Lindqvist & Albert 1999

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Kew Bull. 54: 363-377.

REFNO: 3088

KEYWORDS:

Africa, Cladistics, Conservation, Kenya, Molecular Systematics, Phylogeny, Saintpaulia, Streptocarpus, Tanzania

With compliments
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KEW BULLETIN 54: 363 – 377 (1999)

Phylogeny and conservation of African violets (*Saintpaulia*: *Gesneriaceae*): new findings based on nuclear ribosomal 5S non-transcribed spacer sequences

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Summary. The phylogeny, biogeography, and conservation prioritization of African violet taxa (*Saintpaulia*: *Gesneriaceae*) in East Africa was investigated using parsimony analysis of nucleotide sequences from the 5S nuclear ribosomal DNA (nrDNA) non-transcribed spacer (5S-NTS). Although in substantial agreement with a previous phylogenetic analysis of nrDNA internal transcribed spacer (ITS) sequences, the present study of the faster evolving 5S-NTS locus identified two new, major lineages, the Nguru Mountains and Usambara Mountains/lowland clades. The genetic diversity within the basal-most *Saintpaulia* clade, present in the Nguru, Uluguru, and Ukaguru Mountains, was underscored by addition of an Uluguru Mountains collection of *Saintpaulia pusilla*. Previous studies based on ITS included only a single individual of *Saintpaulia goetzeana*, here represented by two disjunct populations. These accessions were genetically divergent and separated from *Saintpaulia pusilla* by long branches. However, no support could be found for an earlier suggestion that the Uluguru Mountains represent the ancestral area for the genus. Indeed, the Nguru Mountains hold two to three of the four major *Saintpaulia* clades identified, and our conclusion is that this region should be given the highest priority for conservation of African violet genetic and morphological diversity. An additional prioritization suggestion derives from the finding that *Saintpaulia* taxa from Kenyan lowlands form a discrete subclade within the poorly-resolved Usambaras/lowland clade, providing at least one clearly recognizable, geographically distinct, and reproductively isolated lineage of what may have recently constituted only metapopulational variation.

INTRODUCTION

African violets, *Saintpaulia* H. Wendl. (*Gesneriaceae*), comprise 24 recognised specific and subspecific taxa endemic to the Eastern Arc Mountains and lowlands of Kenya and Tanzania. Despite this apparent diversity, *Saintpaulia* species are remarkably uniform morphologically. Indeed, most species and varieties (19 out of the 24) were described rather recently (Burtt 1958, 1964) based largely on vegetative characteristics, most notably leaf indumentum. Floral characters have been considered of limited taxonomic use since Burtt (1958), and plant habit (rosulate versus caulescent) only defines major groups within the genus.

Recent work on *Saintpaulia* phylogeny based on nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) sequences (Möller & Cronk 1997a, b; cf. Soltis & Soltis 1998) has corroborated the morphology-based view of *Saintpaulia* invariance by showing that most species are poorly distinguished at the molecular level. Significantly, however, the two basal clades in *Saintpaulia*, each comprising

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Accepted for publication April 1999.

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single species, were sequence-divergent compared to the remaining taxa (Möller & Cronk 1997b): (1) *Saintpaulia goetzeana*, which has slightly tubular (campanulate), bicoloured flowers, was found to represent the basal clade in the genus, followed by (2) *S. teitensis*, a species with more typical *Saintpaulia* floral morphology, i.e., flat and concolourous (Burtt 1958). Moreover, *Saintpaulia*, with its relatively flat flowers and untwisted fruits, was found to be nested inside *Streptocarpus* Lindl., which has tubular flowers and twisted fruits (Möller & Cronk 1997a).

These findings based on ITS sequences were also significant biogeographically. *Saintpaulia goetzeana* is found in the Uluguru Mountains of Tanzania, where many species of its near relatives in *Streptocarpus* subg. *Streptocarpella* also grow. This fact led earlier authors (Möller & Cronk 1997b) to suggest a possible origin for *Saintpaulia* in the Ulugurus, based largely on the presumed endemicity and demonstrated basal position of *Saintpaulia goetzeana.* However, species of *Streptocarpus* subg. *Streptocarpella* are widespread in the Eastern Arc Mountains (Möller & Cronk 1997b: 1828), *Saintpaulia teitensis* is not from the Ulugurus but from the Taita Hills of Kenya, and no other Uluguru taxa were available for Möller & Cronk (1997b) to solidify their ancestral area hypothesis.

Left undefined by previous molecular systematic work is the phylogenetic status of the genetically ill-defined *Saintpaulia* taxa that inhabit other, geographically delimited areas such as the Nguru and Usambara Mountains of Tanzania, which lie in between the Ulugurus and Taita Hills. Additionally, the relationships of the many lowland taxa to taxa from the Ngurus and Usambaras have gone unaddressed by the ITS sequence studies (Möller & Cronk 1997a, b).

Conservation of *Saintpaulia* taxa, most of which are endangered, has been considered a high priority (e.g., Clarke 1998; Eastwood *et al.* 1998). The ability to recognise *Saintpaulia* taxa accurately is therefore of crucial importance. With species delimitation still unclear on morphological grounds (B. L. Burtt, pers. comm., 1997) and ITS variation insufficient to resolve all but the most basal *Saintpaulia* clades, other markers for taxon identification must be sought. We have used variation in a faster-evolving nrDNA region, the non-transcribed spacer of the 5S array (5S-NTS; Cox *et al.* 1992; Kellogg & Appels 1995; Cronn *et al.* 1996), to highlight hitherto unknown aspects of *Saintpaulia* phylogeny among taxa previously poorly resolved. For this purpose, new collections of *Saintpaulia* were made in Kenya and Tanzania to supplement clonal material already available from botanic gardens. With these collections and 5S-NTS data, we have been able to describe four new patterns of phylogenetic and conservational importance: (1) Addition of a second Uluguru Mountains species, *Saintpaulia pusilla*, breaks up the long branch that previously led only to *S. goetzeana* (Möller & Cronk 1997b), underscoring the genetic diversity within the basal most *Saintpaulia* clade; (2) Two previously unidentified but well-supported sister clades were found, an Nguru Mountains clade, and an Usambara Mountains/lowland clade; (3) *Saintpaulia* taxa from Kenyan lowlands were found to form a discrete subclade within the Usambaras/lowland lineage, and populations from Kachororoni and Mwache, Kenya were together found to be discrete from the two known populations of *S. rupicola*; and (4) The Nguru Mountains harbour two to three of the four major clades of *Saintpaulia*, and thereby may hold the greatest genetic as well as morphological diversity in the genus.

MATERIALS AND METHODS

Plant material

Saintpaulia and *Streptocarpus* leaf specimens were obtained from four sources: cultivated clones grown at (1) Royal Botanic Gardens, Kew, (2) Royal Botanic Garden, Edinburgh, (3) National Museums of Kenya, Nairobi, and (4) new collections made in Kenya and Tanzania by C. Lindqvist and S. Simiyu during January and October – December, 1997 (Table 1). The 1997 field collections comprise 15 of the 24 accessions used. Although not all taxa of *Saintpaulia* are represented, the accessions chosen span the entire range of morphological and geographic variation in the genus. Included are collected specimens from one Uluguru Mountains population of *Saintpaulia pusilla* and two populations of *S. goetzeana*, two populations of the endangered lowland species *S. tongwensis* (see Clarke 1998), the two known populations of *S. rupicola*, and the two newly discovered but undescribed taxa from Kenya, *S.* 'Kachororoni' and *S.* 'Mwache' (both of which are nearly extinct; see Eastwood *et al.* 1998; C. Lindqvist, pers. obs.). Two individuals of *Saintpaulia orbicularis* were used, representing both field-collected and cultivated material. An accession of uncertain identification collected in the Nguru Mountains, *Saintpaulia* cf. *velutina*, was also included (see Eastwood *et al.* 1998: 52); *S. velutina*, which the plant resembles in its leaf indumentum, is otherwise known only from the Usambara Mountains. Another uncertainly identified accession, *Saintpaulia* cf. *nitida*, was included from a collection made in the coastal lowlands of Tanzania; *S. nitida*, which the new collection resembles in its short, appressed leaf hairs, is otherwise known only from the Nguru and Ukaguru Mountains (Burtt 1958; Pócs *et al.* 1990; Eastwood *et al.* 1998). One of the two *Saintpaulia ionantha* accessions included (obtained from the Royal Botanic Gardens, Kew) is from the Mwanihana Forest Reserve in the Uzungwa Mountains (Lovett *et al.* 1988), far to the South of the characteristic Usambaras/lowland range of the species (Clarke 1998; Eastwood *et al.* 1998). The single *Streptocarpus* species, selected based on the ITS study of Möller & Cronk (1997a), was included for outgroup orientation of the phylogenetic tree.

Molecular Methods

DNAs from 23 accessions of *Saintpaulia* and one accession of *Streptocarpus* (Table 1) were isolated from leaves dried in silica gel (Chase & Hills 1991) using c. 0.5 cm^2 of tissue. DNA extraction, PCR amplification, cycle-sequencing reactions, and DNA sequencing were performed exactly as described by Struwe *et al.* (1998) with minor, sequence-specific exceptions. 5S-NTS regions were amplified using primers PI and PII as described by Cox *et al.* (1992) and the following PCR program: hold 94°C, 2 min; 94°C, 1 min; 60°C, 1 min; 72°C, 1 min; repeat 27 cycles; extend 72°C, 4 min; hold 4°C. The same primers were used for cycle-sequencing reactions. Sequences were edited using the software program Sequencher 3.0 (GeneCodes). Sequences reported in this paper are accessioned in the GenBank database under the numbers AF108727–AF108750; voucher information is reported in Table 1.

TABLE 1. Voucher and locality information and GenBank accession numbers for the *Saintpaulia* and *Streptocarpus* 5S-NTS sequences presented in this paper.

Taxon	Voucher/Accession number[a]	Locality information	GenBank accession
Saintpaulia brevipilosa B. L. Burtt	1995-503, K	Tanzania: Nguru Mts, Kanga Forest Reserve	AF108733
Saintpaulia confusa B. L. Burtt	1974-2873, K	Tanzania: Usambara Mts	AF108728
Saintpaulia goetzeana Engl.	(a) Lupanga.1	Tanzania: N Uluguru Forest Reserve, Mbete village, below Lupanga Peak, alt. 1800 m, 16 Nov. 1997	AF108734
	(b) Kilangala.1	Tanzania: S Uluguru Forest Reserve, NE slope of Kilangala.above Bunduki village, alt. 1850 m, 18 Nov. 1997	AF108735
Saintpaulia grotei Engl.	1995-511, K	Tanzania: Usambara Mts	AF108729
Saintpaulia ionantha H. Wendl.	(a) Amboni.I.2	Tanzania: Tanga, Amboni Caves, 22 Oct. 1997	AF108736
	(b) 1983-8132, K	Tanzania: Ulang Distr., Uzungwa Mts, Mwanihana Forest Reserve, Sanje Falls	AF108732
Saintpaulia 'Kachororoni'	Lindqvist 97003, C	Kenya: Kaloleni, Kachororoni Gorge, Jan. 1997	AF108743
Saintpaulia magungensis E. Roberts	Magunga.4	Tanzania: E Usambara Mts, Magunga Forest near Ubiri village, alt. 730 m, 8 Nov. 1997	AF108737
Saintpaulia magungensis E. Roberts var. *minima* B. L. Burtt	1963-42311, K	Tanzania: E Usambara Mts, Amani, Mavoera Estate	AF108738
Saintpaulia 'Mwache'	Pearce 543-94-530.1, National Museums of Kenya	Kenya: Kaloleni, Mwache Forest Reserve	AF108744
Saintpaulia cf. *nitida* B. L. Burtt	Kwamtili.4	Tanzania: Tanga, Kwamgumi Forest Reserve, Kwamtili Estate, alt. 250 m, 24 Oct. 1997	AF108731
Saintpaulia orbicularis B. L. Burtt	(a) 1987-1370, K	Tanzania: W Usambara Mts, Ambangulu Tea Estate	AF108739

	(b) Kwabulu.5	Tanzania: W Usambara Mts, Ambangulu-Kunga Road, Kwabulu, alt. 1100 m, 27 Oct. 1997	AF108740
Saintpaulia pusilla Engl.	Magari.1	Tanzania: N Uluguru Forest Reserve, Tangeni, NW side of Magari Peak, alt. 1500 m, 23 Nov. 1997	AF108741
Saintpaulia rupicola B. L. Burtt	(a) Lindqvist 97002.1, C	Kenya: Kaloleni, Chasimba, Jan. 1997	AF108742
	(b) Simiyu 169-96-1739, National Museums of Kenya	Kenya: Kaloleni, Mwarakaya	AF108745
Saintpaulia shumensis B. L. Burtt	Shume.1	Tanzania: W Usambara Mts, Shume Hill, World's View, alt. 1820 m, 31 Oct. 1997	AF108746
Saintpaulia teitensis B. L. Burtt	Lindqvist 97001, C	Kenya: Mbololo, Taita Hills, Jan. 1997	AF108747
Saintpaulia tongwensis B. L. Burtt	(a) Tongwe.9	Tanzania: Tanga, Tongwe Mt., NW side, Tongwe Forest Reserve, alt. 470 m, 6 Nov. 1997	AF108730
	(b) Pangani Falls.1	Tanzania: Tanga, Pangani Falls, by the Pangani Falls tunnel, 21 Oct. 1997	AF108748
Saintpaulia velutina B. L. Burtt	Mgambo.4	Tanzania: E Usambara Mts, Mgambo village, Mgambo-Handei village catchment forest, alt. 1040 m, 7 Nov. 1997	AF108749
Saintpaulia cf. *velutina* B. L. Burtt	Dibohelo.1	Tanzania: S Nguru Forest Reserve, Mhonda Mission, Kombola village, Dibohelo R., alt. 1200 m, 27 Nov. 1997	AF108750
Streptocarpus caulescens Vatke	1971-1199, E	Tanzania: Uluguru Mts	AF108727

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[a] Names followed by numbers (e.g., Amboni.I.2) indicate particular *Saintpaulia* populations (here, population I from Amboni Caves) and the nth individual (here, individual #2) sampled from these populations. All voucher specimens thus denoted were collected by C. Lindqvist and S. Simiyu and are currently held at the East African Herbarium, National Museums of Kenya, Nairobi, for eventual distribution to DSM, EA, K, and NHT.

Phylogenetic Analysis

Edited sequences were aligned using Sequencher 3.0 with hand adjustments. Inferred insertions/deletions (gaps) were coded as question marks and were not treated further. The data matrix (available at http://www.nybg.org/bsci/res/cullb/dna.html), containing 24 taxa and 378 characters (86 of which were informative), was analyzed under the nonadditive parsimony criterion (Fitch 1971) using PAUP 3.1.1 software (Swofford 1993) and its branch and bound algorithm. The matrix was subsequently analyzed with parsimony jackknifing to estimate the support for internal branches (Farris *et al.* 1996). This was accomplished using the Xac application (by J. S. Farris, unpubl.) with 1000 replicates, SPR branch swapping, and 5 random entry orders per replicate. With Xac, a c. 63% or higher jackknife frequency (63% corresponding to the complement of the character removal rate, e^{-1}), represents (with sampling error) support by the equivalent of one or more uncontradicted synapomorphy (Farris *et al.* 1996); values between c. 63% and ambiguity (i.e., 50%) should have some robustness to extra steps.

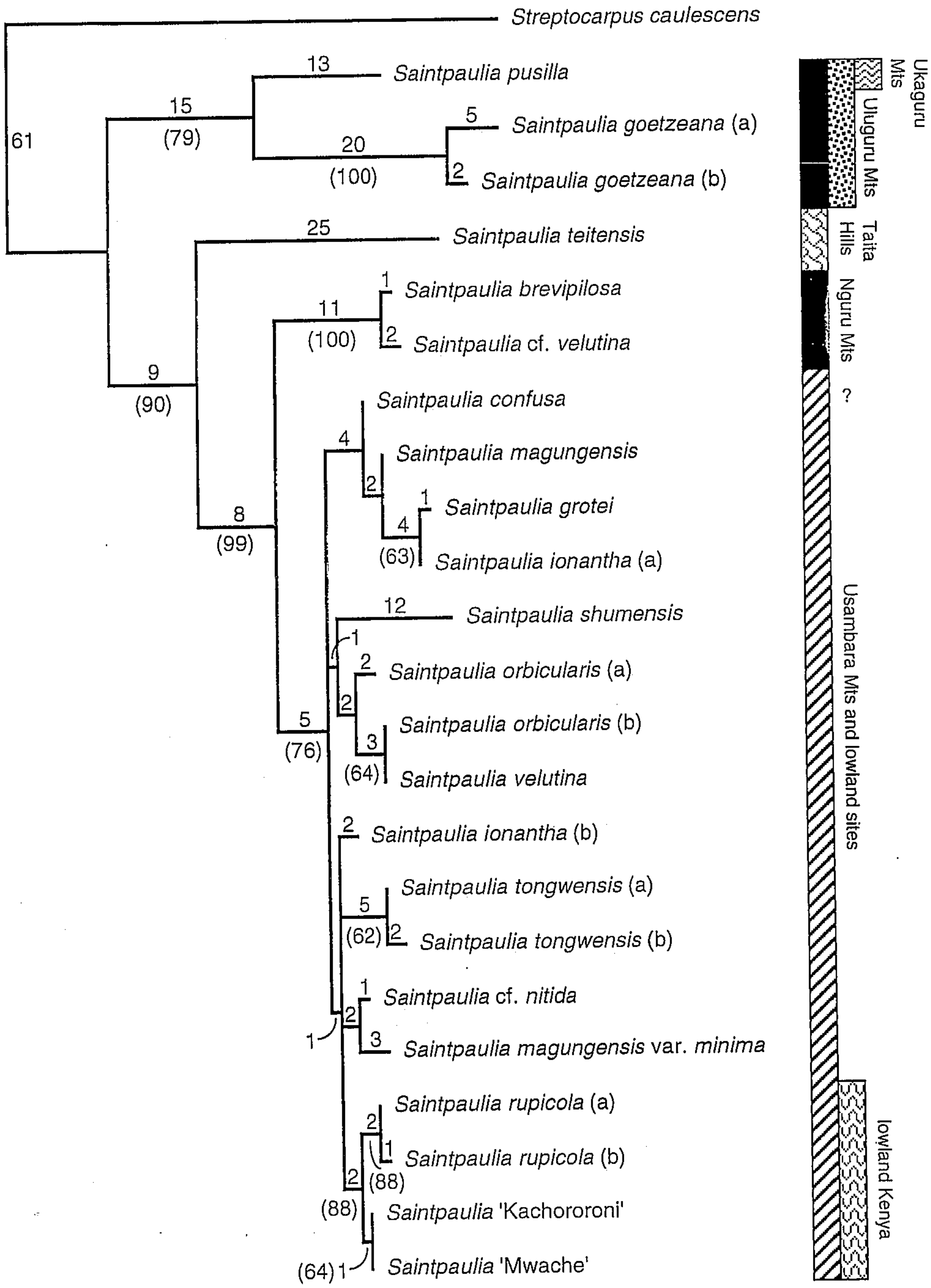
RESULTS AND DISCUSSION

The 5S-NTS sequences obtained from direct sequencing were for the most part monomorphic orthologues. Potentially polymorphic sites (e.g., as many as 7 – 8 in *Saintpaulia brevipilosa* and *S. grotei*, 1 – 4 in many Usambaras/lowland taxa, and 0 – 4 in different accessions of *S. goetzeana*; unpubl.) were observed for a number of accessions, but these appeared in a seemingly random (as opposed to a potentially phylogenetically informative) pattern relative to the cladogram obtained (see below). Alignment of the sequences was unambiguous (see http://www.nybg.org/bsci/res/cullb/dna.html), although inclusion of several insertions/deletions was required to accommodate the outgroup taxon, *Streptocarpus caulescens*, which had a longer 5S-NTS region than *Saintpaulia* (data not shown). Only one major gap, in *Saintpaulia pusilla*, was required to align the ingroup sequences.

Phylogeny and Branch Support

A single most parsimonious tree of *Saintpaulia* and outgroup 5S-NTS sequences was found (Fig. 1); this had a length of 230 steps, a consistency index (*C*) of 0.87, and a retention index (*R*) of 0.84 (Farris 1989). This tree was highly congruent in

FIG. 1. Phylogeny and biogeography of *Saintpaulia*. The single most-parsimonious tree found using 5S-NTS nucleotide sequences from the taxa listed in Table 1. *Saintpaulia goetzeana* and *S. pusilla* comprise the basal-most African violet clade, followed by *S. teitensis*, *S. brevipilosa* plus *S.* cf. *velutina*, and all remaining taxa. These four major clades, all strongly supported by parsimony jackknife analysis, are referred to in the text as the Uluguru Mountains, Taita Hills, Nguru Mountains, and Usambara Mountains/lowland clades, respectively. Although the Ulugurus clade is basal within *Saintpaulia*, consideration of the aggregate distributions of the taxa sampled (shown by the bars to the right of the tree) reveals that the Nguru Mountains (broken black bar) and Ukaguru Mountains are also represented by this lineage. The Ngurus and Usambaras/lowland clades are new findings of this study, and with *Saintpaulia goetzeana*, *S. pusilla*, and possibly *S. confusa* (indicated by the question mark) also native to the Ngurus, this mountain range probably contains the greatest genetic and morphological diversity of African violets. Along with a distinct Usambaras/lowland subclade of described and undescribed Kenyan taxa, saintpaulias from the Ngurus may merit the greatest priority for protection measures. *Streptocarpus caulescens* was included to orient the tree. Branch lengths are proportional to the numbers of ACCTRAN optimised changes (shown above branches). Parsimony jackknife values above 50% are shown in parentheses below branches.


Streptocarpus caulescens
61
13
Saintpaulia pusilla
15
(79)
5
Saintpaulia goetzeana (a)
20
(100)
2
Saintpaulia goetzeana (b)
25
Saintpaulia teitensis
1
Saintpaulia brevipilosa
11
(100)
2
Saintpaulia cf. velutina
9
(90)
Saintpaulia confusa
4
Saintpaulia magungensis
2
1
Saintpaulia grotei
4
(63)
Saintpaulia ionantha (a)
8
(99)
12
Saintpaulia shumensis
1
2
Saintpaulia orbicularis (a)
2
Saintpaulia orbicularis (b)
5
(76)
3
(64)
Saintpaulia velutina
2
Saintpaulia ionantha (b)
Saintpaulia tongwensis (a)
5
(62)
2
Saintpaulia tongwensis (b)
1
Saintpaulia cf. nitida
2
1
3
Saintpaulia magungensis var. minima
Saintpaulia rupicola (a)
2
1
Saintpaulia rupicola (b)
2
(88)
(88)
Saintpaulia 'Kachororoni'
(64)
1
Saintpaulia 'Mwache'
Ukaguru Mts
Uluguru Mts
Taita Hills
Nguru Mts
?
Usambara Mts and lowland sites
lowland Kenya

all major aspects with the previous phylogenetic results based on ITS sequences (Möller & Cronk 1997b) as well as with geographic distribution patterns. However, the ITS analysis provided considerably less topological resolution than 5S-NTS. Whereas the basal Ulugurus clade (so called following Möller & Cronk (1997b), but not the aggregate distributions of its member taxa) and Taita Hills clade were found with both ITS and 5S-NTS data, only 5S-NTS permitted the resolution of the Ngurus and Usambaras/lowland clades (Fig. 1). The Ulugurus and Taita Hills clades, supported by 79% and 90% of jackknife replicates (respectively), were as well supported by 5S-NTS as by ITS, for which the same branchings scored 78% and 100% in a bootstrap analysis (Möller & Cronk 1997b). The Ulugurus clade was also expanded in the present study to include *Saintpaulia pusilla* (79% of jackknife replicates) and two population representatives of *S. goetzeana* (100%). Not only were the Ngurus and Usambaras/lowland clades newly resolved by 5S-NTS, they were strongly supported by parsimony jackknife analysis; the Ngurus clade, comprised of *Saintpaulia brevipilosa* and *S.* cf. *velutina*, was resolved in 99% of jackknife replicates, and the Usambaras/lowland clade (all other non-basal taxa) was found in 76% of jackknife replicates. Branchings within the Usambaras/lowland clade (17 of the 24 taxa studied) were mostly poorly supported, with the exception of pairings of (1) *Saintpaulia grotei* with one *S. ionantha* accession (63% of jackknife replicates), (2) *S. velutina* with one *S. orbicularis* accession (64%), (3) two accessions of *S. tongwensis* (62%), and (4) two accessions of *S. rupicola* (88%) sister to *S.* 'Kachororoni' plus *S.* 'Mwache' (64%), the four of which form a well supported group (88%).

DNA Sequence Divergence

Optimised ACCTRAN branch lengths (Farris 1970; Swofford & Maddison 1987) on the most parsimonious 5S-NTS tree concur with the ITS findings (Möller & Cronk 1997b); the basal Ulugurus and Taita Hills clades are the most sequence divergent, the Usambaras/lowland clade is the least divergent, and the Ngurus clade is intermediate. In the ITS tree shown by Möller & Cronk (1997b: 1833), *Saintpaulia goetzeana* was found to differ from *S. teitensis* by 72 nucleotide changes, which itself differed from all other taxa by a maximum of 31 changes (to *S. orbicularis* var. *purpurea*); the maximum among the remaining saintpaulias was only 14 changes (between *S. brevipilosa* and *S. orbicularis* var. *purpurea*), and indeed 7 taxa were identical to each other. The Ngurus species sampled were a maximum of 14 nucleotide changes different from Usambaras/lowland taxa (again, between *S. brevipilosa* and *S. orbicularis* var. *purpurea*), from which they were not resolved as distinct. The corresponding maximum sequence divergence estimates for the 5S-NTS tree were 74 (*Saintpaulia goetzeana* to *S. teitensis*), 51 (*S. teitensis* to *S. shumensis*), 31 (*S.* cf. *velutina* to *S. shumensis*), and 31 (same as previous) nucleotide changes, respectively. With 5S-NTS, two taxon pairs show zero divergence among their members. This comparison indicates that the 5S-NTS DNA region evolves at least twice as fast as ITS in terminal clades of the phylogenetic tree (i.e., 31/14 changes), and c. 1.0 – 1.6 times faster than ITS in the basal clades (74/72 – 51/31 changes). These relative rate comparisons assume that ITS and 5S-NTS sequences have constant rates of nucleotide change for the taxa sampled and that particular pairwise path lengths are both comparable between ITS and 5S-NTS taxon samples and divergence time coincident.

The Uluguru Mountains Clade and Saintpaulia *Origins*

Möller & Cronk (1997b) suggested that African violets from the Uluguru Mountains of Tanzania be considered for higher conservation priority due to the extensive ITS sequence divergence of *Saintpaulia goetzeana.* Our analysis of 5S-NTS sequences confirms the genetic distinctness of *Saintpaulia goetzeana*, and indeed demonstrates considerable sequence divergence (7 nucleotide changes; Fig. 1) between two geographically disjunct *S. goetzeana* populations (Table 1). Other species with monophyletic population representatives (*Saintpaulia tongwensis* and *S. rupicola*; Fig. 1) varied by only 1 – 2 nucleotide changes.

Another species found in the Uluguru mountains is *Saintpaulia pusilla*, which is morphologically similar to *S. goetzeana* but differs mainly in smaller plant size, rosulate rather than caulescent habit, more campanulate (i.e., tubular) flowers, and shorter, fatter fruits (Burtt 1958; C. Lindqvist, pers. obs.). Of critical biogeographic importance (and partly overlooked by Möller & Cronk 1997b) is that *Saintpaulia pusilla* and *S. goetzeana* are relatively widespread; the former species is known also from the Ukaguru and Nguru Mountains (Pócs *et al.* 1990; *Mabberley* 1362, *Mabberley & Pócs* 1273, E!; *Pócs* 89224/E, *Pócs et al.* 87015/F, *Congdon* 205, K!), and the latter is also known from the Ngurus (Baatvik 1993; *Baatvik* [*Iversen*] 86830, 86831, UPS). Absent from cultivation (Eastwood *et al.* 1998), Ulugurus specimens of *Saintpaulia pusilla* were located by C. Lindqvist and S. Simiyu (Table 1) during an unsuccessful search for *S. inconspicua*, which itself may be extinct. Despite some confusion between *Saintpaulia pusilla* and *S. goetzeana* (Burtt 1958), our finding of a maximum 5S-NTS sequence divergence of 38 nucleotide changes between these species (as opposed to 31 changes between the entire Ngurus and Usambaras/lowland clades; see below) strongly supports their distinction as discrete taxa and highlights genetic diversity within the Ulugurus clade. Significant genetic variation within and between species is important for long-term clade survival (e.g., Huenneke 1991; Gemmill *et al.* 1998), and if allelic diversity can be assumed to correlate with sequence divergence (cf. Humphries *et al.* 1995), the basal clade of saintpaulias, now known to exist in the Ukaguru and Nguru Mountains in addition to the Ulugurus, may have the greatest future evolutionary potential in the genus. The Taita Hills clade is also relatively basal and sequence divergent, but no information exists about genetic structure within the only known population of its single representative, *Saintpaulia teitensis.*

Our 5S-NTS phylogenetic study added no support for an Uluguru Mountains origin for *Saintpaulia* (see Möller & Cronk 1997b). The Ulugurus are included in the basal branch of the tree, but since (1) *Saintpaulia goetzeana* and *S. pusilla* are also known from the Ngurus, (2) *S. pusilla* also occurs in the Ukagurus, and (3) *S. teitensis* is not known from any of these mountain ranges, the ancestral root state for *Saintpaulia* remains undefined following a nonadditive optimization of areas as character states (results not shown; Ronquist 1994; cf. Mickevitch 1981 and Bremer 1992). This finding identifies the Ulugurus, Ukagurus, Ngurus, and Taita Hills all as ancestral areas for *Saintpaulia*, not merely the Ulugurus alone.

The aggregate range of *S. pusilla* (and in part, *S. goetzeana*) implies that the Nguru, Ukaguru, and Uluguru Mountains may together form one major component of the ancestral area. By similar area optimization reasoning, Knox & Palmer's (1998: 125) phylogenetic analysis of giant lobelia accessions concurs in suggesting that the Ngurus and Ulugurus are both components of the ancestral range of *Lobelia* in the Eastern Arc (see their Figure 6). Concerning *Saintpaulia*, an initial North-South disjunction, separating an Uluguru-Ukaguru-Nguru range from the Taita Hills and Usambaras (cf. Pócs *et al.* 1990, Lovett 1993) could be attributed to fragmentation of a previously more continuous distribution (cf. Möller & Cronk 1997b). Long-distance dispersal of *Saintpaulia pusilla* and *S. goetzeana* among the Uluguru, Ukaguru and Nguru Mountains following their floristic isolation from one another seems unlikely because the habitat and reproductive morphology of *Saintpaulia* are not particularly suited for endozoochorous or anemochorous seed dispersal (Möller & Cronk 1997b: 1834). Nevertheless, future research must confirm that the disjunct collections of *Saintpaulia pusilla* and *S. goetzeana* do indeed group with other sequences representing the Uluguru Mountains clade.

The Nguru Mountains and Usambaras/Lowland Clades

As elucidated above, the basal clades of *Saintpaulia* form a major North-South disjunction between the Taita Hills to the North and the Uluguru Mountains to the South. The Ukaguru and Nguru Mountains are potentially included in the southern split because of the known ranges of *Saintpaulia pusilla* and *S. goetzeana*. Previous to this study, the relationships of the basal clades to the rest of the genus remained unresolved. In Möller & Cronk's (1997b: 1833) ITS study, *Saintpaulia* taxa from the Nguru Mountains (other than *S. pusilla* and *S. goetzeana*) were monophyletic yet intermingled with taxa from the Usambaras and lowland regions. The Nguru Mountains, which lie approximately 80 km to the North of the Ulugurus, closer to but Northeast of the Ukagurus, are therefore implicated in both the northern and southern halves of the initial *Saintpaulia* split. *Saintpaulia brevipilosa* and an undescribed taxon similar to *S. velutina* in leaf indumentum (C. Lindqvist & S. Simiyu, pers. obs.; Table 1; Eastwood *et al.* 1998) are endemic to the Nguru Mountains. *Saintpaulia nitida* is known from both the Nguru and Ukaguru Mountains (Pócs *et al.* 1990). Approximately 150 km to the Northeast of the Ngurus lie the Usambara Mountains, where 12 *Saintpaulia* taxa are endemic (Clarke 1998, Eastwood *et al.* 1998). Another 7 taxa (four of which remain undescribed) are endemic to lowland sites, mostly close to the Usambara Mountains, although two *Saintpaulia ionantha* populations are far disjunct to the South (Clarke 1998). *Saintpaulia diplotricha* and *S. grotei* are found in the Usambaras as well as adjacent lowland sites, and *S. confusa* is found in both places plus the Nguru Mountains (Baatvik 1993; Eastwood *et al.* 1998).

As seen with 5S-NTS data (Fig. 1), the clear phylogeographic divergence between the Nguru Mountains, which hold both basal and more terminal *Saintpaulia* taxa, and the Usambaras plus lowland sites may also date to the initial North-South *Saintpaulia* split. The single population of *Saintpaulia teitensis* in the Taita Hills could be interpreted as a relict of cladogenetic events that occurred earlier than the initial North-South split (cf. Möller & Cronk 1997b). From cladistic progression, the formation of discrete Ngurus and Usambaras/lowland clades was synchronous and later than the split from the Taita Hills and could either mark a second fragmentation event in *Saintpaulia* evolutionary history or merely cladogenetic events that occurred when the range of *Saintpaulia* was more continuous. The original North-South fragmentation (and any other fragmentations) could have resulted from range contraction during climatically unfavorable periods (cf. Lovett & Friis 1996). Lowland *Saintpaulia* taxa, which themselves show a patchy distribution in East Africa, may have originated as widespread (dispersed) *Saintpaulia* populations related to Usambara taxa that became range-restricted by still later fragmentation events, some of which may have been human-related.

Relationships Within the Lowland Kenyan Subclade

Saintpaulia rupicola is a critically endangered lowland taxon known from only two coastal forest populations in Kenya (Eastwood *et al.* 1998). Including two closely related but undescribed taxa, *Saintpaulia* 'Kachororoni' and *S.* 'Mwache', the *S. rupicola* group is the best supported subclade within the Usambaras/lowland lineage (Fig. 1). Support for grouping the two *Saintpaulia rupicola* populations is similarly high, but *S.* 'Kachororoni' and *S.* 'Mwache', although resolved together as the sister group to *S. rupicola*, are not distinguished from one another by 5S-NTS. The greater presence of synapomorphic nucleotide variation within these Kenyan populations as opposed to other Usambaras/lowland taxa (as indicated by jackknife support values; Fig. 1) would seem to indicate greater interpopulational differentiation, and perhaps correspondingly, greater divergence times among the Kenyan populations than between them and other Usambaras/lowland taxa. Sequence divergence does not by itself argue for this conclusion, since *Saintpaulia shumensis* from the Usambara Mountains has an autapomorphic branch 12 times longer than that of any sequence sampled for the *S. rupicola* clade (i.e., 12 nucleotide changes versus one in *S. rupicola* [b]). However, *Saintpaulia shumensis* has not been clearly placed with other taxa by 5S-NTS, even though ITS data do support *S. shumensis* as sister to a large polytomy of Usambaras/lowland *Saintpaulia* taxa (Möller & Cronk 1997b).

What distinguishes the lowland Kenyan *Saintpaulia* populations then is their genetic discreteness (through specific nucleotide changes) with clear phylogenetic structure (that these changes are also synapomorphies), features that are lacking from most other taxa of the Usambaras/lowland clade. Whereas the Kenyan populations may have been fixed for synapomorphic traits, other Usambaras/lowland taxa may be in a more active state of evolution, perhaps even deserving the status of a segregating metapopulation (Hanski 1998) rather than a species group. Perhaps the Kenyan taxa were isolated from this hypothetical metapopulation long enough ago to diverge genetically and morphologically, but not long enough ago to lose nucleotide synapomorphies. Fragmentation of the Kenyan populations (as could be suggested by present patterns of habitat destruction alone; Simiyu *et al.* 1996) may have forced them into genetic bottlenecks via restricted gene flow (see Barrett & Kohn 1991), which may well not be the case for sympatric taxa of the Usambaras. The lack of 5S-NTS autapomorphies for the Kenyan taxa could then be reinterpreted to indicate relatively recent population-level differentiation, but with genetic polymorphism low, population size small, and reproductive isolation from other saintpaulias. The evolutionary status of the two, critically endangered coastal forest populations of *Saintpaulia tongwensis* (Clarke 1998; Eastwood *et al.* 1998), which were also resolved with jackknife support (Fig. 1), may also fit this explanation. Likewise, the long autapomorphic branch for *Saintpaulia shumensis* could be taken to indicate evolution in isolation over a longer period of time, perhaps accompanied by extinction of related taxa that would have converted some of its autapomorphies to synapomorphies had they been available for study.

Conservation Priorities

Contrary to the discussion by Möller & Cronk (1997b) and based on all evidence available as of this writing, we suggest that the Nguru Mountains be the area afforded highest conservation priority with respect to *Saintpaulia.* The Nguru Mountains harbour both basal and derived taxa representing two to three of the four major clades identified here (cf. Humphries *et al.* 1995). The Uluguru Mountains hold only species of the basal-most clade, and while these are certainly the most genetically divergent saintpaulias, other clades are not represented there. The Nguru Mountains, on the other hand, share basal and sequence divergent taxa with the Ulugurus (*Saintpaulia pusilla* and *S. goetzeana*), have an endemic *Saintpaulia* clade of their own (here, *S. brevipilosa* and *S.* cf. *velutina*), and may support at least one species of the Usambaras/lowland clade (*S. confusa,* although this must be confirmed molecularly using new Nguru collections). A similar situation occurs among the Eastern Arc giant lobelias, in which accessions from the Nguru Mountains have been found to be closely related to collections from the Ulugurus, East Usambaras, and Ukagurus (Knox & Palmer 1998: 125). The Ukaguru Mountains may also harbour comparable phylogenetic diversity within *Saintpaulia,* with taxa from the Ulugurus clade (*S. pusilla*) and possibly the Ngurus clade (*S. nitida,* not investigated here) both present (Pócs *et al.* 1990). *Saintpaulia nitida* was strongly supported as sister to *S. brevipilosa* based on ITS sequence data (Möller & Cronk 1997b: 1832). However, species-level diversity in the Ukagurus is lower relative to the Ngurus, and no unique clade of *Saintpaulia* taxa, as we have described for the Ngurus, is known from the Ukagurus. These observations nevertheless indicate that both the Ngurus and Ukagurus merit particular attention for further investigation of suitable *Saintpaulia* habitats.

Conservation priority should also be directed at the genetically distinct lowland Kenyan taxa (and perhaps other lowland species such as *Saintpaulia tongwensis*), which may be segregant outposts of a larger Usambaras/lowland metapopulation of *Saintpaulia.* It is difficult to suggest conservation priorities within the Usambara Mountains since so little phylogenetic structure was obtained using 5S-NTS despite the high species diversity in the region. Rather, we suggest that population-level research (e.g., using DNA fingerprinting methods such as amplified fragment length polymorphism—AFLP; e.g., Qamaruz-Zaman *et al.* 1998) be applied to the Usambaras/lowland problem (now undertaken by S. Simiyu, pers. comm.) to detect whether evidence for cladogenesis (speciation) truly exists. Likewise, population-level analysis within species from the sequence-divergent and basal Ulugurus, Taita, and Ngurus clades (C. Lindqvist, unpublished) could provide data on population structure that may be illustrative of past vicariance or dispersal events both within and among highland areas of the Eastern Arc Mountains.

ACKNOWLEDGMENTS

We thank the Centre for Tropical Biodiversity (Denmark), The Lewis B. and Dorothy Cullman Foundation (New York), the Overseas Development Administration (Department for International Development; United Kingdom), and the United States National Science Foundation (grant DBI-9601515) for funding this project. Additional assistance was provided by the National Museums of Kenya. We wish to give particular thanks to S. Simiyu, M. Mbale, and G. Gichuru of the National Museums of Kenya and I. Friis of the Botanical Museum, Copenhagen, for collaboration and assistance in the field. Further logistic or field assistance was provided by W. Mziray of The National Herbarium, Arusha; the staff of the East Usambara Catchment Forest Project; F. Mbago and F. Elias of the University of Dar es Salaam; R. P. C. Temu of Sokoine University of Agriculture; D. Moyer, J. Fjeldså, and N. Burgess of the Centre for Tropical Biodiversity; and P. Clarke, formerly of the Frontier-Tanzania Coastal Forest Research Programme. We also thank COSTECH (Commission for Science and Technology, Tanzania) and the many village elders and local authorities who gave their permission to collect on Tanzanian lands. We are grateful to the Royal Botanic Gardens, Kew, Royal Botanic Garden, Edinburgh (United Kingdom), and the National Museums of Kenya for donations of plant material and for use of their herbaria. I. Friis and O. Seberg (Botanical Laboratory, Copenhagen) provided important organizational and administrative assistance to CL. J. Olsen tended living collections of *Saintpaulia* at the Botanical Garden, Copenhagen. Finally, we thank B. Burtt and M. Möller for helpful discussion.

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