1-1-2011

A Phylogenetic Analysis of the African Plant Genus Palisota (family Commelinaceae) based on Chloroplast DNA Sequences

Grady H. Zuiderveen
Grand Valley State University

Timothy M. Evans
Grand Valley State University

Robert B. Faden
National Museum of Natural History, Smithsonian Institution

A Phylogenetic Analysis of the African Plant Genus *Palisota* (family Commelinaceae) based on Chloroplast DNA Sequences

[1]Grady H. Zuiderveen, [1]Timothy M. Evans, and [2]Robert B. Faden
[1]Biology Department, Grand Valley State University, 232 Henry Hall, 1 Campus Drive, Allendale, Michigan 49401 USA
[2]Department of Systematic Biology-Botany, MRC 166, National Museum of Natural History, Smithsonian Institution, P.O. Box 37012, Washington, DC 20013-7012

ABSTRACT. The plant genus *Palisota* (family Commelinaceae, or spiderwort family) consists of approximately 20 species and is distributed throughout the forests of tropical Africa. The genus exhibits several unusual morphological characteristics, and as a result has been difficult to classify based on morphology. Molecular phylogenetic studies have placed it near the base of Commelinaceae, but the exact placement of *Palisota* within the family is not clear. As the African continent has become more arid in recent geological times, the forests have receded, reducing the habitat for *Palisota* species and potentially impacting speciation and extinction rates within the genus. The goal of this study is to sequence the chloroplast-encoded gene *rbcL* in several additional species of *Palisota* and its relatives in order to: 1) determine the phylogenetic relationship of the genus with respect to other members of Commelinaceae; 2) evaluate phylogenentic relationships among species of *Palisota*; and 3) infer relative speciation/extinction rates within the genus. Additionally, we are exploring the use of other molecular regions for phylogenetic analysis with the genus.

Introduction:

The plant genus *Palisota* is the most prevalent African genus of Commelinaceae (Faden 1998), consisting of approximately 20 species distributed throughout the forests of tropical Africa. The genus is morphologically divergent from other members of the family in several floral and fruit characteristics, and its taxonomic placement within the family has been problematic. Previous molecular studies have placed the genus as sister to the rest of tribe Tradescantieae (Evans et al. 2003), but support for that placement has been relatively weak (i.e. low bootstrap support values). Furthermore, these studies have included only one or two species of *Palisota*, making estimates of relationships among *Palisota* species impossible. This study includes 15 species of *Palisota*, and uses data from previous studies (Evans et al. 2003) to evaluate the relation of *Palisota* to other species within Commelinaceae and to assess relationships among *Palisota* species.

rbcL, a chloroplast gene which codes for the large subunit of ribulose-1,5-biosphosphatecarboxylase/oxygenase (Chase et al. 1993), is a suitable choice for evaluating phylogenetic relationships in *Palisota* for a variety of reasons. First, use of morphological characteristics and the habitat in which the species are found as a basis for determining relationships within *Palisota* has proven difficult (Faden 2007) due to convergent evolution within the group. Second, *rbcL* has been shown to be useful in determining phylogenetic relationships at various taxonomic levels, including relationships among species and genera within Commelinaceae (e.g., Wade et al. 2006; Evans et al. 2003; Chase et al. 1993; Duvall et al. 1993; Givnish et al. 1999; Korall et al. 1999; Setoguchi et al. 1998; Azuma et al. 2000).

The primary goal of this study is to conduct a phylogenetic analysis of *Palisota* and its relatives using nucleotide sequences of *rbcL*. Use of more thorough taxon-sampling in *Palisota* than in previous studies will enable us to examine phylogenetic relationships among species of the genus, and it may help to stabilize the placement of the genus within the family-wide phylogeny, thereby providing a better estimate of its relationship to other Commelinaceae genera. Additionally, we are exploring the use of other molecular regions (i.e. *rps16* and *matK*) for phylogenetic analysis within the genus.

Materials and Methods:

Fifteen species of *Palisota* were selected for inclusion in this study. Multiple accessions of several species were included to accommodate geographic variation within widespread species. DNA was isolated from leaf material using Doyle and Doyle's CTAB procedure (1987) as modified by Smith et al. (1991). *rbcL* was amplified using oligonucleotide primers that anneal to the first 26 nucleotides of the 5' end of the gene and slightly downstream of the 3' end. Internal primers RH1 and 1020-R were used in place of the 5' and 3' primers to amplify the gene in samples for which the other primers did not work. Polymerase chain reactions were performed according to manufacturer (Sigma Aldrich, USA) recommendations. Sequencing reactions were conducted using BigDye version 3.1 chemistry. Nucleotide sequences were obtained with an ABI 3130 Sequencer. Sequences were aligned manually, and phylogenetic analyses were conducted on the DNA sequences using PAUP* and MrBayes software.

Results:

Thus far, *rbcL* has been successfully amplified and sequenced in five species of *Palisota*. Parsimony analysis yielded 45 most parsimonious trees, placing a monophyletic *Palisota* sister to the tribe Tradescantieae (Fig. 1). Branch lengths appear to be similar within *Palisota* species throughout the phylogenies (Fig. 2).

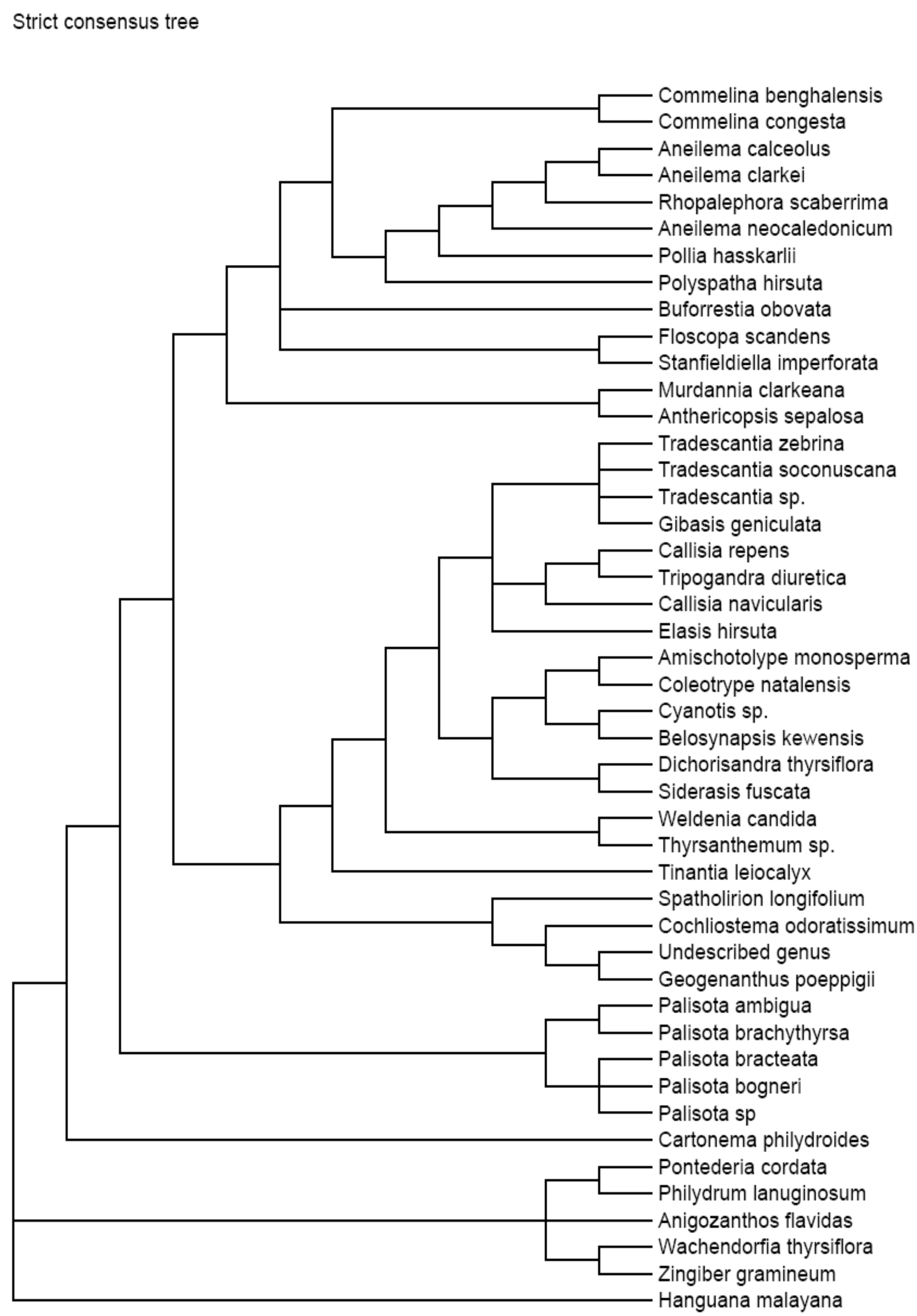

Figure 1. Strict consensus of 45 most parsimonious trees (1187 steps, CI = 0.51) produced by parsimony analysis of *rbcL* sequences in members of Commelinaceae and outgroup genera.

Tree 1

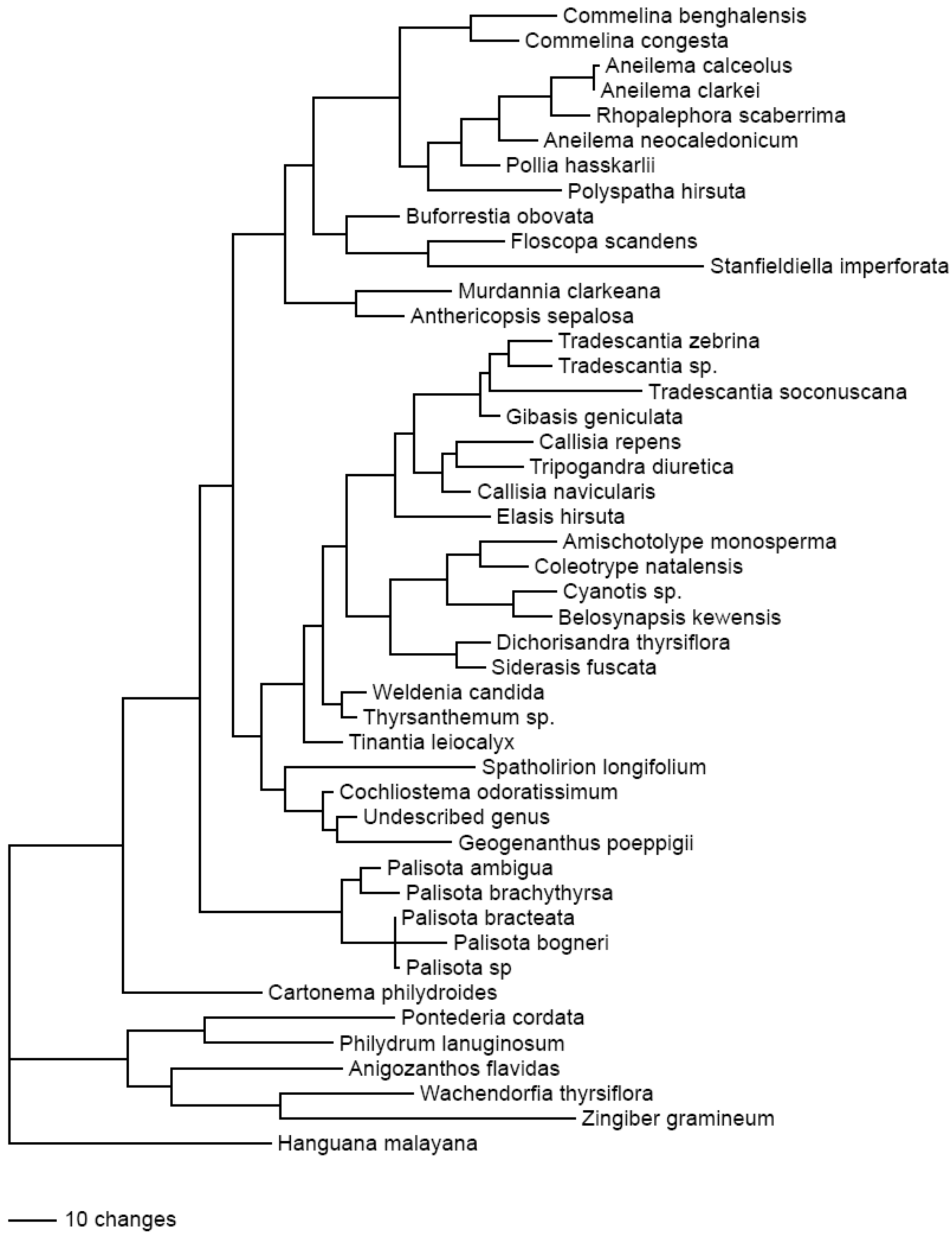

Figure 2. A single representative of the 45 most parsimonious trees produced by parsimony analysis of *rbcL* sequences in members of Commelinaceae and outgroup genera. Branch lengths are proportional to the amount of evolutionary change along each branch.

Discussion:

Currently, for the species of *Palisota* in which sequences have been obtained, there appears to be adequate variability within *rbcL* to resolve relationships within the genus. *Palisota* is monophyletic and consists of two clades (Figs. 1 and 2). Monophyly of *Palisota* is supported by its morphology, which is highly divergent from other members of Commelinaceae. It produces a fleshy berry (instead of the dry capsule produced by other members of the family), and it has several stamen characteristics that are not found in any other Commelinaceae genus (Evans et al. 2003, Tomlinson 1966, 1969).

Along with that, the increased number of *Palisota* samples included within this study increased the confidence in which *Palisota* can be place as sister to the tribe Tradescantieae as found in previous studies (Evans et al. 2003, Wade et al. 2006). These findings contradict previous placement of *Palisota* based solely on morphology (Faden and Hunt, 1991). Their study utilized anatomical and palynological distinctiveness (specifically pollen and stomata characters), instead of the previously used macromorphological characteristics, to place *Palisota* as part of Tradescantieae (Faden and Hunt, 1991).

Evans (unpublished data) has hypothesized that phylogenetic branch lengths may be directly tied to both speciation and extinction rates. Thus, long branches in a phylogeny might reflect either low speciation rates or high extinction rates, whereas short branches are tied to high speciation or low extinction rates. A pattern of short internal branches and long external branches (or “tips” of the phylogeny) have been observed in some Bromeliaceae phylogenies, indicating an elevated rate of extinction in recent time (Evans, unpublished data). A similar pattern in *Palisota* would indicate that it has also undergone changes in evolutionary or extinction rate. No observable differences are found between branch lengths at different regions of the tree (i.e. tips vs. internal branches; Fig. 2), however, suggesting a relatively constant speciation/extinction rate through time in this genus.

Conclusions:

Based on preliminary analysis, *Palisota* appears to be monophyletic and sister to the tribe Tradescantieae, making Tradescantieae *sensu* Faden and Hunt (1991) paraphyletic. Efforts in lab to obtain sequences of the *rbcL* gene within the other species of *Palisota* will be continued to further test the monophyly of the genus and to solidify placement of the genus within Commelinaceae.

Liturature Cited:

Azuma, T., T. Kajita, J. Yokoyama, and H. Ohashi. 2000. Phylogenetic relationships of Salix (Salicaceae) based on rbcL sequence data. American Journal of Botany 87: 67-75.

Chase, M. W., D. E. Soltis, R. G. Olmstead, D. Morgan, D. H. Les, B. D. Mishler, M. R. Duvall, R. A. Price, H. G. Hills, Y-L. Qiu, K. A. Kron, J. H. Rettig, E. Conti, J. D. Palmer, J. R. Manhart, K. J. systsma, H. J. Michaels. W. J. Kress. K. G. Karol, W. D. Clark, M. Hedren, B. S. Gaut, R. K. Jansen, K-J. Kim, C. F. Wimpee, J. F. Smith, G. R. Furnier, S. H. Strauss, Q-Y. Xiang, G. M. Plunkett, P. S. Soltis, S. M. Swensen, S. E. Williams, P. A. Gadek, C. J. Quinn, L. E. Eguiarte, E. Golenberg, G. H. Learn, S. W. Graham, S. C. J. Barrett, S. Dayanandan, V. A. Albert. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene rbcL. Annals of the Missouri Botanical Garden 80: 528-580.

Doyle, J. J. and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11-15.

Duvall, M. R., M. T. Clegg, M. W. Chase, W. D. Clark, W. J. Kress, H. G. Hills, L. E. Eguiarte, J. F. Smith, B. S. Gaut, E. A. Zimmer, G. H. Learn. 1993. Phylogenetic hypotheses for the monocotyledons constructed from *rbcL* sequence data. Annals of the Missouri Botanical Garden 80: 607-619.

Evans, T. M., K. J. Sytsma, R.B. Faden, T. J. Givnish. 2003. Phylogenetic Relationships in the Commelinaceae: II. A Cladistic Analysis of rbcL Sequences and Morphology. Systematic Botany 28: 270-292.

Faden, R. B. 1998. Commelinaceae. In: Kubitzki K. (ed.) The Families and Genera of Vascular Plants 4: 109-128. Springer, Berlin.

Faden, R. B. 2007. Taxonomic problems in the genus Palisota (Commelinaceae) in Tropical East Africa. Kew Bulletin 62: 133-138

Faden, R.B. and D. R. Hunt. 1991. The classification of the Commelinaceae. *Taxon*. 40: 19-31.

Korall, P., P. Kenrick, and J. P. Therrien. 1999. Phylogeny of Selaginellaceae: Evaluation of generic subgeneric relationships based on *rbcL* gene sequences. International Journal of Plant Sciences 160: 585-594.

Setoguchi, H., T. A. Osawa, J. C. Pintaud, T. Jaffre, and J. M. Veillon. 1998. Phylogenetic relationships within Araucariaceae based on *rbcL* gene sequences. American Journal of Botany 85: 1507-1516.

Smith J. F., Sytsma K. J., Shoemaker J. S., and Smith R.L. 1991. A qualitative comparison of total cellular DNA extraction protocols**.** Phytochem Bulletin 23: 2-9.

Tomlinson, F. L. S. 1966. Anatomical data in the classification of Commelinaceae. Botanical Journal of the Linnean Society 59: 371–395.

Tomlinson, F. L. S. 1969. Commelinaceae. Pp. 12–63 in *Anatomy of the monocotyledons, 3.* ed. C. R. Metcalfe. Oxford: Clarendon Press.

Wade, D. J., T. M. Evans, and R. B. Faden. 2006. Subtribal relationships in tribe Tradescantieae (Commelinaceae) based on molecular and morphological data. Pp. 520-526, In: Proceedings for the Third International Symposium on Monocots. Ontario, California.