



Research Networking Programmes

Short Visit Grant or Exchange Visit Grant

(please tick the relevant box)

Scientific Report

The scientific report (WORD or PDF file – maximum of eight A4 pages) should be submitted online within one month of the event. It will be published on the ESF website.

Proposal Title: Taxonomic revision of *Rochefortia* Sw. (Ehretiaceae, Boraginales) based on morphological and molecular data

Application Reference N°: 6317

1) Purpose of the visit

Neotropical *Rochefortia* comprises approximately 10 species and is integral part of the Ehretiaceae (Boraginales). Knowledge about such dioecious plants is poor at present and does not exceed much beyond the first description of species. The aim of this visit was to improve species delimitation and circumscription, each displaying a great morphological variability. Moreover, molecular sequence data were used for the phylogenetic reconstruction of *Rochefortia*, providing an evolutionary scenario of its diversification.

2) Description of the work carried out during the visit

I isolated total genomic DNA from herbarium material (mainly leaves, sometimes seeds) representing the known populations and potential species of *Rochefortia* using the Stratec Invisorb DNA mini kit. The procedure followed the manufacturer's instructions, with an adjustment of the elution buffer to 50µl in order to increase DNA yield. PCR amplification of the nuclear ITS and ETS loci and of the plastidial locus *rps16* was performed in a standard TAQ PCR master mix, occasionally enriched with Q solution. The PCR products were firstly purified by EXO-SAP and then by sepalex column and sequenced on a 3130 Genetic Analyzer from Applied Biosystems. The sequences were edited in Sequencher.

3) Description of the main results obtained

Obtaining amplifiable DNA from dried plant herbarium collections (of which some were older than 50 years) was highly challenging due to a series of independent factors such as specimen preparation, storing conditions, secondary compounds and the presence of trichomes that could have perturbing effects. I tried to surpass this by optimizing some of the PCR parameters (e.g., MgCl₂, T_m values, Q solution) and got a good resolution in terms of sequences, covering all potential species and many of their populations. The resulting molecular tree inferred from concatenated nuclear and plastid loci was mostly well resolved and showed high statistical support for many nodes. *Rocheffortia* was monophyletic, and internal tree topology indicated a strong biogeographical signal. The mainland species (i.e., *R. lundellii* from Central America and *R. spinosa* from northern South America) constituted the sister group of the species distributed over the Caribbean islands. Taxonomic delimitation based on molecular data helped for evaluating the intraspecific morphological variability that was large in the majority of species. As a consequence, many species do not exhibit clear diagnostic morphological traits, but rather show overlaps. I now recognize 8 species of *Rocheffortia* in total, with the highest molecular diversity encountered in Cuba (5 species), followed by Jamaica with 2 species and all the other islands with a single species each. I have annotated all available herbarium specimens with accepted species names and reduced many of the names in *Rocheffortia* to synonyms.

4) Future collaboration with host institution (if applicable)

The *Rocheffortia* project is currently at the stage of completion for publication of the major results. I further consider more taxonomic and phylogenetic work on the woody Boraginales in close collaboration with my hosting institution in future, if applicable as a PhD student.

5) Projected publications / articles resulting or to result from the grant (*ESF must be acknowledged in publications resulting from the grantee's work in relation with the grant*)

I have finished my experimental work in terms of molecular sequencing and morphological investigations. Together with my colleagues at the host institution, I am currently preparing three manuscripts, including a) a molecular phylogeny and its interpretation, b) the description of a new species from eastern Caribbean islands and c) a taxonomic revision of *Rocheffortia*.

6) Other comments (if any)

For the phylogenetic tree of *Rocheffortia* please see the annex.

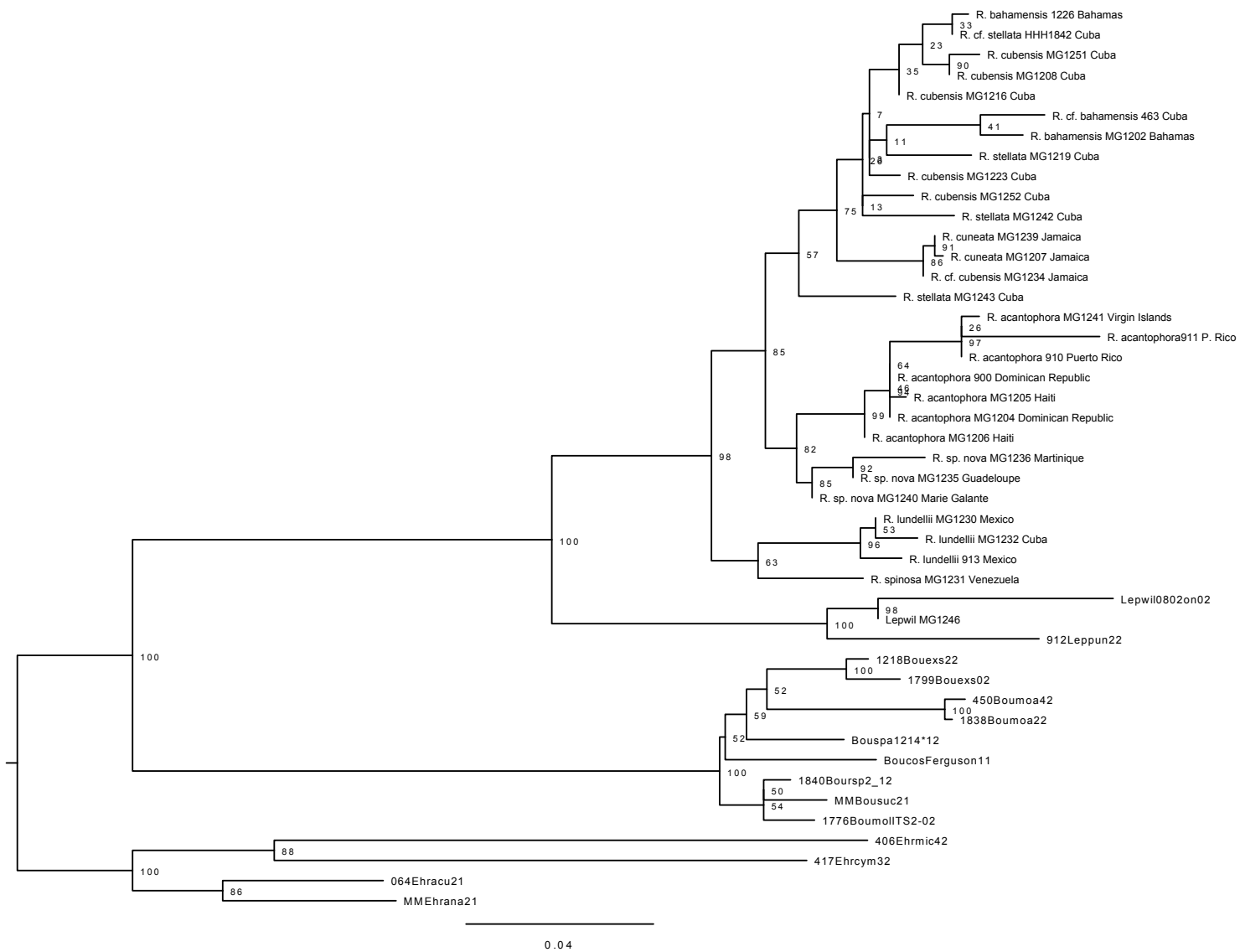


Fig. 1. Concatenated sequence tree of Rochefortia with Lepidocordia and Bourreria as outgroups