Phylogeny and evolutionary distribution patterns in the endemic Hawaiian genus *Charpentiera* (Amaranthaceae)

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BACKGROUND AND INTRODUCTION

The geographic isolation and high degree of floral endemism (89%) has made the Hawaiian archipelago an especially well studied region for biologists (Wagner et al.,1990; Sakai et al., 1995). Extended isolation and alterations in selective pressures and genetic drift following founder events result in the rapid shifting of genes to new coadapted combinations, which reproductively isolate radiating founders from the ancestral population (Barton and Charlesworth, 1984). Altered pollination conditions following insular founder events have favored selection of floral traits promoting selfing. Selfing and inbreeding depression appear to be key factors involved in the evolution of sexual dimorphisms (Barrett et al., 1996). Also significant are the higher frequencies of hybridization observed within insular flora, compared to mainland populations (Rieseberg, 1991).

Of the less studied endemic flora of the Hawaiian Islands, are the species of the genus *Charpentiera* (Amaranthaceae). All species within the genus are endemic to the Hawaiian archipelago, with the exception of *C. australis* found in French Polynesia, which raises questions regarding the evolution and distributional history of the genus (Sohmer, 1972). Due to lack of genetic analysis, all current species are based on morphological and behavioral characteristics, which may vary in differing habitats. With overlapping habitats and widespread distribution, evolutionary radiation patterns are difficult to predict without genetic analysis. To further complicate, previous studies have suggested possible interspecific hybridization between overlapping species of *Charpentiera* (Sohmer, 1973).

Objectives:
Here we apply molecular sequence data from the nuclear and chloroplast genomes and morphological data to reconstruct the phylogeny, infer historical distribution patterns, and identify any potential gene sharing that may be occurring within the genus *Charpentiera.*

METHODS

- Specimens of *Charpentiera* and other genera of Hawaiian and Pacific Basin Amaranthaceae were obtained fresh from wild populations or from 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.
- Nuclear ribosomal Internal Transcribed Spacer (ITS) and the chloroplast *psbA-trnH* spacer 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. Branch support was assessed using 1000 bootstrap replicates.
- A morphological phylogenetic analysis was performed on a matrix of characters derived from Somer (1972; 1973) and Wagner et al. (1990) and analyzed using maximum parsimony in WinClada.

RESULTS

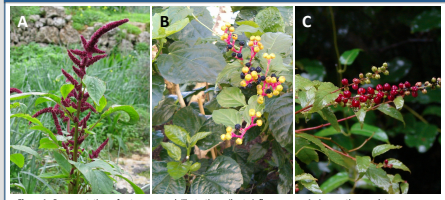


Figure 1. Representatives of outgroups used, illustrating spikeate inflorescences. A. *Amaranthus caudatus*; B. *Pleuropetalum darwinii*; C. *Deeringia amaranthoides.*

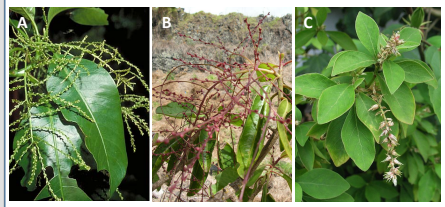


Figure 2. Species of the intergeneric hybridization clade indicated by comparative ITS analysis. A. *Charpentiera australis*; B. *Charpentiera obovata*; C. *Achyranthes mutica.*

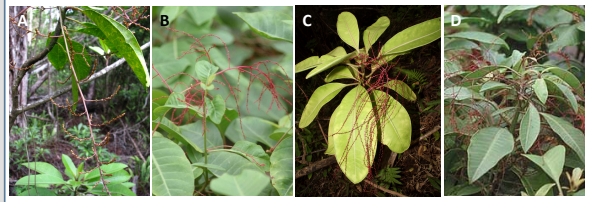
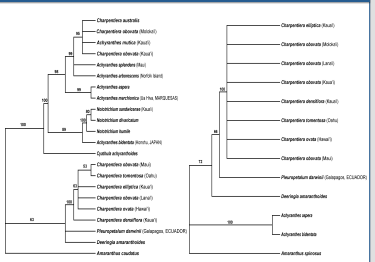


Figure 3. Varieties of *Charpentiera ovata* and *Charpentiera tomentosa* illustrating morphological distinctions.
A. *C. ovata var. ovata*; B. *C. ovata var. niuensis*; C. *C. tomentosa var. tomentosa*; D. *C. tomentosa var. maakuaensis*

Figure 4. Species of *Charpentiera* structural distinctions. A. Height at maturity of *C. elliptica*; B. Height at maturity of *C. densiflora.*


Charpentiera australis
Charpentiera obovata (Moloka'i)
Achyranthes mutica (Kaua'i)
Charpentiera obovata (Kaua'i)
Achyranthes splendens (Niu)
Achyranthes arborescens (Norfolk Island)
Achyranthes aspera
Achyranthes mechistacha (Sa'Hoa, MARQUESAS)
Nototrichium sandwicense (Kaua'i)
Nototrichium divaricatum
Nototrichium humile
Achyranthes bidentata Blumbe, JAPAN)
Cyathula achyranthoides
Charpentiera obovata (Maui)
Charpentiera tomentosa (O'ahu)
Charpentiera elliptica (Kaua'i)
Charpentiera obovata (Lana'i)
Charpentiera ovata (Hawai'i)
Charpentiera densiflora (Kaua'i)
Pleuropetalum darwinii (Galapagos, ECUADOR)
Deeringia amaranthoides
Amaranthus caudatus
100
99
98
100
150
99
100
99
100
99
100