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

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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.

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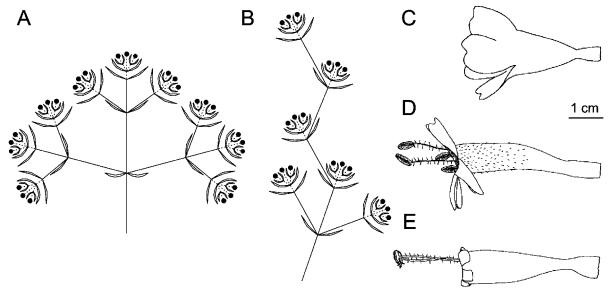

Fig. 1. Inflorescence structures and corolla shapes in *Suessenguthia* and *Sanchezia*. (A) Regular thyrsus as in *Suessenguthia multisetosa*, *S. wenzelii*, and *S. vargasii*. (B) Monochasium as in *S. trochilophila*, *S. barthleniana*, *S. koessleri*, and most species of *Sanchezia*. (C) Short, infundibiliform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

Table 1. Data matrix for the cladistic analysis based on morphological data

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Suessenguthia vargasii Wassh. var. *vargasii*	1	0	0	1	0	0	0	1	1	1	0	0	0	1, 2	1	2	0, 1	0	1	1	0	1	1	2	2	0	1
Suessenguthia vargasii Wassh. var. *hirsuta* Schmidt-Lebuhn	1	0	0	1	0	0, 1	2	1	1	1	0	0	0	1	1	2	2	0	1	1	0	1	1	2	2	0	1
Suessenguthia multisetosa (Rusby) Wassh. & Wood	0, 1	0, 1, 2	0, 1, 2	1	0	0	0, 1, 2	1	0	1	0	0	0	0	0	2	2	0	0	0	0	0	0	0, 1	0	0	0
Suessenguthia wenzelii Schmidt-Lebuhn	1	0	0	1	0	0, 1	1	1	0, 1	1	0	0	0	0	0	2	2	0	1	1	0	1	1	1, 2	0	0	0, 1
Suessenguthia trochilophila Merxm. (Pilcopata, Peru)	1	0, 1, 2	0	1	1	1	2	1	1	0	0	0	1	0, 1	0	2	1	0	1	1	0	1	0	2	1	0	1
Suessenguthia trochilophila Merxm. (Bolivia)	1, 2	0, 1, 2	0	1	1	1	2	1	1	0	0	0	1	0, 1	0	2	1	0	1	1	0	1	1	2	1	0	1
Suessenguthia barthleniana Schmidt-Lebuhn	1, 2	1	1	1	1	1	2	1	1	1	0	1	1	0, 1	0	0, 1	1	0	1	1	0	1	0	1	0	0	0
Suessenguthia koessleri Schmidt-Lebuhn	1	0	0	1	1	1	0	1	1	1	0	0	1	0, 1	0	2	0	1	1	1	1	2	2	2	1	0	1
Sanchezia parvibracteata Sprague & Hutchinson	1	0	0	1	1	1	—	1	0	1	0	0	2	1	0	0	—	1	1	1	1	2	2	0	—	1	1
Sanchezia oblonga R. & P.	1, 2	1	1	1	1	1	—	1	1	1	1	0	2	1	0	0	—	0	1	1	1	2	2	2	0	1	1
Sanchezia skutchii Leonard & Smith	1	0	0	1	1	1	—	1	0	1	0	0	1	1	0	0	—	0	1	1	1	2	2	1	0	1	1
Trichanthera gigantea HBK	0	0	1	0	0	—	0	0	0	1	0	—	—	—	—	0	—	—	0, 1	0	0	0	—	2	0	0	1

Table 2. Characters and character states for the cladistic analysis based on morphological data

	Character	State 0	State 1	State 2	Treatment of multiple states
1.	Growth form	Tree	Shrub	Weak shrub/herb	Ordered
2.	Leaf margin	Entire	Dentate		—
3.	Leaf veins (per side)	Fewer than 10	More than 10		—
4.	Flowers concentrated to headlets	No	Yes		—
5.	Inflorescence	Regularly thyrsoid	Thyrsus with apical monochasia		—
6.	Indumentum of young internodes (vegetative and inflorescence) — length	<0.6 mm	0.6–1.1 mm	>1.1 mm	Ordered
7.	Bracts	Small, scale-like	Large, conspicuous		—
8.	Color bracts/calyx	Green	Reddish		—
9.	Outer bracts — apex	Attenuate	Acute	Rounded	Ordered
10.	Outer bracts — shape	Ovate	Elliptical		—
11.	Outer bracts — base	Adnate	Rounded		—
12.	Inner bracts — apex	Slender, attenuate	Acute	Rounded	Ordered
13.	Inner bracts — shape	Ovate	Elliptical	Obovate	Ordered
14.	Inner bracts — base	Acute	Decurrent		—
15.	Indumentum of bracts — abundance	Absent	Sparse	Dense	Ordered
16.	Indumentum of bracts — length	<0.6 mm	0.6–1.1 mm	>1.1 mm	Ordered
17.	Subhyaline margin of calyx lobes	Absent	Present		—
18.	Corolla tube — length	⩽3.5 cm	>3.5 cm		—
19.	Corolla tube — shape	Infundibiliform	Cylindrical		—
20.	Corolla lobes — length	⩾1.0 cm	⩽0.8 cm		—
21.	Corolla lobes — orientation	Spreading forwards distally from tube	Spreading at right angles to tube	Recurved backwards toward tube	Ordered
22.	Intensity of floral color	Pale (pink)	Medium (pink)	Intense (red, orange, yellow)	Ordered
23.	Indumentum of corolla — abundance	Absent	Sparse	Dense	Ordered
24.	Indumentum of corolla — length	<0.6 mm	0.6–1.1 mm	>1.1 mm	Ordered
25.	Number of fertile stamens	4	2 (plus 2 staminodia)		—
26.	Position of anthers	All included	At least one pair exserted		—

the Thermo Sequenase Sequencing kit with 7-deaza-dGTP (Amersham Pharmacia) using the primers 1800F (Friedl 1996), ITS4 (see above), and ITS2N (Beck et al. 1998), as follows: 120 s at 95 °C, 18 cycles of 18 s at 95 °C, 25 s at 51 °C and 40 s at 70 °C, six cycles of 20 s at 95 °C, and 60 s at 70 °C.

The sequencing reactions were analyzed by gel electrophoresis using a LI-COR LR 4200 DNA-sequencer and employing the e-Seq software 1.1 (LI-COR) and the AlignIR 1.2 software (LI-COR). All sequences were manually aligned using BioEdit (Hall 1999).

AFLP procedure

AFLP procedures followed Mannschreck et al. (2002) and Vos et al. (1995). A part of the restriction fragments produced by digestion of total genomic DNA with two different restriction enzymes are amplified and separated with electrophoresis. For analysis, the different-sized fragments produced by each sample are used to construct a binary data matrix, treating the fragments as absent or present. About 100 ng of genomic DNA were double-digested with the restriction enzymes *Mse*I and *Eco*RI (New England Biolabs). Restriction and

ligation were performed in an 11 µl volume containing 1 × T4 DNA ligase buffer, 0.05 mM NaCl, 0.05 g/l BSA, restriction enzymes *Mse*I (1 U) and *Eco*RI (5 U), 5 pmol of *Eco*RI-adapter, 50 pmol *Mse*I-adapter, and 1 U of T4 DNA ligase (New England Biolabs) which was incubated for 2 h at 37 °C.

The ligation product was diluted with 39 µl of sterile water and then pre-amplified with the primer combination *Eco*RI + A/*Mse*I + C (5-GACTGCGTACCAATT + A-3, 5-GATGAGTCCTGAGTA + C-3). The pre-amplification was performed in a 20 µl volume containing 4 µl of ligation product, 1 × PCR buffer, 1.5 mM $MgCl_2$, 250 µM of each dNTP, 5 pmol of each pre-primer, and 0.5 U Taq DNA polymerase (Silverstar, Eurogentec), with the following temperature profile: 5 min at 94 °C, 20 cycles of 20 s at 94 °C, 30 s at 56 °C, and 120 s at 72 °C.

The pre-amplification product was diluted 10 times with TE buffer, and 3 µl was used as template in the selective amplification taking place in a 20 µl volume containing 1 × PCR buffer, 1.5 mM $MgCl_2$, 250 µM of each dNTP, 5 pmol *Mse*I selective primer, 1 pmol *Eco*RI (IRD700 fluorescence labeled) selective primer, and 0.5 U Taq DNA polymerase (Silverstar, Eurogentec). The thermocycler was run with the following temperature profile: 1 min at 94 °C, 10 cycles of 20 s at 94 °C, 30 s at 65–1 °C, 120 s at 72 °C, 20 cycles of 20 s at 94 °C, 30 s at 56 °C, and 120 + 4 s at 72 °C. For the selective amplification six different primer combinations were tested for their level of variability within and among species with a small number of samples: Eco + A/Mse + CA, Eco + A/Mse + CTA, Eco + AAG/Mse + C, Eco + AAG/Mse + CAT, Eco + ACT/Mse + CTA and Eco + ACG/Mse + CAT. One of them, Eco + ACG (5-GACTGCGTACCAATT + ACG-3)/Mse + CAT (5-GATGAGTCCTGAGTA + CAT-3), was chosen for fingerprinting all samples.

Selective amplification products were separated on an LI-COR LR 4200 DNA-sequencer along with a labeled 50–700 bp sizing standard. Raw AFLP fragment data were analyzed by visual comparison. A binary data matrix was produced scoring fragments between 170 and 700 bp of length as present (1) or absent (0). To test for reproducibility of the results, some samples were analyzed a second time using a different DNA extraction protocol (see above), and in some cases two plants of the same population were analyzed. Reproducibility was very high; only in some cases fragments were missing in a repeated analysis. Because that was clearly due to an overall weak reaction, these selective amplifications were excluded from the analysis.

Principal coordinates analysis

Principal coordinates analysis (PCO) was performed on the AFLP data to visualize genetic distance between the species. A distance matrix was calculated from the binary AFLP data matrix with the genetic distance estimation program of the Treecon software package (Van de Peer and De Wachter 1994) using Nei and Li's (1979) formula. This method was chosen because it takes into account shared fragments only, but not shared absence of fragments, thus reducing the amount of homoplasy. The distance matrix was submitted to PCO on SYSTAT 7.0 for Windows (SYSTAT 1997).

Cladistic analysis

Phylogenetic trees were calculated using PAUP 4.0b5 (Swofford 1997). For morphological data, a heuristic search for maximum parsimony trees with all characters equally weighted was conducted with the following options in effect: MulTrees, tree-bisection-reconnection branch swapping, multistate taxa interpreted as polymorphism, starting trees obtained via random stepwise addition with 10 repetitions, branches collapsed if maximal branch length is zero.

The same parsimony analysis was then repeated using a successive approximation approach to character weighting (Farris 1969, 1988). The characters were re-weighted based on their rescaled consistency (RC) index values calculated in the previous analysis, i.e. characters that showed high homoplasy were given lower weight. The procedure was repeated until the tree length could not be further reduced by re-weighting. Trees obtained from analyses based on morphological data were rooted using *T. gigantea* as outgroup.

A minimum-distance analysis based on AFLP data was conducted with the following options: starting trees obtained by neighbor joining, negative branch lengths allowed, but set to zero for tree score evaluation, distance measure set to restriction site difference (Nei and Li 1979). Search strategy was the same as with the morphological data. The tree obtained from analysis based on AFLP data could not be rooted with *T. gigantea* as outgroup because no molecular sample was available. To facilitate comparison of the results of the two analyses, the tree was rooted using the ingroup species found to be the most basal in the morphological analysis.

In all cases, bootstrap values (Felsenstein 1985) were calculated with 1000 replicates to estimate the support for individual branches.

Results

Morphological analysis

Maximum parsimony analysis of the morphological data with equally weighted characters produced two

equally parsimonious trees (not shown). They differ only in the position of *S. barthleniana* amidst, or as sister to, the two populations of *S. trochilophila*. Tree length was 80 steps, consistency index (CI) = 0.75, retention index (RI) = 0.64, RC index = 0.48. All branches received low bootstrap support values (50–70).

The successive approximations approach produced a single most parsimonious tree identical in topology with one of the trees from the equally weighted approach (Fig. 2), with *S. barthleniana* as sister to *S. trochilophila*. Tree length was reduced to 19.3 steps, CI = 0.88, RI = 0.91, RC = 0.81. This single tree therefore was chosen as the best phylogenetic hypothesis.

The chosen tree (Fig. 2) clearly shows *Suessenguthia multisetosa* as the most basal species of the ingroup. *Suessenguthia wenzelii* and *S. vargasii* diverge next, though the position of *S. wenzelii* receives low bootstrap support. The remaining species form a relatively well-supported clade comprising the poorly supported group of *S. trochilophila* and *S. barthleniana* and a well-supported subclade consisting of *S. koessleri* and *Sanchezia*. The relationship of *S. barthleniana* and *S. trochilophila* to each other and the relationships in *Sanchezia* are rather poorly supported.

ITS rDNA sequence data

The ITS rDNA region of eight specimens included in the project was sequenced completely. In total (ITS1, 5.8S plus ITS2), it comprises 619–621 bp. The region was sequenced partly for three other specimens. Differences between the ITS rDNA sequences of the different species of *Suessenguthia* were few and insufficient for a cladistic analysis. Only very few positions are parsimony informative and even these yield some contradictory information (Table 3). Peruvian *S. trochilophila* (Schmidt-Lebuhn 37; see Appendix A) and all studied species of *Sanchezia* share two nucleotide positions (ITS2 positions 43 and 72), matching the results of the AFLP analysis. They share this feature with the two species of *Ruellia*, but this may be due to homoplasy, because *Ruellia* as a member of a different tribe is related to *Suessenguthia* relatively distantly. *S. multisetosa*, *S. wenzelii*, and *S. vargasii* together are separated from the other species of *Suessenguthia*/*Sanchezia* by a single common substitution (ITS1 position 51). Some variable and informative positions do not match the results of the morphological and the AFLP analysis. A small area at the end of the ITS2 region, including ITS2 positions 205–208, appears to be quite variable, but this may be artifactual as that is also an area of high base ambiguity.

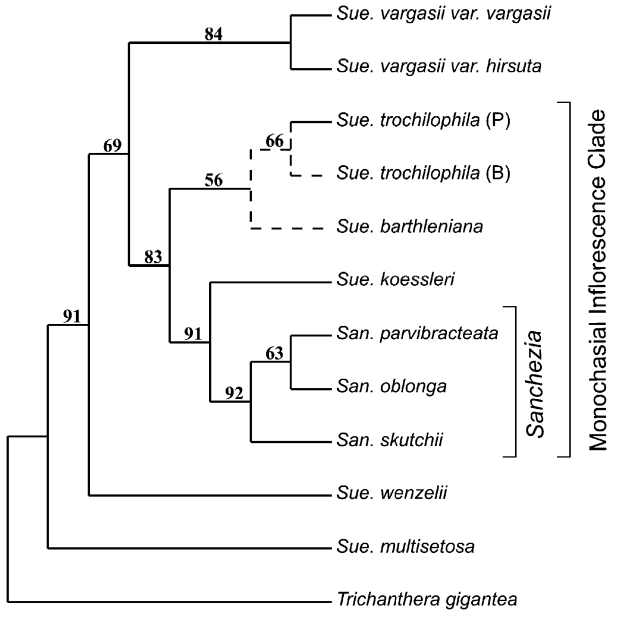

Fig. 2. Single most parsimonious tree based on morphological data, using a successive character re-weighting approach. Bootstrap values are given at the branches. Broken lines indicate branches on which the two equally parsimonious trees from the analysis using equally weighted characters disagreed. B = Bolivia; P = Pilcopata, Peru.

AFLP analysis

The chosen primer combination yielded 165 fragments, 161 of them polymorphic. In a first step, a single unrooted tree was recovered from the minimum-distance analysis. The tree was then rooted using *S. multisetosa*, the most basal species in the cladistic analysis based on morphological data, as an outgroup in order to facilitate comparison between the results produced by the two data sets (Fig. 4).

As in the tree based on morphological data, the first species to diverge are *S. wenzelii* and *S. vargasii*. The remaining species again form one clade, but it is not as well supported and differs in the arrangement of the species belonging to it. *S. barthleniana*, which in the morphological analysis was closest to *S. trochilophila*, has the basal position in this clade. The remainder of the group falls into a well-supported subgroup of Bolivian *S. trochilophila*, and a poorly supported subgroup comprising Peruvian *S. trochilophila* and *Sanchezia*, with the position of *S. koessleri* not resolved. Thus, in this case the different samples of *S. trochilophila*, the most variable species of *Suessenguthia* (Schmidt-Lebuhn 2003), do not form a monophyletic group in the phylogenetic tree. Instead, the only Peruvian specimen for which molecular data are available (Pilcopata) appears as sister to *Sanchezia*, whereas the two morphologically very similar Bolivian populations and *S. koessleri*, the species most strongly resembling *Sanchezia* morphologically, have more distant positions.

Table 3. Variable ITS and 5.8S rDNA base residues among the species included in the molecular analysis

Region	ITS1									5.8S		ITS2						
Position	1	2	31	37	51	66	70	72	107	8	228	43	72	92	205	206	207	208
Ruellia californica	C	G	G	C	T	N	G	T	C	A	C	G	T	G	G	C	G	C
Ruellia puri	?	?	C	T	T	G	G	T	C	A	C	G	T	T	S	-	G	-
Sanchezia speciosa	C	G	G	C	C	G	G	T	C	A	C	G	T	T	G	C	G	C
Sanchezia skutchii	G	A	G	C	C	G	G	T	C	A	C	G	T	C	M	-	G	-
Sanchezia parvibracteata	G	A	A	C	C	G	G	T	C	-	C	G	T	C	M	-	-	C
Suessenguthia trochilophila (Peru)	Y	G	K	C	C	G	G	T	A	A	C	G	T	C	M	-	G	C
Suessenguthia trochilophila (Bolivia)	Y	T	T	A	C	G	T	T	M	A	C	T	C	Y	A	C	G	C
Suessenguthia koessleri	?	?	G	C	C	G	T	T	A	A	C	T	C	T	A	-	G	-
Suessenguthia barthleniana	?	?	?	?	?	?	?	?	?	?	C	K	C	T	A	-	G	C
Suessenguthia wenzelii	C	G	G	C	A	G	G	T	A	-	C	T	C	T	-	C	-	C
Suessenguthia multisetosa (Yungas)	C	R	G	C	A	G	T	C	C	A	M	T	C	Y	M	-	G	C
Suessenguthia multisetosa (Chiquitanía)	G	A	R	A	H	A	K	C	M	A	A	T	C	C	A	C	G	-
Suessenguthia vargasii	S	G	G	A	A	A	K	T	M	G	A	T	C	C	A	-	G	C

Note: To simplify the table, positions uninformative for *Suessenguthia* and *Sanchezia* are excluded. Question marks indicate missing data, hyphens indicate sequence gaps. Position numbers refer to position on sequence of *Suessenguthia multisetosa* from the Bolivian Yungas (Schmidt-Lebuhn 67; see Appendix A).

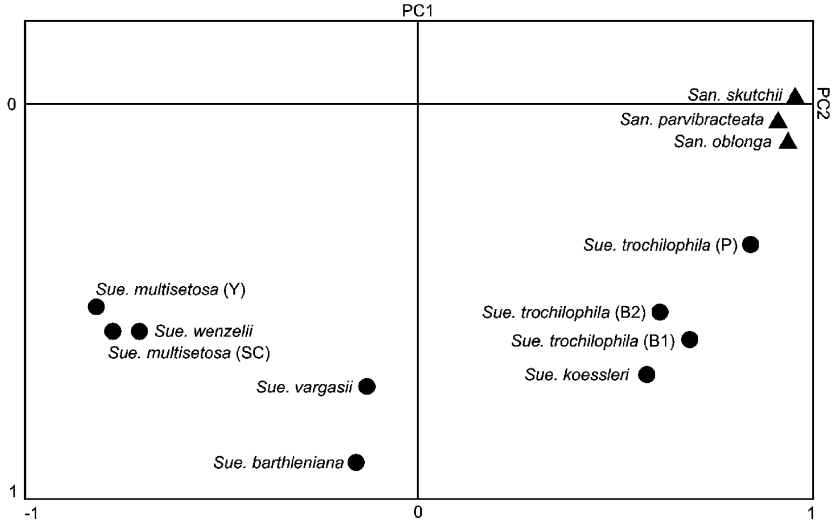

Fig. 3. Principal coordinates plot of the AFLP analysis for the first two Principal coordinates (81.5% of the total variation). *Suessenguthia* species are represented by circles, *Sanchezia* species by triangles. B1 = Tumupasa, Bolivia; B2 = Carmen Florida, Bolivia; P = Pilcopata, Peru; SC = Chiquitanía dry forests, Bolivia; Y = Bolivian Yungas.

Principal coordinates analysis

Variance of the first two principal coordinates accounted for 81.5% of the total variation (Fig. 3). The first axis (52.6%) separated the species included from *Suessenguthia* and *Sanchezia* into three groups, one of *S. multisetosa* and *S. wenzelii*, one of *S. vargasii* and *S. barthleniana* in an intermediate position, and one of *S. trochilophila*, *S. koessleri*, and *Sanchezia*. The second axis (28.9%) showed a greater distance between *S. vargasii* and *S. barthleniana* as well as between *Sanchezia* and a remaining group of *S. trochilophila* and *S. koessleri*. *S. wenzelii* clusters very closely with *S. multisetosa*; *S. koessleri* occupies a position close to *S. trochilophila*. The three accessions of *S. trochilophila* are more dispersed than the three different species of *Sanchezia* included in the analysis.

Comparison of the results based on morphological and AFLP data

If the rooting of the tree based on AFLP data is accepted, the two analyses agree on some important aspects, such as the positions of *S. wenzelii* and *S. vargasii* as the first to diverge following basal *S. multisetosa*. The remaining species (including *Sanchezia*), all characterized by the possible synapomorphy of

inflorescences with most heads arranged in apical monochasia (Fig. 1, Table 4), form a monophyletic group in both analyses. The two analyses resolve relationships within this group differently and disagree in particular with respect to which species of *Suessenguthia* is sister to *Sanchezia*. On the basis of only three species of the latter genus included in this work, no final decision can be made. However, the three examined species of *Sanchezia* form one well-supported clade in both the morphological and the AFLP analysis.

Discussion

Phylogenetic relationship of *Suessenguthia* and *Sanchezia*

Both the morphological and the AFLP analysis placed *Sanchezia* as a monophyletic unit within *Suessenguthia*. This close relationship is not surprising, considering that the genera are similar enough to be readily confused by many collectors and that *Sanchezia* differs morphologically only in having one pair of stamens reduced to staminodia. Because the presence of two pairs of functional stamens is the common and hence presumably ancestral state within Acanthaceae, the emergence of *Sanchezia* from *Suessenguthia* by the reduction of flower organs is logically consistent. *Sanchezia* here is provisionally assumed to be monophyletic on the basis of this synapomorphy and the monophyly of the three species studied in the AFLP analysis.

As a result of *Sanchezia* being nested within *Suessenguthia*, the latter genus becomes a paraphyletic unit. Strictly speaking, *Suessenguthia* therefore can no longer be maintained as a taxon. *Suessenguthia*, *Sanchezia*, and *Trichosanchezia*, a monotypic genus with four fertile but unappendaged stamens, together may form a monophyletic group, *Sanchezia* s. l., possibly supported by the synapomorphy of the flowers concentrated to thyrsoid heads surrounded by showy bracts, versus solitary flowers in *Bravaisia* and *Trichanthera*. However, we refrain from changing the taxonomic status of either *Suessenguthia* or *Sanchezia* until the related genera, particularly four-stamened *Trichosanchezia*, and more species of *Sanchezia* have been included in a phylogenetic analysis. Any taxonomic changes made on the basis of our current understanding certainly would be preliminary and perhaps misleading.

Speciation modes within *Suessenguthia*

The AFLP analysis in combination with the present distribution of the species suggests several events of allopatric/parapatric speciation (Fig. 5). Perhaps the

Table 4. Some important characteristics of flowers and inflorescences of the species of *Suessenguthia*; *Sanchezia skutchii* included for comparison

Taxon	Habitat	Pollination syndrome	Inflorescence	Floral color	Position of anthers	Corolla lobes	Nr. of fertile stamens
Suessenguthia multisetosa	Mostly Chiquitania dry forests	Melittophilous[a]	Regularly thyrsoid	Pale pink	Included	Large, spreading forwards	4
Suessenguthia wenzelii	Along streams in lowland rainforest[c]	Ornithophilous[b]	Thyrsoid	Pink	Usually exserted	Large, slightly curved backwards	4
Suessenguthia vargasii	Lowland rainforest	Ornithophilous[b]	Regularly thyrsoid	Pink	Exserted	Large, curved backwards	4
Suessenguthia barthleniana	Along streams in the Yungas	Unknown	Apically a monochasium	Pale pink	Included	Large, curved backwards	4
Suessenguthia trochilophila	Along streams in lowland rainforest	Ornithophilous[a]	Apically a monochasium	Pink	Usually exserted	Large, spreading at right angles to curved backwards	4
Suessenguthia koesslerí	Along streams in lowland rainforest	Ornithophilous[a]	Apically a monochasium	Red	Exserted	Reduced, curved backwards	4
Sanchezia skutchii	Lowland rainforest	Ornithophilous[b]	Apically a monochasium	Red	Exserted	Reduced, curved backwards	2

[a]Pollinator observed by collectors.
[b]Syndrome inferred from floral characteristics.
[c]Species known from two populations only.

most convincing case is presented by *S. koessleri* which apparently evolved by the divergence of geographically marginal populations of *S. trochilophila*. While present range certainly does not have to coincide with range at time of speciation, the close phylogenetic relationship (as seen in the AFLP analysis) and probably quite recent speciation events (as evidenced by the almost identical ITS sequence data) strongly suggest that the current situation reflects the one at the time of speciation. The close geographical proximity of these species makes it difficult to decide whether speciation took place parapatrically (sensu Grant 1981; allopatric speciation type II sensu Mayr 1963) or allopatrically (type I of Mayr 1963). Further cases of presumed allopatric or parapatric speciation are presented by *S. barthleniana* (whose phylogenetic position is too ambiguous to draw firm conclusions) and by the variety *hirsuta* of *S. vargasii*. This long-haired form is known only from a small, marginal population at the edge of the distribution of *S. vargasii*.

For the origin of *S. wenzelii*, several possibilities are conceivable. The species is morphologically and geographically intermediate between *S. multisetosa* and *S. trochilophila*, combining the bract characteristics of the former with the flower morphology and floral color (and therefore the presumed pollinator) of the latter. The type population is located in the Bolivian Yungas at the northern margin of the local distribution of *S. multisetosa*. *S. trochilophila*, though it has not been found in the same area, occurs in the adjacent Amazonian lowlands and lower Andean foothills, from where it could have migrated to the east along river valleys. Schmidt-Lebuhn (2003) postulated a hybrid origin of *S. wenzelii* on the basis of this morphological and geographical intermediacy. The results of the PCO presented here indicate a very close relationship of *S. wenzelii* to *S. multisetosa*, making this concept less convincing. It thus seems more probable that *S. wenzelii* speciated allopatrically or parapatrically from marginal populations of *S. multisetosa*, but it is also possible that introgression played a role in the origin of this interesting species.

S. trochilophila is by far the morphologically most variable species of the genus, and the three populations sampled for molecular data were placed in two different branches in the AFLP tree (Fig. 4), making it paraphyletic with regard to all of *Sanchezia*. This is mirrored by the dispersion of the accessions in the PCO (Fig. 3), especially in contrast to the clustered position of the clearly distinguishable species of *Sanchezia*. It is thus likely that *S. trochilophila*, as presently construed, includes a number of closely related taxa whose taxonomic delimitation and status, and evolution, can be elucidated only with additional material. While constant in indument, inflorescence structure, bract and bractlet shape, and corolla characteristics, the species shows considerable variability in growth height, leaf margin configuration, and size of the bracts. Presently, *S. trochilophila* is known from only 29 collections from about 13 widely scattered localities, and it is therefore impossible to adequately evaluate the morphological variation seen among the collections.

Overall, the predominance of allopatric/parapatric speciation events found here corresponds well with studies of montane species in the Andes (e.g. Simpson 1983; Kessler 1995) and the Alps (e.g. Hungerer and Kadereit 1998; Zhang et al. 2001; Comes and Kadereit 2003).

Character evolution in *Suessenguthia*

Vegetatively, all species of *Suessenguthia* and *Sanchezia* are fairly similar. The main differences between species are found in the arrangement of the inflorescences, flower and bract morphology and coloration, and pollination type. All these characters are closely correlated according to the phylogenetic hypotheses developed here.

The phylogenetically basal species recovered in our analyses, *S. multisetosa*, has an inflorescence of the heads arranged in regular thyrsus (Fig. 1A), so that many flowers open more or less simultaneously. The flowers are pale pinkish with broadly expanded corolla lobes and an infundibiliform corolla tube, and the stamens are included in the corolla (Fig. 1C). This morphology and field observations all show that this species is insect-pollinated, specifically by large bees. *S. wenzelii* is very similar, differing mainly in its tubular, strongly pink corolla and usually exserted stamens. *S. vargasii*, the next basal species in the phylogenetic analyses, has a similar thyrsoid inflorescence; the flowers are again tubular and of a strong pink coloration. They clearly are pollinated by hummingbirds. All other species of *Suessenguthia*, and most species of *Sanchezia*, have their heads arranged in inflorescences mainly consisting of monochasia (Fig. 1B), thus opening the flowers consecutively (in one species of *Sanchezia* the heads are arranged monopodially instead of sympodially, but this is a rare exception). This arrangement enables the plants to open a few flowers in each inflorescence sequentially over a lengthy time period. This presumably is an adaptation to pollination by hummingbirds, which require a steady supply of nectar and are unable to efficiently use large amounts of nectar produced over a short-time period, as in bee-pollinated *S. multisetosa* (Bawa 1990; Endress 1994). Within the hummingbird-pollinated species of *Suessenguthia*, the species with the most highly derived corolla is *S. koessleri*, carrying narrow, bright red flowers with small, recurved corolla lobes. This corolla shape and a very intense color (usually yellow, orange, red) are also

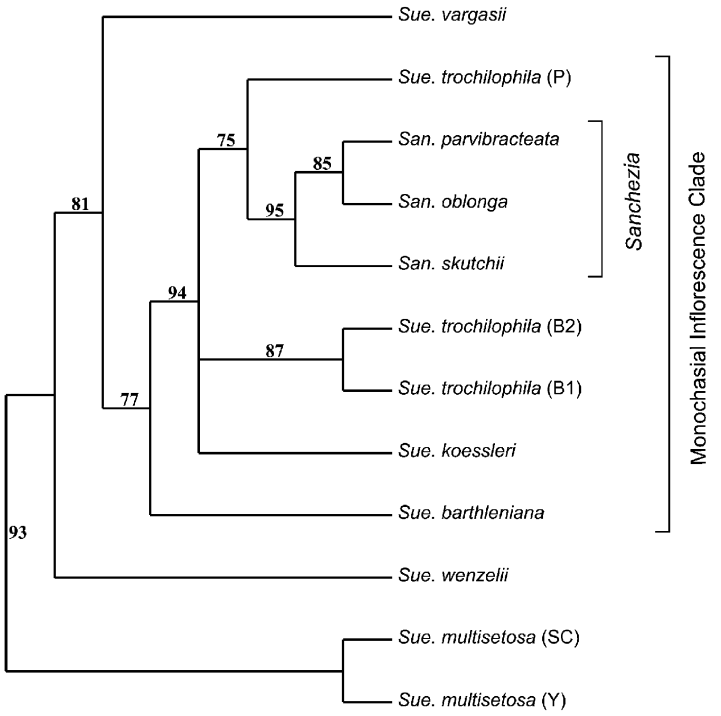

Fig. 4. Bootstrap consensus tree of the minimum-distance analysis based on AFLP data. The tree is rooted with *S. multisetosa* as outgroup to facilitate comparison with the tree based on morphological data (Fig. 2). Bootstrap values are given at the branches. B1 = Tumupasa, Bolivia; B2 = Carmen Florida, Bolivia; P = Pilcopata, Peru; SC = Chiquitanía dry forests, Bolivia; Y = Bolivian Yungas.

typical of *Sanchezia* (Fig. 1E), which additionally often has brightly colored bracts subtending the flowers. These showy inflorescences have made some species of *Sanchezia* popular in cultivation. In *Suessenguthia*, the bracts are normally tainted reddish at the apex or in the upper half, but remain green or whitish in the lower part.

Biogeography and evolution of *Suessenguthia* and *Sanchezia*

Summing up all of the above considerations, we can draw an evolutionary and biogeographical scenario for *Suessenguthia* and *Sanchezia*. Overall, the geographic distribution of the species of *Suessenguthia* shows a gradient from fairly dry, seasonal habitats in central Bolivia to more humid and less seasonal habitats in northern Bolivia, southern Peru, and adjacent Brazil (Fig. 5). Most species of *Sanchezia* are found in the even more humid and less seasonal region extending from central Peru to Ecuador. This climatic gradient corresponds to the morphological trend from insect-pollinated *S. multisetosa* to well-adapted hummingbird-pollinated species such as *S. koessleri* and all species of *Sanchezia*. This pollinator shift presumably reveals adaptations to the regionally most common and/or suitable pollinators. Hummingbirds are fairly uncommon in drought-deciduous forests because of the seasonal shortage of nectar (Stiles 1981; Arizmendi and Ornelas 1990). In contrast, insects readily survive nectar-free periods and can reappear en masse during the flowering season. Correspondingly, insect pollination is more common in dry-forest regions (Gentry 1995; Kessler and Krömer 2000). In humid regions, pollination by birds appears to be advantageous, and many plant groups shift toward this pollination type in evergreen tropical forest habitats (Gottsberger 1986). This shift is plainly evident in *Suessenguthia*. Field observations show that populations of *Suessenguthia* and *Sanchezia* usually are small and scattered, a situation requiring efficient, far-ranging pollinators such as hummingbirds. Notable exceptions are *S. multisetosa* and the wide-ranging group of *Sanchezia oblonga* R. & P. and its allies.

Intriguingly, in our study group hummingbird pollination correlates with much higher speciation rates than insect pollination. Even though *S. multisetosa* is the presumed sister to all other species of *Suessenguthia* and *Sanchezia*, it has not speciated. Thus, we are faced with two equally old lineages, one with a single insect-pollinated species and the other with >60 hummingbird-pollinated species. A parallel situation, with higher species richness among animal-dispersed fleshy-fruited plants than among their dry-fruited sister groups, has been documented by Smith (2001). This was interpreted as evidence that key evolutionary innovations enabling

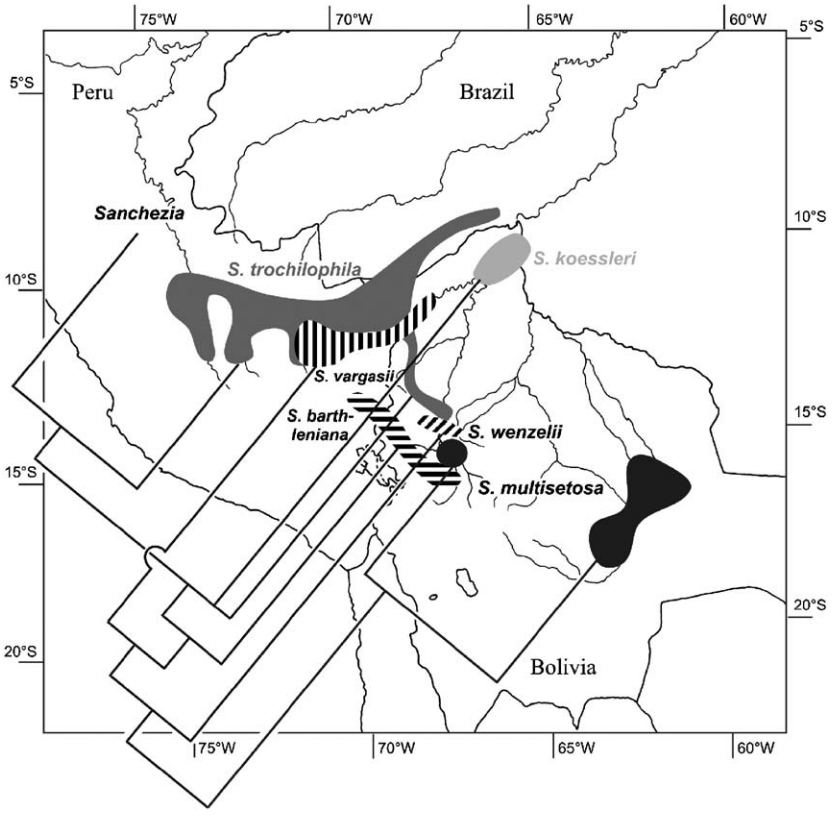

Fig. 5. Phylogenetic hypothesis based on AFLP data, correlated with the currently known areas of distribution of the different species of *Suessenguthia*, presuming *S. multisetosa* to be the most basal species. The tree from Fig. 4 has been simplified here by uniting *Sanchezia* and Bolivian *S. trochilophila* to respective single branches. Note the general tendency of migration from the seasonal, dry-forest habitats of eastern Bolivia to increasingly tropical, non-seasonal habitats to the northwest.

plants to utilize previously unexploited ecological niches, in this case fleshy fruits permitting animal dispersal, promote speciation (see also Bremer and Eriksson 1992; Futuyma 1998). This hypothesis certainly could be applied to the situation in *Suessenguthia* and *Sanchezia* as well. However, it is also conceivable that animal pollination and dispersal influence the genetic population structure of the plant species in some way that promotes speciation, for example by reducing gene flow between populations. Finally, it is possible that the high species richness of hummingbird-pollinated species of *Suessenguthia* and *Sanchezia* represents only a spurious correlation, and that speciation rates in these genera are determined by their biogeographical settings. For example, *S. barthleniana* and many species of *Sanchezia* are restricted to isolated valleys on the lower eastern Andean slopes. Their distributions might have been influenced by successive climatic cycles, leading to population expansions and fragmentations. More detailed studies of the population structure of closely related bird- and insect-pollinated plant species are needed to evaluate the contribution of pollination ecology to the speciation rates of tropical plants.

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Appendix A. Alphabetical list of taxa included in the molecular analyses, with voucher specimens and GenBank accession numbers

Ruellia puri (Nees) Mart. ex Jackson. Origin: Bolivia, Dep. Santa Cruz, Prov. Velasco; voucher: *Schmidt-Lebuhn 18* (GOET, LPB, US); use: ITS; GenBank No. AY530731.

Sanchezia oblonga R. & P. Origin: Peru, Dep. Cuzco, Pilcopata; voucher: *Schmidt-Lebuhn 33* (GOET, US); use: AFLP.

Sanchezia parvibracteata Sprague & Hutchinson. Origin: cultivated at Botanical Garden of the University of Göttingen; voucher: *Schmidt-Lebuhn 249* (GOET); use: AFLP, ITS; GenBank No. AY530733.

Sanchezia skutchii Leonard & Smith. Origin: cultivated at Botanical Garden of the University of Göttingen; voucher: *Schmidt-Lebuhn 95* (GOET); use: AFLP, ITS; GenBank No. AY530732.

Suessenguthia barthleniana Schmidt-Lebuhn. Origin: Bolivia, Dep. La Paz, Yolosa; voucher: *Schmidt-Lebuhn 30* (GOET); use: AFLP.

Suessenguthia barthleniana Schmidt-Lebuhn. Origin: Bolivia, Dep. La Paz, Yolosa; voucher: *Schmidt-Lebuhn 31* (GOET); use: AFLP, ITS; GenBank No. AY530737.

Suessenguthia koessleri Schmidt-Lebuhn. Origin: Bolivia, Dep. Pando, Agua Dulce; voucher: *Schmidt-Lebuhn 50* (GOET, LPB, US); use: AFLP.

Suessenguthia koessleri Schmidt-Lebuhn. Origin: Bolivia, Dep. Pando, near Riberalta; voucher: *Schmidt-Lebuhn 55* (GOET, LPB, US); use: AFLP.

Suessenguthia koessleri Schmidt-Lebuhn. Origin: Bolivia, Dep. Pando, near Riberalta; voucher: *Schmidt-Lebuhn 56* (GOET); use: AFLP, ITS; GenBank No. AY530736.

Suessenguthia multisetosa (Rusby) Wassh. & Wood. Origin: Bolivia, Dep. Santa Cruz, Prov. Velasco; voucher: *Schmidt-Lebuhn 24* (GOET, LPB, US); use: AFLP, ITS; GenBank No. AY530740.

Suessenguthia multisetosa (Rusby) Wassh. & Wood. Origin: Bolivia, Dep. La Paz, Caranavi; voucher: *Schmidt-Lebuhn 67* (GOET, LPB, US); use: ITS; GenBank No. AY530739.

Suessenguthia multisetosa (Rusby) Wassh. & Wood. Origin: Bolivia, Dep. La Paz, Caranavi; voucher: *Schmidt-Lebuhn 68* (GOET); use: AFLP.

Suessenguthia trochilophila Merxmüller. Origin: Peru, Dep. Cuzco, Pilcopata; voucher: *Schmidt-Lebuhn 37* (GOET, US); use: AFLP, ITS; GenBank No. AY530734.

Suessenguthia trochilophila Merxmüller. Origin: Bolivia, Dep. La Paz, Tumupasa; voucher: *Schmidt-Lebuhn 46* (GOET, LPB, US); use: AFLP.

Suessenguthia trochilophila Merxmüller. Origin: Bolivia, Dep. La Paz, Tumupasa; voucher: *Schmidt-Lebuhn 47* (GOET); use: AFLP, ITS; GenBank No. AY530735.

Suessenguthia trochilophila Merxmüller. Origin: Bolivia, Dep. Beni, Carmen Florida; voucher: *Schmidt-Lebuhn 61* (GOET, LPB, US); use: AFLP.

Suessenguthia vargasii Wasshausen var. *vargasii*. Origin: Peru, Dep. Cuzco, Pilcopata; voucher: *Schmidt-Lebuhn 35* (GOET, US); use: AFLP, ITS; GenBank No. AY530741.

Suessenguthia wenzelii Schmidt-Lebuhn. Origin: Bolivia, Dep. Beni, Puente Rio Quiquibey; voucher: *Schmidt-Lebuhn 64* (GOET, LPB); use: AFLP, ITS; GenBank No. AY530738.

Suessenguthia wenzelii Schmidt-Lebuhn. Origin: Bolivia, Dep. Beni, Puente Rio Quiquibey; voucher: *Schmidt-Lebuhn 66* (GOET); use: AFLP.

References

Arizmendi, M.C., Ornelas, J.F., 1990. Hummingbirds and their floral resources in a tropical dry forest in Mexico. Biotropica 22, 172–180.

Bawa, K.S., 1990. Plant–pollinator interactions in tropical rain forests. Annu. Rev. Ecol. Syst. 21, 399–422.

Beck, A., Friedl, T., Rambold, G., 1998. Selectivity of photobiont choice in a defined lichen community: inferences from cultural and molecular studies. New Phytol. 139, 709–720.

Bremer, B., Eriksson, O., 1992. Evolution of fruit characters and dispersal modes in the tropical family Rubiaceae. Biol. J. Linn. Soc. 47, 79–95.

Comes, H.P., Kadereit, J.W., 2003. Spatial and temporal patterns in the evolution of the flora of the European Alpine System. Taxon 52, 451–462.

Daniel, T.F., 1988. A systematic study of *Bravaisia* DC. (Acanthaceae). Proc. Calif. Acad. Sci. 45, 111–132.

Endress, P.K., 1994. Diversity and Evolutionary Biology of Tropical Flowers. Cambridge University Press, Cambridge, UK.

Farris, J.S., 1969. A successive approximations approach to character weighting. Syst. Zool. 18, 374–385.

Farris, J.S., 1988. Hennig86, Version 1.5 Manual. Port Jefferson Station, New York.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Friedl, T., 1996. Evolution of the polyphyletic genus *Pleurastrum* (Chlorophyta): inferences from nuclear-encoded ribosomal DNA sequences and motile cell ultrastructure. Phycologia 35, 456–469.

Futuyma, D.J., 1998. Evolutionary Biology. Sinauer, Sunderland, MA.

Gentry, A.H., 1995. Diversity and floristic composition of neotropical dry forests. In: Bullock, S.H., Mooney, H.A., Medina, E. (Eds.), Seasonally Dry Tropical Forests. Cambridge University Press, Cambridge, UK, pp. 146–194.

Gottsberger, G., 1986. Some pollination strategies in neotropical savannas and forests. Plant Syst. Evol. 152, 29–45.

Grant, V., 1981. Plant Speciation, second edition. Columbia University Press, New York.

von Hagen, K.B., Kadereit, J.W., 2001. The phylogeny of *Gentianella* (Gentianaceae) and its colonization of the southern hemisphere as revealed by nuclear and chloroplast DNA sequence variation. Org. Divers. Evol. 1, 61–79.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Hellwig, F.H., Nolte, M., Ochsmann, J., Wissemann, V., 1999. Rapid isolation of total cell DNA from milligram plant tissue. Haussknechtia 7, 29–34.

Hodkinson, T.R., Renvoize, S.A., Ni Chonghaile, G., Stapleton, C.M.A., Chase, M.W., 2000. A comparison of ITS nuclear rDNA sequence data and AFLP markers for phylogenetic studies in *Phyllostachys* (Bambusoideae, Poaceae). J. Plant Res. 113, 259–269.

Holmgren, P.K., Holmgren, N.H., Barnett, L.C., 1990. , eighth edition. Index Herbariorum, vol. 120. Regnum Vegetabile, New York.

Hungerer, K.B., Kadereit, J.W., 1998. The phylogeny and biogeography of *Gentiana* L. sect. *Ciminalis* (Adans.) Dumort.: a historical interpretation of distributional ranges in the European high mountains. Perspect. Plant Ecol. Evol. Syst. 1, 121–135.

Kessler, M., 1995. Polylepis-Wälder Boliviens: Taxa, Ökologie, Verbreitung und Geschichte. J. Cramer, Berlin/Stuttgart.

Kessler, M., Krömer, T., 2000. Patterns and ecological correlates of pollination modes among bromeliad communities of Andean forests in Bolivia. Plant Biol. 2, 659–669.

Leonard, E.C., Smith, L.B., 1964. *Sanchezia* and related American Acanthaceae. Rhodora 66, 313–343.

Manktelow, M., McDade, L.A., Oxelman, B., Furness, C.A., Balkwill, M.-J., 2001. The enigmatic tribe Whitfieldieae (Acanthaceae): delimitation and phylogenetic relationships based on molecular and morphological data. Syst. Bot. 26, 104–119.

Mannschreck, B., Fink, T., Melzer, A., 2002. Biosystematics of selected *Chara* species (Charophyta) using amplified fragment length polymorphism. Phycologia 41, 657–666.

Mayr, E., 1963. Animal Speciation and Evolution. Harvard University Press, Cambridge, MA.

McDade, L.A., Moody, M., 1999. Phylogenetic relationships among Acanthaceae: evidence from noncoding trnL–trnF chloroplast DNA sequences. Am. J. Bot. 86, 70–80.

McDade, L.A., Masta, S.E., Moody, M.L., Waters, E., 2000. Phylogenetic relationships among Acanthaceae: evidence from two genomes. Syst. Bot. 25, 106–121.

Merxmüller, H., 1953. Eine neue Gattung der Acanthaceen. Mitt. Bot. Staatssammlung München 6, 175–181.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858.

Nei, M., Li, W.H., 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76, 5269–5273.

Schmidt-Lebuhn, A.N., 2003. A taxonomic revision of the genus *Suessenguthia* (Acanthaceae). Candollea 58, 101–128.

Simpson, B.B., 1983. A historical biogeography of the high Andean flora. Rev. Chil. Hist. Nat. 56, 109–122.

Smith, J.F., 2001. High species diversity in fleshy-fruited tropical understory plants. Am. Nat. 157, 646–653.

Stiles, F.G., 1981. Geographical aspects of bird-flower coevolution, with particular reference to Central America. Ann. Mo. Bot. Gard. 68, 323–351.

Swofford, D.L., 1997. PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods), version 4.0. Sinauer, Sunderland, MA.

SYSTAT, 1997. SYSTAT for Windows, Statistics, Version 7.0. SPSS Inc., Chicago.

Van de Peer, Y., De Wachter, R., 1994. TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comput. Appl. Biosci. 10, 569–570.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Freuters, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M., 1995. AFLP, a new technique for DNA fingerprinting. Nucleic Acids Res. 23, 4407–4414.

Wasshausen, D.C., 1970. A synopsis of the genus *Suessenguthia* (Acanthaceae). Rhodora 72, 119–125.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innes, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: a Guide to Methods and Applications. Academic Press, New York, pp. 315–322.

Young, K.R., Ulloa Ulloa, C., Luteyn, J.L., Knapp, S., 2002. Plant evolution and endemism in Andean South America: an introduction. Bot. Rev. 68, 4–21.

Zhang, L.-B., Comes, H.P., Kadereit, J.W., 2001. Phylogeny and quaternary history of the European montane/alpine endemic *Soldanella* (Primulaceae) based on ITS and AFLP variation. Am. J. Bot. 88, 2331–2345.