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Unusual morphological and anatomical features of two woody Madagascan endemics, *Streptocarpus papangae* and *S. suffruticosus* (Gesneriaceae), and their potential taxonomic value

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Abstract

As members of a small group of caulescent Madagascan endemics, *Streptocarpus papangae* and *Streptocarpus suffruticosus* are distinctive in being branched woody shrubs in a genus largely of herbaceous habit. The present study is the first detailed comparative investigation of any woody Madagascan *Streptocarpus* species and draws attention to certain notable morphological and anatomical characteristics, particularly their non-coherent anthers, foliar and stem sclereids, nodal anatomy (split-lateral or semi-girdling traces), and ovule morphology, features previously inadequately examined in *Streptocarpus* and their potential taxonomic value overlooked. For example, in a family where anatropous ovules were previously thought to be typical, the presence of hemi-anatropous ovules in *Streptocarpus* is confirmed and here recorded for the first time in the two species. Another feature is the occurrence of split-lateral traces, a distinctive nodal feature generally uncommon among angiosperms. Macrosclereids, rare among mainland African *Streptocarpus* have been observed in the stem, petiole, leaf lamina and receptacle of *S. papangae* but only near the base of the petiole in *S. suffruticosus*. The possible significance of these and other characters to the taxonomy and phylogenetic systematics of *Streptocarpus* is discussed.
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Keywords: Hemi-anatropous ovules; Leaf structure; Non-cohering anthers; Ovule starch pattern; Sclereids; Shoot structure; Split-lateral traces

1. Introduction

Streptocarpus Lindl. (Gesneriaceae) consists of ca. 140 species (Weber, 2004). Its centre of distribution is southern Africa, its range extending into East and West tropical Africa (Hilliard and Burtt, 1971). A few species of doubtful affinity have been recorded from Asia. Thirty-seven species are endemic to Madagascar and the Comoro Islands (Hilliard and Burtt, 1971; Humbert, 1971). A diversity of growth forms, many of highly unusual architecture, occurs in the genus, ranging from the familiar acaulescent rosulate *Streptocarpus rexii* (Hook.) Lindl. (Cape Primrose) and single-leafed *Streptocarpus grandis* N.E.Br. to caulescent species, such as *Streptocarpus caulescens* Vatke with opposite decussate leaves borne on aerial shoots (Hilliard and Burtt, 1971; Jong, 1970, 1973, 1978; Jong and Burtt, 1975).

A greater morphological diversity, however, is now known among Madagascan members of *Streptocarpus* through the work of Humbert (1955, 1967, 1971) and Hilliard and Burtt (1971). Some of the species there have a rosette growth habit with long-petioled leaves, bearing a striking resemblance to *Saintpaulia* H.Wendl. (African violet) of mainland Africa (Hilliard and Burtt, 1971; Humbert, 1971; Möller and Cronk, 2001a), but more spectacular are the woody species.

Species with a shrubby woody habit are rare in the genus and form only a small group of seven species endemic to Madagascar: *Streptocarpus campanulatus* B.L.Burtt, *Streptocarpus coursii* Humbert, *Streptocarpus glabrifolius* Humbert, *Streptocarpus macropodus* B.L.Burtt, *Streptocarpus papangae* Humbert,

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Streptocarpus suffruticosus Humbert, and *Streptocarpus tsaratananensis* B.L.Burtt (Hilliard and Burtt, 1971; Humbert, 1971). As little detailed anatomical information is available for any such members of the genus, *S. papangae* and *S. suffruticosus*, the only two representatives successfully cultivated at the Royal Botanic Garden Edinburgh (RBGE), form the subject of this investigation. Both occur in montane evergreen forest, and *S. papangae* is also found among ericoid shrubs on mountain tops. *S. papangae* is known only from the Col de Beampingaratra in South Madagascar, growing as a shrub up to 120 cm, whereas *S. suffruticosus* occurs in central, northern and eastern Madagascar as shrubs 1–2 m tall (Hilliard and Burtt, 1971). With regard to the latter our own field observations in the North indicate that it occurs mainly as an epiphyte.

Streptocarpus comprises two subgenera, *Streptocarpella* consisting of caulescent species and *Streptocarpus* of predominantly acaulescent ones. The woody species have thus been placed in the subgenus *Streptocarpella* by Hilliard and Burtt (1971), but they differ in so many respects from other members of this subgenus that their assignment may have to be re-evaluated when more is known about them. In their book on the genus, Hilliard and Burtt (1971, chapter 9) allocated a separate chapter to Madagascan *Streptocarpus*, "recognising three groupings that are not represented on the mainland" placing together the shrubby woody species into their group (iii) characterised by short filaments, with seeds "long pointed at both ends and distinctly larger than in the other species" (Hilliard and Burtt, 1971).

Phylogenetic studies on *Streptocarpus* have increased our understanding of the evolution of the genus (Möller and Cronk, 1997; Smith et al., 1998) and demonstrated that woody Madagascan species are not closely related to herbaceous caulescent ones (Möller and Cronk, 2001a,b; MacMaster et al., 2005). Molecular phylogenetic work indicated that the subgenus *Streptocarpella* is not a monophyletic group, with only the African and Madagascan herbaceous caulescents forming a closely related alliance (clade I in Möller and Cronk, 2001a,b). All acaulescents of subgenus *Streptocarpus*, fall in clade II of Möller and Cronk (2001a,b). The woody caulescent species also fell in this clade (Möller and Cronk, 2001a,b; MacMaster et al., 2005). The two clades are fully congruent with the basic chromosome number, being $x=15$ for the caulescent members in clade I, and $x=16$ for the acaulescent species in clade II (Möller and Cronk, 2001a). It should be noted that the shrubby caulescent Madagascan species *S. papangae* and *S. suffruticosus* also have $x=16$ (Jong and Möller, 2000; Möller and Kiehn, 2004). Within clade II these species form a monophyletic clade that is most closely related to acaulescent Madagascan species, such as *Streptocarpus ibityensis* Humbert, *Streptocarpus itremensis* B.L.Burtt and *Streptocarpus lanatus* MacMaster (Möller and Cronk, 2001a,b; MacMaster et al., 2005). Thus, it seems that members of the shrubby woody group constitute a coherent group, separate from herbaceous caulescent species of both Madagascar and mainland Africa; their evolutionary and morphological affinities may lie with other Madagascan acaulescent species.

Apart from their shrubby habit and similarity in basic chromosome number noted above, *S. papangae* and *S. suffruticosus* are clearly distinguishable anatomically from each other, as for example differences in the abundance and distribution of sclereids, structure of stomata and indumentums (Hilliard and Burtt, 1971). These features, together with new observations on other aspects of shoot, floral and ovular structure are highlighted in the present study, alongside a consideration of their relevance and potential value to the taxonomy of the woody species and the genus.

2. Materials and methods

2.1. Plant materials

This investigation was based on specimens cultivated at the RBGE. Two genotypes of *S. papangae* were examined, one with dark pink flowers (four individuals), the other creamy white (six individuals) (Möller 9718, E, cult. RBGE 19972886 referred to as A and B, respectively). One accession with eight plants of *S. suffruticosus* var. *suffruticosus* was available for study (Möller 9877, E, cult. RBGE 19990122).

S. papangae was propagated from cuttings collected at the Col de Beampingaratra, whereas *S. suffruticosus* was raised from seed, collected on the Marojejy Mountains, Madagascar. The accessions thrived in cultivation; at the time of writing, specimens of *S. papangae* have reached ca. 140 cm in height, with several woody branches arising from the base, and often flowering profusely. *S. suffruticosus* has a pendulous form, conforming to its epiphytic habit in the wild. For the benefit of horticulturists interested in growing these unusual plants, some notes on their cultivation are appended. Both species, interestingly, failed so far to set seed under glasshouse conditions, despite numerous attempts at artificial pollination.

2.2. Preparation of stem, node and leaf sections

Freshly collected shoot samples were fixed in FAA (9 parts 70% ethanol, 0.5 parts glacial acetic acid and 0.5 parts formaldehyde, Johansen, 1940) or in Farmer's Fluid (3 parts absolute ethanol to 1 part glacial acetic acid, Johansen, 1940) for 1 h to overnight, then stored in 70% ethanol for later use. No notable difference in fixation was detected between the two fixatives. Observations were made on free hand sections of stem and nodes as well as on freezing microtome sections. Rotary microtome sections of paraffin wax-embedded stem and leaf material were prepared after dehydration in a tertiary butyl alcohol series according to the procedure in Johansen (1940), except for the substitution of the toxic xylene with Histoclear in the final steps. Parawax of 56 °C melting-point was used in embedding. Microtome sections were taken at 12–15 μm, and stained with dilute aqueous Safranin O (0.05%) (Johansen, 1940).

Fixed material for clearing of nodes was transferred to water (through an alcohol series). Thick longitudinal as well as transverse sections were then placed in 5% NaOH at 30–40 °C for 5 or more days, washed in several changes of distilled water and transferred to saturated chloral hydrate until the material was transparent. The vascular tissues became clearly visible, although staining (about 5 min) in 0.01% aqueous Safranin O improved the details.

Cleared material was viewed under incident light against a dark background under a Stemi 2000C dissecting microscope (Zeiss, Welwyn Garden City, UK) and photographed with an AxioCam MRc5 digital camera. Microtome sections were viewed under a Zeiss Axioskop brightfield microscope and images recorded with a similar digital camera as above.

2.3. Preparation of ovary and anther sections

Freshly collected floral samples were fixed in FAA as above (with 50% ethanol instead of 70% for the more delicate material). Rotary microtome sections of plastic-embedded material (Technovit 7100, Heraeus Kulzer, Wehrheim, Germany) were prepared according to the manufacturer's protocol, and sectioned at 10 μm. Sections were double-stained with Delafield's Haematoxylin and dilute Safranin O according to the rapid technique of Jong (1970) that is essentially similar to the protocol of Dean (1940). The microtome sections were observed with an Axioskop brightfield microscope and the images were captured as above.

2.4. Whole mounts of ovules

Newly opened flowers fixed in Farmer's Fluid were first transferred to distilled water through an ethanol series. An ovary segment was then placed in a drop of water on a microscope slide, one side cut open longitudinally to expose the ovules. Under a dissecting microscope, ovules were carefully detached with a dissecting needle or pointed scalpel, unwanted tissues removed, excess water blotted off, and a drop of Melzer's Reagent applied (see below). The material was left for about 5 min before applying a coverslip, and sealing it with rubber solution or clear nail varnish. The preparations were observed under a brightfield Axioskop microscope and the images were recorded as above.

Melzer's Reagent is basically a mycological reagent first introduced by Melzer in 1924. It was adopted as a routine histological mountant by Jong (1970) and now found to be of great advantage to the present study. In addition to the staining of cellulose cell walls a yellow colour, and lignified tissues a light to darker orange hue, Melzer's chloral hydrate component conferred a useful degree of clearing, while its iodine and potassium iodide ingredients revealed the presence of starch. As this reagent is not widely used in angiosperm histology, its composition as published in Wakefield and Dennis (1950, p. 37) is here included: distilled water — 100 ml, chloral hydrate — 100 g, potassium iodide — 5 g, and iodine — 1.5 g. One of the drawbacks of this reagent is that the reactions observed, particularly the starch reaction, fade within 5 days or so. It is therefore advisable to photograph suitable views soon after slide preparation.

2.5. Scanning electron microscopy of leaves and floral structures

Freshly collected leaf and floral samples were fixed in 50% ethanol FAA overnight at room temperature, then dehydrated through an ethanol series: 15 min at 50%; 15 min at 70%; 10 min at 95%; 5 min at 100%; 2 × 5 min at 100% dry acetone. They were then dried using an Emitech K850 critical point dryer (Quorum Technologies Ltd, Ashford, UK). Ovules were first dissected from the ovary using a razor blade prior to fixation to assist their handling once dry. Samples were mounted on aluminium stubs with double sided sticky carbon tabs and coated with platinum for 2 min at 25 mA using an Emitech K575x sputter coater (Quorum Technologies Ltd, Ashford, UK). The material was scanned using a LEO Supra 55VP SEM (Zeiss, Cambridge, UK). Images were captured at 5 kV using secondary electron signal detection.

3. Observations

Although *S. papangae* and *S. suffruticosus* are remarkable in being woody shrubs (Fig. 1A, B) in a largely herbaceous genus, they possess other distinctive morphological and anatomical characteristics. While they have some features in common, they also differ in numerous notable respects from each other, not only in gross morphology, but also in their anatomy (summarized in Table 1). To avoid unnecessary repetition, characteristics for *S. papangae* are given in fuller detail than those for *S. suffruticosus* and generally only where they differ from *S. papangae*.

Stems erect (*S. papangae*, Fig. 1A) or pendulous (*S. suffruticosus*, Fig. 1B), almost glabrous, inconspicuous eglandular trichomes on the first and second internodes (*S. papangae*) or densely pubescent with predominantly long uniseriate multicellular glandular and eglandular trichomes, and some scattered short two-celled glandular ones (*S. suffruticosus*); young internodes slightly flattened, older ones cylindrical (*S. papangae*) or shoots always round (*S. suffruticosus*); white patches at nodes often containing stomata; periderm superficial with lenticels in both species; in transverse sections (TS), stem cortical cells not in a neatly layered organisation (Fig. 2A); outer cortex of 6 or 7 layers of collenchyma (*S. papangae*) or 7 or 8 neatly layered rectangular parenchyma cells (*S. suffruticosus*); simple starch grains in the parenchyma and endodermis, notably abundant in cortical layers adjoining the endodermis (*S. papangae*) or simple starch grains present in cortical tissues as well as in endodermis and pith (*S. suffruticosus*); Casparian strip indistinct in places; sclereids scattered in outer cortex, simple, rhomboidal to oblong (*S. papangae*) (Fig. 2B, C) or sclereids absent (*S. suffruticosus*); druses and other forms of crystals in pith; vascular bundles (with pericyclic fibre caps) initially separate become joined by lignified interfascicular tissue.

Nodes trilacunar with split-lateral traces, i.e. in addition to a single median leaf trace that enters the associated leaf directly, two lateral traces arising 90° from the leaf insertion point, each partially encircling or girdling the stem before reaching the leaf it supplies. In both species, these laterals can arise through bifurcation of a single vertical trace at the level of leaf insertion (Fig. 3A, B) or as parallel vertical traces running down the internode (Fig. 3C, D); median trace with prominent associated single gap (Fig. 3E); lateral trace gaps small and often indistinct (Fig. 3B); split-lateral traces observed in both young and old nodes; sclereids abundant in the outer regions, with their long axes parallel to the stem axis (*S. papangae*) (Fig. 2C) or sclereids absent (*S. suffruticosus*).

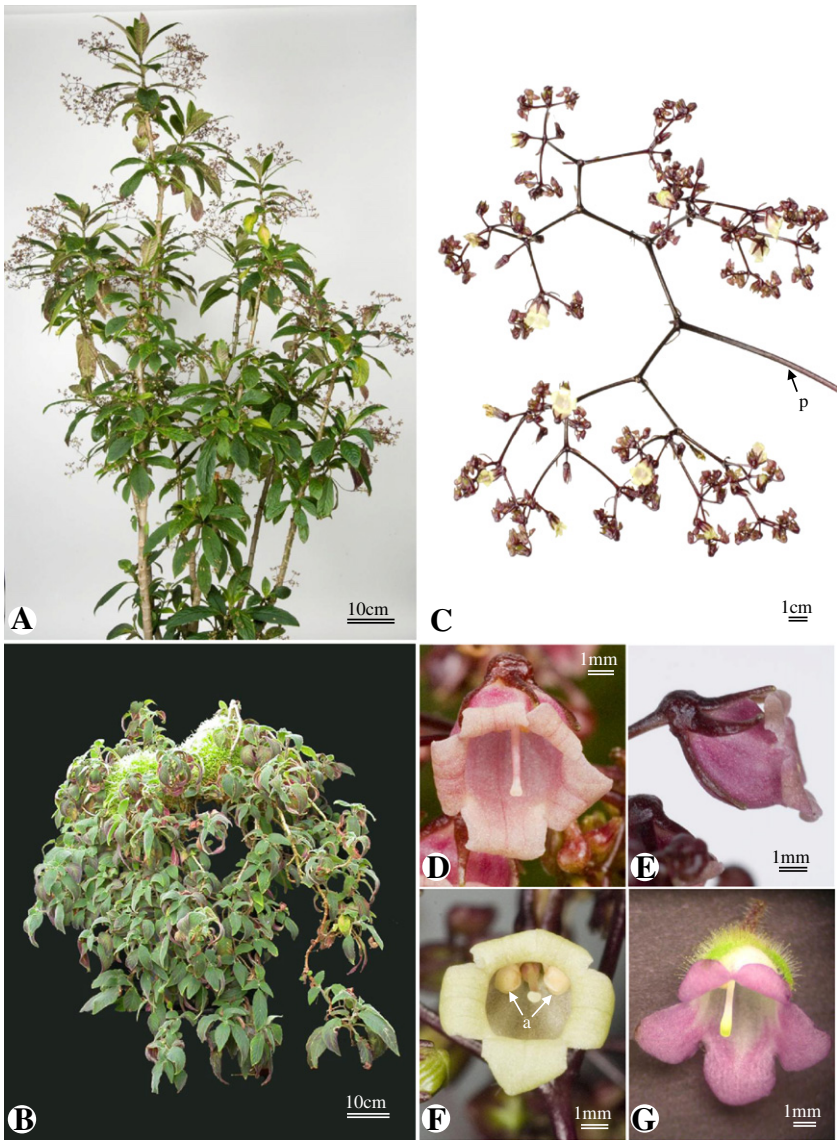

Fig. 1. General morphology. *Streptocarpus papangae*: (A) habit; (C) much branched sympodial inflorescence; (D) front view and (E) side view of the pink flower, note glabrous calyx; (F) front view of the creamy white flower showing non-coherent anthers. *Streptocarpus suffruticosus*: (B) habit; (G) flower, note dense pubescence. a — anther, p — peduncle.

Leaves opposite decussate, petioles 10–20(30) mm long (*S. papangae*) or 10–15 mm long (*S. suffruticosus*); lamina 115–160 × 40–60 mm (*S. papangae*) or 65–100 × 30–40 mm (*S. suffruticosus*); unequal at base; thick, coriaceous, largely glabrous except for a few glandular trichomes, each with a single-celled stalk and one to four-celled heads in depressions on both upper and lower epidermis (Fig. 5E), only a few inconspicuous multicellular eglandular trichomes on petioles, sometimes on young leaves of the first and second nodes (*S. papangae*) (Figs. 4A; 5A, C) or thin (Fig. 4B), densely pubescent with long trichomes, uniseriate multicellular glandular and eglandular (tipped with a thick-walled pointed terminal cell), trichome-base with conspicuous multicellular foot (Fig. 5B), some short glandular ones scattered among the long glandular ones on both surfaces (*S. suffruticosus*) (Fig. 5D, F); leaf margins smooth, gently undulating, with inconspicuous hydathodes (*S. papangae*) or finely toothed, each tooth with a conspicuous hydathode (*S. suffruticosus*); adaxial epidermal cells mostly of irregular isodiametric shape, surface conspicuously convex (*S. papangae*) (Figs. 4A; 5A) or with flat surface (*S. suffruticosus*) (Fig. 5B); stomata adaxially absent; abaxial epidermis glabrous, cells not convex (*S. papangae*) or obscured by the dense presence of trichomes and stomata (*S. suffruticosus*) (Fig. 5D); stomata anisocytic with three unequal subsidiary cells surrounded by an additional ring of larger narrow subsidiary cells (*S. papangae*) (Fig. 5C) or anisocytic stoma conspicuously raised on usually two to three rings of subsidiary cells of varying size (*S. suffruticosus*) (Figs. 4B, C; 5D, F); lamina 450–500 μm thick (*S. papangae*) or 300–350 μm (*S. suffruticosus*); hypodermis a single layer in genotype with cream-coloured flowers, two in genotype with pink flowers (*S. papangae*) (Fig. 4A), number of layers constant within a plant irrespective of leaf age, sclereids present in the inner layer

Table 1
Morphological and anatomical differences between the shrubby Madagascan *Streptocarpus papangae* and *S. suffruticosus*.

Character	*S. papangae*	*S. suffruticosus*
Gross morphology	Erect terrestrial shrub; glabrous	Pendulous epiphyte; densely pubescent
Stem and node		
Sclereids	Present	Absent
Internodes	1st and 2nd somewhat flattened	Always round
Leaf		
Petiole	10–20(30) mm long	10–15 mm long
Lamina	115–160 × 40–60 mm	65–100 × 30–40 mm
Thickness	450–500 μm	300–350 μm
Spongy mesophyll	Ca. 250 μm thick	Ca. 150 μm thick
Margins	Smooth, with inconspicuous hydathodes	Finely toothed, each tooth with a conspicuous hydathode
Trichomes	Scattered short glandular trichomes on both leaf surfaces	Dense indumentum of long glandular and eglandular trichomes on both surfaces
Adaxial epidermis	Cell surface distinctly convex	Cell surface flat
Stomata	Level with epidermal cells	Raised on turrets
Hypodermis	One or two layers	Absent
Sclereids	In the hypodermis, midrib and outer regions of the petiole; astrosclereids in spongy mesophyll	Confined to basal region of petiole
Floral features		
Peduncle	70–90 mm long	20–40 mm long
Pedicel	Ca. 6–8 mm long, obliquely attached to flowers	Ca. 6 mm long, with straight attachment to flowers
Trichomes	Few short glandular trichomes on the lower region of inflorescences, on bracts and bracteoles	Peduncle and pedicel densely pubescent, long uniseriate multicellular glandular and eglandular trichomes; some short glandular trichomes scattered among long ones
Calyx lobes	Ca. 3 mm long, some short glandular trichomes on outer surface	Ca. 4 mm long, long glandular and eglandular multicellular trichomes on outer surface
Sclereids	Present in receptacle	Absent
Stamens	Filament ca. 1 mm long, without apical tooth	Filament ca. 1–1.5 mm long, each with apical tooth
Stigma	Subcapitate	Sublingulate
Ovary	Ca. 2.5 mm long, bottle-shaped, glabrous	Ca. 1.5 mm long, with short multicellular glandular trichomes
Ovules	Straight micropylar tip	Slightly curved micropylar tip

with long axes perpendicular to epidermis, also abundantly in the midrib and the outer regions of the petiole (*S. papangae*) (Figs. 3B; 4A, D) or hypodermis absent (*S. suffruticosus*); palisade single layer; spongy mesophyll ca. 250 μm thick, with scattered astrosclereids that resemble lignified mesophyll cells (*S. papangae*) (Fig. 4E) or ca. 150 μm thick, lower epidermal cells with abundant simple starch grains, sclereids notably confined to basal region of petiole, slightly longer than short, otherwise not very different in size from the surrounding parenchyma (*S. suffruticosus*) (Figs. 2D; 3B, D).

Inflorescences axillary in the upper part of the shoot (Fig. 1A), branching repeatedly and symmetrically up to eight levels of dichasial cymose units (Fig. 1C), fully developed inflorescence carries remarkably about 500 flowers (at least in cultivated specimens; few-flowered in the field according to the description of *S. papangae* in Hilliard and Burtt, 1971). Peduncles 70–90 mm long (*S. papangae*) or 20–40 mm long (*S. suffruticosus*); pedicels ca. 6–8 mm long, obliquely attached to flowers (*S. papangae*) (Fig. 1E) or ca. 6 mm long, straight attachment to flowers (*S. suffruticosus*); trichomes few, inconspicuous, multicellular eglandular on lower regions of inflorescence, on bracts and bracteoles (*S. papangae*) or on peduncle and pedicel long, uniseriate multicellular glandular and eglandular, some short, glandular, scattered among long ones (*S. suffruticosus*).

Calyx lobes narrowly linear-lanceolate (*S. papangae*) (Fig. 1E) or lanceolate (*S. suffruticosus*); ca. 3 mm long (*S. papangae*) or ca. 4 mm long (*S. suffruticosus*); a few short one to four-celled glandular (*S. papangae*) or long glandular and eglandular multicellular trichomes (*S. suffruticosus*); sclereids scattered in receptacle (*S. papangae*) or absent (*S. suffruticosus*).

Corolla pouch-like (Fig. 1D–G); glabrous; short corolla tube ca. 4–5 mm long, corolla face ca. 7–8 mm wide (*S. papangae*) or ca. 6–8 mm wide (*S. suffruticosus*); in some plants dark pink (Fig. 1D, E), creamy white (Fig. 1F) in others (*S. papangae*) or light pink (*S. suffruticosus*) (Fig. 1G); lobes reflexed.

Stamens 2, in anterior positions; filaments short, 1 mm long (*S. papangae*) (Fig. 5G) or ca. 1–1.5 mm long and each with a prominent apical tooth (*S. suffruticosus*) (Fig. 5H); free to base; glabrous; anthers non-coherent, each situated on either side of the ovary (Figs. 1F, 5G, H); filaments short, curved, arising from a pad of tissue at the corolla base; initially separate pollen sacs becoming confluent at maturity; wall layers comprise the epidermis, one middle layer, a typical endothecium with secondary thickening and single layered tapetum; stomium subapical; introrse dehiscence (Figs. 5H, 6A); staminodes 2 (Fig. 5G, H).

Ovary bottle-shaped, ca. 2.5 mm long (*S. papangae*) (Fig. 5G) or ca. 1.5 mm long (*S. suffruticosus*) (Fig. 5H); glabrous (*S. papangae*) or with short multicellular glandular trichomes similar to those on leaves (*S. suffruticosus*) (Fig. 5H); unilocular; bifid intrusive parietal placenta at right angles to the dorso-ventral floral axis; ovules only at tips of the incurved placental surface (Fig. 6B); nectary disc a ring.

Style ca. 2.5 mm long (*S. papangae*) or ca. 3 mm long (*S. suffruticosus*). *Stigma* stomatomorphic with a deep cleft; subspatulate (*S. papangae*) (Fig. 5I) or sublingulate, somewhat bilaterally flattened (*S. suffruticosus*) (Fig. 5J); surface papillate; papillae bottle-shaped, dimple-tipped with callose, covering only ca. 200 μm length at the tip (*S. papangae*) (Fig. 5I) or papillae covering relatively larger stigmatic area ca. 800 μm (*S. suffruticosus*) (Fig. 5J).

Ovules hemi-anatropous, micropyle facing obliquely outwards (Fig. 6C); ovules straight (*S. papangae*) (Fig. 6D) or with slightly curved micropyle (*S. suffruticosus*) (Fig. 6E); integument single; funiculus short; in newly opened flowers ovules with a regular pattern of starch granule distribution in the shape of an exclamation mark, showing distinct polarity, the greatest quantity of

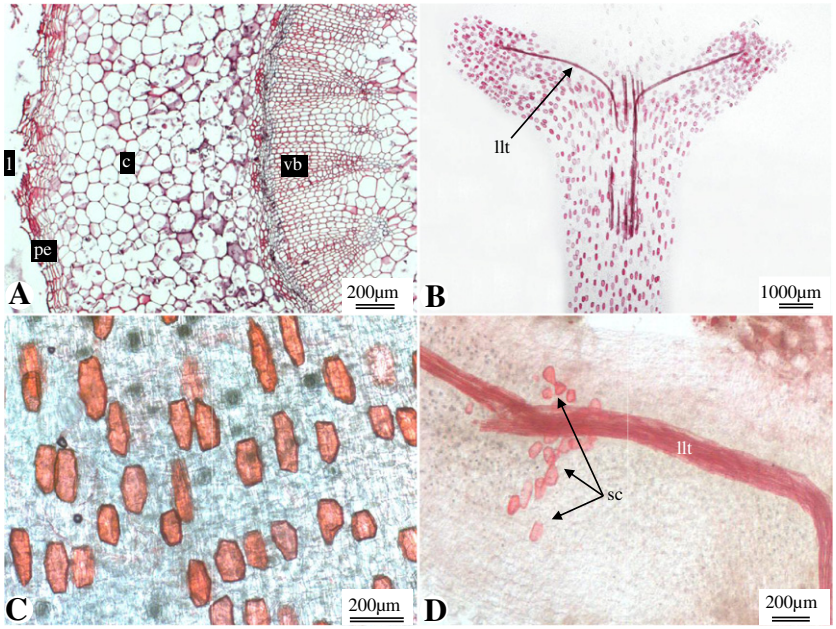

Fig. 2. Shoot anatomy and sclereid distribution. *Streptocarpus papangae*: (A) transverse sections of a young stem with superficial periderm and lenticel, outer cortex and arrangement of vascular bundles; (B) stem longitudinal section of the fourth node showing sclereid (stained red) distribution in the cortex and petioles, and split-lateral traces; (C) longitudinal section of the fourth node with sclereids (stained red). *Streptocarpus suffruticosus*: (D) longitudinal section of the fourth node base of the petiole showing split-lateral trace, sclereids only in the petiole base (arrows). l — lenticel, llt — lateral leaf trace, c — cortex, pe — periderm, sc — sclereids, vb — vascular bundle.

starch at micropylar end, sparser towards the chalaza, usually followed by a clear zone, ending in a small dot-like group (Fig. 6D, E); starch granules mainly compound at the micropylar pole, much larger than those at the chalazal end where the grains tend to be simple; in microtome sections, starch grains clearly visible within the embryo-sac itself as well as in the nucellus (Fig. 6C).

4. Discussion

4.1. Secondary growth

Woodiness is perhaps the feature that clearly differentiates the shrubby Madagascan species examined here from any other group of *Streptocarpus* species recognised by Hilliard and Burtt (1971). Secondary growth through the activity of a vascular cambium, however, is not unique to *S. papangae* and *S. suffruticosus*, as it also occurs in other caulescent but non-shrubby *Streptocarpus*, as for example in the herbaceous perennial *S. caulescens* and annual *Streptocarpus nobilis* C.B.Clarke of mainland Africa (Jong, 1970). In her study of the former, Sahasrabudhe (1970) observed that secondary growth is restricted to the vascular bundles of the eustele. Secondary tissues are also formed to a limited extent in acaulescent species, as in the unifoliate *S. grandis* (Jong, 1970; Schenk, 1942). The woodiness of the two Madagascan shrubs is clearly the result of the relatively larger amounts of secondary vascular tissue formed. Woodiness is a character shared by all shrubby Madagascan species, and since they appear to form a natural group (MacMaster et al., 2005), this character is diagnostic for these species.

4.2. Indumentum

The indumentum is probably the most variable character in plants. While Solereder (1908) subdivided the trichomes of Gesneriaceae into two categories, glandular and eglandular, Sahasrabudhe and Stace (1974) distinguished five categories, (1) short glandular trichomes, (2) stalked glandular trichomes, (3) unbranched eglandular trichomes, (4) branched eglandular trichomes and (5) seedling glandular trichomes. The trichomes of the two woody *Streptocarpus* species here conform to the above first three categories. However, even though they have many characters in common (e.g. non-coherent anthers, chromosome number and woodiness) and are phylogenetically closely related to each other (MacMaster et al., 2005), they differ markedly in their indumentums (Table 1). This difference is of diagnostic value, though its ecological significance is unclear; *S. papangae* is a terrestrial plant in ericoid shrubland whereas *S. suffruticosus* a facultative epiphyte in evergreen rainforest. However, many other evergreen rainforest epiphytes are glabrous, as in most species of the genus *Aechynanthus* Jack of the Gesneriaceae.

The basal cells of the uniseriate trichomes of *S. suffruticosus* are distinctly multi-celled, a feature that occurs also in other Gesneriaceae, as for example in the New World species such as *Kohleria grandiflora* L.P.Kvist & L.E.Skog (as *Capanea grandiflora* in Wiehler, 1983). The glandular trichomes of *S. suffruticosus* produce a sticky exudate, also visible as yellow-brown droplets in Melzer stained preparations. Short glandular trichomes situated in depressions on the adaxial leaf surface encountered in *S. papangae* (Fig. 5A, C) have also been reported for some *Aeschynanthus* species (e.g. *Aeschynanthus parvifolius*

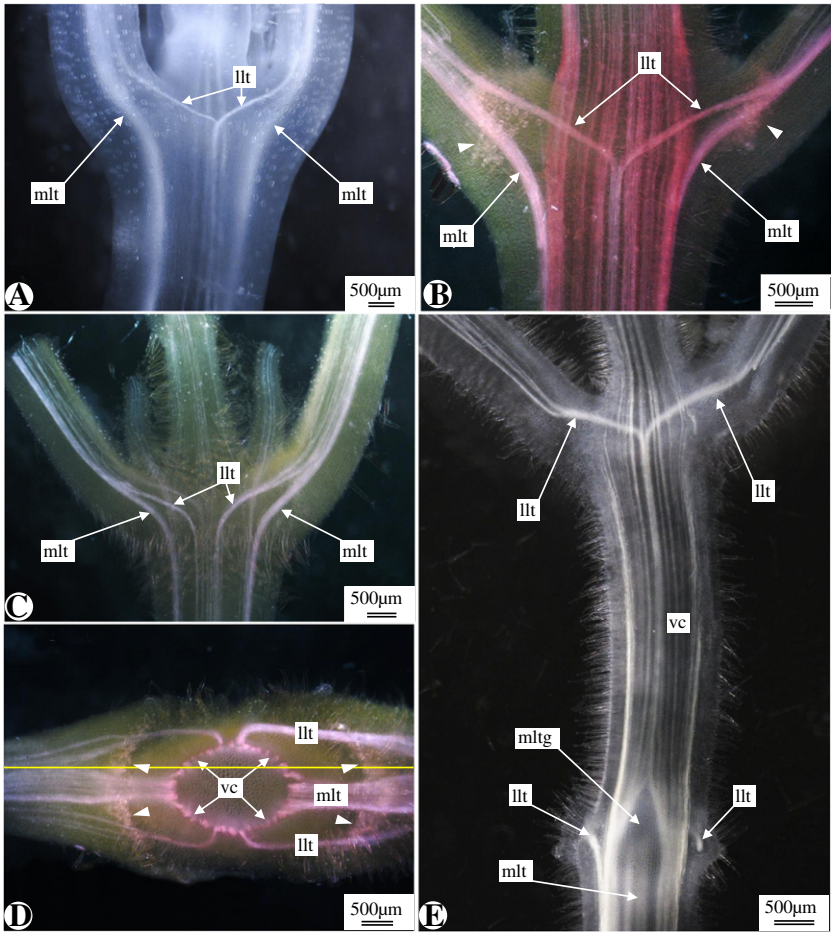

Fig. 3. Shoot vasculature. *Streptocarpus papangae*: (A) longitudinal section through young stem observed from the outside, median leaf traces and split-lateral leaf traces with split configuration. *Streptocarpus suffruticosus*: (B) longitudinal section through the fourth node external view, showing median leaf traces and split-lateral leaf traces with split configuration and sclereids at the base of the petiole (arrowheads); (C) young node with median leaf traces and split-lateral leaf traces with parallel configuration; (D) transverse section of the 4th node with the median leaf and split-lateral leaf traces in parallel configuration, and band of sclereids only at the base of the petioles (arrowheads); yellow line indicates the plane of sectioning of the stem shown in E; (E) stem longitudinal section at the fourth and fifth node internal view, showing a spatial relationship between the median leaf traces and the split-lateral leaf traces, split configuration with continuing vascular strands. llt — lateral leaf trace, mlt — median leaf trace, mltg — median leaf trace gap, vc — vascular cylinder.

R.Br.) (Metcalf and Chalk, 1950) and the Sri Lankan *Championia reticulata* Gardner, *Chirita* D.Don and *Didymocarpus* Wall. (Herat and Theobald, 1979). The species from Sri Lanka of the latter two genera are now included in *Henckelia* Spreng. (Weber et al., 2011). Too few observations across the Gesneriaceae are available to make any sound deduction on the systematic value of these indumentum features.

4.3. Stomata

Both the Madagascan species studied here possess basically anisocytic stomata. As the three unequal subsidiary cells are surrounded by one or more rings of narrow cells, these stomata may be classified as cyclocytic in the terminology of Stace (1965). The characteristic domed structures topped by a single stoma above a large substomatal chamber in *S. suffruticosus* (Figs. 4B, C; 5D, F), termed "stomatal turrets" by Hilliard and Burtt (1971), are clearly very different from those of *S. papangae*, where the stomata are more or less level with the epidermis.

Turrets with a single stoma similar to those of *S. suffruticosus* are also found in New World Gesneriaceae, in most members of the tribe Gloxinieae (e.g. *Kohleria* Regel and *Moussonia* Regel, Wiehler, 1983) and in a few of the Old World, for example *Henckelia humboldtiana* (Gardner) A.Weber & B.L.Burtt, *Henckelia floccosa* (Thwaites) A.Weber & B.L.Burtt and the monotypic *Championia reticulata* (Herat and Theobald, 1979). Stomatal turrets consisting of groups of stomata instead of just a single stoma have been reported in a few species of the Old World (e.g. *Boea* Lam., *Cyrtandra* J.R.Forst. & G.Forst and on the petiole-like structure (petiolode in the terminology of Jong, 1970) of the unifoliate *S. grandis*) and the New World (e.g. *Napeanthus* Gardner and the *Gesneria* alliance) (Jong, 1970; Sahasrabudhe and Stace, 1974; Skog, 1976). The occurrence of

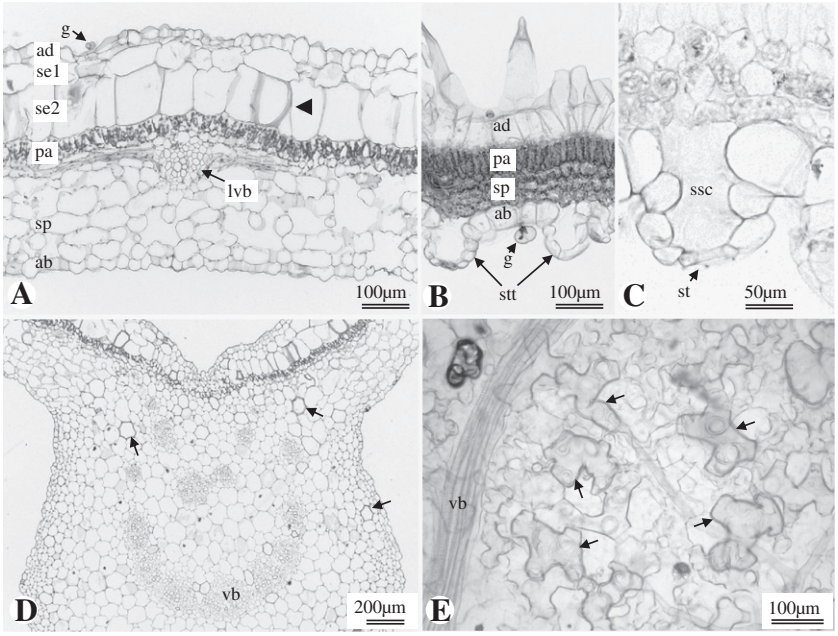

Fig. 4. Leaf structure. *Streptocarpus papangae*: (A) transverse section of leaf adaxial epidermis with convex epidermal cells, and sclereid (arrowhead) in the hypodermis; (D) transverse section of the midrib with vascular bundles, and scattered sclereids (some indicated with arrows); (E) cleared lamina segment, astrosclereids in the spongy mesophyll (arrows). *Streptocarpus suffruticosus*: (B) leaf transverse section, enlarged upper and lower epidermal cells, and stomatal turrets; (C) magnified stomatal turret showing a ring-like arrangement of the subsidiary cells and large sub-stomatal chamber. ab — abaxial epidermis, ad — adaxial epidermis, g — short trichome with single celled stalk and two-celled head, lvb — lateral vascular bundle with smaller branches, pa — palisade layer, se1 and se2 — hypodermis layers 1 and 2, sp — spongy parenchyma, ssc — sub-stomatal chamber, st — stoma, stt — stomatal turrets, vb — vascular bundle.

such stomata in a wide range of genera across the family suggests that they may have evolved several times independently. The most recent molecular phylogenetic study clearly supports this inference, as the above-mentioned genera of both the Old World and the New World belong to unrelated major clades (Möller et al., 2009).

4.4. Hypodermis

In the Gesneriaceae the hypodermis is generally regarded as the water storage tissue (Metcalf and Chalk, 1950; Rosser and Burtt, 1969; Wiehler, 1983). Within a genus its presence can be consistent but the number of layers can vary greatly, and was found for example, to be between one and nine layers among 32 species of *Aeschynanthus* analysed by Rosser and Burtt (1969).

The presence or absence of hypodermal layers has been reported for the genera in both Old World and New World (Metcalf and Chalk, 1950; Sahasrabudhe, 1970; Wiehler, 1983), reflecting Cutler's view that "because of the sporadic occurrence of the distinct hypodermis in the taxa of vascular plants, its presence or absence is of little taxonomic and small diagnostic value (except at the species level)" (Cutler et al., 2007, p. 61). And although Wiehler (1983) suggests that the absence/presence of a hypodermis can have a taxonomic value in separating genera such as *Alloplectus* Mart. and *Nematanthus* Schrad. and *Alsobia* Hanst. from *Episcia* Mart., he gives examples of variation within a genus as in *Gloxinia*. This was also found for *Streptocarpus* by Sahasrabudhe (1970) who showed that all acaulescent species she examined lack a hypodermis, but not all caulescent herbaceous species possessed a hypodermis. Notably it was absent in the African *S. nobilis* and the Madagascan *Streptocarpus hilsenbergii* R.Br. We only investigated two woody Madagascan species here, and these differ in the presence of a hypodermis. This illustrates the low diagnostic value of this characteristic for this group of *Streptocarpus* species.

4.5. Split-lateral leaf traces

Girdling traces are generally uncommon among vascular plants. They are characteristic of cycads but otherwise not known in other gymnosperms (Crane, 1985). They have been recorded in just a few angiosperm families (Dickison, 1980; Howard, 1970; Puff, 1978).

Among the first studies on the nodal pattern of such lateral traces specifically in the family Gesneriaceae was in fact that of Hollstein. His work was contained in his unpublished dissertation in 1878 which was traced by Wiehler (1983) to the only known copy in the Library of Congress, Washington DC. Wiehler's (1983) own extensive nodal survey covered over 340 species largely of neotropical Gesneriaceae, confirming the presence of split-lateral traces only in members of the tribe Episcieae. The nodal pattern in all samples from other tribes was unilacunar with one trace per leaf. Also included in his survey were several genera from the Old World. Split-lateral traces were observed in six species of caulescent *Streptocarpus* and in a few species each of *Saintpaulia*, *Chirita* and *Cyrtandra*, but not in any of

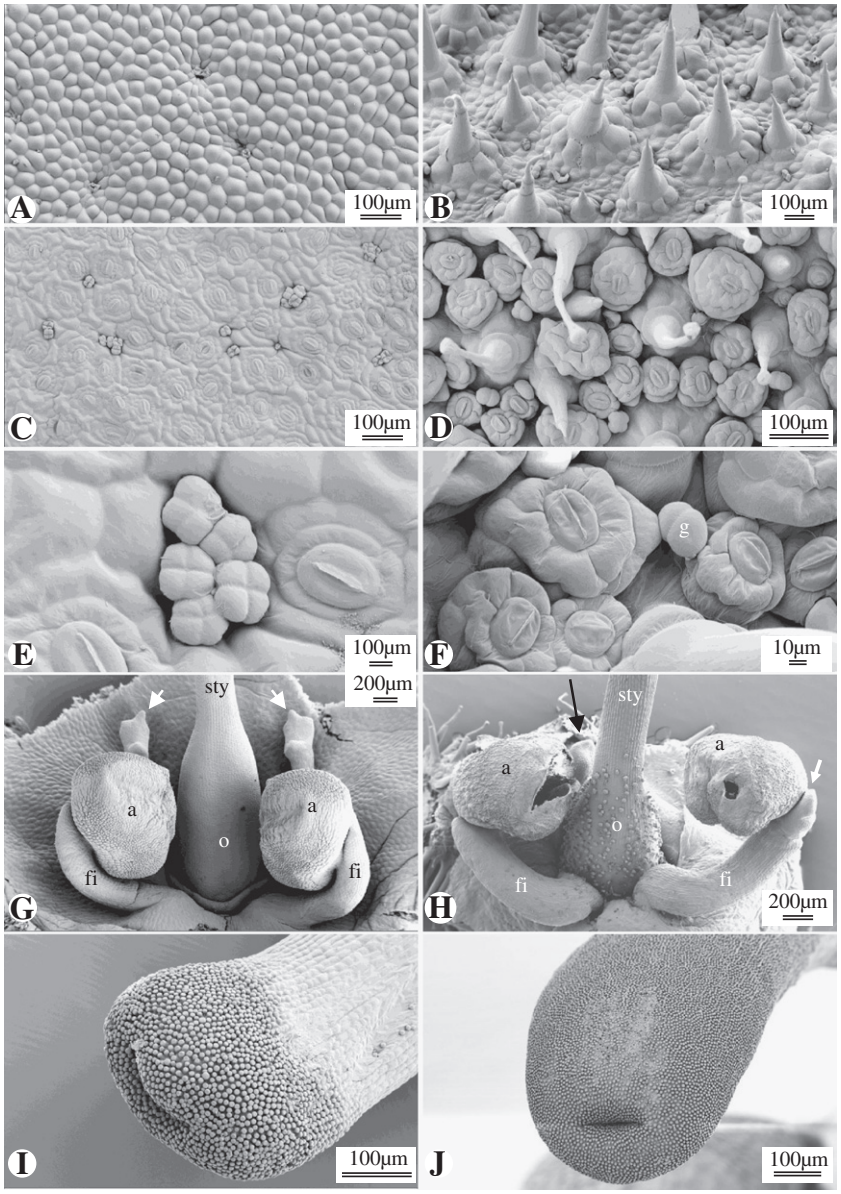

Fig. 5. Leaf and floral morphology SEM images. *Streptocarpus papangae*: (A) adaxial epidermal surface view with convex epidermal cells and short trichomes with multicellular heads in depressions; (C) abaxial epidermis with anisocytic stomata and groups of short trichomes with multicellular heads in depressions; (E) magnified view of C; (G) ovary, stamens and staminodes (arrows); (I) clefted papillate stigma. *Streptocarpus suffruticosus*: (B) adaxial epidermal surface view with glandular and eglandular trichomes with conspicuous multicellular foot; (D) abaxial epidermal surface view with glandular and eglandular trichomes and stomatal turrets of varying size; (F) higher magnification of D; (H) ovary with short glandular trichomes, stamens with toothed filament (white arrow), only one staminode visible in the figure (black arrow); (J) clefted papillate stigma. a — anther, fi — filament, g — short trichome with single celled stalk and two-celled head, o — ovary, sty — style.

the 10 species of *Aeschynanthus* nor in *Boea hygroscopica* F. Muell. where the nodes were single traced and unilacunar.

In *Streptocarpus*, split-lateral traces were first recorded by Jong (1970) for the herbaceous caulescent *S. nobilis* of West Tropical Africa. In this species and in the two woody species examined here, the lateral trace or traces originate from lower down the internode. Most commonly as a single trace splitting into two at the node, and in some nodes, two parallel independent traces each journeying separately to the relevant leaf. Such variation has also been observed by Wiehler (1983) e.g. in the New World *Columnea repens* (Hook.) Hanst.

It is clear that split-lateral traces occur in both Old World and New World Gesneriaceae. In *Streptocarpus*, they are present in the woody as well as non-woody caulescent species of both Madagascar and mainland Africa. Without further investigation of the extent of their occurrence in the family, it is too early to judge their possible taxonomic and biogeographical importance. From their scattered presence in several unrelated angiosperm families (e.g. Chloranthaceae, Rhizophoraceae, Zygophyllaceae, Gentianaceae, Adoxaceae, Gesneriaceae) Wiehler (1983) concluded "that this nodal type arose many times independently in the dicotyledons".

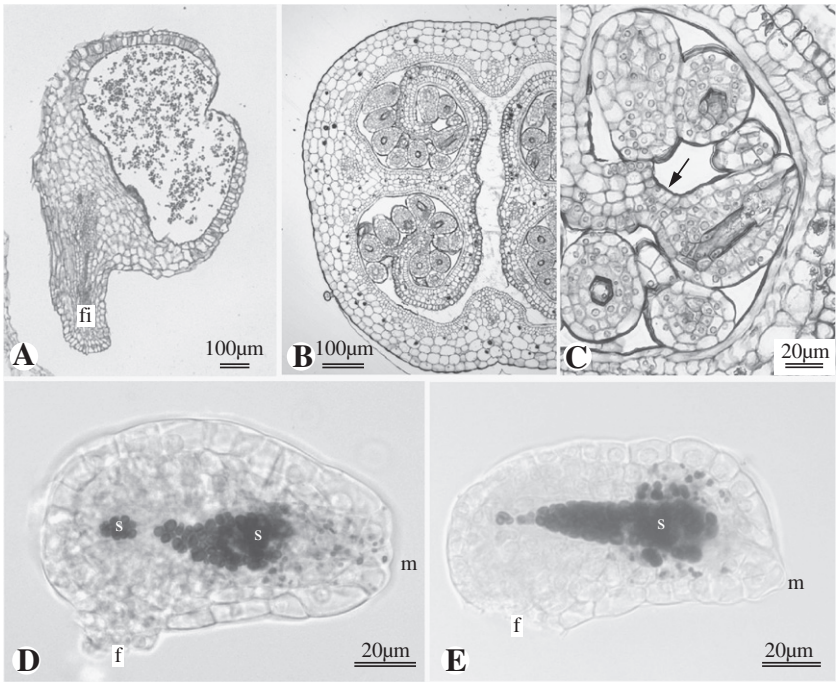

Fig. 6. Stamen, ovary and ovules. *Streptocarpus papangae*: (A) transverse section of the mature anther with confluent sporangia; (B) transverse section of the ovary showing ovules on recurved placenta; (C) hemi-anatropous ovule with short funicle (arrow); (D) whole mount ovule with starch pattern after Melzer staining (starch grains stained blue-black), note funicle damaged during preparation. *Streptocarpus suffruticosus*: (E) ovule with curved apex, ovular starch pattern after Melzer staining, note funicle damaged during preparation. f — funicle, fi — filament, m — micropylar end, s — starch grains.

4.6. Sclereids

Sclereids have long been known to have a taxonomic value in many plant groups as exemplified in *Memecylon* L. (Melastomataceae) (Rao, 1957) and palms (Tomlinson, 1959). They are rare in the family Gesneriaceae, previously recorded for only a few genera, including *Hemiboea* C.B.Clarke (Wang et al., 1998), *Loxocarpus* R.Br. (Sahasrabudhe, 1970) and *Aeschynanthus* (Rosser and Burtt, 1969). One of the most extensive surveys of foliar sclereids in the family is that on *Cyrtandra* (Bokhari and Burtt, 1970; Burtt and Bokhari, 1973). In contrast to the great diversity of sclereids reported for *Cyrtandra*, only a few sclereid types have so far been observed in *Streptocarpus*. The presence of sclereids in this genus was first mentioned by Hilliard and Burtt (1971), referred to as osteosclereids (columnar with enlarged ends, according to Dickison, 2000) in the leaf hypodermis of *S. papangae* and in another shrubby Madagascan endemic, *S. glabrifolius*. In addition, they also reported the presence of astrosclereids (radiately branched) in the spongy mesophyll of the latter species. The present study confirms the presence of sclereids in both the species examined here, but their distribution is very different from each other (Table 1). In *S. papangae*, their somewhat elongated shape (wider in the middle) with even wall thickness are more appropriately classed as macrosclereids, which according to the terminology in Dickison (2000) are somewhat elongated with uneven secondary walls. Bokhari and Burtt (1970) adopted a rather broader concept of osteosclereids which included columnar to squat shaped forms. Our observations are therefore not contradictory.

It is worth noting that "exceptionally large sclereids (with strong circular thickenings)" were observed by Sahasrabudhe (1970) in the leaf midrib of the African *Linnaeopsis subscandens* B.L.Burtt. On the basis of the molecular data of Möller and Cronk (2001a,b), species of this genus have been recently transferred to *Streptocarpus* (Darbyshire, 2006). They are phylogenetically unrelated to the woody Madagascan shrubs (Möller and Cronk, 2001a,b), and the existence of such sclereids in the Madagascan and African species is probably the result of convergent evolution.

Sahasrabudhe (1970) also reported that "all *Streptocarpus* species [examined] show sclereids with circular to horse-shoe-shaped thickenings randomly scattered in the pith and cortex of the older stems or some cells may show depositions of lignin at the corners". The shape of these cells scarcely differs from those of the surrounding parenchyma cells. Sclereids of the type here described for the Madagascan species are so far unknown among African mainland *Streptocarpus*. It would be of great interest to discover whether or not sclereid occurrence and type are associated with growth form and geographical distribution of *Streptocarpus*.

4.7. Non-cohering anthers

One of the unusual features of the two woody species examined here is their possession of non-cohering anthers each attached to short and stocky filaments (Fig. 5G, H). Besides *S. suffruticosus*, Hilliard and Burtt (1971) observed the presence of such anthers in several other Madagascan shrubs, namely *S. coursii* and *S. campanulatus*. They also noted that very

short filaments occur near the base of the corolla tube in the "herb or subshrub" *S. macropodus* and the woody shrub *S. glabrifolius*, respectively 1.5 mm and ca. 1 mm long, as in *S. papangae* and *S. suffruticosus*. It is not known whether *S. glabrifolius* and *S. macropodus* also have non-coherent anthers. Short filaments need not necessarily lead to non-coherent anthers, as for example in the herbaceous caulescent *Streptocarpus levis* B.L.Burtt of Madagascar, where the filaments are ca. 1.5 mm in length, and the anthers are coherent. It remains to be determined, however, whether non-cohering anthers are characteristic of all the Madagascan woody species, a feature not known elsewhere in *Streptocarpus*. If this turns out to be the case, it would be a taxonomically useful synapomorphy for this species group.

4.8. Ovule morphology

The ovule in both *S. papangae* and *S. suffruticosus* is of the hemi-anatropous type (Fig. 6C). Published information for several genera of the Gesneriaceae, as for example *Rhynchoglossum* Blume and *Klugia* Schltdl. (subsequently subsumed into *Rhynchoglossum*, Burtt, 1962) indicates that the ovule is of the anatropous type, which had been considered typical for the whole family (e.g. Batygina, 2006; Davis, 1966; Weber, 2004). The occurrence of another ovule type in *Streptocarpus* was first illustrated by Anders (1966), and described in the unpublished work of Sahasrabudhe (1970). The current study demonstrates the presence of hemi-anatropous ovules also in the woody *Streptocarpus*. A broad survey (unpublished) of ovular morphology in the genus and across the family thus far confirms the presence of hemi-anatropous ovules in the >20 *Streptocarpus* species examined, suggesting that this may well be characteristic for the entire genus. This ovular feature would be another notable character whose possible taxonomic significance has hitherto been overlooked.

It may be concluded that the woody *S. papangae* and *S. suffruticosus* differ from each other in certain anatomical–morphological features, such as trichome and sclereid distribution, stomata structure and hypodermis, but they also possess a unique set of characters that unite them (i.e. woodiness, non-cohering anthers, macrosclereids) each readily differentiating them from herbaceous caulescent *Streptocarpus*. They also possess relatively large seeds, 0.9 to 1.2 mm (Hilliard and Burtt, 1971; M. Möller, pers. observ.), compared to non-woody species, 0.3 to 0.7 mm (Beaufort-Murphy, 1983; M. Möller, pers. observ.). They also differ cytologically in their basic chromosome number, x = 16, from other caulescent members of the subgenus *Streptocarpella* with x = 15. Furthermore, we demonstrated that they possess hemi-anatropous ovules and split-lateral leaf traces, although these are also found in *Streptocarpus* of the African mainland.

The sample of woody Madagascan species investigated is too small to deduce that the prominent woody habit, non-cohering anthers, macrosclereids and large seeds are the only features that characterise Hilliard and Burtt's group (iii) of Madagascan shrubby *Streptocarpus* (Hilliard and Burtt, 1971). Our work points to the necessity for further studies not only on this group but also on other species of Madagascar and the Comoro Islands where our knowledge is still scant. This account is but a first fascinating glimpse of what remains to be discovered, and points to the importance for future work to include herbaceous species of *Streptocarpus* that molecular results indicate as possible allies of the woody species.

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Appendix A

Notes on cultivation of *S. papangae* and *S. suffruticosus*.

S. papangae

The specimens growing at Edinburgh are 1.4 m tall and multi-stemmed. They are kept at a minimum night temperature of 12 °C and have a minimum day temperature of 17 °C. Humidity varies depending on the time of year but rarely falls below 65% relative humidity. Day length also varies during the year from 6.5 h in mid winter to 18 h at the height of summer; the plants are also shaded during this time to prevent scorching. Supplementary lighting is used in part of the glasshouses during the winter months but this can lead to soft, weak growth and direct lighting is best avoided. Flowering usually occurs around June/July and can last for 2 months. Further flowering can also occur in late autumn if sunny and warm. *S. papangae* is grown in plastic pots, the deeper the better, using a peat free, bark based compost. This mix provides an open, light and free draining medium. Nutrients are added by

incorporating a small amount of nine month Osmocote to provide an initial boost and thereafter liquid feeding on a weekly or fortnightly basis with a 1:1:1 fertilizer (underfeeding rather than overfeeding). The plants are re-potted on a bi-annual basis or sooner if required. The only pest problem to date has been mealy bug which can be very damaging if left untreated.

S. suffruticosus

S. suffruticosus is grown alongside *S. papangae* and the conditions it requires are basically similar, the main difference is the compost and how the plants are grown. The species is generally known to be an epiphyte and therefore requires very open compost, bark based, and a free draining pot or pond basket for it to grow in. These plants are never very tall and usually droop and hang down over their container. Flowering can occur several times in a year with spring and autumn being the main period. Feeding and re-potting is the same as in *S. papangae*, but being slower growing it may not need re-potting as often. There have been few if any pest problems with *S. suffruticosus*, only the occasional mealy bug attack. It is a brave insect who gets too close to this plant as the whole plant is covered in sticky glandular trichomes and any insect that comes into contact with it will have a short life.

Propagation

In principle, the same propagation methods and techniques can be used for both species. *S. papangae* plants were introduced to the RBGE's living collection from wild collections in Madagascar in 1997. In the field cuttings were taken, kept as cool as possible and wrapped in moist tissue to prevent drying out. At RBGE cuttings can be taken any time of the year but late spring is usually best when they have just started to grow. The cuttings should be from semi-ripe stems and about 10 cm long, all but the top leaves are removed; the remaining leaves may also need reducing to prevent water loss. The cuttings are placed in medium grade perlite in small pots and watered in well. No rooting hormone treatment is required. The propagation cases should provide high humidity and bottom heat, set to a temperature of 25 °C. New roots will start to appear after a week or so. Supplementary lighting is also beneficial and will speed up the rooting process.

Plants of *S. suffruticosus* were raised from seeds collected in Madagascar in 1998. At RBGE we use a simple method of sterilised compost in a small pot which is then placed inside a resealable plastic bag; this can then be placed at 25 °C. Compost used for sowing is a fine bark grade, with added charcoal which is put into a 7 or 8 cm pot with boiling water put through it. The pot is allowed to cool over night and is ready for seed sowing the next morning. Seed is evenly scattered over the surface and the pot labelled. There is no need to cover the seed as it is small (ca. 0.7 mm) and light will aid germination. The reason for the plastic bag is to prevent the pot from drying out and from pests getting to the seed before and after germination. Once the seeds have germinated they are fertilised weekly with a dilute solution of a high potash fertiliser (i.e. tomato plant food). The pot should never become too wet.

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