Available online at www.sciencedirect.com
SCIENCE DIRECT®
Organisms, Diversity & Evolution 5 (2005) 1–13
ORGANISMS DIVERSITY & EVOLUTION
www.elsevier.de/ode

Evolution of *Suessenguthia* (Acanthaceae) inferred from morphology, AFLP data, and ITS rDNA sequences

Alexander N. Schmidt-Lebuhn[a,*], Michael Kessler[a], Julia Müller[b]

[a]*Abt. Systematische Botanik, Albrecht-von-Haller-Institut für Pflanzenwissenschaften, Universität Göttingen, Untere Karspüle 2, 37073 Göttingen, Germany*
[b]*Abt. Experimentelle Phykologie und Sammlung von Algenkulturen, Albrecht-von-Haller-Institut für Pflanzenwissenschaften, Universität Göttingen, Untere Karspüle 2, 37073 Göttingen, Germany*

Received 26 January 2004; accepted 29 April 2004

Abstract

The phylogeny and evolution of *Suessenguthia* (Acanthaceae), a genus of six species from the Andean foothills and adjacent Amazonia in Bolivia, Peru and western Brazil, are discussed based on morphological and molecular (amplified fragment length polymorphism, ITS rDNA) data. *Suessenguthia* forms a paraphyletic group at the base of the larger genus *Sanchezia*. The non-overlapping geographical distribution of closely related species suggests that parapatric or allopatric speciation is the major mode in the genus. A major evolutionary tendency promoting diversification of the group presumably was a change from bee- to hummingbird pollination, resulting in a successive adaptation of flower morphology and inflorescence structure.

Keywords: Acanthaceae; *Suessenguthia*; *Sanchezia*; Flower morphology; Andes; AFLP

Introduction

The tropical Andes and adjacent lowlands are one of the world's botanically richest regions and support a very large number of endemic plant species (Myers et al. 2000). Yet, relatively little is known about the evolutionary pathways and mechanisms that have led to the proliferation of the flora (Young et al. 2002). Very few species-level phylogenetic studies of tropical Andean plants have been published, and these treat mostly high-montane taxa, e.g. *Gentianella* (von Hagen and Kadereit 2001) or *Polylepis* (Kessler 1995).

In the present study, we have analyzed the evolution of *Suessenguthia* (Acanthaceae), a genus with six species. *Suessenguthia* was described by Merxmüller (1953), and revised by Wasshausen (1970) and Schmidt–Lebuhn (2003). It differs from the larger and better known genus *Sanchezia* R. & P. by having four functional stamens, versus one pair of stamens reduced to staminodia (Leonard and Smith 1964) (Fig. 1D and E). Both genera belong to tribe Trichanthereae, together with *Bravaisia* DC., *Trichanthera* HBK and *Trichosanchezia* Mildbraed (Daniel 1988). This tribe is characterized by bicolporate pollen grains with a characteristically banded surface sculpturing, bipolarly pointed cystoliths, and an almost radially symmetric corolla. It is also remarkable in the mostly herbaceous to shrubby family Acanthaceae for including several tree species.

*Corresponding author.
E-mail address: schmidtleb@yahoo.de (A.N. Schmidt-Lebuhn).

1439-6092/$ - see front matter © 2005 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ode.2004.04.006

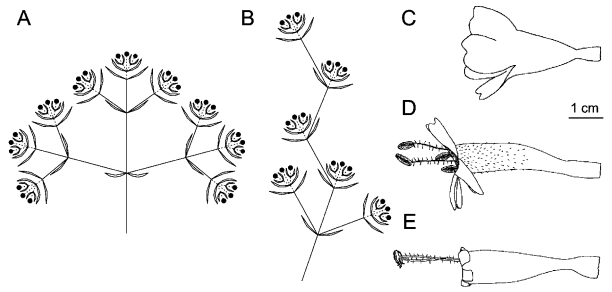

Fig. 1. Inflorescence structures and corolla shapes in *Suessenguthia* and *Sanchezia*. (A) Regular thyrsus as in *Suessenguthia multisetosa*, *S. wenzelii*, and *S. vargasii*. (B) Monochasium as in *S. trochilophila*, *S. barthleniana*, *S. koessleri*, and most species of *Sanchezia*. (C) Short, infundibiliform corolla of *S. multisetosa*. (D) Tubular corolla of *S. trochilophila*. (E) Corolla of *Sanchezia skutchii*, with reduced lobes.

Suessenguthia was selected as a study group because of its tractable size and relatively restricted distribution, which enabled us to study all species in the field. Our work consisted of a taxonomic revision (Schmidt-Lebuhn 2003) and a phylogenetic analysis aimed at elucidating the evolution and biogeography of the genus. Specific questions were: What is the phylogenetic relationship of *Suessenguthia* to *Sanchezia*? Which speciation mechanisms have been prevalent in *Suessenguthia*? Can the phylogenetic history of the genus be linked to morphological character changes? We based our phylogenetic analysis on morphological characters and data obtained from ITS rDNA sequences and amplified fragment length polymorphism (AFLP). AFLP data have been used successfully for population genetics as well as phylogenetic analyses at the level of closely related species (e.g. Hodkinson et al. 2000; Zhang et al. 2001). So far, phylogenetic studies of Acanthaceae using molecular methods have focussed on the main evolutionary lineages within the family (McDade and Moody 1999; McDade et al. 2000; Manktelow et al. 2001) rather than on species-level relationships.

Material and methods

Morphological data

Populations of all species of *Suessenguthia* were studied and sampled in Bolivia and Peru in July–September 2000. Herbarium specimens were borrowed from M, MO, NY, and US (acronyms according to Holmgren et al. 1990). In addition, the collections at B, CUZ, LPB, M, and USZ were studied on site. Altogether, herbarium specimens from a total of 120 different collections served as the basis for the morphological analysis.

The morphological data matrix (Table 1) includes all species of *Suessenguthia*, and three species of *Sanchezia*. The two varieties of *S. vargasii* as well as two geographically distant populations of the variable species *S. trochilophila* were included separately. *Trichanthera gigantea* (HBK) Nees, a representative of the type genus of the Trichanthereae, was added to the data matrix for comparison (voucher specimens: *Dodson 5846* [AAU], *Holm-Nielsen et al. 26040* [AAU], *Løjtnant & Molau 15241* [AAU], *Zak & Jaramillo 2326* [MO]).

From a total of about 50 morphological characters examined in the course of the taxonomic revision, 26 characters with two or three character states each were selected for the cladistic analysis (Table 2). All characters with three possible states were regarded as ordered (Wagner parsimony), because an unambiguous evolutionary order was evident, with one character state intermediate between the two most different states. Polymorphic character states were scored as such; characters not existing in the outgroup were scored as missing data.

Sampling and DNA extraction for molecular analysis

Plant material (young leaves) was collected freshly during fieldwork and dried with silica gel. A total of 15 specimens representing all species of *Suessenguthia* and three species of *Sanchezia* were studied with molecular methods (Appendix A). For comparison, a specimen of *Ruellia puri* (Nees) Mart. ex Jackson was included, and sequences for the ITS rDNA regions of *Sanchezia speciosa* Leonard and *Ruellia californica* (Rose) I.M. Johnston were obtained from GenBank (accession numbers AF169835 and AF167704, respectively; McDade et al. 2000).

Total genomic DNA extraction followed the protocol of Hellwig et al. (1999). Some samples were extracted anew for AFLP analysis, using the "Puregene DNA Isolation Kit" (Gentra Systems) as recommended by the manufacturer, in order to test whether the previous results would be reproduced.

Sequencing of the ITS rDNA region

Parts of the rDNA containing the ITS1, 5.8S, and ITS2 regions were amplified with the primers NS7m (Friedl 1996) and ITS4 (White et al. 1990). Amplifications were performed in 50 µl volumes containing 2 mM $MgCl_2$, 1% DMSO, 1 × PCR buffer, 0.75 U Taq DNA polymerase (Silverstar, Eurogentec), 0.2 µM primer, 50 µM of each dNTP, and 1 µl of genomic DNA, as follows: 300 s at 95 °C, 33 cycles of 40 s at 94 °C, 30 + 2 s at 50 °C and 120 + 2 s at 72 °C, six cycles of 40 s at 94 °C, and 120 s at 72 °C.

PCR products were purified by a precipitation of at least 1 h with 1 vol isopropanol and 0.1 vol sodium-acetate (pH 4.0). Cycle sequencing was carried out with