EDINBURGH JOURNAL OF BOTANY 60 (3): 389–408 (2004)

DOI: 10.10M/S0960428603000301

PLASTID INHERITANCE IN *STREPTOCARPUS* (*GESNERIACEAE*) AND AN INFERRED HYBRID ORIGIN FOR A POPULATION OF *S.* AFF. *PRIMULIFOLIUS* FROM IGODA RIVER, SOUTH AFRICA

M. MÖLLER*, K. J. BROOKS* & M. HUGHES*

A length polymorphism in the *trn*L/*trn*F intergenic spacer was used as a marker to determine the mode of chloroplast inheritance in *Streptocarpus* (*Gesneriaceae*). Exclusively maternal inheritance was recorded for all the F_1 progeny of reciprocal intraspecific crosses between *S. primulifolius* and a population referred to as *S.* aff. *primulifolius* from the Igoda River mouth, Eastern Cape, South Africa, and for interspecific crosses between *S. rexii* and *S. dunnii*. A combination of molecular and morphological data was used to clarify the origin of *S.* aff. *primulifolius*, which possesses *S. rexii*-type cpDNA and rDNA, while the morphological data suggest an intermediate position between *S. rexii* and *S. primulifolius*. The distribution of *S. rexii* and *S. primulifolius*, combined with molecular and morphological data, supports the hypothesis that the *S.* aff. *primulifolius* population is a hybrid between *S. rexii* and *S. primulifolius*, with *S. rexii* as the maternal parent, and that substantial molecular but limited morphological introgression into *S. primulifolius* has taken place.

Keywords. cpDNA, *Gesneriaceae*, length polymorphism, maternal inheritance, speciation, *Streptocarpus*, *trn*L/*trn*F intergenic spacer.

INTRODUCTION

Unusual vegetative morphology, developmental processes and evolutionary patterns, not to mention an enticing destination for collectors, have generated considerable interest in the genus *Streptocarpus* Lindl. (*Gesneriaceae–Cyrtandroideae–Didymocarpeae*), a genus largely confined to Africa and Madagascar. The two subgenera, *Streptocarpus* Fritsch and *Streptocarpella* Fritsch, are found on the African continent and Madagascar, with subgenus *Streptocarpus* extending in Africa from Ethiopia southwards to the Cape Region of South Africa. Hilliard & Burtt (1971) show that the range and distribution of the genus is partially correlated with altitude and partially with climate and terrain, and they hypothesize that it was a highly diversified genus prior to its spread southwards from East Africa. Indeed, the presence of highly divergent ITS sequences in *Streptocarpus* could be interpreted as an indication that the genus is comparatively old (Möller & Cronk, 1997).

In the most recent comprehensive revision of the genus (Hilliard & Burtt, 1971), 132 species were recorded. However, delimitation of species and subspecies is often

* Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh EH3 5LR, UK.

difficult. *Streptocarpus* is a genus with poorly developed breeding barriers, and interbreeding between species is frequently observed (Hilliard & Burtt, 1971). Thus, widespread highly polymorphic species are typical of the currently accepted taxonomy and 'species concepts are based on the consistency and degree of phenotypic differences' (Weigend & Edwards, 1994). Consequently, taxa within the genus are in a state of flux.

Recent investigations using chloroplast DNA revealed a molecular marker that showed intraspecific variation within the *S. rexii* aggregate (*S. rexii* Lindl., *S. primulifolius* Gand., *S. cyaneus* S. Moore and *S. parviflorus* Hook.f.). The marker is a length polymorphism in the intergenic spacer between the *trn*L(UAA)3′ and *trn*F(GAA) genes (*trn*L/*trn*F intergenic spacer) (Fig. 1). A short form of the *trn*L/*trn*F intergenic spacer was found in the chloroplasts of all accessions of *S. rexii* sampled, and was also recorded in *S.* aff. *primulifolius* collected from a population at Igoda River. All true *S. primulifolius* and a range of other *Streptocarpus* species examined (except *S. baudertii* L.L. Britten) have a long version of the spacer (M. Möller, unpublished data).

```
                      10         20         30         40         50         60
                       .          .          .          .          .          .
S. rexii              GGTTCAAGTC CCTCTATCCC CAAAAAAATC CTATAATTGA CTCCTAAAAT ATTTATCCTA
S. primulifolius      GGTTCAAGTC CCTCTATCCC CAAAAAAATC CTATAATTGA CTCCYAAAAT ATTTATCCTA

                      70         80         90         100        110        120
                       .          .          .          .          .          .
S. rexii              TCCGCTTTGT TCGTTAAAGG TTCAAAATTC CTTTATCTTT CTAATTCTTT TAGAAATGTC
S. primulifolius      TCCGCTTTGT TCGTTAATGG TTCAAAATTC CTGTATCTTT CTAATTCTTT TAGAAATGTC

                      130        140        150        160        170        180
                       .          .          .          .          .          .
S. rexii              TTTGGGCGTA AATGACTTTC TCTTTGATAT AGAATACACA TTCAAATGAA GCAAGGAATC
S. primulifolius      TTTGGGCGTA AATGACTTTC TCTTTGATAT AGAATACACA TTCAAATGAA GCAAGGAATC

                      190        200        210        220        230        024
                       .          .          .          .          .          .
S. rexii              CCTATTGGAA TAATTCACAA TCAATAGCAT TACGCATACT GACACTTAGA AAGTCGTCTT
S. primulifolius      CCTATTGGAA TAATTCACAA YCAATAGCAT TACGCATACT GACACTTAGA AASTCGTCTT

                      250        260        270        280        290        300
                       .          .          .          .          .          .
S. rexii              TTTAAAGATC CAAG------ ---------- ---------- ---------- ----------
S. primulifolius      TTTCAAGATC CAAGAGATTA GAGGACTTGG AGAAAACTTT GTAATTTGAT CTTGTCCCTT

                      310        320        330        340        350        360
                       .          .          .          .          .          .
S. rexii              ---------- ---------T CCTCTAATAA AATGAGGATG GGATGATACA TTAGGAATGG
S. primulifolius      TAATTGACAT AGACCCCAGT CCTCTAATAA AATGAGGATG GGATGATACA TTAGGAATGG

                      370        380        390        400        410
                       .          .          .          .          .
S. rexii              TCGGGATAGC TCAGCTGGTA GAGCAGAGGA CTGAAAATCC TCGTGTCACC AGTTCAAAT [354]
S. primulifolius      TCGGGATAGC TCACCTGGTA ?AGCAGARGA CTGAAAATCC TCGTGTCACC AGTTCAAAT [419]
```

FIG. 1. Sequence data of the *trn*L/*trn*F cpDNA spacer, illustrating the length polymorphism between *Streptocarpus rexii* and *S. primulifolius* subsp. *primulifolius*. Missing data are coded as '?' and hyphens denote alignment gaps; primer sequences are in bold.

The presence of the cpDNA *trn*L/*trn*F intergenic spacer length polymorphism in *Streptocarpus* provides the opportunity to study the mode of chloroplast inheritance using reciprocal crosses within a species (Mogensen, 1996). The study of chloroplast inheritance will contribute towards untangling relationships in the genus by providing information on the direction of hybridization. This will help to clarify the origin of the Igoda River population, which was initially identified as *S. rexii* by B.L. Burtt and O.M. Hilliard (pers. comm.), but on the basis of further morphological study on material growing at the Royal Botanic Garden Edinburgh (RBGE) was later treated as *S.* aff. *primulifolius*. Morphometric measurements and ITS sequencing of the species putatively involved in the origin of this population – *S. rexii*, *S. primulifolius* subsp. *primulifolius* and *S. primulifolius* subsp. *formosus* Hilliard & B.L. Burtt – should provide data on the extent of potential introgression. The mapping of known geographic ranges of these species will additionally highlight other potential areas of hybridization.

Materials and methods

Plant material

For determination of the direction of cpDNA inheritance, 16–20 F_1 plants from crosses cultivated at RBGE were analysed; these were reciprocal crosses between *S. rexii* (short spacer) and *S. dunnii* Hook.f. (long spacer), and between *S. primulifolius* subsp. *primulifolius* (long spacer) and *S.* aff. *primulifolius* (short spacer) (Table 1). Fifteen *S.* aff. *primulifolius* individuals from the Igoda River population were also assayed for the *trn*L/*trn*F polymorphism, using silica gel-dried material kindly provided by A.U. Batten.

Table 1. Plant material (RBGE accession numbers in parentheses) used for investigating inheritance of the *trn*L/*trn*F intergenic spacer and cpDNA type in F_1 progenies

Maternal		Paternal		F_1 progeny	
	cpDNA type		cpDNA type	cpDNA type	No. of plants analysed
S. primulifolius subsp. *primulifolius* (19660432)	Long	*S.* aff. *primulifolius* (19912192)	Short	Long	20
S. aff. *primulifolius* (19912192)	Short	*S. primulifolius* subsp. *primulifolius* (19660432)	Long	Short	20
S. rexii (19870333)	Short	*S. dunnii* (19972909)	Long	Short	18
S. dunnii (19972909)	Long	*S. rexii* (19870333)	Short	Long	15

TABLE 2. *Streptocarpus* accessions used in ITS sequence comparisons

Taxon	Locality (or distribution)	RBGE accession no.*	DNA no.
S. aff. *primulifolius*†	SA: E Cape, Igoda River mouth	19912192	S 17
S. aff. *primulifolius*	SA: E Cape, Igoda River mouth	–	191p
S. aff. *primulifolius*	SA: E Cape, Igoda River mouth	–	192p
S. aff. *primulifolius*	SA: E Cape, Igoda River mouth	–	193p
S. primulifolius Gand. subsp. *primulifolius*	SA: KwaZulu/Natal, Table Mountains	19660432	S 64
S. primulifolius Gand. subsp. *primulifolius*	SA: Natal, Creighton	19660431	S 68
S. primulifolius Gand. subsp. *formosus* Hilliard & B.L. Burtt†	SA: Natal, Umzinto Dist., Umgaya between Dumisa and Umzinto	19690444	S 55
S. primulifolius Gand. subsp. *formosus* Hilliard & B.L. Burtt	SA: Natal, Umtamvuna Gorge	19923046	S 34
S. primulifolius Gand. subsp. *formosus* Hilliard & B.L. Burtt	(SA: E Cape, S Natal)	19972036	SF 2
S. rexii Lindl.†	SA: SE Cape, Grahamstown, Faraway Estate	19870333	S 5
S. rexii Lindl.	SA: E Cape, Transkei, near Umtata, Mhlahlane	19912545	S 53

*The RBGE accession numbers were also used as voucher numbers.
†Sequences from Möller & Cronk (2001).

For ITS sequencing fresh plant material came from RBGE and dried material from A.U. Batten, including samples of *S. rexii*, *S. primulifolius* subsp. *primulifolius*, *S. primulifolius* subsp. *formosus* and *S.* aff. *primulifolius* (Table 2). Voucher specimens were taken for all taxa analysed and deposited at E. Additional sequences were taken from Möller & Cronk (2001).

Measurement and analysis of floral macromorphological characters were carried out on fresh and pickled plant material from RBGE on the same range of taxa (Table 3).

DNA extraction and PCR amplification

Total genomic DNA of fresh or dried leaf material was extracted according to a modified CTAB method (Doyle & Doyle, 1987).

The *trn*L/*trn*F intergenic spacer length polymorphism is caused by a 65-bp deletion in *S. rexii*, relative to the 418-bp long spacer in *S. primulifolius* (Fig. 1); this length polymorphism can be visualized conveniently on agarose gel. This method was used to determine the plastid type present in the progeny of the reciprocal crosses. Universal primers *trn*L e and *trn*L f, designed by Taberlet *et al.* (1991), were used to PCR amplify the *trn*L/*trn*F intergenic spacer. The PCR reaction mix and conditions were identical to those used by Möller & Cronk (1997). Two microlitres

TABLE 3. *Streptocarpus* accessions used in macromorphological study

Species	Locality	Accession no.
S. aff. *primulifolius*	Igoda River	19972192
S. primulifolius subsp. *primulifolius*	Bashee River, Mpozolo	C5112*
S. primulifolius subsp. *primulifolius*	Cape Province	19660432
S. primulifolius subsp. *primulifolius*	Creighton, Natal Province	19660431
S. primulifolius subsp. *primulifolius*	Creighton, Natal Province	C5089*
S. primulifolius subsp. *primulifolius*	East London	C5090*
S. primulifolius subsp. *primulifolius*	Moheni River, Bashee, The Haven	C5175*
S. primulifolius subsp. *primulifolius*	Mtwalume Valley, Umzinto	C6075*
S. primulifolius subsp. *primulifolius*	Unknown	19981627
S. primulifolius subsp. *formosus*	Excelsior near Uvango	C5081*
S. primulifolius subsp. *formosus*	Umgaye, Umzinto Dist., Natal	10690444
S. primulifolius subsp. *formosus*	Umtamvuna	C4798*
S. primulifolius subsp. *formosus*	Unknown	19972933
S. primulifolius subsp. *formosus*	Unknown	19972036
S. rexii	Faraway Estate, Grahamstown, Cape Province	19870333
S. rexii	Grahamstown, E Cape	C2014*
S. rexii	Katberg Pass, Natal Province	19773290
S. rexii	Kambi, Umtata, Transkei	C5022*
S. rexii	Mount Ngeli	C4953*

*From spirit collection.

of PCR product were analysed by electrophoresis at 80 V for 1 hour in a 1.6% agarose gel using a 1 × TBE buffer.

The procedure for PCR amplification and sequencing of the complete ITS region followed previously published protocols (Möller & Cronk, 1997). The sequences were compared in Sequence Navigator (Applied Biosystems Division, Foster City, CA, USA).

Morphometric measurements

The terminology used for trichome characteristics follows Hewson (1988). Flower size has previously played a crucial role in species delimitation, even though size differences in flower parts may be correlated to general flower size. Potential correlation in size of floral parts was tested and visualized graphically through the comparison of measurements from different floral organs. Care was taken to ensure that measurements were recorded consistently (Fig. 2).

Biogeography

Where available, localities of all specimens of *S. rexii*, *S. primulifolius* subsp. *primulifolius*, *S. primulifolius* subsp. *formosus* and *S.* aff. *primulifolius* held in the

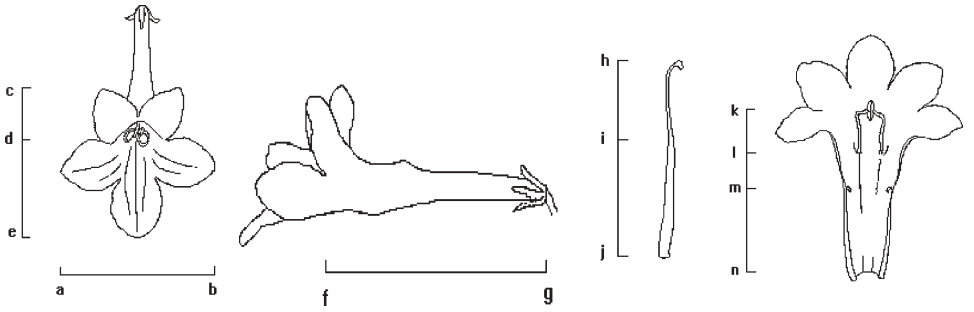

FIG. 2. Delimiters used in morphometric measurements: a–b, corolla face width; c–e, corolla face length; d–e, abaxial lobe; f–g, corolla tube; i–j, ovary; h–j, style; k–n, total filament; k–l, free filament; l–n, attached filament; m–n, staminode. Adapted from an illustration of accession no. C4634.

herbarium and research collections at RBGE (Table 4) were plotted using ArcView version 3.0a (Environmental Systems Research Institute, Inc., CA, USA).

RESULTS

Chloroplast types and inheritance

In all F_1 progenies of reciprocal crosses between *S. primulifolius* and *S.* aff. *primulifolius* and between *S. rexii* and *S. dunnii* tested, only one cpDNA fragment is present, in each case the respective maternal type (Fig. 3A,B). Further, all 15 individual specimens of *S.* aff. *primulifolius* from the Igoda River population exhibit the short form of the *trn*L/*trn*F intergenic spacer (Fig. 3C).

ITS sequence variation

To investigate variation in the Igoda River population, three additional individuals were sequenced, as well as RBGE accession 19912192. All four sequences obtained from *S.* aff. *primulifolius* were identical (Fig. 4).

Between the taxa, ITS sequence divergence is low. Across the 478-bp long matrix (ITS1 = 236bp, ITS2 = 242bp), 10 positions are variable, excluding ambiguous sequencing results. Positions 180, 236, 435 and 440 are consistently different between *S. rexii* and *S. primulifolius* subsp. *primulifolius* or *S. primulifolius* subsp. *formosus*. The other six positions are all autapomorphies: three in *S. rexii* (positions 70, 102 and 201) and three in *S. primulifolius* subsp. *formosus* SF2 (positions 215, 229 and 231) (Fig. 4).

At all four positions consistently differentiating *S. rexii* from *S. primulifolius*, all *S.* aff. *primulifolius* samples are identical to *S. rexii*. Only one peak is visible at the

TABLE 4. Accessions used to plot distributions of *Streptocarpus rexii*, *S. primulifolius* subsp. *primulifolius*, *S. primulifolius* subsp. *formosus* and *S.* aff. *primulifolius*

Species	Locality	Latitude and longitude	Accession no./ collection no.
S. aff. *primulifolius*	Igoda River	33°06′S, 27°46′E	19972192
S. primulifolius subsp. *primulifolius*	Bashee River, Mpozolo	32°10′S, 28°45′E	C5112
S. primulifolius subsp. *primulifolius*	Creighton	30°02′S, 29°50′E	19660431
S. primulifolius subsp. *primulifolius*	East London	33°00′S, 27°55′E	C4938, C4939
S. primulifolius subsp. *primulifolius*	Harding, Alfred Dist., Natal	30°35′S, 30°00′E	*Hilliard* 1360
S. primulifolius subsp. *primulifolius*	Inchanga, Camperdown Dist.	29°43′S, 30°32′E	*Hilliard* 861
S. primulifolius subsp. *primulifolius*	Inchanga Hill	29°45′S, 30°40′E	*Hilliard & Burtt* 3796
S. primulifolius subsp. *primulifolius*	Kentani	32°30′S, 28°30′E	*Strey* 6652
S. primulifolius subsp. *primulifolius*	Moheni River, Bashee, The Haven	32°14′S, 28°53′E	C5175
S. primulifolius subsp. *primulifolius*	Mtwalume Valley, Umzinto	30°27′S, 30°38′E	C6075
S. primulifolius subsp. *primulifolius*	Rooivaal, near Harding Walat	30°35′S, 29°53′E	C5381
S. primulifolius subsp. *primulifolius*	Port St Johns	31°35′S, 29°25′E	*Hilliard & Burtt* 3529
S. primulifolius subsp. *formosus*	Esperanza Rd., Natal	30°20′S, 30°39′E	C5096
S. primulifolius subsp. *formosus*	Excelsior near Uvango	30°50′S, 30°23′E	*Hilliard & Burtt* 3813
S. primulifolius subsp. *formosus*	Izingolweni Rd., Port Edward	31°03′S, 30°13′E	*Hilliard* 1133
S. primulifolius subsp. *formosus*	Jolivet, Dumisa	30°17′S, 30°21′E	*Hilliard* 3122
S. primulifolius subsp. *formosus*	Oribi, Port Shepstone Dist.	30°45′S, 30°20′E	*Hilliard* 2791
S. primulifolius subsp. *formosus*	Umgaye, Umzinto Dist.	30°20′S, 30°35′E	19690444
S. primulifolius subsp. *formosus*	Umtamvuna	30°56′S, 30°09′E	*Burtt* 3010 (C4798)
S. rexii	2mi from Engcobo on Elliot Rd., Engcobo Dist.	31°45′S, 28°05′E	*Hilliard & Burtt* 3714
S. rexii	Faraway Estate, Grahamstown	33°18′S, 26°32′E	19870333
S. rexii	Kambi Forest Reserve, Umtata Dist.	31°45′S, 28°30′E	*Hilliard & Burtt* 3722
S. rexii	Kologho Forest, E Cape	32°32′S, 27°25′E	*Hilliard & Burtt* 3534
S. rexii	Mount Baziya	31°32′S, 28°24′E	*Hilliard & Burtt* 13876
S. rexii	Mount Frere	30°45′S, 28°42′E	*Hilliard & Burtt* 3709
S. rexii	Ntywenka, Tsolo Dist., E Cape	31°09′S, 28°33′E	*Hilliard & Burtt* 3733
S. rexii	Tabankulu Forest, E Cape	30°59′S, 29°21′E	*Hilliard* 2494

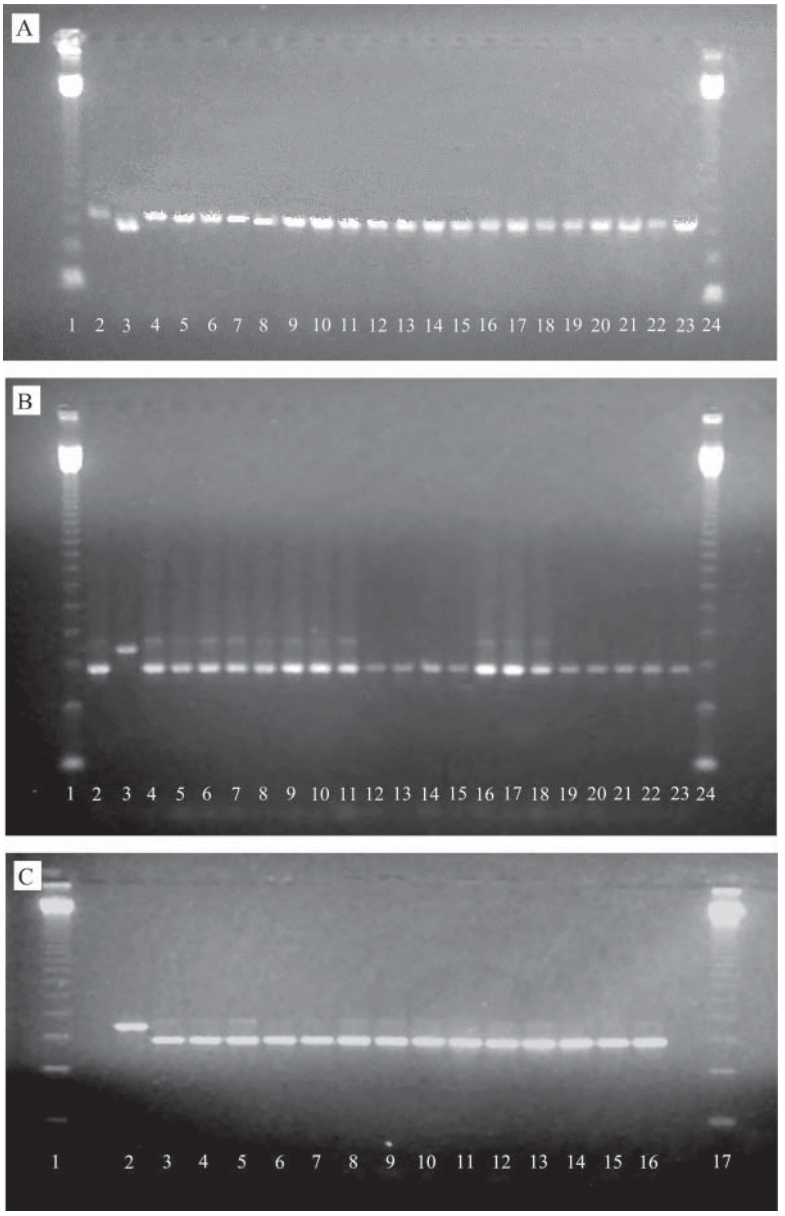

FIG. 3. Agarose gels of PCR-amplified *trn*L/*trn*F intergenic spacer DNA. A and B, 40 F_1 progenies from crosses between *Streptocarpus primulifolius* subsp. *primulifolius* (19660432, long spacer) and *S.* aff. *primulifolius* (19912192, short spacer). A (lanes from left to right): 1 and 24, molecular marker 123bp; 2, *S. primulifolius* subsp. *primulifolius* (maternal parent); 3, *S.* aff. *primulifolius* (paternal parent); 4–23, F_1 progeny. B (lanes from left to right): 1 and 24, molecular marker 123bp; 2, *S.* aff. *primulifolius* (maternal parent); 3, *S. primulifolius* subsp. *primulifolius* (paternal parent); 4–23, F_1 progeny. C (lanes from left to right): 1 and 17, molecular marker 123bp; 2, *S. primulifolius* subsp. *primulifolius* (19660432); 3–16, *S.* aff. *primulifolius*.

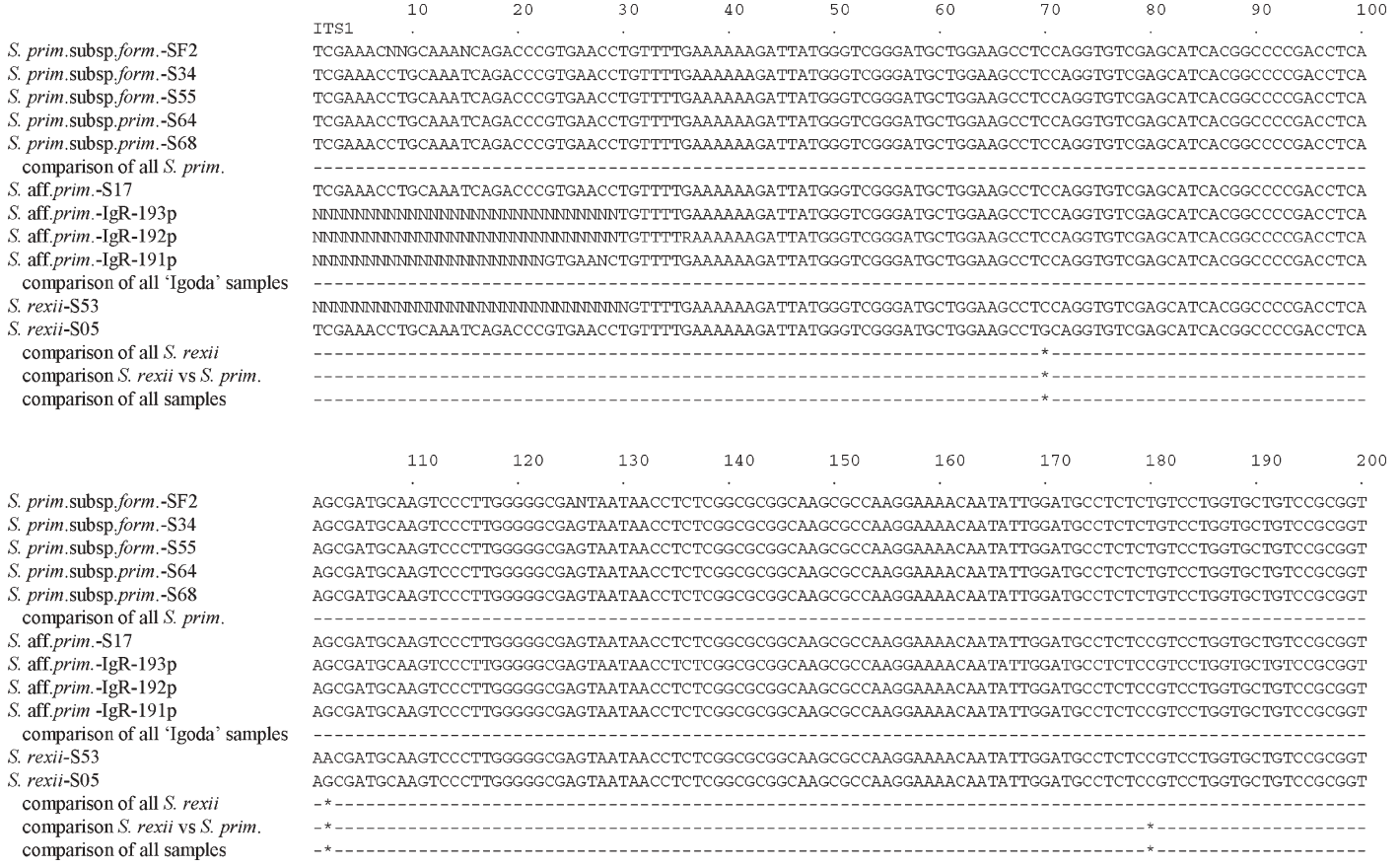

FIG. 4. The ITS sequences of *Streptocarpus rexii*, *S.* aff. *primulifolius*, *S. primulifolius* subsp. *primulifolius* and *S. primulifolius* subsp. *formosus* accessions. Sequences are displayed from 5′ end to 3′ end. Uncertain nucleotide states are coded according to general conventions: N = A/C/G/T, K = G/T, M = A/C, R = A/G, S = C/G, W = A/T and Y = C/T, hyphens denote alignment gaps, and * denotes difference between accessions.

	210 . 220 . 230 . 240 ITS2 . 250 . 260 . 270 . 280 . 290 . 300 .
S. prim.subsp.*form*.-SF2	ACCCAAGGCGTGACAAGGAACGTCTATAAATTACGTATCTCGTCGCCACCCCCACAGACAGATCTTCGGTTCCCCACCACAAGTGTCGGGAGCGGAAGCA
S. prim.subsp.*form*.-S34	ACCCAAGGCGTGACGAGGAACGTCTATAGAGTACGTATCTCGTCGCCACCCCCACAGACAGATCTTCGGTTCCCCACCACAAGTGTCGGGAGCGGAAGCA
S. prim.subsp.*form*.-S55	ACCCAAGGCGTGACGAGGAACGTCTATAGAGTACGTATCTCGTCGCCACCCCCACAGACAGATCTTCGGTTCCCCACCACAAGTGTCGGGAGCGGAAGCA
S. prim.subsp.*prim*.-S64	ACCCAAGGCGTGACGAGGAACGTCTATAGAGTACGTATCTCGTCGCCACCCCCACAGACAGATCTTCGGTTCCCCACCACAAGTGTCGGGAGCGGAAGCA
S. prim.subsp.*prim*.-S68	ACCCAAGGCGTGACGAGGAACGTCTATAGAGTACGTATCTCGTCGCCACCCCCACAGACAGATCTTCGGTTCCCCACCACAAGTGTCGGGAGCGGAAGCA
comparison of all *S. prim.*	--------------*-------------*-*---
S. aff.*prim*.-S17	ACCCAAGGCGTGACGAGGAACGTCTATAGAGTACGCATCTCGTCGCCACCCCCACAGACAGATCTTCGGTTCCCCACCACAAGTGTCGGGAGCGGAAGCA
S. aff.*prim*.-IgR-193p	ACCCAAGGCGTGACGAGGAACGTCTATAGAGTACGCATCTCGTCGCCACCCCCACAGACAGATCTTCGGTTCCCCACCACAAGTGTCGGGAGCGGAAGCA
S. aff.*prim*.-IgR-192p	ACCCAAGGCGTGACGARGAACGTCTATAGAGTACGCATCTCGTCGCCACCCCCACAGACAGATCTTCGGTTCCCCACCACAAGTGTCGGGAGCGGAAGCA
S. aff.*prim*.-IgR-191p	ACCCAAGGCGTGACGAGGAACGTCTATAGAGTACGCATCTCGTCGCCACCCCCACAGACAGATCTTCGGTTCCCCACCACAAGTGTCGGGAGCGGAAGCA
comparison of all 'Igoda' samples	--
S. rexii-S53	TCCCAAGGCGTGACGANGAACGTCTATAGAGTACGCATCTCGTCGCCACCCCCACAGACAGATCTTCGGTTCCCCACCACAAGTGTCSGGAGCGGAAGCA
S. rexii-S05	ACCCAAGGCGTGACGAGGAACGTCTATAGAGTACGCATCTCGTCGCCACCCCCACAGACAGATCTTCGGTTCCCCACCACAAGTGTCGGGAGCGGAAGCA
comparison of all *S. rexii*	*---
comparison *S. rexii* vs *S. prim.*	*-------------*-------------*-*----*--
comparison of all samples	*-------------*-------------*-*----*--

	310 . 320 . 330 . 340 . 350 . 360 . 370 . 380 . 390 . 400 .
S. prim.subsp.*form*.-SF2	TGCTGGGGAGTGTGCGGATATTGGCCTCCCGTTACCTCGTGTWKCGGCTGGCCCAAACAGGATACCGTGTCGATGGATGTCACACGATACGTGGTGGTTG
S. prim.subsp.*form*.-S34	TGCTGGGGAGTGTGCGGATATTGGCCTCCCGTTACCTCGTGTAGCGGCTGGCCCAAACAGGATACCGTGTCGATGGATGTCACACGATACGTGGTGGTTG
S. prim.subsp.*form*.-S55	TGCTGGGGAGTGTGCGGATATTGGCCTCCCGTTACCTCGTGTAGCGGCTGGCCCAAACAGGATACCGTGTCGATGGATGTCACACGATACGTGGTGGTTG
S. prim.subsp.*prim*.-S64	TGCTGGGGAGTGTGCGGATATTGGCCTCCCGTTACCTCGTGTAGCGGCTGGCCCAAACAGGATACCGTGTCGATGGATGTCACACGATACGTGGTGGTTG
S. prim.subsp.*prim*.-S68	TGCTGGGGAGTGTGCGGATATTGGCCTCCCGTTACCTCGTGTAGCGGCTGGCCCAAACAGGATACCGTGTCGATGGATGTCACACGATACGTGGTGGTTG
comparison of all *S. prim.*	--
S. aff.*prim*.-S17	TGCTGGGGAGTGTGCGGATATTGGCCTCCCGTTACCTCGTGTAGCGGCTGGCCCAAACAGGATACCGTGTCGATGGATGTCACACGATACGTGGTGGTTG
S. aff.*prim*.-IgR-193p	TGCTGGGGAGTGTGCGGATATTGGCCTCCCGTTACCTCGTGTANCGGCTGGCCCAAACAGGATACCGTGTCGATGGATGTCACACGATACGTGGTGGTTG
S. aff.*prim*.-IgR-192p	TGCTGGGGAGTGTGCGGATATTGGCCTCCCGTTACCTCGTGTANCGGCTGGCCCAAACAGGATACCGTGTCGATGGATGTCACACGATACGTGGTGGTTG
S. aff.*prim*.-IgR-191p	TGCTGGGGAGTGTGCGGATATTGGCCTCCCGTTACCTCGTGTANCGGCTGGCCCAAACAGGATACCGTGTCGATGGATGTCACACGATACGTGGTGGTTG
comparison of all 'Igoda' samples	--
S. rexii-S53	TGCTGGGGAGTGTGCGGATATTGGCCTCCCGTTACCTCGTGTANCGGCTGGCCCAAACAGGATACCGTGTCGATGGATGTCACACGATACGTGGTGGTTG
S. rexii-S05	TGCTGGGGAGTGTGCGGATATTGGCCTCCCGTTACCTCGTGTAGCGGCTGGCCCAAACAGGATACCGTGTCGATGGATGTCACACGATACGTGGTGGTTG
comparison of all *S. rexii*	--
comparison *S. rexii* vs *S. prim.*	--
comparison of all samples	--

FIG. 4. (*Cont'd*).

```
                                410       420       430       440       450       460       470       [478]
                                .         .         .         .         .         .         .
S. prim.subsp.form.-SF2         GATTCCACAACTCTCGTAACTGTCGTGTGAGACCTCATCGAGACACGAGCACGACCCCGTGGCACCGGCTGCCCTCGA
S. prim.subsp.form.-S34         GATTCCACAACTCTCGTAACTGTCGTGTGAGACCTCATCGAGACACGAGCACGACCCCGTGGCACCGGCTGCCCTCGA
S. prim.subsp.form.-S55         GATTCCACAACTCTCGTAACTGTCGTGTGAGACCTCATCGAGACACGAGCACGACCCCGTGGCACCGGCTGCCCTCGA
S. prim.subsp.prim.-S64         GATTCCACAACTCTCGTAACTGTCGTGTGAGACCTCATCGAGACACGAGCACGACCCCGTGGCACCGGCTGCCCTCGA
S. prim.subsp.prim.-S68         GATTCCACAACTCTCGTAACTGTCGTGTGAGACCTCATCGAGACACGAGCACGACCCCGTGGCACCGGCTGCCCTCGA
  comparison of all S. prim.    ----------*---------*---------*---------*---------*---------*---------*----------
S. aff.prim.-S17                GATTCCACAACTCTCGTAACTGTCGTGTGAGACCGCATCAAGACACGAGCACGACCCCGTGGCACCGGCTGCCCTCGA
S. aff.prim.-IgR-193p           GATTCCACAACTCTCGTAACTGTCGTGTGAGACCGCATCAAGACACGAGCACGACCCCGTGGCACCGGCTGCCCTCGA
S. aff.prim.-IgR-192p           GATTCCACAACTCTCGTAACTGTCGTGTGAGACCGCATCAAGACACGAGCACGACCCCGTGGCACCGGCTGCCCTCGA
S. aff.prim.-IgR-191p           GATTCCACAACTCTCGTAACTGTCGTGTGAGACCGCATCAAGACACGAGCACGACCCCGTGGCACCGGCTGCCCTCGA
  comparison of all 'Igoda' samples ----------*---------*---------*---------*---------*---------*---------*----------
S. rexii-S53                    GATTCCACAACTCTCGTAACTGTCGTGTGAGACCGCATCAAGACACGAGCACGACCCCGTGGCACCGGCTGCCCTCGA
S. rexii-S05                    GATTCCACAACTCTCGTAACTGTCGTGTGAGACCGCATCAAGACACGAGCACGACCCCGTGGCACCGGCTGCCCTCGA
  comparison of all S. rexii    ----------*---------*---------*---------*---------*---------*---------*----------
  comparison S. rexii vs S. prim. ----------*---------*---------*---------*---------*---------*---------*----------
  comparison of all samples     ----------*---------*---------*---------*---------*---------*---------*----------
```

FIG. 4. (*Cont'd*).

associated electropherogram sites, an indication that these sites are not polymorphic and probably highly homogenized.

Morphology

Apart from differences in the size of flower parts, which more or less correlate with differences in flower size, only six characters are potentially informative (Table 5). A single trichome type (eglandular) on the lower lamina and pedicel is present in *S. rexii*, *S.* aff. *primulifolius* and *S. primulifolius*. The only consistent character separating *S. rexii* from all *S. primulifolius* accessions is the presence of a beard of long eglandular multicellular hairs at the mouth of the upper throat in *S. primulifolius*. This beard is also present in *S.* aff. *primulifolius* from Igoda River, and it can be seen in many photographs of the population (data not shown).

Plotting flower part measurements consistently produces the same picture: *S. primulifolius* and *S. primulifolius* subsp. *formosus* form a cluster in the upper size range, with *S. rexii* in the lower size range. The extent of overlap within these ranges depends on the measurements plotted. However, all the relationships plotted correlate to some extent with flower size. Interestingly, the taxa do not form discrete size groups but rather there is a gradient between the smaller *S. rexii* accessions and the larger *S. primulifolius* subsp. *formosus* (Fig. 5).

While corolla face size varies between species, largely separating *S. rexii* from *S. primulifolius*, it also varies considerably within taxa. However, corolla proportions are well correlated within all taxa sampled: corolla width is dependent on length (Fig. 5A), and an increase in total corolla face length corresponds to a proportionately shorter abaxial lobe (Fig. 5B). In contrast, the proportions of the corolla tube and corolla face width are relatively variable within species (Fig. 5C). Similar proportional correlations are found between corolla tube length and length of attached filament. These are closely correlated with an increase in corolla length, corresponding to a proportionately shorter length of attached filament (Fig. 5D). Likewise, the length of the attached stamen increases proportionately to total stamen length (Fig. 5E). The ratio between style and ovary is the most variable measurement intraspecifically. However, more or less discrete clusters are formed, with the taxa being separated by both size and to a degree the ratio between the two measurements: in *S. rexii* the ratio between style and ovary length is around 1:2, in *S. primulifolius* less (Fig. 5F). Interestingly, *S.* aff. *primulifolius* consistently falls just outside the *S. rexii* upper range and at the lower end of the *S. primulifolius* range, except for the ratio between ovary and style length.

Biogeography

Plotting the distribution of each taxon reveals the parallel distributions of *S. rexii* and *S. primulifolius* (Fig. 6). Populations of *S. primulifolius* subsp. *formosus* form a discrete group along the coast, surrounded on three sides by *S. primulifolius*, and cluster in three distinct localities: Umtamvuna, Oribi and Dumisa. One

TABLE 5. Morphological characters used in the delimitation of *Streptocarpus primulifolius* subsp. *formosus*, *S. primulifolius* subsp. *primulifolius*, *S.* aff. *primulifolius* and *S. rexii*

	S. primulifolius subsp. *formosus*	*S. primulifolius* subsp. *primulifolius*	*S.* aff. *primulifolius*	*S. rexii*
Lower lamina trichome type	Eglandular (few glandular on veins and occasionally between veins)	Eglandular	Eglandular	Eglandular
Pedicel trichome type	Glandular/few to many eglandular	Glandular/eglandular	Glandular/eglandular	Glandular/eglandular
Corolla tube and face internal markings	Purple speckles at anther insertion or deep purple or red stippling; 7 mauve lines on lower lobes	7 deep purple lines on lower lobes	7 distinct to blurred violet lines on lower lobes	7 parallel deep violet lines on lower lobes
Corolla tube				
Internal colour	Pale bluish mauve to white or pale greenish yellow	Deep mauve to white	Mauve darkening to violet	White or pale mauve
Presence of heavily pigmented area on lower throat	Weak to strong yellow between anther insertion	Deep violet, present or absent	None	Deep violet
Trichomes at mouth of upper throat	Present, eglandular	Present, eglandular	Present, eglandular	Absent

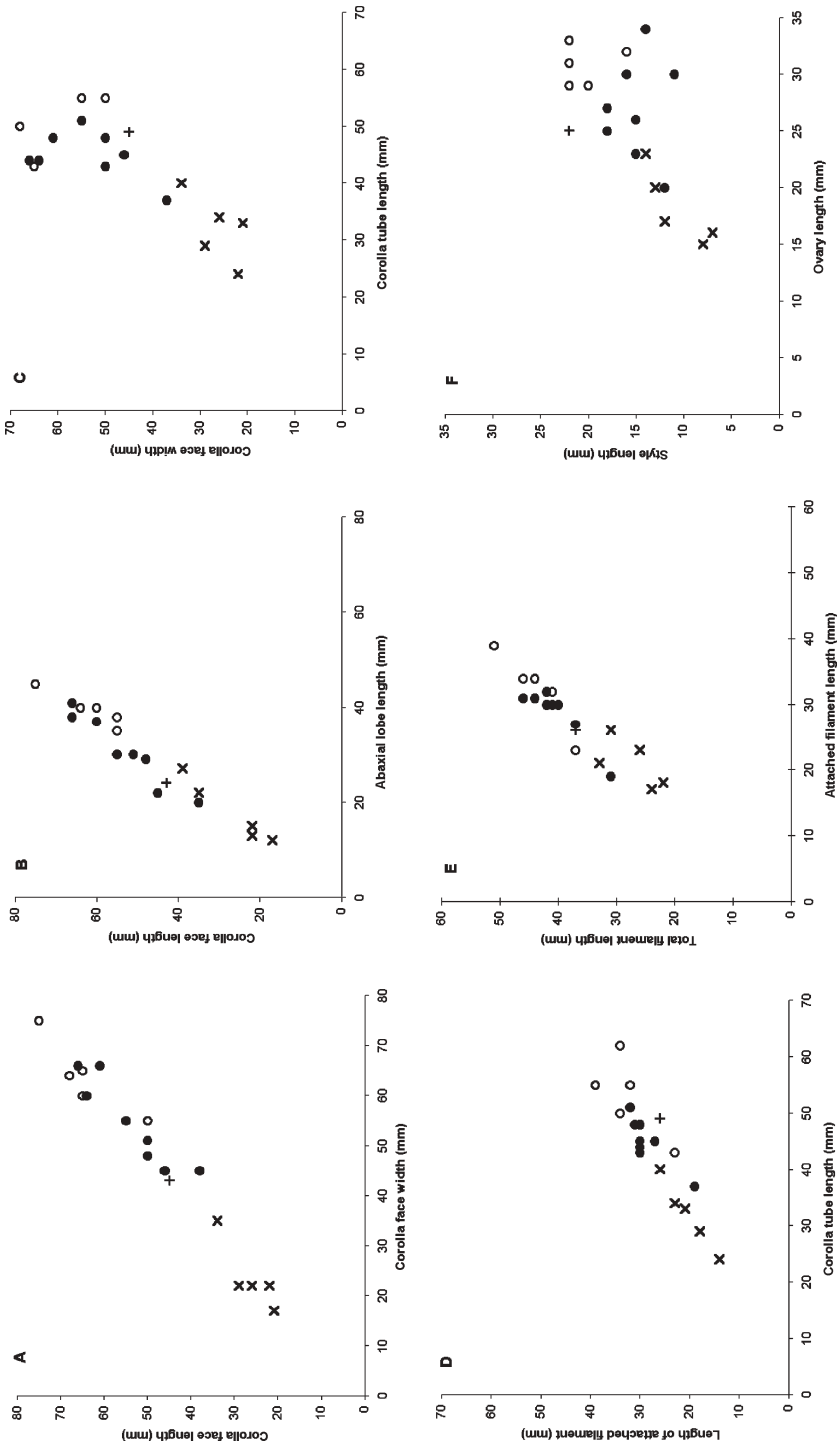

FIG. 5. Comparisons of floral morphology measurements for *Streptocarpus rexii* (×), *S. primulifolius* subsp. *primulifolius* (●), *S. primulifolius* subsp. *formosus* (○) and *S.* aff. *primulifolius* (+). A, corolla face length versus face width; B, corolla face length versus abaxial lobe length; C, corolla face width versus corolla tube length; D, length of attached filament versus corolla tube length; E, total filament length versus attached length; F, style length versus ovary length.

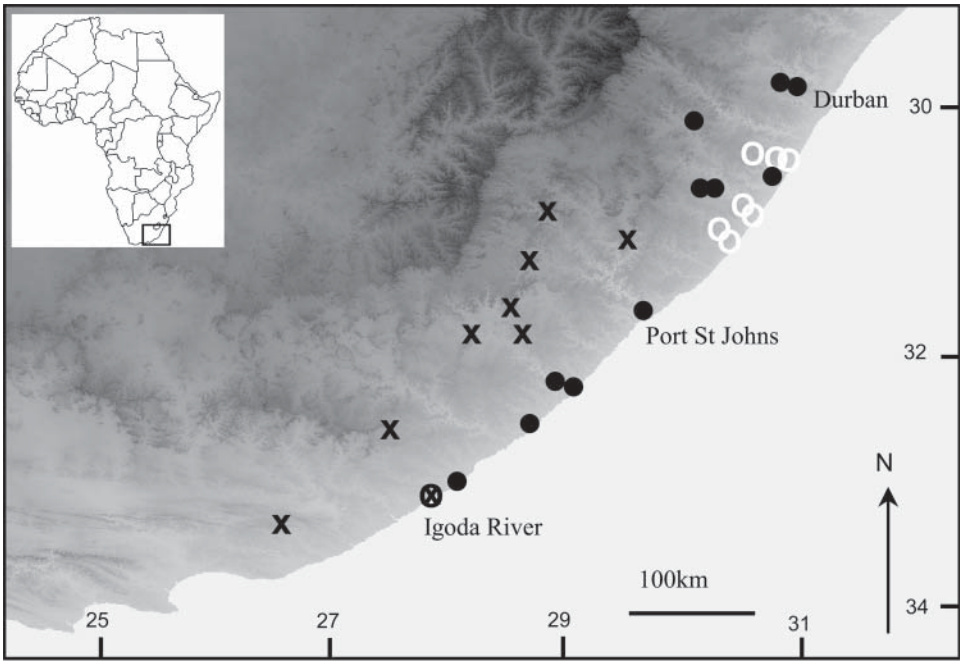

FIG. 6. Distribution of *Streptocarpus rexii* (×), *S. primulifolius* subsp. *primulifolius* (●), *S. primulifolius* subsp. *formosus* (○) and *S.* aff. *primulifolius* (⊗) from living and herbarium accessions at RBGE (Table 4).

accession (C6075) of *S. primulifolius* was also collected within this range near Umzinto. Populations of *S. rexii* and *S. primulifolius* abut near the northern end of their ranges. The two species then diverge following a roughly parallel course, *S. primulifolius* along the coast, *S. rexii* approximately 30km inland. *Streptocarpus primulifolius* reaches its lower limit at East London, the vicinity of the Igoda River population, while *S. rexii* swings towards the coast along the Ngeli Range and continues southwest towards Grahamstown.

The Igoda River population of *S.* aff. *primulifolius* is located at the extreme south of the *S. primulifolius* range. Several specimens of *S. primulifolius* have also been collected from around East London (*Burtt* 3539, 3543 and 3544; *Hilliard* 3544), some 20km north of the Igoda River. The nearest collection of *S. rexii* to the Igoda River population was made some 60km inland at Kologha Forest (*Hilliard & Burtt* 3534 and 3533).

DISCUSSION

Chloroplast inheritance in Streptocarpus

A lack of intraspecific molecular markers means that many recent studies of chloroplast inheritance involve interspecific crosses (Soltis *et al.*, 1992). However,

without knowing the mechanism of plastid inheritance it is impossible to predict if plastome–genome interactions will result in atypical inheritance (Chiu & Sears, 1993; Reboud & Zeyl, 1994). Therefore, data from intraspecific inheritance studies are desirable, because mechanisms of exclusion and plastome–genome interactions are behaving normally. Although reciprocal crosses between *S. primulifolius* and *S.* aff. *primulifolius* may not be strictly intraspecific, it is the closest possible cross between these taxa. In all such crosses analysed in this study, the inheritance of chloroplast markers was maternal. In the more distantly related *S. rexii* and *S. dunnii* (Möller & Cronk, 2001), the exclusive maternal inheritance recorded indicates that the breakdown of the mechanisms of paternal organelle exclusion in *Streptocarpus* occurs relatively rarely, if at all. Thus, it can be deduced that chloroplast inheritance is maternal in *Streptocarpus*.

Chloroplast type in Streptocarpus *aff*. primulifolius

Some authors suggest that sampling strategies for molecular data are biased against the detection of intraspecific variation (Soltis *et al*., 1992; Ennos *et al*., 1999). Failure to sample widely for chloroplast type, especially in suspected cases of chloroplast capture, risks overlooking a possible polymorphism within the species or population in question. This can subsequently affect assumptions made regarding gene flow, hybridization and introgression. Thus, a large proportion of the *S.* aff. *primulifolius* population was sampled at random for chloroplast type analysis. The occurrence of the short *trn*L/*trn*F intergenic spacer type in all plants sampled suggests that the whole population is homogenous for the *S. rexii* chloroplast type.

In a survey of more than 20 species of *Streptocarpus* subgenus *Streptocarpus*, including all species belonging to group C (including the *S. rexii* aggregate), group D and the *S. meyeri* B.L. Burtt alliance of Hilliard & Burtt (1971), only *S. rexii* and *S. baudertii* accessions were found to possess the short spacer. The latter has a 'keyhole' flower, with a laterally flattened, curved corolla tube and a centric rosette (Hilliard & Burtt, 1971; Jong, 1978), which makes it unlikely that this species is involved in the origin of *S.* aff. *primulifolius*. Given the maternal inheritance mode found in both species, it can be postulated that *S. rexii* was the maternal parent in the initial, possibly single, hybridization event.

ITS sequence variation

Inter- and intraspecific sequence variation was observed in both *S. rexii* and *S. primulifolius* subsp. *formosus*. Because the samples represented different discrete populations, this may be an indication that the populations are genetically isolated. However, more extensive studies at population level are necessary to substantiate this hypothesis.

The low ITS sequence variation between *S. rexii* and *S. primulifolius* (five to nine changes = 1–1.9%) may indicate very recent divergence. Calculated on the basis of

divergence rates for comparable herbaceous plants (Richardson *et al.*, 2001), the event may have occurred in the Quaternary, 0.7–1.2 million years ago (see the *Biogeography* section of this discussion).

The fact that all four individuals of *S.* aff. *primulifolius* analysed possessed ITS sequences typical of *S. rexii*, without any signs of heterogeneities, suggests a complete replacement of the *S. primulifolius* ITS gene cluster. There is evidence that multicopy rDNA within nucleolar organizer regions may be differentially inherited as a single allele with no sequence interchange between parental copies, leading to complete ITS replacements in backcrosses of hybrid plants (Denduangboripant, 2001).

Morphology

Micromorphological pollen studies have failed to provide any informative characters to separate *S. rexii* from *S. primulifolius* (Weigend & Edwards, 1996), and the same was true in a preliminary SEM study of vegetative and reproductive organs in *S. rexii* and *S. primulifolius* (Brooks, 1998). The study of macromorphology was more successful, highlighting some consistent variation in size and proportion of flower parts. However, reliance on size of flower parts as a defining characteristic for species of the *S. rexii* aggregate may be unwise, given that only a few measurements behave independently of each other while others are variable within a taxon. Indeed, in *Streptocarpus* the range of large- and small-flowered variants within species is considerable (Hilliard & Burtt, 1971), an indication that corolla size evolution is continuous. Other species-defining characters are the presence of yellow pigmentation on the floor of the tube and the type of marking, the occurrence of glandular and eglandular hairs, and in *S. rexii* the notable absence of a beard of multicellular hairs at the mouth of the upper corolla throat. These characters have been the mainstay of previous investigations into the group. However, revealing the identity of the Igoda River population was the prime goal of this study. This accession appears to be intermediate in corolla size, shape, colour and markings between *S. rexii* and *S. primulifolius*, although corolla markings are highly variable. All three (*S. rexii*, *S. primulifolius* and *S.* aff. *primulifolius*) are distinct from *S. primulifolius* subsp. *formosus* in the consistent absence of glandular hairs on the underside of the leaves. The introgression of *S. rexii* characters into *S. primulifolius* is favoured by Hilliard & Burtt (1971) as the likely origin of the Igoda River population. Morphological evidence loosely supports their conclusion, the presence of the beard of hairs being the strongest character. However, substantial introgression of DNA markers is also evident.

Biogeography

A north–south range is apparent in the distributions of *S. rexii* and *S. primulifolius*. In the north *S. rexii* abuts with *S. primulifolius*; further south the two species are more separated, forming two nearly linear parallel distributions. The distributions

then converge at the southern end of the range of *S. primulifolius* near East London and represent an opportunity for hybridization. Limited ITS sequence differences suggest a recent divergence of *S. rexii* and *S. primulifolius* that fits well with Quaternary isolation. Although not subjected to glaciation, palaeological evidence suggests that changes in the relative abundance of vegetation in southern Africa during the glacial and interglacial periods of the Quaternary were widespread (Williams, 1985; Williams *et al*., 1993; Eeley *et al*., 1999). It is plausible that climatic fluctuations during this period resulted in the isolation of refugial populations, the precursors of *S. rexii* and *S. primulifolius*. Their preference for different altitudes is conceivably the product of ecological differentiation.

Altitudinal conditions are an important delimiter of species boundaries in *Streptocarpus*, but where other suitable ecological conditions occur, such as in river gorges or kloofs, populations of species usually found on higher slopes may occur near the coast. Indeed, preference for river gorge habitats may explain the presence of *S.* aff. *primulifolius* at Igoda River. Hilliard & Burtt (1971) believe that the population of *S. primulifolius* recorded near East London is also intermediate. If so, the closest recorded true breeding populations of either *S. rexii* or *S. primulifolius* are approximately 60–80km away from the area. That raises the question of how hybridization might have occurred between geographically distant, insect-pollinated species with passive seed dispersal. One of two plausible scenarios is that seed from *S. rexii* was carried downstream into or near to a population of *S. primulifolius*, and that germination followed by cross-pollination and subsequent backcrossing produced the intermediates observed. Alternatively, scattered and undetected or long-since vanished plants of *S. rexii* colonized the river gorges and hybridized with *S. primulifolius*, resulting in the postulated gene flow between the two species. Hybrid vigour, simple stochasticity or the singularity of the hybridization event may account for dominance of the *S. rexii* chloroplast type. The direction of river drainages in the region (Bowmaker *et al*., 1978) precludes the possibility of *S. primulifolius* being transported into the region in the same manner.

Even if the above hypothesis is untrue, the fact remains that all accessions of *S. rexii* sampled, and the recognized intermediates at Igoda River, have a chloroplast type different from that of *S. primulifolius*. The presence of this chloroplast type in the Igoda River population, a population strongly resembling *S. primulifolius*, supports observations that these plants are intermediate between the two species. Because the suspected chloroplast capture and introgression is the result of gene flow between *S. rexii* and *S. primulifolius*, it is now important to study the distribution, ecology and biology of these two species. Populations in areas of potential hybridization, where the species ranges abut (such as the East London region where *S. rexii* and *S. primulifolius* meet), should be targeted for further study.

Acknowledgements

Streptocarpus geography has many pitfalls and the authors thank Dirk Bellstedt and Quentin Cronk for helpful comments. We also thank Hans Sluiman, Caroline

Guihal and Jill Preston for technical support; Steve Scott, David Mitchell and David Rae for maintenance of the *Gesneriaceae* collection; and RBGE for provision of research facilities. We thank Auriol Batten and Jessada Denduangboripant for providing plant and DNA material. The receipt of Leverhulme Trust Award no. F/00 771A-0735 is gratefully acknowledged.

References

Bowmaker, A. P., Jackson, P. B. N. & Jubb, R. A. (1978). Freshwater fish. In: Werger, M. J. A. (ed.) *Biogeography and Ecology of Southern Africa*. The Hague: Dr W. Junk.

Brooks, K. J. (1998). *Phylogenetic relevance of plastid types in* Streptocarpus *(Gesneriaceae), with special reference to* S. *agg.* rexii *and the Igoda River population.* MSc thesis, University of Edinburgh.

Chiu, W. L. & Sears, B. B. (1993). Plastome–genome interactions affect plastid transmission in *Oenothera*. *Genetics* 133: 989–997.

Denduangboripant, J. (2001). *Molecular evolution of nuclear ribosomal DNA in* Aeschynanthus *and* Streptocarpus *(Gesneriaceae).* PhD thesis, University of Edinburgh.

Doyle, J. J. & Doyle, J. L. (1987). A rapid isolation procedure for small quantities of fresh leaf material. *Phytochem. Bull. Bot. Soc. Amer.* 19: 11–15.

Eeley, H. A. C., Lawes, M. J. & Piper, S. E. (1999). The influence of climate change on the distribution of indigenous forest in KwaZulu-Natal, South Africa. *J. Biogeogr.* 26: 595–617.

Ennos, R. A., Sinclair, W. T., Hu, X. S. & Langdon, A. (1999). Using organelle markers to elucidate the history, ecology and evolution of plant populations. In: Hollingsworth, P. M., Bateman, R. M. & Gornall, R. S. (eds) *Molecular Systematics and Plant Evolution*. London: Chapman & Hall.

Hewson, H. J. (1988). *Plant Indumentum. A Handbook of Terminology*. Canberra: Australian Government Publishing Service.

Hilliard, O. M. & Burtt, B. L. (1971). Streptocarpus: *An African Plant Study*. Pietermaritzburg: University of Natal Press.

Jong, K. (1978). Phyllomorphic organisation in rosulate *Streptocarpus*. *Notes Roy. Bot. Gard. Edinburgh* 36: 369–396.

Mogensen, H. L. (1996). The hows and whys of cytoplasmic inheritance in seed plants. *Amer. J. Bot.* 83: 383–404.

Möller, M. & Cronk, Q. C. B. (1997). Origin and relationships of *Saintpaulia* (Gesneriaceae) based on ribosomal DNA internal transcribed spacer (ITS) sequences. *Amer. J. Bot.* 84: 956–965.

Möller, M. & Cronk, Q. C. B. (2001). Phylogenetic studies in *Streptocarpus* (Gesneriaceae): reconstruction of biogeographic history and distribution patterns. *Syst. Geogr. Pl.* 71: 545–555.

Reboud, X. & Zeyl, C. (1994). Organelle inheritance in plants. *Heredity* 72: 132–140.

Richardson, J. E., Pennington, R. T., Pennington, T. D. & Hollingsworth, P. M. (2001). Rapid diversification of a species-rich genus of Neotropical rain forest trees. *Science* 293: 2242–2245.

Soltis, D. E., Soltis, P. S. & Milligan, B. G. (1992). Intraspecific chloroplast DNA variation: systematic and phylogenetic implications. In: Soltis, P. S., Soltis, D. E. & Doyle, J. J. (eds) *Molecular Systematics of Plants*, pp. 117–150. New York: Chapman & Hall.

TABERLET, P., GIELLY, L., PAUTOU, G. & BOUVET, J. (1991). Universal primers for the amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1109.

WEIGEND, M. & EDWARDS, T. J. (1994). Notes on *Streptocarpus primulifolius* (Gesneriaceae). *S. African J. Bot.* 60: 169–174.

WEIGEND, M. & EDWARDS, T. J. (1996). The palynology of *Streptocarpus* and the other African and Malagasy Gesneriaceae and its systematical implications. *Bot. Jahrb. Syst.* 118: 59–80.

WILLIAMS, M. A. J. (1985). Pleistocene aridity in Africa, Australia and Asia. In: DOUGLAS, I. & SPENCER, T. (eds) *Environmental Change and Tropical Geomorphology*, pp. 219–233. London: Allen & Unwin.

WILLIAMS, M. A. J., DUNKERLEY, D. C., DE DECKKER, P., KERSHAW, A. P. & STOKES, T. (1993). *Quaternary Environments*. London: Edward Arnold.

An invited contribution to the Festschrift for B.L. Burtt's ninetieth birthday