, the evoluti-

onary tree is not robust. The reasons of inconsistent nodes support the strict consensus of parsimony trees and ML trees should be due to the total characteristics are not long enough (461 bp) because the parsimony informative characteristics have 33% of the total characters. The reason for the inconsistent nodes supports of the strict consensus of parsimony trees and ML trees on the data set of combined *trnL-F* and *psbA-trnH* should be due to too few parsimony informative characters (10% of total characters) not due to short sequences.

From the current biogeography literature, it is still not clear how *Pleomele* genus emerged from *Dracaena* or how *Dracaena* dispersal from the Africa-Arabic Peninsula to the South Asia and Southeast Asia. In the previous study, no geographical barrier was seen between mainland Southeast Asia and the western part of Malesia until the Pliocene (Hall 1998), and the southern Yunnan, mainland Southeast Asia, and the western part of Malesia during the Tertiary when it formed a landmass (Morley 1998). It shows that the flora in southern Yunnan, China should have been derived from tropical Asia due to climatic warming after Tertiary when the Himalaya started to uplift and monsoon forming began (Zhu 2008). Several *Dracaena* and *Pleomele* species are native or endemic to Yunnan and to Myanmar (Kurz 1974; Xinqi and Turland 2000). The Eastern Himalaya could be the northern barrier for the *Dracaena* and *Pleomele* migration due to cooler climate but also could be undertaken into the southern Yunnan flora model to include the species of these two genera in the near future according the climate change evidence and its theory.

Dracaena and *Pleomele* belong to tropical and subtropical plants. If in one location, some species belonging to both genera exist then this location can be interpreted as "warm" area. Therefore, these plants can be used as an index for climate change in the specific location and broader area. For example, these plants should not occur in the Himalaya regions. But if they begin to appear in Himalaya regions either by direct introduction or cultivated methods, it can be assumed the plants are adapting into the region's warming climate. Eastern Himalaya is one of the 25 biodiversity hotspots (Myers *et al*. 2000), but its flora data is not complete yet. According to *Dracaena*/*Pleomele*'s biological information, it is possible to find those species in the Eastern Himalaya in the subtropical area. Further plant identification and survey in this area should be done. Once the database is set up, the related strategies for conservation can be carried out.

Conclusion

This study shows that *Pleomele* is not monophyletic and could be placed into *Dracaena*. It can be concluded that *Pleomele* and *Dracaena* as circumscribed are both paraphyletic groups. Even though *Pleomele* is resolved to be nested within *Dracaena*, the support for this relationship remains not strong enough. *Pleomele* is still possible to form a monophyletic group only in Hawaii (become endemic to the Hawaii Archipelago) and the remaining species under this genus should be replaced into *Dracaena* in other places in the world. Further work should include more taxa of *Dracaena* and *Pleomel*e and focus on other genetic regions to investigate better resolution and statistic supports of phylogeny for establishing a robust evolutionary relationship within and between the genera *Dracaena* and *Pleomele*.

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