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# ORIGIN AND RELATIONSHIPS OF *SAINTPAULIA* (GESNERIACEAE) BASED ON RIBOSOMAL DNA INTERNAL TRANSCRIBED SPACER (ITS) SEQUENCES[1]

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Phylogenetic relationships of eight species of *Saintpaulia* H. Wendl., 19 species of *Streptocarpus* Lindl. (representing all major growth forms within the genus), and two outgroups (*Haberlea rhodopensis* Friv., *Chirita spadiciformis* W. T. Wang) were examined using comparative nucleotide sequences from the two internal transcribed spacers (ITS) of nuclear ribosomal DNA. The length of the ITS 1 region ranged from 228 to 249 base pairs (bp) and the ITS 2 region from 196 to 245 bp. Pairwise sequence divergence across both spacers for ingroup and outgroup species ranged from 0 to 29%. *Streptocarpus* is not monophyletic, and *Saintpaulia* is nested within *Streptocarpus* subgenus *Streptocarpella*. *Streptocarpus* subgenus *Streptocarpus* is monophyletic. The ITS sequence data demonstrate that the unifoliate *Streptocarpus* species form a clade, and are also characterized by a unique 47-bp deletion in ITS 2. The results strongly support the monophyly of (1) *Saintpaulia,* and (2) *Saintpaulia* plus the African members of the subgenus *Streptocarpella* of *Streptocarpus.* The data suggest the evolution of *Saintpaulia* from *Streptocarpus* subgenus *Streptocarpella.* The differences in flower and vegetative characters are probably due to ecological adaptation leading to a relatively rapid radiation of *Saintpaulia.*

**Key words:** Gesneriaceae; internal transcribed spacers; molecular phylogeny; nuclear ribosomal DNA; *Saintpaulia*; *Streptocarpus.*

The genus *Saintpaulia* (Gesneriaceae, subfamily Cyrtandroideae) is endemic to Eastern Africa. It forms a geographically restricted aggregate of ~20 species ranging from the Teita Hills in the south of Kenya to the Uluguru Mountains in eastern Tanzania, with the center of species diversity being the Usambara Mountains of northeast Tanzania (Burtt, 1958). *Saintpaulia* species were first introduced to cultivation in Europe in 1892 by Baron Walter von Saint Paul, and within years became a popular house plant, and are now the basis of a large horticultural industry (Robey, 1988).

The relationships of *Saintpaulia* to other genera of Gesneriaceae have been disputed. This is partly a result of the genus being characterized by an almost rotate corolla and the near absence of a corolla tube, which are comparatively unusual features in the Gesneriaceae, although they occur in a few other species, such as *Boea, Petrocosmea, Platystemma,* and *Ramonda.* Ivanina (1966) even suggested that *Saintpaulia* occupied its own tribe, Saintpaulieae. However, Hilliard and Burtt (1971, pp. 114–115) wrote: ''Until recently it was difficult to see any approach to *Saintpaulia* amongst species of *Streptocarpus.* The idea, already entertained, that all the African genera were more closely related amongst themselves than they were to the Asiatic gesneriads had the most tenuous foundations. However, Professor Humbert's publication of many new species of *Streptocarpus* from Madagascar (Humbert, 1967) completely altered the picture here. Amongst these newly described plants were three species, all rosette plants with long petiolate leaves having suborbiculate blades, all having axillary inflorescences with long peduncles, all with short, rather wide, corollas and with fruits that are spirally twisted but notably short for the genus. The whole facies of these species is highly reminiscent of *Saintpaulia.* The corollas, admittedly, are not flat in the limb as a typical *Saintpaulia* such as *S. ionantha*; they are more campanulate as in *S. pusilla* and in the allied genus *Linnaeopsis.* Nevertheless the resemblance is remarkably close: both *Saintpaulia* and these Madagascan *Streptocarpus* have verruculose seeds.''

These observations suggest that the relationship of *Saintpaulia* might lie with *Streptocarpus,* tribe Didymocarpeae. In order to investigate the monophyly of *Streptocarpus* and its relationship with *Saintpaulia* we undertook a molecular investigation using the internal transcribed spacers (ITS) of the nuclear ribosomal DNA genes.

## MATERIALS AND METHODS

***Origin of plant material***—All plant material was taken from living plants of the Research Collection held at the Royal Botanic Garden Edinburgh (RBGE). Identifications were kindly confirmed by B. L. Burtt. For all taxa analyzed, voucher herbarium specimens were prepared, flowers were preserved in Kopenhagen mixture in a spirit collection (both deposited in the RBGE herbarium), and photographs of

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TABLE 1. Accessions of *Saintpaulia, Streptocarpus, Haberlea rhodopensis,* and *Chirita spadiciformis* examined for ITS 1 and ITS 2 sequence variation.

| Taxon | Origin (distribution) | RBGE accession no.[a] |
|---|---|---|
| *Chirita spadiciformis* W. T. Wang | (China) | 1995 1205 |
| *Haberlea rhodopensis* Friv. | (Europe, Greece) | 1975 4106 |
| *Saintpaulia brevipilosa* B. L. Burtt | Tanzania, Kanga Forest, Mt. Kanga (Nguru Mts.) | 1970 0909 |
| *Saintpaulia diplotricha* B. L. Burtt | Tanzania, NE Usambara Mts. | 1987 2172B |
| *Saintpaulia grandifolia* B. L. Burtt | Tanzania, W Usambara Mts. | 1985 0678 |
| *Saintpaulia* cf. *ionantha* H. Wendl. | Tanzania, Tanga, Sigi River | 1971 0860 |
| *Saintpaulia nitida* B. L. Burtt | (Tanzania, Nguru Mts.) | 1992 3186 |
| *Saintpaulia orbicularis* B. L. Burtt | Tanzania, W Usambara Mts. | 1958 3586B |
| *Saintpaulia rupicola* B. L. Burtt | Kenya, Kaloleni | 1992 3184 |
| *Saintpaulia velutina* B. L. Burtt | (Tanzania, NW Usambara Mts.) | 1987 2179 |
| *Streptocarpus candidus* Hilliard | South Africa, Zululand | 1977 1204 |
| *Streptocarpus caulescens* Vatke | Tanzania, Uluguru Mts. | 1971 1199 |
| *Streptocarpus cyaneus* S. Moore | Swaziland, Mbabane, Sebebe Mts. | 1991 1950 |
| *Streptocarpus dunnii* Mast. | Swaziland, N Nlbabare | 1994 1745 |
| *Streptocarpus eylesii* S. Moore | Zimbabwe, Mt. Nyangoi, (S. Tanzania) | 1993 2790 |
| *Streptocarpus glandulosissimus* Engl. | (East Africa) | 1965 2118 |
| *Streptocarpus hilsenbergii* R. Br. | Madagascar, Mandrake Valley | 1963 1505 |
| *Streptocarpus holstii* Engl. | (Tanzania, E Usambara Mts.) | 1959 2272 |
| *Streptocarpus johannis* L. L. Britten | South Africa, Natal | 1969 0450 |
| *Streptocarpus kirkii* Hook.f. | Tanzania, E. Usambara Mts. (Kenya, Teita Hills; Uluguru Mts.) | 1994 1332 |
| *Streptocarpus modestus* L. L. Britten | South Africa, Transkei, Magwa Falls | 1994 3058 |
| *Streptocarpus porphyrostachys* Hilliard | South Africa, Transkei, Mkabati Nature Reserve | 1990 2311 |
| *Streptocarpus primulifolius* Gand. | South Africa, East Cape, Igoda River | 1991 2192 |
| *Streptocarpus rexii* Lindl. | South Africa, Cape Province, Grahamstown | 1987 0333 |
| *Streptocarpus saxorum* Engl. | Tanzania, Usambara, (Kenya, Teita Hills; Nguru, Uluguru Mts.) | 1972 1499 |
| *Streptocarpus* sp. (Madagascar) | Madagascar, Fianarantsoa, Itremo Massif | 1993 1445 |
| *Streptocarpus stomandrus* B. L. Burtt | Tanzania, Nguru Mts. | 1971 1392 |
| *Streptocarpus thompsonii* R. Br. | Madagascar | 1994 1334 |
| *Streptocarpus wittei* De Wild. | Malawi, Nyika | 1987 1695B |

[a] These numbers were also used as voucher numbers.

flowering specimens were taken, and deposited in the RBGE library. *Streptocarpus* sp. (Madagascar) is an as yet unidentified species, which may be undescribed, collected in the wild in Madagascar in 1993 by R. Clement.

***Outgroup taxa***—To investigate the relationship between *Saintpaulia* and *Streptocarpus,* appropriate outgroup selection is critical. The outgroup taxa should be systematically close enough to the genera under observation to allow sequence alignment and yet distantly enough related to enable unequivocal rooting of the tree. The shared advanced characters of diandry and the rapidly degenerating one-celled and uninucleate chalazal haustorium (Holmqvist, 1964) are strong indications of the monophyly of *Streptocarpus* and *Saintpaulia* with respect to other genera of the Didymocarpeae. Possible exceptions are the other African genera *Acanthonema, Linnaeopsis, Schizoboea,* and *Trachystigma,* and *Colpogyne* from Madagascar. The facts that these genera are unavailable in cultivation, and DNA notoriously difficult to extract from herbarium material of these taxa (Möller, unpublished data), led us to exclude these African genera from outgroup choice. Instead, the unequivocally distant tetrandrous European genus *Haberlea* was used along with the diandrous genus *Chirita* (Asian, lacking the specialized chalazal features). Two outgroup taxa rather than one were used as an added check on the correct rooting of the trees. It was not possible to use further outgroup taxa because they introduced alignment ambiguities throughout the data matrix.

As an additional check on the monophyly of *Saintpaulia* and *Streptocarpus* with respect to *Haberlea* and *Chirita* a molecular confirmation was sought, based on an initial sequence analysis of the chloroplast gene *trnL* (Möller and Cronk, unpublished data). The greater conservation of this spacer allowed rooting on a more distantly related genus: *Niphaea* (in a different subfamily, Gesnerioideae, tribe Gloxinieae). Analysis of these sequences confirms the monophyly of *Streptocarpus* and *Saintpaulia* with respect to *Chirita* and *Haberlea.*

***Ingroup taxa***—Eight species of *Saintpaulia,* representing all but the most southern geographical distribution and the greater part of the morphological diversity of this genus, and 19 species of *Streptocarpus,* representing the diverse growth forms and geographical patterns found in this genus (unifoliate: *S. dunnii, S. eylesii, S. wittei*; rosulate: *S. candidus, S. cyaneus, S. johannis, S. modestus, S. porphyrostachys, S. primulifolius, S. rexii*; African caulescent: *S. caulescens, S. glandulosissimus, S. holstii, S. kirkii, S. saxorum, S. stomandrus*; Madagascan caulescent: *S. hilsenbergii, S. thompsonii, S.* sp.) were chosen for this study (Table 1).

***DNA extraction***—Fresh leaf material of one plant representing each species was used for total DNA extraction using a modified CTAB procedure of Doyle and Doyle (1987), with no further purification.

***PCR amplification and conditions***—The complete ITS region was amplified with the polymerase chain reaction (PCR), using primers based on published data by White et al. (1990) and partly modified primers according to published sequence data of *Daucus carota* L. and *Vicia faba* L. (Yokota et al., 1989). These primers are ''ITS 5P''—GGA AGG AGA AGT CGT AAC AAG G; ''ITS 4''—TCC TCC GCT TAT TGA TAT GC; and ''ITS 8P''—CAC GCT TCT CCA GAC TAC A. Primer pairs ''ITS 4'' and ''ITS 5P'' amplified double-stranded DNA of ~720–750 base pairs (bp) length, and primer combination ''ITS 5P'' and ''ITS 8P'' yielded a fragment 117 bp longer. For certain DNA extractions the latter primer combination was more successful in the PCR. The PCR reaction mixture contained 28.5–32.5 μL distilled sterile water, 5.0 μL of 10× Dynazyme™ reaction buffer (1×: 10 mmol/L Tris-HCl, pH 8.8 at 25°C, 1.5 mmol/L $MgCl_2$, 50 mmol/L KCl, 0.1% Triton X-100; Finnzymes Oy, Espoo, Finland), 1.0 μL of a mix of each dNTP at 10 mmol/L (final concentration 200 μmol/L) (Sigma Chemicals, Poole, Dorset, UK), 0.5 μL (1U) of Dynazyme™ II thermostable DNA polymerase (Finnzymes Oy, Espoo, Finland), 5.0 μL of each primer

(final concentration 1 μmol/L) (Oswel DNA Service, Southampton, UK), and 1–5 μL aliquots of total genomic DNA. PCR cycle parameters were set as follows after an initial denaturation step for 3 min at 94°C: denaturation of template DNA for 1 min at 94°C, primer annealing for 1 min at 55°C, primer extension for 1.5 min at 72°C. After 30 cycles a final extension step of 5 min at 72°C was added to allow completion of unfinished strands. A negative control (sterile distilled water instead of DNA) was added to each set of reactions. Two microlitres of PCR product was checked for successful amplification and quantified by electrophoresis at 60 V for 2 h in a 2% agarose (Promega, Madison, WI, USA) gel using 1× TBE as the gel buffer.

The PCR products were purified prior to sequencing using the QIAquick™ PCR purification kit (Qiagen Ltd, Dorking, Surrey, UK), eluting the DNA in 30–50 μL sterile distilled water at the final step to obtain DNA concentrations between 10 and 30 ng/μL.

***Sequencing protocol***—Purified PCR products were sequenced using a dye terminator cycle-sequencing ready-reaction kit (Perkin Elmer, Applied Biosystems Division, Foster City, CA, USA), with AmpliTaq® DNA Polymerase, FS, according to the manufacturer's recommendations. Sequencing products were analyzed on an ABI 377 Prism Automatic DNA Sequencer (Perkin Elmer, Applied Biosystems Division, Foster City, CA, USA), according to the manual supplied. For each taxon forward and reverse sequencing reactions were performed for sequence confirmation. Sequencing primers were identical to those used for PCR (e.g., forward primer ''ITS 5P,'' reverse primer ''ITS 4'' or ''ITS 8P''). Additionally, two shorter reactions were carried out sequencing ITS 1 and ITS 2 from within the 5.8S coding region. The annealing sites of the primers ''ITS 3P'' and ''ITS 2G'' used were located at the far end, respectively, of the conserved 5.8S region. When directed forwards and reverse, they covered the conserved 5.8S region in each reaction, facilitating sequencing of ITS 1 and ITS 2, respectively. Primer ''ITS 3P'' sequence was based on White et al. (1990) and modified according to published data (Yokota et al., 1989), whereas the sequence for primer ''ITS 2G'' was obtained during the course of this work. All primers were synthesized by, and purchased from, Oswel DNA Service, Southampton, UK. The primer sequence for ''ITS 3P'' was GCA TCG ATG AAG AAC GTA GC, and for primer ''ITS 2G'' was GTG ACG CCC AGG CAG ACG T.

***Sequence analysis***—Sequence boundaries of both internal transcribed spacers of all taxa were determined by comparison with published rDNA sequence data for *Daucus carota* and *Vicia faba* (Yokota et al., 1989). Both ITS regions were aligned using the CLUSTAL option in the multiple alignment program Sequence Navigator™ Version 1.0.1 software package (Perkin Elmer, Applied Biosystems Division, Foster City, CA, USA), with minor manual adjustments. The G + C content was determined by inspection, and transition/transversion ratios using MacClade Version 3.01 (Maddison and Maddison, 1992). Sequence divergence among taxa was calculated using the DISTANCE MATRIX option in PAUP Version 3.1.1 (Swofford, 1993), based on unambiguously alignable regions (Fig. 1). All sequences used in this study are available from the authors.

***Phylogenetic analysis***—Phylogenetic trees were generated from unordered character states using PAUP Version 3.1.1 (Swofford, 1993), run on a Power Macintosh 6100/60 computer. In view of the large number of taxa included in this study the following heuristic search strategies were employed to find the most parsimonious trees: SIMPLE addition sequence with TBR (tree bisection-reconnection) swapping, and 500 replicates of RANDOM addition sequence with no swapping. This was followed by TBR swapping on the resulting trees. Random addition sequence has been suggested as a means to detect any multiple islands of most parsimonious trees (Maddison, 1991). The options MULPARS, STEEPEST DESCENT, COLLAPSE, and ACCTRAN optimization were selected.

Bootstrap analyses (Felsenstein, 1985) were performed using PAUP, set to HEURISTIC search option and SIMPLE addition sequence. Bootstrap values were calculated using 1000 replicates with MAXTREE set to 1000. Decay indices (DI: Bremer, 1988; Donoghue et al., 1992) for individual clades were obtained by comparing the strict consensus of all equal-length trees up to six steps longer than the shortest tree, using SIMPLE addition sequence and TBR in PAUP. Descriptive statistics reflecting the amount of phylogenetic signal in the parsimony analyses were given by the consistency index (CI: Kluge and Farris, 1969), retention index (RI: Farris, 1989), and the resulting rescaled consistency index (RC: Swofford, 1993). Additionally, the $g_1$ statistic (Hillis and Huelsenbeck, 1992) was obtained by calculating the tree-length distribution of 10 000 random trees using RANDOM TREES under PAUP to assess the amount of phylogenetic signal in the data set, in comparison to random noise.

Only combined ITS 1 and ITS 2 sequence data were subjected to phylogenetic analyses. For all analyses of sequence data, gaps (indels) were treated as missing data (Soltis and Kuzoff, 1995; Susanna et al., 1995; Downie and Katz-Downie, 1996). Indels were scored as a separate presence/absence character and added to the sequence data matrix (Wojciechowski et al., 1993; Oxelman and Lidén, 1995). To investigate the effect of these additional data, a separate analysis without a gap matrix was undertaken. Ambiguous regions that allowed alternative alignment interpretations were excluded from phylogenetic analyses (Wojciechowski et al., 1993; Downie and Katz-Downie, 1996). To determine the phylogenetic implications of the exclusion of a 54-bp region in ITS 2, due to a 47-bp deletion in three *Streptocarpus* species, a separate analysis was conducted including this area. Character state changes were weighted equally, except for one analysis in which character-state weighted parsimony was implemented: transversions were weighted over transitions by a factor of 1.6, corresponding to the higher value of the transition/transversion ratio in ITS 1, using PAUP (applying a user-defined step matrix set to 16:10 for transition:transversion events, as PAUP does not accept decimals in this option). Only the resulting tree length was adversely affected by this modification, being perceived by PAUP as ten times longer.

## RESULTS

***Sequence analysis***—Alignment of internal transcribed spacer sequences of the 29 taxa analyzed resulted in a 521-bp long data matrix (Fig. 1); their characteristics (including average G + C content) are summarized in Table 2. The number of unresolved bases ranged from 0 to 3 bp per sequence. It is likely that these unresolved bases represent genuine polymorphisms, as they occur in both forward and reverse sequencing reactions.

The length of ITS 1 and ITS 2 was, on average, 239.7 and 230.4 bp. Alignment of all taxa required the insertion of 31 gaps of 1–5 bp length (excluding one 47-bp deletion), 18 in ITS 1 and 13 in ITS 2, of which 13 and 10, respectively, were potentially informative. The lengths of aligned ITS 1 and ITS 2 regions were 265 and 256 bp, respectively. Due to alignment ambiguities (where alternative alignments were possible or large gaps present), 79 sites were excluded (identified by asterisks in Fig. 1) in all but one of the phylogenetic analyses: these comprised 22 sites in ITS 1 and 57 in ITS 2, of which one included a 47-bp deletion shared by three *Streptocarpus* taxa. Of the remaining 442 unambiguously aligned sites, 213 (48.2%) were constant, 161 (36.4%) were potentially informative phylogenetically, and 68 (15.4%) were autapomorphies, unique to individual taxa (Table 2).

Within the ingroup accessions, sequence divergence of unambiguously alignable positions of ITS 1 ranged from

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                          10        20        30        40        50        60        70        80        90
taxon          ITS 1      .         .         .         .         .         .         .         .         .
                          .         .         .    **** .   12    3  4 5    .         .6        7.        .
Hab.rhodopensis           TCGAAACCTGCAAAGCAGACCCGCGAACATGTTTAATGAAAAA-TATTGTGGCC-GTGGCGTCGGATGC-----TTCG-GTGCCCGGCAT
Ch.spadiciformis          TCGAAACCTGCAAAGCAGACCCGCGAACATGTTTA---AAATACGCTTG-CGTTCGCGACGTTGGATGC-----TTTT-GTGTCCAATGC
Sa.orbicularis            TCGAAACCTGCAAAGCAGACCAGTGAACCTGTTCC---ACACTAGATTGTGGTT-GAGATGCTTGATGCC----TTTTGGGGTCGAGCAT
Sa.velutina               TCGAAACCTGCAAAGCAGACCAGTGAACCTGTTCC---ACACTAGATTGTGGTT-GAGATGCTGGAACGCC----TTTTGGGGTCGAGCAT
Sa.rupicola               TCGAAACCTGCAAAGCAGACCAGTGAACCTGTTCC---ACACCAGATCGTGGTT-GAGATGCTGGACGCC----TTTTGGGGTCGAGCAT
Sa.diplotricha            TCGAAACCTGCAAAGCAGACCAGTGAACTTGTTCC---ACACTAGATTGTGGTT-GAGATGCTGGACGCC----TTTTGGGGTCGAGCAT
Sa.cf.ionantha            TCGAAACCTGCAAAGCAGACCAGTGAACCTGTTCC---ACACTAGATTGTGGTT-GAGATGCTGGACGCC----TTTTGGGGTCGAGCAT
Sa.grandifolia            TCGAAACCTGCAAAGCAGACCAGTGAACCTGTTCC---ACACTAGATkGTGGTT-GAGATGCTGGATGCC----TTTTGGGGTCGAGCAT
Sa.brevipilosa            TCGAAACCTGCAAAGCAGACCAGTGAACCTGTTCC---ACACCAGATTGTGGTT-GAGATGCTGGATGCC----TTTTGGGGTCGAGCAT
Sa.nitida                 TCGAAACCTGCAAAGCAGACCAGTGAACATGTTCC---ACACCAGATTGTGGTT-GAGATGCTGGACGCC----TTTTGGGGTCGAGCAT
Str.porphyrostachys       TCGAAACCTGCAAATCAGACCCGTGAACCTGTTTT--GAAAAA-GATTGTGGTTCGGGATGCTGGAAGCC----TCCGGGTGTCGAGCAT
Str.candidus              TCGAAACCTGCAAATGAGACCCGTGAACCTGTTTT--GAAAAA-GATTGTGGGTCGGGATGCTGGAAGCC----TCCAGGTGTCGAGCAT
Str.johannis              TCGAAACCTGCAAATCAGACCCGTGAACCTGTTTT--GAAAAAAGATTATGGGTCGGGATGCTGGAAGCC----TCCAGGTGTCGAGCAT
Str.rexii                 TCGAAACCTGCAAATCAGACCCGTGAACCTGTTTT--GAAAAAAGATTATGGGTCGGGATGCTGGAAGCC----TGCAGGTGTCGAGCAT
Str.primulifolius         TCGAAACCTGCAAATCAGACCCGTGAACCTGTTTT--GAAAAAAGATTATGGGTCGGGATGCTGGAAGCC----TCCAGGTGTCGAGCAT
Str.cyaneus               TCGAAACCTGCAAATCAGACCCGTGAACCTGTTTT--GAAAAAAGATTATGGGTCGGGATGCTGGAAGCC----TCCAGGTGTCGAGCAT
Str.modestus              TCGAAACTTGCAAATCAGACCCGTGAACCTGTTTT--GAAAAAAGATTATGGGTCGGGATGCTGGAAGCC----TCCAGGTGTCGAGCAT
Str.dunnii                TCGATACCTGCAAATCAGACCCGTGAACCTGTTTT---AAAAAAGACTGTGG-TCGGGATGCTCGAAGCC----TCCGGGTGTCGAGCTT
Str.eylesii               TCGAGACCTGCAAATCAGACCCGTGAACCTGTTTT---AAAAAA-ACTGTGG-TCGGGATGCTCGATGCC----TCTGGGTGTCGAGCTT
Str.wittei                TCGAGACCTGCAAATCAGACCCGTGAACCTGTTTT---AAAAAA-ACTGTGG-TCGGGATGCTCGATGCC----TCCGGGTGTCGAGCTT
Str.caulescens            TCGAAACCTGCAAAGCAGACCCGTGAACATGTTTT---AAACAAGATTGTGG-TCGAGATGCTGGACGCC----TTCGGGGGTCGAGCAT
Str.holstii               TCGAAACCTGCAAAGCAGACCCGTGAACATGTTTT---AAACAAGATTGTGG-TCGAGATGCTGGACGCC----TTCGGGGGTCGAGCAT
Str.glandulosissimus      TCGAAACCTGCAAAGCAGACCCGTGAACATGTTTT---AAACAAGATTGTGG-TCGAGATGCTGGACGCC----TTCGGGGGTCGAGCAT
Str.stomandrus            TCGAAACCTGCAAAGCAGACCCGTGAACATGTTTT---AAACAAGATTGTGG-TCGAGATGCTGGACGCC----TTGGGGAGTCGAGCAT
Str.kirkii                TCGAAACCTGCAAAGCAGACCCGTGAACATGTTTT---AAACAAGATTGTGG-TCGAGATGCTGGACGCC----TTTGGGAGTCGAGCAT
Str.saxorum               TCGAAACCTGCAAAGCAGACCCGTGAACATGTTTT---AAACAAGATTGTGG-TCGAGATGCTGGATGCC----TTGGGGGGTCTAGCAT
Str.thompsonii            TCGAGACCTGCAAAGCAGACCCGTGAACCAGTTT----AAACAATATATGCGGCCGAGGTGCCGGACGCCCCACTCCGGGAGTCGAGCAC
Str.sp.(Madagascar)       TCGAGACCTGCAAAGCAGACCCGTGAACCAGTTTC---AAACAATATATGCGGCCGAGGTGCCGGACGCCCCACTCCGGGAGTCGAGCAC
Str.hilsenbergii          TCGAGACCCGCAAGGCAGACCCGTGAACCGGTwTA---AAACGAGATATTTGGCCGAGGTGTTGGACGCCCGACTCCGGGGGTCGAGCAC

                          100       110       120       130       140       150       160       170       180
taxon                     .         .         .         1         .         .         .         .         1
                    **    .         .    8    . 9       0       *****       .         .         .         1
Hab.rhodopensis           CAC-GGCCCCGGTCCCAAGCAGCGCCAAGTTGGCTTGGG--CGTAC-----TAACCTCTCGGCGCGGCAAGCGCCAAGGAAAACCATAT-
Ch.spadiciformis          CAC-GACCTCGACCCTAAGTGGCG-CAAGTC-TCTTGGG--CGTGC-----TAACCTCTCGGCGCGAAAAGCGCCAAGGAAAAGCGTAC-
Sa.orbicularis            CA-TGGCTCCGACCCCAAGCGATG-AAAGTC-GTTTGGG--TGAGTAA---TAACCTCTCGGCGCAGCAAGCGCCAAGGAAAACCATAC-
Sa.velutina               CAC-GGCTCCGACCCCAAGCGATG-AAAGTC-GTTTGGG--TGAGTAA---TAACCTCTCGGCGCAGCAAGCGCCAAGGAAAACCATAC-
Sa.rupicola               CACTGGCTCCGACCCCAAGCGATG-AAAGTC-GTTTGGG--TGAGTAA---TAACCTCTCGGCGCAGCAAGCGCCAAGGAAAACCATAC-
Sa.diplotricha            CAC-GGCTCCGACCCCAAGCGATG-AAAGTC-GTTTGGG--TGAGTAA---TAACCTCTCGGCGCAGCAAGCGCCAAGGAAAACCATAC-
Sa.cf.ionantha            CAC-GGCTCCGACCCCAAGCGATG-AAAGTC-GTTTGGG--TGAGTAA---TAACCTCTCGGCGCAGCAAGCGCCAAGGAAAACCATAC-
Sa.grandifolia            CA-TGGCTCCGACCCCAAGsGATG-AAAGTC-GTTTGGG--TGAGTAA---TAACCTCTCGGCGCAGCAAGCGCCAAGGAAAACCATAC-
Sa.brevipilosa            CAC-GGCTCCGACCCCAAACGATG-AAAGTC-GTTTGGG--TGAGTAA---TAACCTCTCGGCGCAGCAAGCGCCAAGGAAAACCATAC-
Sa.nitida                 CAC-GGCTCCGACCCCAAACGATG-AAAGTC-GTTTGGG--TGAGTAA---TAACCTCTCGGCGCAGCAAGCGCCAAGGAAAACCATAC-
Str.porphyrostachys       CAC-GGCCCCGACCTCAAGCGATG-CAAGTC-CCTTGGGGGCGAGTAA---TAACCTCTCGGCGTGGCAAGCGCCAAGGAAAACAATAC-
Str.candidus              CAC-GGCCCCGACCTCAAGCGATG-CAAGTC-CTTTGGGGGCGAGTAA---TAACCTCTCGGCGCGGCAAGCGCCAAGGAAAACAATAC-
Str.johannis              CAC-GGCCCCGACCTCAAGCGATG-CAAGTC-CCTTGGGGGCGAGTAA---TAACCTCTCGGCGCGGCATGCGCCAAGGAAAACAATAT-
Str.rexii                 CAC-GGCCCCGACCTCAAGCGATG-CAAGTC-CCTTGGGGGCGAGTAA---TAACCTCTCGGCGCGGCAAGCGCCAAGGAAAACAATAT-
Str.primulifolius         CAC-GGCCCCGACCTCAAGCGATG-CAAGTC-CCTTGGGGGCGAGTAA---TAACCTCTCGGCGCGGCAAGCGCCAAGGAAAACAATAT-
Str.cyaneus               CAC-GGCCCCGACCTCAAGCGATG-CAAGTC-CCTTGGGGGCGAGTAA---TAACCTCTCGGCGCGGCAAGCGCCAAGGAAAACAATAT-
Str.modestus              CAC-GGCCCCGACCTCAAGCGATG-CAAGTC-CCTTGGGGGCGAGTAA---TAACCTCTCGGCGCGGCAAGCGCCAAGGAAAACAATAT-
Str.dunnii                CAC-GGCCCCGACCTCAAGAGACG-CAAGTC-CCTTGGG--CGAGTAATAATAACCTCTCGGCGCGGGAAGCGCCAAGGAAAACAATACG
Str.eylesii               CAC-GGCCCCGACCTCAAGAGACG-CAAGTC-CCTTGGG--CGAGTAA---TAACCTCTCGGCGCGGGAAGCGCCAAGGAAAACAATACA
Str.wittei                CAC-GGCCTCGACCTCAAGAGACG-CAAGTC-CCTTGGG--CGAGTAA-AATAACCTCTCGGCGTGGGAAGCGCCAAGGAAAACAATACA
Str.caulescens            CA-TGGCTCCGACCCCAAGCGATG-AAAGTC-GCTTGGG--TGAGTAA--ACAACCTCTCGGCGCAGCAAGCGCCAAGGAAAACCTTAC-
Str.holstii               CA-TGGCTCCGACCCCAAGCGATG-AAAGTC-GCTTGGG--TGAGTAA--ACAACCTCTCGGCGCAGCAAGCGCCAAGGAAAACCTTAC-
Str.glandulosissimus      CA-TGGCTCCGACCCCAAGCGATG-AAAGTC-GCTTGGG--TGAGTAA--ACAACCTCTCGGCGCAGCAAGCGCCAAGGAAAACCTTAC-
Str.stomandrus            CAC-GGCTCCGATCCCTAGCGATG-CGAGTC-GCTTGGG--TGAGTAT---TAACCTCTCGGCGCAGTAAGCGCCAAGGAAAACCATAC-
Str.kirkii                CAC-GGCTCCGATCCCTAGCGATG-CGAGTC-GCTTGGG--TGAGTAT---TAACCTCTCGGCGCAGTAAGCGCCAAGGAAAACCATAC-
Str.saxorum               CAC-GGCTCCGATCCCTAGCGATG-CGAGTC-GCTCGGG--TGAGTAA---TAACCTCTCGGCGCAGTAAGCGCCAAGGAAAACCATAC-
Str.thompsonii            CGC-GGCCCCGACCCCGAGCGACG-AGAGTC-GTTCGGG--CGAGTAA---CAAACACTCGGCGCAGCGCGCGCCAAGGAAGAACATAC-
Str.sp.(Madagascar)       CGC-GGCCCCGACCCCGrGCGACG-AGAGTC-GTTCGGG--CGAGTAA---CAAACACTCGGCGCAGCGCGCGCCAAGGAArAACATAC-
Str.hilsenbergii          CGC-GGCCCCGACCCCGGGCGACG-TGAGTC-ATTCGGG--CGAGCAA---CAAACACTCGACACAACACGCGCCAAGGAAGAACATAC-
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Fig. 1. Sequence data matrix of aligned ITS 1 and ITS 2 regions of nuclear ribosomal DNA of 29 taxa of Gesneriaceae. Nucleotide sequence displayed from 5′ to 3′. ITS 1 ranges from site 1 to 265 and ITS 2 ranges from site 266 to 521. Uncertain nucleotide states are coded according to PAUP conventions (Swofford, 1993): n = A/C/G/T, k = G/T, r = A/G, s = C/G, w = A/T, y = C/T; hyphens denote alignment gaps; * = nucleotide sites excluded from part of the phylogenetic analyses; numbers in italic print above nucleotide matrix, ranging from 1 to 31, indicate the number and position of alignment gaps; numbers in square brackets at the end of sequences indicate the actual spacer length of the combined region of ITS 1 plus ITS 2.

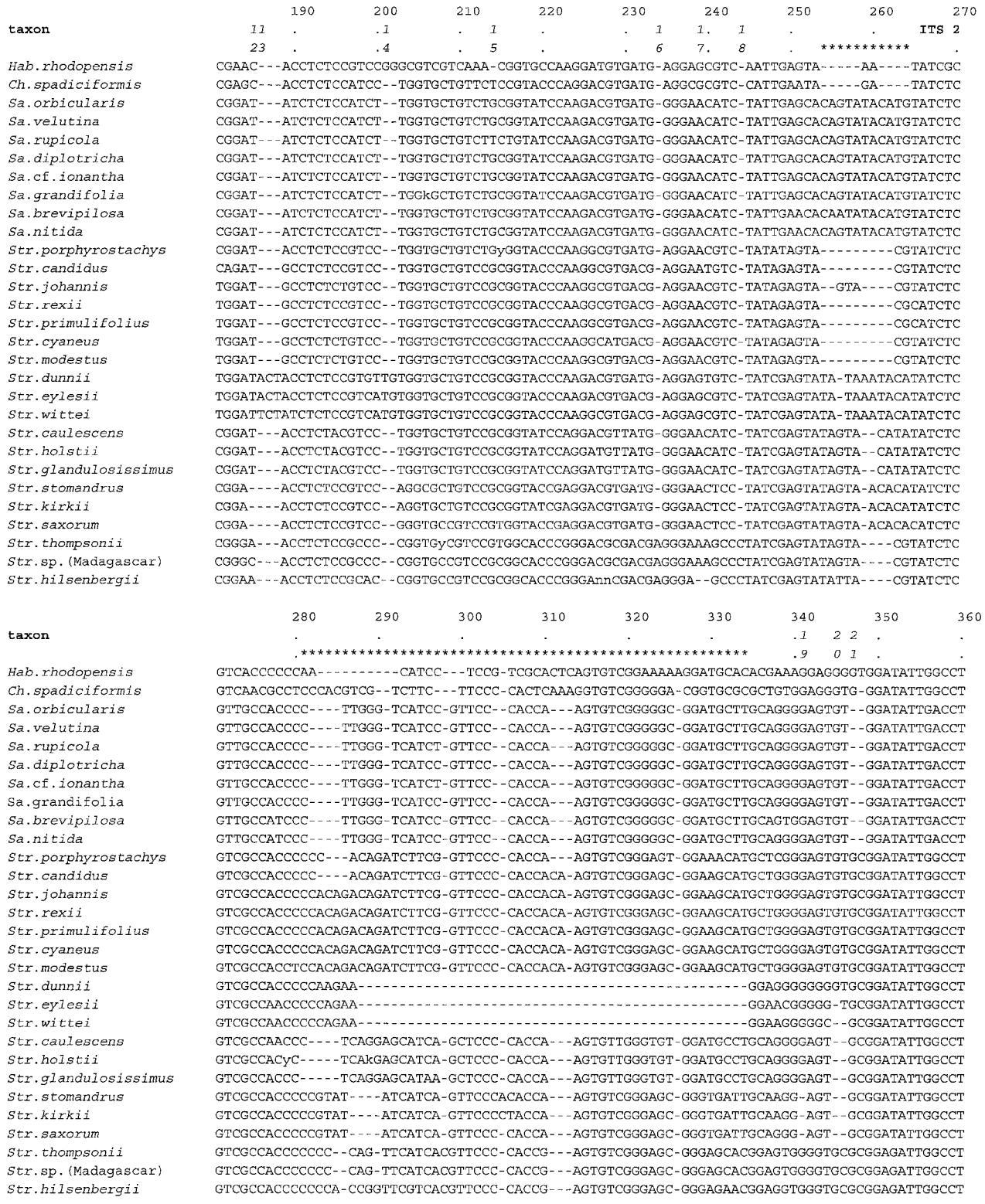


Fig. 1. Continued.

0 to 27.2%, and from 22.0 to 32.9% between ingroups and outgroups. ITS 2 was less variable and only up to 19.6% divergence was observed between ingroup taxa, while 12.7 to a maximum of 24.6% sequence divergence in pairwise comparisons was evident between ingroups and outgroups. Pairwise comparisons of individual taxa across both spacer regions revealed 0–23.5% sequence divergence within the ingroup, and 20.0–29.0% divergence between ingroup and outgroup taxa analyzed (Table 2). Little sequence variation was observed among

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                          370        380        390        400        410        420        430        440        450
taxon                     .          .          .          2          .          . 22       .          . 2        .
                          . ***      .          .          2          .          . 34       .          . 5        .
Hab.rhodopensis           CCCGTTATCTT---GTGTAGCGGCCGGCCCAAATAGGAT-ACCGTGTCGATGGACGTCGCACGATACGTGGTGGTTGGATTTCTCAACTT
Ch.spadiciformis          CCCGTTATCCTTGCGCGTAGCGGCTGGCCCAAACAACAT-ACCGTGCCGAAGGAAGTCGCACGATACGTGGTGGTTGGATTCCTCAACTT
Sa.orbicularis            CCCGTCACCTA---GTGTAGCGGCTGGCCCAAATAGTAT-ACCGTGTCGATGTATCACACTT-ATACGTGGTTGTTGTATT-CACGACTT
Sa.velutina               CCCGTCACCTA---GTGTAGCGGCTGGCCCAAATAGTAT-ACCGTGTCGATGTATCACACTT-ATACGTGGTTGTTGTATT-CACGACTT
Sa.rupicola               CCCGTCACCTA---GTGTAGCGGCTGGCCCAAATAGTAT-ACCGTGTCGATGTATCACACT--ATACGTGGTTGTTGTATT-CACGACTT
Sa.diplotricha            CCCGTCACCTA---GTGTAGCGGCTGGCCCAAATAGTAT-ACCGTGTCGATGTATCACACTT-ATACGTGGTTGTTGTATT-CACGACTT
Sa.cf.ionantha            CCCGTCACCTA---GTGTAGCGGCTGGCCCAAATAGTAT-ACCGTGTCGATGTATCACACTT-ATACGTGGTTGTTGTATT-CACGACTT
Sa.grandifolia            CCCGTCACCTA---GTGTAGCGGCTGGCCCAAATAGTAT-ACCGTGTCGATGTATCACACTT-ATACGTGGTTGTTGTATT-CACGACTT
Sa.brevipilosa            CCCGTCACCTA---GTGTAGCGGCTGGCCCAAATAGTAT-ACCGTGTCGATGTATCACACT--ATACGTGGTTGTTGTATT-CACGACTT
Sa.nitida                 CCCGTCACCTA---GTGTAGCGGCTGGCCCAAATAGTAT-ACCGTGTCGATGTATCACACT--ATACGTGGTTGTTGTATT-CACGACTT
Str.porphyrostachys       CTCGTTACCTCT-CGTGTAGCGGCTGGCACAAACAGGAT-ACCGTGTCGATGGATGTCACACGATACGTGGTGGTTGGATTCCACAACTC
Str.candidus              CCCGTTACCTC---GTGTAGCGGCTTGCCCAAACAGGAT-ACCGTGTCGATGGATGTCACACGATACGTGGTGGTTGGATTCCACAACTC
Str.johannis              CCCGTTACCTC---GTGTAGCGGCTGGCCCAAACAGGAT-ACCGTGTCGATGGATGTCACACGATACGTGGTGGTTGGATTCCACAACTC
Str.rexii                 CCCGTTACCTC---GTGTAGCGGCTGGCCCAAACAGGAT-ACCGTGTCGATGGATGTCACACGATACGTGGTGGTTGGATTCCACAACTC
Str.primulifolius         CCCGTTACCTC---GTGTAGCGGCTGGCCCAAACAGGAT-ACCGTGTCGATGGATGTCACACGATACGTGGTGGTTGGATTCCACAACTC
Str.cyaneus               CCTGTTACCTC---GTGTAGCGGCTGGCCCAAACAGGAT-ACCGTGTCGATGGATGTCACACGATACGTGGTGGTTGGATTCCACAACTC
Str.modestus              CCCGTTACCTC---GTGTAGCGGCTGGCCCAAACAGGAT-ACCGTGTCGATGGATGTCACACGATACGTGGTGGTTGGATTCCACAACTC
Str.dunnii                CCCGTCACCTC---GTGTAGCGGCTGGCCCAAACAGGAT-ACCGTGTCGATGGATGTCACACGATACGTGGTGGTTGGATTCCACAACTT
Str.eylesii               CCCGTCACCTC---GTGTAGCGGCTGGCCCAAACAGGAT-ACCGTGTCGATGGATGTCACACGATACGTGGTGGTTGGATTCCACAACTT
Str.wittei                CCCGTCACCTC---GTGTAGCGGCTGGCCCAAACAGGAT-ACCGTGTCGATGGATGTCACACGATACGTGGTGGTTGGATTCCACAACTT
Str.caulescens            CCCGTCACCTC---GTGTAGCGGTTGGCTCAAATAGGAT-ACCGTGTCGATGGATGTCACACGATACGTGGTGGTTGGATT-CACAACTT
Str.holstii               CCCGTCACCTC---GTGTAGCGGTTGGCTCAAATAGGAT-ACCGTGTCGATGGATGTCACACGATACGTGGTGGTTGGATT-CACAACTT
Str.glandulosissimus      CCCGTCACCTC---GTGTAGCGGTTGGCTCAAATAGGAT-ACCGTGTCGATGGATGTCACACGATACGTGGTGGTTGGATT-CACAACTT
Str.stomandrus            CCCGTCACCTC---GTGTGGCGGCTGGCCCAAATAGGAT-ACCGTGTCGATGGATGTCACACGATACGTGGTGGTTGGAAT-CACAACTT
Str.kirkii                CCCGTCACCTC---GTGTGGCGGCTGGCCCAAATAGGAT-ACCGTGTCGATGGATGTCACACGATACGTGGTGGTTGGAAT-CACAACTT
Str.saxorum               CCCGTCACCTC---GTGTGGCGGCTGGCCCAAATAGGATAACCGTGTCGATGGATGTCACACGATATGTGGTGGTTGGATT-CACAACTT
Str.thompsonii            CCCGTCACCCC--CGAGTAGCGGCTGGCCCAAATAGGTT-ACCGTGTCGATGGATGTCACGCGATACGTGGTGGTTGGATCCCACAACTC
Str.sp.(Madagascar)       CCCGTCACCCC--CGAGTAGCGGCTGGCCCAAATAGGTT-ACCGTGTCGATGGATGTCACGCGATACGTGGTGGTTGGATCCCACAACTC
Str.hilsenbergii          CCCGTCACCCCC-CGAGTAGCGGCTGGCCCAAATATGTT-ACCGTGTCGATGGATGTCACGCGATACGTGGTGGTCGGATCCCACGACTC

                          460        470        480        490        500        510        520
taxon                  2  .       2  2.         .          2.         . 3        . 3        .
                       6  .       7  8.         .          9.         . 0        . 1        .
Hab.rhodopensis           GCG-AACTGTCGTGT-GGGACTGCCTCGAGCTACGGGC---ACGGCCCAAT-GGCACCCGG-TGCCCTCGA   [464]
Ch.spadiciformis          GCG-AGCTATCGTGTAGGGACTGCATCGAGCCACGGAC---ACGACCCAAC-GGCAAAAGATTGCCCTCGA   [470]
Sa.orbicularis            GCG-AACTATCGTTC-GA----ACATCGAGCCACGGGC---ACGACCCAATAGGCACAAGC-TGTCCTCGT   [467]
Sa.velutina               GCG-AACTGTCGTTC-GA----ACATCGAGCCACGGGC---ACGACCCAATAGGCACAAGC-TGTCCTCGT   [467]
Sa.rupicola               GCG-AACTGTCGTTC-GA----ACATCGAGCCACGGGC---ACGACCCAATAGGCACAAGC-TGTCTTCGG   [467]
Sa.diplotricha            GCG-AACTGTCGTTC-GA----ACATCGAGCCACGGGC---ACGACCCAATAGGCACAAGC-TGTCCTCGT   [467]
Sa.cf.ionantha            GCG-AACTGTCGTTC-GA----ACATCGAGCCACGGGC---ACGACCCAATAGGCACAAGC-TGTCCTCGT   [467]
Sa.grandifolia            GCG-AACTGTCGTTC-GA----ACATCGAGCCACGGGC---ACGACCCAATAGGCACAAGC-TGTCCTCGT   [467]
Sa.brevipilosa            GCG-AACTGTCGTTC-GA----ACATCGAGCCACGGGC---ACGACCCAATAGGCACAAGC-TGTCCTCGG   [466]
Sa.nitida                 GCG-AACTGTCGTTC-GA----ACATCGAGCCACGGGC---ACGACCCAATAGGCACAAGC-TGTCATCGG   [466]
Str.porphyrostachys       TCGTAACTGTCGTGT-GAGACCGCATCGAGACACGGGC---ACGACCCCGT-GGCATCGGC-TGCCCTCGA   [474]
Str.candidus              TCGTAACTGTCGTGT-GAGACCGCATCGAGACACGGGC---ACGACCCCGT-GGCACTGGC-TGCCCTCGA   [473]
Str.johannis              TCGTAACTGTCGTGT-GAGACCTCATCGAGACACGAGC---ACGACCCCGT-GGCACCGGC-TGCCCTCGA   [481]
Str.rexii                 TCGTAACTGTCGTGT-GAGACCGCATCAAGACACGAGC---ACGACCCCGT-GGCACCGGC-TGCCCTCGA   [478]
Str.primulifolius         TCGTAACTGTCGTGT-GAGACCGCATCAAGACACGAGC---ACGACCCCGT-GGCACCGGC-TGCCCTCGA   [478]
Str.cyaneus               TCGTAACTGTCGTGT-GAGACCGCATCGAGACACGAGC---ACGACCCCGT-GGCACCGGC-TGCCCTCGA   [478]
Str.modestus              TCGTAACTGTCGTGT-GAGACCTCATCGAGACACGAGC---ACGACCCCGT-GGCACCGGC-TGCCCTCGA   [478]
Str.dunnii                TCG-AACTGTCGTGT-GAGACCGCATCGAGACACGGGC---ACTACCCCAT-GGCACCGGT-TGCCCTCGA   [447]
Str.eylesii               TTG-AACTGTCGTGT-GAGACCGCATCGAGACACGGGC---ACGACCCCAT-GGCACCTGC-TGCCCTCGA   [442]
Str.wittei                TCG-AACTGTCGTGT-GAGACCGCATCGAGACACGAGC---ACGACCCCAT-GGCACCTGC-TGCCCTCGA   [443]
Str.caulescens            GCG-AACTGTCGTGT-GAGACCACATCGAGCCACGGGA---ATGACCCAATAGGCACAAGC-TGCCCTCGG   [473]
Str.holstii               GCG-AACTGTCGTGT-GAGACCACATyGAGCCACGGGA---ATGACCCAATAGGCACAAGC-TGCCCTCGG   [472]
Str.glandulosissimus      GCG-AACTGTCGTGT-GAGACCACATCGAGCTACGGGA---ATGACCCAATAGGCACAAGC-TGCCCTCGG   [472]
Str.stomandrus            GCG-AACTGTCGTGT-GAGATCACATCGAGGCACGTGC---ATGACCCAATAGGCACAAGA-TGCCCTCGG   [472]
Str.kirkii                GCG-AACTGTCGTGT-GAGATCACATCCAGGCACGTGC---ATGACCCAATAGGCACAAGA-TGCCCTCGG   [472]
Str.saxorum               GCG-AACTGTCGTAT-GATATCACATCGAGGCACGTGC---ATGACCCAATAGGCACAAGA-TGCCCTCGG   [472]
Str.thompsonii            TCG-AACCGTCGCGT-GAGACCGCATCGATACACGGGCAAAAAGACCCAACAGGCACAAGC-TGCCCTCGG   [485]
Str.sp.(Madagascar)       TCG-AACCGTCGCGT-GAGACCGCATCGATACACGGGCAAAACGACCCAACAGGCACAAGC-TGCCCTCGG   [486]
Str.hilsenbergii          TCG-AACCGTCGCGT-GAGACCGCATCGATACACGGGAAAAACGACCCAACAGGCACAAGC-TGCCCTCGG   [487]
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Fig. 1. Continued.

*Saintpaulia* accessions. The maximum of 2.2% divergence (nine character changes) occurred between *Saintpaulia brevipilosa* and *Saintpaulia rupicola,* whereas the sequences of *Saintpaulia* cf. *ionantha* and *Saintpaulia velutina* were identical. Low sequence divergence was also present between (1) *Streptocarpus rexii* and *Streptocarpus primulifolius* (1 bp), (2) *Streptocarpus holstii, Streptocarpus glandulosissimus,* and *Streptocarpus caulescens* (1–3 bp), and (3) *Streptocarpus thompsonii* and *Streptocarpus* sp. (Madagascar) (3 bp). The greatest pair-

TABLE 2. Sequence characteristics of ITS 1 and ITS 2 regions of 29 taxa of Gesneriaceae.

| Parameter | ITS 1 | ITS 2 | ITS 1 and ITS 2 |
|---|---|---|---|
| Length range (total) (bp) | 228–249 | 196–245 | 442–487 |
| Length mean (total) (bp) | 239.7 | 230.4 | 470.1 |
| Length range (ingroup) (bp) | 235–249 | 196–245 | 442–487 |
| Length mean (ingroup) (bp) | 240.3 | 229.9 | 470.2 |
| Length range (outgroup) (bp) | 228–233 | 231–242 | 464–470 |
| Length mean (outgroup) (bp) | 230.5 | 236.5 | 467.0 |
| Aligned length (bp) | 265 | 256 | 521 |
| G + C content range (%) | 50.2–65.3 | 54.4–63.7 | 52.3–64.3 |
| G + C content mean (%) | 54.3 | 57.9 | 56.1 |
| Number of excluded sites (%) | 22 | 57 | 79 |
| Sequence divergence (ingroup) (%)[a] | 0–27.2 | 0–19.6 | 0–23.5 |
| Sequence divergence (in/outgroup) (%)[a] | 22.0–32.9 | 12.7–24.6 | 20.0–29.0 |
| Number of indels (ingroup)[a] | 5 | 2 | 7 |
| Number of indels (total)[a] | 18 | 13 | 31 |
| Size of indels (ingroup)[a] | 1–4 | 1–4 | 1–4 |
| Size of indels (total)[a] | 1–5 | 1–4 | 1–5 |
| Number of sites after exclusion[a] | 243 | 199 | 442 |
| Number of variable sites[a] | 138 | 91 | 229 |
| Number of constant sites (%)[a] | 105 (43.2) | 108 (54.3) | 213 (48.2) |
| Number of informative sites (%)[a] | 99 (40.7) | 62 (31.1) | 161 (36.4) |
| Number of autapomorphic sites (%)[a] | 39 (16.1) | 29 (14.6) | 68 (15.4) |
| Transitions (minimum)[a] | 142 | 75 | 217 |
| Transversions (minimum)[a] | 86 | 64 | 150 |
| Transitions/transversions[a] | 1.65 | 1.17 | 1.45 |
| Skewness of tree-length distribution ($g_1$ value for 10 000 random trees)* | −0.522 | −0.677 | −0.566 |

[a] Based on alignment excluding ambiguous sequence sites.

wise distance among the *Streptocarpus* accessions was 21% (87 bp) between the Malagasy *Streptocarpus hilsenbergii* and the African *Streptocarpus porphyrostachys*.

***Phylogenetic analysis***—Parsimony analysis of unambiguously aligned ITS sequences yielded one most parsimonious tree when the coded gaps were added to the data matrix (Fig. 2). When all uninformative characters were included the tree had a length of 419 steps, and 336 steps with autapomorphies excluded, with CIs of 0.788 and 0.737, respectively. This is considerably higher than the value of 0.441 calculated for 29 taxa from the study of this statistic by Sanderson and Donoghue (1989). The RI was 0.902, and thus the RC was 0.711 with, and 0.665 without, uninformative characters.

The high negative value of −0.566 in the $g_1$ statistic of 10 000 random trees based on the sequence data indicated a significant skewness of the length distribution, rather than a normal random distribution ($g_1 = -0.09$ for 250 variable positions and 25 or more taxa; $P < 0.01$), suggesting that the data matrix contains considerable phylogenetic signal, rather than random noise (Hillis and Huelsenbeck, 1992). The average number of nucleotide substitutions per character was 0.886, with seven out of 473 sites changing four or five times. This is lower than was observed in a previous study on Apioideae (Downie and Katz-Downie, 1996), indicating a relatively low saturation of mutation in the observed rDNA gene spacers. It is, therefore, unlikely that phylogenetic signal has become obscured by multiple substitutions. The homoplasy index (HI) of the present data matrix was low (HI = 0.212). The bootstrap values for individual clades ranged from 62 to 100% (Fig. 2).

Twenty-eight base substitutions and one insertion event separated the outgroup taxon *Chirita spadiciformis* from *Streptocarpus* and *Saintpaulia*. These ingroup taxa formed two major clades, one consisting of unifoliate and rosulate *Streptocarpus* [bootstrap value (BS) = 93%, DI = +5], the other consisting of *Saintpaulia* and all caulescent *Streptocarpus* (BS = 86%; DI = +4). Within the unifoliate/rosulate clade the unifoliate species form a sister clade to the rosulate types (BS = 100%; DI = >+6). The caulescent *Streptocarpus* species originating from Madagascar (BS = 100%; DI = >+6) were separated from the African caulescent *Streptocarpus* and *Saintpaulia* species (BS = 95%; DI = +5). *Saintpaulia* is a sister group to the African caulescent *Streptocarpus* species (BS = 100%; DI > +6). The phylogenetic analyses also resolved relationships within the *Saintpaulia* clade, tentatively separating *Saintpaulia brevipilosa* and *Saintpaulia nitida* (BS = 91%; DI = +3) from *Saintpaulia rupicola* and those three taxa from the other *Saintpaulia* accessions analyzed (BS =70%; DI = +1).

Reanalyzing the combined ITS 1 and ITS 2 data matrix without the gap matrix yielded three most parsimonious trees of 382 steps (CI excluding uninformative characters = 0.731; RI = 0.897; RC = 0.702). The strict consensus tree was identical to the tree shown in Fig. 2, except for the collapse of a branch separating *Saintpaulia rupicola* and *Saintpaulia brevipilosa*/*S. nitida* from the rest of the *Saintpaulia* accessions. Also, decay indices were generally lower, and for the branch supporting the clade of African caulescent *Streptocarpus* the index was reduced to one step, the branch supporting the African caulescent *Streptocarpus* and *Saintpaulia* reduced to three steps, and the branch separating all caulescent *Streptocarpus* and *Saintpaulia* from rosulate and unifoliate *Streptocarpus* was reduced to two steps. Of the 23 potentially infor-

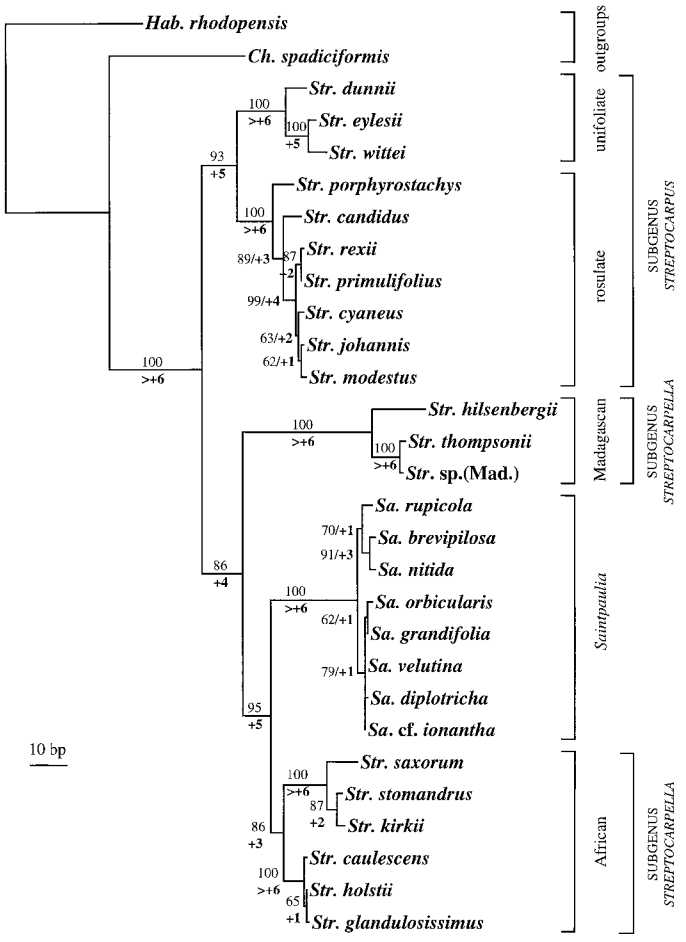


Fig. 2. Most parsimonious tree for *Streptocarpus* and *Saintpaulia* of 419 steps length based on parsimony analysis of the combined ITS 1 and ITS 2 sequence data plus the alignment gap matrix. Upper numbers are bootstrap values of 1000 replicates. Lower (boldface) numbers are decay indices (the number of steps necessary to cause collapse of monophyletic groups).

mative indels, all but five were congruent with the tree topology when superimposed onto the strict consensus tree. Four of those incongruent characters were 1- or 2-bp deletions that occurred twice on the tree, shared mostly between one ingroup and one of the outgroups. Only one 1-bp deletion (alignment gap 1 in Fig. 1) occurred in three independent lineages. These homoplastic indels may indicate the relatively large phylogenetic distance of the two outgroups from the ingroup.

Inclusion of the 54-bp gap region in ITS 2 in phylogenetic analyses resulted in six most parsimonious trees of 449 steps (CI excluding uninformative characters = 0.782; RI = 0.895; RC = 0.700). The strict consensus tree was identical to the strict consensus tree obtained where the gap region was excluded, and inclusion of this region did not improve the resolution between taxa.

The transition/transversion ratio was 1.65 for ITS 1 and 1.17 for ITS 2, and 1.45 for the combined data matrix. Altering the character weights to 1.6:1 to accommodate the transition ratio and reanalyzing the data (without the gap matrix attached) in a weighted parsimony analysis gave three most parsimonious trees of 472 steps. The resulting strict consensus tree had an identical topology to the strict consensus tree (gap matrix excluded) for the unweighted parsimony analysis.

## DISCUSSION

***Molecular evolution of ITS***—The size of ITS 1 and ITS 2 regions reported here are within the range observed for terrestrial plants in previous studies (Suh et al., 1993; Wojciechowski et al., 1993; Downie and Katz-Downie, 1996). In *Streptocarpus* and *Saintpaulia,* these spacers evolved not only by base substitutions but also by a comparatively large number of insertion and deletion events. The indel events appeared to be congruent with sequence substitution pattern, as only one difference separated the most parsimonious tree based on sequence and gap character and the strict consensus tree based on solely the sequence data, and addition of the gap matrix resulted in markedly higher decay indices. A remarkably large deletion in ITS 2 is shared among the three unifoliate *Streptocarpus* taxa. The significance of this deletion in terms of transcription is not known. Although the internal transcribed spacers are thought to be important in posttranscriptional processing and thus conserved to some extent, the levels of sequence variation between the taxa examined are considerable and similar to intergeneric levels found in other angiosperms. For example, in Brassicaceae sequence differences between *Sinapis alba* L. and *Arabidopsis thaliana* (L.) Heynh. were 24.3% for ITS 1 and 18.9% for ITS 2 (Rathgeber and Capesius, 1989); in Asteraceae subtribe Madiinae the range of sequence divergence ranged from 0.4 to 19.2% in ITS 1 and 0 to 12.9% in ITS 2 (Baldwin, 1992); in Apiaceae subfamily Apioideae from 0.5 to 33.2% in ITS 1 and 0 to 33.2% in ITS 2 (Downie and Katz-Downie, 1996). Maximum sequence divergence within *Streptocarpus* was 26% for ITS 1 and 15.7% for ITS 2, which is much higher than the maxima of 10.2% for ITS 1 and 7.9% for ITS 2 in a group of *Astragalus* (Wojciechowski et al., 1993), 4.3 and 3.0% of the same spacers in *Calycadenia* (Baldwin, 1993), or 3.8% in *Heuchera* or 8.4% in *Mitella* over both spacers (Soltis and Kuzoff, 1995), but similar to *Centaurea* (Susanna et al., 1995). The high sequence divergence for *Streptocarpus* may indicate that either the genus is comparatively old [Hilliard and Burtt (1971) suggested that the occurrence of the genus in both Africa and Madagascar may reflect a continuous distribution prior to continental breakup], or that ITS sequences evolve particularly fast in this genus. However, within *Saintpaulia* the sequence divergence was too low (0–0.2%) for clear phylogenetic resolution of some species (*Saintpaulia diplotricha–velutina–*cf. *ionantha* group). This may indicate a rapid radiation in *Saintpaulia* as discussed below.

***Phylogenetic relationships within and between Streptocarpus and Saintpaulia***—The molecular phylogenetic analyses indicate that the genus *Streptocarpus* is not monophyletic, rather a monophyletic *Saintpaulia* is nested within a paraphyletic *Streptocarpus* (Fig. 2). The results presented here indicate that *Saintpaulia* has evolved from within the genus *Streptocarpus* subgenus *Streptocarpella.* This supports Hilliard and Burtt (1971), who suggested a close relationship among African genera of

Gesneriaceae, including *Streptocarpus* and *Saintpaulia*. Some earlier taxonomists placed *Streptocarpus* and *Saintpaulia* in separate tribes: Fritsch (1893–1894) recognized a tribe Streptocarpeae and placed *Saintpaulia* in the Ramondeae. Ivanina (1966) retained *Streptocarpus* as a subtribe of Didymocarpeae and recognized an independent tribe Saintpaulieae based on carpel morphology. However, Burtt (1963) and Burtt and Wiehler (1995) merged both in the tribe Didymocarpeae, re-emphasising their similarities. Sister to *Saintpaulia* is a group consisting of African members of *Streptocarpus* subgenus *Streptocarpella,* split into two clades of three species each. In the phylogenetic analyses neither of the two groups seems to be more closely related to *Saintpaulia,* as bootstrap values and decay indices for the support of the respective branches are similar (Fig. 2). The close relationship between *Saintpaulia* and *Streptocarpus* subgenus *Streptocarpella* is supported by morphological features, such as the fact that both have verruculose seeds, and cytologically, as both have the same chromosome number ($2n = 30$). In contrast, subgenus *Streptocarpus* has reticulate seeds and a chromosome number of $2n = 32$ (Ratter, 1975). Another possible link between subgenus *Streptocarpella* and *Saintpaulia* is the presence of an orange disc, which is characteristic of *Saintpaulia,* in certain caulescent Madagascan *Streptocarpus* species (R. Smith and B. L. Burtt, personal communications, Honorary Associates, RBGE).

The genus *Streptocarpus* is, in vegetative morphology at least, one of the more diverse in the plant kingdom. The phylogenetic analyses here clearly separate the species analyzed into the two subgenera: the unifoliate/rosulate group (subgenus *Streptocarpus*) and the caulescent forms from Africa and Madagascar (subgenus *Streptocarpella*) (Hilliard and Burtt, 1971). Moreover, the two growth forms within subgenus *Streptocarpus,* the unifoliate (*Streptocarpus dunnii, Streptocarpus eylesii* and *Streptocarpus wittei*), and the rosulate forms, were clearly separated into two distinct clades. Hilliard and Burtt (1971), on the basis of floral morphology and other characters, placed *Streptocarpus porphyrostachys* apart from the rest of the taxa included in that clade, which is supported by the molecular data (Fig. 2). The phylogenetic clustering of *Streptocarpus johannis* and *Streptocarpus modestus* is also congruent with the systematic position as they are placed as intermediates between group C (*Streptocarpus rexii, Streptocarpus primulifolius, Streptocarpus cyaneus*), group B (*Streptocarpus candidus*) and group D by Hilliard and Burtt (1971). To resolve the clade of rosulate *Streptocarpus* more reliably more extensive sampling is needed; only 10 out of the estimated 90 species of subgenus *Streptocarpus* described were included in this study.

***Evolution of Saintpaulia***—The main differences between typical *Streptocarpus* subgenus *Streptocarpella* and *Saintpaulia* are the absence of a marked aerial stem (*Saintpaulia* mainly being a rosette herb) and the absence of a marked corolla tube in the latter. These features may be related to the habitat. The chasmophytic (wet cliff) habitat of many *Saintpaulia* species may have been an evolutionary force. Many chasmophytes (e.g., *Saxifraga*) are typically rosette plants. The absence of a marked corolla tube may be associated with the loss in the past of specialist long-tongued pollinators and a switch to generalist short-tongued insects, as has been implicated in the evolution of dioecy (Bawa, 1994). Also, the two large exserted yellow anthers of *Saintpaulia* imply that it is a pollen flower rather than a nectar flower, further supporting a pollinator switch theory. Another significant difference between *Saintpaulia* and *Streptocarpus* is the absence of a twisted fruit in *Saintpaulia.* It is a generic character of *Streptocarpus,* but is also found in *Boea, Dichiloboea, Ornithoboea, Paraboea, Rhabdothamnopsis,* and *Trisepalum.* These genera do not show close relationships with *Streptocarpus* in other characters, and the character of twisted fruit may have been derived independently several times in the family. It is interesting that *Streptocarpus capuronii* has only a very slight or absent twist. The presumed loss of the twisting of the fruit during the evolution of *Saintpaulia* may be associated with the shortening of the fruit (they are almost globose in some species) and ecological differences: twisting of the fruit may extend the period of seed dispersal (Hilliard and Burtt, 1971).

*Streptocarpus* subgenus *Streptocarpella* includes ~15 species in tropical mainland Africa, and ~20 species occurring in Madagascar. This may indicate an origin for the subgenus in Madagascar and the evolution in two lines on the African mainland, one being *Saintpaulia.* The topology of the most parsimonious tree in Fig. 2 reflects the geographical distribution of the genus *Saintpaulia.* For example, *Saintpaulia rupicola* originates from Kaloleni in south Kenya, whereas *Saintpaulia brevipilosa* and *Saintpaulia nitida* are distributed in the Nguru Mountains in eastern Tanzania. All three species are geographically distant from the rest of the species included in this study, which all are concentrated in or around the Usambara Mountains. The relative uniformity of the *Saintpaulia* sequences may indicate a comparatively recently adaptive radiation in the geographically restricted area of distribution (Tanzania and Kenya). This adaptive radiation appears to have been associated with key changes in floral and vegetative morphology, probably driven by ecological factors suggested above (change of habitat and pollinator).

It is interesting to note that three Madagascar species of *Streptocarpus* subgenus *Streptocarpella* (*S. andohahelensis* H. Humbert, *S. beampingaratrensis* H. Humbert, and *S. mandrerensis* H. Humbert) approach the *Saintpaulia* morphology in having suborbicular leaves, rosette habit, and shortened corolla tubes (Humbert, 1971). Unfortunately, these potentially informative species have been infrequently collected and are currently unavailable in cultivation. However, it would be interesting to study them, as well as the other African and Madagascan genera of Didymocarpeae (*Acanthonema, Colpogyne, Linnaeopsis, Schizoboea,* and *Trachystigma*), in the light of the findings reported here.

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