CLUSIA SCARIOSEPALA (CLUSIACEAE), A DISTINCT SPECIES OF *CLUSIA* SECT. *ANANDROGYNE* ENDEMIC TO THE VENEZUELAN ANDES

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Abstract. *Clusia* sect. *Anandrogyne* is the most species-rich section in the genus and it includes approximately 70 species. Taxonomic boundaries within the group have been difficult to establish due to high degree of homoplasy in morphological characters. In order to make informed taxonomic decisions, an evaluation of both morphological (fruit morphology) and molecular (nrDNA ITS) data was made among species included in *Clusia* sect. Anandrogyne. It was found that *Clusia multiflora* subsp. *scariosepala* has strikingly distinctive fruit morphology and, furthermore, it is phylogenetically more closely related to other species of *Clusia* than to *Clusia multiflora*. Therefore, raising this taxon to the rank of species is proposed herein. Conservation status assessment for the species is also provided.

Resumen. *Clusia* sect. *Anandrogyne* es la sección con mayor número de especies en el género e incluye aproximadamente 70 especies. Límites taxonómicos dentro del grupo han sido difíciles de establecer debido a alto grado de homoplasia en caracteres morfológicos. Con el fin de realizar decisiones taxonómicas fundamentadas, se evaluaron caracteres morfológicos (forma de fruto) y moleculares (nrDNA ITS) entre especies incluidas dentro de *Clusia* sect. *Anandrogyne*. Se encontró que *Clusia multiflora* subsp. *scariosepala* posee una forma de fruto notablemente distintiva y que además, está filogenéticamente más relacionada con otras especies de *Clusia* que con *Clusia multiflora*. Por lo tanto, se propone elevar este taxon al rango de especie. Se provee una evaluación del estado de conservación de la especie.

Keywords: *Clusia* sect. *Anandrogyne*, Andes, Venezuela

The Neotropical genus *Clusia* L. (ca. 300 spp., Clusiaceae) comprises woody hemiepiphytes, shrubs, and trees with remarkable physiological and morphological plasticity. The genus includes species known to use the C3, CAM and facultative C3/CAM photosynthetic pathways (Lüttge, 2006). *Clusia* is the only genus of trees in which some species produce floral resins as a reward for pollinators (Bittrich and Amaral, 1996). Nearly all species are dioecious and some are apomictic (Maguire, 1976; Pipoly and Graff, 1995). The center of diversity of the genus is northern South America, specifically the montane forests of the Northern Andes, the Guyana Highlands, and the Amazonian lowlands (Gustafsson et al., 2007). Taxonomic work on *Clusia* began with Planchon and Triana (1860), Baillon (1877: 392–425), Vesque (1893), and Engler (1925); all of these authors based their infra-generic groupings mainly on the highly variable androecial characteristics. *Clusia* sect. *Anandrogyne* Planch. & Triana was defined to include species with 4–5-merous calyx, 5–6-merous corolla, staminate flowers with multiple free stamens inserted in a prominent receptacle, anthers linear with longitudinal dehiscence, and pistillate flowers with 5–10 anantherous staminodia. This section is considered the largest within the genus and includes approximately 70 species (Pipoly, 1995). Modern phylogenetic inferences based on molecular data have confirmed the monophyly of *Clusia* sect. *Anandrogyne*, although evolutionary relationships among closely related taxa are still unresolved (Gehrig et al., 2003; Gustafsson et al., 2007).

Within *Clusia* sect. *Anandrogyne*, authors have recognized the "multiflora complex," an informal group that includes the widely polymorphic species *Clusia multiflora* Kunth and its closely related taxa (Maguire, 1979; E. Medina, pers. comm.). In this group, taxonomic boundaries are difficult to establish due to a high degree of intergradation among morphological characters (Hammel, 1986).

As part of an in-depth systematic analysis of *Clusia*, extensive sampling of taxa across the genus is currently been undertaken. A number of species from *Clusia* sect. *Anandrogyne*, including taxa from the *C. multiflora* complex, have been sampled. Preliminary phylogenetic analysis was performed in order to assess evolutionary relationships among taxa and to make informed taxonomic decisions.

Material and Methods

Herbarium specimens of taxa included in *Clusia* sect. *Anandrogyne* were studied from collections housed at F, HUH, MO, NY, US, and VEN (acronyms following Thiers, continuously updated). In order to assess phylogenetic relationships, plant material from representatives of *Clusia* sect. *Anandrogyne* was collected in the field, including collections from the type localities of *C. multiflora* and *Clusia multiflora* subsp. *scariosepala* Maguire. DNA extraction was performed using standard CTAB protocol (Doyle and Doyle, 1987). The entire internal transcribed spacer (ITS) of nuclear ribosomal repeat was amplified via polymerase chain reaction (PCR) using primers N-nc18S10

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and C26A (Wen and Zimmer, 1996). PCR products were sequenced on the ABIxl sequencer facility at Rancho Santa Ana Botanic Garden following the BigDye® terminator protocol. Additional ITS sequences from taxa included in *Clusia* sect. *Anandrogyne*, as well as outgroups taxa, were downloaded from GenBank. All sequences were edited, assembled and aligned using MUSCLE algorithm as implemented in Geneious® 8.1.8 (Biomatters Ltd., Aukland, New Zealand). Model of evolution was estimated using ModelTest in Phangorn package (Schleip and Paradis, 2013) in R statistics environment. The Akaike Information Criterion (AIC; Akaike et al., 1974) was used to choose among substitution models. Maximum likelihood (ML) analyses was conducted using PhyML (Guindon and Gasquel 2003) with 1000 nonparametric bootstrap replicates to estimate support values. Bayesian inference (BI) analyses was performed in MrBayes 3.2.6 (2001) using 10.000.000 cycles of MCMC algorithm with four heated chains, 1000 burn-in samples and a sampling frequency of 10.000 generations.

Results

Observation of qualitative morphological characters of taxa included in *Clusia* sect. *Anandrogyne* showed extensive homogeneity, particularly in vegetative characters. Nonetheless, fruit morphology of *C. multiflora* subsp. *scariosepala* exhibited remarkable distinct characteristics. Capsules of *C. multiflora* subsp. *scariosepala* have 6–8 deep longitudinal grooves or slits and distally-free style vestiges. Capsules in other taxa from *Clusia* sect. *Anandrogyne*, including *C. multiflora*, have smooth surface and connate style vestiges (Fig. 1).

The alignment of the ITS sequence resulted in a matrix of 39 accessions and 674 bp of which 459 are variable and 293 and potentially parsimony informative. Maximum likelihood and Bayesian analyses resulted in similar reconstructions of phylogenetic relationships. Moreover, phylogenetic reconstructions based on 207 terminals, including representatives of all sections within *Clusia* (data not shown) results in the same tree topology for section *Anandrogyne*. Monophyly of this section is strongly supported (0.98 posterior probability, 100% bootstrap support). Within the section, two main clades are resolved; one formed by *C. salvinii* Donn. Sm. and *C. alata* Planch. & Triana; and a second clade formed by all the remaining species in the section. Evolutionary relationships among taxa included in this second clade are not well resolved, particularly among species closely related to *C. multiflora* (the multiflora complex). Phylogenetic position of *C. multiflora* subsp. *scariosepala* was resolved with strong support in a clade that includes *C. ducu* Benth, *C. elliptica* Kunth, *C. trochiformis* Vesque, and *C. melchiorii* Gleason (Fig. 1).

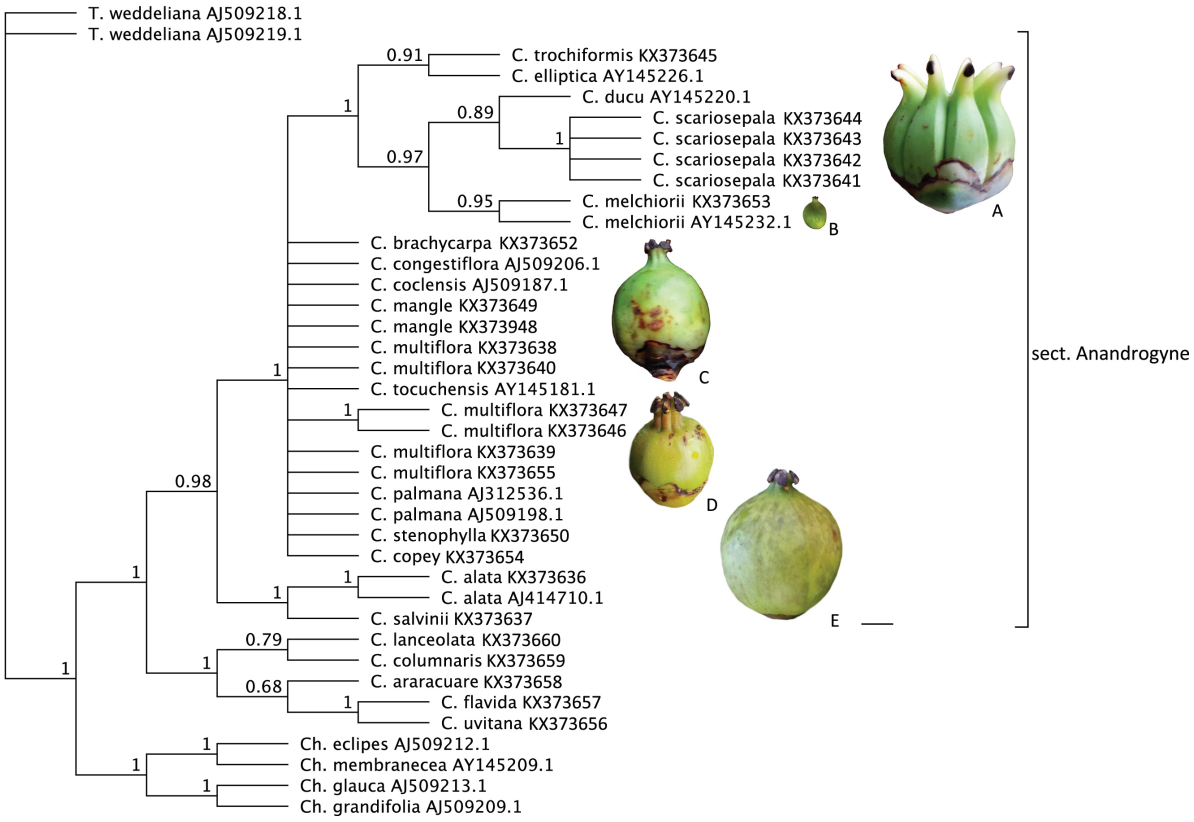

Figure 1. Bayesian analysis consensus tree of ITS region from *Clusia* sect. *Anandrogyne*. Numbers above branches indicate Bayesian posterior probabilities. Terminals are taxa with corresponding GenBank accession codes. Pictures of fresh mature capsules are shown. **A**, *C. scariosepala* (KX373642); **B**, *C. melchiorii* (KX373653); **C**, *C. multiflora* (KX373638); **D**, *C. stenophylla* (KX373650); **E**, *C. salvinii* (KX373637). Scale bar = 1 cm.

Discussion

Observation of qualitative morphological characters of taxa in *Clusia* sect. *Anandrogyne* showed that fruit morphology in *C. multiflora* subsp. *scariosepala* is remarkably distinctive in the group, and that it is different from the fruits of *C. multiflora*. Moreover, phylogenetic analyses of ITS sequences provide evidence that *C. multiflora* subsp. *scariosepala* is phylogenetically more closely related to *C. ducu*, *C. elliptica*, *C. trochiformis,* and *C. melchiorii* than to *C. multiflora*.

Based on a morphological species concept wherein "species are the smallest group that are consistently and persistently distinct and distinguishable by ordinary means" (Cronquist 1978), *C. multiflora* subsp. *scariosepala* should be considered a distinct species within *Clusia* sect. *Anandrogyne*, since it represents a biological entity morphologically different from *C. multiflora*. Furthermore, analysis of molecular data suggests that *C. multiflora* subsp. *scariosepala* is more closely related to other species within sect. Anandrogyne than to *C. multiflora*, which indicate that *C. multiflora* subsp. *scariosepala* belongs in a lineage apart from *C. multiflora*. In order to make taxonomic classification to reflect the evolutionary relationships in *Clusia*, a taxonomic status change is here proposed.

Although ITS data have shown to be useful to estimate the phylogenetic position of *Clusia* species, additional molecular data including more variable markers, as well as more extensive taxon sampling, are needed to better resolve evolutionary relationships within *Clusia* sect. *Anandrogyne*.

Taxonomic treatment

Clusia scariosepala (Maguire) Luján *stat. nov.* TYPE: VENEZUELA. Mérida: frequent small tree, in temperate forest at 2200–2400 m, La Carbonera, 25 km NW [from] Ejido, 19 October 1953, *B. Maguire 39446* ♂ (holotype: NY [LAPI 00842426]).

Etymology: Following ICN recommendation from Article 41.2 (McNeill et al. 2012), the specific epithet is based on the former infraspecific taxon name, which refers to the shriveled appearance of the sepal margins.

Conservation status: *Clusia scariosepala* is endemic to the Venezuelan Andes, and it is found in montane forests in the Cordillera de Mérida at 1850–2700 m (Fig. 2). *Clusia scariosepala* have been collected in two areas: Sierra de La Culata and Sierra Nevada. Collections from the

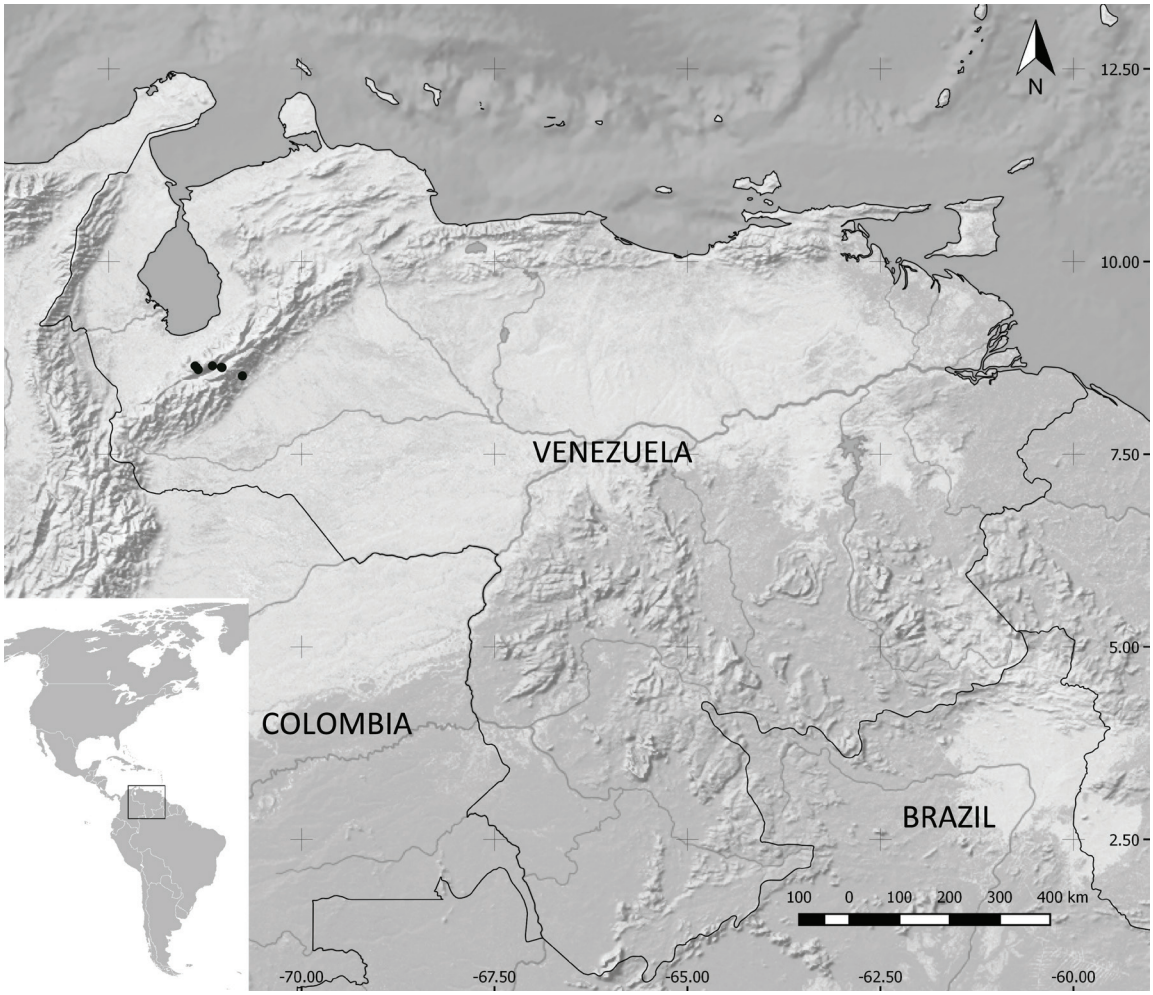

Figure 2. Geographical distribution of *C. sacariosepala* (Maguire) Luján based on the collections cited in the text.

latter have been made in Sierra Nevada National Park, therefore, conservation of the species in the area should be assured. Nonetheless, montane forests in the Sierra de la Culata area have been severely affected by deforestation and habitat transformation due agricultural activities (Rodríguez-Morales et al. 2009). Moreover, extensive fieldwork was done in the Sierra de La Culata area on 2013 and only two populations of *Clusia scariosepala* were found. Therefore, conservation status of *Clusia scariosepala* is here assessed as Vulnerable under criterion B1 a, b (iii) of IUCN (2014) with an estimated extent of occurrence less than 20,000 km^2, severely fragmented habitat and decline of habitat quality.

Additional specimens examined: VENEZUELA. Mérida: La Carbonera, 25 km NW of Ejido, 2200–2400 m, 19 October 1953, *B. Maguire 39445* ♂, *39447* ♀, (MO, NY); VENEZUELA. Barinas: Dtto. Pedraza, W of El Carrizal, La Escaza, N of río Canagua, in área locally known as La Reynosa, Los Laureles and Los Granates, P. N. Sierra Nevada, 8°31'N, 70°46'W, 27 June 1988, *L. J. Dorr, L. C. Barnett and M. Guerrero 5726* (NY, PORT [not seen], VEN); Mérida: La Mucuy, 2600 m elev., August 1958, *L. Aristiguieta 3318* (F, NY, VEN); Cerro Las Flores, 2000–2100 m, 26 April 1953, *L. Bernardi 444* (NY); Bosque nublado San Eusebio, La Carbonera, 2250–2600 m, 1971, *J. P. Veillon 18* (NY, VEN); 38 km W of Mérida, low forest near Las Cruces, 1850 m, 16 November 1966, *J. De Bruijn 1288* (VEN); Selva de musgos, prope Mérida, April 1957, *V. Vareschi 4442* (VEN); Camino a Sierra Nevada, 10 km E de Mérida, 2700 m elev., 30 August 1953, *E. L. Little Jr. 15492* (VEN); La Carbonera, 20 km NW de Ejido, 2000 m, 19 October 1953, *E. L. Little Jr. 15775* (VEN); La Carbonera, 15 km NW de Ejido, 2200 m, 4 July 1953, *E. L. Little Jr. 15276* (VEN); Dtto. Libertador, Mcpo. Tabay, P. N. Sierra Nevada, La Mucuy, vía a la laguna La Coromoto, 9°54'N, 71°03'W, 2125 m, 2 February 1986, *A. Castillo 1905* (VEN); P. N. Sierra Nevada, La Mucuy, 9°54'N, 71°03'W, 2150 m, 6 December 1980, *A. Castillo 1030* (VEN).

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