# Paraphyly, hybridization, and multiple introductions in the origin and evolution of the endemic Amaranthaceae of the Hawaiian Islands (genera *Achyranthes*, *Charpentiera* and *Nototrichium*)


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## INTRODUCTION

The Hawaiian Islands are well known for their endemic plant taxa, many of which provide striking examples of adaptive radiation following their introduction to the islands. Some groups including the Silversword alliance, lobeliods and violets have been well-studied, while others we know little about. One such little-studied group are the native Amaranthaceae represented by six genera and 24 species with four of these genera contributing the 11 endemic species (Wagner et al., 1990). Similarities in floral form and distribution have led to the development of hypotheses of origin however no explicit tests of these hypotheses have ever been made.

*Nototrichium* is a group of shrubs to small trees circumscribed into three species, *N. sandwicense* (A. Gray) Hillebr., *N. divaricatum* Lorence and *N. humile* Hillebr. Affinities to other Amaranthaceae have been difficult to determine due to the unique morphology, but it has been believed that the genus has an Austral origin. The predominant hypothesis suggests a close relationship to the widespread tropical genus *Achyranthes*, which has been reinforced by recent large-scale phylogenetic studies (Müller & Borsch, 2005).

*Charpentiera* is a group of large shrubs to trees to 12 m represented by five endemic species, *C. densiflora* Sohmer, *C. elliptica* (Hillebr.) A. Heller, *C. obovata* Gaud., *C. ovata* Gaud., and *C. tomentosa* Sohmer. The genus also includes a single species, *C. australis* Sohmer in the Austral Islands (Sohmer, 1972). Affinities and origins of *Charpentiera* have been very difficult to deduce (Sohmer, 1976). The gynodioecious breeding system is rare in the family although its pollen is reminiscent of other core amaranths (Eliasson, 1988). Molecular phylogenetic studies additionally place *Charpentiera* within a basal grade in the family with the genus *Bosea* (Müller & Borsch, 2005).

*Achyranthes* are shrubs represented by three endemic and one introduced species. The endemic species, *A. atollensis* St. John, *A. mutica* A. Gray, and *A. splendens* Mart. Ex Moq. are all quite rare in the islands. *Achyranthes atollensis* is extinct and prior to its rediscovery in 1992, *A. mutica* was thought to have gone extinct as well. A single origin from wide-ranging *Achyranthes* is believed to have given rise to these species (Eliasson, 2004).

Lastly one species of the widely introduced genus *Amaranthus* is endemic to the islands. *Amaranthus brownii* Christoph. & Caum is restricted to the island of Nihoa where there are likely less than 50 individuals in existence. Due to the lack of available material we were unable to evaluate this taxon.

Here we apply molecular sequence data from the nuclear and chloroplast genomes to reconstruct the phylogeny of the native Hawaiian Amaranthaceae to understand the relationship to putative ancestors across the Pacific Basin.

## METHODS

- Specimens from Hawaii and the Pacific Basin were obtained from wild populations, botanical gardens, herbarium specimens and DNA libraries.
- Total DNA was extracted from samples using a QIAGEN DNeasy kit. Difficult specimens used a modified incubation in β-mercaptoethanol and proteinase K.
- The nuclear ribosomal Internal Transcribed Spacer (ITS) and the chloroplast *rpl32-trnL* region were amplified and sequenced.
- Sequences were assembled and aligned using Geneious Ver. 6. and Clustal X Ver. 2.1
- Equally weighted parsimony tree searches were conducted for the nuclear and plastid data using 1,000 tree-bisection-reconnection (TBR) searches in PAUP* 4.0b10. Maximum Likelihood trees were also generated in PAUP* using the best-fit model of nucleotide substitution selected using AIC with ModelTest ver. 3.7 . Branch support was assessed using 100 bootstrap replicates.

## RESULTS

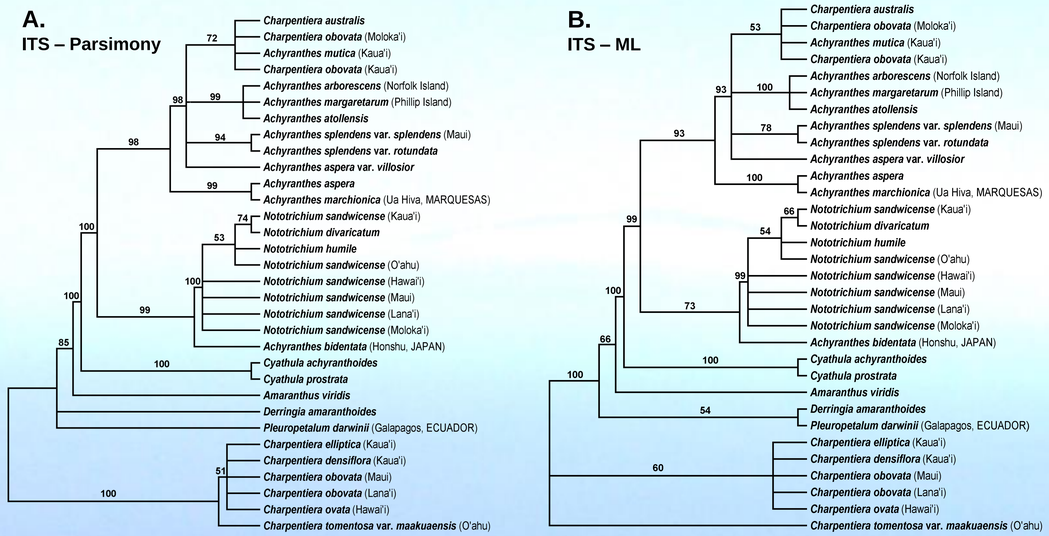


**Figure 1.** Results of phylogenetic analysis of the *nr* ITS region in Pacific Amaranthaceae. **A.** Parsimony. Bootstrap consensus (length = 472, CI = 0.794, RI = 0.913, RC = 0.725) **B.** Maximum Likelihood. Bootstrap consensus (ln = 3382.7542 – SYM+G Model) Analyses strongly support the monophyly of *Nototrichium* embedded within a larger *Achyranthes* clade. Within *Nototrichium* the widespread *N.* sandwicense is shown to be clearly paraphyletic and species relationship is based principally on geography. Multiple introductions to Hawaii from Achyranthoid ancesters are suggested. *Charpentiera* was shown to be polyphyletic with two species associating with the Hawaiian endemic *Achyranthes mutica*.

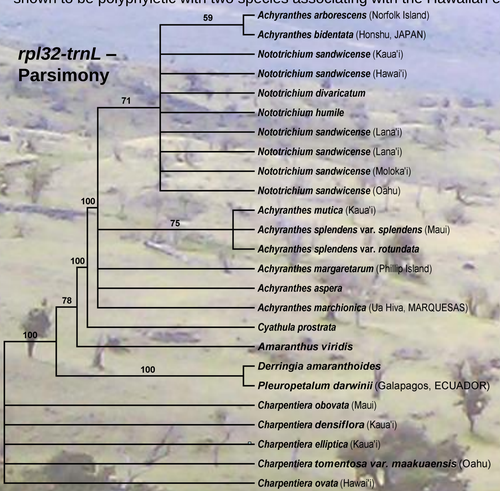


**Figure 2.** Results of phylogenetic analysis of the *cp rpl32-trnL* region in Pacific Amaranthaceae. Parsimony bootstrap consensus (length = 586, CI = 0.298, RI = 0.964, RC = 0.895) (Maximum likelihood analysis gave an identical tree topology and is not presented) Analysis also supports the monophyly of *Nototrichium* embedded within a larger *Achyranthes* clade. Limited amplification of cp DNA supports two introductions of an Achyranthoid ancester.

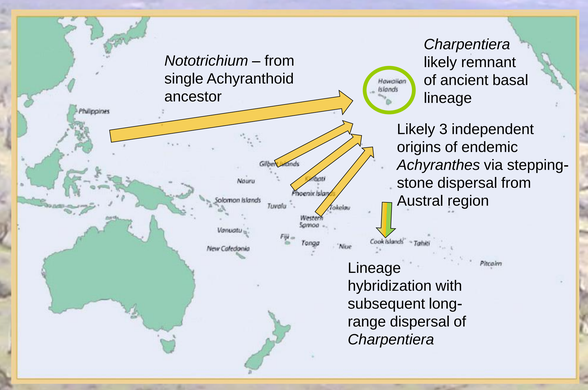


**Figure 3.** Hypothesized patterns of origin and relationship in the endemic Hawaiian Amaranthaceae. Multiple introductions of the genus *Achyranthes* (orange arrows) have given rise to variably widespread and restricted taxa and to the unique *Nototrichium*. *Charpentiera* is likely a remnant of a now-extinct lineage of Amaranthaceae which radiated across the archipelago (green). An apparent gene exchange between *Charpentiera* and *Achyranthes* gave rise to a secondary radiation of *Charpentiera* in Hawaii and led to long-range dispersal to the Austral Islands.

***Achyranthes mutica***
Kohala Mountains, Hawai'I, historically from Kaua'i
Photo © David Eickhoff

***Achyranthes atollensis***
Extinct, Kure Atoll
Photo © David Eickhoff

***Achyranthes splendens***
O'ahu, Molokai, Maui, & Lana'i

## CONCLUSION AND FUTURE DIRECTIONS

Our preliminary work has uncovered a complex and dynamic pattern of introduction and subsequent diversification within the Hawaiian Amaranthaceae. The Achyranthoid clade is of particular importance contributing multiple independent introductions. The distinct *Nototrichium* is one of these introductions which radiated and diversified. This finding is troublesome taxonomically as recognition of *Nototrichium* results in a paraphyletic *Achyranthes*. This would need to be examined in the context of revisionary work in *Achyranthes*. From a standpoint of character evolution the unique characters segregating *Nototrichium* from *Achyranthes* (lack of pseudostaminodia and ascending flowers) and near unique characters (arborescence and 4-merous flowers) appear to be of less taxonomic importance and represent inherent variability within the *Achyranthes* clade.

The basal position of *Charpentiera* makes determining origin and migration difficult. Its unique morphology and reproductive biology are likely the result of long-term isolation and segregation from a now-extinct ancestor. The apparent intergeneric gene exchange between *Charpentiera* and *Achyranthes mutica* was unexpected and further work to verify this pattern and better understand the mechanism for gene exchange is necessary.

Currently we are surveying additional chloroplast regions to better test these patterns of diversification and provide stronger evidence of phylogenetic relationships.

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***Nototrichium sandwicense***
Widespread on all main islands. Highly variable in morphology
Photo © David Eickhoff

***Nototrichium humile***
Rare, Wai'anae Mts, O'ahu & Lualai'ilua Hills, Maui
Photo © John Obata

***Nototrichium divaricatum***
Rare from Nā Pali coast, Kaua'i
Photo © K.R. Wood

***Charpentiera ovata***
O'ahu, Moloka'I, Maui & Hawai'i
Photo © Jupiter Nielsen

***Charpentiera obovata***
All main islands
Photo © David Eickhoff

***Charpentiera tomentosa***
Principally O'ahu, scattered on other islands
Photo © Joel Lau

***Charpentiera elliptica***
Common on Kaua'I
Photo © J. Price

***Charpentiera densiflora***
Kaua'i. Trees to 12 m
Photo © W. L. Wagner

Background: *Charpentiera* habitat, slopes of Haleakalā, East Maui