

Recent insights in the evolution of epiphytic Cactaceae from DNA sequence data

Nadja Korotkova¹, Thomas Borsch², Nigel P. Taylor³, Dietmar Quandt¹, Kai F. Müller⁴, Wilhelm Barthlott¹

¹Nees Institute for Biodiversity of Plants, University of Bonn, Germany; ²Botanischer Garten und Botanisches Museum Berlin-Dahlem, Berlin, Germany; ³Royal Botanic Gardens Kew, UK; ⁴Institute for Evolution and Biodiversity, University of Münster, Germany

Background

Our research is part of a project dealing with the evolution of the epiphytic cacti. Using fast evolving regions from the chloroplast genome (*trnK/matK*, *psbA-trnH*, *trnQ-rps16*, *rpl16*, *trnS-G* spacer, *trnG* intron and *rbcL*) we are studying the phylogenetic relationships in the group.

I. The genus *Pfeiffera*

Background and aims: Prior to our study, three tribes containing epiphytic genera were accepted in Cactaceae. The two largest groups are the Rhipsalideae and the predominantly Mesoamerican Hylocereeae. After the first phylogenetic study of the Cactaceae, a part of *Lepismium* was found to be an independent lineage from either Rhipsalideae or Hylocereeae (Nyffeler 2002, Amer. J. Bot.). As a consequence, the genus *Pfeiffera* was reinstated in the New Cactus Lexicon (Hunt 2006) with 9 species of epiphytic, terrestrial and epilithic cacti. But only 3 of the species had been included in Nyffeler's analysis. So our aim was to redefine the circumscription of *Pfeiffera*.

Results: Two unrelated groups containing species classified as *Pfeiffera* in the New Cactus Lexicon were found. *Pfeiffera* sensu Hunt (2006) is therefore polyphyletic. One group contains 6 species, including the type species *P. ianthothele* and 5 further species. The other group contains two *Pfeiffera* species and *Lepismium incachacatum* and is found to be the sister group of *Calymmanthium substerile*.

Conclusions: Our results justify generic status for this newly found group. Since it includes the type species of the earlier-proposed monotypic genus *Lymanbensonia*, we recently suggested the reinstatement of the latter in an amplified circumscription (Korotkova et al. 2010). Our results further support the establishment of a separate tribe *Lymanbensonieae* N. Korotkova & Barthlott, to contain *Lymanbensonia* and *Calymmanthium*. The results imply that epiphytism evolved more frequently in Cactaceae than hitherto assumed. There are four geographically distinct lineages containing epiphytic species: The Mesoamerican *Hylocereeae*, the predominantly Brazilian *Rhipsalideae*, the Bolivian/Argentinian *Pfeiffera* and the newly found Peruvian/Bolivian *Lymanbensonia*.

Future work: The closest relatives of *Pfeiffera* are still not known with confidence and remain among the open questions in Cactaceae phylogenetics. Although *Corryocactus* (incl. *Erdisia*) and *Eulychnia* have been found putatively close to *Pfeiffera*, their exact position is unresolved and generic limits of *Corryocactus* also need further evaluation.

Reference: Korotkova, N., Zabel, L., Quandt, D., Barthlott, W. (2010). A phylogenetic analysis of *Pfeiffera* and the reinstatement of *Lymanbensonia* as an independently evolved lineage of epiphytic Cactaceae within a new tribe *Lymanbensonieae*. Willdenowia 40: 151–172.

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II. Resolving relationships and identifying species in the Rhipsalideae

Background and aims: The tribe Rhipsalideae was the main focus of this study. The aim was to resolve relationships within the tribe, especially between the species. Our analysis included 51 of the 52 species currently accepted in the New Cactus Lexicon, including also all the subspecies and forms. The second aim was to evaluate whether species are monophyletic or not. For that purpose, we have included several accessions from the morphologically variable and widely distributed species, such as *Rhipsalis micrantha* or *R. baccifera*. So not only was the whole tribe sampled, the morphological variation was covered as well. We were especially interested in how well we could identify the species using sequence data. This relates to a new methodological approach termed DNA barcoding. It aims at identifying species using a DNA sequence – the DNA barcode. The relevance of that is that first, all Rhipsalideae are CITES-listed; therefore accurate species identification is actually desirable and relevant. And second, as DNA barcoding has not been yet attempted for the Cactaceae, we wanted to provide a first study of that kind. And finally, we wanted to compare how the different DNA regions would perform for the species identification – as DNA barcodes.

Results: We found the Rhipsalideae to be monophyletic. Also, the currently and traditionally recognized genera were found to be natural groups. The only exception is *Hatiora*: its two subgenera *Hatiora* and *Rhipsalidopsis* are not closely related. We therefore propose to recognise *Rhipsalidopsis* at generic level again. Unfortunately, we could not fully resolve the relationships between the genera. The subgenera of *Rhipsalis* were also found with maximal statistical support and largely correspond to the ones established by Barthlott & Taylor (1995, Bradleya). Only slight changes to the subgenera are suggested. Most important is that we were able to resolve the species level with very good statistical support. Then we found that all but two species and subspecies can also be identified using DNA sequences. Finally, we found that the markers used are variously successful in identifying the OTUs. It is remarkable that many of the morphologically variable and widely distributed species that are not easy to define morphologically are also not easy to define using sequence data.

Future work: This study is only based on chloroplast markers. But there may be hybrids, so the aim is to sequence nuclear markers as well. For those species where species-limits are less clear, population-level studies would be needed.

Reference: Korotkova, N., Borsch, T., Quandt, D., Taylor, N.P., Müller, K.F., Barthlott, W. (*in press*). How much does it take to resolve relationships and to identify species with molecular markers? An example from the epiphytic Rhipsalideae (Cactaceae). *Amer. J. Bot.*

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