

Phylogeny of the genus *Artocarpus* (Moraceae)



CHICAGO
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GARDEN

using plastid markers
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ABSTRACT

The Moraceae family contains 37 genera and 1,050 species. These genera are distributed throughout tropical climates, particularly Southeast Asia and Oceania. *Artocarpus*- the third largest genus- is particularly interesting because it contains several economically important fruit bearing plants including breadfruit (*A. altilis*) and jackfruit (*A. heterophyllus*). Morphological classifications of the genus *Artocarpus* have shown different results than those of genetic studies. Evolution of inflorescence structure, pollination syndromes, and other features have clouded the taxonomy of the genus at the subgenus level and below. We addressed the question: is the morphological classification supported by genetic data? We evaluated two plastid regions for 137 taxa representing a comprehensive sampling of *Artocarpus* as well as several closely related outgroups. We analyzed the data using Bayesian inference and compared the trees to previous work. Polytomies persisted in both trees and monophyly of traditional subgenera were not supported. The addition of more DNA regions may help resolve the trees and inform a revision of the genus.

INTRODUCTION

Moraceae (mulberry family) comprises 37 genera and ~1,050 species (Zerega et al. 2010). The genus *Artocarpus* is particularly important because several economically important fruit bearing species such as breadfruit (*Artocarpus altilis*) and jackfruit (*Artocarpus heterophyllus*) are found within the genus. For example the breadfruit is an important source of protein and carbohydrates for populations throughout Southeast Asia, the Pacific Islands, Oceania, and Papua New Guinea (Zerega et al. 2005). These species can be cultivated and have been domesticated for human use. The use of such fruits is particularly important in countries that have high rates of malnourishment.

Historically, *Artocarpus* has been classified using morphological data. This morphological data suggests that *Artocarpus* is monophyletic, but due to enormous diversity in the genus the taxonomy of the subgenera, sections, and series has been complicated (Zerega et al. 2010). Zerega et al. (2010) proposed 4 subgenera based on morphology and two regions (ITS and trnL F); *Artocarpus*, *Pseudojaca*, *Califfiori*, and *Prainea* (Fig. 1).

The purpose of this study is to test the taxonomic classifications of the genus *Artocarpus* by using genetic data. The goal of the study is to produce a comprehensive phylogenetic tree. We achieved this by using genetic sequences of plastid regions *rbcl* and *matK*. The final phylogenetic tree was generated using Bayesian inference. We compared the phylogenetic tree to previously collected data, morphological divisions from Jarrett (1959, 1960) and the tree from Zerega et al. (2010).

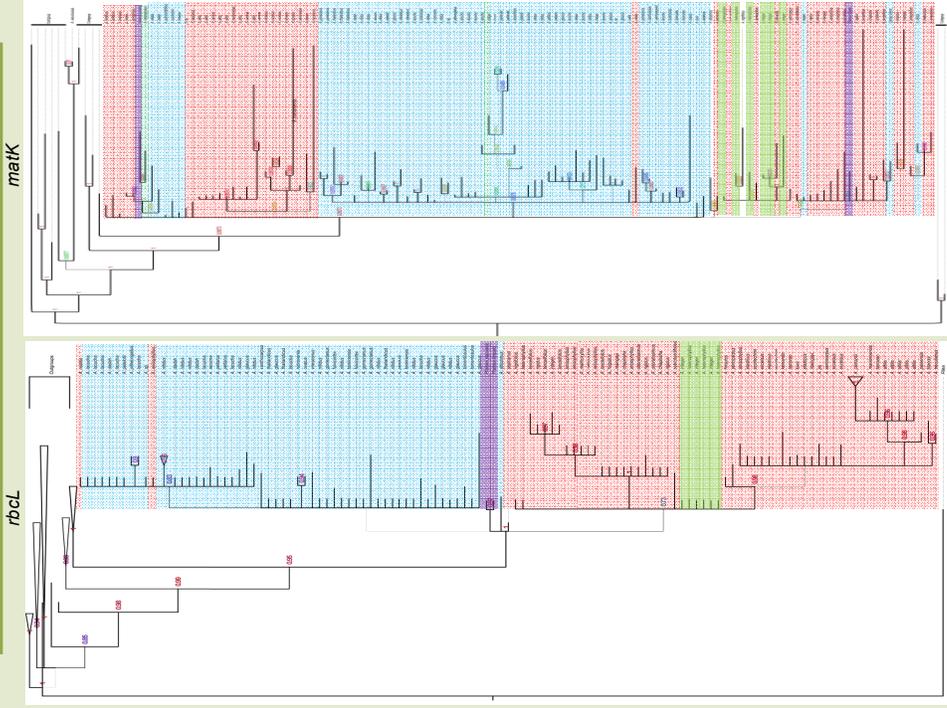
We hypothesize that the phylogenetic tree based on *rbcl* and *matK* sequences will more closely resemble the trees from Zerega et al. (2010) than the traditional morphological divisions.



METHODS & MATERIALS

- Taxon Sampling:** 137 *Artocarpus* taxa, 2 species of *Prainea*, and 10 outgroups
- Collected by Zerega and colleagues in Southeast Asia and collaborating herbariums
- Contains both fresh leaf tissue dried in silica gel and herbarium specimens
- DNA Extraction:** Extracted chloroplast and genomic DNA using a Qiagen DNeasy Plant Kit
- Used gel electrophoresis and a nanodrop spectrophotometer to confirm that the extraction was successful
- PCR:** Amplified plastid regions *rbcl* and *matK* using primers *rbcl* F, *rbcl* R, *kim* F, and *kim* R
- Used the following PCR reaction recipe for all the samples (5ul of Mastermix, 3ul of water, 0.5ul of 10mM of both the forward and reverse primers, and 1ul of DNA)
- Ran the samples on a 1.5% agarose gel to verify if PCR was successful
- Cleaned the products using ethanol
- Cycle Sequencing:** 3ul of water, 1ul of ABI Big Dye, 3ul of 100x BIG Dye Buffer, 1ul of either of the primers, and 5ul of PCR product
- Cleaned the samples with an ethanol/EDTA protocol
- Sequencing and Phylogenetic Analysis:** Re-sus-pended the samples in HIDI formamide and sequenced the samples on an ABI 3730 sequencer
- Trimmed the sequence traces in CodonCode 4.2.2, assembled contigs and aligned the sequences in CodonCode 4.2.2 on Maatf. The alignment was also checked and optimized by hand to ensure accuracy
- Analyzed the alignment using Bayesian inference in MrBayes 3.2.1 with 1,000,000 generations, 25% burnin, and 2 runs with 4 chains.

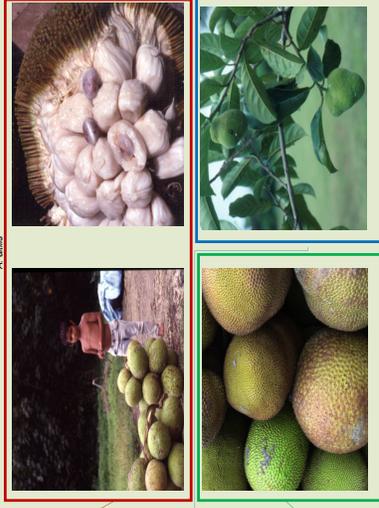
RESULTS



DISCUSSION

Artocarpus has historically been difficult to classify. Zerega et al. (2010) proposed four subgenera: *Artocarpus*, *Pseudojaca*, *Prainea*, and *Califfiori*. Some unresolved issues within the genus are monophyly of the subgenera and lack of support for traditional sections and series. The data does not provide resolution among the sections and series, but the data suggests that traditional monophyly is not supported. The *rbcl* tree serves as a backbone for the genus; providing strong support values for the clades (>80% p.p.). The *matK* data provides more variability among the genus but without strong support values. This variability was unexpected and may suggest contamination among some samples.

The addition of more regions, including plastid and nuclear regions, may provide resolution within the subgenus level and below. Also the use of different analyses and different analytical programs may paint a clearer picture. The exploration of more aspects such as biogeography may also be necessary.



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- Jarrett, F. M. 1959a. Studies in *Artocarpus* and allied genera. I. General considerations. Journal of the Arnold Arboretum 40: 1-29.
 - Zerega, N.J.C., et al. 2010. Phylogeny and re-description of *Artocarpus* (Moraceae) with a focus on *Artocarpus*. Sys. Bot. 35: 4
 - Zerega, N.J.C., et al. 2005. Systematics and species limits of Breadfruit (*Artocarpus*, Moraceae). Sys. Bot. 30(3).
- All photos by Nyree Zerega unless noted

- rbcl* acts as a backbone region for the genus
- matK* produces more variability, but without strong support
- matK* shows some samples in unexpected clades that suggest contamination