A CLADISTIC ANALYSIS OF *ndh*F SEQUENCES FROM REPRESENTATIVE SPECIES OF *SAINTPAULIA* AND *STREPTOCARPUS* SUBGENERA *STREPTOCARPUS* AND *STREPTOCARPELLA* (GESNERIACEAE)

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Two African genera of the Gesneriaceae, *Saintpaulia* and *Streptocarpus*, are similar in many respects. Both genera have blue to purple flowers, pollen of similar shape and exine sculpting, embryos with one-celled, uninucleate chalazal haustoria, shared vegetative structures among some species, and are among the few genera in the Gesneriaceae which include species with chromosome counts of $n = 15$. The similarity of these features has indicated that the two genera are likely to be closely related. This study examines the sequences of the chloroplast gene *ndh*F among several representative species of *Saintpaulia* and representatives of *Streptocarpus* subgenera *Streptocarpella* and *Streptocarpus*. The results of this analysis are congruent with those of a previous analysis based on the nuclear ribosomal region, ITS. *Saintpaulia* was found to be nested within *Streptocarpus* subgenus *Streptocarpella*. The results raise the possibility of the taxonomic revision of these genera, and this is discussed. Comparisons between the data sets are made regarding utility of the two regions, sample size and outgroup.

Keywords. African violet, Cape primrose, cladistic analysis, *ndh*F.

INTRODUCTION

The majority of species in the Old World subfamily of Gesneriaceae, Cyrtandroideae, are distributed in south-east Asia and China with a few genera in Africa and Europe (Wang et al., 1992; Burtt & Wiehler, 1995). Among the African genera are the widely cultivated African violet (*Saintpaulia* Wendl.) and the Cape primrose (*Streptocarpus* Lindl.). These two genera have been thought to be closely related on the basis of numerous characters. Among these are shared pollen shape and exine sculpting (Weigend & Edwards, 1996), one-celled, uninucleate chalazal haustoria in embryos (Holmqvist, 1964), rosulate herbs with orbicular, petiolate leaves common to *Saintpaulia* and some species of *Streptocarpus* (Hilliard & Burtt, 1971) and (in subg. *Streptocarpella*) chromosome counts of $n = 15$ (Skog, 1984) (although *Streptocarpus* subg. *Streptocarpus* has $n = 16$).

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Early interpretations of relationships among African genera of the Gesneriaceae indicated neither unity nor a strong relationship between *Saintpaulia* and *Streptocarpus*. However, as additional species were described and the characters of the African Gesneriaceae examined more carefully, a closer relationship among all of the African genera of Gesneriaceae, in particular *Saintpaulia* and *Streptocarpus*, became apparent (Hilliard & Burtt, 1971).

Saintpaulia and *Streptocarpus* have a similar embryology (Holmqvist, 1964). In these two genera the chalazal haustorium is always one-celled and uninucleate, degenerating very quickly (Holmqvist, 1964). In Asiatic genera (*Boea* Comm. ex Lam. and *Chirita* Ham ex G. Don) the haustorium is large, unicellular and binucleate (Holmqvist, 1964).

Both *Saintpaulia* and *Streptocarpus* have similar pollen structure. The pollen of all African species examined was found to be eurypalynous, prolate to spheroidal to oblate, triporate or tricolpate to tricolporate, and the colpus margin is not differentiated (Weigend & Edwards, 1996). The similarity of the pollen more than likely reflects a monophyletic lineage of all African genera (Weigend & Edwards, 1996). Additionally, some *Streptocarpus* species share similar pollen types to *Saintpaulia* although exact relationships between these genera cannot be made with pollen data alone (Weigend & Edwards, 1996).

A cladistic analysis of the tribes of the Gesneriaceae based on morphological data included both *Saintpaulia* and *Streptocarpus* (Smith, 1996). A strict consensus of the trees from this analysis placed both genera in a polytomy, although the majority of trees placed *Streptocarpus* as the sister to *Ornithoboea* Parish ex C.B. Clarke on the basis of twisted carpels common to these two genera. In a similar analysis, based on comparison of *ndh*F sequences, Smith et al. (1997a) found that the single species of *Saintpaulia* included was the sister to *Streptocarpus holstii. Streptocarpus saxorum* was the sister to both of these two species. Thus, *Saintpaulia* was nested within *Streptocarpus*.

The close relationship of *Saintpaulia* and *Streptocarpus* based on the above data prompted further investigations using the chloroplast DNA (cpDNA) encoded *ndh*F gene, the results of which are presented in this paper. Möller & Cronk (1997) examined the relationship of *Saintpaulia* to the subgenera and sections of *Streptocarpus* using the nuclear ribosomal DNA (nrDNA) ITS region. They concluded that *Saintpaulia* was nested within *Streptocarpus* subg. *Streptocarpella* Fritsch and resolved relationships among other sections of *Streptocarpus* (Möller & Cronk, 1997).

The present paper presents a cladistic analysis of the cpDNA data and makes comparisons with the results of Möller & Cronk (1997). This paper examines a much more thorough sampling of *Saintpaulia* and *Streptocarpus* with the *ndh*F gene than has been examined previously (Smith et al., 1997a).

The source of molecular data for this analysis is the chloroplast DNA encoded gene *ndh*F which in tobacco encodes a protein of 740 amino acids presumed to be a subunit of an NADH dehydrogenase (Sugiura, 1992). The use of *ndh*F sequences

for systematic studies has provided a far greater number of characters to resolve relationships than studies using *rbc*L (Olmstead & Sweere, 1994; Clark et al., 1995; Olmstead & Reeves, 1995; Scotland et al., 1995; Smith et al., 1997a, b). In general, the *ndh*F gene is 50% larger than *rbc*L and has a nucleotide substitution rate approximately two times higher (Sugiura, 1989). The larger number of variable characters makes *ndh*F sequences ideal for taxonomic groups that have not been resolved well using *rbc*L data, such as members of the Asteridae and Lamiales s.l. (Olmstead et al., 1992, 1993; Chase et al., 1993).

MATERIALS AND METHODS

The gene sequences used in this analysis were generated by thermal cycle sequencing (Innis et al., 1988) of previously amplified *ndh*F regions. The *ndh*F gene was amplified in two overlapping sections (positions 1–1350, and 972–2044) from genomic DNA isolated from fresh or frozen material (Smith et al., 1992). Amplification and sequencing procedures followed that of Smith et al. (1997a) used for other members of the Gesneriaceae. The species used in the analysis, voucher information, and Genbank accession numbers are shown in Table 1. Outgroups included two members of the Buddlejaceae, selected on the basis of recent molecular analyses that indicate the Buddlejaceae to be the sister to the Gesneriaceae (Smith et al., 1997b). Other members of the Old World subfamily Cyrtandroideae were included in this analysis to verify that neither *Streptocarpus* nor *Saintpaulia* had any affinities with other genera.

Phylogenetic trees were reconstructed using PAUP (Phylogenetic Analysis Using Parsimony) version 3.1.1 (Swofford, 1993) to implement parsimony (Farris, 1970; Farris et al., 1970; Swofford & Maddison, 1987). In this analysis, trees were generated using the general heuristic option, saving minimal trees only, with the collapse zero-length branches, and ignore uninformative characters options in effect. Because of the large number of taxa in this analysis, the branch and bound and exhaustive search options would have consumed an excessive amount of time. Therefore, the trees presented here are best approximations and not exact solutions. The manner in which the program reconstructs phylogenetic sequences is sensitive to the order of taxa presentation in the data matrix, frequently finding islands of equally parsimonious trees depending on the order (Maddison, 1991). Therefore, it is important to repeat the analysis several times, each time with a different starting place in the data matrix. To do this, the search strategy of Olmstead & Palmer (1994) was implemented searching for 1000 trees each using the 'random addition sequence' in five subsequent analyses with the nearest neighbour interchange (NNI) search option in effect and mulpars 'off'. Each of the results from the five NNI searches was used as the starting tree(s) for a search with tree bisection reconnection (TBR) and mulpars 'on'. The mulpars option, when 'on', will save all equally parsimonious trees.

Branch support analysis was performed to examine trees that were three or fewer steps longer than the most-parsimonious tree (Bremer, 1988, 1994; Donoghue et al., 1992). This type of analysis provides an indication of the robustness of the data by

TABLE 1. Species sequenced in this study with Genbank submission numbers and voucher specimens. SI, Smithsonian Institution. Letters in parentheses indicate herbarium where vouchers are deposited.

Species	Voucher	Genbank Number
Aeschynanthus micranthus C.B. Clarke	Smith et al. 1997a	U62169
Agalmyla parasitica (Lam.) Kuntze	Smith et al. 1997a	U62171
Anna mollifolia (W.T. Wang) W.T. Wang & K.Y. Pan	Smith et al. 1997a	U62188
Boea hygroscopica F. Muell.	Smith et al. 1997a	U62205
Chirita sinensis Lindl.	Smith et al. 1997a	U62189
Cyrtandra hawaiensis C.B. Clarke	Smith et al. 1997a	U62172
Cyrtandra umbellifera Merr.	Smith et al. 1997a	U62165
Cyrtandromoea acuminata Benth. & Hook.	Smith et al. 1997a	U62173
Didissandra frutescens Clarke	Smith et al. 1997a	U62190
Didymocarpus albomarginata Hemsl.	Smith et al. 1997a	U62207
Hemiboea henryi C.B. Clarke	Smith et al. 1997a	U62180
Lysionotus pauciflorus Maxim.	Smith et al. 1997a	U62182
Monophyllaea hirticalyx Franch.	Smith et al. 1997a	U62168
Opithandra primuloides (Miq.) B.L. Burtt	Smith et al. 1997a	U62183
Ornithoboea wildeana Craib.	Smith et al. 1997a	U62166
Paraboea rufescens (Franch.) Burtt	Smith et al. 1997a	U62206
Petrocosmea flaccida Craib	Smith et al. 1997a	U62184
Primulina tabacum Hance	Smith et al. 1997a	U62167
Ramonda myconi (L.) Rchb.	Smith et al. 1997a	U62185
Rhynchoglossum notonianum (Wall.) B.L. Burtt	Smith et al. 1997a	U62179
Saintpaulia difficilis B.L. Burtt	Smith 3602 (SRP)	AFO12837
S. diplotricha B.L. Burtt	Smith 3601 (SRP)	AFO12838
S. grotei Engl.	Smith 3598 (SRP)	AFO12839
S. grandifolia B.L. Burtt	Smith 3702 (SRP)	AFO12840
S. pendula B.L. Burtt	no voucher	AFO12841
S. rupicola B.L. Burtt	Smith et al. 1997a	U62176
Streptocarpus cyaneus S. Moore	SI 94-596	AFO12843
S. fanniniae Harvey ex C.B. Clarke	SI 94-137	AFO12844
S. holstii Engl.	Olmstead & Reeves 1995	L36415
S. johannis L.L. Britten	SI 94-223	AFO12845
S. porphyrostachys Hilliard	SI 94-141	AFO12846
S. primulifolius Gandoger	SI 94-096	AFO12847
S. saxorum Engl.	Smith et al. 1997a	U62170
S. sp.	SI 94-500	AFO12849
S. vandeleurii E.G. Baker & S. Moore	SI 95-130	AFO12848
Titanotrichum oldhamii (Hemsl.) Soler.	Smith et al. 1997a	U62187
Outgroups		
Buddleja davidii Franch.	Olmstead & Reeves 1995	L36394
Nicodemia diversifolia (Vahl.) Ren.	Olmstead & Reeves 1995	L36405

determining which clades persist in a consensus tree as parsimony is relaxed. This analysis was performed by saving all trees three steps longer than the most-parsimonious trees and then examining subsets of trees one to three steps longer with the filter option of PAUP.

Bootstrap analysis (Felsenstein, 1985) was performed using 1000 replicates. An estimate of how well the data fit the tree was determined by calculating the g_1 statistic (Hillis & Huelsenbeck, 1992) using 10,000 random trees.

RESULTS

A total of 2223 base pairs of *ndh*F were sequenced for each species in this analysis. Of these base pairs, 408 were variable and phylogenetically informative among the species used in this analysis (Table 1). An insertion at position 1440 of 13 base pairs was found in all species examined. The sequence of this insertion was not used in the cladistic analysis. Only two base pairs differ among all of the species of *Saintpaulia* and *Streptocarpus* included in this analysis, therefore the choice to exclude this insertion (due to possible lack of homology) is not likely to have altered the results.

The cladistic analysis of *ndh*F sequences resulted in 253 trees of 2904 steps each, consistency index (CI) = 0.31, retention index (RI) = 0.38. The strict consensus of these trees is presented in Fig. 1. *Saintpaulia* and *Streptocarpus* form a monophyletic group. However, neither *Saintpaulia* nor *Streptocarpus* are monophyletic. The *Saintpaulia* species are in a polytomy with the two species of *Streptocarpus* subg. *Streptocarpella* (Fig. 1). There is significantly more structure than random data within this analysis as reflected by the g_1 statistic (-0.392). Extrapolation from the charts generated by Hillis & Huelsenbeck (1992) for the number of characters and taxa used in this analysis indicates that the g_1 is clearly significant at $P < 0.01$.

The general results of this analysis are found to be completely congruent with those of Möller & Cronk (1997) based on ITS sequences. Because the sampling between the two studies was not identical, this study does not resolve relationships among the different sections of *Streptocarpus* and in general provides less resolution among the included taxa than does the ITS region.

DISCUSSION

Cladistic analysis of the cpDNA gene *ndh*F indicates that *Saintpaulia* is closely related to *Streptocarpus* subg. *Streptocarpella* (Fig. 1). Poor resolution of the relationship between *Saintpaulia* and *Streptocarpus* subg. *Streptocarpella* prevents further speculation on the exact relationship between these two taxa. For example, *Saintpaulia* could be a monophyletic group that is a sister to subg. *Streptocarpella*, or be paraphyletic. Regardless, the data provide evidence that *Saintpaulia* is derived from within *Streptocarpus* and in particular shows a close affinity to subg. *Streptocarpella* (Fig. 1).

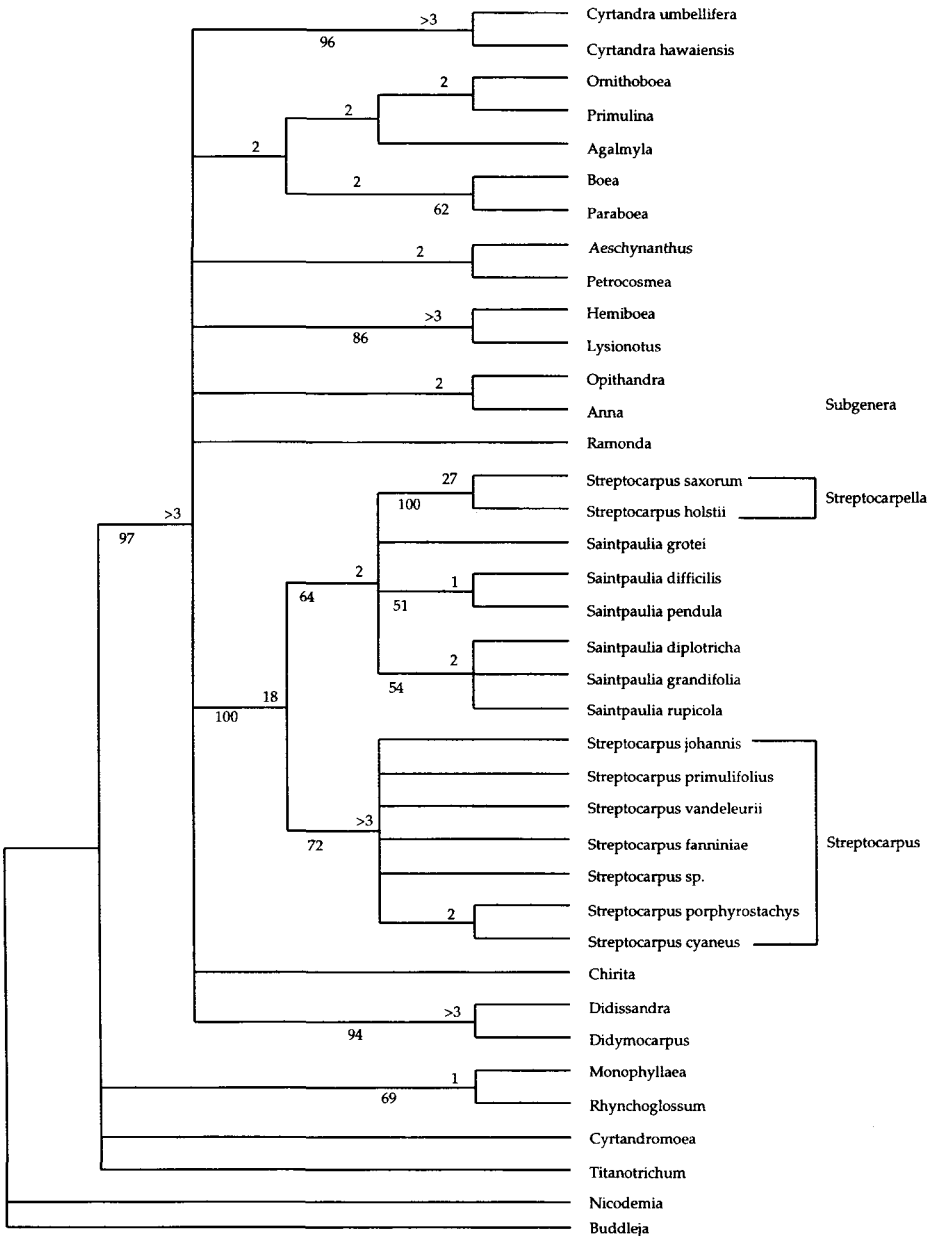Further evidence for the relationship of *Saintpaulia* to *Streptocarpus* subg.

FIG. 1. Strict consensus of 253 trees of 2904 steps each (CI = 0.31, RI = 0.38) based on cpDNA *ndh*F gene sequences. Numbers above clades are decay values, numbers below clades are bootstrap values. Subgenera of *Streptocarpus* are indicated on the figure.

Streptocarpella comes from ITS sequence data (Möller & Cronk, 1997). The results from the ITS sequencing are congruent with the results of the *ndh*F sequencing presented here with the benefit of higher resolution among the species examined (Fig. 2; Möller & Cronk, 1997). The ITS data places *Saintpaulia* as a monophyletic group nested within subg. *Streptocarpella* (Fig. 2).

Lastly, the close relationship between *Saintpaulia* and subg. *Streptocarpella* is supported by data from pollen analysis (Weigend & Edwards, 1996) and chromo-
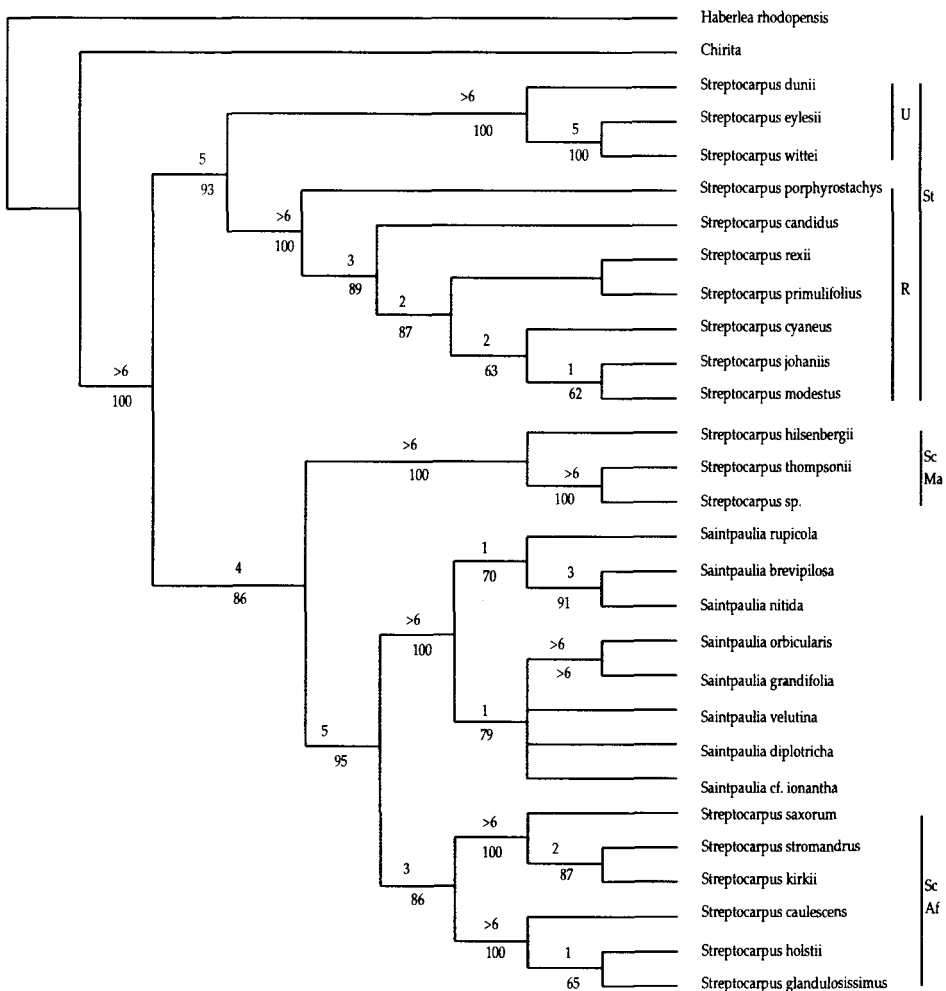

FIG. 2. Most-parsimonious tree of 419 steps (CI=0.79, RI=0.90) based on nrDNA ITS sequences including aligned gaps. Redrawn from Möller & Cronk (1997). Numbers above clades are decay values, numbers below clades are bootstrap values. Subgenera of *Streptocarpus* are indicated and abbreviated as follows: St, subg. *Streptocarpus*; Sc, subg. *Streptocarpella*; Ma, Madagascan species; Af, African species. Sections within subg. *Streptocarpus* are indicated and abbreviated as U, unifoliate; R, rosulate.

some numbers; *Saintpaulia* and *Streptocarpus* subg. *Streptocarpella* are among the few species of the Gesneriaceae with $n = 15$ chromosomes (Skog, 1984).

Despite different sampling strategies, the results presented here based on *ndh*F sequence data (Fig. 1) and ITS sequence data (Fig. 2; Möller & Cronk, 1997) are congruent. The major difference between the two analyses is the lower level of resolution obtained from the cpDNA (Fig. 1). Both the cpDNA and nrDNA indicated that *Saintpaulia* is closely related to *Streptocarpus* subg. *Streptocarpella* (Figs 1, 2). The greater sampling from subg. *Streptocarpella* in the ITS study identifies two clades within this subgenus, one from mainland Africa and the other from Madagascar (Fig. 2; Möller & Cronk, 1997). Both the *ndh*F and ITS data indicate that subg. *Streptocarpella* (including *Saintpaulia*) is sister to the remainder of *Streptocarpus* (Figs 1, 2). The greater resolution obtained from the ITS study indicates, as had been previously noted (Baldwin et al., 1995), that this region is better suited to resolve phylogenetic questions at the species and generic level.

A taxonomic revision of *Streptocarpus* and *Saintpaulia* is likely to be inevitable in order to reflect monophyletic genera, although no revision is recommended at this time. One possibility is to combine *Saintpaulia* into *Streptocarpus* subg. *Streptocarpella*; the other is to apply the name of *Streptocarpella* at the generic level to one of the clades and erect a new genus for the second (Fig. 2). This latter system is likely to be preferable in order to reflect the morphological distinctiveness of the species placed in *Saintpaulia* from *Streptocarpus*, and, since *Saintpaulia* is the most widely cultivated member of the family, would result in less confusion among horticulturists. Furthermore, the division of *Streptocarpus* subg. *Streptocarpus* into two sections, one the rosulate form, the other unifoliate, receives support from the ITS data (Fig. 2; Möller & Cronk, 1997), although these sections were abandoned earlier on the basis of not being tenable by Hilliard & Burtt (1971). To more fully resolve the nomenclatural issues within *Streptocarpus*, additional studies will be necessary to increase sampling among the species of this large and morphologically variable genus.

The major morphological shifts from *Streptocarpus* to *Saintpaulia* are the loss of the twisted fruit, the shortening of the corolla tube, and the loss of caulescence in *Saintpaulia* (although some species of *Saintpaulia* have a creeping stem rather than being rosulate and some *Streptocarpus* are rosulate), and the shift from nectar reward to pollen reward for pollinators (Hilliard & Burtt, 1971; Möller & Cronk, 1997). The loss of the twisted fruit may be the result of developmental constraints since the fruits of *Saintpaulia* are more globose in shape. The shift in fruit type is probably the result of selection pressures due to the habitat shift (most species of *Saintpaulia* occur on steep cliffs). The occurrence of twisted fruit, although long used to define *Streptocarpus*, is not unique to this genus. Other members of the Cyrtandroideae possess a twisted fruit, such as *Boea, Ornithoboea, Paraboea* (C.B. Clarke) Ridley, *Rhabdothamnopsis* Hemsl. and *Trisepalum* C.B. Clarke. Three of these genera (*Boea, Paraboea* and *Ornithoboea*) were included in an analysis of the tribal relationships of the Gesneriaceae along with two species of *Streptocarpus* (Smith et al., 1997a) and none showed any close affinity to *Streptocarpus*. A morphological analysis that

did not include *Boea* or *Paraboea* did result in a close affinity of *Streptocarpus* and *Ornithoboea* but this relationship was only weakly supported (Smith, 1996). Therefore, the occurrence of the twisted fruit is likely to have several origins within the Cyrtandroideae.

The change in corolla shape and pollen reward probably reflects a shift in pollinator from *Streptocarpus*, albeit little is known regarding the natural pollinators of these plants (Hilliard & Burtt, 1971). The change in habitat, for instance, from rock outcrops to steep cliffsides would probably result in a change in the pollen vector pool, and subsequent changes in the morphology of the flowers would be expected.

A recent analysis of pollen morphology among African Gesneriaceae indicated that the African genera *Streptocarpus*, *Saintpaulia*, *Acanthonema* Hook., *Linnaeopsis* Engler, *Schizoboea* (Fritsch) B.L. Burtt (*Didymocarpus*) and *Trachystigma* C.B. Clarke are likely to be a monophyletic group distinct from Asiatic taxa (Weigend & Edwards, 1996). Since this present study has confirmed previous hypotheses on the close relationship of *Saintpaulia* and *Streptocarpus* subg. *Streptocarpella*, the inclusion of these additional genera in a molecular analysis may provide further support for the hypothesis generated from the pollen data. Unfortunately most of these genera are rarely collected and are currently not available for molecular analysis.

A comparison of the trees generated for these taxa based on different DNA sequences provides not only a chance to gain further confidence regarding the relationship of these genera (Donoghue, 1994), but also an opportunity to compare the utility of the two genomes. Sampling within *Streptocarpus* was much greater for the ITS region (Möller & Cronk, 1997; Fig. 2) than it was for the *ndh*F gene (Table 1, Fig. 1) and the sampling among the genera of the Cyrtandroideae was greater for the latter. Regardless, the results of the two analyses resolve the relationship of *Saintpaulia* to *Streptocarpus* consistently, indicating that the level of sampling was adequate for both studies in order to resolve this particular question. Further resolution among the different sections of *Streptocarpus* and among the species within these clades was obtained with ITS data that was not resolvable with the more conserved sequences of the *ndh*F gene (Figs 1, 2). Furthermore, the outgroups for both studies were different. Two genera from a related family (Buddlejaceae) were used for the cpDNA study (Fig. 1) and two genera within the Cyrtandroideae (*Haberlea* Friv. and *Chirita*) were used for the ITS study (Möller & Cronk, 1997; Fig. 2). Based on a wider sampling of genera within the Cyrtandroideae (Smith et al., 1997a) *Chirita* is not closely related to the *Streptocarpus/Saintpaulia* clade. The congruence of the two analyses regarding the relationship of *Streptocarpus* and *Saintpaulia* indicates that the particular choice of outgroup was less important in resolving this question.

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