Research

Phylogenetcs 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 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
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 (APG II 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; Kubitzki 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; Kubitzki 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; Kubitzki 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; Kubitzki 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 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; Kubitzki 1998; Staples and Herbst 2005). Therefore, the purpose of this study is to provide molecular phylogenetic evidence for the classification of *Draceaena* and *Pleomele* and resolve the systematic problems between *Pleomele* and *Draceaena* at the genetic level.

**Materials and Methods**

**SAMPLE COLLECTION**

Leaf tissues from species of *Draceaena* 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* intergenic 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. augustofolia* 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. aurea*, *P forbesii*, and *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 characters). Strict consensus tree obtained from 153 retained trees with L=430 (CI=0.744, and RI=0.476; both CI and RI were calculated including parsimony uninformative characters).

On the combined data set all of the nodes have similar patterns on DI, PP and BP (Fig. 4). The strict consensus trees also recovered a polyphyletic 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 Pleomele 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 synapomorphy characters. Therefore, the evoluti-
Conclusion

This study shows that *Pleomele* is not monophyletic and could be placed into *Dracaena*. It can be concluded that *Dracaena* and *Pleomele* 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 *Pleomele* 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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References


