

# DNA and Palm Evolution

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Palms have always been regarded as special plants with many distinctive features, so it comes as no surprise that botanists continue to recognize them as a well-defined family. The fossil record indicates that palms have been around for at least 65 million years, but we still don't know how the major groupings within the family are related to one another and to other plants.

Only recently, botanists have gained insight into some of the early evolutionary changes that may have given rise to the palms from within a group of related flowering plants (the monocotyledons). This research has been fueled by recent advances in phylogenetics, the field of biology that examines relationships among living things. Relationships are represented in the form of phylogenetic trees, which are branching diagrams of species groupings (e.g., Fig. 1E, Fig. 2, Fig. 4). The newest trees of monocot relationships (e.g., Chase et al. in press) show that the palms share an ancestor with familiar cultivated plants including spiderworts, ginger, bromeliads and grasses.

As phylogenetic research helps to pinpoint the origin of palms, the same techniques allow us to explore the pattern of evolution that gave rise to the incredible diversity within the family. This is an exciting time to be involved with palm evolutionary studies, because we now have the ability to access information stored within the genetic material of palms and to analyze this information using specialized computer software. We are now poised to answer questions about how the 189 genera of palms (Dransfield & Uhl 1998)

arose in relation to one another, and we can also begin to tell the story of how the genera attained their present distribution across the world.

Evolutionary trends in the palm family have been investigated previously (e.g., Moore 1973, Moore & Uhl 1982), and the ideas laid down in these and other papers formed the foundation for the classification scheme in *Genera Palmarum* (Uhl & Dransfield 1987). At that time, however, the authors did not have access to the molecular techniques and the powerful computer technology that are available so readily today.

## Why study DNA?

Like all other organisms, palms have a finite number of observable structural characteristics. Features such as stem, leaf, and inflorescence structure are relatively easy to observe, but more detailed morphological and anatomical features can be physically and technically difficult to study. Many researchers (e.g., Barfod et al. 1999, Henderson 1999, Pintaud 1999) have overcome these difficulties, collecting vast amounts of structural information and producing valuable phylogenetic trees. Still, these studies are limited by a shortage of data and by complexities in their

interpretation. It is clear that structural features alone cannot answer all of our questions about palm evolution.

The main advantage of studying DNA is that the available data are almost limitless. Each DNA molecule is a long chain of chemical units called nucleotides. Only four kinds of nucleotides (abbreviated A, C, T and G) are used to build the chain, and the sequence (arrangement) of these nucleotides is a code containing an organism's genetic information. We can access small amounts of this information by reading DNA sequences, such as the one shown in Figure 1C. Although today's technology allows us to explore only a tiny fraction of a plant's total DNA, we are able to gather sequences containing hundreds of nucleotide positions.

Changes have arisen in DNA sequences throughout evolutionary history, so we often find different sequences in different species. For example, one nucleotide may be substituted for another, or nucleotides can be added, removed, or rearranged within a sequence. These changes can be seen when sequences from a set of species are compared (Fig. 1D). When we see a particular change shared by two or more species, we have evidence that those species may be related.

### DNA Sequencing

The first, and often the most difficult step, is to collect palm specimens suitable for DNA study. Each specimen consists of two parts: (1) a small amount of leaf tissue preserved by rapid freezing or rapid drying, so that DNA is held intact within the cells, (2) a larger sample collected as a voucher, consisting of leaves, inflorescences, fruits and any other distinguishing organs. The voucher is stored in a herbarium, allowing future researchers to verify the specimen's identification, and to repeat experiments when necessary.

DNA is extracted from leaf samples using a combination of mechanical and chemical procedures, resulting in a pure solution of DNA (Fig. 1A). Specific pieces of DNA can be isolated using a method called the polymerase chain reaction (PCR), which produces millions of copies of the specified region of DNA (Fig. 1B). The sequence of nucleotides in the region can then be determined using automated DNA sequencing equipment (Fig. 1C).

For each DNA region studied, sequences from many species are gathered together and aligned with one another in a matrix (Fig. 1D). The matrix allows sequence data to be viewed in tabular form, so similarities and differences between species can be detected. Specialized computer programs

evaluate all of the different ways the species can be related to one another, searching for the arrangements that most closely match the sequence data. The best of these alternatives are used to build the final phylogenetic tree for the species (Fig. 1E).

### What are we learning from DNA?

We now have complete data from three DNA regions, and our current knowledge about palm evolution based on these data is summarized in a phylogenetic tree (Fig. 2). The tree confirms many of the groupings defined in *Genera Palmarum*, but there are several surprises which we discuss below.

### Relationships among subfamilies within the palm family

Each branch in Figure 2 represents a separate lineage, or a line of evolution containing all descendants of a common ancestor. For example, subfamilies Calamoideae (yellow triangle) and Phytelephantoideae (orange triangle) are well-defined lineages in the tree of relationships. However, subfamilies Ceroxyloideae (blue triangles), Arecoideae (red triangles) and Coryphoideae (green triangles) do not appear to be single lineages because they do not form discrete groups derived from a shared ancestor. On this tree, subfamily Phytelephantoideae (orange triangle) is related to parts of subfamily Ceroxyloideae (blue triangle). There are still areas of doubt and ambiguity that may be clarified with the addition of further data. These include the relative positions of the Calamoideae and the Nypoideae, and the relationships among the five lineages of subfamily Coryphoideae.

### Hyophorbeae in Arecoideae

Tribe Hyophorbeae (*Chamaedorea*, *Gaussia*, *Hyophorbe*, *Synechanthus* and *Wendlandiella*) has a peculiar Neotropical and Indian Ocean distribution, yet it is a clearly-defined group of palms. Among other things, the group is characterized by flowers that are usually arranged in rows, although the flowers of *Chamaedorea* are generally solitary. Subfamily Arecoideae, on the other hand, is characterized by flowers usually arranged in groups of three composed of a central female flower flanked by two male flowers (triads). One of the surprising results of DNA analysis has been the placement of the Hyophorbeae within subfamily Arecoideae, rather than with *Pseudophoenix* and tribe Ceroxyleae (e.g., *Ceroxylon*, *Juania*, *Oraniopsis* and *Ravenea*), which also lack triads. As a result we are reconsidering the concepts of inflorescence evolution in this interesting group of palms.

### Affinities of caryotoid palms

The three genera *Caryota*, *Arenga* and *Wallichia* form a group that Uhl and Dransfield (1987) recognized as tribe Caryoteae. Any caryotoid palm can be recognized instantly by the presence of pinnate leaves (twice pinnate in the case of *Caryota*) with induplicate leaflets (leaflets V-shaped in section) with jagged tips. This remarkable group of palms has long been recognized as a distinct lineage, and yet its affinities with other palms have remained uncertain. In *Genera Palmarum*, Uhl and Dransfield placed the caryotoids in the Arecoideae, a decision based on the shared characteristic of flowers arranged in triads.

Despite the morphological evidence placing the caryotoid palms within the Arecoideae, the new DNA data indicate that the Caryoteae is most closely related to a lineage of familiar palmate-leaved palms that includes tribe Borasseae (e.g., *Borassodendron*, *Lodoicea*, *Hyphaene* and *Satranala*) and the genus *Corypha*. This revolution in palm classification has profound implications not only for our concepts of palm relationships, but also for our ideas of palm morphology. Our confidence in this link between caryotoid and borassoid palms is increasing as more DNA data are collected and yet few morphological connections can be made. The mutual possession of induplicate leaflets in both Caryoteae and Borasseae could be construed as a link, but anatomical differences argue against this. Further shared characters can be found only in the anatomy of female reproductive structures. This novel relationship cries out for closer examination.

### Relationships among calamoid palms

Few palm botanists would dispute the fact that the calamoid palms constitute a lineage derived from a single common ancestor. The beautifully scaly fruit is the most striking feature common to all 22 genera of subfamily Calamoideae, indicating a close relationship between them. However, within the subfamily, almost all possible palm growth forms are found, namely tree palms (e.g., *Mauritia*, *Raphia*, *Pigafetta* and *Metroxylon*), shrub palms (e.g., *Lepidocaryum*), stemless palms (e.g., *Salacca*, *Eleiodoxa*) and climbing palms or rattans (e.g., *Calamus*, *Plectocomia*, *Korthalsia*, *Laccosperma*). Furthermore, there is a great diversity of inflorescence structure ranging from the congested heads of catkin-like flowering branches in *Eleiodoxa* through to the immensely extended, whip-like inflorescences of *Calamus* that may grow to a length of 8 m or more. To cap it all, though the majority have pinnate leaves, three genera, *Mauritia*, *Mauritiella* and *Lepidocaryum*, have palmate leaves. These patterns of variation conflict

with one another, and the relationships among these palms are by no means obvious.

Recent efforts aimed specifically at clarifying this confusion using a combination of DNA and morphological evidence have disclosed three major lineages within the Calamoideae, rather than the two proposed by Uhl and Dransfield (1987) based on the presence of either pinnate or palmate leaves. The new scheme indicates a group comprising all African and American calamoids, a second comprising all Asian calamoids (except for the extraordinary genus, *Eugeissona*) and a third consisting of *Eugeissona* alone (Baker et al., in press a).

The 13 rattan genera are of special interest because they are not as closely related to one another as one might have expected. Rather the geographical division of calamoid genera divides the rattans into at least two groups (Fig. 3), separating the three African genera (*Laccosperma*, *Eremospatha* and *Oncocalamus*) from the remaining Asian genera (e.g., *Calamus*, *Plectocomia* and *Korthalsia*). These two groups are not close relatives within Calamoideae and consequently it seems that the climbing habit, for which the subfamily is so well known, has evolved independently on at least two occasions (Fig. 3). There is good morphological evidence for these two distinct groups; for example, the climbing whip, or cirrus, that extends from the end of the leaf is quite different in the two groups of taxa. In the African rattan genera, the cirrus is armed with reflexed thorns derived from reduced leaflets, whereas the cirrus of Asian rattans lacks such thorns and possess only grapnel-like spines, usually arranged in groups like a cat's claws.

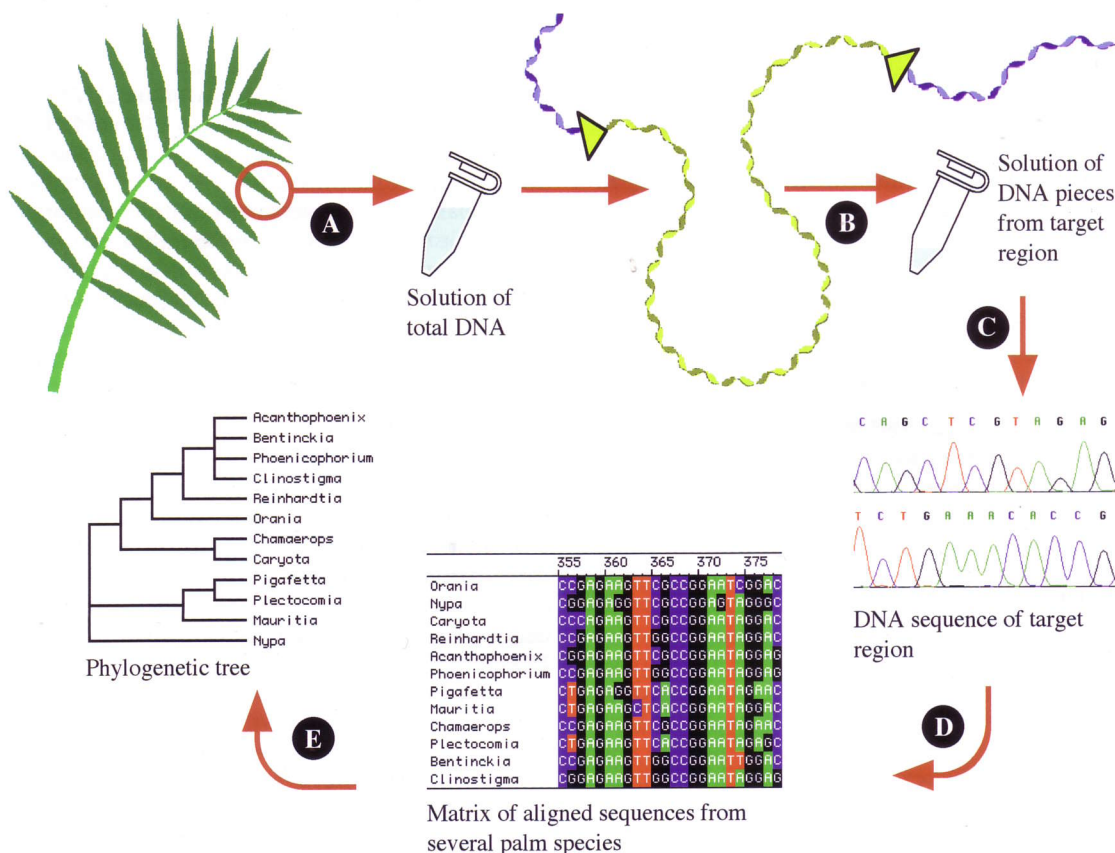
### Challenges

Progress in molecular studies of the palm family has been limited by an unusually slow rate of DNA evolution in the family. It has been necessary to gather sequences from several different DNA regions in order to find enough nucleotide changes to resolve relationships. The choice of which regions to use is difficult, and requires consideration of various aspects of DNA evolution (see Soltis & Soltis, 1998 for a review).

It is important to find DNA regions that evolve at a useful rate. If a region evolves too slowly, there will be too few nucleotide changes to provide information about relationships. If a region evolves too rapidly, the overwhelming number of changes makes it difficult to find comparable regions among sequences from different species.

For phylogenetic studies of the entire palm family (e.g., Baker et al. 1999, Asmussen et al. in press,





1. Overview of DNA sequencing and analysis: A. DNA is extracted from leaf tissue. B. The target region is isolated and copied using PCR. C. Copies of the target region are sequenced. D. Sequences from different species are aligned together in a matrix. E. Computer software is used to select the best tree or trees of relationships for the species.

Asmussen & Chase in press), the most useful data have come from regions of DNA located on the single chromosome within the chloroplasts of plant cells. Certain regions of DNA from the nuclei of plant cells may also be appropriate for these studies (Lewis & Doyle submitted, Hahn 1999) and for analyses of more recent lineages, such as groups of species or genera (Baker et al. in press b, Lewis & Martinez submitted).

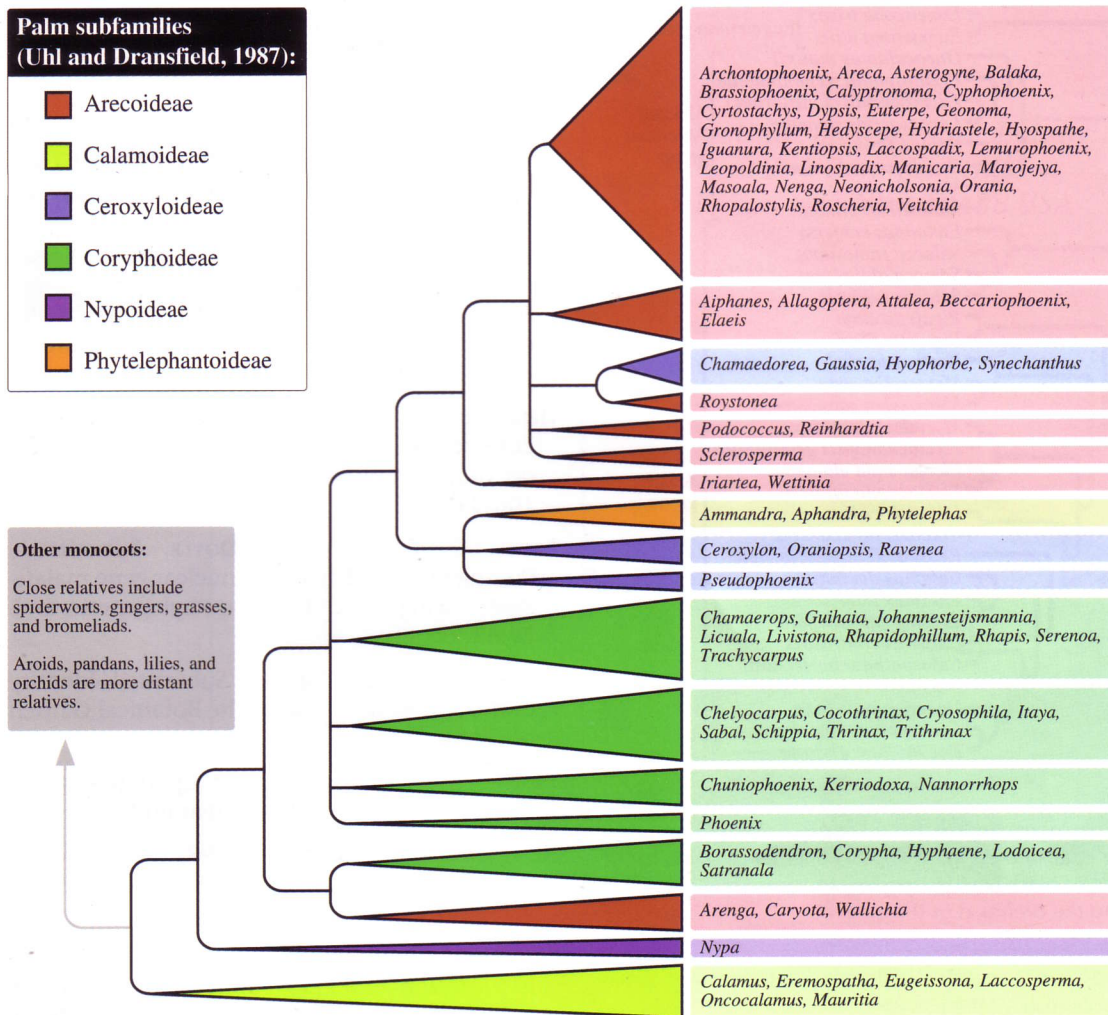
Another challenge that we face in this research is the limited availability of specimens. Access to wild populations requires extensive travel and collaboration abroad, both of which can be difficult to arrange. For many years, collectors have worked under laws that restrict access to biodiversity in individual countries. Now, precise guidelines on the collection and transport of specimens have been formalized on a global scale in the Convention on Biological Diversity (CBD), ratified by the United Nations in 1992. The CBD stipulates that each country has control over the genetic resources within its borders, and that

collectors must make clear arrangements with local governments before collecting and exporting biological samples. These guidelines were designed to protect countries from unrestricted removal and exploitation of their genetic resources. As a result of the CBD, however, collectors often face complex application procedures and long waiting periods before they can obtain permission to work in a country.

We are fortunate that palms are represented well in cultivation, and many of our DNA samples have been gathered (with permission) from botanic gardens. There is also a great diversity of species grown by hobbyists, but it is often difficult for researchers to gain access to private collections. We hope to begin working with palm growers as we gather samples for future research projects.

### Conclusion

As we continue to build and analyze data sets of DNA sequences, a clearer picture of palm evolution is emerging. The new DNA-based phylogenetic



2. A simplified DNA-based phylogenetic tree showing the relationships of representative palms sampled from across the family (after Asmussen & Chase in press). Each triangle represents a lineage derived from a common ancestor, and the branches of the tree show how these lineages are related to one another.

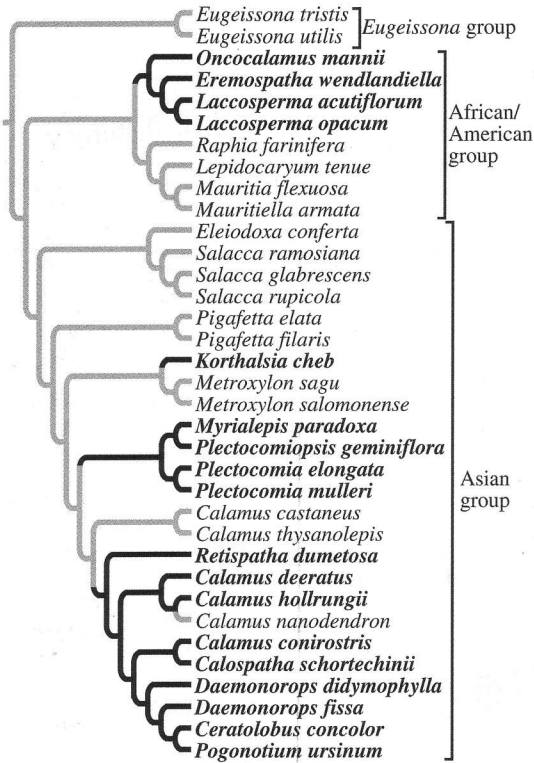
trees largely confirm the groupings proposed in recent classifications, but interesting relationships among some genera are indicated for the first time.

The technology for phylogenetic research has improved dramatically over the past decade, and we can assume that new advances will continue to arise at the same pace. We will find it easier to gather data as DNA sequencing methods become more streamlined, and new sources of data will become available as we learn more about plant genes. So far we have explored just a tiny fraction of the total DNA information, and our future phylogenetic research will focus on the unexplored palm DNA that still holds a wealth of information about palm evolution.

#### LITERATURE CITED

- ASMUSSEN, C. B., W. J. BAKER AND J. DRANSFIELD. In press. Phylogeny of the palm family (Arecaceae) based on *rps16* intron and *trnL-trnF* plastid DNA sequences. In Wilson, K. L. and D. A. Morrison [eds.], *Systematics and Evolution of Monocots*. CSIRO Publishing, Australia.
- ASMUSSEN, C. B. AND M. W. CHASE. In press. Coding and noncoding plastid DNA in palms. *American Journal of Botany*.
- BAKER, W. J., C. B. ASMUSSEN, S. BARROW, J. DRANSFIELD AND T. A. HEDDERSON. 1999. A phylogenetic study of the palm family (Palmae) based on chloroplast DNA sequences from the





4. A phylogenetic tree for subfamily Calamoideae (after Baker et al. in press a). It appears that the climbing habit, shown as black lines in the diagram, arose multiple times during the evolution of the group.

*trnL-trnF* region. Plant Systematics and Evolution 219:111–126.

BAKER, W. J., J. DRANSFIELD AND T. A. HEDDERSON. In press a. Phylogeny, character evolution, and a new classification of the calamoid palms. Systematic Botany.

BAKER, W. J., T. A. HEDDERSON AND J. DRANSFIELD. In press b. Molecular phylogenetics of *Calamus* (Palmae) and related rattan genera based on 5S nrDNA spacer sequence data. Molecular Phylogenetics and Evolution.

BARFOD, A. S., F. ERVIK AND R. BERNAL. 1999. Recent evidence on the evolution of Phytelphantoid palms (Palmae). In A. Henderson and Borchsenius, F. [eds.], Evolution, variation, and classification of palms, 265–277. New York Botanical Garden Press, New York, USA.

CHASE, M. W., D. E. SOLTIS, P. S. SOLTIS, P. J. RUDALL, M. F. FAY, W. J. HAHN, S. SULLIVAN, J. JOSEPH, M. MOLVRAJ, P. J. KORES, T. J. GIVNISH, K. J. SYTSM AND J. C. PIRES. In press. Higher-level systematics of the monocotyledons: An assessment of current knowledge and a new classification. In Wilson,

K. L. and D. A. Morrison [eds.], Systematics and Evolution of Monocots. CSIRO Publishing, Australia.

DRANSFIELD, J. AND N. W. UHL. 1998. Palmae. In Kubitzki, K. [ed.], The Families and Genera of Vascular Plants Vol. IV. Springer-Verlag, Berlin, Germany.

HAHN, W. J. 1999. Molecular systematic studies of the Palmae. In A. Henderson and Borchsenius, F. [eds.], Evolution, variation, and classification of palms, 47–60. New York Botanical Garden Press, New York, USA.

HENDERSON, A. 1999. A phylogenetic analysis of the Euterpeinae (Palmae; Arecoideae; Areceae) based on morphology and anatomy. Brittonia 51: 106–113.

LEWIS, C. E. AND J. J. DOYLE. Submitted. Phylogenetic utility of the nuclear gene malate synthase in palms. Molecular Phylogenetics and Evolution.

LEWIS, C. E. AND N. MARTINEZ. Submitted. Identity of the *Hyophorbe* palms at the Botanical Garden of Cienfuego, Cuba. PALMS.

MOORE, H. E. 1973. The major groups of palms and their distribution. Gentes Herbarum 11: 27–141.

MOORE, H. E. AND N. W. UHL. 1982. Major trends of evolution in palms. The Botanical Review 48: 1–69.

PINTAUD, J.-C. 1999. A cladistic analysis of the Archontophoenicinae (Palmae, Areceae) based on morphological and anatomical characters. In A. Henderson and Borchsenius, F. [eds.], Evolution, variation, and classification of palms, 279–284. New York Botanical Garden Press, New York, USA.

SOLTIS, D. E. AND P. S. SOLTIS. 1998. Choosing an approach and an appropriate gene for phylogenetic analysis. In D. E. Soltis, P. S. Soltis, and J. J. Doyle [eds.], Molecular systematics of plants II: DNA sequencing, 1–42. Kluwer Academic Publishers, Boston, Massachusetts, USA.

UHL, N. W. AND J. DRANSFIELD. 1987. Genera Palmarum: a classification of palms based on the work of H. E. Moore, Jr. International Palm Society and L. H. Bailey Hortorium, Lawrence, Kansas, USA.