Genera delimitation and floral evolution in the Cyclanthera clade (Cucurbitaceae, Sicyoeae)

Candidate Juana De Egea (PhD Student)

Introduction

The Cucurbitaceae family includes around 97 genera distributed throughout temperate, subtropical and tropical areas around the world (Schaefer & Renner 2011a). Recent phylogenetic analyses based on 14 molecular markers support the monophyly of Cucurbitaceae and group their genera into 14 tribes (Schaefer & Renner 2011b). The Cucurbitaceae originated in Asia during the Late Cretaceous, and their current distribution is a result of several episodes of long-distance dispersion between Asia and Africa, and finally between Africa and America, where the most derived clades originated (Schaefer et al. 2009).

One of the most interesting aspects of the Cucurbitaceae is the modifications that are observed in the structure of the androecium and the gynoecium (Pozner 1993, 1994; Matthews & Endress 2004; Schaefer & Renner 2011a). The ancestral structure of the androecium is considered to be of 5 dithecal, free stamens, with straight thecas (Kocyan et al. 2007; Schaefer & Renner 2011a), observing within the derived species about 22 different structures of androecium (Pozner 1993), which are defined according to the presence of 5, 3 or 2 stamens, free or joined filaments, anthers with 1 or 2 thecas, free or joined anthers, vertical or horizontal anthers, and straight, curved, sigmoid or circular thecas. There has still been no speculation on the ancestral structure of the gynoecium, which also has a great morphological variation depending on the number of fertile carpels in the ovary (1 to 5), the number of carpels present in the style and stigma (2 to 5) free or fused in a unique style, the number and position of the ovules, the production (or not) of "cincinnoid" proliferations of ovules (Pozner 1993), and of the development (or not) of carpellary ovuliferous foveas (Pozner 1994; Matthews & Endress 2004).

Of the 14 tribes that are currently recognized within the family (Schaefer & Renner 2011b), the Sicyoeae includes genera with the most particular floral morphology: *Cyclanthera* (ca. 25 spp), *Pseudocyclanthera* (1 sp), *Rytidostylis* (ca. 16 spp), and *Hanburia* (7 spp), all with American distribution and currently included in the Cyclanthera clade (Sebastian 2011). This clade is characterized by species with fleshy, explosive fruits, with a ballistic dispersion of seeds, and its genera have historically been distinguished by their floral characters. Among these, *Cyclanthera* stands out for its androecium constituted by a single horizontal, annular, and continuous theca resembling a wheel; while *Rytidostylis* stands out for its floral tube with an invaginating base that surrounds the apex of the ovary simulating the lower half of the style (or the floral pedicel apex simulating the lower half of the staminal column) (Pozner 2004).

According to the most recent phylogenetic analyses of the Sicyoeae (Schaefer & Renner 2011b; Sebastian 2011; Atakaya 2014), *Rytidostylis* and *Pseudocyclanthera* have been included under the *Cyclanthera* synonymy on the basis of three questionable phylogenetic analyses: two of them include only 1 species of *Rytidostylis* and 3 species of *Cyclanthera*, and the third includes 11 samples of *Rytidostylis* (with some missing species determinations) and 8 species of *Cyclanthera*, but the low clade support does not justify a nomenclatural change. Furthermore, morphological differences between genera were not taken into account. To a large extent, this lack of support and resolution in the analyses is due to the recent and rapid diversification of the American Cucurbitaceae in general, and of the American Sicyoeae in particular (Schaefer et al.

2009), which makes it difficult to find sufficient genetic variation to solve phylogenetic analyses (Belgrano 2012). Due to the poorly representative sampling of the *Cyclanthera* and *Rytidostylis* genera, and the disregarded evidence of floral morphology, we suggest that the synonymy of *Rytidostylis* and *Pseudocyclanthera* under *Cyclanthera* is premature, and that the delimitation of the genera within the Cyclanthera clade needs a phylogenetic analysis that is better represented, and includes other genetic markers that provide greater variation and more informative data to the analysis. Likewise, it is necessary to explain the origin of such particular floral structures present in different species of this clade, such as the floral tubes with an invaginating base, and continuous annular horizontal anthers.

Consequently, this PhD project proposes the reconstruction of a phylogenetic hypothesis that explains the origin and diversity of the particular floral morphology of the species of this clade, and at the same time, reevaluates the limits of its constituent genera.

General Objective of the Project

To develop a hypothesis of the phylogeny and floral morphology evolution for the Cyclanthera clade (*Cyclanthera* + *Pseudocyclanthera* + *Rytidostylis* + *Hanburia*, Cucurbitaceae, Sicyoeae) in order to reassess the limits of its constituent genera and explain the origin and diversity of the particular floral morphology of the species within this clade.

Justification for the visit to the MEXU Herbarium

The 15-day stay at the MEXU Herbarium will be carried out within the framework of my PhD project "Genera delimitation and floral evolution in the Cyclanthera clade (Cucurbitaceae, Sicyoeae)" which I am developing under the doctoral programme from the Northeastern National University (Corrientes, Argentina). The taxonomic revision to be carried out at the MEXU Herbarium aims to generate highly relevant data for this project; within this timeframe, taxonomic studies based on morphology will be carried out, and appropriate specimens will be selected for subsequent studies applying molecular methodologies.

Cucurbitaceae have one of the most particular and variable floral morphologies among Angiosperms, and the understanding of their origin and evolution has been the subject of much debate among botanists of the twentieth century (Pozner 1993). The data generated by this project will contribute towards the greater objective of elucidating a part of the complex floral evolution of this family, with relevance for Angiosperms in general.

Materials and Methods

The study of the Cyclanthera clade in the MEXU herbarium will be carried out in three stages:

1. Revision of herbarium specimens of the genera of interest applying traditional taxonomy methods, the use of dichotomous keys, the observation of taxonomic characters through stereoscopic microscopy, among others.
2. Recording of revised samples in a database tailored to the PhD project requirements, where previously studied collections at other herbaria (BM, CTES, FCQ) are already stored.
3. Selection of samples for subsequent morphological, anatomical and molecular studies at the Darwinion Institute (SI) of Buenos Aires (Argentina), under the supervision of Dr. Raúl Pozner, PhD project Director. The loan of these selected

herbarium specimens will be arranged between MEXU and SI, following the procedures established by the host institution.

The taxonomic studies carried out at MEXU will subsequently allow, within the framework of the PhD project: 1) to obtain molecular markers (nucleotide sequences of the rbcL gene and the trnL-F spacer of the chloroplast, and the nuclear ITS); 2) the interpretation of the structure of the perianth, the gynoecium and androecium through the morphological study of representative species of the Cyclanthera clade, in order to establish primary homologies and appropriate characters for the phylogenetic analysis; and 3) the phylogenetic analysis of the Cyclanthera clade based on all data obtained with parsimony, maximum likelihood and bayesian analysis methods.

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