

Molecular phylogeny of *Chamaedorea* based on the low-copy nuclear genes PRK and RPB2



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Abstract

We conducted the first phylogenetic analysis of *Chamaedorea* (Arecaceae: Ceroxyloideae: Hyophorbeae), the largest neotropical genus of palms, using molecular data from the low-copy nuclear genes PRK and RPB2. The monophyly of the genus and its placement within its tribe Hyophorbeae have not been systematically tested, nor have more than two of the approximately 100 species of *Chamaedorea* previously been included in a phylogenetic study of the palms. The prevalent current taxonomy of *Chamaedorea* recognizes eight subgenera (Hodel 1992), all based on floral characters, which provide a useful starting point to explore species-level systematics. Sequence data from 63 species, including representatives of all eight subgenera were analyzed using maximum parsimony and Bayesian inference optimality criteria. *Chamaedorea* is resolved as monophyletic with strong support in all PRK, RPB2 and combined analyses. However, at least two of the subgenera are resolved as non-monophyletic, including the two largest, *Chamaedorea* and *Chamaedoropsis*, which are distinguished from each other by the degree of connation in the staminate petals. Phylogenetic resolution among members of these two subgenera shows no pattern of congruence between relatedness and degree of staminate petal connation. Many of the well-supported clades resolved by these gene regions are unsupported by existing morphological evidence and define groups never before proposed within *Chamaedorea*. PRK is found to be more informative than RPB2, but both have proven useful for interpreting species-level relationships in palms. A paralogue of the desired copy of PRK is identified that could be a valuable source of phylogenetic information for future studies.

Key Words: Arecaceae; *Chamaedorea*; character transformation; classification; evolution; low-copy nuclear genes; molecular systematics; paralogue; PRK; RPB2.

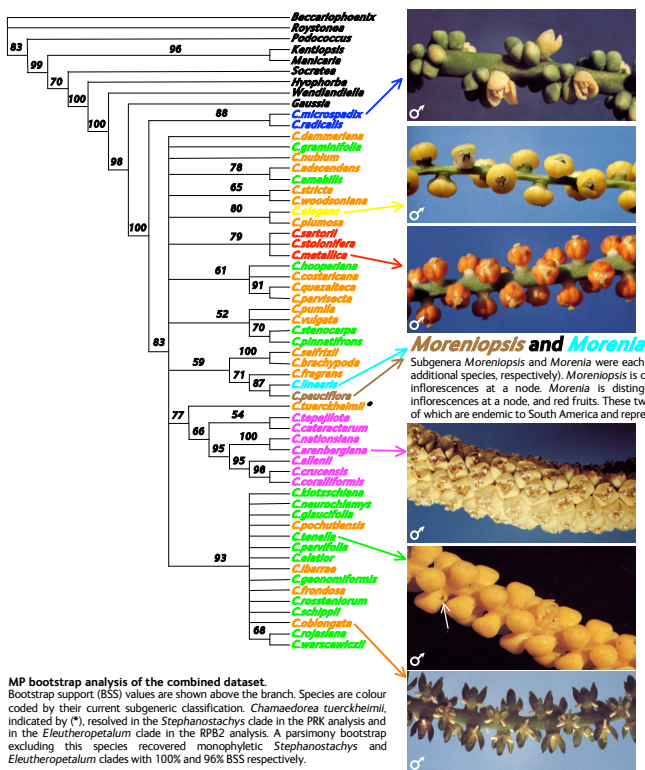
Objectives

- Test the monophyly of the genus *Chamaedorea*
- Determine its position within the tribe Hyophorbeae
- Explore and refine the current morphologically-based subgenera

Materials and Methods

- Included 63 species of *Chamaedorea*, including representatives of all 8 subgenera
- Included the four other genera of tribe Hyophorbeae
- Six outgroup genera chosen from the Arecoid line (from three tribes and six subtribes)
- Non-coding regions of the genes phosphoribulokinase (PRK) and RNA polymerase II (RPB2) were amplified
- Sequence data were analyzed using maximum parsimony (MP) and Bayesian inference (BI) optimality criteria

Results



Moreniella - monophyletic

Subgenus *Moreniella* is characterized by its staminate flowers in clusters (acervuli). Inflorescences are solitary at a node and fruits are red. The group contains only two species and was found to be monophyletic and basal to the rest of *Chamaedorea*.

Collinia - monotypic (*C. elegans*)

Subgenus *Collinia* is characterized by its staminate and pistillate flowers with fused petals, the flower opening through a tiny apical aperture. *C. elegans* was found to be most closely related to *C. plumosa* (a member of *Chamaedoropsis*) – a relationship never previously suggested.

Eleutheropetalum - monophyletic

Subgenus *Eleutheropetalum* is characterized by its orange, fleshy, hooded, bilobed staminate flowers. The group was found to be monophyletic and includes *C. rhizomatosa* (not included in the combined analysis) and possibly the wildcard taxon *C. tuerckheimii*.

Moreniopsis and *Morenia*

Subgenera *Moreniopsis* and *Morenia* were each represented by one species in this study (they contain one and two additional species, respectively). *Moreniopsis* is characterized by its solitary staminate flowers and multiple staminate inflorescences at a node. *Morenia* is distinguished by its staminate flowers in acervuli, multiple staminate inflorescences at a node, and red fruits. These two species formed a moderately-supported clade with *C. fragrans*, all of which are endemic to South America and represent the southern limit of the range of *Chamaedorea*.

Stephanostachys - monophyletic

Subgenus *Stephanostachys* is characterized by its contiguous staminate flowers and often densely packed pistillate flowers. The group formed a well-resolved monophyletic clade. Some members of other subgenera, such as *C. adscendens*, exhibit densely packed (but not contiguous) staminate flowers; these species were clearly distinguished from the *Stephanostachys* clade.

Chamaedorea - polyphyletic

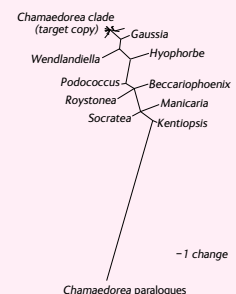
Subgenus *Chamaedorea* is characterized by its apically connate staminate flowers, which open through small lateral pores (highlighted in this photo with a white arrow). The group was found to be highly polyphyletic, its members resolving variously throughout the tree, usually in clades with members of subgenus *Chamaedoropsis*.

Chamaedoropsis - polyphyletic

Subgenus *Chamaedoropsis* is characterized by its free and spreading staminate petals and solitary (not contiguous) staminate flowers. The group was found to be highly polyphyletic, its members frequently occurring in clades with members of subgenus *Chamaedorea*.

A paralogue of PRK

A paralogue of the target copy of PRK was identified in five species of *Chamaedorea*. For all but one of these species, the target copy of PRK was also recovered. Despite being similar in length to the target copy of PRK, the paralogue is easily distinguished in a sequence alignment. In *C. dammeriana*, for example, the paralogue and the target copy have an uncorrected pairwise distance of 0.410, whereas the largest distance between the target copy of *C. dammeriana* and any other taxon in the study, outgroup taxon *Roystonea oleracea*, is only 0.142. Most of this variation is contained within the intron, but even the coding regions of the two copies are easily distinguished. The paralogue is apparently functional, having no stop codons or frame shifts. A translated BLAST search of the coding regions of paralogue 3 found it to be most similar to the PRK gene in *Aralidopsis thaliana* (BLAST score = 1e-31), *Triticum aestivum* (2e-31), *Oryza sativa* (5e-31), *Pisum sativum* (6e-31) and *Beta vulgaris* (3e-30), followed by the rest of the palms in the database (2e-29). The paralogous sequences were not included in the final analyses, but the new paralogue of PRK identified in this study showed strong potential to be phylogenetically informative, as it exhibited several variable and informative mutations across the five species from which it was amplified. Paralogue-specific primers have been designed and attempts are currently being made to amplify this DNA region from additional *Chamaedorea* species.



Phylogram of the translated sequence data for the target and paralogous copies of PRK.

Conclusions

It is evident from this study that the subgeneric classification of genus *Chamaedorea* requires substantial revision. Although the data support monophyly of the smaller subgenera *Moreniella*, *Eleutheropetalum* and *Stephanostachys*, subgenera *Chamaedorea* and *Chamaedoropsis*, which contain over 80% of the species described in the genus, are clearly polyphyletic. Additional information on aspects such as floral anatomy, reproductive biology, autecology and karyotypes should improve understanding of species-level relationships within *Chamaedorea*. Future population studies should shed more light on the apparent existence of morphologically cryptic species complexes, biogeography and degrees of endemism. A crucial step towards a more complete understanding of *Chamaedorea* phylogenetics will be the construction of a rigorous morphological matrix for the genus. There is also enormous potential for additional molecular studies on *Chamaedorea*, as the development of new molecular markers, including low-copy nuclear genes, expands the number and variety of DNA regions available for phylogenetic analysis.

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