Phylogenetics of tribe Exaceae (Gentianaceae) based on molecular, morphological and karyological data, with special emphasis on the genus *Sebaea*.

-

Taxonomic treatment of *Exochaenium*, *Lagenias* and the new genus *Klackenbergia*

Photography of the type locality of *Sebaea fernandesiana*, Zambia 2004

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IMPRIMATUR POUR LA THESE

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à mes grands-parents : Liliane et Pierre-Michel, Hélène et Charles

à mes parents : Marie-Claude et Jean-Luc

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TABLE OF CONTENTS

Tribe Exaceae, focusing on *Sebaea*

Taxonomy and taxonomic problems

Perspectives

Appendices

Keyword – Africa, Exaceae, *Belmontia*, biogeography, chromosome numbers, *Exacum*, *Exochaenium*, Gentianaceae, *Gentianothamnus*, Gynoecium, karyology, *Klackenbergia*, *Ornichia*, Madagascar, *Lagenias*, *Parasia*, phylogeny, polyploidy, *Sebaea*, seed, stigma, systematics, *Tachiadenus*, taxonomy

SUMMARY

In an attempt to understand the evolutionary history of the poorly studied genus *Sebaea* and its relationship to other genera of tribe Exaceae (Gentianaceae), intensive morphological and karyological character optimization based on robust molecular phylogeny was performed.

Phylogenetic reconstructions support the monophyly of Exaceae, and further reveal a polyphyletic *Sebaea*, including four well-supported clades, hereafter treated as separate genera, based on non-molecular synapomorphies. The first clade contains the single species *Lagenias pusillus*, characterized by its medifix anthers, inserted at the base of the corolla tube and its seed testa cells (polygonal). The second clade, *Sebaea s. str.*, contains most of the South African species having secondary stigmas and bilateral seeds, with rectangular testa cell. The third clade, *Exochaenium*, contains exclusively tropical African species, characterized by a stylar polymorphism and a papillose clavate stigma (versus smooth and bilobed). Finally the fourth clade, *Klackenbergia*, contains two species characterized by inflorescences with axillary sub-sessile flowers. Based on these results, the taxonomic reinstatement of *Exochaenium* (23 species) and *Lagenias* (1 species), along with the establishment of a new genus *Klackenbergia* (2 species), are proposed.

In the light of the new phylogenetic relationships found within the Exaceae, new views on the evolution of (1) karyological and (2) morphological characters are proposed. Finally, the historical biogeography of the tribe is reevaluated (3).

1. Intensive chromosome counts based on material collected and fixed in the field (157 population and c. 60 species), and exhaustive literature survey, reveal a broad set of chromosome numbers ($2n$ = 18, 28, 32, 34, 36, 42, 52, 54, 56, 60, 62, 64, 68), and the occurrence of polyploid systems within *Exacum* and *Sebaea*. These results allow us to postulate $x = 7$, 8, or 9 as the possible base chromosome numbers for the Exaceae. Karyological reconstruction, based on the molecular phylogeny, suggest a basic number of $x=7$ for the Exaceae, followed by dysploidy event leading to secondary base number of $x=8$ and $x=9$, and several polyploidization events.

2. Optimization of morphological characters suggests that the most recent common ancestor of Exaceae (MRCA) was similar to *Lagenias pusillus* by having pentamerous yellow actinomorphic flowers, with anthers included in the corolla tube and dehiscing by longitudinal slits, a bilobed stigma, and the absence of secondary stigmas, and cubical seeds with polygonal testa cells. This MRCA might have then developed particular floral syndromes as indicated by long corolla tubes or presence of enantiostyly in the tribe.

3. Dating analyses and dispersal-vicariance reconstructions suggest that the Exaceae evolved c. 32 million years ago in Africa and subsequently spread to Madagascar. The colonization of Australia, New-Zealand, and Asia involved at least three long-distance dispersals. Early diversification of Exaceae in Africa might be the consequence of the development of a temperate with dry summer climate, in the Cape region (South Africa), while the Quaternary climatic variation might explain most of the species diversity of *Sebaea* and *Exochaenium*.

At the generic level, molecular phylogenies of *Sebaea*, based on chloroplastic and nuclear DNA markers, reveals five well-supported clades. *Sebaea sulphurea* seems to have evolved early, and is distinct from all the remaining extant species. Each clade is supported by several characters (morphological, vegetative, phenological or geographical), and a preliminary infrageneric classification is proposed.

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Gentianaceae – Brief Overview

The Gentianaceae are a cosmopolitan family of over 1650 species and 87 commonly accepted genera, absent only from Antarctica. The family was described by Jussieu, but the name *Gentiana* is accredited to Tournefort (1700) who used a Dioscoridean name commemorating the Illyrian king Gentius (180-167 BC) (Gunther, 1934).

In his *Genera et Species Gentianearum*, Grisebach (1839) published the first complete account of all genera and species of the family, followed five years later by his treatment of Gentianaceae in De Candolle's Prodromus (Grisebach, 1845). Since then, other treatments of the Gentianaceae have been proposed (e.g. Endlicher, 1838; Bentham, 1876; Huxley, 1888; Baillon, 1888-1891; Gilg, 1895).

More recently a new tribal and subtribal classification has been defined by Struwe et al. (2002) (Fig. 1). It integrates a considerable amount of molecular and also morphological results. Notably, the Saccifoliaceae were included in Gentianaceae (tribe Saccifolieae), as well as the Potalieae often considered as part of the Loganiaceae since (Bentham, 1876). *Mitreola* L., *Spigelia* L., and the Menyanthaceae, often retained in the Gentianaceae were excluded as well.

Yet, in spite of the indubitable monophyly of the Gentianaceae in their current definition (Struwe, Albert, and Bremer, 1994; Thiv et al., 1999; Struwe et al., 2002), there is no particularly striking synapomorphic diagnostic feature confined to the entire family (Struwe and Albert, 2002; Chassot, 2003) except, maybe, the presence of a combination of specific secondary metabolites (xanthone and secoiridoids) (Mandal, Das, and Joshi, 1992; Rodriguez et al., 1998; Jensen and Schripsema, 2002).

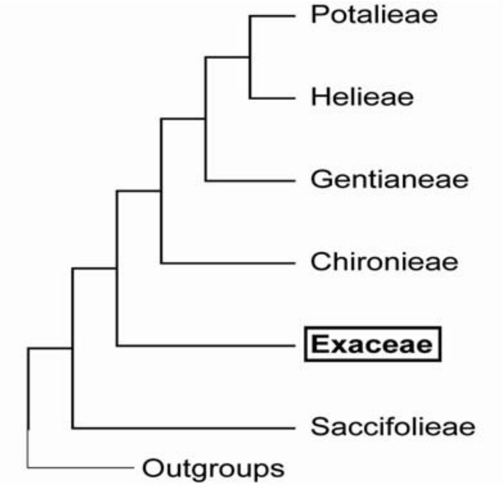
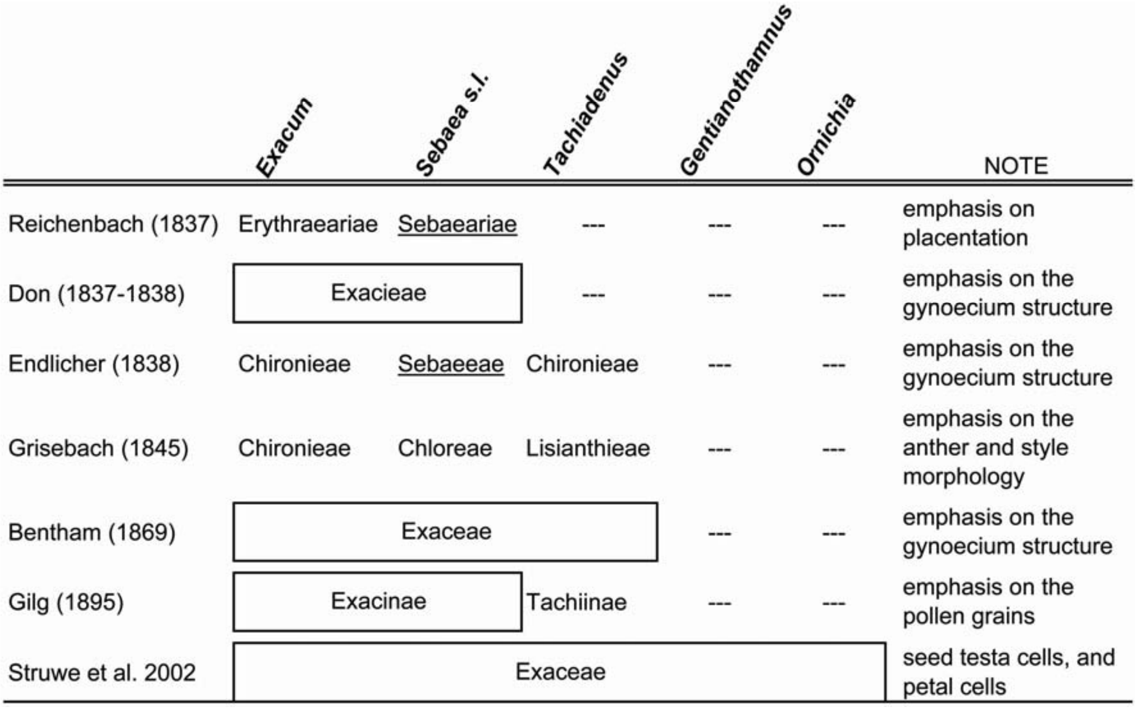


Figure 1: Summarized relationship of the Gentianaceae-tribes from (Struwe et al., 2002)

Tribe Exaceae

The Exaceae are a small tribe of, at present, five recognised genera: *Exacum*, (including *Cotylanthera*), *Gentianothamnus*, *Ornichia*, *Sebaea* and *Tachiadenus* (Struwe et al, 2002). The genera *Sebaea* and *Exacum* make up the majority of species in the tribe Exaceae (*c*. 165 spp). The tribe Exaceae was already recognized in the first infrafamilial classification of Gentianaceae proposed by Colla (1834) in his description of the plants kept in the Turin herbarium. In the early classification of the family Gentianaceae, *Exacum* and *Sebaea* (the other genera of the tribe were not described at that time) have been usually placed together in tribe Exaceae (table 1). However, depending on the character used, *Sebaea* has sometimes been placed in its own tribe. Most authors have focused on the bilocular structure of the ovary to circumscribe the tribe and therefore often misplaced *Tachiadenus*, which presents a "pseudobilocular ovary" (Klackenberg, 1985, 1987a). Moreover this character is the common state in plausible outgroups to Gentianaceae (e.g. Rubiaceae and Loganiaceae). Consequently this structure should be regarded as a symplesiomorphy and cannot be used to characterize Exaceae (Klackenberg, 1985). Recently, Klackenberg (2002) proposed both the particular star-shaped testa cells of the seeds and the shape of the petal epidermis cells (basically rounded and convex versus elongated and flat) as possible synapomorphies for the tribe, because these characters have not been observed elsewhere in the family.

Table 1: Summary of the taxonomical history of the Exaceae, indicating the respective position of its genera in infra-familial classifications.



	Exacum	*Sebaea s.l.*	*Tachiadenus*	*Gentianothamnus*	*Ornichia*	NOTE
Reichenbach (1837)	Erythraeariae	Sebaeariae	---	---	---	emphasis on placentation
Don (1837-1838)	Exacieae		---	---	---	emphasis on the gynoecium structure
Endlicher (1838)	Chironieae	Sebaeeae	Chironieae	---	---	emphasis on the gynoecium structure
Grisebach (1845)	Chironieae	Chloreae	Lisianthieae	---	---	emphasis on the anther and style morphology
Bentham (1869)	Exaceae			---	---	emphasis on the gynoecium structure
Gilg (1895)	Exacinae		Tachiinae	---	---	emphasis on the pollen grains
Struwe et al. 2002	Exaceae					seed testa cells, and petal cells

Taxa of Exaceae have a paleotropical and southern African (temperate) distribution. The centre of generic diversity of the tribe is in Madagascar, where all the genera are present, but species diversity is concentrated in Continental Africa (*Sebaea* and two *Exacum* species), especially in the Cape region and the Drakensberg Mountains. Recently, intensive studies on the Malagasy genera have been undertaken by Klackenberg (1985, 1986, 1987, 1990, 2002). He monographed the large genus *Exacum* (Klackenberg 1985), revised the genus *Tachiadenus* (Klackenberg 1987), described the new genus *Ornichia* (Klackenberg 1986) and finally wrote the Gentianaceae account for "Flore de Madagascar et des Comores" (Klackenberg, 1990). Those important works have shaped the current circumscription of tribe Exaceae, and were summarised in *Gentianaceae Systematics and Natural History* (Struwe et al., 2002).

Conversely, no general work on the African Exaceae (*Sebaea*) has been undertaken since the last monograph of Schinz (1906) one century ago, even if it appears that *Sebaea* with its 60 (Mabberley, 1997) to c. 150 (Boutique, 1972) or c. 159 (Paiva and Nogueira, 1990) species is the largest genus in Exaceae.

Furthermore, despite series of systematic works performed on the Exaceae (Rafinesque, 1837; Meyer, 1838; Grisebach, 1845; Bentham, 1876; Schinz, 1891, 1903, 1906; Gilg, 1895a, 1898; Hill, 1908a), the taxonomic circumscription of *Sebaea* remains controversial. Most of the confusion relies on the frequent use of taxonomic inconsistent characters (Marais, 1961) including the presence of "bi-glandular swelling" on the style, appendages (glands) on the anthers, the form (straight or recurved) and disposition (free or connate) of the anthers, and the height of the filament insertion.

In addition to its poorly understood systematic history, the phylogenetic position of *Sebaea* is still a matter of speculation. Recent molecular inferences support the inclusion of the genus in the tribe Exaceae (Struwe et al., 1998; Thiv et al., 1999; Thiv, Struwe, and Kadereit, 1999; Yuan et al., 2003), and a possible polyphyletic status (Yuan et al., 2003). Nevertheless, only a reduced subset of the genus diversity (5 out of c. 60-159 species) was included in these studies, preventing further phylogenetic or systematic conclusions.

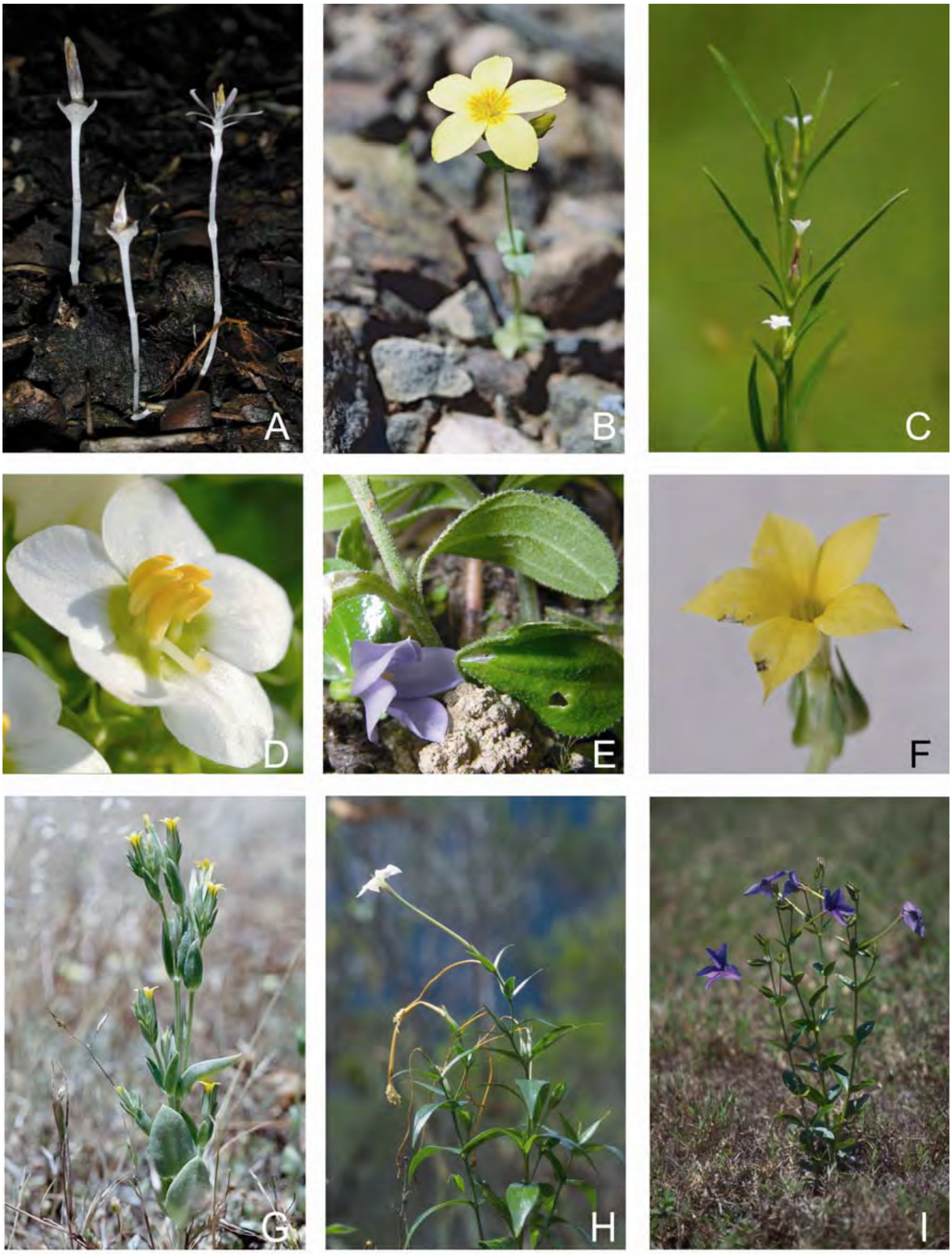


Figure 1: **A**: *Exacum tenue* (a saprophytic Exaceae), **B**: *Sebaea exacoides*, **C**: *Sebaea madagascariensis*, **D**: *Exacum affine* (white cultivar), **E**: *Ornichia madagascariensis*, **F**: *Sebaea clavata*, **G**: *Sebaea affinis membranaceae*, **H**: *Tachiadenus longiflorus*, *I*: *Tachiadenus carinatus*.

Genera of the Exaceae

***Exacum* L.**

The genus *Exacum* currently comprises 65 recognised species (Klackenberg 1985, 1990) and shows a typical paleotropical distribution (Klackenberg, 1985; Thulin, 2001; Klackenberg, 2002). *Exacum* has two main centres of diversity, namely Madagascar and the area including Southern India and Sri Lanka, and only a few species occur in Socotra (and the Arabian peninsula), in the Himalayas, Southeast Asia, New Guinea, and in the extreme northern Australia. *Exacum* species have a wide spectrum of habitat preferences. Taxa are found from sea level up to the highest mountains tops in Madagascar (*c.* 2800 m elevation) , and up to *c.* 2000 m in the Himalayas, South India, and New Guinea. Most species occur in lowland and montane rainforest areas, although they usually grow in full sun (Klackenberg, 1985, 1990, 2002).

Synapomorphies supporting *Exacum* are finely perforated endothecial walls of the anthers, and the anther opening mechanism by pores (versus by slits for the rest of the tribe) (Klackenberg, 1985, 2002). Most species of *Exacum* are annual herbs. The flowers are tetra- or pentamerous, actinomorphic to zygomorphic by having the anthers connivent into a cone above a bent style. The calyx lobes are usually furnished with a keeled or broad wing that enlarges in fruit. The corolla is white to pale blue to violet (a few millimetres to up 7 cm in diameter). For a detailed taxonomic history of *Exacum*, one can refer to Klackenberg (1985, 2002)

More recent studies on the phylogeny and biogeography of the genus were performed by Yuan and al. (2003, 2005), showing a Malagasy origin for the genus (Yuan and al. 2005), and the paraphyly of *Exacum* towards *Cotylanthera* (Yuan et al., 2003). The genus *Cotylanthera* was described by Blume (1826), and contains until recently four small, almost leafless, saprophytic Southeast Asian herbs (Hara, 1975). *Cotylanthera* shares with *Exacum* two synapomorphic anther characters, viz. anthers opening by pores and finely perforated endothecial walls (Klackenberg 2002), while two important autapomorphies for *Cotylanthera* are the mode of anthers opening (with one pore only) and saprophytism. Klackenberg (2002) remarked that no apomorphy is known for *Exacum* that excludes the species of *Cotylanthera*, and therefore merged all *Cotylanthera* species in *Exacum* (Klackenberg, 2006).

As shown by Yuan et al. (2005), *Exacum* has experienced multiple out-of-Madagascar dispersals. The most important is the long dispersal to Sri-Lanka / South-India, which resulted in the extensive radiation of the Socotra-Arabia and other Asian lineages in the northern India Ocean basin regions (Yuan et al., 2005). More recent out-of-Madagascar journeys include single dispersal of *E. oldenlandioides* to the African mainland, or several dispersals to other islands around Madagascar including Comores (*E. stenopterum*), or the volcanic island of Mauritius (*E. quinquenervium*) (Klackenberg, 1985).

Karyological studies on the genus *Exacum* are unfrequent. To date, the most important work has been performed by Mallikarjuna (1987) who counted 12 Indian species, and obtained chromosomal numbers of 2n= 34, 56, 62, 68. The Socotran species, *E. affine* was counted to 2n= 36 (Sugiura, 1936a, 1936b; Post, 1967), while less known work has been done by Subramanian (1980) and Borgmann (1964). More recently (Riesman et al., 2006) evaluated the chromosome numbers and the sexual reproduction in the *E. trinervium* complex and related species. The counted numbers were for *E. trinervium* subsp. *trinervium*, 2n = 60; subsp. *ritigalensis*, 2n = 60; subsp. *macranthum*, 2n = 54; and subsp. *pallidum*, 2n = 52 and for the closely related species *E. pedunculatum*, 2n = 56, while they confirmed the chromosome number of *E. affine* (2n = 36).

Gentianothamnus **Humbert**

The only known species in this genus, *G. madagascariensis*, occurs in isolated populations from northern to southern Madagascar growing in the humid sclerophyllous montane forests above 1000 m altitude (Klackenberg, 1997, 1990, 2002). *Gentianothamnus* is an erect, sparsely branched subshrub up to 2 m in height, and is easily recognised by its large (2.5-4.0 cm) yellow to orange corolla, with a long, narrowly funnel-shaped tube. Other characteristics of *Gentianothamnus* are the long slender filaments inserted at the base of the corolla tube and the *ovary* placed on a small shallowly 5-lobed disk (apparently unilocular but in fact "pseudo-bilocular" with parietal placentas protruding to the centre). From both a morphological (Klackenberg, 2002) and molecular (Yuan et al., 2003; Yuan et al., 2005) point of view, *Gentianothamnus* is closely related to the genus *Tachiadenus*.

Ornichia **Klack.**

This is a small Malagasy endemic genus of three species (Klackenberg, 1986) which occurs in the eastern more humid part of the island, where it grows mainly in evergreen forest up to 1500 m (Klackenberg 2002). Hairy leaves represent an apomorphic character state for *Ornichia* (Klackenberg, 1986), which is not seen in any other species within tribe Exaceae.

Tachiadenus **Griseb**.

This is a small homogenous genus of 11 species, all native of Madagascar (Klackenberg, 1987). The species occur over a large part of the more humid East Malagasy phytogeographic region, except for the higher mountains (Klackenberg, 1987, 1990, 2002). Some species are widely distributed along eastern lowlands, growing in different habitats, while other common species are restricted to the central plateau above 800 m altitude and are often found in grassland (Klackenberg, 1987b, 1990, 2002). *Tachiadenus* can be distinguished by its large flowers with long corolla tubes and the “pseudo-bilocular” ovary. However those characteristics are also present in *Gentianothamnus* (Klackenberg, 1990, 2002). Finally, large flowers with long corolla tube also occur in some *Sebaea* species (e.g. *Sebaea thomasii*).

Sebaea **Sol. ex R.Br.**

The genus *Sebaea* comprises, according to Mabberley (1997) or Dyer (1975) c. 60 species, while its number has been estimated to *c*. 100 species by Adams (1996) or Wielgorskaya (1995), and up to c. 150-159 species by Paiva and Nogueira (1990) or Boutique (1972). *Sebaea* has three main centres of diversity, including (i) the Cape and (ii) the Drakensberg regions of South Africa, and (iii) the Katanga plateau region in tropical Africa. Only a few species occur in Madagascar, Australia-New-Zealand, and Indo-Malaysia.

Sebaea species have a wide spectrum of habitat preferences. Taxa are found from sea-level up to the highest mountain tops of South Africa (c. 3500 m elevation) and up to 2000 m elevation in the Himalayas. They grow in a wide variety of habitats, in e.g. dense tropical forests, grasslands and savannas, marshes or waterlogged areas, on wet rocks, sandy riverbanks or sand dunes, but they usually grow in wet naturally disturbed habitats where competition is reduced.

Currently no synapomorphies are known to support the current circumscription of *Sebaea*, except maybe the flower colour (from white to bright yellow, or salmon)

Taxonomical history of Sebaea

Solander erected the genus *Sebaea* in a manuscript that was published by R. Brown (1810). The genus was dedicated to the Amsterdam-based pharmacist Albertus Seba (1665-1736), well-known for his “Cabinet of Curiosities”. Solander transferred three yellow-flowered South African species namely *Sebaea albens, S. aurea* and *S. cordata* (= *S. exacoides*) and one Australian species, *S. ovata* (the type species), from *Exacum* to the newly proposed genus *Sebaea*. He distinguished *Sebaea* from *Exacum* by several morphological characters such as the anther opening mechanism (longitudinal slits for *Sebaea*), the form of the style (straight for *Sebaea*), and the form of the stigma (“undivided” for *Exacum*). Later, Rafinesque (1837) erected the genus *Parasia* for the single species *S. cordata* (= *S. exacoides*), but that genus was ignored by latter authors. Meyer (1838) split *Sebaea* into three genera, namely *Belmontia*, *Sebaea* and *Lagenias*. Meyer transferred three species (*S. grandis*, *S. cordata* (= *S. exacoides*) and *S. spathulata*) to *Belmontia*, and *S. pusilla* to *Lagenias*. *Sebaea* was distinguished from *Belmontia* based on (i) exserted anthers becoming recurved, and (ii) stigma being capitate and bilobed with a secondary division (“stigmate capitato-didymo”). It is interesting to note that *S. exacoides* (which was placed in *Belmontia*) clearly presents, when mature, bilobed stigma. However, when the plant is at a young stage, the immature part (which will develop into a mature bilobed stigma) appears somewhat linear to clavate. Recently, in the *Nomina Generica Conservanda et Rejicienda* of the Tokyo Code (1994), it was decided to conserve the name *Belmontia* towards *Parasia*. Therefore *Parasia* is now a homotypic synonym of *Belmontia*. Finally, *Lagenias* was characterized by having the anthers recurved (like in *Sebaea*) and the filaments inserted in the corolla tube (like in *Belmontia*).

Grisebach (1845) further split *Belmontia* and erected one more monotypic genus based on *S. grandis*. The newly proposed genus – *Exochaenium* – was based on the anthers being connate and forming a tube around the style. At that time *Sebaea* was split into four genera.

In *Genera plantarum*, Bentham (1876) included *Lagenias* and *Exochaenium* into *Belmontia*, which was kept distinct from *Sebaea*. Schinz (1891) recognized both *Lagenias* and *Belmontia* as separated from *Sebaea*, but included *Exochaenium* species in *Belmontia*. Gilg (1898) followed Schinz's classification with minor modifications at the species level. Unexpectedly, Schinz (1903) revised his previous arguments and claimed that it was impossible to discriminate between *Sebaea* and *Belmontia*. He therefore proposed a single genus *Sebaea* with two sections: *Eusebaea* and *Belmontia*, where he placed all species with anthers inserted in the corolla tube. Three years later, Schinz (1906) revised his own classification by accepting *Exochaenium*, to which he transferred most species of *Sebaea* sect. *Belmontia*, and merged *Lagenias* with *Sebaea*. *Exochaenium* was characterized by having both included anthers and a disk of glands between the calyx and the corolla. Hill (1908), when preparing *Flora Capensis*, followed Schinz's classification, adding one more character to *Exochaenium*, i.e. the style not having a "biglandular swelling" - secondary stigma on the style (Hill, 1913; Marloth, 1909) - like in *Sebaea*.

However, Marais (1963) argued that the characters used to uphold *Exochaenium* as distinct from *Sebaea* were unreliable, and synonymized *Exochaenium* with *Sebaea*. Marais (1961) observed that: (i) *Sebaea thomasii* sometimes have, sometimes not, a glandular disc between the calyx and the corolla (a characteristic of *Exochaenium*) ; (ii) *Sebaea micrantha* and *S. exacoides* have stamens included in the corolla tube (a characteristic of *Exochaenium*) and further present a "glandular swelling" on the style (a feature of *Sebaea*). Taylor (1963) for Flora of Tropical West Africa, Boutique (1972) for the Flore d'Afrique Centrale, Klackenberg (1990) for Flore de Madagascar et des Comores, Paiva & Nogueira (1990) for Flora Zambesiaca, and Sileshi (2002) for Flora of Tropical East Africa followed Marais.

The floral morphology of *Sebaea* is outstanding. The presence of secondary stigmas on the style (Hill, 1913; Marloth, 1909) characterized many species, while the presence of a stylar polymorphism (Hill, 1908; Raynal, 1967; Welwitsch, 1869), or an extended stigmatic region (sometimes the style is absent and replaced by a long style-like stigma), can be found in others.

Some species present striking apical glands and/or basal glands on the anthers. In some cases, the apical gland is larger and longer than the anthers themselves. Despite those particularities, previous taxonomical work failed to identify synapomorphies supporting *Sebaea* or its "satellite" genera. As a consequence, *Sebaea* became a "grab-bag" for all problematic species in Exaceae.

Table 2: Synopsis of the taxonomical history of *Sebaea*, in red, the species used as a type for the different genera.

1810, Brown	***Sebaea*** *S.ovata* …				
1837, Rafinesque	***Sebaea*** *S.ovata* …	***Parasia*** *P. cordata* (=*S. exacoides*)			
1838, Meyer	***Sebaea*** *S.ovata* …		***Belmontia*** *B. cordata* (=*S. exacoides*) …	***Lagenias*** *L. pusillus*	
1845, Grisebach	***Sebaea*** *S.ovata* …		***Belmontia*** *B. cordata* (=*S. exacoides*) …	***Lagenias*** *L. pusillus*	***Exochaenium*** *E. grande*
1876, Bentham	***Sebaea*** *S.ovata* …		***Belmontia*** *B. cordata* (=*S. exacoides*) *B. pusilla* *B. grandis* …		
1891, Schinz	***Sebaea*** *S.ovata* …		***Belmontia*** *B. cordata* (=*S. exacoides*) *B. grandis* …	***Lagenias*** *L. pusillus*	
1903, Schinz	***Sebaea*** *S.ovata* *S. grandis* *S. cordata* (= *S. exacoides*) …			***Lagenias*** *L. pusillus*	
1906, Schinz	***Sebaea*** *S.ovata* *S. pusilla* *S. exacoides* …				***Exochaenium*** *E. grande* …
1908, Hill	***Sebaea*** *S.ovata* *S. pusilla* *S. exacoides* …				***Exochaenium*** *E. grande* …
1961, Marais	***Sebaea*** *S.ovata* *S. pusilla* *S. exacoides* *S. grandis* …				
1994, Tokyo Code		*Parasia* is rejected for *Belmontia*			

CONTEXT OF THE STUDY

Taxon sample

The investigations carried out in this thesis were initially aimed at exploring the extent of polyphyly in the genus *Sebaea*, as suggested the results of Yuan et al. (2003). This has led to efforts to collect fresh material suitable for DNA extraction, as species accessible from various herbaria were mostly represented by material collected during the first half of the past century or even before, and from which DNA of satisfactory quantity and quality could only rarely be obtained.

In order to achieve a substantial collection of *Sebaea* species, three dedicated expeditions were carried out in Zambia in 2004 and South Africa / Lesotho (2005 and 2006). Material was also collected by friends and colleagues from both Africa and Madagascar. Unfortunately, we were not able to obtain fresh material for the two *Sebaea* species from Australia. All vouchers are deposited at the herbarium of Neuchâtel, and represent nearly all the described *Sebaea* species.

Previous works on large genera of the Gentianaceae (Chassot, 2003; Hagen and Kadereit, 2001; Mansion and Struwe, 2004; Yuan et al., 2003), have shown the polyphyly of all the investigated groups, and the necessity of extensive sampling at the tribal level. For the Exaceae, this effort has been considerably facilitated by the previous works (Yuan et al. 2003, 2005) performed on *Exacum*, the second largest genus of the Exaceae.

Genes sequenced and phylogenetic inference

The DNA regions sequenced in this work, along with their respective rates of evolution, and their adequacy at a given taxonomic level, have been extensively reviewed (Olmstead and Palmer, 1994; Soltis and Soltis, 1998; Shaw et al., 2005; Shaw et al., 2007). In this study, coding and non-coding fragments of both nuclear and chloroplast regions were analysed, which ultimately allowed us to explain potential phylogenetic incongruence caused by different phenomena, including hybridization, introgression, chloroplast capture, paralogy, or incomplete lineage sorting (Wendel and Doyle, 1998).

Among the chloroplast regions, the *trn*L intron and the *trn*L-F spacer, as well as the *atp*B-*rbc*L spacer were chosen, because of the occurrence of well-developed universal primers, and the fact that most of them are single copy gene (Palmer, 1995). Disadvantages of the chloroplast genome include: (i) their monoparental, usually maternal (Corriveau and Coleman, 1988), inheritance, with the risk of not detecting relationships biased by chloroplast capture; (ii) their rather slow evolutionary rate, and (iii) the fact that mutations can come from structural changes (making homology assessment problematic). However, these three chloroplastic regions, which provided sufficient information at generic level, did not permit to resolve the topology between the main clade. Therefore a part of the *mat*K gene, a region evolving slower than the other regions was also sequenced to resolve basal polytomy.

The nuclear region sequenced consists of the 5.8S gene, and its two flanking internal transcribed region (ITS1 and ITS2). This region was chosen because ITS sequences have been used successfully in Gentianaceae and many other studies, and that the mechanism of concerted evolution among the multiple tandem repeats of this region allows in most cases to consider these as single copy gene (Hillis and Dixon, 1991; Hamby and Zimmer, 1992).

In order to infer molecular phylogenies, two methods were used. The first one, based on maximum parsimony, yielded to well resolved, and robust topologies, but does not take in consideration parameters such as unequal base frequencies, different substitution rates between nucleotides, and site-dependant rate heterogeneity. To confirm the parsimony based results, we choose among alternative methods (e.g. distance based method, maximum likelihood), a Bayesian approach. Bayesian inference uses a Markov chain Monte Carlo (MCMC) procedure to sample trees from a posterior probability distribution. The advantages of this method include its time efficiency and the integration of full substitution models. Among the disadvantages, it seems that trees found by MCMC are strongly autocorrelated and, as they may only reflect local optima, the procedure must be repeated several times varying the starting parameters. In addition, the posterior probability of a branch in the phylogeny, in terms of statistical support for that branch, is sensibly higher than as estimated by resampling techniques (Douady et al., 2003). Overall, Bayesian inference was used here as an alternative to parsimony in order to verify the consistency of phylogenies inferred from molecular data.

Karyology

Cytotaxonomy in the Gentianaceae has been widely and intensively studied since the1950', especially in the tribes Chironieae and Gentianeae. These studies have generally yielded useful information on relationship at species or even genus levels. Conversely, karyological studies of Exaceae remain poor, focusing mainly on the Indian and Sri-Lankan species of *Exacum*.

The abundant material collected and fixed in Madagascar by Dr. Louis Zeltner, allowing chromosome counts, has rendered a karyological study at the tribal level possible. Consequently, in order to obtain an idea of the overall karyological variability of the tribe, and to give further support and explanations to the results obtained with either molecular or morphological data, young flower buds were fixed in the field, if possible at the meristematic stage (suitable for the determination of chromosome numbers), using Carnoy solution. In some cases, counts were realised from the roots tips of cultivated specimens growing in the botanical garden of Neuchâtel.

Gross morphology and characters evolution

As no morphological analyses were carried out on *Sebaea*, emphasis was laid to identify synapomorphic morphological characters supporting molecular subdivisions. These characters mainly included flower morphology, life form, and seed morphology. Finally, the incorporation of previously documented morphological characters from other genera of Exaceae allowed the acquirement of relevant morphological intra-tribal synapomorphies.

Historical Biogeography

In order to develop a hypothesis explaining the historical biogeography of the tribe, the patterns of species distribution were identified from herbarium labels (more than 3000 samples), literature and field work. Ancestral area reconstruction and dating analysis were conducted to identify the likely biogeographical scenario.

Thus the main targets of the thesis are:

1. to investigate the phylogenetic relationship within tribe Exaceae, focusing on its largest genus: *Sebaea*
2. to investigate the chromosomal variation and evolution within Exaceae
3. to evaluate the evolution of several morphological characters by optimizing them on robust molecular phylogenies
4. to gain insights into the origin and biogeographic history of the tribe
5. to clarify the taxonomy of *Sebaea*

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CYTOGEOGRAPHY OF THE GENTIANACEAE-EXACEAE IN AFRICA, WITH A SPECIAL FOCUS ON *SEBAEA*

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THE POSSIBLE ROLE OF DYSPLOIDY AND POLYPLOIDY IN THE EVOLUTION OF THE TRIBE

Cytogeography of the Gentianaceae-Exaceae in Africa, with a special focus on *Sebaea*

The possible role of dysploidy and polyploidy in the evolution of the tribe

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Abstract – Unlike other tribes of the Gentianaceae, the Exaceae have received so far little attention regarding their karyological evolution. Indeed, only 29 chromosome number counts have been referenced to date, representing only a negligible fraction of the tribe diversity. In this paper, we performed an intensive chromosome count on material collected in the field (South and central Africa, plus Madagascar), encompassing 157 populations and ca. 60 species from four genera of *Exaceae*, including *Exacum*, *Ornichia*, *Sebaea*, and *Tachiadenus*. Fifty-four species (14 *Exacum*, 1 *Ornichia*, 40 *Sebaea* and 2 *Tachiadenus*) were examined for the first time, revealing a broad set of chromosome numbers ($2n$ = 18, 28, 32, 36, 42, 56), and the occurrence of polyploid systems within *Exacum* and *Sebaea*. These results allow us to postulate x = 7, 8, or 9 as the possible base chromosome numbers for the Exaceae, and emphasize the importance of both dysploidy and polyploidy processes in the evolution of the tribe.
Finally, chromosome numbers appear to be associated to some morphological, geological or geographic traits, suggesting new systematic combinations and likely active speciation patterns in the group.

Keyword – chromosome numbers, dysploidy, karyology, *Sebaea*, *Exacum*, *Ornichia*, *Tachiadenus*, polyploidy

INTRODUCTION

The particular knowledge of chromosome number in plants is primordial to detect processes enabling abrupt speciation such as polyploidy or aneuploidy / dysploidy (Briggs and Walters, 1997). Polyploidy is an extremely important phenomenon in plants and occurs in e. g. 97 % of ferns or ca. 70 % of angiosperms (Averett, 1980; Grant, 1981). Indeed the knowledge of chromosome numbers within a species or a polyploid system may help to differentiate between allopolyploidy (i.e. the merging of genomes that have diverged from one an other before episodes of polyploidization) or autopolyploidy (i. e. the merging of similar genomes before polyploidization) (Stebbins, 1947). Furthermore, establishing extensive karyological surveys on particular taxa may allow the detection of particular changes in chromosome number such as aneuploidy / dysploidy processes (loss or gain of chromosomes in a genome). The occurrence of both polyploidy and dysploidy has been recently demonstrated for certain groups of angiosperms, including e. g. *Borago*, *Nonea* (Boraginaceae; Selvi, Coppi, and Bigazzi, 2006), *Hypochaeris* (Asteraceae; Cerbah et al., 1998), *Centaurium*, *Gentiana*, or *Zeltnera* (Yuan, Küpfer, and Zeltner, 1998; Mansion, Zeltner, and Bretagnolle, 2005).

Despite the importance of karyological studies in the understanding processes of the evolution in the Gentianaceae or establishing systematic treatments (Favarger, 1949; Rork, 1949; Favarger, 1952), especially in the Gentianeae (Shigenobu, 1983; Yuan, 1993; Yuan and Kupfer, 1993; Yuan and Küpfer, 1993; Küpfer and Yuan, 1996; Yuan, Küpfer, and Zeltner, 1998; Liu, Ho, and Chen, 2002; Chassot, 2003) and the Chironieae (Favarger, 1960; Zeltner, 1970; Küpfer and Yuan, 1996; Zeltner and Mansion, 2003; Mansion and Zeltner, 2004), only a few chromosome counts have been performed so far on the tropical Exaceae (Annex 1).

Exaceae is a small tribe of the Gentianaceae, with ca. 180 species and five genera (Struwe et al., 2002) Klackenberg 2006): *Exacum* L. (including the saprophytic *Cotylanthera* Blume) comprises 65 spp., distributed in tropical Africa, Madagascar, and eastern Asia (Yuan et al., 2003); *Gentianothamnus* Humbert is a monotypic genus occurring in Asia (Yuan et al., 2003); *Ornichia* Klack. contains three endemic species to Madagascar (Klackenberg, 1986); *Sebaea* Sol. is so far the most species-rich genus with ca. 90-150 species in South Africa, tropical Africa, and Madagascar (Schinz, 1906; Boutique, 1972; Paiva and Nogueira, 1990); finally, *Tachiadenus* Griseb. comprises 11 species endemic to Madagascar (Klackenberg, 1987).

To date, most karyological studies have focused on *Exacum* (Riseman, Sumanasinghe, and Craig, 2006), but not including African or Malagasian taxa. Early work on *Exacum tenuis* (under *Cotylanthera tenuis*; Oehler, 1927) failed to unambiguously establish a definite number ($2n = 32\text{-}36$). So far, several species from India (Borgmann, 1964; Subramanian, 1980; Mallikarjuna, Scheriff, and Krishnappa, 1987; Riesman, Sumanasinghe, and Craig, 2006) or Socotra (Sugiura, 1936a, 1936b; Post, 1967) have been examined, showing an extensive variation in their chromosome number range, e. g. 2n = 18, 30, 34, 36, 52, 54, 56, 62, 68. More recently Riesman et al. (2006) reported karylogical data in the *Exacum trinervium* complex, showing some ability of species to produce in vitro viable hybrids, despite a difference in chromosome valence. Finally, only two chromosome counts are currently available for the large genus *Sebaea*, including *S. brachyphylla* from Africa (2n=22) (Thulin, 1970) and *S. ovata* from New-Zealand (n = *c*. 27) (Beuzenberg and Hair, 1983).

In this context, the main goals of the present paper are to: (i) fill the gap and check uncertainties in the karyological data available for the Exaceae, with a focus on the poorly investigated *Sebaea*; (ii) check the systematic relevance of the chromosome groups obtained; and (iii) infer patterns of chromosome evolution and speciation mechanisms within the tribe.

MATERIALS & METHODS

Most of the species come from wild populations collected in the field in 1995 and 2003-06 (Annex 1). All voucher specimens are deposited in the herbarium of the University of Neuchâtel, Switzerland (NEU). For karyological studies, flower buds were collected and directly fixed in the field in a Carnoy solution (3/1 glacial acetic acid/absolute ethanol). When buds were not available, root-tips of species cultivated in the botanical garden of Neuchâtel were used. In that case, suitable root tips were first pre-treated with a saturated aqueous solution of α-bromonaphthalene for 1 hr and 20 min, fixed in Carnoy for two weeks, and finally stained with aceto-carmine, and squashed on temporary slides.

Chromosome observations were performed either on meiotic plates from pollen mother cells (fixed buds), or on mitosis plates from young cells of the ovary wall (fixed buds or root tips). In few cases chromosomes were counted from second pollen mitosis. Most of the chromosomes were of small size and showed some tendency to merge, karyograms were performed for a few representative accessions only (*Sebaea filiformis*, *S. sedoides* and *S. macrophylla*). Microphotographs were taken, and drawings were made with a camera lucida apparatus. Each chromosome number was determined from at least 4 different preparations by two of us (JK and LZ).

RESULTS AND DISCUSSION

A total of 157 accessions representing 56 species and four genera (*Exacum*, *Ornichia*, *Sebaea* and *Tachiadenus*) collected in Lesotho, Madagascar, South Africa and Zambia was analyzed (Annex 1). Chromosome valence ranged from n = 9 (*Sebaea madagascariensis*) to n = 28 (e.g. *Sebaea brachyphylla*, *S. macrophylla*), with most variability occurring in *Sebaea* (n = 9, 14, 21, 28) and *Exacum* (n = 8, n = 9). Chromosomes numbers for 54 taxa are reported for the first time, encompassing species of *Exacum* (14 out of 69), *Ornichia* (one out of three), *Sebaea* (ca. 40 out of c.95; Schinz, 1906), and *Tachiadenus* (two of eleven species). In the following we provide an enumeration of the chromosome numbers hitherto found in the Exaceae, and interpret them in the light of the present results.

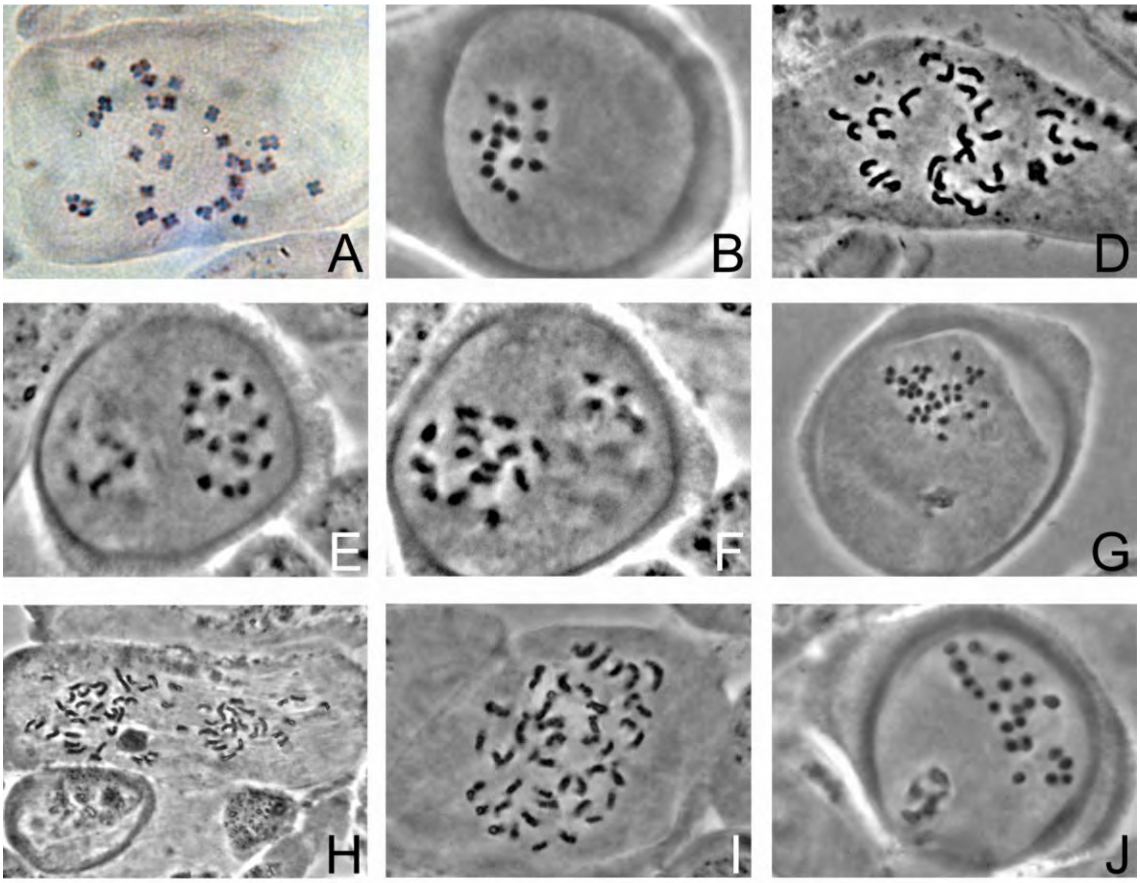


Figure 1 – Chromosome of *Sebaea*. **A**: *S. thomasii*, mitotic metaphase of root-tip, 2n=28; **B**: *S. bojeri*, meiotic anaphase I, n=14; **C**: *S. filiformis*, mitotic metaphase, 2n=28; **D**: S. leiostyla, meiotic metaphase II, n=14; **E**: *S. repens*, meiotic metaphase II, n=14; **F**: *S. micrantha*, meiotic anaphase I, n=28; **G**: *S. macrophylla*, Mitotic metaphase, 2n=56; **H**: *S. sedoides*, mitotic metaphase, 2n=42; **I**: *S. rehmanii*, meiotic anaphase I, n=21.

Karyological review of Exaceae

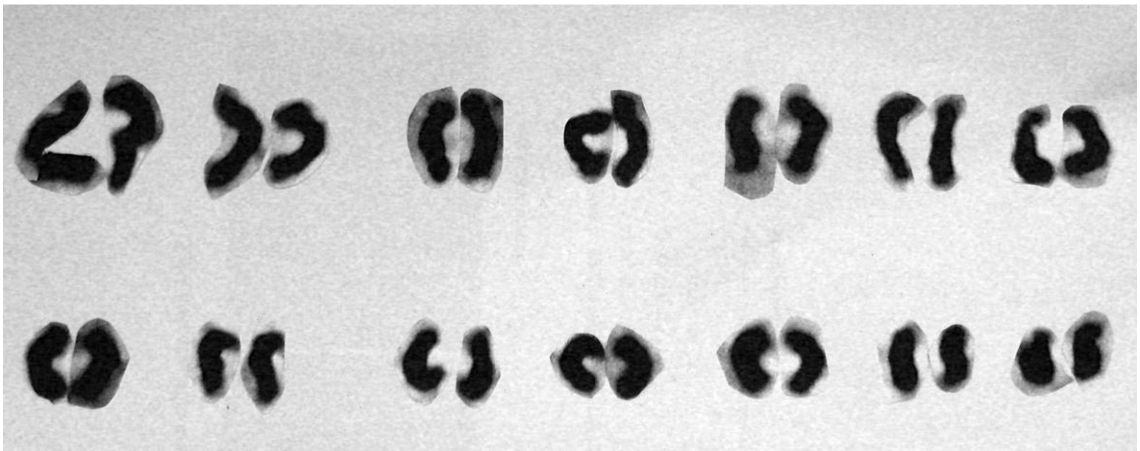
Based on new chromosome numbers evidenced in this study, several basic numbers can be proposed for the Exaceae: x = 7 (n = 14, n = 21, n = 28), x = 8 (n = 16), and x = 9 (n = 9, n = 18). The haploid numbers n = 17, 26, 27, 31, and 34 reported in the literature (Annex 2), might be viewed with caution as the result of dysploidy and/or allopolyploidy; the latter processes being relatively frequent in the evolution of some Gentianaceae including e. g. *Centaurium* (Zeltner, 1970; Mansion, Zeltner, and Bretagnolle, 2005), *Exacum* (Darlington and Wylie, 1995;

Riseman, Sumanasinghe, and Craig, 2006), or *Gentiana* sect. *Chondrophyllae* (Yuan, Küpfer, and Zeltner, 1998) and reported here for the first time within *Sebaea*.

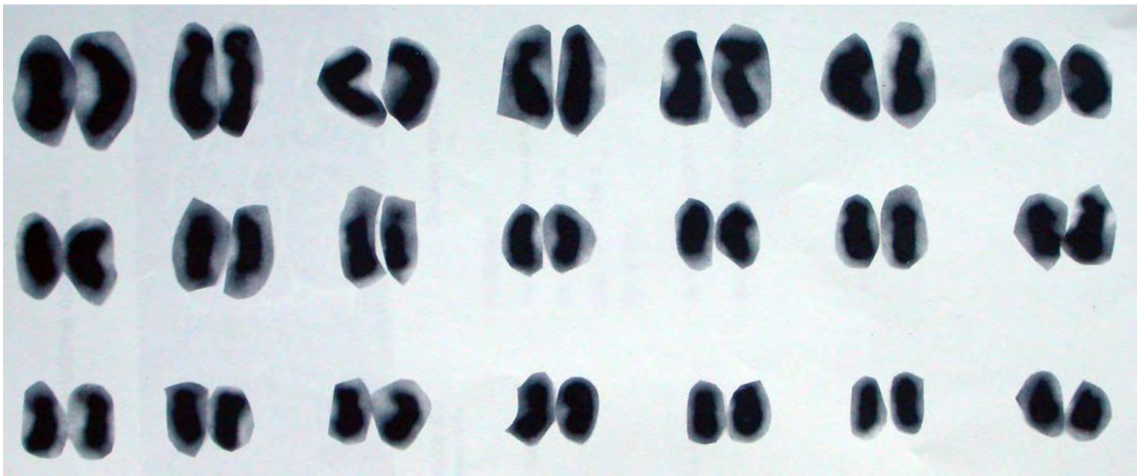
Base chromosome number x = 7. The chromosome number n = 14 is frequent within *Sebaea* (26 species investigated) but also occurs in *Ornichia* (one species), for which it is the first published report. Fourteen species of *Sebaea* from Zambia and South Africa share the haploid number n = 21, while one species, *S. brachyphylla* was found with either n = 21 (Madagascar) or n = 28 (Drakensberg). In both cases our results differ from a previous report from Kenya (n = 22, Thulin 1970). Finally, n = 28 is specific to two species of *Sebaea* collected in South Africa. In the absence of populations with n = 7, we can propose n = 14 to be either a tetraploid number or a secondary diploid one, in a series based on x = 7. In the latter case, the genus is presently represented by tetraploid (n = 14), hexaploid (n = 21), and octoploid (n = 28) species.

Examination of the different *Sebaea* karyotypes reveals rather asymmetric karyotypes at all the ploidy levels. The tetraploid *S. filiformis* (Fig. 2) shows two large pairs of submetacentric chromosomes, six middle-sized pairs (submeta- to metacentrics), and six small ones. The hexaploid *S. sedoides* (Fig. 2) shows six large pairs (meta- to subtelocentrics), four middle-sized and ten small pairs. Finally, the octoploid *S. macrophylla* (Fig. 2) presents four large pairs (meta- to subtelocentrics), four middle-sized and 20 small pairs. The presence of such asymmetric karyotypes, with subtelocentric chromosomes might support ancient episodes of ploidy (with a putative diploid species presenting such a large pair of chromosomes), and intensive chromosomal reorganization. Nevertheless, our small sampling does not allow us to further speculate.

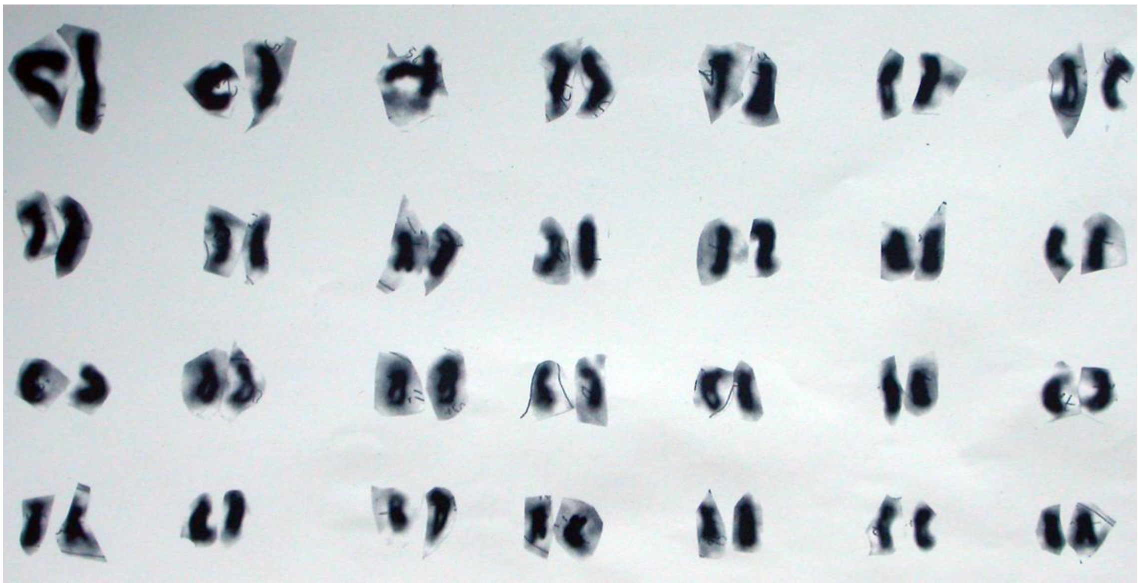
Figure 2 – Karyotypes of three *Sebaea*.



S. filiformis, mitotic metaphase, 2n=28



S. sedoides, mitotic metaphase, 2n=42



S. macrophylla, Mitotic metaphase, 2n=56

Base chromosome number x = 8. This number, new for the Exaceae, is reported here with the haploid chromosome number n = 16, and seems to characterize Malagasian species of *Exacum* (14 species). However, one species of *Exacum* from Indonesia was reported to have n = 16-18 (Annex 1, under *Cotylanthera*; Oehler 1927). Two species of the Malagasian endemic *Tachiadenus* are reported here with the haploid number n=16.

Base chromosome number x = 9. The haploid number n = 9 was previously reported for *Exacum tetragonum* from India (Borgmann 1964) but was not confirmed by Mallikarjuna et al (1987), who found 2n = 62, a chromosome number more frequent in *Exacum* (cf. below). Our results support the occurrence of n = 9 in only one species of *Sebaea* (*S. madagascariensis*), endemic to Madagascar. The haploid number n = 18 occurs in two species of *Exacum* from Madagascar. This study also confirms the previous reports of n = 18 for *Exacum affine* from Socotra (Sugiura, 1936a, 1936b; Post, 1967; Riesman, Sumanasinghe, and Craig, 2006).

Other chromosome numbers. The number n = 17, reported for *Exacum atropurpureum* from India (Mallikarjuna et al., 1987), has not been found in the present study where mostly Malagasian species of *Exacum* were investigated. The series n = 26, n = 27, and n = 30, published recently for several subspecies of *Exacum trinervium* from Sri-Lanka (Riseman, Sumanasinghe, and Craig, 2006), underlie the great chromosome instability of this taxon. This report might also support x = 10 and x = 13 as possible base number for the Exaceae. Finally, n = 31, n = 34 have been reported for eight species of *Exacum* from India (Mallikarjuna et al., 1987) supporting n = 17 as another possible base number for the tribe.

Chromosome number and systematics of the Exaceae

***Exacum*.** In the more recent monograph of the genus (Klackenberg, 1985), *Exacum* has been divided in two sections based on phenotypic and biogeographic evidence. Section *Exacum* (21 spp, including four saprophytes previously included in *Cotylanthera*) is restricted to India, Ceylon, and the Himalayas. This section, comprising biennial robust plants with large flowers (range = c. 2 - 7 cm in diameter; Klackenberg, 1985), is characterized by a wide range of published chromosome numbers (n = 9, 16, 17, 18, 26, 27, 28, 30, 31, and 34). If we excluded the approximate n = 16, reported with caution for *Exacum tenuis* (under *Cotylanthera*) and whose confirmation is needed, and the intraspecific variation (n = 26, 27, 30) observed in the *E. trinervium* complex, which might be the result of allopolyploidy / dysploidy events (Riseman, Sumanasinghe, and Craig, 2006), the more frequent haploid numbers observed in Exacum are n = 17, 18, 28, 30, 31 and 34. Possible primary or secondary base numbers for this section might then be x = 7, 9, 10 and 17 (31).

Section *Africana* (44 spp.) is distributed in Madagascar, Socotra and the African mainland. All the investigated members of this section, generally small annual plants with a tiny corolla (range size = c. 0.8 - 1.5cm in diameter; reference), show either n = 16 (14 spp, Madagascar) or n = 18 (1 sp., Socotra), except the variable *E. quinquinervium* (n = 16 or 18), a fact which supports x = 8 and x = 9 as possible base numbers for this African group. Some systematic support can be drawn if the respective Madacascan and Socotran groups appear to be monophyletic (morphology or molecular data).

Overall, the range in base chromosome number detected in the genus, x = 7, 8, 9, 10 and 17 (31), does not allow us to propose an unambiguous scenario of karyotype evolution within *Exacum*. Nonetheless, the high karyotypic diversity encountered in section *Exacum* might indicate rapid evolutionary episodes within this group, a fact supported by recent phylogenetic studies (Yuan et al., 2005), showing multiple out-of-Madagascar dispersals of *Exacum* species, with further extensive radiation into central Asia.

Sebaea. Although the morphologically variable *Sebaea* represents the most important genus in term of species number (ca. 95 spp; Schinz, 1906), no convincing taxonomic treatment has been proposed so far, mainly due to a lack of global studies integrating phylogenetic hypotheses, biogeographic data, and cytologic evidence. The present karyological investigation and the range of chromosome variation detected so far (n = 9, 14, 21, 28) put some light on the possible evolutionary history of the genus.

The smallest chromosome number detected so far in *Sebaea* (n = 9), indeed in the Exaceae, occurs in *S. madagascariensis*. This species, endemic to the north-western part of Madagascar, differs from all the remaining *Sebaea* by having "raceme-like" inflorescence and particular floral features (Klackenberg, 1990). Furthermore, recent phylogenetic studies (Yuan et al., 2003) support the exclusion of *S. madagascariensis* from *Sebaea*, and further affinities with *Ornichia* and *Exacum*. As also evidenced by our karyological data, *Sebaea madagascariensis* likely deserves a generic ranking (Chapter 2 & 4).

The most common chromosome number in *Sebaea* (n = 14, 2n = 28; Annex 1) is found in a group of species distributed from the Cape region of South Africa to the Drakensberg mountains. These species are morphologically characterized by a bilobed stigma, and a flowering period between September and December. Members of this group formed a clade deriving first in a phylogenetic analysis of the tribe based on cpDNA and nrDNA markers (Yuan et al., 2003).

Species of *Sebaea* with n = 21 generally occur in the Zambesian region of tropical Africa, are morphologically characterized by a clavate stigma, and come into flower in March (Paiva and Nogueira, 1990).

Finally, *S. brachyphylla* shows intraspecific variation, with n = 21 found in African populations and n = 28 in the Malagasian ones (Annex 1), overall reflecting the confusing taxonomy reported within this taxon (Hedberg, 1955). Hence, at least four different taxa are refered to as *S. brachyphylla* (Boutique, 1972; Paiva and Nogueira, 1990), with the type species described from Madagascar. It would be of interest to increase chromosome counts in both tropical Africa and Madagascar, including the type locality, to confirm or discuss the present karyological pattern. Finally, the report from Thulin (1970) for *S. brachyphylla* (2n = 22) is not confirmed by our study, neither in *Sebaea* nor in other Exaceae, and might be taken with caution.

Overall, if we exclude *S. madagascariensis* from *Sebaea* (Yuan et al., 2003); chapter 2 & 4), we can postulate a base chromosome number for x = 7 for *Sebaea*, with a group of tetraploid species (n = 14) centred in South Africa, and a group of hexa-tetraploid species in Central

Africa. The role of polyploidy in the evolution of *Sebaea* is highlighted for the first time in this study.

Karyotype evolution in the Exaceae

In our review, including present and past karyological reports for the Exaceae, the following haploid chromosome numbers have been detected so far: n = 9 (*Sebaea*, 1 sp, *Exacum*, 1 sp.), 14 (*Sebaea*, 29 spp; *Ornichia*, 1 sp), 16 (*Exacum*, 14 spp.; *Tachiadenus*, 2 spp.), 17 (*Exacum*, 1spp), 18 (*Exacum*, 2 spp;), 21 (*Sebaea*, 12 spp.), 26 (*Exacum*, 2 spp.), 27 (*Exacum*, 3 spp.), 28 (*Sebaea*, 2 spp.; *Exacum*, 1 spp.), 30 (*Exacum*, 1 sp.), 31 (*Exacum*, 4 spp.), 34 (*Exacum*, 5 spp.). These numbers confirm the polybasic and dysploid nature of both *Exacum* and *Sebeae* s.l. karyotypes. Hence, several intraspecific cytotypes have been detected for e.g. *Sebaea brachyphylla*, *S. minutiflora*, *Exacum trinervium*, or *E. pedunculatum*, which may indicate cryptic speciation in the absence of detectable morphological variation. Our current sampling does not allow us further speculation on the topic.

Considering the present data, we can propose a combination of both dysploidy and polyploidy events in the karyotypic evolution of the Exaceae. If we accept x = 7 as a possible base number for *Sebaea* (n = 14, 21, 28), *Exacum* (n = 28), and *Ornichia* (n = 14), and x = 8 as another base number for *Exacum* (sect. *Africana*) – these haploid numbers not being detected yet – we can infer a primarily dysploid series x = 7, 8, and 9 for the Exaceae. In the absence of strong phylogenetic hypotheses and complete karyotype reconstruction for all investigated species, both the determination of a putative ancestral number and the polarity of dysploid series within Exaceae remain challenging. Nevertheless, external evidence can lead us to postulate x = 7 as a possible ancestral number for the Exaceae, and thus an ascending dysploid series for the tribe: (1) the haploid numbers n = 14 to 28 (x = 7) occur in most of the species investigated in this study (30 of 51), followed by n = 16 (x = 8; 16 of 51), and n = 9, 18 (x = 9; 5 of 51); (2) Exaceae species based on x = 7 generally show a wide distribution; and (3) x = 7 occurs in morphologically well-distinct genera, including *Sebaea*, *Exacum* and *Ornichia*. This hypothesis is further supported by molecular data indicating that members of *Sebaea* with n = 14 form a primarily derived, well-supported clade, in a global phylogeny of the tribe (Yuan et al., 2003).

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THE POLYPHYLETIC GENUS *SEBAEA* AND NEW INSIGHTS ON THE MORPHOLOGICAL AND KARYOLOGICAL EVOLUTION OF THE TRIBE EXACEAE (GENTIANACEAE)

THE POLYPHYLETIC GENUS *SEBAEA* AND NEW INSIGHTS ON THE MORPHOLOGICAL AND KARYOLOGICAL EVOLUTION OF THE TRIBE EXACEAE (GENTIANACEAE): INFERENCE OF MOLECULAR PHYLOGENY

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Abstract – To better understand the evolutionary history of the genus *Sebaea* and its relationships with other genera of tribe Exaceae (Gentianaceae), a phylogeny based on three plastid genes was built. In addition, morphological and karyological characters were mapped on the tree to detect possible non-molecular synapomorphies. Phylogenetic reconstructions support the monophyly of the Exaceae, but reveal a polyphyletic *Sebaea*, with four well statiticaly supported clades. The first clade consists of a single species (*Sebaea pusilla*), which differs from the other species of *Sebaea s.l.* by its medifixed anthers, and its seed testa cells (polygonal). The second clade contains mostly South African species characterized by more or less bilateral seeds, with rectangular testa cell, and a secondary stigma, and corresponds to *Sebaea* Sol ex. R.Br.. The third clade contains exclusively tropical African species, characterized by a stylar polymorphism and papillose clavate stigma, and deserves generic status. Finally the fourth clade only contains *Sebaea madagascariensis*, a species characterized by its inflorescence (axillary flower) and unique base chromosome number (n=9 versus n=14, 21, 28 for the other clades). Karyological reconstruction suggests a basal number of x=7 for the Exaceae, followed by dysploidy leading to secondary base number of x=8 and 9.

Keyword – Africa, Exaceae, *Exacum*, Gentianaceae, *Gentianothamnus*, karyology, *Ornichia*, Madagascar, phylogeny, *Sebaea*, *Tachiadenus*

INTRODUCTION

The Exaceae (Gentianaceae) in their current circumscription (Struwe et al., 2002; Klackenberg, 2006) are a small tribe that includes ca. 184 species distributed in the respective genera *Exacum* L. (69 spp.) (together with *Cotylanthera* Blume) (Klackenberg, 2006), *Gentianothamnus* Humbert (1 sp), *Ornichia* Klack. (3 spp.), *Sebaea* Sol. ex R. Br. (c. 100 spp.), and *Tachiadenus* Griseb (11 spp.) (Schinz, 1906; Hill, 1909; Klackenberg, 1985, 1986, 1987a, 1990, 2002) (Fig.1). Exaceae mainly differ from the five other tribes of the gentian family by some synapomorphies including the star-shaped testa cells of the seeds (Klackenberg, 1985; Bouman et al., 2002; Klackenberg, 2002) and the shape of the epidermis cells of petals (basically rounded and convex versus elongated and flat) (Klackenberg, 1985, 2002). Detailed morphological studies on Exaceae have been carried out on *Exacum* (Klackenberg, 1985) and possible close relatives, namely *Ornichia* and *Tachiadenus* (Klackenberg, 1986, 1987a). Klackenberg (1985) further divided the large *Exacum* in two sections, section *Africana* and section *Exacum*.

A wide range of morphological variation has been found within the tribe (Klackenberg, 2002), including: habit (herb versus subshrub), gynoecium structure (ovary truly or peudo-bilocular, style straight or bent downwards), androecium (insertion of filaments, opening mechanism of the stamens) flower colour (white, yellow, orange, blue and purple), merosity (4 or 5), size of the corolla (a few mm up to 7 cm in diameter), length of the corolla tube (a few mm up to 20 cm), etc.

Moreover, recent karyological studies and a literature survey (Chapter 1) show a large variation in chromosome numbers within Exaceae ($n=9$, 14, 16, 17, 18, 21, 26, 27, 28, 30, 31, and 34), and suggest a base chromosome number of $x=7$ for the Exaceae.

Furthermore, two molecular studies have also been performed on the tribe, with a special focus on *Exacum* (Yuan et al., 2003; Yuan et al., 2005), revealing a monophyletic *Exacum*, and a basal position for *Sebaea* (apparently polyphyletic), followed by a clade including *Tachiadenus* and *Gentianothamnus* sister to *Exacum* and *Ornichia* plus one of the sampled *Sebaea*.

Conversely, systematic studies on *Sebaea*, the most species-rich genus of the tribe, remained anecdotic at either phenotypic or molecular levels (Klackenberg, 2002; Yuan et al., 2003). For example, little is known on the polarity of vegetative or floral characters states, or the karyological evolution of the genus.

Sebaea comprises annual to perennial, erect to procumbent herbs, or more rarely achlorophyllous saprophytes (*S. oligantha*), and is mostly characterized by the absence of synapomorphic features characterising the other genera of Exaceae. Currently no synapomorphy has been found to support *Sebaea*, although this genus is apparently homogenous.

According to various estimates (Schinz, 1906; Boutique, 1972; Dyer, 1975; Paiva and Nogueira, 1990b; Wielgorskaya, 1995; Adams, 1996; Mabberley, 1997) the range in species number is quite broad in *Sebaea* (from 60 to ca.159). Our estimates of ca. 100 species, based on field observations and examination of many herbaria collections (more than 3000) make the genus the most species-rich within the Exaceae.

Sebaea is widely distributed in the Old World, with two hot spots of diversity centred in Tropical Africa and South Africa (Schinz, 1906; Marais and Verdoorn, 1963). The tropical species are mainly distributed on the Katanga plateau (a region situated between the Democratic Republic of Congo, Angola and Zambia), while the South African species (*ca.* 70 spp.) mostly occur in the Cape and the Drakensberg escarpment (Lesotho and South Africa). Four species extend their range eastward to Madagascar, two of them being endemic to the island. Finally, two species are endemic to the Australia/New Zealand region, whereas one species extends its range from Africa to the Indo-Malaysian area.

Sebaea species grow from sea level (e. g. *S. minutiflora*, *S. ambigua*) to 3000-3500 m altitude in the Drakensberg (e. g. *S. marlothii*, *S. thodeana*) and in the East African mountains (e. g. *S. brachyphylla* and *S. leiostyla*). They can be encountered in most parts of Sub-Saharan Africa in a wide variety of habitat e.g., in dense tropical forests, grasslands and savannas, in marshes or waterlogged areas, alpine meadows, and on wet rocks and sandy riverbanks or sand dunes. They usually grow in open, wet, sometimes disturbed habitats, including e.g. recently burned places or sandy stream banks, where competition is generally reduced (Kissling, pers. obs.).

Despite series of systematic works performed on the Exaceae (Rafinesque, 1837; Meyer, 1838; Grisebach, 1845; Bentham, 1876; Schinz, 1891; Gilg, 1895a, 1898; Schinz, 1903, 1906; Hill, 1908a), the taxonomic circumscription of *Sebaea* remains controversial. Most of the confusion relies on the frequent use of taxonomically inconsistent characters (Marais, 1961) including the presence of “bi-glandular swelling” on the style, appendage (glands) on the anthers, the form (straight or recurved) and disposition (free or connate) of the anthers, and the height of the filament insertion. Moreover, specific circumscription within *Sebaea* is difficult because a large amount of morphological plasticity occurs due to varying environmental conditions that may obscure species boundaries (Hedberg, 1955). Therefore the high intra- or interspecific variation has led to the naming of many species in *Sebaea*, resulting in confusing redundant or synonymous combinations and a global taxonomical uncertainty (e.g. Chamisso and Schlechtendal, 1826; Rafinesque, 1837; Meyer, 1838; Grisebach, 1849; Bentham, 1876; Schinz, 1891; Knochblauch, 1894; Gilg, 1899; Schinz, 1903, 1906; Hill, 1908b, 1908a; Marais, 1961; Marais and Verdoorn, 1963).

In addition to its poorly understood systematic history, the phylogenetic position of *Sebaea* is still a matter of speculation. Recent molecular inferences support the inclusion of the genus in the tribe Exaceae (Struwe et al., 1998; Thiv et al., 1999b; Thivet al., 1999a; Yuan et al., 2003), and a possible polyphyletic status (Yuan et al., 2003). Nevertheless, only a reduced subset of the genus diversity (5 species out of c. 100) was included in these studies, preventing further phylogenetic or systematic conclusions.

In this paper, we used an extensive dataset of *Sebaea* species collected in the field to provide a phylogenetic hypothesis aimed at revealing the morphological and karyological evolution of the genus. More specifically, the goals of this study are to: (1) confirm the monophyly of the tribe *Exaceae*, taking into account the large geographical diversity of *Sebaea*; (2) test the monophyly vs. polyphyly of the genus; (3) identify synapomorphic characters supporting molecular clades; and (4) reconstruct the evolution of relevant non-molecular characters within the tribe.

MATERIALS AND METHODS

Taxon sampling and outgroup choice

Sebaea species were collected in Africa (South Africa and Zambia) in 2004 and 2005; *Exacum* and *Tachiadenus* species were collected in Madagascar in 2005. All species of *Sebaea* were determined by the first author following current local flora (Marais and Verdoorn, 1963; Boutique, 1972; Klackenberg, 1990; Paiva and Nogueira, 1990b; Nemomissa, 2002). Voucher specimens are deposited in the herbarium of the University of Neuchâtel, Switzerland (NEU). Material for DNA extraction was obtained from both collected samples and herbarium specimens (BR, C, and PRE).

All the genera included in the *Exaceae* sensu Struwe et al. (2002) are represented, except for *Cotylanthera*, which was recently transferred to *Exacum* (Klackenberg, 2006) based on both molecular and morphological data. Sampling of *Sebaea* species was maximized to cover the geographical (southern Africa, tropical Africa and Madagascar), ecological (savannah, forest, high altitude meadow, etc.) and morphological (habit, inflorescence, merosisty, etc.) variation of the genus. All the ecological and morphological groups in this taxon are sampled. A total of 174 (*trn*L intron sequences) operational taxonomic units (OTUs) representing the six tribes of Gentianaceae were included in this study as ingroup taxa in order to check the monophyly of the tribe.

Nine species representing all other four families of the order Gentianales (Struwe et al., 1994; Backlund et al., 2000; Bremer et al., 2002; APG, 2003) were included as outgroups. They were *Labordia tinifolia* A. Gray and *Mitreola petiolata* (Walt.) Torr. et Gray of Loganiaceae, *Nerium oleander* L. and *Plumeria obtusa* L. of Apocynaceae, *Emmenopterys henryi* Oliv., *Gardenia taitensis* DC., *Guettarda boliviana* Standl. and *Mitragyna inermis* (Willd.) K. Schum. of Rubiaceae, and *Gelsemium sempervirens* Ait. of Gelsemiaceae.

Because a preliminary analysis indicated that the tribe Exaceae was poorly resolved by the *trn*L intron sequence, a subset of Exaceae species (67 taxa) (Annex 3) was sequenced for the *atp*B-*rbc*L intergenic spacer and *trn*L-F intergenic spacer. In addition to Exaceae, 49 *trn*L-F intergenic spacer sequences, representing outgroups and other tribes of the Gentianaceae family were retrieved from GenBank and added to the matrix. In the same way, 12 *atp*B-*rbc*L intergenic spacer sequences were also added to the matrix. Some of the 67 accessions of the Exaceae subset were not sequenced for one of the two additional chloroplast markers due to difficulties in PCR amplification. Sequential exclusion of species lacking one of the additional markers from the data set was tested, and showed that the inclusion of those incomplete sequences did not interfere with the robustness of the trees obtained.

The species included in this study are listed in Annex 3 (species retrieved from GenBank are indicated with asterisks following their accession numbers) and the data matrix is available from the corresponding author.

Molecular methods (DNA extraction, amplification, and sequencing)

Total DNA was extracted from silica gel dried leaves, or from leaf tissue taken from herbarium sheets, by using the DNeasy Plant Mini Kit (Qiagen, Switzerland), according to the manufacturer‟s instructions. Extracted DNAs were electrophoresed on 0.8% agarose gels with size markers and visualized with ethidium bromide to control their quantity and quality.

Polymerase Chain Reactions (PCR) were performed on a Biometra thermocycler using the following program: one cycle of 3 min at 94°C linked to 30 cycles of 10 s at 94°C, 20 s at 50–55°C, 1.5 min at 72°C, and then 4 min at 72°C to complete primer extension. PCR were done in a 25µl total reaction volume containing 2.5µl 10X PCR buffer (with 1.5mM $MgCl_2$), 0.5µl 10mM dNTPs, 0.5µl of 10mM each forward and reverse primers, 0.2µl (1U) HotStar Taq DNA polymerase (Qiagen AG, Basel), 19.8µl H2O, and 1µl (ca. 10–20ng) genomic DNA.

The *trnL* intron and the *trnL-trnF* intergenic spacer were amplified as a single PCR fragment using the two universal primers „c‟ and „f‟ of Taberlet et al. (1991). The *atpB-rbcL* intergenic spacer was amplified using universal primers „Oligo 2‟ and „Oligo 5‟ (Manen et al., 1994).

The PCR products were checked on 0.8% agarose gels. Successfully amplified DNA fragments were then purified prior to sequencing using the QIAquick PCR purification kit (Qiagen AG, Basel) following the manufacturer‟s protocol. Cycle sequencing reactions were performed using the dye-terminator chemistry as implemented in the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, USA) in a Biometra thermal cycler. The same primers used for amplification were used for sequencing for both *atp*B-*rbc*L and *trn*L-F (UAA) intron and spacer. Protocols and cycling parameters suggested by the sequencing kit were followed except that the reaction volumes were scaled down to 5µl. The cycle sequencing products were cleaned using the ethanol/sodium acetate precipitation method as suggested by the manufacturer of the sequencing kit. The purified sequencing products were resuspended in 13 µl TSR or Hi-Di Formamide (supplied by Applied Biosystems, Foster City, USA) and then analyzed on an ABI310 automated sequencer using a 47 cm capillary and polymer POP-6 (Applied Biosystem, Foster City, USA). Sequences were subsequently checked manually against the electropherograms using the software ChromasPro Version 1.33 (Technelysium Pty Ltd).

Sequence alignment, congruence test, and phylogenetic analysis

Alignment was performed using the program Clustal W (Thompson et al., 1994) as implemented in the program BioEdit 7.0.1 with subsequent manual improvement. For the *trn*L intron, the alignment was straightforward and unambiguous except for two simple sequence repeat (SSR) regions of multiple As (44 and 21 bp, respectively in our aligned data matrix). These ambiguously alignable SSR regions (65 bp in total) were excluded in subsequent analyses. Similarly, three ambiguously alignable regions (35, 18, and 58 bp, respectively in our aligned data matrix, 111 bp in total) were excluded for the *trn*L-F spacer. The *atp*B-*rbc*L spacer was aligned without ambiguities.

Potentially informative and unambiguously assessable indels were scored as binary characters regardless of their length and were added to the data matrix (which gave a total of 112 additional characters for the combined data matrix, 36 for the *trn*L intron, 34 for the *trn*L-F spacer and 42 for the *atp*B-*rbc*L spacer).

To assess the level of congruence between the *trn*L intron, *trn*L-F spacer and *atp*B-*rbc*L spacer data sets, we analysed each data set independently to see if they produced a similar topology. We also performed an incongruence length difference (ILD) test of Farris et al. (1995), implemented in PAUP* 4.0b10 (Swofford, 2002) as the partition–homogeneity test after assuring ourselves that the properties of the data did not lend themselves to biasing this test (see results). 1000 replicates of partition of *trn*L intron versus *trn*L-F spacer, versus *atp*B-*rbc*L spacer were conducted with heuristic searches (simple sequence addition and TBR branch–swapping). The three data sets were confirmed as congruent P = 0.92) and were then combined for all further analyses. The resulting combined matrix contained 165 accessions, representing the six tribes of the Gentianaceae and 9 outgroups.

Phylogenetic analyses were performed using maximum parsimony (MP), and Bayesian inference. MP analyses were performed using PAUP* 4b10 (Swofford, 2002). Heuristic searches were conducted using 1000 random taxon-addition-order replicates with the tree-bisection-reconnection (TBR) branch swapping option, saving multiple trees (MULTREES) at each step. Thousand best trees per replicate were saved. Branch lengths were derived under ACCTRAN optimization. Clade support was assessed by bootstrap analysis (Felsenstein, 1985) with 1000 replicates of heuristic searches applying the same search parameters as those in the parsimony analysis. The complete analysis was limited to 100 trees saved per replicate due to memory overrun. All clades with at least 70% bootstrap values could be considered as well supported (Hillis and Bull, 1993). The amount of phylogenetic signal was reflected by the classical descriptive statistics (consistency index [CI] and retention index [RI]). Bayesian inference was conducted using MrBayes version 3.1.2 (Huelsenbeck et al., 2001; Ronquist and Huelsenbeck, 2003) and data were partitioned by genes for the combined analysis.

The most appropriate model of sequence evolution for each dataset was determined using MrModeltest version 2.2. (Posada and Crandall, 1998; Nylander, 2004; Posada and Buckley, 2004) to be the general time reversible model (GTR + I + G) for the *trn*L-F spacer, and the GTR + R model for both *trn*L intron and *atp*B-*rbc*L spacer dataset. Default priors and a dirichlet distribution were used for the base frequency parameters. Two independent analyses each with four Markov chains, three heated and one cold, starting from a random tree were run simultaneously for five million generations with trees sampled every 100 generations. Trees generated prior to the four Markov chains reaching stationarity (the burn-in) were discarded. The remaining trees were used to construct a 50% majority rule consensus tree. Although studies have shown Bayesian methods can overestimate support for nodes (Suzuki et al., 2002; Cummings et al., 2003), we considered such an analysis useful here to provide a model-based estimate of the phylogeny for a data set too large to feasibly conduct a standard maximum likelihood analysis. Internodes with posterior probabilities ≥ 95% were considered statistically significant.

Morphological data and character state optimization

A total of 23 morphological characters (table 1) were surveyed. The characters were selected on the basis of, a) the diagnostic characters of the currently defined genera (5 characters), b) diagnostic characters used in previous taxonomy of *Sebaea* (2 characters), and c) personal observation on the reproductive system (13 characters) of *Sebaea* and relative and vegetative characters (3 characters). The character states for *Sebaea* was based on direct observations on living and herbarium material; for other genera literature resources were used (Klackenberg, 1985, 1986, 1987a, 2002) with additional observation and corrections.

Character state evolution was reconstructed with MESQUITE 1.12 (Maddison and Maddison, 2005) by using two methods: (*i*) maximum-likelihood-based discrete Markov *k*-state 1 parameter models (Lewis, 2001; Maddison and Maddison, 2005) and (*ii*) a maximum parsimony approach (Maddison and Maddison, 2000).

Table 1: Morphological characters and character states used in this study

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1. Seed form: 0=oval, ridged with a more bilateral symmetry; 1= polyhedral to angled
2. Testa cell: 0= polygonal; 1= ± rectangular; 2= star-shaped or ± isodiametric
3. Testa cell arrangement: 0= not in row; 1= in row
4. Anther opening mechanism: 0=by longitudinal slits; 1= by apical pores; 2= by apical pores that finally widen to slits
5. Anther appendix: 0= gland or papilla absent; 1= with a conspicuous apical gland (± = anthers size); 2= with a small apical gland (<<anther size); 3 = with a small papilla near the apex
6. Anthers furnished by two basal glands: 0= absent; 1= present
7. Staminal insertion: 0= in the sinus, or at a short distance from the sinus; 1= in the corolla tube; 2= at the base of the corolla tube
8. Anther fixation: 0=medifix; 1= basifix
9. Ovary: 0= truly bilocular, at least towards the base; 1= ovary pseudo-bilocular
10. Style: 0= straight; 1= bent downwards below the anthers, or markedly curved
11. Stigma division: 0=bilobed; 1=slightly bilobed; 2= apparently entire
12. Stigma form: 0= capitate; 1=capitate to slightly clavate; 2=linear to clavate; 3="flat" to capitate
13. Stigma texture: 0= smooth; 1=papillate
14. Secondary stigma on the style: 0= absent; 1= present
15. Five-lobed disk below the ovary: 0=absent; 1=present
16. Corolla colour: 0= bright yellow; 1= white; 2 = blue-violet; 3=salmon-orange; 4=yellow to orange with at least the lobes red
17. Corolla merism: 0= pentamerous; 1= tetramerous
18. Corolla tube: 0=long; 1= short (nearly absent); 2= extremely long (< 6-10 cm)
19. Enantiostyly: 0=absent; 1=present
20. Stylar polymorphism (short styled versus long styled flowers): 0= absent; 1= present
21. Bracteoles: 1=much longer than the whole flower; 0= shorter than the whole flower
22. Inflorescence: 0=dichasium; 1=monochasium; 2= solitary flowers; 3= raceme-like; 4=panicle; 5=terminal or axillary umbel-shaped (in fact condensed cymes)
23. Leaves: 0= glabrous; 1= hairy
24. Life form: 0= herb; 1= suffrutescent; 2= shrubby; 3=crassulescent herb
25. Chromosomes base numbers: 0=7; 1=8; 2=9; 3=17

The likelihood-based Markov *k*-state 1 model does not consider any particular plesiomorphic state at the root of the tree, and a character state can change to any other state on any branch of the tree with equal probability. We report proportional likelihood (P.L.) values of states scaled so that the sum of all states is 1. We used a decision threshold of 2.0 in MESQUITE (Maddison and Maddison, 2005) for statistical considerations, while parsimony analyses considered character state transformations unordered.

The Bayesian topology based on the combined data was chosen for the tracing because it showed a high resolutions for internal nodes of the tribe Exaceae. The basal trichotomy of the Bayesian topology was artificially resolved by assigning a branch length of 0.00001, according to the MP trees for this relevant node.

Karyological data optimization

Karyological data were obtained from a recent survey of the tribe, including more than 160 populations collected in the field (chapter 1). This study suggests x=7, 8, 9, and 17 as possible base numbers, which were here coded as follow a: x=7, b: x=8, c: x=9, and d: x=17, implying that no distinctions were made between polyploid species with the same basal number. Character state reconstruction was performed using MP method described before.

RESULTS

Sequence and Alignment Characteristics, Congruence among data sets

All newly obtained sequences have been submitted to EMBL/GenBank databases. The accession numbers of the sequences used in this study are listed in Annex 3.

The *trn*L intron sequences ranged from 334 to 539 bps in length. Within Gentianaceae, the Exaceae, and the Saccifolieae are characterized by longer sequences (from 449 to 539 bps). In the aligned matrix a long insertion of 122 bps characterize the Exaceae and Saccifolieae.

The *trn*L-F spacer sequences ranged from 335 to 478 bps in length. Aligned sequences show important variation, due to 112 indels; the most important one is a deletion of 93 bps, occurring within a group of *Sebaea* species. The *atp*B-*rbc*L spacer sequences ranged from 710 to 781 bps in length.

The three sets of sequences were revealed as congruent by the ILD test, (P = 0.92). However recent studies and simulations suggested that the ILD test could fail to detect congruence due to different noise levels of the data sets (Dolphin et al., 2000; Yoder et al., 2001) or incongruence (Darlu and Lecointre, 2002; Dowton and Austin, 2002) due to large difference in sizes and evolutionary conditions of the data partitions. Our data sets had similar sizes (702 characters in *trn*L, 643 characters in *trn*L-F, and 964 characters in *atp*B-*rbc*L), and all of them, when analysed separately, revealed similar topology with minor incongruences on terminal branches at species level. Incongruent nodes on terminal branches are at best moderately supported by bootstrap analysis, which probably suggest limited rather than conflicting signal. Thus we consider that our data sets were not suffering from these limitations, and, therefore, were combined. The combined data matrix had 2309 bp sequence characters plus 112 binary indels characters. Of the 2309 bp combined sequence, 176 bp (7.6%) ambiguously aligned *trn*L and *trn*L-F sequence were excluded from phylogenetic analyses. The number of variable characters was 1116 (46.1%), of which 786 (32.5%) were informative.

Phylogenetic Analysis

Results of the heuristic searches on the separate *trn*L intron, *trn*L-F intergenic spacer, and *atp*B-*rbc*L intergenic spacer data set are summarized in table 2 and the recovered relationships within Exaceae of each data set are summarized in Figure 1.

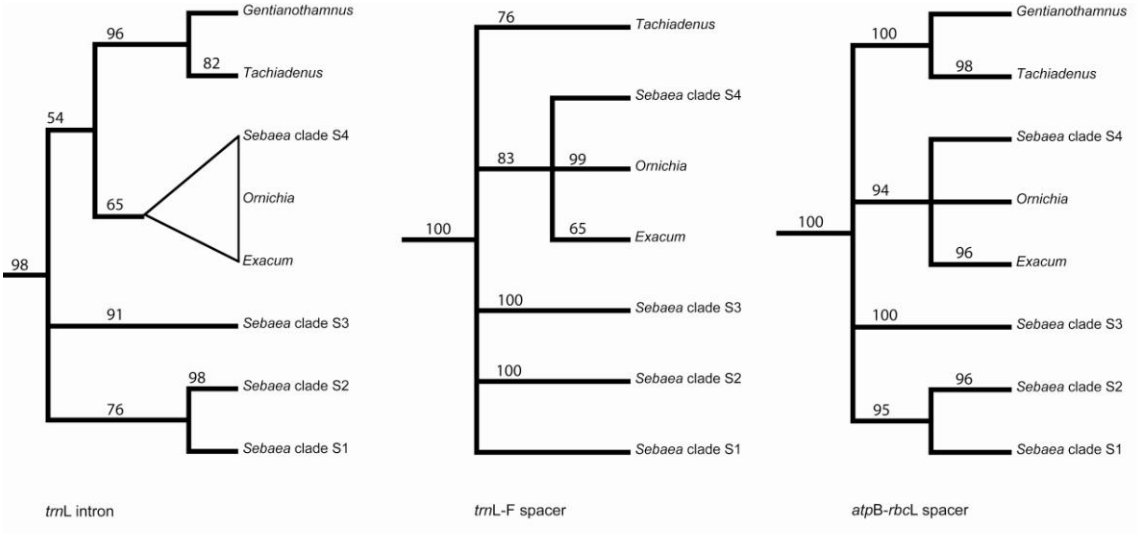

Figure 1: Simplified strict consensus tree obtain from MP analysis of each data set, showing the genus relationship within the tribe Exaceae. Numbers above the branches are bootstrap values supporting the corresponding branch when greater than 50%. *Sebaea* is polyphyletic. *Sebaea* clade S1 corresponds to *S. pusilla*. *Sebaea* clade S2 includes mainly South African *Sebaea*. *Sebaea* clade S3 contains mostly tropical African *Sebaea*, and finally *Sebaea* clade S4 corresponds to *S. madagascariensis*, an endemic species from Madagascar. In the *trn*L intron data set, the genus *Exacum* is paraphyletic towards a clade formed of *Ornichia* + *Sebaea* clades S3.

Table 2: Characteristics of the four cpDNA data sets.

Data set	Aligned positions	No. excluded characters	No. constant sites	No. Informative sites	No. Uninformative sites	Indels	Tree length	CI	RI
trn L intron	702	65	335	223	115	36	733	0.6385	0.9131
trn L-F spacer	643	111	240	233	93	34	711	0.654	0.917
atp B-*rbc* L spacer	964	0	554	330	122	42	759	0.7615	0.9027
combined dataset	2309	176	1129	786	330	112	2222	0.6809	0.9098

Table 3: Summary of the Bayesian inferences.

	Base frequencies				substitution rate matrix						invariable site	gama distribution parameter	
Data set	A	C	G	T	A-C	A-G	A-T	C-G	C-T	G-T		alpha	m
trn L intron	0.3928	0.1536	0.1735	0.2798	0.1312	0.2114	0.048	0.1104	0.3163	0.1824		0.9485	0.0583
trn L-F spacer	0.3074	0.1556	0.1556	0.3645	0.1795	0.228	0.0534	0.1243	0.2512	0.1672	0.144	0.0889	2.236
atp B-*rbc* L spacer	0.3226	0.1936	0.1424	0.3412	0.1464	0.2235	0.0763	0.1145	0.2453	0.1892		3.7216	0.0977
combined dataset												77.0693	0.034

Heuristic searches on the combined data set resulted in 2000 equally most parsimonious trees of 2222 steps (CI= 0.617, excluding autapomorphic sites, RI= 0.910) (Fig. 2.). The combined consensus tree was well resolved and highly supported toward the base of the tree (the root), while the upper branches (the tips) were poorly resolved or received less significant support. The monophyly of all tribes and subtribes was highly supported (71–100%).

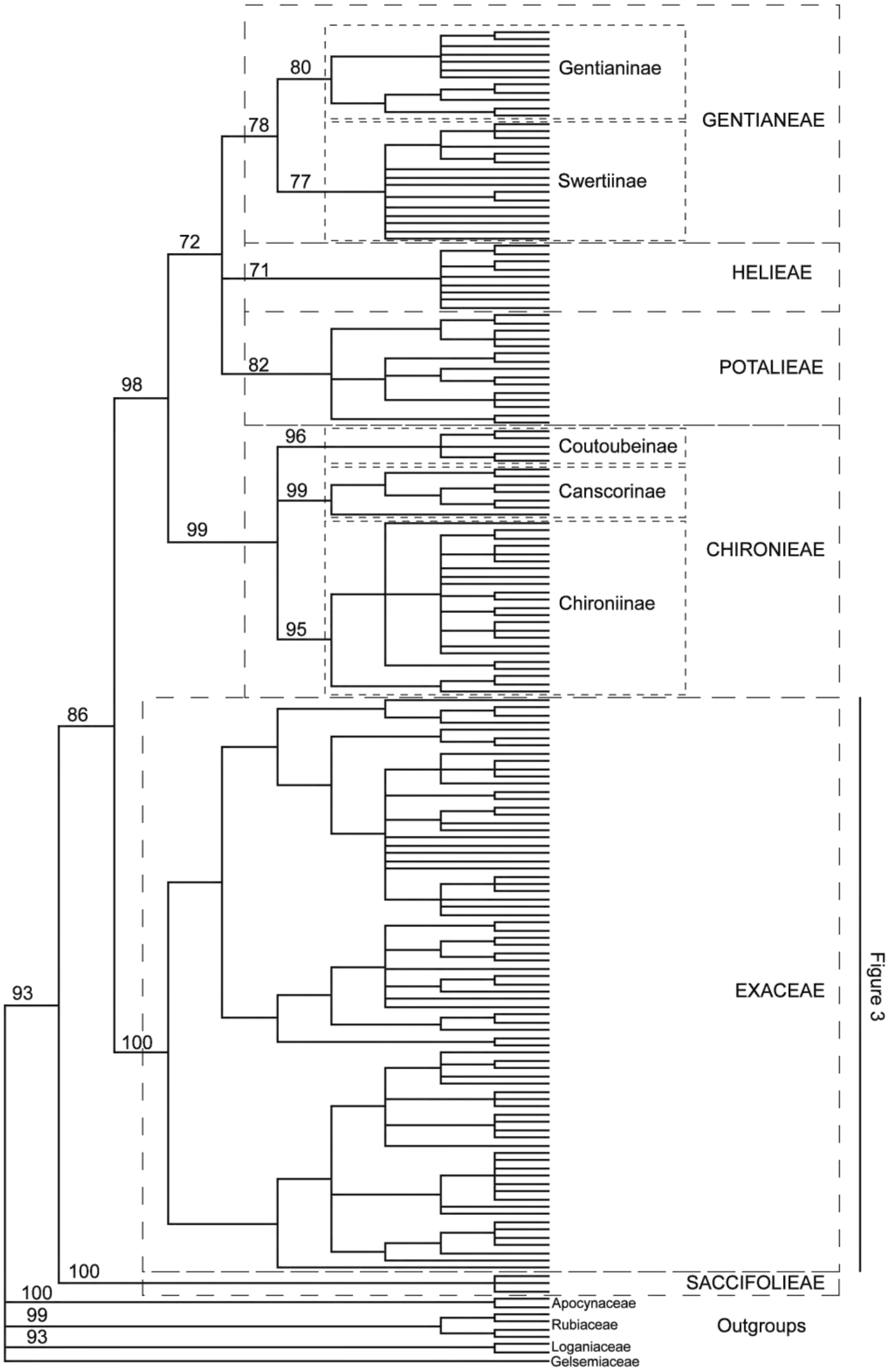

Figure 2: The combined dataset (*trn*L intron, *trn*L-F, and *atp*B-*rbc*L spacer) strict consensus tree. Numbers above the branches are bootstrap values supporting the corresponding branch when greater than 50%.

The tribe Saccifolieae was resolved as the most basal clade of the family Gentianaceae with high support (100%), followed by the tribe Exaceae (100%) and tribe Chironieae (99%). The remaining three tribes formed an unresolved trichotomy.

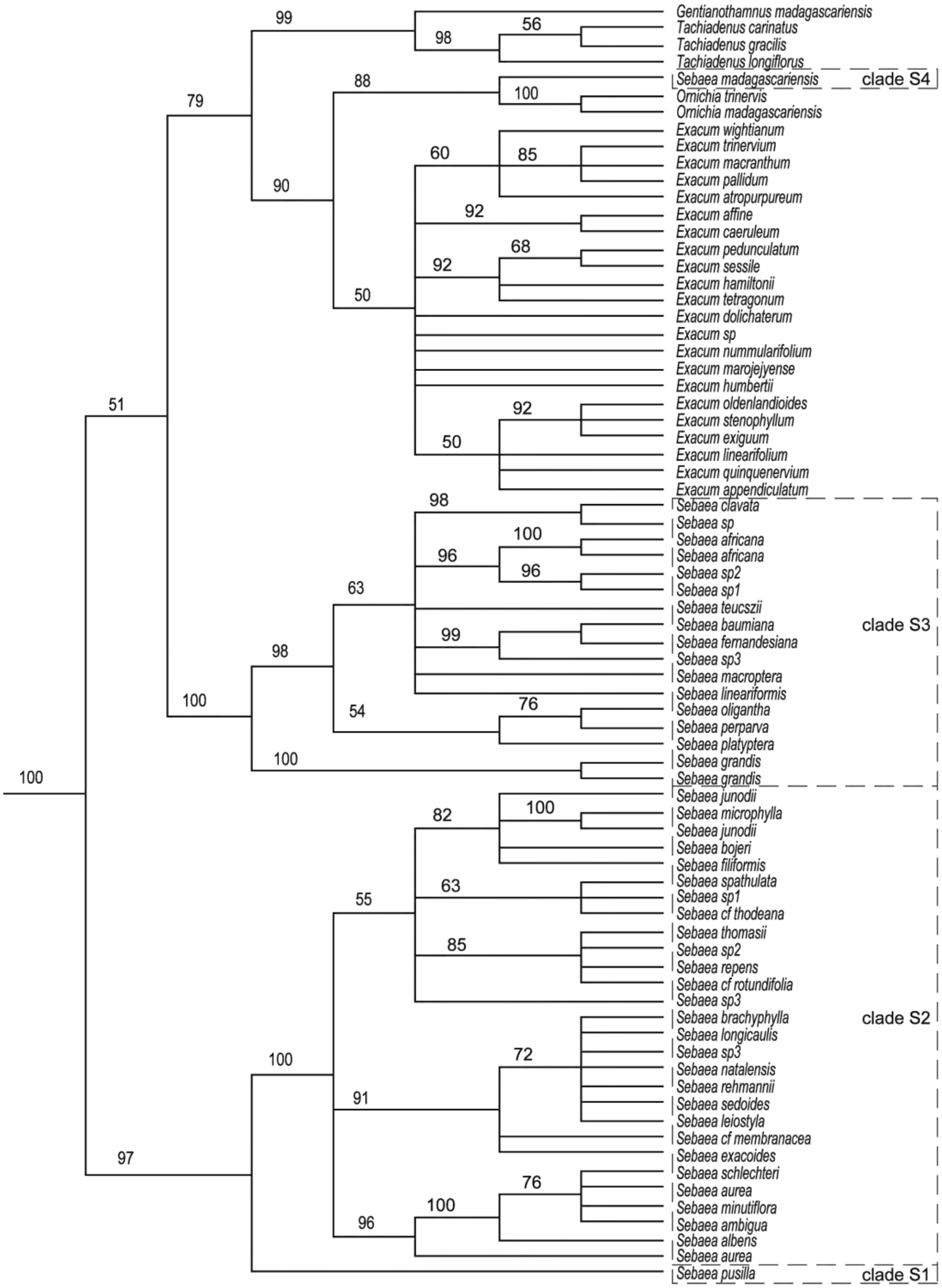

Figure 3: A focus on the Exaceae representative from the strict consensus tree obtained with the combined dataset (fig. 2). Numbers above the branches are bootstrap values supporting the corresponding branch when greater than 50%.

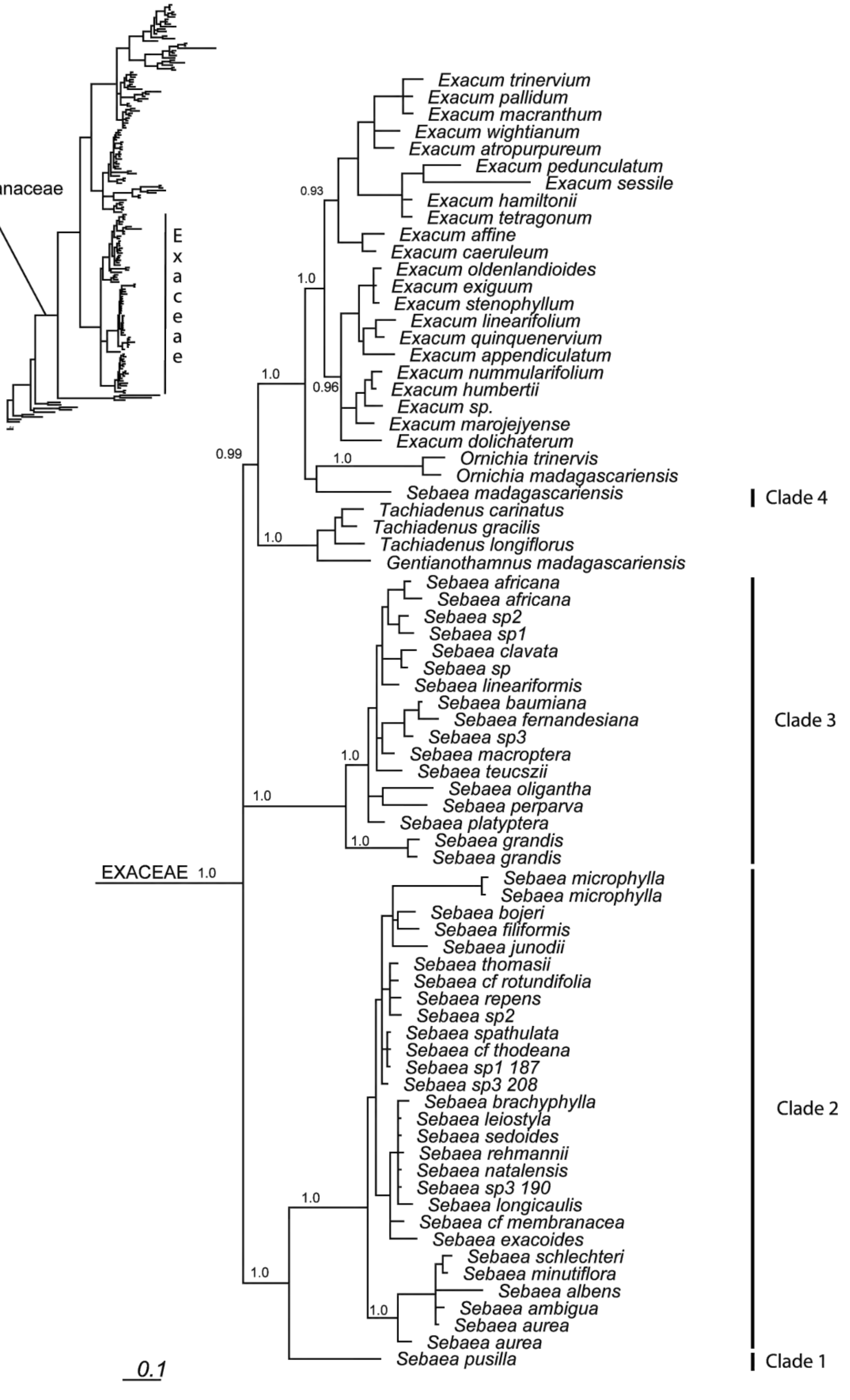

Figure 4: The 50% majority rule consensus trees from Bayesian inference analyses of the combined data set. Branch support values are posterior probabilities from a 50% majority rule consensus tree derived from a Bayesian inference analysis.

The tribe Exaceae was well resolved (Fig. 3). The genus *Sebaea* was shown polyphyletic with four clades named as S1, S2, S3 and S4 hereafter. Clade S1 contains only *S. pusilla*, and is sister to Clade S2 which formed a highly supported clade (97%). Clade S1 + S2 are sister to all the remaining genera of the tribe. The highly supported clade S3 (100%) comprises of tropical species of *Sebaea*. The clade S4 comprises the single species *S. madagascariensis*, and was shown to be sister to the genus *Ornichia* (88%). The species of *Exacum* formed a weakly supported monophyletic clade sister to the *Ornichia*-S4 clade. The Madagascan endemic genera *Gentianothamnus* and *Tachiadenus* grouped together as a highly supported clade (99%).

For the Bayesian inference, the GTR + G molecular evolution model was selected for both *trn*L intron and *atp*B-*rbc*L spacer data sets, while the GTR + I + G model was selected for *trn*L-F spacer. The parameters obtained from the Bayesian inferences for each data partition are summarized in table 3. The majority rule consensus tree obtained from the Bayesian analyses (Fig. 4) is congruent with the strict consensus tree of the combined MP analysis. The posterior probabilities are in accordance with the MP bootstrap values as well. Despite the high support for the monophyly of the tribe Exaceae, the Bayesian tree could not confidently resolve the relationships among the monophyletic clades (S1-S2), S3 and the monophyletic clade formed by the rest of the genera plus S3. However, the resolution within each resolved clade is significantly higher than the MP consensus tree.

Phenotypic character variation and reconstruction of ancestral floral traits

Most of the 22 phenotypic characters investigated (Table 2), are highly consistent on to the molecular phylogeny when optimized on the cladogram. The found apomorphies supporting the different clades within Exaceae are summarized in Figure 5.

Both maximum likelihood (ML) and MP ancestral reconstruction give similar results. ML ancestral reconstruction of floral traits indicated that the ancestral form in the tribe Exaceae was a glabrous (P.L.=0.99) herb (P.L.=0.99) with a few solitary (P.L.=0.94) pentamerous flowers (P.L.=0.99). Flowers had a mid-size corolla tube (P.L.=0.99) with stamen inserted in the middle of the corolla tube (P.L.=0.57). The anthers were basifixed (P.L.=0.99), opening by slits (P.L.=0.99), and were furnished with a small apical gland (P.L.=0.73). The ovary was bilocular (P.L.= 0.99) with a straight style (P.L.=0.99), without secondary swelling (P.L.=0.98). The stigma was capitate (P.L.=0.65) and either bilobed (P.L.=0.50) or entire, but smooth (P.L.=0.98). Flowers were homostylous (P.L.=0.99), and the colour might have been yellow (P.L.=0.57). The seeds were cubical (P.L.=0.99). The testa cells could have been star shaped (P.L.=0.59). The combination of characters suggests the putative ancestral species had a corolla very similar to that of *S. pusilla*.

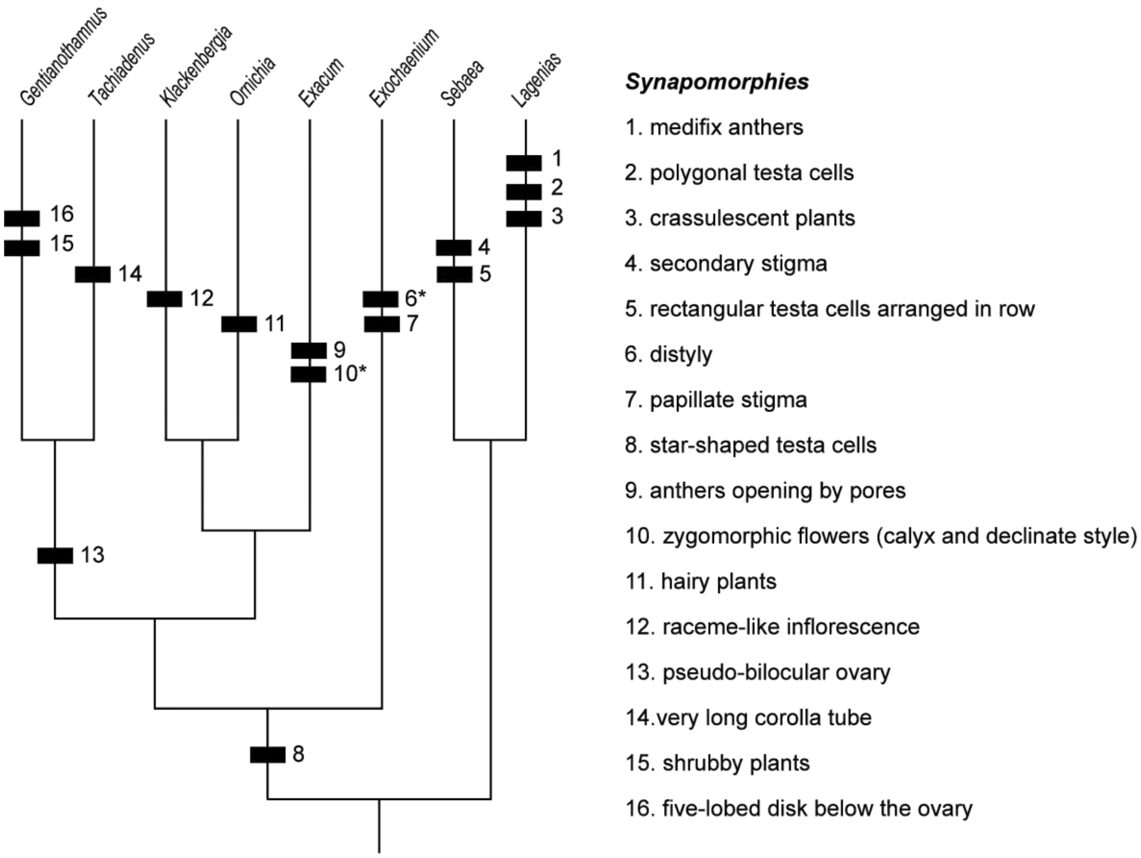

Figure 5: Distribution of synapomorphic characters in Exaceae. An asterisk indicates that the characters were not observed in all the species.

Karyological reconstruction

Maximum parsimony reconstruction supports x=7 as the ancestral base chromosome number for the whole tribe and x=8 for *Tachiadenus* and *Exacum*. Reconstructions at the other nodes remain ambiguous. (fig 6).

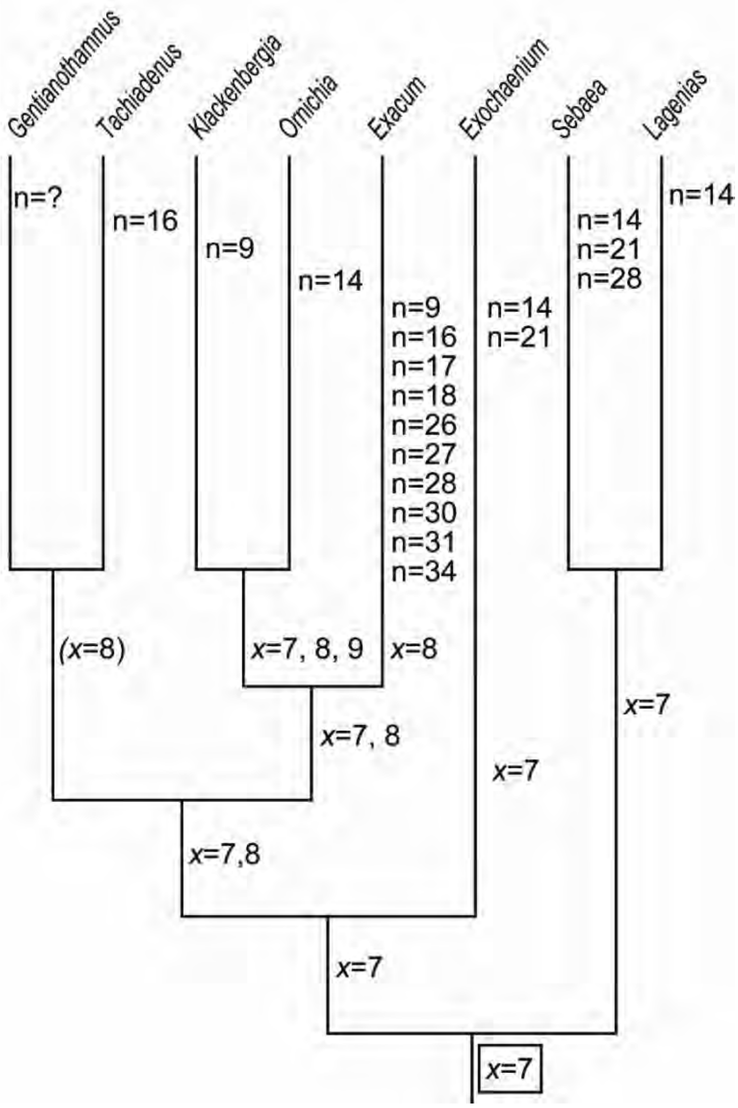

Figure 6: Summary of the maximum parsimony reconstruction of the ancestral basic chromosome numbers of Exaceae

DISCUSSION

By extending the sampling of taxa that were not available for previous studies, our present analyses provided strong evidence and statistical support for the monophyly of the tribe Exaceae. One of the most striking results of this study is the polyphyly of *Sebaea s. l.*. Since the treatment of Marais (1961), this genus has been regarded as a morphologically well-delimited assemblage by most recent authors (e.g. Marais and Verdoorn, 1963; Taylor, 1963; Boutique, 1972; Klackenberg, 1990; Paiva and Nogueira, 1990a; Nemomissa, 2002). Interestingly, four genera (*Exochaenium*, *Belmontia*, *Lagenias* and *Sebaea s. str.*) belonging to the *Sebaea s. l.* assemblage were recognized in the early significant classifications (Brown, 1810; Meyer, 1838; Grisebach, 1845). However, the characters used for upholding those genera were overlapping, and therefore those genera were merged into *Sebaea s. l.* by Marais (1961).

Generic delimitation of Sebaea sensu lato *and taxonomic implications*

Our molecular analyses depict four major clades S1, S2 S3, and S4 (Figure 3).

Clade S1, represented by a single accession of *S. pusilla*, is characterized by apomorphic characters such as medifixed anthers, polygonal testa cells, and a crassulescent habit.

Clade S2 mainly contains South African species of *Sebaea s. l.*, some tropical African and Malagasy species, along with the paleotropical *S. microphylla*. Two apomorphies were found to support this clade i.e.: the arrangement of seed testa cells and the presence of a secondary stigma on the style.

Clade S3 comprises most of the tropical African species of *Sebaea s. l.*, and is characterized by its stigma texture (papillate), and the presence of a stylar polymorphism (short-styled versus long-styled flowers).

Sebaea madagascariensis (clade S4) was previously found to be more closely related to the *Exacum – Ornichia* clade than to *Sebaea* (Yuan et al., 2003). However, there is no phenotypic or karyological support to include *S. madagascariensis* within *Ornichia*. Leaf pubescence is a synapomorphy for *Ornichia* (Klackenberg, 1986), a feature not shared by *S. madagascariensis*. Moreover, the stigma of *Ornichia* is small and capitate to slightly bilobed, while in *S. madagascariensis*, the stigma is linear to very slightly clavate. The flower colour of *Ornichia* is violet to blue, while it is white in *S. madagascariensis*. The solitary flowers or lax inflorescence of *Ornichia* contrast with the axillary flowers and “raceme-like” inflorescence of *S. madagascariensis*. Recent cytological work (Chapter 1) emphases the difference in chromosome numbers between *Ornichia* (n=7) and *S. madagascariensis* (n=9).

Accepting a monophyletic genus *Sebaea s. l.* would imply the inclusion of all other genera of the tribe within *Sebaea*. Such a reduction is apparently a drastic option regarding morphological or karyological evidence. Consequently, the splitting of *Sebaea s. l.* seems necessary in order to maintain well-supported genera.

A new taxonomic treatment with nomenclatural changes and a complete revision on the taxonomic history of *Sebaea s. l.* is presented in Chapter 4 and 5. In this classification, several taxonomic changes are made as a result of the findings from this research. The monotypic genus *Lagenias* E. Mey, described by Meyer (1838) is rehabilitated and includes *L. pusillus* (clade S1). Clade S2 can be defined as *Sebaea* Sol. ex R.Br., because it contains the type species of the genus (Brown, 1810). Clade S3 corresponds to *Exochaenium*, a genus described by Grisebach (1845) on the basis of *E. grande* (*Sebaea grandis*). Finally, a new genus *Klackenbergia* is proposed for the Malagasy species corresponding to *S. madagascariensis* (Chapter 4). This new taxonomic treatment, dividing *Sebaea* into four genera, is followed in the subsequent discussions.

Phylogenetic relationship within Exaceae

Previous phylogenetic analyses of the Gentianaceae (Struwe et al., 1998; Thiv et al., 1999b) using the *trn*L intron covered six species of the tribe Exaceae representing four genera (*Exacum*, *Ornichia*, *Sebaea*, and *Tachiadenus*). The main relationships of the tribe were drawn: *Sebaea s. l.* was basal, and *Tachiadenus* was sister to a clade formed by *Ornichia* and *Exacum*. Yuan et al. (2003), in their study on the monophyly of the tribe Exaceae, added one more genus, *Gentianothamnus*, but it only includes five representatives of *Sebaea*, four of them being morphologically closely related. Despite the small sampling of *Sebaea*, the possible polyphyly/paraphyly of the genus was emphasized.

Our present study sampled all the morphological and ecological diversity of *Sebaea s. l.* as well as that of the tribe Exaceae, and demonstrated that *Sebaea s. l.* was polyphyletic. The relationship of *Sebaea s. str.* (S2) with *Lagenias pusillus* (S1) is shown for the first time (Fig. 3 & 4). Both genera occur in the Western Cape region, and share some common morphological characters such as the shape of the stigma (bilobed) and the colour of the flower (yellow).

The genus *Exochaenium* (S3) was also sampled for the first time, and was shown to be sister to the Malagasy genera *Exacum*, *Gentianothamnus*, *Klackenbergia* (S4), *Ornichia*, and *Tachiadenus*. This relationship is weakly supported (bootstrap support (BS) = 51%) by the molecular data, but well supported by seed morphology (see discussion below) (Fig. 5 & 7).

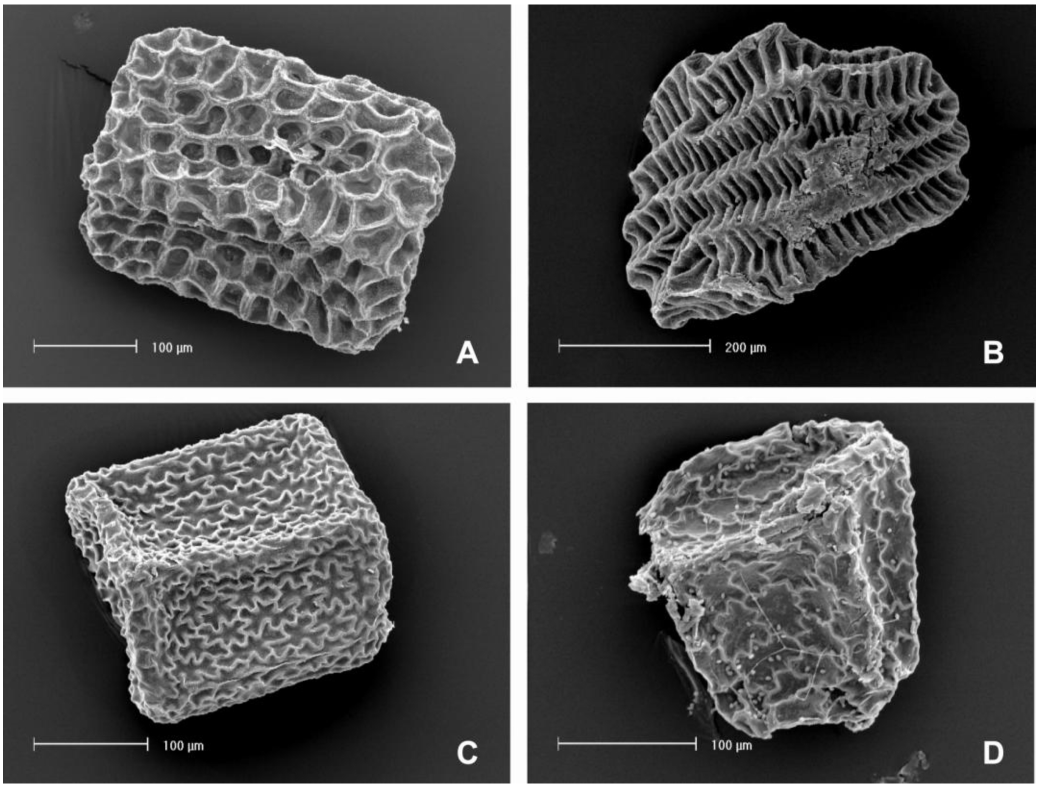

Figure 7: Seeds of representative species from the four clades depicted in *Sebaea s.l.*: **A**: *S. pusilla* (clade S1); **B**: *S. aurea* (clade S2); **C**: *S. macroptera* (clade S3); **D**: *S. madagascariensis* (clade S4).

Within the Malagasy clade, our molecular phylogeny corroborated most of the relationships suggested by Klackenberg (2002), and Yuan et al. (2003). *Tachiadenus* is closely related to *Gentianothamnus* (BS = 99%) and forms a clade sister to the rest of the Malagasy genera (*Exacum*, *Klackenbergia* and *Ornichia*). Klackenberg (2002) included *Gentianothamnus*, which was originally included within the subtribe Chironiinae by Humbert (1937) in the tribe Exaceae and suggested its close relationship with *Tachiadenus* based on morphological and anatomical characters such as pseudo-bilocular ovary and pollen morphology. Previously, Yuan et al. (2003) did not find a statistical support for the relationship of *Klackenbergia* and *Ornichia*. In this study *Klackenbergia* and *Ornichia* form a well supported clade (bootstrap value of 88%), which is sister to *Exacum*. Our study was not able to find morphological or karyological synapomorphies supporting this clade.

Morphological character evolution

1. Micromorphological characters

Seed structure of the Exaceae has been studied by Klackenberg (1983, 1985, 1986, 1987b), but mostly focusing on the Malagasy taxa (*Exacum*, *Gentianothamnus*, *Ornichia* and *Tachiadenus*). Bouman et al. (2002) recently made a comparative study of the morphology of seeds of Gentianaceae, including some *Sebaea s. l.* Our present study considers the whole range of diversity within the tribe Exaceae.

With the exception of *Sebaea s. str.* (S2) and the species of *Cotylanthera*, which were recently placed within *Exacum* (Klackenberg, 2006), the seeds of Exaceae closely resemble one another and are discernible from those of the other tribes of Gentianaceae by the shape of the testa cells (Bouman et al., 2002). The seeds are numerous, small and polyhedral to angled. In most taxa the testa cells are star-shaped in surface (Fig. 7).

The seeds of *Exochaenium* (Fig. 7) resemble more those of the Malagasy genera of the tribe (*Exacum*, *Gentianothamnus*, *Klackenbergia*, *Ornichia*, and *Tachiadenus*), thus defining a clade (Fig. 3). Star-shaped testa cells are here identified as an apomorphy for this clade (Fig. 5), and not for the whole Exaceae as suggested by Klackenberg (2002)

The seeds of *Sebaea s. str.* are often ridged and show a more bilateral symmetry (Fig. 7). Testa cells are more or less rectangular, elongated perpendicular to the length of the seed, and arranged in regular longitudinal rows, making *Sebaea s. str.* distinct from the rest of the tribe.

Seed shape and testa cell arrangement and form are constant within the genus and are considered an apomorphy (Fig. 5). One exception should be noticed: *S. exacoides* has seeds that lack ridges or fringes, and present slightly star shaped testa cells. Nonetheless testa cells are arranged in rows (personal observation). It is not clear yet whether star-shaped testa cells are ancestral for Exaceae, *S. exacoides* seeds sharing ancestral characters, or if the star-shaped testa cells of this species are a convergence with the Malagasy genera. However, the functional role of the star-shaped testa cells (if there is one) is currently unknown.

Although, the sister species of *Sebaea s. str.*, *Lagenias pusillus* has a distinct reticulate pattern of polygonal cells with straight cell walls (Fig. 7), resembling in this more to the seeds of the tribe Chironieae than to any Exaceae, the cubical shape of the seeds is more reminiscent of other members of Exaceae. Seed structure shows an extensive diversity in micromorphology within Gentianaceae and is often characteristic at the tribal, subtribal, or generic level in the family (Bouman et al., 2002). Like in other tribes of the family Gentianaceae, seed shape, testa cell form and arrangement are phylogenetically informative to define large clades within Exaceae, and support well the segregation between *Exochaenium*, *Lagenias* and *Sebaea s. str*.

2. Macromorphological characters

Most of the Exaceae are annual herbs. The suffrutescent habit has developed only once in the clade containing *Tachiadenus* and *Gentianothamnus*, this last one having evolved into a small shrub-tree. The basal woodiness in some *Tachiadenus* could be interpreted as secondary, and has probably evolved as a consequence of the perennial life cycle or as an adaptation to its environment. The “arbustive” habit of *Gentianothamnus* is not surprising in Gentianaceae, where several groups have evolved in to a tree form (e.g. *Anthocleista*, *Fagraea*, *Macrocarpaea*), probably as a result of adaptation to specific environment. Most of the arbustive genera of Gentianaceae are found in humid forest and unsurprisingly *Gentianothamnus* is growing in the humid sclerophyllous montane forest, above 1000 m, of Madagascar (Klackenberg, 1990, 2002). The presence of hairs (trichomes: simple and one-celled (Klackenberg, 1986)) on the whole plants of *Ornichia* is sufficiently rare in Gentianaceae to be mentioned. Within Gentianaceae only a few genera, e.g., *Orphium* (Chironieae), *Macrocarpaea* (Helieae) or a few species (e.g. *Swertia racemosa* or *Metagentiana souliei* in the Gentianeae) are also hairy. Trichomes might have different functions, like insulation from heat or sunlight, salt removal, defence against herbivores, water absorption, but it is often unclear (Callow, 2000), and our current knowledge of *Ornichia* does not permit us to hypothesise on any functions for those hairs. However, trichomes are known to occur often at the base of the calyx of many Gentianaceae (Renobales et al., 2001 and ref. therein), including in Exaceae (Schinz, 1906; Hill, 1908a; Kissling, unpublished data). They are usually not found on the whole plant like in *Ornichia* or *Orphium*. The secretory nature of those intracalycinal trichomes have been only recently demonstrated in a few species belonging to the tribe Gentianeae (Renobales et al., 2001) but their function remains unknown.

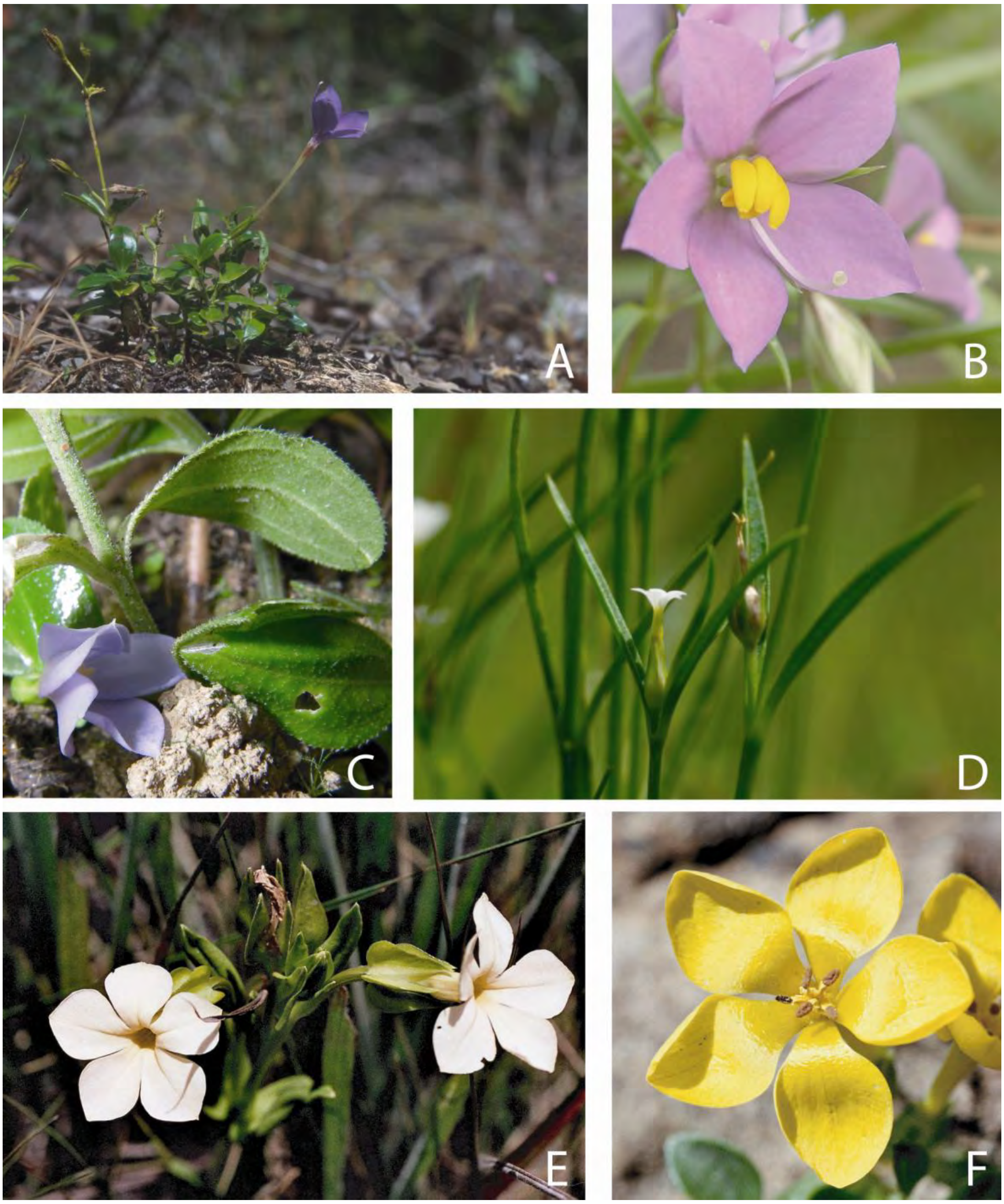

Figure 9: Photographs of different Exaceae representative **A** *Tachiadenus carinatus*; **B** *Exacum sp.*; **C**. *Ornichia madagascariensis*; **D**, *Klackenbergia stricta*, **E**. *Exochaenium grande*; **F.** *Sebaea marlothii*.

Corolla colour varies significantly within Exaceae (Klackenberg, 2002) (Fig. 9). Yellow corollas appear to be a plesiomorphic state, and blue to lilac corollas are an apomorphy for *Ornichia* and *Exacum*. On the one hand, pentamerous corollas seem to be the most frequent character state within the tribe Exaceae; this character has been regarded as the ancestral merosity state for the whole family (Mészàros et al., 2002). On the other hand, tetramerous flowers are also consistently reported within the two most species rich genera of the Exaceae

(*Exacum* and *Sebaea s. str.*). Our analysis supports the pentamery of the corolla as a plesiomorphic state for the Exaceae. This pattern is supported for the Chironieae (Mansion and Struwe, 2004), and seems to be the rule for other tribes of Gentianaceae as well (Struwe et al., 2002; Chassot, 2003) Nevertheless, tetramerous flowers appear to be a synapomorphic character state for some lineages within *Sebaea s. str.* and *Exacum*.

The size of the corolla tube varies greatly within the tribe, from a few millimetres long in some *Exacum*, up to 20 cm long in some *Tachiadenus* species. *Exacum* and *Ornichia* have very short corolla tube, while *Tachiadenus*, *Gentianothamnus*, some *Exochaenium* species and *Sebaea thomasii* present impressively long corolla tube. Our results suggest a mid-size corolla tube length as the ancestral state and an independent evolution within different genera towards long corolla tube. This character trait is often associated with particular pollinators (e.g. Faegri and van der Pijl, 1966; Goldblatt and Manning, 2000; Johnson and Steiner, 2000; Fenster et al., 2004), those with a long proboscis for examples. In Exaceae it is certainly a convergence linked to pollination adaptation. In Madagascar, all *Tachiadenus* species present this pollination syndrome with a long corolla tube. It is yet unclear whether this adaptation has led to the radiation of this genus, but in Africa, only one *Sebaea* and two *Exochaenium* present similar long corolla tubes, indicating a more recent adaptation to specialized pollinators.

Enantiostyly (a plant sexual polymorphism in which female sexual organs are deflected to the left or right, resulting in „mirror-image" flowers) has evolved once in Exaceae (*Exacum*) but is also known in Chironieae (*Chironia*), and is known to promote cross-pollination in bee-pollinated plants (Endress, 1994). *Exacum* is particularly interesting, because it presents a wide range of intermediate between non-enantiostyle flowers from “straight-styled”, monomorphic (left- and right-styled flowers) to dimorphic (only one stylar direction) flowers (Kissling, pers. obs.), suggesting a relatively recent evolution, or a gradual reversion to a “non-enantiostyle” system.

The androecium is of particular interest for the taxonomy of the tribe because most classic systematic treatments within the tribe were based on this organ complex (Meyer, 1838; Grisebach, 1839, 1845). The anthers are generally basifixed, (except for *L. pusillus*), this character being the ancestral state for the tribe. Tribes Saccifolieae (Thiv et al., 2002), Chironieae (Mansion and Struwe, 2004) and Helieae have also basifixed anthers, while in more derived tribe, such as Gentianeae, medifix anthers are more abundant (Ting-nong and Shang-wu, 2001; Chassot, 2003) and may play an important role in pollination. No comprehensive study has yet focused on the evolution of anther fixation within Gentianaceae, but the distribution of basifixed anthers in the family suggests it as a plesiomorphic state. Our study shows that filament insertion inside the corolla tube is the ancestral state of the tribe. However, this result has to be regarded with caution; level of insertion of the anthers filament has proved to be highly homoplastic within Gentianaceae (Hagen and Kadereit, 2002; Chassot, 2003; Kadereit and Hagen, 2003), and previous attempts to use this characters as a distinctive criterion within Exaceae (Meyer, 1838; Grisebach, 1845; Schinz, 1891; Gilg, 1898) were shown to be misleading (Marais, 1961).

In Exaceae, pollen is released following anther opening either by pores (*Exacum*), or by slits (all the other genera). Opening by pores is an apomorphy for the genus *Exacum*, while opening by slits is a plesiomorphy for the tribe. Presence of apical or basal glands on the anthers do not show any taxonomic information at the generic level, but the presence of these glands or appendices on the anthers is striking. Schinz (1903) describes the glands found on the anthers of *Sebaea* as containing sugar, and emphasises that it might be linked with pollination of the flowers by means of insects. Marloth (1909) observed insect, belonging to the Thripsidae (thrips), on the flowers of *S. exacoides* and said that thrips "would eagerly suck the sweet contents of these bodies (the glands)". Anther glands are reported to function in pollination in certain taxa (Chaudhry and Vijayaraghavan, 1992; Endress, 1994) but there is no evidence to support this assertion. Chaudhry and Vijayaraghavan (1992) studied the development of anther glands in *Prosopis juliflora* and found that they are made up of secretory cells that produce a protein/carbohydrate exudate. The cells lyse, and the exudate is passed through cuticular openings to the exterior of the gland. They also noted that anther glands function in pollination by providing a food source to attract pollinators, which is apparently also the case in *Sebaea*. The variation in gland size, colour, and structure (Kissling, unpublished data) suggest a strong adaptation to different pollinators; however, no pollination studies have been yet conducted on *Sebaea* and furthermore on the other genera of the tribe.

The gynoecium of the Exaceae is bicarpellate, with a superior bilocular ovary, surmounted by a style of variable length. The stigma ranges from linear to clavate (*Exochaenium*, *Gentianothamnus*, *Klackenbergia* and *Tachiadenus*) to more or less capitate (*Exacum*, *Ornichia* and *Sebaea*), entire, slightly divided or strongly bilobed, to sometimes bifid (*Exochaenium pygmaeum*.) The stigma morphology has proved to be of importance in the systematics of Gentianaceae (e.g. Marais and Verdoorn, 1963; Mansion, 2004; Mansion and Struwe, 2004) and here this feature is particularly useful to discriminate between *Exochaenium* and *Sebaea* species. A character of importance in the systematics of the African Exaceae is the stigma texture. Our reconstruction suggests a smooth stigma as the ancestral state, and therefore the very papillate stigma of *Exochaenium* has to be regarded as an apomorphy for this genus.

Within Exaceae, a stylar polymorphism (long-styled, versus short-styled flowers), like in distylous plants, is only present in the genus *Exochaenium*. This stylar polymorphism is in some cases also associated with a reciprocal position of the anthers, like in heterostylous flowers, as in *E. grande* (Welwitsch, 1869; Schinz, 1906; Hill, 1908a) or *E. oliganthum* (Raynal, 1967). In

Gentianaceae, heterostyly has been reported in most genera of tribe Saccifolieae (Gilg, 1895b; Thiv et al., 2002), and is also known to occur in other Gentianales families (e.g. Pailler and Thompson, 1997; Faivre and McDade, 2001; Leege and Wolfe, 2002; Naiki and Nagamasu, 2004; Ornelas et al., 2004). It is therefore difficult to settle whether the stylar polymorphism is a symplesiomorphy or an apomorphy for *Exochaenium*.

Heterostyly has been widely studied (Barrett et al., 2000; Barrett, 2002) and thus in different genera e.g. *Primula* (e.g., Guggisberg et al., 2006; Mast and Conti, 2006; Mast et al., 2006), *Narcissus* (e.g. Graham and Barrett, 2004; Perez-Barrales et al., 2006), *Turnera* (e.g. Barrett and Shore, 1987; Shore, Arbo, and Fernandez, 2006) etc. But the stylar polymorphism of *Exochaenium* merits a particular interest because of the presence of many particularities such as huge apical glands on the anthers, sometimes bigger than the anthers themselves or the cohering anthers in the short-styled form which are free in the long-styled form. *Exochaenium grande*, basally positioned in our phylogeny, presents both monomorphic and dimorphic populations (Kissling, pers. obs.), and might help to better understand how this stylar polymorphism could have evolved within this genus.

The presence of the striking secondary stigma on the style of *Sebaea s. str.* species has already been used as a diagnostic character for this genus (Hill, 1908a, 1909), and our analysis also supports this as an apomorphy for the genus. To our knowledge, this particular feature of *Sebaea s. str.* has not been found elsewhere in Gentianaceae or Gentianales, and seems to be unique in the angiosperms (Chapter 7). Hill (1913) and Marloth (1909) suggested that the functional role of the secondary stigma was a direct help to self-pollination, but does not exclude cross-pollination, either by the apical stigma or by the secondary stigma. However, this role has not yet been tested. The secondary stigma (a synapomorphy for the genus) might explain the current success of *Sebaea* in Africa.

Karyotype evolution

Distribution of base chromosome numbers correlates relatively well with taxonomic groups. The clade *Lagenias*-*Sebaea*, *Exochaenium*, and *Ornichia* have x=7, while *Tachiadenus* has x=8. *Exacum* has been divided in two sections by Klackenberg (1985). All the species of section *Africana* have a basic chromosome number of x=8 except one with x=9, while section *Exacum* has a wide range of basic chromosome numbers, i.e., x=7, x=8, x=9, and x=17 (chapter 1).

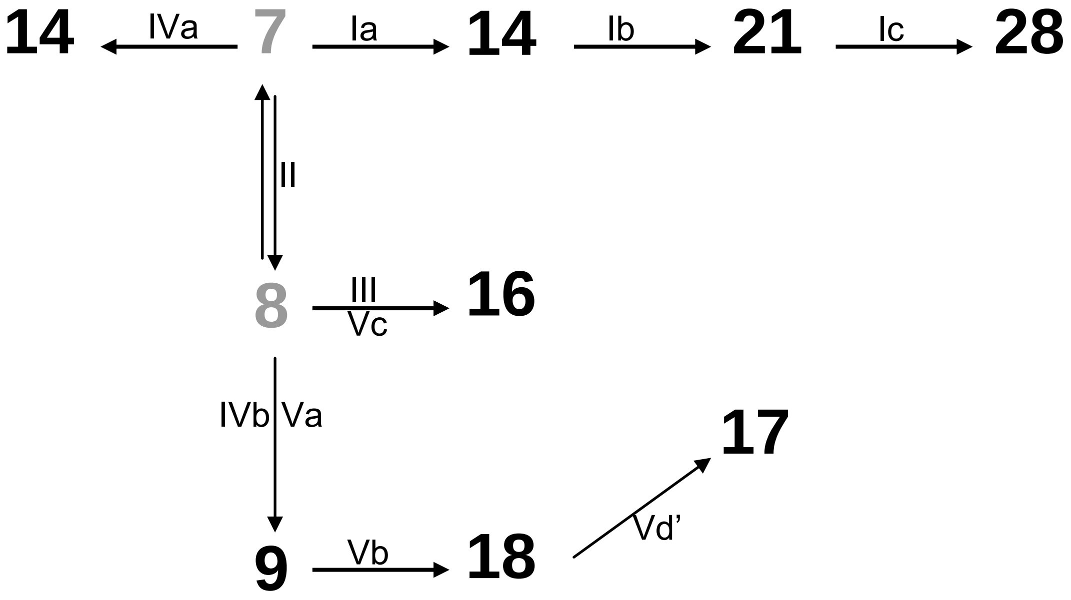

Figure 8: Scheme of possible pathway of base chromosome number evolution in Exaceae. Black numbers are chromosome numbers presently found in the tribe. Gray numbers are hypothesized ancestral numbers. Bold arrows indicate possible episodes of polyploidization; thin arrows dysploidization. See discussion for details (numbers I to V).

Evolution of basic chromosome numbers

In chapter 1 we proposed a combination of both dysploidy and polyploidy events in the karyotypic evolution of the Exaceae, without determining the polarity of dysploid series, due to the lack of strong phylogenetic hypotheses. Here, we present a model, that minimizes the number of dysploidization steps at the expense of polyploidization steps, which seems to be more likely in Exaceae for two reasons: (1) the basic chromosome numbers found today are very stable within groups, with the exception of *Exacum* sect. *Exacum*, which might experiment rapid evolutionary episodes (chapter 1; Yuan et al., 2005); (2) polyploidy occurs independently in several lineages within Exaceae and seems to be a relatively frequent evolutionary event within Gentianaceae (e.g. Küpfer and Yuan, 1996; Yuan et al., 1998; Mansion et al., 2005).

Based on x=7 as the base chromosome number for Exaceae, polyploidization of x=7 would lead to x= 2x =14 (*Lagenias* and *Sebaea*), 3x = 21(*Exochaenium* and three *Sebaea*), and finally 4x = 28 (two *Sebaea*). The clade *Lagenias–Sebaea* (n=14, 21, 28) has a basic tetraploid number, and it is more parsimonious to consider a single paleopolyploidization event for their common ancestor (**Ia**, **Ib**, **Ic**).

In the common ancestors of the clade comprising *Exacum*, *Gentianothamnus*, *Klackenbergia*, *Ornichia*, and *Tachiadenus*, a first dysploidy event might have occurred (x=7 -> x=8), giving two basic chromosome numbers (x=7 and x=8) (**II**). A first polyploidization of x=8 occurred (*Tachiadenus*, n=16) (**III**), later followed by a polyploidization of x=7 (*Ornichia*, n=14) (**IVa**).

The common ancestor of the clade formed by *Klackenbergia* and *Ornichia* experienced one more dysploidy event (x=8 -> x=9), leading to *Klackenbergia* (n=9) (**IVb**).

Although we lack resolution in our phylogeny of *Exacum*, a putative scenario based on the resolved tree of *Exacum* obtained by Yuan et al. (2005), and on our result suggesting x=8 as a basic number of the common ancestor of *Exacum*, is proposed here. *Exacum* sect. *Africana* occurs in Madagascar and contains species with n=16 only, with a single exception *E. quinquenervium*, found to have both n=16 and n=18. *Exacum* sect. *Exacum* occurs in Asia and has a considerable variation of possible basic chromosome numbers, but two (x=9, x=17) are more abundant. Furthermore Yuan et al. (2005) suggest a Madagascan origin for *Exacum*, followed by multiple out-of-Madagascar dispersals, with further extensive radiation into Asia. Based on this scenario, a first dysploidy event (x=8 -> x=9) (**Va**) occurred in Madagascar and eventually the x=9 ancestor dispersed in Sri-Lanka / South India (*E. tetragonum* has been reported to be n=9 (Borgmann, 1964)). Further polyploidization events of x=8 and x=9 happened in Madagascar (**Vb** & **Vc**). The base chromosome number, x=17, found in several *Exacum* species from Southern India-Sri Lanka is explained by one more dysploid event n=2x=18 giving x=17 (**Vd**).

Intraspecific ploidy

The presence of more than one ploidy level is known for two *Sebaea* species, and several *Exacum* species of sect. *Exacum*.

Sebaea minutiflora (n=14, n=21) might have experiment autopolyploidisation, while a correlation of cytotype distribution with the presence of biogeographically defined races might be present in *Sebaea brachyphylla* (n=21 in Africa and n=28 in Madagascar). However, more data are needed for a sounder evaluation of cytotype distribution and possible correlation with the presence of different races.

In *Exacum* sect. *Exacum* (n = 9, 16, 17, 18, 26, 27, 28, 30, 31, and 34), Riesman et al. (2006) reported karylogical data in the *E. trinervium* complex, showing some ability of species to produce in vitro viable hybrids, despite a difference in chromosome valence. The high karyotypic diversity encountered in section *Exacum* might indicate rapid evolutionary episodes within this group, a fact supported by recent phylogenetic studies (Yuan et al. 2005), showing multiple out-of-Madagascar dispersals of *Exacum* species, with further extensive radiation into central Asia.

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HISTORICAL BIOGEOGRAPHY OF THE PALEOTROPICAL TRIBE EXACEAE (GENTIANACEAE)

HISTORICAL BIOGEOGRAPHY OF THE PALEOTROPICAL TRIBE EXACEAE (GENTIANACEAE)

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Abstract – The tribe Exaceae (Gentianaceae), comprising eight genera (c. 180 species) distributed in the paleotropics, was analysed to elucidate its historical biogeography. cpDNA and nrDNA sequences representing half of the tribe (100 spp.) were combined and analysed phylogenetically. We reconstructed an almost fully resolved phylogenetic tree and were thus able to make a meaningful inference of inter- and intrageneric historical biogeography. Ancestral areas were inferred using parsimony-based methods, and the ages of clades within Exaceae were estimated using the penalized likelihood method with a wider Gentianaceae cpDNA phylogeny. Exaceae evolved c. 32 million years ago in Africa and spread to Madagascar. The colonization of Australia, New-Zealand, and Asia involved at least three long-distance dispersals.

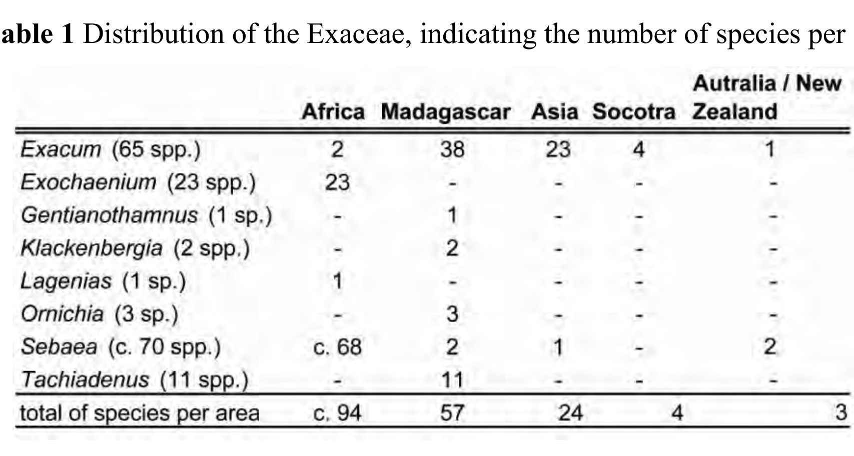
Keyword – Africa; Biogeography; Exaceae; *Exacum*; Gentianaceae; *Gentianothamnus*; *Klackenbergia*; *Lagenias*; *Ornichia*; Madagascar; Phylogeny; *Sebaea*; *Tachiadenus*

INTRODUCTION

The Exaceae are a small tribe of the gentian family, with c. 180 spp. and eight genera, recently supported as monophyletic clades in several phylogenetic analyses (chapter 2; Yuan et al., 2003). The main representatives are *Sebaea* Sol. ex. R. Br. (c. 70 spp.) and *Exacum* L. (65 spp.), which make up the majority of species in the Exaceae (*c.* 155 spp), followed by *Tachiadenus* Griesb. (23) and *Exochaenium* Griesb. (11 spp.), while *Gentianothamnus* Humbert (1 sp.), *Klackenbergia* Kissling (2 spp.), *Lagenias* E. Mey. (1 sp.), *Ornichia* Klack. (3 spp.) are represented by relatively few species.

Species of Exaceae mainly occur in paleotropical and temperate regions of Africa (Tab. 1), with most species distributed in Africa (c. 94 spp. and two endemic genera) and Madagascar (57 spp. and four endemic genera). *Sebaea* has a broad distribution throughout tropical and subtropical Africa, with two main centres of diversity: the Western Cape (c. 25 spp.) and the Drakensberg (c. 28 spp.). Two species (*S. brachyphylla* and *S. bojerii*) are found in Madagascar (Klackenberg, 1990), two (*S. albidiflora* and *S. ovata*) from Australia to NewZealand (Adams, 1996; Champion et al., 2003), and one (*S. microphylla*) in the Drakensberg to South-east Asia (Marais and Verdoorn, 1963; Paiva and Nogueira, 1990; Ho and Pringle, 1995; Suksathan and Sasirat, 2000; Nemomissa, 2002). *Exacum* has two main centres of diversity, in Madagascar and in the Western Ghats region of India plus Sri-Lanka (Klackenberg, 1985, 2002, 2006). This disjunct distribution may be the result of long-distance dispersal from Madagascar to India, followed by extensive radiation and subsequent dispersal west to Socotra and the Arabian Peninsula, and east to the Himalaya and the Indo-Malaysian Peninsula (Yuan et al., 2005). Finally, the middle-sized genus *Tachiadenus* is endemic to Madagascar, along with *Gentianothamnus*, *Klackenbergia*, *Ornichia*, while *Exochaenium* occurs in subtropical and tropical Africa, mainly on the Katanga plateau (a region situated between the Democratic Republic of Congo, Angola and Zambia), and the monotypic *Lagenias* in the western Cape region only (Chapter 5).

Table 1 Distribution of the Exaceae, indicating the number of species per area.



	Africa	Madagascar	Asia	Socotra	Autralia / New Zealand
Exacum (65 spp.)	2	38	23	4	1
Exochaenium (23 spp.)	23	-	-	-	-
Gentianothamnus (1 sp.)	-	1	-	-	-
Klackenbergia (2 spp.)	-	2	-	-	-
Lagenias (1 sp.)	1	-	-	-	-
Ornichia (3 sp.)	-	3	-	-	-
Sebaea (c. 70 spp.)	c. 68	2	1	-	2
Tachiadenus (11 spp.)	-	11	-	-	-
total of species per area	c. 94	57	24	4	3

A preliminary biogeographic hypothesis to explain the current distribution of Exaceae was postulated by Klackenberg (2002), who suggested a Gondwanan origin for the tribe, and explained its current distribution, as resulting from the break-up of Gondwana. Paleomagnetic data and tectonic reconstruction suggested that the Gondwana breakup initiated ca. 180 million years ago (Mya) (Storey, 1995), and the Madagascar–Seychelles–India block began to separate from the Africa–South America block ca. 165 Mya, with movement ending by 130-118 Mya (Rabinowitz et al., 1983; Harland et al., 1990; Seward et al., 2004). Since then, Madagascar has remained in its position with respect to Africa (Coffin and Rabinowitz, 1988), whereas Australia and Antarctica separated from the Madagascar–Seychelles–India block ca. 132 Mya (Barron, 1987). India separated from Madagascar ca. 88 Mya (Storey, 1995).

This hypothesis has been recently challenged by phylogenetic studies, which proposed a more recent age for the Exaceae (Yuan et al., 2003; Yuan et al., 2005). Even if uncertainties remain concerning the age of the respective Gentianales, Gentianaceae, or Exaceae, the Gondwanian hypothesis seems to be rejected by all the available estimations. For example, the age of the Gentianales has been estimated to be c. 60 Mya, based on fossil pollen (Muller, 1984), or c. 108 Mya (Bremer et al., 2004), and c. 83-89 Mya (Wikström et al., 2001), based on different dating methods and data sets, while the age of the Gentianaceae has been estimated to be c. 50 Mya (Yuan et al., 2003). Whatever the respective differences in those estimates, they all refute the Gondwanan hypothesis.

Alternative hypotheses, including either post-Gondwanan vicariance events or random dispersal, are highly needed to explain the historical biogeography of the tribe.

Here, we conducted phylogenetic and molecular dating analyses of Exaceae aimed at: (1) inferring the age of Exaceae, based on molecular dating analyses; (2) elucidating the biogeographic history of Exaceae, and (3) understanding the relative success of widespread genera (*Sebaea* and *Exacum*) vs. geographically restricted (*Gentianothamnus*, *Klackenbergia*, *Lagenias*, *Ornichia*, and *Tachiadenus*) genera.

MATERIALS AND METHODS

Taxon and accession sampling, DNA regions and outgroup choice

Two datasets were used, the first one, here called “Gentianaceae-dataset” included most representatives of the Gentianaceae and Gentianales and was aimed at dating the Exaceae and most of its representatives, the second one here called “Exaceae-dataset”, included only Exaceae representatives, and was aimed at reconstructing the biogeographical history of the tribe.

The “Gentianaceae-dataset” is composed of 10 Apocynaceae, 8 Rubiaceae, 4 Loganiaceae, *Gelsemium sempervirens* and 141 Gentianaceae (72 Exaceae) representing the main tribes of each family from the Gentianales. One species, *Nicotiana glutinosa*, from the neighbouring order Solanales (Bremer et al., 2001), was selected as outgroup. The "Gentianaceae-dataset" was built using both published (Genbank accessions, labelled with a * in Annex 1), or newly produced accessions (Annex 1 and Annex 1, Chapter 2). This first data set included 165 accessions of the *trn*L intron. Due to the poor resolution within the Exaceae in analyses based on the *trn*L intron only, a subset of the Exaceae species (72 accessions) and two outgroup species were sequenced for the *trn*L-F intergenic spacer, *atp*B-*rbc*L intergenic spacer, and partial *mat*K gene, in order to resolve this clade for the dating.

The "Exaceae-dataset" includes 104 operational taxonomic units (102 representatives of Exaceae and 2 outgroup species). Sequences were obtained for plastid regions *mat*K gene (partial), *atp*B-*rbc*L spacer, *trn*L intron, and *trn*L-F spacer, as well as for the nuclear ribosomal transcribed spacer (ITS) regions, including the 5.8 S gene, and the flanking internal transcribed spacers ITS1 and ITS2 (Annex 1).

Sampling of Exaceae species were maximised to represent all areas of endemism (South-Africa, Tropical Africa, Madagascar, Socotra-Arabia, India/Sri-Lanka, Australia/New-Zealand) and morphological diversity within the tribe. At least half of the taxa were sampled from each delimited geographic area, including the eight genera of Exaceae. Thirty species of *Exacum* (42% of the genus), 31 of *Sebaea* (*c.* 50% of the genus), 12 of *Exochaenium* (52% of the genus), four of *Tachiadenus* (36% of the genus), two of *Ornichia*, and one of each of the monotypic genera *Klackenbergia*, *Gentianothamnus*, and *Lagenias*, were included in this analysis. Finally, two species of the Chironieae were used as outgroup.

Molecular methods

Procedures of DNA extraction, amplification by polymerase chain reaction (PCR), purification of PCR products, and DNA sequencing followed Chapter 2. Primers ITS5 and ITS4 (White et al., 1990) were used for the amplification of the ITS region. Primer *mat*K8 and *mat*K503 (Endress et al., 1996) were used to amplify a part of the *mat*K gene. All sequences have been deposited in GenBank (Appendix 2).

Sequence alignment

Alignment was performed using the program ClustalW (Thompson et al., 1994) as implemented in BioEdit 7.0.1, with subsequent manual improvement.

For the "Gentianaceae-dataset" the alignment was straightforward and unambiguous except for two simple sequence repeat (SSR) regions of multiple "As", located within the *trn*L intron. These ambiguously alignable SSR regions (65 bp in total) were excluded in subsequent analyses. Within the *trn*L-F spacer (sequenced only for the "Exaceae subset"), two SSR regions of multiple "AT", were similarly removed from the analyses (91 bp in total).

Concerning the "Exaceae-dataset", the alignment was straightforward and unambiguous. The same ambiguous regions as those found in the Gentianaceae-data set were excluded from the analyses.

Phylogenetic analyses

For the "Gentianaceae-data set", Bayesian inference was conducted using MrBayes version 3.1.2 (Huelsenbeck et al., 2001; Ronquist and Huelsenbeck, 2003) and the data were partitioned by genes. The most appropriate model of sequence evolution for each partition was determined using MrModeltest version 2.2. (Posada and Crandall, 1998; Nylander, 2004; Posada and Buckley, 2004) to be the general time reversible model (GTR + I + G) for the *trn*L intron, and the GTR + R model for the *trn*L-F spacer, *atp*B-*rbc*L spacer, and the partial *mat*K gene dataset. Default priors and a dirichlet distribution were used for the base frequency parameters. Two independent analyses each with four Markov chains, three heated and one cold, starting from a random tree were run simultaneously for five million generations with trees sampled every 100 generations. Trees generated prior to the four Markov chains reaching stationarity (the burn-in) were discarded. The remaining trees were used to construct a 50% majority rule consensus tree.

For the "Exaceae-dataset", each partition (*trn*L intron, *trn*L-F spacer, *atp*B-*rbc*L spacer, partial *mat*K gene, 5.8S gene, the ITS1 and the ITS2) was first analyzed separately under maximum parsimony (MP) optimization. Direct visual comparisons between the strict consensus trees obtained from the respective reduced datasets revealed no strongly supported (bootstrap values > 54%) topological incongruence, thus two large dataset, a cpDNA (*trn*L intron, *trn*L-F spacer, *atp*B-*rbc*L spacer, partial *mat*K gene) and a nrDNA (5.8S gene, the ITS1 and the ITS2) were built. For each of the two datasets (cpDNA and nrDNA), we further performed an incongruence length difference (ILD) test (Farris et al., 1995), implemented in PAUP* 4.0b10 (Swofford, 2002) as the partition–homogeneity test. Heuristic searches (simple sequence addition and TBR branch–swapping) with 1000 replicates, and a maximum tree limit to 1000 was used. The four cpDNA data sets were confirmed as congruent ($P = 0.37$) as well as the three nrDNA data sets ($P = 1.0$). A last ILD test was finally performed on the combined dataset (cpDNA + nrDNA), revealing some incongruence ($P=0.007$). But the properties of the data could lead themselves to biasing this test (Dolphin et al., 2000; Yoder et al., 2001; Darlu and Lecointre, 2002; Dowton and Austin, 2002). As no strongly supported topological incongruence was found between the two datasets (cpDNA and nrDNA), a total evidence approach (Kluge, 1989; Nixon and Carpenter, 1996) was followed.

Following a total evidence approach, data partitions were combined into a „global matrix“ that was analyzed with MP using the beta 10 version of PAUP* 4.0 (Swofford, 1999). Heuristic searches were performed with character states weighted equally, gaps treated as missing data and the following parameters: TBR branch swapping, Steepest Descent ON, Mulpars ON and Collapse branches option ON for branches with a minimum length of zero. Two hundred searches were performed under these conditions, after randomizing the order of taxon addition. Five trees per replicate were saved and used as starting trees for a further round of branch swapping with TBR, now saving all the trees. Statistical support for each branch of the most parsimonious trees was assessed with a bootstrap analysis conducted on the original data matrix by generating 1000 pseudoreplicates and by using the above mentioned heuristic search strategy.

For analyses using Bayesian inference, the "Exaceae-dataset" was partitioned by genes. The most appropriate model of sequence evolution for each partition was determined using MrModeltest version 2.2. (Posada and Crandall, 1998; Nylander, 2004; Posada and Buckley, 2004) to be the general time reversible model (GTR + G) for the *trn*L intron, the *trn*L-F spacer, *atp*B-*rbc*L spacer, the partial *mat*K gene, and ITS1 data set, the GTR + I + G model for the ITS2 dataset, and finally the K80 model for the 5.8S gene. MrBayes was run as described for the "Gentianales data set".

Molecular dating and node calibration

We used molecular dating methods to obtain approximate timing of branching events within *Exaceae*. We first tested for clocklike evolution of the DNA sequence data using the likelihood ratio statistics, $-2(\ln L_{\text{clock}} - \ln L_{\text{non-clock}})$. Likelihood scores were calculated, using PAUP*, for the best scoring MrBayes tree and the best fitting substitution model as selected by ModelTest, with and without the constraint of a molecular clock. Twice the difference between these two scores was compared to the χ^2 distribution with n–2 degrees of freedom (n = number of taxa) (Muse and Weir, 1992). Clock-like sequence evolution was rejected ($P < 0.01$), the MrBayes 50% majority rule tree was thus rate-smoothed to make branch-lengths proportional to time. To do so, we used the penalized likelihood (PL) approach (Sanderson, 2002a) implemented in the program r8s (Sanderson, 2002b). Penalized likelihood produces a clock-like (ultrametric) tree from a non-clock-like tree by allowing substitution rates to vary across branches, under the assumption that changes in rate are autocorrelated, i.e. that more closely related taxa have more similar rates of evolution. Under PL, the degree of change of rates between lineages is limited to a degree determined by the so-called „smoothing parameter“, λ. The optimal value for λ was determined using the data-driven cross-validation procedure implemented in r8s.

The ages of a number of nodes were constrained in accordance with evidence from the fossil record, geology, and a previous molecular dating estimate. Such calibration points are used in order to place the relative ages of clades, as represented by the ultrametric tree, in absolute time. Where fossils could unambiguously be identified on the basis of morphology to be descendents of particular nodes, those nodes were constrained to be at least as old as the fossil. Three such nodes were thus calibrated: the stem node of the Gentianales at 60 mya [fossil pollen; (Muller, 1984)]; stem node of *Lisianthus* at 40 mya [fossil pollen; (Graham, 1984)]; and *Gentiana cruciata* at 5 mya [fossil seeds; (Mai and Walther, 1998)]. We used the age of the subtribe Swertiinae (15 Ma), estimated by Hagen and Kadereit (2001, 2002), as a secondary calibration point and the age of the most recent common ancestor of *Ixanthus* and *Blackstonia* [15 mya, based on geological evidence (Mansion and Struwe, 2004)] as a maximum age constraint. Point estimations were obtained for the ages of nodes of interest by rate-smoothing the tree with ML branch lengths based on the original data. Error margins for those estimates (representing stochasticity in character sampling, as reflected in branch-length estimations) were obtained by summarising the values obtained by rate smoothing 100 trees with branch-lengths calculated from bootstrap-resampled data matrices.

Ancestral area reconstruction

In order to reconstruct the historical biogeography of the Exaceae, the dispersal-vicariance approach, implemented in DIVA (Ronquist, 1997), was performed on the combined Exaceae 50% Majority rule Bayesian tree. Based on the analyses of the distribution of Exaceae species we recognised two sets of areas of endemism. The first set comprised only 6 wide areas of endemism, including Africa, Madagascar, Southern India/Sri-Lanka, Socotra/Southern Arabian Peninsula, Asia, and Australia/New Zealand. The second, more precise, dataset included 10 zones: Cape region, Drakensberg, Katanga plateau, Guineo-Congolian region, Madagascar, Sri-Lanka and South-India, Socotra and Southern Arabian Peninsula, The Himalayas, mainland Southeast Asia, and an area comprising Australia and New Zealand. Terminal taxa were scored according to their distribution across the above areas to generate the distribution data matrix, and this data matrix was subsequently optimized onto the Bayesian 50% Majority rule Exaceae tree. Optimizations of both, unconstrained and area-constrained to a maximum of two, were conducted following the reasoning of Ronquist (1997) and Donoghue et al. (2001).

Ancestral ecological and climatological reconstructions

In an attempt to obtain a scenario for the climatological and ecological history of the Exaceae, both Dispersal-vicariance analysis [implemented using DIVA 1.1; (Ronquist, 1997), and MP reconstruction (implemented in Mesquite 1.12; (Maddison and Maddison, 2006)] was performed to determine the likely ancestral climate and ecology for each internal node of the phylogeny of the Exaceae. These two methods were chosen because they allow a polymorphic state reconstruction for the ancestor (individual species are often ecologically variable).

Based on analyses of the geographical data of Exaceae, and using a modified Köppen system to define climatic zones (Köppen, 1931; National-Geographic-Society, 2006), we recognised four geographical area (Africa, Australia + New Zealand, Madagascar, and Indomalesia) and three general climates, each with two to three further subdivisions. (A) a humid equatorial climate subdivided in (Af) no dry season, (Am) short dry season, and (Aw) dry winter; (B) a dry climate subdivided in (Bs) semiarid and (Bw) arid; and (C) a humid temperate climate subdivided in (Cf) no dry season, (Cw) dry winter, and (Cs) dry summer. By combining, the climatological data with the geographic distribution we identified 15 regional climatic areas (6 climatological regions were recognised for Africa, 1 in Australia + New Zealand, 4 in Madagascar, and 4 in Indo-Malesia).

Macroecological preferences of Exaceae were recognised based on field observations of numerous populations and on label data from herbarium sheets (BOL, BR, C, G, K, MO, NEU, PRE, S, WAG). These preferences were grossly divided into four broadly defined types of vegetations: (W) woodland vegetation (including tropical forest); (X) grassland vegetation; (Y) sandy wet areas where competition is reduced, e.g. stream/river banks; and (Z) ericoid vegetation. Terminal taxa were respectively scored according to their climatological and ecological distribution, creating a data matrix that was subsequently optimized with DIVA (both unconstrained and constrained to a maximum of two ancestral states), following the reasoning of Ronquist (1997) and Donoghue et al. (2001), and using MP reconstruction, following the reasoning of Maddison and Maddison (2006). For the climatological or ecological preference of the Saccifolieae, a possible outgroup of Exaceae (Thiv et al., 1999; Struwe and Albert, 2002), data were coded as being found in all possible climate and ecology states.

RESULTS

Congruence assessment

The inspection of tree topologies obtained from the respective datasets (*mat*K gene (partial), *atp*B-*rbc*L spacer, *trn*L intron, and *trn*L-F spacer; 5.8 S gene, ITS1 and ITS2; cpDNA and nrDNA) revealed strong congruence. The respective ILD tests supported this topological congruence, except for the combined dataset where significant heterogeneity between the cpDNA and nrDNA partitions was found ($P = 0.007$). Sequential exclusion of the “problematic” species and groups of species, did not improve the ILD test. Recent studies and simulations suggested that the ILD test could fail to detect congruence due to different noise levels or large difference in size and evolutionary conditions between the data partitions (Darlu and Lecointre, 2002; Dowton and Austin, 2002). Our data sets varied in size (2450 characters in the cpDNA data set vs 686 characters in the ndDNA data set) and in number of informative characters (18.4% for the cpDNA data set vs 40.7% for the ITS one). As the topologies of the different datasets did not display supported incongruence, we combined them.

Phylogenetic Analysis

The 50% majority rule tree obtain from the Bayesian analysis of the "Gentianaceae- dataset" (Fig. 1) (arithmetic mean of the –log likelihood (*L*) of trees sampled after the burn-in = -14713.59; harmonic mean = -14820.58) supports monophyletic families (Apocynaceae, Gelsemiaceae, Gentianaceae, Loganiaceae, and Rubiaceae). Within the Gentianaceae, the tribe Saccifolieae is basal, while the Exaceae are the second most basal tribe, followed by the Chironieae. The tribe Gentianeae, Helieae and Potalieae form an unresolved clade (Fig. 1). The obtained topology is in agreement with the relationships previously inferred in Gentianaceae using smaller taxonomic samplings of cpDNA and nrDNA (Thiv et al., 1999; Struwe et al., 2002; Yuan et al., 2003).

For the "Exaceae-dataset", the main characteristics of the data sets used for the present study, along with the corresponding tree statistics, are summarized in Table 2. Parsimony analyses performed on the global data set (cpDNA + nrDNA) produced 1796 MP trees (length (L) = 2223, consistency index (CI) = 0.620, retention index (RI) = 0.887; not shown). The strict consensus tree generated from the global data set supported the monophyly of each of the Exaceae genera. The 50% majority rule tree obtained from Bayesian inference (Fig. 2) (arithmetic mean of the –log likelihood (*L*) of trees sampled after the burn-in = −17942.03; harmonic mean = −18021.30) is similar to the strict MP consensus tree. In both trees, the *Sebaea*-*Lagenias* clade (BS 100) diverged first, followed by *Exochaenium* (100% BS) which is sister to a large (*Exacum*-*Gentianothamnus*-*Klackenbergia*-*Ornichia*-*Tachiadenus*) clade. Within the latter, *Gentianothamnus* and *Tachiadenus* form a well supported clade (100% BS), followed by a clade consisting of both *Klackenbergia* and *Ornichia* (BS 100%), in turn sister to *Exacum* (100% BS).

Table 2 Characteristics of the data sets used.

Data set	Aligned positions	No. constant sites	No. Informative sites	No. Uninformative sites	Number of trees	Tree length	CI	RI
trn L intron	571	413 (72.3%)	95 (16.7%)	63 (11.0%)	1642	236	0.784	0.948
trn L-F spacer	479	312 (65.2%)	119 (24.8%)	48 (10.0%)	1474	251	0.805	0.929
atp B-*rbc* L spacer	931	679 (72.9%)	172 (18.5%)	80 (8.6%)	1998	370	0.792	0.934
mat K gene (partial)	469	373 (79.5%)	65 (13.9%)	31 (6.6%)	20	125	0.888	0.974
cpDNA	**2450**	**1777 (72.5%)**	**451 (18.4%)**	**222 (9.1%)**	**2000**	**1013**	**0.778**	**0.933**
5.8S	168	143 (85.1%)	14 (8.3%)	11 (6.6%)	11	28	0.893	0.985
ITS-1	258	84 (32.5%)	140 (54.3%)	34 (13.2%)	218	576	0.495	0.845
ITS-2	260	107 (41.1%)	125 (48.1%)	28 (10.8%)	444	552	0.505	0.866
nrDNA	**686**	**334 (48.7%)**	**279 (40.7%)**	**73 (10.6%)**	**740**	**1176**	**0.502**	**0.857**
combined data	**3136**	**2111 (67.3%)**	**730 (23.3%)**	**295 (9.4%)**	**1796**	**2223**	**0.62**	**0.887**

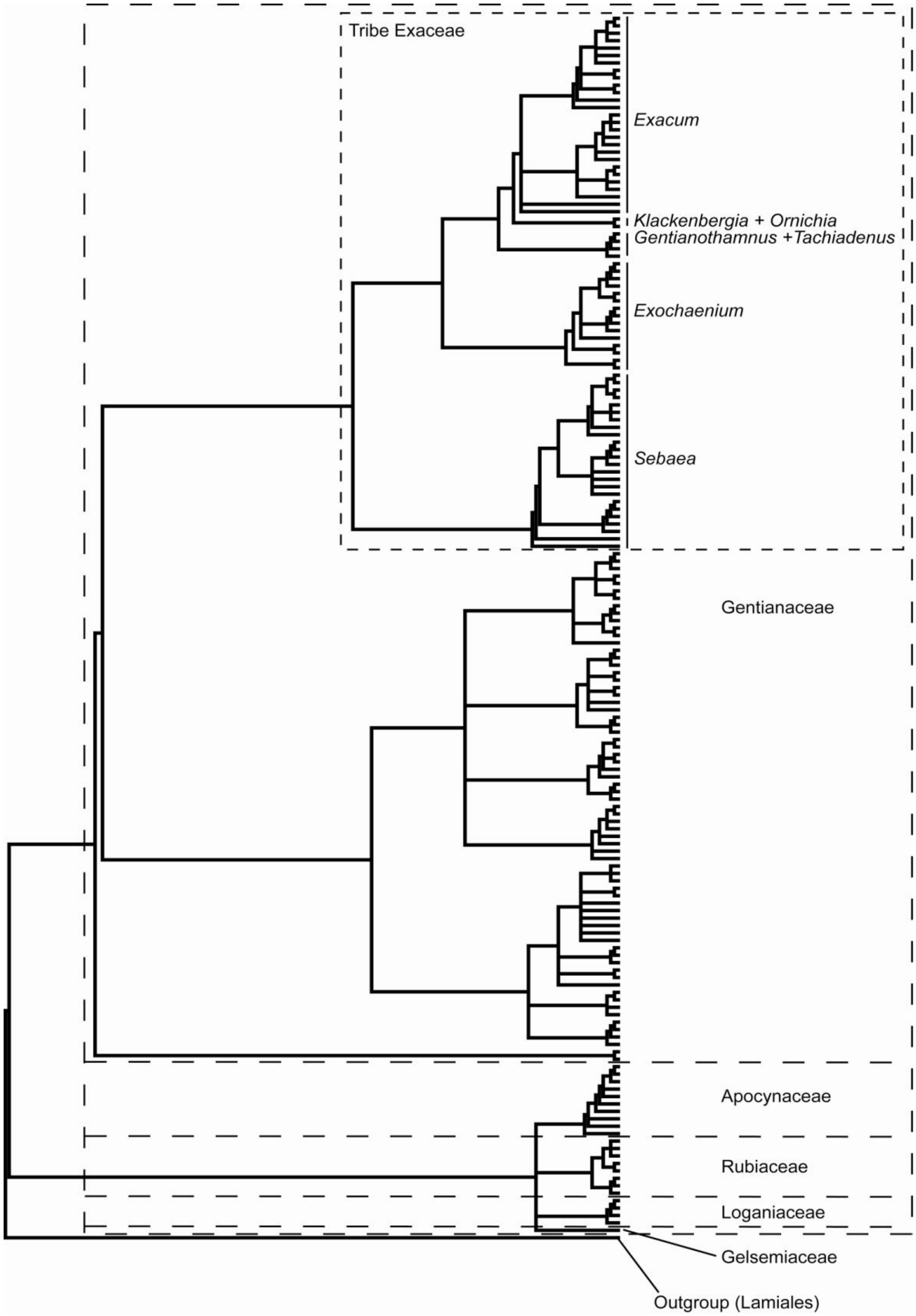


Figure 1 The 50% majority rule consensus tree from Bayesian inference analyses of the "Gentianaceae data set". Only the major clades are indicated. Branch support values are posterior probabilities from a 50% majority rule consensus tree derived from a Bayesian inference analysis.

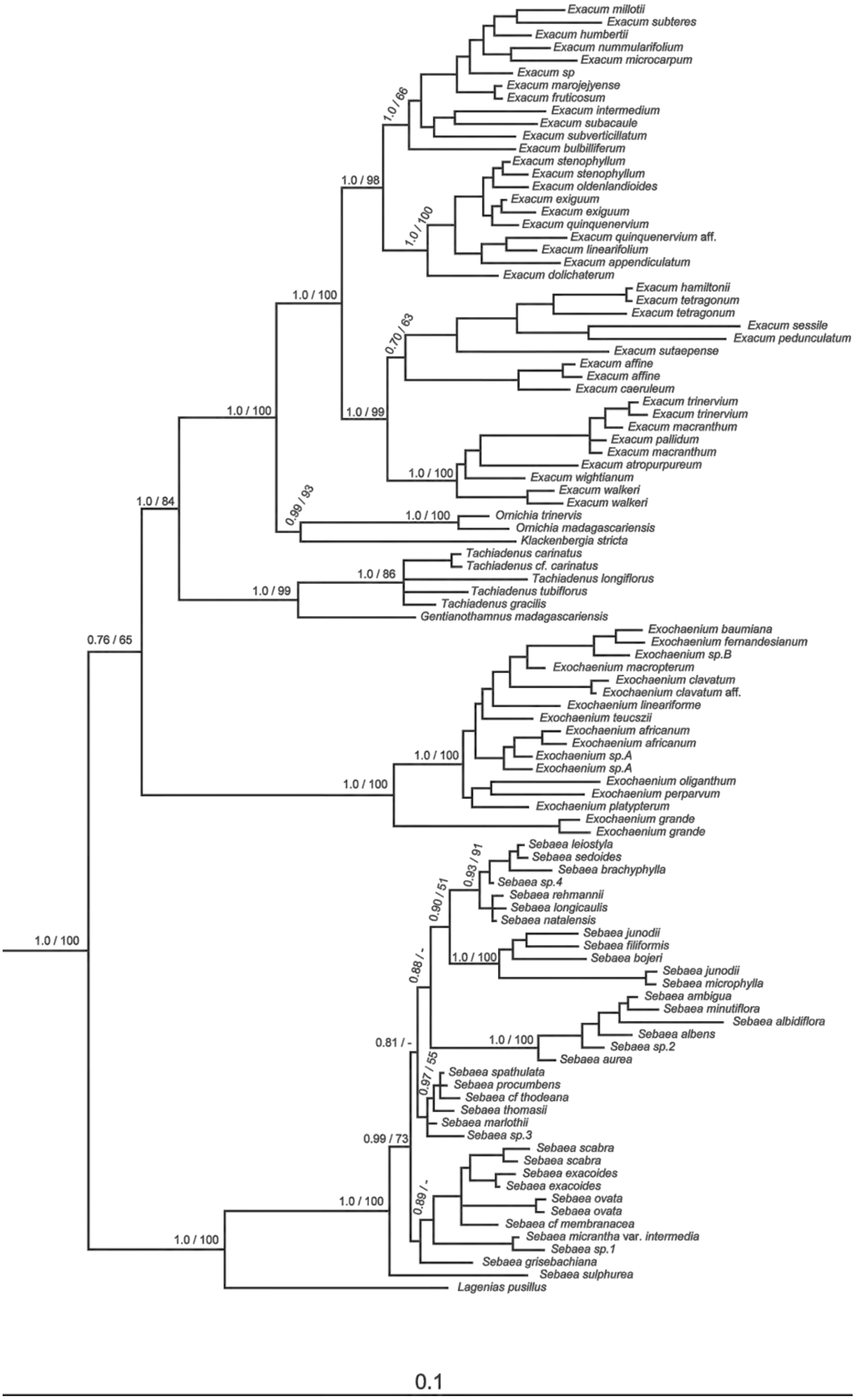


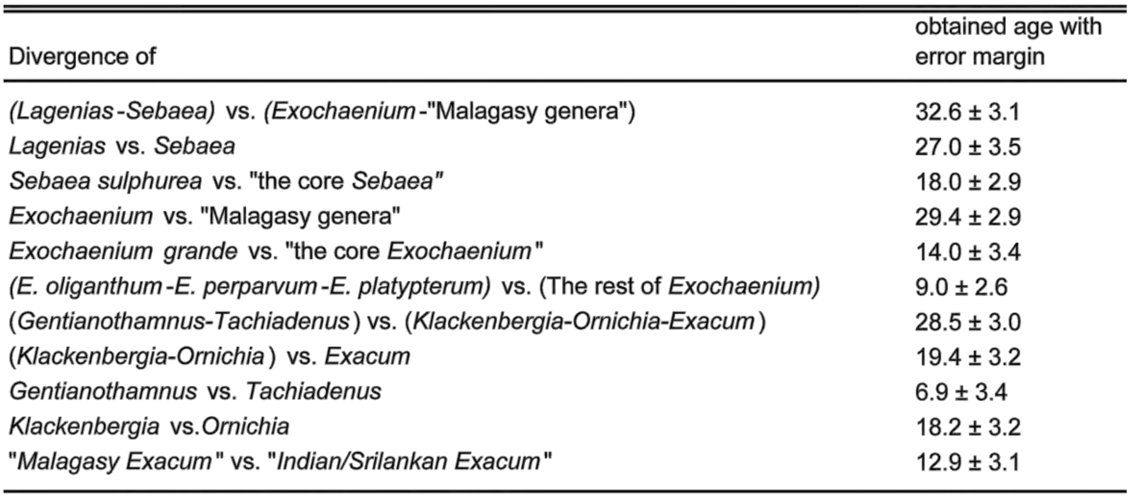
Figure 2 The 50% majority rule consensus tree from Bayesian inference analyses of the combined cpDNA and nrDNA of the "Exaceae data set". Branch support values are posterior probabilities from a 50% majority rule consensus tree derived from a Bayesian inference analysis and bootstrap values.

Divergence calibration and Dating

Age estimates and standard deviations of well-supported clades, obtained from the PL analyses, are shown in Table 3, and summarized in figure 3. Among the clades of interest, (*Sebaea*-*Lagenias*) diverged first during the late Oligocene (32.6 ± 3.1 Mya). *Exochaenium* diverged from the large (*Exacum*-*Gentianothamnus*-*Klackenbergia*-*Ornichia*-*Tachiadenus*) clade in the late Oligocene (29.4 ± 2.9 Mya), while the clade (*Gentianothamnus*-*Tachiadenus*) diverged from (*Exacum*-*Klackenbergia*-*Ornichia*) also during this period (28.5 ± 3.0 Mya). The clade (*Klackenbergia*-*Ornicha*) diverged from *Exacum* during the early Miocene (19.4 ± 3.2 Mya).

Sebaea diverged from *Lagenias* during the Late Oligocene (27.0 ± 3.5 Mya). *Tachiadenus* diverged from *Gentianothamnus* during the late Miocene (6.9 ± 3.4 Mya), while *Klackenbergia* diverged from *Ornichia* much earlier, during the early Miocene (18.2 ± 3.2 Mya). Finally the diversification of *Sebaea* (18 ± 2.9 Mya), *Exochaenium* (14.0 ± 3.4 Mya) and *Exacum* (12.9 ± 3.1) starts all three during the early-middle Miocene.

Table 3 Estimated timing of divergence based on PL rat-smoothening approach.



Divergence of	obtained age with error margin
(Lagenias-Sebaea) vs. *(Exochaenium*-"Malagasy genera")	32.6 ± 3.1
Lagenias vs. *Sebaea*	27.0 ± 3.5
Sebaea sulphurea vs. "the core *Sebaea*"	18.0 ± 2.9
Exochaenium vs. "Malagasy genera"	29.4 ± 2.9
Exochaenium grande vs. "the core *Exochaenium*"	14.0 ± 3.4
(E. oliganthum-E. perparvum-E. platypterum) vs. (The rest of *Exochaenium)*	9.0 ± 2.6
(*Gentianothamnus-Tachiadenus*) vs. (*Klackenbergia-Ornichia-Exacum*)	28.5 ± 3.0
(*Klackenbergia-Ornichia*) vs. *Exacum*	19.4 ± 3.2
Gentianothamnus vs. *Tachiadenus*	6.9 ± 3.4
Klackenbergia vs.*Ornichia*	18.2 ± 3.2
"*Malagasy Exacum*" vs. "*Indian/Srilankan Exacum*"	12.9 ± 3.1

Ancestral area reconstruction analysis

Ancestral area reconstruction for Exaceae using DIVA 1.1 (Ronquist, 1997), and restricting the ancient distribution to two geographical areas, indicates an African origin for the tribe and subsequent migration through Madagascar and Asia (Fig 3). DIVA failed to unambiguously choose between tropical (Katanga) or South Africa (Cape) regions for the MRCA of Exaceae. The Cape region was assigned as a common ancestral area for *Lagenias* and *Sebaea*, while *Exochaenium* originated in the Katanga Region, and a Malagasy origin was inferred for the large clade (*Exacum*-*Gentianothamnus*-*Klackenbergia*-*Ornichia*-*Tachiadenus*), hereafter called the “Malagasy genera”.

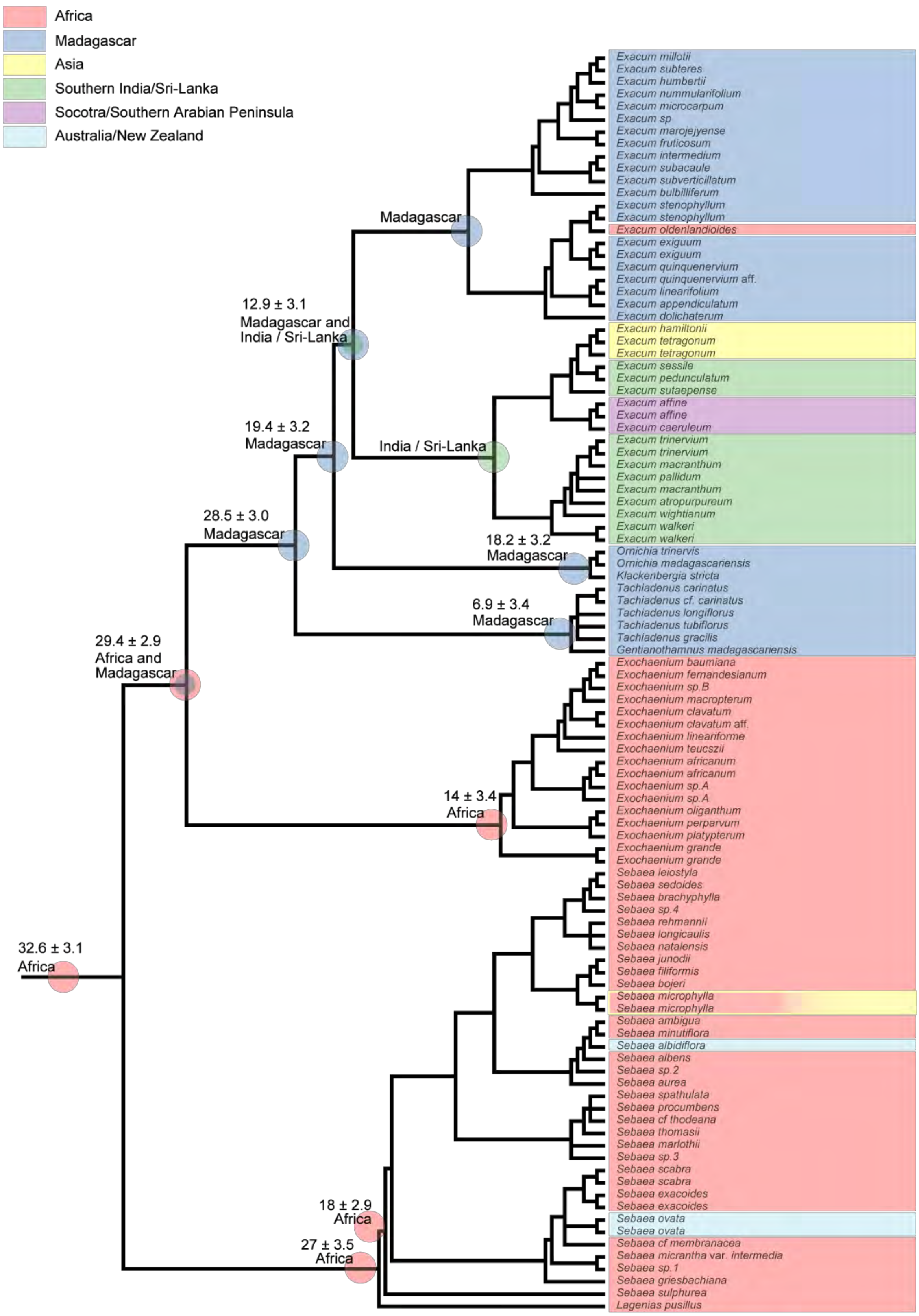


Figure 3 Ancestral areas reconstructed for each basal node of the phylogeny shown in Figure 2 using DIVA with two-area optimization. The inferred divergence time for the corresponding nodes expressed in million years ago (Mya) are also indicated.

Exacum originated in Madagascar, and dispersed from Madagascar to Sri Lanka and south India.

Sebaea originated in the Cape region, and experimented a single dispersal event to the Drakensberg, and from there, to tropical Africa (e.g. *S. brachyphylla*) or Madagascar (*S. bojeri* and *S. brachyphylla*). Two distinct long-distance dispersals from the Cape to Australia–NewZealand also occur (*S. albidiflora* and *S. ovata*), while a single long-distance dispersal from either the Drakensberg or tropical Africa to Asia explain the current distribution of *S. microphylla*.

Ancestral ecological and climatological reconstructions

Climatotolgy:

Reconstruction based on MP suggested either a Malagasy humid equatorial climate without a dry season, or an African temperate climate with either a dry summer or a dry winter, for the MRCA of Exaceae (Fig. 4). The clade formed by *Lagenias* and *Sebaea* originated in an African temperate climate with a dry summer, while *Exochaenium* originated in an African temperate climate with a dry winter. The large clade formed by the Malagasy genera (*Exacum*, *Gentianothamnus*, *Klackenbergia*, *Ornichia* and *Tachiadenus*) originated in a Malagasy humid equatorial climate without dry season.

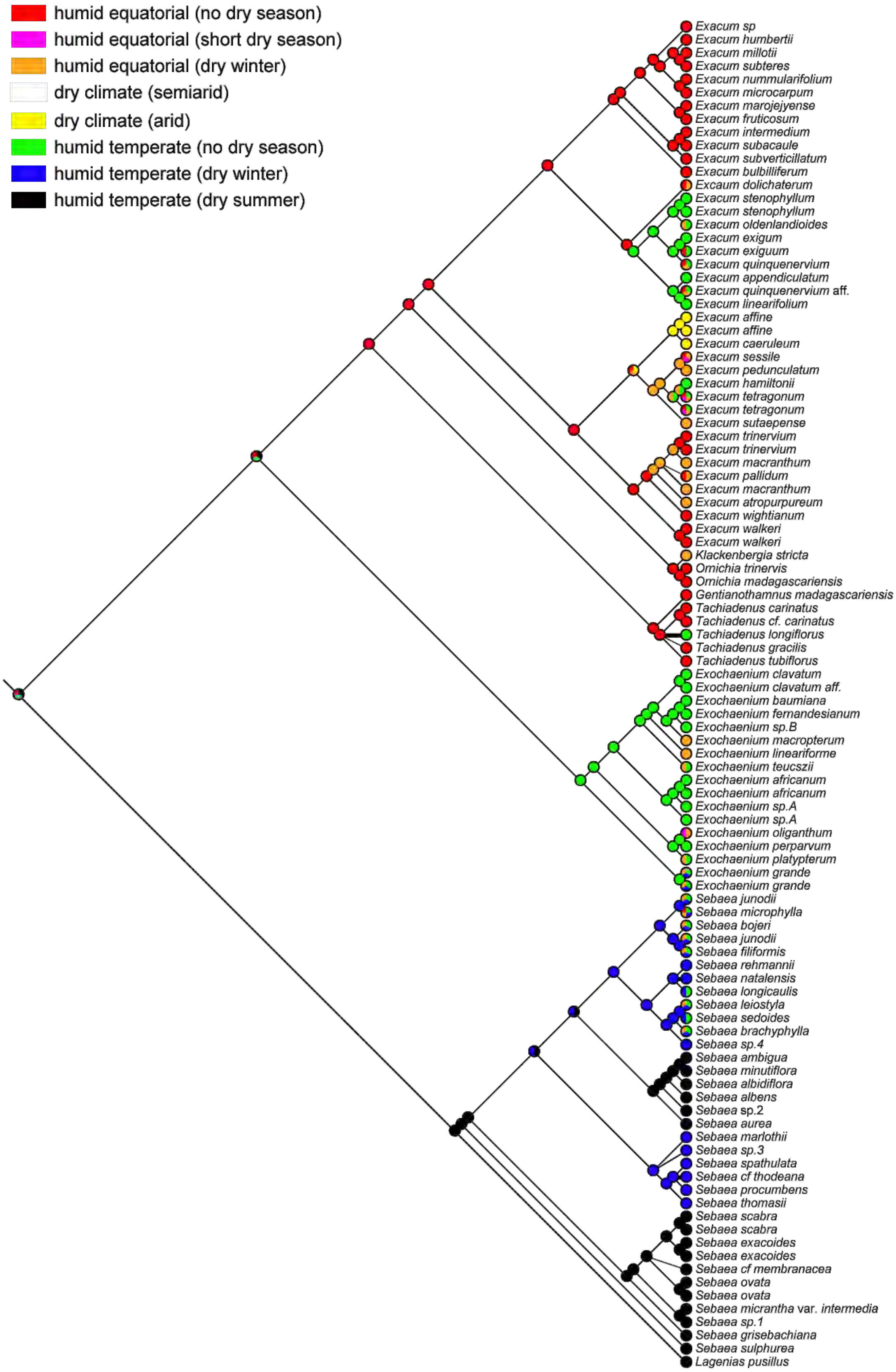


Figure 4 Ancestral climatological state reconstructed for each basal node of the phylogeny shown in Figure 2 using MP optimization.

Ecology

Both dispersal-vicariance analysis of ecological patterns for Exaceae using DIVA (Ronquist, 1997) and MP reconstruction (Maddison and Maddison, 2006) indicate that the MRCA of Exaceae was found in area of "reduced competition" (Fig 5). All the genera originated in a similar environment, except for *Gentianothamnus* and *Ornichia*, found in woodland; *Klackenbergia*, found in grassland; and *Tachiadenus*, found on sandy wet soil and woodland.

Several clades or species of *Sebaea* dispersed from sandy wet soil to grassland or ericoid vegetations, while a few species became adapted to woodland. For *Exacum*, several clades or species dispersed from sandy wet soil to woodland, grassland or ericoid vegetation. Two *Exochaenium* species adapted to a unique, and thus "unclassified", habitat: inundated lakeshore; and one species adapted to woodland.

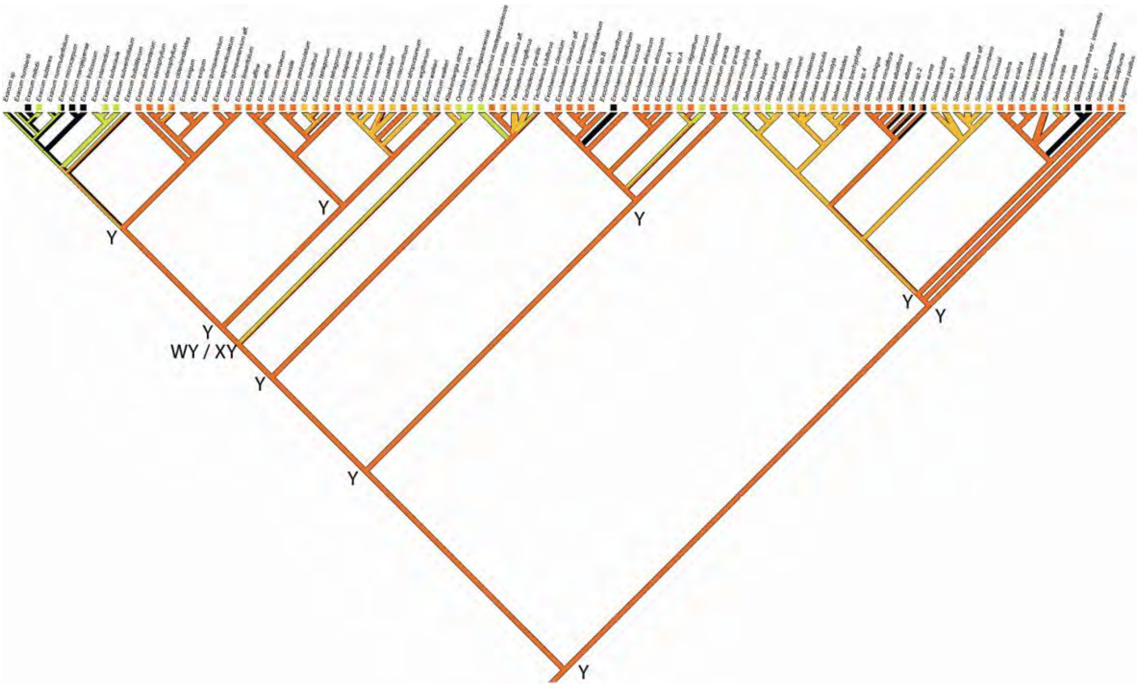


Figure 5 Ancestral ecological state reconstructed for each basal node of the phylogeny shown in Figure 2 using both MP optimization (coloured branches) and DIVA (only indicated for the main nodes) with two-area optimization. **Green** and **W**=woodland / **orange** and **X**=grassland / **red** and **Y**=sandy wet areas where competition is reduced / **black** and **Z**=ericoid vegetation.

DISCUSSION

Phylogeny of Exaceae

Tree topologies inferred from both the BI and MP criteria are congruent with previous published results, mainly focusing on *Exacum* (Yuan et al., 2003; Yuan et al., 2005; chapter 2).

Estimation of the clade ages

Molecular dating, based on the PL approach, suggests an early Oligocene origin for the Exaceae (32.6 ± 3.1 Mya), in congruence with previously published studies (Yuan et al., 2003), thus also rejecting the Gondwanan hypothesis (Klackenberg, 2002). Our data further suggest that the three main lineages of Exaceae (*Sebaea–Lagenias*), *Exochaenium*, and the "Malagasy genera" diverged rapidly within a relatively short period of time (c. 3 million years), in areas corresponding to the Western Cape (South Africa), the Katanga plateau (Tropical Africa) and Madagascar. In Madagascar, the main clades and genera of the tribe show a more or less continuous diversification from the late Oligocene to the late Miocene, followed by possible radiation for the most species-rich genera (*Exacum* and *Tachiadenus*) during the Miocene (Fig. 3), suggesting a continuous "speciation" process.

In Africa, *Exochaenium* and *Sebaea* also possibly radiate during the early-middle Miocene, which is regarded as the geological epoch with the largest turnover of plant taxa in the Cape Floristic Region (Coetzee et al., 1983).

Climatological and ecological reconstruction

Maximum Parsimony optimizations of the climatology character consistently assigned (i) a temperate climate, with dry summer for the MRCA of the *Sebaea–Lagenias* clade, (ii) a temperate climate with dry winter for an *Exochaenium*-like ancestor, and (iii) a tropical climate for the MRCA of the "Malagasy clade".

Climatological reconstruction is more problematic at the deeper nodes, i.e., the MRCA of the Exaceae, and of the clade *Exochaenium*–"Malagasy genera". For example, the MRCA of Exaceae could be found in both temperate climate with a dry season (either winter or summer) or tropical without dry season climates. However, with the exception of a single species, *E. oliganthum*, none of the African species of either *Exochaenium*, *Lagenias*, or *Sebaea* are currently found in a tropical climate, unlike most of the Malagasy species. Paleoclimatic reconstructions at the inferred time of origin of the Exaceae (c. 32 Mya), suggest the occurrence of a climate similar to the modern, with extensive glaciations of Antarctica (Zachos et al., 2001), and so better support a temperate climate for the MRCA of Exaceae, followed by one or several shifts to more tropical climates.

Both MP and DIVA optimization of the Ecological character consistently assigned sandy wet places with reduced competition for the MRCA of the Exaceae, and those of all the major clades (Fig. 5) (e.g. *Sebaea–Lagenias*, *Exochaenium*, "Malagasy Genera"), followed by subsequent shifts from this environment to others (e.g. grassland, woodland).

Biogeographical history of Exaceae

One characteristic of DIVA is that it results in an increasing number of possible distribution areas towards the basal nodes in a tree when no constraints for the maximum number of distribution areas are imposed (Ronquist, 1997). For example, DIVA provides an optimization of the basal node to the occurrence of Exaceae in all distribution areas inhabited by Exaceae today (except Australia/New-Zealand). This is a very unlikely scenario with regard to continental positions 32 Mya ago. Furthermore, DIVA does not take into account the relative or absolute nodal age in the tree and the relative geographical position of the specified distribution areas when assigning vicariance versus dispersal events. Given these factors, the constrained optimization of DIVA offers a more plausible estimation of the historical biogeography of Exaceae, particularly with regard to the divergence of the basal lineage.

Based on this analysis, we can infer that the Exaceae originated in Africa, c. 32 Mya ago, and were most widely distributed on wet and sandy places, where plant competition is generally reduced. Then, in the early Oligocene, with the possible onset of a summer dry, winter rain climate in the southwestern Africa (Linder, 2003), the MRCA of the *Lagenias*-*Sebaea* clade adapted to new environmental conditions. The monotypic *Lagenias*, which currently occurs in mountainous refuges, might be seen as representative of these past, non-tropical conditions. The MRCA of *Exochaenium* and the "Exaceae Malagasy genera" likely occurred in mainland Africa, in places with reduced competition, similar to the present day "dambo[1] island", and were adapted to long rainfall, with or without a dry period.

Based on our results, we can hypothesize a single event of dispersal from Africa to Madagascar, during the early Oligocene (29.4 ± 2.9 Mya).

At that time, Madagascar had already reached its current position (Rabinowitz et al., 1983; Harland et al., 1990; Seward et al., 2004), and the climate was quite similar to the present day (Wells, 2003). This Oligocene African-Madagascan dispersal pattern is supported by many studies, based on either animal or plants (Yoder and Nowak, 2006, and ref. therein).

We can postulate that, during the Oligocene, the Exaceae had a similar pattern to that encountered today, with the MRCA of *Sebaea* and *Lagenias* found in Southwestern (Mediterranean) Africa, the MRCA of *Exochaenium* in tropical / temperate Africa, and the MRCA of the "Malagasy genera" starting to diversify in Madagascar.

Early diversification of *Sebaea* started in the middle Miocene (18 ± 2.9 Mya), a wetter and warmer period than the Oligocene (Linder, 2003), and one in which the eastern Escarpment[2]

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[1] Dambo: seasonally waterlogged, predominantly grass-covered, depressions bordering headwater drainage lines (MACKEL, R. 1985. Dambos and related landforms in Africa; an example for the ecological approach to tropical geomorphology. *Zeitschrift für Geomorphologie, Supplementband* 52: 1-23.)

was uplifted by about 250 m (Partridge and Maud, 2000). Currently, different clades of *Sebaea* appear to be either restricted to the Western Cape, with some extension in the Southern Cape, or endemic of the Drakensberg, with some extension in Tropical Africa. According to recent studies (Cowling et al., 1999), the Southern Cape might have experienced alternate dry climates during the Quaternary period, possibly linked to glaciation events, in contrast to the Western Cape, with wet winters (Linder, 2003). In the Southern Cape, wetter climates could have acted as temporary stepping stone between the Western Cape and the Drakensberg, explaining the current disjunct distribution within *Sebaea* clades.

The clade from the Drakensberg subsequently dispersed to the Afrotemperate region, in concordance with a general biogeographical pattern for Cape clades, as demonstrated for e.g. the African Restionaceae, the *Irideae* p.p., or the "*Pentaschistis* clade" (Galley et al., 2007).

Recent episodes of long-distance dispersal, between Africa and Australia, better explain the current distribution of *S. albidiflora* and *S. ovata*. According to our age estimates, these events seem to be recent (the earliest age for *Sebaea* diversification being c. 18 Mya). Seed dispersal has been poorly studied for *Sebaea* (see Annex 1), but the respective size and shape of the seeds would perfectly fit random dispersal by either birds, mammals, or vegetal rafts, as hypothesized for other gentians, e.g *Centaurium* dispersed by camels along the silk road (L. Zeltner and G. Mansion, pers. comm.); or by birds for the long-distance dispersal of *Exacum* (Yuan, 2005).

The origin of *Exochaenium* dates back from the middle Miocene (14 ± 3.4 Mya), which corresponds to the beginning of the grass-dominated savannah biome expansion in Africa (Jacobs, 2004; Jacobs and Herendeen, 2004), a favourable environment for most Exaceae. Most of the diversification of *Exochaenium* started in the late Miocene (c. 9 Mya, Table 3), corresponding with the tectonic uplift of the East African Rift System, leading to an aridification of Africa, and a larger expansion of grasslands (Sepulchre et al., 2006).

The current diversity of *Exochaenium* could perhaps be explained by the alternation of wet and dry periods during the Pliocene-Pleistocene (Demenocal, 1995, 2004), leading to a cyclic expansion of woodland biomes (isolating *Exochaenium* populations in e.g. dambo) followed by expansion of grassland that permitted the isolated species to meet again (via a facilitated wind-dispersion (Annex 1) of the seeds, due to the absence of a woody barrier). This scenario of speciation can explain why so many *Exochaenium* species are currently found in sympatry.

The diversification of Exaceae in Madagascar dates back from the late Oligocene (28.5 ± 3.0 Mya). At that time, the island of Madagascar has reached its current position with respect to Africa (Coffin and Rabinowitz, 1988), and was climatically characterized by orographic

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[2] Geomorphologically, southern and south-central Africa are dominated by a major uplift, with much of the subcontinent raised to an altitude well above 1000 m. This plateau is bounded by a large and often abrupt escarpment (the Great Escarpment) dropping to rather narrow coastal (Partridge & Maud, 1987 in Linder, 2003)

precipitation more or less similar to that currently encountered in the eastern rainforest (Wells, 2003). The initiation of monsoon system is thought to have begun (or to have intensified greatly) at only c. 8 Mya in India (Prell et al., 1992; Burchfield, 1993). It seems safe to assume that the monsoons started small and local and gradually grew in strength and extent (Wells, 2003), thus heavy seasonal rains to the north-western Sambirano region (Madagascar) may have begun some time later. This might explain the diversification of the *Tachiadenus*-*Gentianothamnus* clade.

Factors other than biogeography and climates, might also have triggered the impressive diversification of the Madagascar clade. On the one hand, adaptation to new pollinators might have played a crucial role in floral diversification (see chapter 2), as indicated for many insular groups of plants. Indeed the length of the corolla tube in *Tachiadenus* strongly suggests some adaptation to long-proboscis pollinators, while the typical buzz-pollination syndromes encountered in *Exacum* (poricidal anthers and bent downwards styles), is only found in the Malagasy Exaceae. On the other hand edaphic and/or climatic specialisation might also explain the Malagasy Exaceae diversity. Madagascar is geologically complex, with extensive areas of sedimentary (e.g. sandstones, limestones, lake deposit) and metamorphic (e.g. basalt, marble, quartzite) rocks (Du Puy and Morat, 2003). The climate exhibits a conspicous seasonality and sharp ecological gradients due to both the latitudinal range of the island and its mountainous nature (Jury, 2003). This allows great potential scope for edaphic or climatic specialisation.

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KLACKENBERGIA (GENTIANACEAE – EXACEAE): A NEW ENDEMIC GENUS FROM MADAGASCAR

KLACKENBERGIA (GENTIANACEAE - EXACEAE): A NEW ENDEMIC GENUS FROM MADAGASCAR

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Abstract – Several lines of evidence (morphological, karyological and molecular) support the exclusion of both *Sebaea madagascariensis* (Schinz) Klack. and *S. condensata* Klack. from *Sebaea* (Gentianaceae – Exaceae), resulting in the establishment of a new genus *Klackenbergia* Kissling, and two new species combinations: *K. stricta* (Schinz) Kissling and *K. condensata* (Klack.) Kissling. *Klackenbergia* is endemic to Madagascar, and is morphologically recognized by its inflorescence with characteristic long bracts and bracteoles and sub-sessile-flowers arranged in axillary fascicles at each node. Affinities with the other genera of the tribe (*Exacum*, *Gentianothamnus*, *Ornichia*, *Sebaea*, and *Tachiadenus*) are discussed.

Keyword – *Belmontia*, Exaceae, Gentianaceae, Madagascar, *Sebaea*, systematics, taxonomy

INTRODUCTION

The tribe Exaceae in its current circumscription (Struwe et al., 2002; Klackenberg, 2006) includes ca. 186 species distributed into five genera, namely *Exacum* L. (including *Cotylanthera* Blume; (Klackenberg, 2006), *Gentianothamnus* Humbert, *Ornichia* Klack., *Sebaea* Sol. ex R. Br. and *Tachiadenus* Griseb. Exaceae differ from the other tribes of the gentian family by some synapomorphies including the star-shaped testa cells of the seeds (Klackenberg, 1985, 2002; Bouman et al., 2002) and the shape of the epidermis cells of petals (basically rounded and convex versus elongated and flat) (Klackenberg, 1985, 2002).

The genus *Sebaea* contains most of the species diversity of the tribe Exaceae, even though the exact number of species is not yet properly established. Based on field work and examination of different herbarium collections (more than 3000 specimens), a good estimate of the actual species number appears to be around 95 (Kissling, unpublished data). The genus *Sebaea* has a paleotropical distribution, but most of the species are endemic to continental Africa, including the Cape region of South Africa, the Drakensberg, and the Zambesian basin (Marais and Verdoorn, 1963; Taylor, 1963; Boutique, 1972; Paiva and Nogueira, 1990; Nemomissa, 2002). Four species are found in Madagascar (Klackenberg, 1990), two in Australia / New Zealand (Adams, 1996; Champion et al., 2003), and one species has a wide distribution from Africa to South-East Asia (Marais and Verdoorn, 1963; Paiva and Nogueira, 1990; Ho and Pringle, 1995; Suksathan and Sasirat, 2000; Nemomissa, 2002). *Sebaea* comprises annual to perennial, erect to procumbent herbs, or more rarely achlorophyllous saprophytes, and is mostly characterized by the absence of synapomorphic features characterising the other genera of Exaceae. Currently no synapomorphy has been found to support *Sebaea*, although this genus is apparently morphologically homogenous.

Phylogenetic analyses based on sequences of both *cp*DNA and *nr*DNA (Yuan et al., 2003) emphasized that *S. madagascariensis* was more closely related to *Ornichia*, than to *Sebaea*. Furthermore, a survey of the chromosome numbers of some African Exaceae, including *Sebaea* (Chapter 1) revealed that *S. madagascariensis* has a chromosome number of n=9, while all the other *Sebaea* (n=14, 21, and 28) and *Ornichia* (n=14) species have a base number of x=7.

Morphologically, *S. madagascariensis* can be easily distinguished from the rest of the genus by its inflorescence, with characteristic long bracts and bracteoles and sub-sessile flowers arranged in axillary fascicles at each node (Fig. 1), a feature found neither in the remaining *Sebaea* (except *S. condensata*) nor in the Exaceae.

Figure 1: Photographs of *Klackenbergia stricta*. **A**: details of the flowers, showing the typical long bracteoles of the genus; **B** & **C**: the raceme-like inflorescence (*Piso J.C. & al. MO17, 18,* NEU).

A putative sister taxon would be *Ornichia*, which differs from *S. madagascariensis* by the presence of hairs on the leaves.

During the eventful taxonomical history of *Sebaea*, four taxa previously included in *Sebaea* (*Belmontia* E.Mey., *Exochaenium* Griesb., *Lagenias* E. Mey., and *Parasia* Raf.) were upheld at the generic level. However, the different morphological characters used for upholding those genera were highly overlapping. As a consequence, all these genera were merged into a single genus *Sebaea* by Marais (1961), even though some of the previous combinations might have reflected actual differences between the respective taxa. *Sebaea madagascariensis* was initially described as belonging to *Belmontia*, but was not chosen as a type species for this genus.

In this study, we discuss the taxonomic status of *S. madagascariensis* and *S. condensata*, two taxa that cannot be assigned to any of the current genera described in the Exaceae. We propose the creation of a new genus (*Klackenbergia* Kissling), including *S. madagascariensis* and likely *S. condensata*, the two species being morphologically similar, and sharing synapomorphies not found in other members of Exaceae.

MATERIAL AND METHODS

Material was examined from BM, G, K, MO, NEU, P, TAN and Z (abbreviations according to Holmgren & al., 1981). Historical collections lacking geographic coordinates were post-facto georeferenced using web-based “Gazetteer to Malagasy Botanical Collecting Localities” (Schatz & Lescot, 2005; http://www.mobot.org/MOBOT/Research/madagascar/gazetteer/), and are surrounded by square brackets in the citation of specimens examined. Species distribution maps were created using ESRI ArcView software (1999). Distributions were mapped over the five simplified bioclimatic zones of Madagascar (Cornet, 1974). Authors of plant names follow Brummit & Powell (1992).

TAXONOMICAL TREATMENT

While upholding *S. madagascariensis* in a new genus, the epithet status of this species should change according to article 11.4 of the International Code of Botanical Nomenclature (Vienna Code). Indeed *S. madagascariensis* was first described by Schinz (1891) as *Belmontia stricta* based on a Malagasy specimen (*Hildebrandt 3428*). The genus *Belmontia* was created by E. Meyer (1837) on the basis of three species, *B. grandis*, *B. cordata* and *B. spathulata*, which were later included with *Sebaea* (Schinz, 1903). Schinz (1903), while merging *Belmontia* with *Sebaea* preferred keeping the epithet *stricta* for his species, although Gilg had already published a *Sebaea* from Zulu-Natal, South Africa, with the epithet *stricta* (Gilg, 1899). In 1986, Klackenberg, while working on the tribe *Exaceae* of Madagascar, noticed this taxonomical contradiction and gave a new name for Schinz's Malagasy species (Klackenberg, 1986a), *Sebaea madagascariensis*. According to the Code (Art. 11.4), while transferring *S. madagascariensis* in *Klackenbergia*, the epithet *stricta* should be conserved.

***Klackenbergia* Kissling, gen. nov**. - Typus generis: *Klackenbergia stricta* (Schinz) Kissling (Fig. 1)

Diagnosis. - *Genus novum* Sebaeae *affine sed differt inflorescentiis sessilibus ac subsessilibus, axillaribus, insertis fascibus, bracteis florigenis longioribus; a genere* Ornichia *differt foliis angustioribus glabrisque ; a genere* Exaco *antheris rimis dehiscentibus differt.*
Herba glabra, erecta, non vel paulum ramificata, caule subtereti quadrangulari, foliis decussatis, integris, linearibus; inflorescentiis axillaribus ; floribus pentameris, actinomorphis, subsessilibus ; bracteis longioribus quam sunt flores ; staminibus in tubo sub sinibus insertis; antheris non exsertis et in annulo plus minusve cohaerentibus; ovario biloculari ovulis numerosis, dispositis in placentis axilibus; stylo filiformi, recto; stigmate integro, paulum papillato; fructu capsulari; seminibus numerosis, minutis, angulatis; parietibus cellularum testae stellatis.

Description. – A small erect typically unbranched to slightly branched herb, 2-25 cm high, glabrous. Stem subterete, slightly quadrangular to quadrangular. Leaves entire, linear, acute, decussate, 1(-3) nerved. Inflorescence formed of sub-sessile flowers arranged in fascicules at each node (in fact only one branch of the cymes is developing, with a large internode constituting the prolongation of the stem, the other branch is inexistent or both lateral branches of the cyme are developed but one of them is condensed bearing the flowers closely set to each other near the node). Bracts and bracteoles much longer than the flowers, 1-2(-2.5) times longer. Flowers pentamerous, actinomorphic. Pedicels very short, < 1mm long, much shorter than the internodes below, erect. Calyx actinomorphic; sepals coalescent only at the very base, lobes overlapping at the base, ovate to elliptic, gradually tapering at the apex, acuminate, with a few parallel veins near the mid-nerve, keeled to narrowly winged. Corolla white with the petals basally connate, forming a long narrow tube several times longer than the lobes; tube rather long; lobes upright or nearly so, narrowly ovate to elliptic, acute. Stamens inserted at the middle of the corolla tube, and never exserted from the corolla mouth; filaments very short, free; anthers usually coherent in a ring, with a gland at the apex (formed of small glands), straight, dehiscing by slits to the base. Ovary bilocular, many ovules on axile placentas; style filiform, straight; stigma entire, linear to very slightly clavate, slightly papillate. Fruit a capsule, ovate, about as long as or longer than the calyx, attenuate at the apex, coriaceous and septicidally 2-

valved; septum partially coriaceous. Seeds numerous, minute, angular (cubical); testa cells with more or less star-shaped walls (Fig. 2).

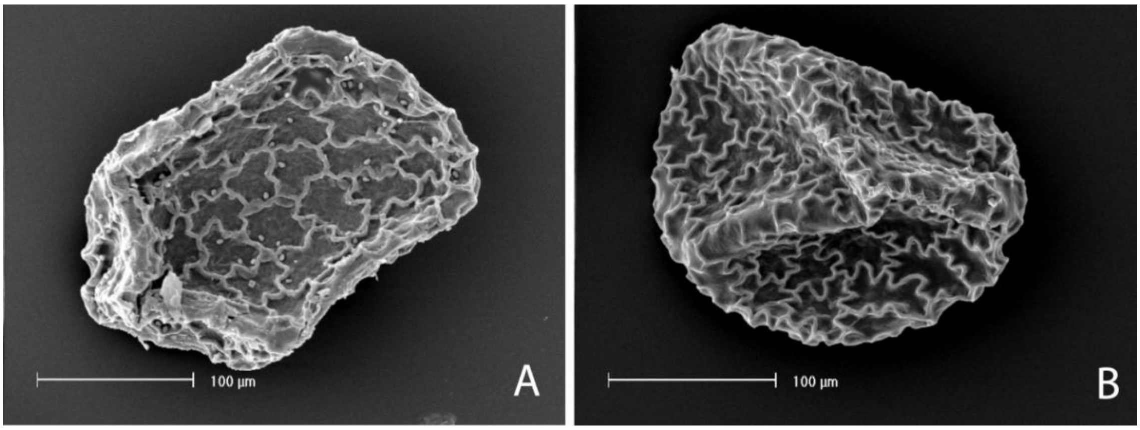

Figure 2: SEM photography of the seed of *Klackenbergia stricta* (A) (*Piso & al. MO17, 18*, NEU) and *Klackenbergia condensata* (B) (*Croat 32108*, MO).

Etymology. – The genus *Klackenbergia* is named after Jens Klackenberg (1951-) from the Swedish Museum of Natural History, Stockholm, who actively contributed to the systematics of *Exaceae* with several important papers on the Exaceae (e.g. Klackenberg, 1985, 1986, 1987, 1990).

Key to the species:

Plants 17-25 cm high; 1-(2) flower at each leaf node…………..…………..…*K. stricta*

Plants 5-10 cm high; 3 to several flowers at each leaf node ....…....*K. condensata*

K. condensata is morphologically more closely related to *K. stricta,* than to any other genera of the tribe, by sharing with *K. stricta* a particular inflorescence, with characteristic long bracts and bracteoles and sub-sessile axillary flowers, making them distinguishable at a glance from the rest of *Sebaea* and furthermore the tribe. Moreover, the flowers of the two species are very similar in color, size and general shape. *Klackenbergia condensata* differs from *K. stricta* by its size, generally 5-10 cm, the numbers of flowers at each node (2-several), the capsule, generally as long, or longer than the lobes of the calyx, while they are distinctly shorter in *K. stricta*, and finally by the geographical distribution.

As we cannot confirm the chromosome number and phylogenetic position of *Sebaea condensata*, a species extremely rare in the field, we tentatively include it in *Klackenbergia* based on morphological grounds only. This species is currently known from five collections, and the last collection is from 1975. In spite of considerable joint efforts, we were not able to collect this species again in the highlands of Madagascar, where it was described first. Those highlands have undergone substantial human pressure (Meyers et al., 2000), resulting in a drastic rarefaction of the ecological niches available for *K. condensata*.

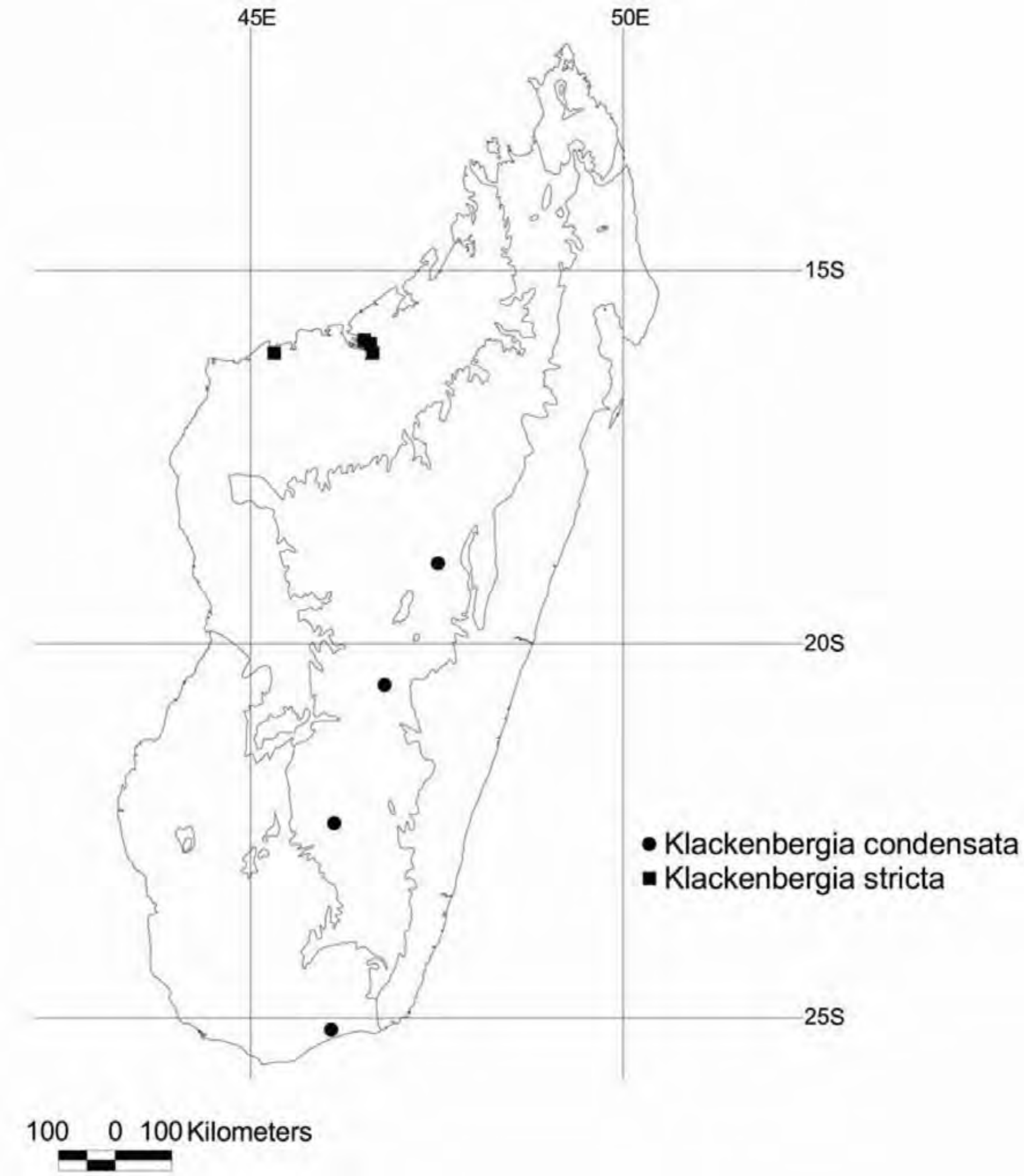

Figure 3: Distribution of *Klackenbergia stricta* and *K. condensata* mapped on the bioclimatic zones of Madagascar (Cornet, 1974).

Klackenbergia stricta (Schinz) Kissling, *comb. nov.* ≡ *Belmontia stricta* Schinz, Vierteljahrsschr. Naturforsch. Ges. Zürich 36: 334 (1891).). ≡ *Sebaea stricta* (Schinz) Schinz nom. illeg. Bull. Herb. Boissier, Ser 2 6(9): 738 (1906), non *Sebaea stricta* (E. Mey.) Gilg, Bot. Jahrb. Syst. 26: 90 (1899). ≡ *Sebaea madagascariensis* Klack. nom. nov. Taxon 35(3): 595 (1986). - Type: **Madagascar.** Prov. Mahajanga, Marovoay, [82 m, 16°06'00''S, 46°37'60''E], mai 1880, fr. & fl., *Hildebrandt 3428* (Hololectoype: Z!; Isolectotype BM!, G!, K!, P!, Z!). (2n=18).
Icon: Flore de Madagascar et des Comores, Famille 168, 111, Pl. 33 (1990).

Distribution, Ecology and Phenology

Klackenbergia stricta is distributed in the northwest of Madagascar, especially near Majunga (Fig. 3). It grows in swampy open places up to 100 m altitude. Flowering specimens have been found from March to May.

Specimens studied

Madagascar. Prov. Majunga. Ambongo-Soalala, [0m, 16°06'S, 45°19'E], Apr. 1904, fl., *Perrier de la Bâthie 1701* (P) ; Maevarano près de Majunga, [178m, 15°56'S, 46°31'E], Mar. 1908, fl., *Perrier de la Bâthie 8443* (P) ; Mahajunga, [124m, 15°43'S, 46°19'E], Apr. 1925, fl., *Perrier de la Bâthie 17259* (P) ; Miadana, route de Maevarana, 58m, 15°60'S, 46°37'E, 01 Apr. 1995, fl. & fr., *Piso J.C. & al. MO17, 18* (NEU, TAN).

Without localities: Madagascar, June 1906, *Hildebrandt s.n.* (MO).

Klackenbergia condensata (Klack.) Kissling, *comb. nov.* ≡ *Sebaea condensata* Klack. Bull. Mus. Natn. Hist. Nat., Paris, 4e sér., **9**, section B, *Adansonia* 2: 133 (1987). – Type : **Madagascar.** Prov. Fianarantsoa, Ambatofinandrahana, [1453 m, 20°33'00''S, 46°47'60''E], Apr. 1919, fl., *Perrier de la Bâthie 12564* (Holotype : P!; Isotype P!).

Icon: Bull. Mus. Natn. Hist. Nat., Paris, 4e sér., **9**, section B, *Adansonia* 2: 135, Fig 1 (1987). Flore de Madagascar et des Comores, Famille 168, 111, Pl. 33 (1990).

Distribution, Ecology and Phenology

Klackenbergia condensata is distributed along the central plateau of Madagascar but also in the very south of the island, and has been collected from near Antananarivo in the north to Ambovombe in the south (Fig. 3). It grows in open places in sand and has been collected on cliffs and river embankments up to 1400 m altitude. Flowering specimens have been found from March to June.

Specimens studied:

Madagascar. Prov. Antananarivo. Antananarivo, [1288 m, 18°55'S, 47°31'E], Mai 1926, fl., *Perrier de la Bâthie 17639* (P) ; Prov. Toliara. Along RN13 from Betroka to junction with RN10 near Ihosy, [900 m, 22°23'60''S, 46°07'00''E], 27 Febr. 1975, fl., *Croat 32108* (MO); Ambovombe, [149 m, 25°10'00''S, 46°04'60''E], 05 May 1924, fl., *Decary 2707* (P); Prov. Fianarantsoa. Env. d'Ihosy, [759 m, 22°23'60''S, 46°07'00''E], June 1933, fl., *Perrier de la Bâthie 19277* (P).

AFFINITIES

Klackenbergia is morphologically closely related to the large and widely distributed genus *Sebaea* Sol. ex. R.Br., as well as to the Malagasy endemic *Ornichia* Klack. (Yuan & al., 2003). *Klackenbergia* differs from *Sebaea* by having an unusual inflorescence with axillary sub-sessile flowers at each node (vs. dichasium, or solitary flower in *Sebaea*) and bracts and bracteoles much longer than the flowers (vs. much shorter in *Sebaea*). *From Ornichia* it differs by having glabrous and thinner leaves, very short pedicels as well as a linear stigma (vs. capitate more or less bilobed stigma).

Klackenbergia is easily distinguished from the other genera of the tribe. It differs from *Tachiadenus* and *Gentianothamnus* by its small flowers (up to 1 cm), while both *Tachiadenus* and *Gentianothamnus* have very long corolla tube (up to 20 cm), and its truly bilocular ovary (vs. pseudo-bilocular ovary), and from *Exacum* by the septicidal (vs. poricidal) dehiscence of the anthers.

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We would like to thank especially Philippe Küpfer (University of Neuchâtel), for the supervision of this work. We are much obliged to Nicole and Louis Zeltner for making available their photographs, specimens and personal observations of *Klackenbergia stricta*, as well as for many comments and help with Latin grammar. Thanks are also due to Philippe Chassot for comments and help with Latin grammar, Sébastien Wohlhauser (University of Neuchâtel) for providing photographs of *Klackenbergia stricta*, Lena Struwe (Rutgers University) for comments and advice. We thank the curators of the following herbaria for allowing access or loans of specimens: BM, G, K, MO, NEU, P, TAN and Z, and the University of Neuchâtel for funding this research.

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TAXONOMY OF *EXOCHAENIUM* AND *LAGENIAS*: TWO RESURRECTED GENERA OF THE TRIBE EXACEAE (GENTIANACEAE)

Taxonomy of *Exochaenium* and *Lagenias*: two Resurrected Genera of the Tribe *Exaceae* (Gentianaceae)

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Abstract – Several lines of evidence (molecular and morphological) have recently revealed the polyphyly of *Sebaea* (Gentianaceae – Exaceae), and the possible establishment of four new genera corresponding to highly supported clades. Here we propose the reinstatement of both *Exochaenium* and *Lagenias* as two segregate genera from *Sebaea*. *Exochaenium* consist of 23 species distributed in tropical Africa, and is characterized by papillate stigmas, and cubical seeds with star-shaped testa cells. *Lagenias* is a monotypic genus endemic to the Western Cape in South Africa, which differs from *Exochaenium* and *Sebaea* by its medifixed anthers, the insertion of the filaments in the corolla, and the cubical seeds with polygonal testa cells.

Keywords – Africa, *Belmontia*, Exaceae, *Exochaenium*, Gentianaceae, *Lagenias*, *Parasia*, *Sebaea*, systematics, taxonomy

INTRODUCTION

Gentianaceae sensu Struwe & al. (2002) comprise 87 genera and about 1650 species grouped in six tribes. *Sebaea*, with c. 95 species, is the largest genus in the *Exaceae* and is currently distributed in Africa (c. 90 spp.), Madagascar (2 spp.), Australia (2 spp.), New Zealand (1 sp.), and India and Thailand (1 sp.). A considerable number of species are endemic to the Cape region (e.g. *Sebaea albens*, *S.ambigua*, *S. exacoides*) or to the Drakensberg (e.g. *S. marlothii*, *S. spathulata*, *S. thomasii*), while others are only found in tropical Africa (e.g. *S. grandis*, *S. teucszii*, *S. clavatum*).

Solander described the genus *Sebaea* in a manuscript that was published by R. Brown (1810). It was dedicated to the Amsterdam-based pharmacist Albertus Seba (1665-1736), well-known for his "Cabinet of Curiosities". Solander transferred three yellow-flowered South African *Exacum* species namely *Exacum albens*, *E. aureum* and *E. cordatum* (= *S. exacoides*) and one Australian species – the type species – *E. ovatum* to the new genus *Sebaea*. He based the new genus on the anther opening mechanism (longitudinal slits for *Sebaea*) the form of the style (straight for *Sebaea*), and the form of the stigma ("undivided" for *Exacum*). Rafinesque (1837) described the genus *Parasia* for the single species *S. cordata* (= *S. exacoides*), but *Parasia* was not recognized by later authors. Meyer (1838) split *Sebaea* into three genera, namely *Belmontia*, *Sebaea* and *Lagenias* and transferred three species (*S. grandis*, *S. cordata* [= *S. exacoides*] and *S. spathulata*) to *Belmontia*. *Sebaea* was distinguished from *Belmontia* by exserted anthers becoming recurved and the stigma being capitate and bilobed with a secondary division ("stigmate capitato-didymo").

It is interesting to note that *S. exacoides* (which was placed in *Belmontia*) clearly presents a bilobed stigma, when mature. However, in young flowers, the part that will develop into a mature stigma appears somewhat linear to clavate. Therefore Meyer probably made his observation on immature flowers.

Recently, in the *Nomina Generica Conservanda et Rejicienda* of the Tokyo Code (1994) it was decided to conserve the name *Belmontia* over *Parasia*. Therefore *Parasia* is a homotypic synonym of *Belmontia*. Meyer (1838) also established the monotypic genus *Lagenias* for *Sebaea pusilla*. *Lagenias* was characterized by having the anthers recurved like in *Sebaea* and the filaments inserted in the corolla tube like in *Belmontia*. Later, Grisebach (1845) erected a new genus from *Belmontia*, based on *B. grandis*, and named it *Exochaenium*. The main character for separating *Exochaenium* from *Belmontia* was anthers being connate to form a tube around the style, in addition of being included. At that time the genus *Sebaea* was split in four genera: *Belmontia*, *Exochaenium*, *Lagenias*, and *Sebaea*. In *Genera plantarum*, Bentham (1876) synonymised *Lagenias* and *Exochaenium* under *Belmontia*, which was kept distinct from *Sebaea*. Schinz (1891) recognized both *Lagenias* and *Belmontia* as separate from *Sebaea*, but included species of *Exochaenium* in *Belmontia*. Gilg (1898) followed Schinz's (1891) classification with minor modifications at species level. Later on, Schinz (1903) contradicted his previous work and put *Sebaea* and *Belmontia* in the same genus *Sebaea*, further subdivided into two sections: *Eusebaea* and *Belmontia*. These sections differed by anther insertion (inside the tube for section *Belmontia*, and at the corolla sinus for section *Eusebaea*. Only three years later, Schinz (1906) revised his own classification by accepting *Exochaenium*, to which he transferred most species of *Sebaea* sect. *Belmontia*, and merged *Lagenias* with *Sebaea*. *Exochaenium* was characterized by having both included anthers and a disk of glands between the calyx and the corolla. Hill (1908), when preparing *Flora Capensis*, followed Schinz's (1906) classification, adding one more character to *Exochaenium*, i.e. the style not having a "biglandular swelling" (secondary stigma on the style (Marloth, 1909; Hill, 1913)), in contrast to *Sebaea*. However, Marais (1961) argued that the character used to uphold *Exochaenium* as distinct from *Sebaea* were unreliable, and synonymized *Exochaenium* with *Sebaea*. Taylor (1963) for Flora of Tropical West Africa, Boutique (1972) for the Flore d'Afrique Centrale, Klackenberg (1990) for Flore de Madagascar et des Comores, Paiva & Nogueira (1990) for Flora Zambesiaca, and Sileshi (2002) for Flora of Tropical East Africa followed Marais (1961). As stated by Klackenberg (2002) "The systematics of *Sebaea* sensu lato is confused, and no satisfactory classification of the genus currently exists, let alone any phylogenetic analysis."

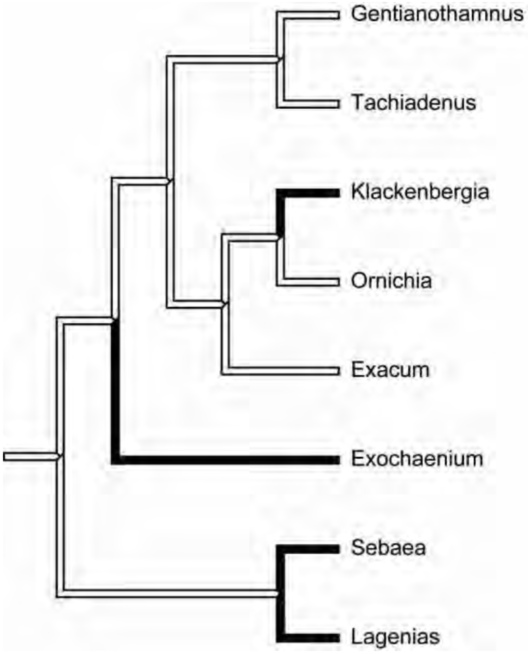


Fig. 1. Simplified phylogenetic relationship within the tribe Exaceae (from chapter 2). Black branches represent segregate genera of the *Sebaea* complex.

Recently, a global phylogenetic study of the tribe Exaceae focusing on the genus *Sebaea* has been performed (Chapter 2; Fig 1) and includes most of the diversity of the known *Sebaea* species. This work based on the analysis of chloroplast DNA sequence regions revealed the polyphyly of *Sebaea*, and supported its segregation into four well-supported clades. The first clade (*Sebaea* s. str.) in addition to containing the type species of *Sebaea* (*S. ovata*) also contains the type species of *Belmontia* (*S. exacoides*). The second clade contains only the type species of *Lagenias* (*L. pusillus*), while the type species of *Exochaenium* (*E. grande*) belongs to the third clade. The fourth clade only contained a Malagasy species, recently described as a new genus (chapter 4). Furthermore, this study found several micro- and macro-morphological characters supporting each clade (Chapter 2).

In this paper I will focus only on the taxonomical reinstatement of *Lagenias* and *Exochaenium*

MATERIAL AND METHODS

For the morphological observations, herbarium material from BM, BOL, BR, C, G, K, MO, NEU, PRE, S, WAG, Z (abbreviations according to Holmgren & al., 1981) and personal collections of L. Zeltner were used. Flowers and fruits were rehydrated with boiling water and preserved in a 1:1 solution of 70% ethanol and distilled water. Fresh material was collected when possible in the field and preserved in a 70% ethanol solution. Authors of plant names follow Brummitt & Powell (1992).

TAXONOMY

Key to the genera of the tribe Exaceae:

1.
Anthers dehiscing by terminal pores…………………………………….…………………***Exacum***
Anthers dehiscing by a slits along each theca……..…………………………………….………2.

2.
Corolla tube longer than 1.5 cm, Madagascar………………………………………………..3.
Corolla tube shorter than 1.5 cm, or, if longer, Continental Africa……………………….…....4.

3.
Flowers blue or white; stamen filaments less than 2 mm long………………...……***Tachiadenus***
Flowers yellowish; stamen filaments longer than 5 mm……………...……....***Gentianothamnus***

4.
Flowers usually blue to purple, rarely white; leaves ± hairy, at least presence of unicellular trichomes on the leaves nerve (inferior face)…………….……………………...……...***Ornichia***
Flowers yellow or white; leaves glabrous……………………………………….………………5.

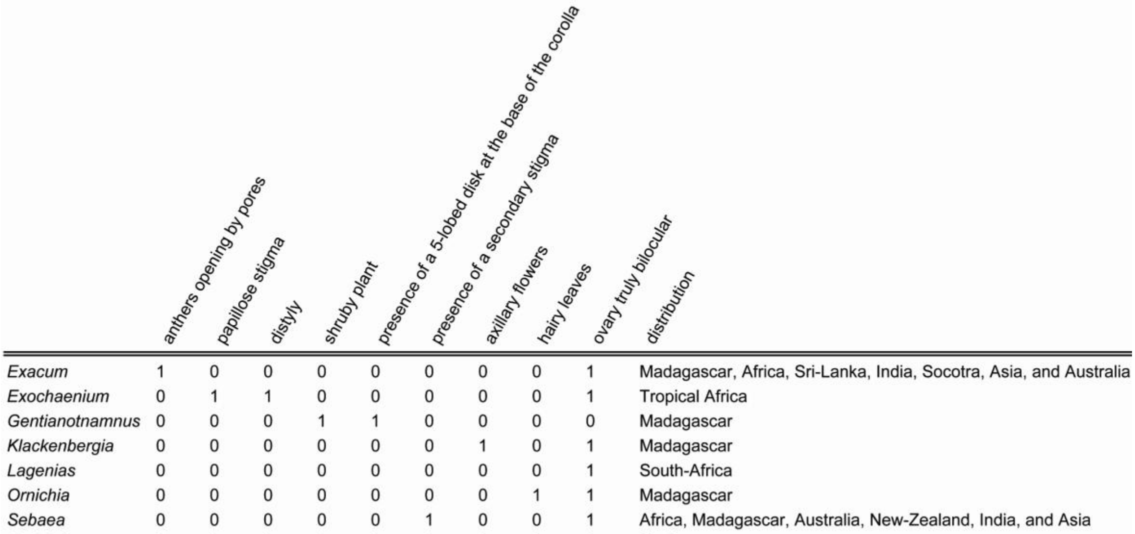
5.

Flowers white, subsessile; bracts much longer (1-1.5 time) than the flowers; axillary flowers (solitary or in fascicule)………………….………......…...………………….……….……***Klackenbergia***
Flowers white, yellow or salmon, clearly pedicellate; bracts shorter than or rarely as long as the flowers; inflorescence a dense or lax dichasium, or solitary…..………………..………………….6.

6.
Plants more or less crassulescent, anthers dordifixed, inserted at the base of the corolla tube……………………………………………………………………….……………...***Lagenias***
Plants usually herbaceous, anthers basifixed, inserted at mid-length of the corolla tube or above…………….……………………………………………………………….….………..7.
7.
Stigma papillate, entire, usually slightly clavate; stamens and apical glands always included in the corolla tube; style without secondary stigma...………....................................…***Exochaenium***
Stigma smooth, bilobed; stamens often exserted; style usually with a secondary stigma……………………………………………………………………….……………..***Sebaea***

The main morphological features distinguishing the genera of the tribe are summarized in Table 1.

Table 1. Morphological characters of *Exochaenium* and related genera of the tribe Exaceae.



	anthers opening by pores	papillose stigma	distyly	shruby plant	presence of a 5-lobed disk at the base of the corolla	presence of a secondary stigma	axillary flowers	hairy leaves	ovary truly bilocular	distribution
Exacum	1	0	0	0	0	0	0	0	1	Madagascar, Africa, Sri-Lanka, India, Socotra, Asia, and Australia
Exochaenium	0	1	1	0	0	0	0	0	1	Tropical Africa
Gentianotnamnus	0	0	0	1	1	0	0	0	0	Madagascar
Klackenbergia	0	0	0	0	0	0	1	0	1	Madagascar
Lagenias	0	0	0	0	0	0	0	0	1	South-Africa
Ornichia	0	0	0	0	0	0	0	1	1	Madagascar
Sebaea	0	0	0	0	0	1	0	0	1	Africa, Madagascar, Australia, New-Zealand, India, and Asia
Tachiadenus	0	0	0	0	0	0	0	0	0	Madagascar

TAXONOMIC TREATMENT OF *EXOCHAENIUM* GRISEB.

***Exochaenium* Griseb**., in DC. Prod. 9: 55 (1845). –Type: *Exochaenium grande* (E. Mey.) Griseb., in DC. Prod. 9: 55 (1845).

Description – Annual, erect or dwarf herbs, rarely parasitic or saprophytic. *Stems* simple or branched, usually tetragonal, more or less 4-ridged or winged. *Leaves* well-developed or reduced and scale-like, sessile, opposite, linear-lanceolate to suborbicular. *Flowers*, pedicellate, 5-merous, terminal, solitary, usually white, sometimes yellow (*E. brevicaulis*, *E. clavatum*, *E. caudatum*, *E. dimidiatum*, *E. leariformis*, *E. wildemanianum*) or salmon (*E. grande*), often pendant or inclined, generally with a stylar polymorphism (short- and long-styled flowers), sometimes with a reciprocal position of the anthers (*E. grande*, *E. oliganthum*). *Calyx* almost free or forming a short tube; lobes linear-lanceolate to ovate or obovate; dorsally keeled or winged. *Corolla* tube cylindrical or infundibuliform, the lower portion enlarged in fruit; lobes oblong-obovate, obtuse at the apex or acuminate; when the flower is white then the inner part of the corolla tube is more or less yellowish and striate. *Filaments* filiform, inserted at mid-length of the corolla tube. *Anthers* oblong, basifixed, included in the corolla tube, with a conspicuous apical stipitate gland, with or without 2 basal minute glands. *Ovary* ovoid or obovoid or almost globose, bilocular, placentas axile, ovules numerous. *Style* filiform, included, never with secondary stigmas (in contrast to *Sebaea s. str.*). *Stigma* straight or clavate, entire or very slightly bilobed (*E. grande*), rarely bifid (*E. pygmaeum*), papillate. *Capsule* ovoid or obovoid, membranous or coriaceous, septicidally bivalved. *Seeds* minute, very numerous, cubical; testa cells star-shaped (Fig. 2).

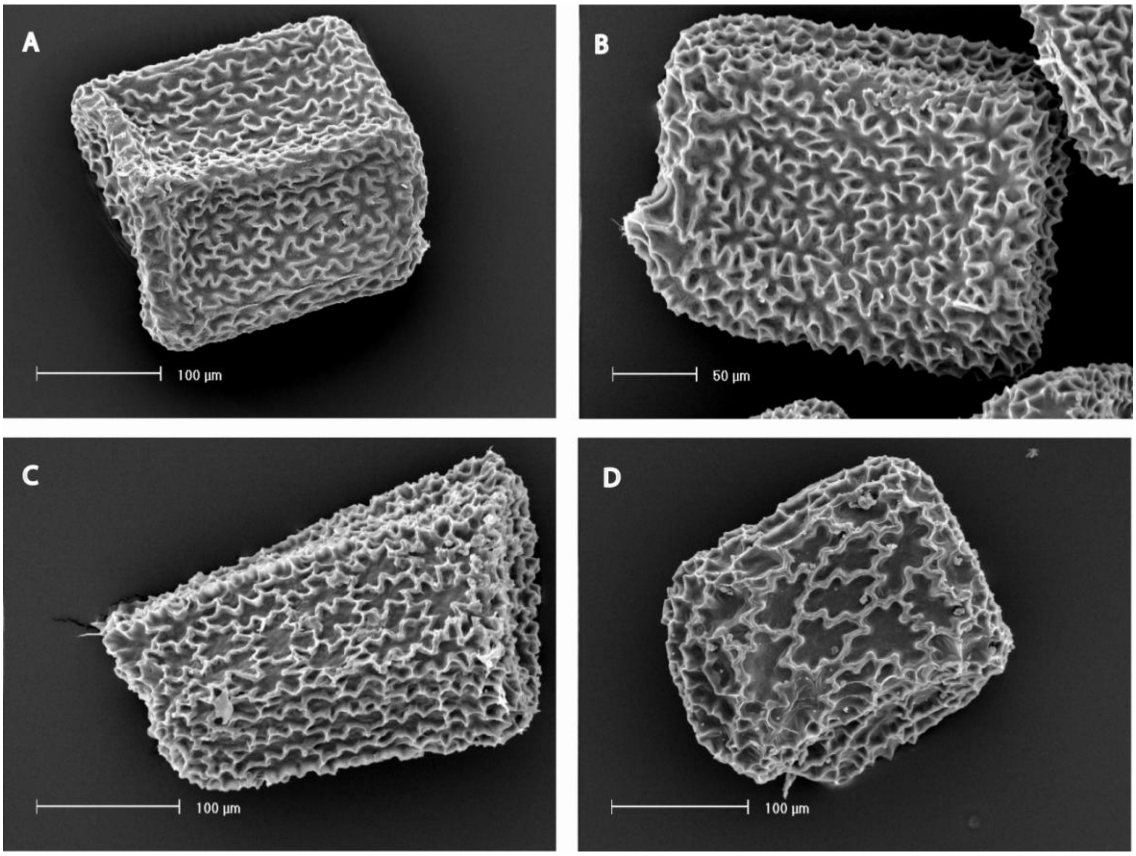


Fig. 2. Seeds of *Exochaenium* (SEM micrographs) showing polyhedric seeds with distinctly star-shaped testa cells. **A**, *Exochaenium macropterum* (*Bidgood et al., 4012*) **B**, *E. teucszii* (*Dessein et al., 701*), **C**, *E. clavatum* (*Dessein et al., 543*) **D**, *E. oliganthum* (*Dessein et al., 499*).

Remark – Twenty three species, all endemic to Africa, are recognized here. Most of the species occur on the Katanga plateau (Angola, Congo, and Zambia) many extending their distribution in the Sudano-Zambesian domain sensu White (1986) (Fig. 3), except *E. oliganthum* distributed in the Guineo-Congolian region sensu White (1986). The current species number might be underestimated, due to the very few available collections from countries of Tropical Africa presenting favorable ecological environments for most *Exochaenium*.

Fig. 3. Distribution of *Exochaenium*. Grey pattern shows the total distribution of the genus.

Affinities – *Exochaenium* differs from *Sebaea* by several morphological features. The seeds of *Exochaenium* have star-shaped testa cells (Fig. 2), while *Sebaea* has seeds with testa cells ± rectangular aligned in rows. The stigma of *Exochaenium* is papillate and entire, or very slightly bilobed (*E. grande*) while the stigma of *Sebaea* is smooth and clearly bilobed (exception: *S. thomasii*, *S. spathulata*). Most species of *Sebaea* have a secondary stigma on the style (Hill, 1913; Marais and Verdoorn, 1963), except, *S. thomasii* and *S. minutiflora* while *Exochaenium* never has such features. The stamens of *Exochaenium* are always inserted at the mid-length of the corolla tube, while the stamens of *Sebaea* are usually near or in the corolla sinus (exception: *S. thomasii*, *S. exacoides*, *S. minutissima, S. membranacea*).

Exochaenium differs from *Lagenias* by its seed morphology (star-shaped testa cells versus polygonal testa cells), by its anther fixation (basifixed versus medifixed), and also by its habit (*Lagenias* presents a crassulescent habit).

Exochaenium can be easily distinguished from *Tachiadenus*, by it ovary structure (bilocular for *Exochaenium* versus pseudobilocular (Klackenberg, 1987) for *Tachiadenus*), and its distribution (tropical Africa for *Exochaenium* versus Madagascar for *Tachiadenus*). The long corolla tube and the robust aspect of the flowers of *Tachiadenus* are also useful to distinguish between the two genera. However some species of *Exochaenium* (*E. teucszii*, *E. chionanthum*) present also rather long corolla tubes.

The remaining genera of tribe Exaceae (*Exacum*, *Gentianothamnus*, *Klackenbergia*, and *Ornichia*) are easily distinguished from *Exochaenium*. *Exacum* has poricidal anthers, and are usually blue-purple flowers, while *Exochaenium* has anthers dehiscing by slits, and generally white flowers. *Gentianothamnus* is a subshrub up to 2 m in height, endemic to Madagascar, while *Exochaenium* species are all herbs. *Gentianothamnus* is also characterized by the presence of a shallowly 5-lobed disk, supporting the ovary (Klackenberg, 2002), a feature that is absent in *Exochaenium* species. *Klackenbergia* species, endemic to Madagascar, with their unique spike-like inflorescence are distinguishable at a first glance. *Ornichia*, also endemic to Madagascar, has hairs on the leaves, while *Exochaenium* species are glabrous.

Key to the species of *Exochaenium* Griseb.

1.
Achlorophyllous, saprophytic or rarely parasitic plant ………………..…….. 16. *E. oligantham*
Chlorophyllous plant…………………………………………………...……….………..2.

2.
Plant small (<0.8 mm)………………………..……………..……….…… 17. *E. perparvum*
Plant taller >1 cm……………………………………………………….…………..…..……3.

3.
Basal rosette present from which several long pedicellate (c. 50mm) flowers develop ………………………………..…………………………………………..……………….…..…4.
Basal rosette absent……………………….................……………………………….....……5.

4.
Pedicel and calyx hyaline when dry; calyx narrowly winged (<0.3 mm wide)…….. 13. *E. hockii*
Pedicel and calyx green when dry; calyx conspicusly winged (1.9-2.5 mm wide)………………………………………………………………….... 15. *E. macropterum.*

5.
Corolla tube cylindrical, not exceeding 3 millimeters in diameter, 15-35 mm long; flower white…………………………………………………………………………………..6.

Corolla tube infundibuliform, 3-15 mm long, when longer: flower yellow or salmon....………7.

6.
Corolla 20-45 mm in diameter……... 22. *E. teucsziii*
Corolla 5-10 mm in diameter.. 5. *E. chionthatum*

7.
Plant with the basal leaves oriented downwards, and adhering to the stem when dried………..8.
Plant with basal leaves normally oriented upwards or horizontally, not adhering to the stem when dried…………………………………………………………………………..……...9.

8.
Anthers tailed at the base, not having basal glands……………………...………. 4. *E. caudatum*
Anthers not tailed at the base, having two basal glands…………………….……. 6. *E. clavatum*

9.
Pedicels long (>50mm) or much longer than the internode below……………….…………10.
Pedicels short (<50mm), equal to or shorter than the internode below……….....................…11.

10.
Calyx wing 1-1.5 mm wide; anthers 1.3 mm long……………..……..……… 3. *E. brevicaulis*
Calyx wing 0.3-0.8 mm wide; anthers 0.7-1mm long………………...… 10. *E. fernandesianum*

11.
Stigma bifid…………….. 21. *E. pygmaeum*
Stigma entire………........…..…....12.

12.
Corolla 15- 30 mm in diameter..13.
Corolla <10 mm in diameter...17.

13.
Calyx wing narrow, c.0.5 mm broad; cauline leaves linear to lanceolate. ….. 14. *E. leariforme*
Calyx wing 1-2 mm broad and wider near the base; cauline leaves ovate, elliptic to lanceolate……………………………………………………………………………….....14.

14.
Flowers bright yellow…………………………………………...………… 8. *E. dimidiatum*
Flowers salmon, rarely white………………………….……………………….……………15.

15.
Plant robust; flowers salmon rarely white, when so growing in South Africa; calyx segment dorsally broadly winged (1-2mm); style present; stamen inserted near the middle of the corolla tube, ….…………………………….………………………………………………......16.
Plant slender; flower usually white; calyx segment dorsally winged (up to 1 mm), style absent (stigma emerging directly from the ovary); stamen inserted near the base of the corolla tube, ……………………………………………………………………..……... 1. *E. alatum*

16.
Plant tall (>15 cm); stem not branching at the base. ..……………….…………… 12. *E. grande*
Plant small (<15 cm), stem branching at the base.……………………..… 19. *E. primulaeflorum*

17.

Plant filiform, taller than 20 cm; stem usually unbranched, bearing a single small flower; leaves linear, often as large as the stem……………………..……………………...… 2. *E. baumianum*
Plant slender, smaller than 20 cm; stem usually branched, with a few flowers (1)-3-(5); leaves linear to elliptic, larger than the stem...……………………………………………………...18.

18.
Anthers without basal glands; calyx wing 0.7-1.2 mm; flowers always inclined to pendulous………………………………………………………………..… 18. *E. platypterum*
Anthers with or without basal glands; calyx wing 0.25-0.5 mm; flowers usually erected, or slightly pendent..……………………………………………………………….……………19.

19.
Herbs 5-15(20) cm high; cauline leaves linear to lanceolate…………………….……….……20.
Herbs 1.5 -4 (6) cm high; cauline leaves ovate to elliptic....…………………………….………..21.

20.
Corolla tube slightly longer than or as long as the calyx; anthers without two basal glands ……..………………………………………………………………………..….. 11. *E. gracile*
Corolla tube longer than the calyx; anthers with two minute and globose basal glands……..…………………………………………………………….…..... 9. *E. exiguum*

21.
Leaves markedly 3 nerved, petiolate; flower yellow..……………...……. 23. *E. wildemanianum*
Leaves 1 nerved, sessile; flower white……………………………………….….…..……....22.

22.
Corolla lobes c. 1/5 of the corolla tube in length.…………………...............….. 20. *E. pumilum*
Corolla lobes longer than half the size of the corolla tube…………………….……. 7. *E. debilis*

New combinations in *Exochaenium* Griseb.:

1. ***Exochaenium alatum*** (Paiva & Nogueira) Kissling, **comb. nov.** ≡ *Sebaea alata* Paiva & Nogueira, in Anal. Jard. Bot. Madrid **47**, 1: 96, Fig. 5. (1990). – Type: Zambia, Chishinga Ranch, near Luwinga, 27 April 1961, *Astle 545* (Holotype: K!).

Distribution – Zambia, in dambo, March-April

Remark – This species is morphologically related to *E. exiguum*, from which it differs by the size of the whole plant (c. 10-17cm for *E. alatum* versus c. 3.5-10 cm for *E. exiguum*), the flowers, (c. 1.5 cm long for *E. alatum* versus c. 1 cm long for *E. exiguum*), and the plant aspect (slender for *E. exiguum*, versus more robust for *E. alatum*).

Due to the intensive record of hybridization within the Gentianaceae as a whole (Pringle, 1965; Zeltner, 1978; Ubsdell, 1979; Guggisberg et al., 2006; Riesman et al., 2006), reticulation patterns, including introgression or hybridization, might explain the overlapping of some selected characters (e.g. plant robustness, size of the calyx wing, leaf shap)

2. ***Exochaenium baumianum*** (Gilg) Schinz, in Bull. Herb. Boiss. Ser. II. vi. 716 (1906). ≡ *Belmontia baumiana* Gilg, In Baum, Kunene. – Samb. – Exped. 331 (1903). ≡ *Sebaea baumiana* (Gilg) Boutique, in Fl. Afr. Centr., Gentianaceae: 46 (1972). – Type: Angola, am Longa oberhalb des Quirri, an Sumpfrändern, 1250m, *Baum 692* (Holotype: K!; Isotype: Z!).
Distribution – Angola, Southern Congo-Kinshasa, Zambia. It grows in damp soil, among grasses. Flowering time: (December)-February-March-(April)
Remark – This species is morphologically related to *E. teucszii* and *E. chionanthum*, from which it differs by the size of the flowers (> 1cm for E. baumianum, < 1.5 cm for *E. teucszii* and *E. chionanthum*).

This species has been poorly collected; however it is present nearly in all dambos visited by the author. This might be explained, by its extremely slender, filiform habit, rendering it difficult to spot among grasses.

3. ***Exochaenium brevicaulis*** (Sileshi) Kissling, **comb. nov.** ≡ *Sebaea brevicaulis* Sileshi, Kew Bull. 55, 1: 213-214, Fig. 1. (2000). – Type: Tanzania, Mapanda Distr., 56 km on Uvinza – Mpanda road, 27 May 1997, *Bidgood, Sitoni, Vollesen & Whitehouse 4121* (Holotype: K!).
Distribution – Only known from the type, Tanzania, Mpanda District, on Uvinza-Mpanda road. The label indicates that it grows in seepage areas, in wet grasslands. Flowering time: May
Remark – see *E. fernandesianum*

4. ***Exochaenium caudatum*** (Paiva & Nogueira) Kissling, **comb. nov.** ≡ *Sebaea caudata* Paiva & Nogueira, in Anal. Jard. Bot. Madrid 47, 1: 89-92, Fig. 2. (1990). – Type: Zambia, Mapanza, *E. Robinson 1251* (Holotype: K !).
Distribution – Only known from the type: Zambia, Mapanza, Simansunda Dambo. It was indicated as growing in Dambo. Flowering time: April
Remark – Despite recent joint efforts, this species has only been collected once.

This species is morphologically related to *E. clavatum*, from which it differs only by its caudate anthers.

Fig. 4. Selected species of *Exochaenium*. **A-B**, *E. grande*; **C** & **G**, *E. teucszii*; **D**, *E. exiguum*; **E**, *E. clavatum*; **F**, *E. fernandesianum*; **H**, haustorium of *E. oliganthum*; **I**, Root system of the minute *E. perparvum*; **J**, a typical dambo area where *Exochaenium* species could be found. Here is the type locality of *E. fernandesianum*; *E. baumianum* is found in sympatry in the same locality (Zambia, Zambezi rapids near Kalene Hill) (all photography by the author).

5. ***Exochaenium chionanthum*** (Gilg) Schinz, Schinz in Bull. Herb. Boiss., Sér 2, **6**: 716 (1906). ≡ *Belmontia chionantha* Gilg in Baum, Kunene.-Samb.-Exped.: 332 (1903). – Type: Angola, am Lazingna, auf Moorboden zwischen Gras, ziemlich selten, 1300 m, 19 apr. 1900, *Baum 838* (Lectotype: Z!). A Lectotype is chosen here among the three paratypes (Art. 9.10) viz. *Baum 316*, *Baum 702*, and *Baum 838*

= *Exochaenium gentilii* De Wild. , in Ann. Mus. Congo, Bot., Sér. 5, 2: 336 (1908). ≡ *Sebaea gentilii* in Fl. Afr. Centr., Gentianaceae: 48 (1972). ≡ *Belmontia gentilii* De Wild in Th. & H.

Dur., Syll.: 373 (1909). – Type: République Démocratique du Congo, Bas-Katanga, Kanda Kanda, May 1902, *Gentil s.n.* (Holotype : BR?)

Distribution – Angola, Democratic Republic of Congo, Sierra-Leone. Flowering time: January, (May), (September).

Remark – This species is related to *E. teucszii*, from which it differs by the size of its corolla diameter (5-10 mm for *E. chionanthum* versus 20-45 mm for *E. teucszii*).

Although the ecology of both species is in some point similar, the distribution of *E. chionanthum* seems to be restricted to the western part of the Soudano-Zambesian region, while *E. teucszii* has a widespread distribution

6. ***Exochaenium clavatum*** (Paiva & Nogueira) Kissling, **comb. nov.** ≡ *Sebaea clavata* Paiva & Nogueira, in Anal. Jard. Bot. Madrid 47, 1: 97, Fig. 6. (1990). – Type: Zambia, Mbala, the Dambo, 15 miles along the road from Senga Hill to Mporokoso, fl. & fr. 08 June 1956, *E. Robinson 1736* (Holotype: K!).

Distribution – Zambia. Growing in *c.* 30 cm water, in swamp or along inundated lake shores. It is only known from four localities. Flowering time: March-April

Remark – This species together with *E. caudatum* forms a clearly distinct group. Both are easily recognizable by their size (c. 50 cm), and their bright yellow flowers. In herbaria, the basal leaves are massed together with the stem, and the roots are reddish. *Exochaenium clavatum* might be the only species of the genus that grows in water (helophyte). More information concerning the potential ecology niche of its related species *E. caudatum* is needed. Some species like *E. teucszii* can also sometimes grow in c. 20 cm water, but it is not the ecological preference of this species. The red roots of *E. clavatum* and *E. caudatum*, are most probably not related with the helophyte habit as they are also found in a few other *Exochaenium* (e.g. *E. platypterum*).

7. ***Exochaenium debile*** Welw., in Trans. Linn. Soc. 27: 48 (1869). ≡ *Sebaea debilis* (Welw.) Schinz, in Bull. Herb. Boiss., Sér. 2, **6**: 734 (1906) ≡ *Belmontia debilis* (Welw.) Benth. & Hook. In Viertelj. Nat. Gesellsch. Zürich, **36**: 332 (1891) ≡ *Parasia debilis* (Welw.) Hiern, in Cat. Welw. Afr. Pl. 1, **3**: 708 (1898). – Type: Angola, Pungo Andongo, *Welwitsch 1511* (Holotype: K!).

Distribution – Angola and Namibia (Caprivi), growing in savannah, on quartzic soil, March-April.

Remark – This minute species is closely related to *E. pygmaeum*, from which it differs by its stigma (entire for *E. debile* versus bifid for *E. pygamaeum*). *Exochaenium debile* also resembles

E. pumilum, but can be distinguished from the latter by its longer corolla lobes (c. 1/5 of the corolla tube for *E. pumilum* versus c. 1/3 for *E. debile*)

8. ***Exochaenium dimidiatum*** (Sileshi) Kissling, **comb.nov.** ≡ *Sebaea dimidiata* Sileshi, in Kew Bull. 55, 1: 214-217, Fig. 2. (2000). – Type: Tanzania, Manyoni Distr., Kazi Kazi, near Lambo ya Mulengali, 17 May 1932, *Burtt 3632* (Holotype: K!).
Distribution – Tanzania, marshy sites in grassland. Flowering time: June-September.
Remark – By its particularly large flowers this species resembles *E. grande* and *E. primulaeflorum*, but differs from both by its flower color (yellow), and the more fragile aspect of the petals. The stem is also somewhat winged.

9. ***Exochaenium exiguum*** A. W. Hill, in Kew Bull. 1909: 50 (1909). ≡ *Sebaea minuta* Paiva et Nogueira, in Anal. Jard. Bot. Madrid 47, 1: 99 (1990). –Type: Zimbabwe, Bulawayo, *F. Eyles & W. Johnson 1032* (Holotype: GRA; Drawing from the type: K!)
= *Sebaea africana* Paiva & Nogueira, in Anal. Jard. Bot. Madrid **47**, 1: 94, Fig. 4. (1990). – Type: Zambia, Kwambwa-Mbereshi Rd., fl. & fr. 19. April. 1957, *M. A. Richards 9337* (Holotype: K!).
Distribution – Widespread in the Zambesian region. In dambo, damp open grassland, stream banks on sandy soil. Flowering time: depends on the rain season, usually c. one month after the end of the raining season.
Remark – Paiva & Nogueira, when describing the new species *S. africana*, only compared their new species to *E. pumilum*, and not with *E. exiguum*. From the type of *S. africana*, one can only retain that the plants are somewhat smaller than *E. exiguum*. During a field trip in Zambia, I visited the locality of the type species of *S. africana*, and found that this plant was growing abundantly in the whole region, showing sometimes extreme range of variation, especially in size, within each population. As I am not able to find any differences between *E. exiguum* and *S. africana*, I believe that it is in fact a single species, and therefore synonymise *S. africana* with *E. exiguum*.

10. ***Exochaenium fernandesianum*** (Paiva & Nogueira) Kissling, **comb. nov.** ≡ *Sebaea fernandesiana* Paiva & Nogueira, in Anal. Jard. Bot. Madrid **47**, 1: 92, Fig. 3. (1990). – Type: Zambia, Mwinilunga, Zambesi Rapids, c. 6 km. N. of Kalene Hill, *Hooper & Townsend 256* (Holotype : K !)
Distribution – Tanzania and Zambia, in damp soil over rocky outcrops. Flowering time: February-May.

<u>Remark</u> – This species is closely related to *E. brevicaulis*, but differs in the size of the calyx wing and the anthers. Due to the lack of available material, I was not able to check the morphological variability of this group of species and therefore cannot make a decision to merge *E. brevicaulis* with *E. fernandesianum*. In doubt I maintain both species.

11. ***Exochaenium gracile*** (Welw.) Schinz, in Bull. Herb. Boiss., sér. 2, **6**: 716 (1906). ≡ *Belmontia gracilis* Welw., in Trans. Linn. Soc. 27: 47 (1869). ≡ *Parasia gracilis* (Welw.) Hiern, Cat. Pl. Welw. 1: 708 (1898). ≡ *Sebaea gracilis* (Welw.) Paiva & Nogueira nom. illeg., in Anal. Jard. Bot. Madrid **47**, 1: 100 (1990), non *Sebaea gracilis* Spreng., in Syst. 4 Cur. Post. (1827), 338. – Type: Angola, Huila, river Monino, Apr. 1860, *F. Welwitsch 1524* (Holotype: K!)

= *Belmontia luteo-alba* A. Chev., in journ. de Bot. xxii. 119 (1909). ≡ *Sebaea luteo-alba* (A. Chev.) P. Taylor, in Hutchinson & Dalziel, Fl. W. Trop. Afr., ed. 2, ii. 298 (1963); and in Taxon, xii. 294 (1963). –Type: Guinea, Fouta Djalon, Sept. 1907, *Chevalier s.n.* (Holotype: P, isotype: K!)

<u>Distribution</u> – Angola, Zambia, also found in West Africa. In dambo, marsh soil and shallow soil over rocks. Flowering time depends on the raining season, usually c. one month after the end of the raining season.

<u>Remark</u> – I found no differing morphological characters to support *E. luteo-album* as a distinct species from *E. gracile*. *Exochaenium gracile* is closely related to *E. exiguum*, but differs by the absence of basal glands on the anthers.

12. ***Exochaenium grande*** (E. Mey.) Griseb. in DC., Prodr. 9: 55 (1845). ≡ *Sebaea grandis* (E.Mey.) Steud. in Nom. Bot., ed. 2, 4: 550 (1841). ≡ *Belmontia grandis* E. Mey., Comm. 183 (1837). ≡ *Parasia grandis* (E. Mey.) Hiern, in Cat. Welw. Afr. Pl. 1, 3: 707 (1898). ≡ in part, *Exochaenium macranthum* Hill., Kew Bull. 339 (1908). – Type: South Africa, Cape, between Umtentu R. & Umzimkulu R., *Drege s.n.* (Lectotype: K!, design by Sileshi, 2002)

= *Exochaenium grande* var. *major* (S. Moore) Schinz, in Bull. Herb. Boiss. Sér. 2, **6**: 802 (1906). ≡ *Parasia grandis* var. *major* S. Moore, in Journ. Bot. 40: 384 (1902). –Type: South Africa, Transvaal, Pienaarsport, *Pateshall Thomas s.n.* (Holotype: BM!)

= *Belmontia natalensis* Schinz, in Bull. Herb. Boiss. Sèr. 2, 220, (1894). ≡ *Sebaea natalensis* (Schinz) Schinz, Bull. Herb. Boiss. Sér. 2, **6**: 736 (1906) non Schinz (1895). = *Exochaenium grande* var. *homostylum* Hill, in Kew Bull 1908: 338 (1908). – Type: South Africa, Natal, Clairmont, *Schlechter 3060*. (Holotype: K)

= *Belmontia zambesiaca* Baker., Kew Bull. 25 (1894). ≡ *Sebaea zambesiaca* (Baker) Schinz in Bull. Herb. Boiss. Sér. 2, **6**: 744 (1906) – Type: Zambia, in the valley of the Leshumo, *Holub s.n.* (Holotype: K)

Distribution – Widespread throughout tropical Africa, from Ethiopia to north-eastern South Africa, and from West Africa (Guinea-Conakry) to Kenya. Wide type of habitat, usually growing in open grassland, among rocks and clayey or sandy soil. Flowering time depends on the raining season, usually c. one to two months after the end of the raining season.

Remark – This well-defined species is of particular interest because its floral color can vary between areas, from pure white to bright yellow, but most of the plants have salmon flowers. The flower morphology variation was studied by Hill (1908), who demonstrated the stylar polymorphism of that species. Two “forms” of stylar polymorphism occur within the species: in the first “form” the anthers remain at the same level inside the corolla tube, while in the second “form”, there is a reciprocal position of the anthers, which are connate with each together, in the long-styled form. Currently no work has been performed to demonstrate the distyly/heterostyly of this interesting species.

13. ***Exochaemium hockii*** (De Wild) Kissling, **comb. nov.** ≡ *Belmontia hockii* De Wild., in Bull. Jard. Bot. Etat, Brux., 3: 266 (1911). ≡ *Sebaea hockii* (De Wild) Boutique in Fl. Afr. Centr., Gentianaceae: 48 (1972). –Type: Congo, Katanga, Monts Kundelungu, June 1910, *Hock s.n.* (Holotype: BR!)

Distribution – Congo-Kinshasa (Haut-Katanga), open grassland, on quartzic soil, Flowering time: found in March and September

Remark – This remarkable species is recognizable at a first glance. It has basal leaves in a rosette surmounted by many long-pedicellate flowers, and its small size is unique within the genus. In herbarium specimens, the calyx and pedicels are hyaline.

14. ***Exochaenium leneariformis*** (Sileshi) Kissling, **comb. nov.** ≡ *Sebaea leneariformis* Sileshi, in Kew Bull. 54, 1: 193, Fig. 2. (1999). –Type: Tanzania, Nkansi Distr.: 5 km N of Namanyere, 2 May 1997, *Bidgood, Sitoni, Vollesen & Whitehouse 3630* (Holotype: K!)

Distribution – Western Tanzania, seepage areas in grassland or woodland, flowering time: May-Aug.

Remark – This species resembles *E. alatum*, but the leaves are much broader and longer. The flowers are yellow (versus white for *E. alatum*), and the calyx is narrowly winged (versus broadly winged and larger at the base for *E. linearoformis*).

15. ***Exochaenium macropterum*** (Sileshi) Kissling, **comb. nov.**, ≡ *Sebaea macroptera* Sileshi in Kew Bull. 54, 1: 191-193, Fig. 1. (1999). Type: Tanzania, Mpanda Distr.: 48 km on Mpanda-Uvinza road, 18 May 1997, *Bidgood, Sitoni, Vollesen & Whitehouse 4012* (Holotype: K!)

Distribution – Western Tanzania, Mpanda District, only known from the type, Shallow soil over rocks in *Barchystegia* woodland, flowering time: May.

Remark – *E. macropterum* shares with *E. hockii* a basal rosette, but has a green peduncle and calyx (versus hyaline for *E. hockii*)

16. ***Exochaenium oliganthum*** (Gilg) Schinz, in Bull. Herb. Boiss., Ser 2, **6**: 736 (1906). ≡ *Belmontia oligantha* Gilg, in Bot. Jahrb. **26** : 102 (1898). ≡ *Sebaea oligantha* (Gilg) Schinz, in in Bull. Herb. Boiss., sér. 2, **6**: 736 (1906). – Type: Gabun, Sibange-Farm, auf einem steinhart getretenen Fusspfade unter Ölpalmen wachsend; im Januar 1880 blühend, *H.Soyaux s.n.* (Holotype: B, delet.). – NeoType: Kamerun, im Urwalde bei Moliwe, Aug. 1905, *R. Schlechter* 15721 (Holotype: P!; Isotype: K!, design by A. Raynald, 1967).

= *Sebaea Mildbraedii* Gilg, in Wiss. Ergebn. 2. Deutsch. Zentr.-Afr. Exped. 1910-11. **2**: 98 (1922). – Type: *Mildbraed* 6048 (Holotype: B, delet.)

= *Exochaenium Evardii* A.Rob., in Bull. Jard. Bot. Etat Bruxelles **32**: 472 (1962). -Type: *Evard* 6265 (Holotype: BR!)

= *Exochaenium bugandense* A. Rob., in Bull. Jard. Bot. Etat Bruxelles **32**: 473 (1962). -Type: *Dawkins 695* (Holotype: K!)

= *Belmontia sp*., in Andrews, Fl.Pl. Sudan **3**: 64 (1956). –Type: not defined

Note: Isotype of *H.Soyaux s.n.* were not found by A. Raynal in BM, C, FI, G, K, HBG, W, Z. An isotype was also searched in the following herbaria without success by the author: BR, S, MO, PRE, NEU, BOL, Dar es Salam, Lusaka.

Distribution – The distribution is mainly following the Guineo-Congolian region. It is found in forest among dead leaves, or in moist places. It has also been collected in the Sudano-Zambesian region, and is found in moist site amongs grasses. Flowering time: throughout the year.

Remark – The ecology and particular morphology of this species is described by Raynal (1967). It is said to be a saprophyte, but was often found to parasitize roots of other species (mainly Cyperaceae or Poaceae). The species is achlorophyllous, however I found within a population some green individuals (*Dessein et al. 499*, NEU). No studies focusing on the transition between saprophytism to parasitism in *E. oliganthum* have been currently performed. It might involve similar transition as that know for some Orchidaceae species (Julou et al., 2005). Nemomissa (2002) said that the phyllotaxy of this species tends to be alternate as in certain other Gentianaceae representatives. I agree with his observation.

17. ***Exochaenium perparvum*** (Sileshi) Kissling, **comb. nov.** ≡ *Sebaea perparva* Sileshi, in Fl. Trop. E. Afr., Gentianaceae (2002). Type: Tanzania, Ufipa District, Sumbawanga, Mbizi Mts, 29 April 1997, *Bidgood et al. 3586* (Holotype: K!)

Distribution – Democratic Republic of the Congo, Tanzania, Zambia. This species probably has a wide Zambesian region distribution like most *Exochaenium* species, but due to its small size (< 8 mm) it has probably not been found often. Growing on black clay in damp grassland, or

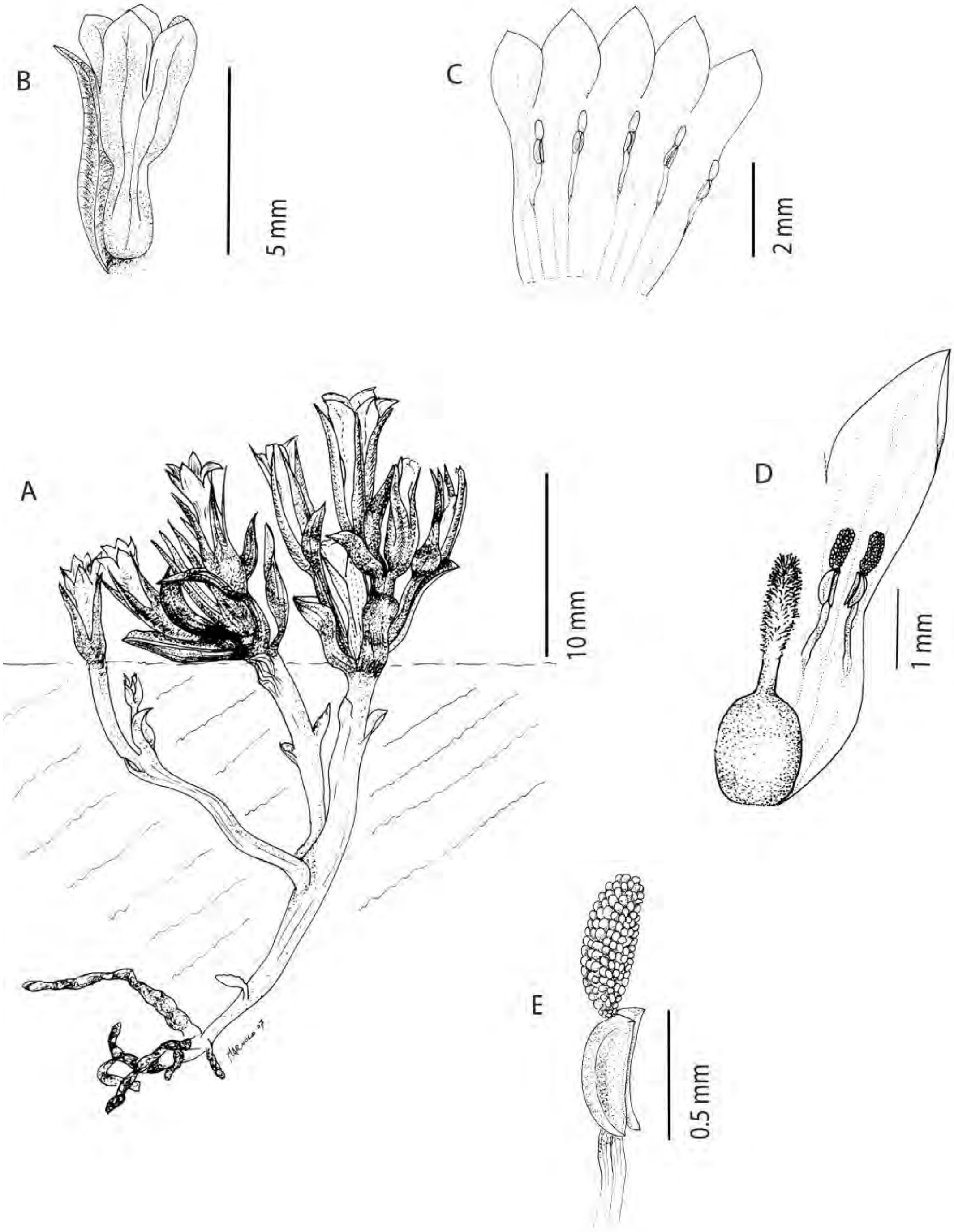


Fig. 5. *Exochaenium perparvum*. **A**: habit; **B**: flower with one sepal; **C**: corolla opened out; **D**: an opened corolla view; **E**: anther. From *Dessein, Archer, Kissling, Luwingu & Tembo 728* (NEU, BR). Illustrated by Maeva Arnold.

temporarily inundated grassland. It has once been found on lateritic soil (*Dessein et al. 851*, NEU). Flowering time: (June)-March-April

Remark – This dwarf species forms very small mat of a few flowers. Its impressive underground shoot system (Fig. 4 & 5) is unique within the genus. It can be easily distinguished from the two other dwarf species of the genus. It has an entire stigma (versus bifid stigma in *E. pygmaeum*) and is chlorophyllous (versus achlorophyllous for *E. oliganthum*).

18. ***Exochaenium platypterum*** (Baker) Schinz, in Bull. Herb. Boiss., sér. 2, **6**: 716 (1906). ≡ *Sebaea platyptera* (Baker) Boutique in Fl. Afr. Centr., Gentianaceae: 48 (1972). ≡ *Belmontia platyptera* Baker in Kew Bull. **1894**: 25 (1894). ≡ *Parasia platyptera* (Baker) Hiern in Cat. Pl. Afr. Welw. **1**, 3: 706 (1898). –Type: Angola, Province Huila, *Welwitsch 1524* (Holotype: K! – but see note)

Note: Hiern (Cat. Pl. Afr. Welw. 1, 3: 706 (1898)) states that the no. 1524 on the sheet is a mistake-it should read 1512. We agree with him. *Welwitsch 1524* is the type of *E. gracile.*

Distribution – Zambesian region, growing in damp soil, usually in open forest. Flowering time: March-April (June)

Remark – This species is easily recognizable from the rest of the *Exochaenium* species, by its pendant, very small flowers, with a conspicuously winged calyx.

19. ***Exochaenium primulaeflorum*** Welw., in Trans. Linn. Soc. London 27: 47 (1869) ≡ *Sebaea primulaeflora* (Welw) Sileshi in Kew Bull. 55, 1: 217 (2000). ≡ *Belmontia primulaeflora* (Welw.) Engl., in Abh. Königl. Wiss. Berlin 2: 336 (1892). ≡ *Belmontia primulaeflora* sensu Schinz, in Vierteljahrsschr. Naturf. Ges. Zürich 37: 332 (1896). ≡ *Exochaenium primulaeflorum* var. *nanum* Welw., in Trans. Linn. Soc. London 27: 48 (1869) ≡ *Parasia primuliflora* var. *nana* (Welw.) Hiern, in Cat. Afr. Pl. 1: 708 (1896). –Type: Angola, Huilla, towards Humpata, *Welwitsch 1513* (Lectotype: K!, designated by Sileshi (Nemomissa, 2000)).

Distribution – Angola

<u>Remark</u> – This species resembles *E. grande*, but differs by its general habit. The plant is smaller, branched from the base, and the inflorescence is formed of many flowers (5-10), while *E. grande* has a typical erect habit, with a robust stem, surmounted by solitary flowers.

20. ***Exochaenium pumilum*** (Baker) Hill, in Kew Bull. **1908**: 336 (1908). ≡ *Belmontia pumila* Baker in Kew Bull. **1894**: 25 (1894). ≡ *Sebaea pumila* (Baker) Schinz in Bull. Herb. Boiss., Sér. 2, **6**: 731 (1906). –Type: Nigeria, swamp at Nupe, near mouth of Niger, *Barter 1680*, (Holotype. K! –but see note)

Note: in the description of the new species, Baker, defines the type species, as "Swamps. Near the mouth of Niger, *Barter*". It is clear that Baker was making reference to the herbarium sheet quoted as *Barter 1680*.)

<u>Distribution</u> – Angola, Democratic republic of Congo, Kenya, Mozambic, Nigeria, Sudan, Tanzania, Uganda, Zambia. Widespread in the Sudano-Zambesian region.

<u>Remark</u> – see. *E. debile*

21. ***Exochaenium pygmaeum*** Milne-Redhead, in Kew Bull. **4**: 377 (1951). ≡ *Sebaea perpusilla* Paiva & Nogueira, in Anal. Jard. Bot. Madrid **47**, 1: 99 (1990). –Type: [Zambia], Mwinilunga, Sinkabalo Dambo, *E. Milne-redhead 3579* (Holotype: K!)

<u>Distribution</u> – Only known from the type, Zambia, Mwinilunga, Sinkabalo Dambo

<u>Remark</u> – see *E. debile*

22. ***Exochaenium teucszii*** (Schinz) Schinz, in Bull. Herb. Boiss. Sér 2, **6**: 716 (1906). ≡ *Belmontia teucszii* Schinz in Viertlj. Nat. Gesellsch. Zürich. **36**: 335 (1891). ≡ *Sebaea teuczii* (Schinz) Taylor, in Taxon **12**, 8:294 (1963). – Type: Angola, Malange, *Teucsz 387* (Holotype: B?; isotype: K!)

= *Belmontia teucszii* var. *angustifolia* De Wild. In Bull. Jard. Bot. Etat. Brux., **3**: 279 (1911). –Type: [Democratic Republic of Congo], Kundelungu, 13 March 1908, *Th. Kassner 2584* (Holotype: ?)

= *Tachiadenus continentalis* Baker in Kew Bull. 70 (1895). –Type: Zambia, Fwambo, 1894, *A. Carson 33* (Holotype: K!)

= *Belmontia mechowiana* Schinz, in Viertlj. Nat. Gesellsch. Zürich. 36: 333 (1891). ≡ *Exochaenium mechowianum* (Schinz) Schinz, in Bull. Herb. Boiss. Sér 2, **6**: 716 (1906). ≡ *Tachiadenus mechowianus* (Schinz) Hill, in Kew Bull. **1908**: 337 (1908). –Type: Angola, Benguella, bank of Hamba, near Gambo, Jul. 1880, *Mechow 503* (Holotype: Z!)

= *Belmontia chevalieri* Abbayes & Schnell in Bull. Soc. Bot. Fr. **96**: 204 (1950). –Type: Guinea, Fouta-Djalon, Chevalier s.n. (P)

Note: The protologue and all subsequent treatments use the epithet *teuszii*, and mention the collector as Teusz. Sileshi (2002) mentions that the correct spelling of the name is Teucsz, and so corrected the species name to teucszii (Article 60 of the Code)

Distribution – Distributed in the Sudano-Zambesian region. In swamps, inundated savannah, along lake shores, usually growing in very wet places. Flowering time: (January)-February-April-(June) in the Zambesian region; however this species was found to flower throughout the year. Its flowering time depends on water availability.

Remark – This species is related to *E. chionanthum*, but differs by its much larger flowers. It was once placed within the genus *Tachiadenus*, because of its long corolla tube (a few centimeters). This character is a convergence. *Tachiadenus* is endemic of Madagascar, while *Exochaenium* is endemic of Tropical Africa. There are very few morphological differences between the two genera. The ovary of *Exochaenium* is truly bilocular, while that of *Tachiadenus* species is pseudo-bilocular (Klackenberg 1985; 2002).

23. ***Exochaenium wildemanianum*** (Boutique) Kissling, **comb. nov.** ≡ *Sebaea wildemaniana* Boutique, in Bull. Jard. Bot. Nat. Belg., 41: 262 (1971). ≡ *Exochaenium wildemanianum* Gilg ex De Wild. In Ann. Mus. Congo. Bot., ser 5, 2: 336 (1908); ubique nomen nudum. –Type: République Démocratique du Congo, Kasai, rive du Kasai, *Luja 222* (Holotype: BR!)

Distribution – Southern Democratic Republic of the Congo, growing in marsh grassland, riverbanks. Flowering time: March

Remark – This species has marked 3-nerved leaves, like sometimes *E. grande*, but differs by it size (a few cm) versus 10-20 (30) cm for *E. grande*. The flowers of *E. wildemanianum* are pure white, while *E. grande* has usually salmon flowers.

Incertae Sedis

Sebaea species A sensu Sileshi, in FTEA, Gentianaceae, 21 (2002) – Kenya, Northern Frontier District: Moyale, 3 July 1952, Gillett 13475 (K!). This species has not been formally described and, according to the description, has to be placed in *Exochaenium*. It is, unlike any other *Exochaenium,* an annual herb of about 4 cm high. The stem is simple, leaves are sessile, the flower is 5-merous, yellow, the Filaments are very short and stamens are inserted into the corolla tube, the stigma is clavate, papillate. The material is insufficient to be described as a new species.

Distribution – Only known from the type: Kenya, Northern Frontier District, Moyale. Flowering time: July

Taxonomy and systematics of *Lagenias* E. Mey.

***Lagenias* E. Mey.**, in Comm. Pl. Afr. Austr. 186 (1837). –Type: *Lagenias pusillus* (Eckl. ex Cham.) E. Mey., in Comm. Pl. Afr. Austr. 186 (1837). ≡ *Sebaea pusilla* Eckl. ex Cham., in Linneaea 6: (1831).

Description – Annual, erect herbs (1.5-10 cm). *Stems* simple or branched, slightly 4-ridged or not. *Leaves* well-developed, sessile, opposite, ovate-lanceolate or elliptic, acute, somewhat fleshy. *Inflorescences* (1)-3-5-flowered, with relatively conspicuous bracts, *Flowers* pedicellate, 5-merous, terminal, solitary, yellow. *Calyx* free lobes long, linear-lanceolate. *Corolla* tube cylindrical, 5-10 mm long; lobes orbicular or elliptic-obovate, rounded or usually subacute at the apex. *Filaments* filiform, with a particular S-shape (Fig. 6), inserted in the corolla tube, at the upper level of the ovary. *Anthers* oblong, medifixed, included in the corolla tube, with an apical gland and with 2 basal glands. *Ovary* ovoid or obovoid or almost globose, bilocular, placentas axile, ovules numerous. *Style* long (c. 2 mm), filiform, included, never with secondary stigma. *Stigma* capitate, slightly bilobed. *Capsule* ovoid or obovoid, membranous or coriaceous, septicidally bivalved. *Seeds* minutes very numerous, cubical with polygonal testa cell.

Affinities – *Lagenias* can easily be distinguished from other genera of Exaceae, by both its medifixed anthers, and its seeds (cubical with polygonal testa cells). *Lagenias* appears to be more closely related to the genus *Sebaea* (chapter 2), as exemplified by a common bilobed stigma. *Lagenias* differs from *Sebaea* by its included stamen (versus exserted), and the absence of a secondary stigma on the style, which is a feature unique to *Sebaea*. It differs from *Exochaenium* by its stigma shape (bilobed versus entire for *Exochaenium*), and from *Exacum*, *Gentianothamnus*, *Klackenbergia*, *Ornichia*, and *Tachiadenus* by its yellow flower (versus white or blue-purple).

Lagenias pusillus (Eckl. ex Cham.) E. Mey., in Comm. Pl. Afr. Austr. 186 (1837). ≡ *Sebaea pusilla* Eckl. ex Cham. In Linneaea 6: 346 (1831). –Type: South Africa, Cape, Table mountain, *Ecklon s.n.* (Isotype: K!, C!)

= *Sebaea pusilla* var. *major* Hill in F.C. 4, 1: 1133. –Type: South Africa, Cape, Clanwilliam, *Leipoldt 654* (Holotype: K!)

Distribution – *Lagenias* is endemic of South-Western South Africa; it was found from the Table Mountain up to Nieuwoudtville and Calvinia and mostly grows in moist sand soil areas.

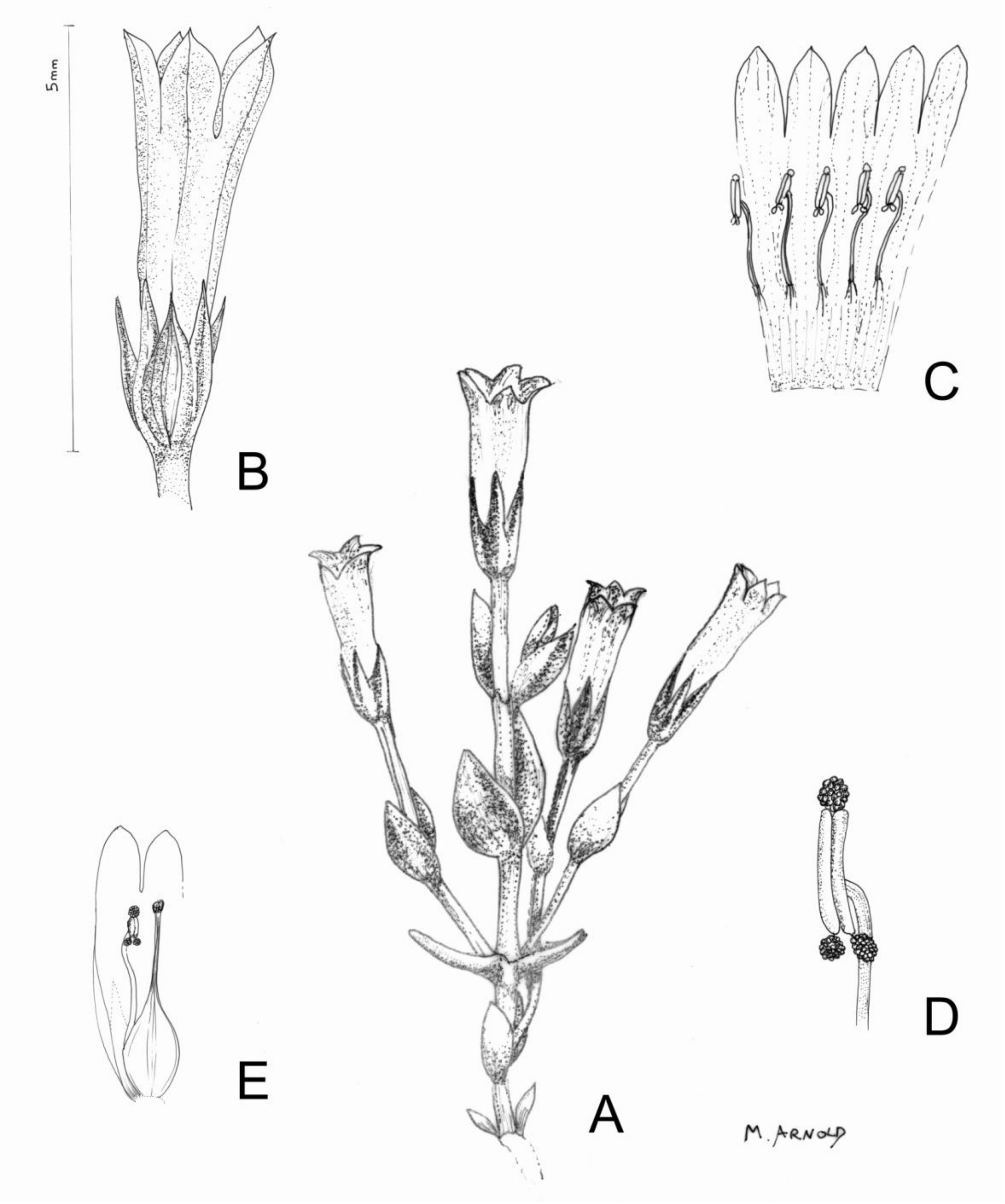


Fig. 6. *Lagenias pusillus*. **A**: habit; **B**: flower; **C**: corolla opened out; **D** respective position of the anthers and the stigma; **E** anther and glands details. From *Kissling J. & Zeltner L. 64* (NEU, BOL). Illustrated by Maeva Arnold.

Notes on *Belmontia* E.Mey. and *Parasia* Raf. as synonyms of *Sebaea* R.Br. ex Sol.

In my work on the African Exaceae, I have come to the conclusion that both *Belmontia* and *Parasia* (Tokyo Code, 1994), are inseparable from *Sebaea* and should be treated as synonyms of the latter (Chapter 2). Apomorphies delimitating *Sebaea* are seed shape and testa cell (chapter 2) and the presence of a secondary stigma (Hill, 1913). *Sebaea exacoides* (the type species of *Belmontia* and *Parasia*) share with all the remaining *Sebaea* the presence of a secondary stigma, and a general floral plus vegetative bauplan (e.g. annual herb, dichasial inflorescence, exserted basifix stamens). Furthermore recent molecular phylogeny, based on three chloroplastic markers (chapter 2), show that *S. exacoides* is nested deeply inside *Sebaea*. Therefore the genus *Belmontia* ≡ *Parasia* has to be treated as synonymous with *Sebaea*.

SEBAEA R.Br. Ex Sol.

Sebaea R. Br. Ex Sol., Prod. 451 (1810). –Type : *Sebaea ovata* (Labill.) R. Br., in Prodr. 451 (1810). ≡ *Exacum ovatum* Labill., in Novae Hollandiae plantarum 1. Paris 38 (1805)

= *Belmontia* E. Mey, in Comm. Pl. Afr. Austr. 183 (1837). ≡ *Parasia* Raf., in Fl. Tellur. 3: 78 (1836) (Tokyo Code, 1994). –Type: *Sebaea exacoides* (L.) Schinz, in Bull. Herb. Boiss., Sér. 2, **6**: 728 (1906) ≡ *Gentiana exacoides* L., in Sp. Pl. Ed. 2: 332 (1762). ≡ *Exacum cordatum* L.f., in Suppl. 124 (1781). ≡ *Sebaea cordata* (L.f.) Roem et Schult., Syst. Veg. 3: 161 (1818). ≡ *Parasia cordata* (L.f.) Rafin., in Fl. Tellur. 3: 78 (1836). ≡ *Belmontia cordata* (L.f.) E. Mey., in Comm. 183 (1837).

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INFRAGENERIC PHYLOGENY OF *SEBAEA S.STR.* (GENTIANACEAE – EXACEAE)

INFRAGENERIC PHYLOGENY OF *SEBAEA s.str.* (GENTIANACEAE: EXACEAE)

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Abstract – The present molecular phylogeny of *Sebaea* support fire well supported clades and a basal species: *S. sulphurea*. Each clade is supported by several characters (morphological, phenological or geographical), and a preliminary infrageneric classification is proposed. The phylogeny also revealed many species as polyphyletic. Floral morphological evolution and new insight on the biogeography of the genus are discussed.

Keyword – South Africa, Exaceae, Gentianaceae, *Sebaea*, phylogeny, systematics

INTRODUCTION

In the previous chapters, *Sebaea s. l.* has been revealed as polyphyletic, and was further split into four genera, based on molecular, karyological and morphological data. In the present chapter, we will focus on *Sebaea s.str.* The monophyly of a restricted genus *Sebaea* Sol. ex. R.Br. is highly supported by both molecular and morphological synapomorphies, including the presence of a secondary stigma on the style, seed shape, and testa cell arrangement

Sebaea Sol. ex R.Br. comprises c. 70 species, which are mainly distributed in continental Africa. Its taxonomic history is closely linked to that of *Exochaenium* and *Lagenias*, and the last monograph of the genus *Sebaea s. l.* was made one century ago by the Swiss botanist, Hans Schinz (Schinz, 1906). In his work, Schinz recognised both *Sebaea* and *Exochaenium* as distinct genera, with 92 species described for *Sebaea* and 8 for *Exochaenium*. Schinz further divided *Sebaea* into the two sections *Eusebaea* and *Belmontia*. *Eusebaea* was characterized by having exserted stamens versus included ones in *Belmontia*. *Eusebaea* was in turn divided into two subsections, T*etrandria*, and *Pentandria*, depending on the merism of the flowers.

Two years later, in the treatment of the Flora Capensis, Hill (1908, 1909) followed Schinz (1906) in generic circumscriptions, and accepted both *Sebaea* and *Exochaenium* as distinct genera. Hill (1908) also described ten new species, subspecies or varieties, while he synonymised some species of Schinz (1906), making a total of 71 *Sebaea* species for the Flora Capensis. Hill (1908) divided the genus into two sections, *Tetrandria* and *Pentandria* according to whether the flowers are tetramerous or pentamerous. The subsection P*entandria* of Schinz, was therefore elevated at the section rank and enlarged to include Schinz"s section *Belmontia*. Hill (1908) argued that the distinction between section *Eusebaea* and *Belmontia* "seems somewhat artificial" and divided this section into four subsections, and each again into several groups. Half a century later while working for the Flora of southern Africa, (Marais, 1961 and Marais and Verdoorn, 1963) merged *Exochaenium* with *Sebaea* arguing that the characters used to distinguish the two genera were unreliable (Marais, 1961). Marais (1961) for his treatment of the southern African *Sebaea*, considerably reduced the number of species, by synonymising most of them. However, he also described a new species. More recently, several new species for *Sebaea s. str.* have been described (Hilliard and Burtt, 1982, 1983; Oliver and Beyers, 2001).

Sebaea species are annual, biennial, or perennial herbs, with erect or, more rarely procumbent simple or branched stems. The roots are generally small and fleshy, but some species have a well developed tap-root. The inflorescence show considerable variation (e.g. dichasium, panicle, capitulum-like or solitary flowers). The corolla colour is generally yellow, but some species are white. In a few species the corolla is marked by orange-red lines, indicating the centre of the flowers (personal observation). The calyx-segments are keeled or more or less conspicuously winged. Furthermore, floral features are of particular interest, because of several reproductive particularities. The style is furnished with two secondary stigmas (Marloth, 1909; Hill, 1913), which in a few species are more or less confluent with the stigma (e.g., *S. micrantha*, *S. thomasii*). The stigma is usually exserted or more rarely included in the tube, but except in a few species (e.g. *S. thomasii*) is always situated above the anthers. The anthers may be situated in the sinuses of the corolla or in some rare cases deeply in the tube, and are usually furnished with apical, and sometimes also with paired basal glands. Those glands are usually yellow, but in several species they are black, brown, red or orange (Schinz, 1903; personal observation). It is also interesting to note that several species of *Sebaea* species have scented flowers. The variation of perfume is particularly striking in a small group of species where it is reminiscent of jasmine, clover, curry or rose frangrances (personal observation).

As far as we know, the phenology of *Sebaea* has never been studied. Our field observations as well as herbarium label data indicate that the flowering time varies consistently within groups of species. Species growing in the Cape flower mostly in September-November, while the species found in the Drakensberg flower either in October-December or March-May.

Sebaea are growing in a wide range of ecological environments, ranging from sea-level up to 3500 m in the Drakensberg. They can be found either in sandy places, along streams, in alpine meadows, within woodland or ericoid vegetation, or along road sides.

Sebaea are principally distributed in the Cape region and the Drakensberg (South Africa), with only two species endemic to Australia and New Zealand. A few species extend their distribution range from the Drakensberg to tropical Africa or/and Madagascar, and a single species (*S. microphylla*) from Africa to Asia.

These morphological, phonological, ecological, or geographical characters have not been examined in the context of possible synapomorphies to support relationships between species groups.

Current investigation – In our previous studies on Exaceae, combined chloroplastic DNA, as well as nuclear DNA analyses, strongly support the sister position of *Sebaea* and *Lagenias* to a large clade encompassing the remaining genera of the Exaceae.

Here we increased the sampling of *Sebaea s.str.* species to c. 40 spp., and performed parsimony and Bayesian analyses on both sequences from the chloroplast DNA (cpDNA: *trn*L intron, *trn*L-F spacer, *atp*B-*rbc*L spacer) and nuclear region (nrDNA) of the internal transcribed spacer region (ITS-1, 5.8S, and ITS-2). Both these regions have been successfully used in inferring the phylogeny of Exaceae (chapter 2, chapter 3; Yuan et al., 2003; Yuan et al., 2005) or other tribes of the Gentianaceae (Chassot et al., 2001; Hagen and Kadereit, 2001, 2002; Mansion and Struwe, 2004; Mansion and Zeltner, 2004).

In this study, we intend to (1) produce an infrageneric phylogeny of *Sebaea*, (2) evaluate the monophyly of several species complexes, (3) identify clades requiring further study, and (4) identify synapomorphies supporting the obtained clades.

MATERIAL AND METHODS

Plant material

Sebaea species were collected in South Africa in 2005 and 2006, and determined by the author. Voucher specimens were deposited in the herbarium of the University of Neuchâtel, Switzerland (NEU), and/or in the personal Gentianaceae collection of Dr. L. Zeltner, and/or the Bolus Herbarium, University of Cape Town (BOL). The species included in this study are listed in Annex 1. Material for DNA extraction was obtained from both collected samples and herbarium specimens (PRE). Species of *Sebaea* and outgroup specie (*Lagenias pusilla*) that were retrieved from GenBank are indicated with an asterisk following their accession number in Annex 1. Sampling of *Sebaea* species was maximized to cover ecological preferences (alpine meadow, sand dune, etc.) and morphological (habit, flower size, leaf shape etc.) variation of the genus. All the ecological and morphological groups in this taxon are sampled. A total of 62 plant accessions representing 40 of the c. 70 species currently ascribed to *Sebaea* and accessions of *Lagenias pusilla* as outgroups were included in this study. Sequences were generated from four regions of the chloroplast DNA (cpDNA), the *trn*L intron*, trn*L-F spacer, *atp*B-*rbc*L spacer, and partial *mat*K gene, as well as three regions from the ITS nuclear DNA (nrDNA) region, which encompasses the 5.8S gene and the flanking internal transcribed spacers ITS1 and ITS 2.

Molecular techniques

Total genomic DNA was extracted from silica gel dried leafs tissue, or from leaf tissue taken from herbarium sheets, by using the DNeasy Plant Mini Kit (Qiagen, Switzerland), according to the manufacturer‟s instructions. Polymerase Chain Reaction (PCR), purification, sequencing reaction, purification of sequencing products and sequencing, followed the procedure described in CHAPTER 2. The *trnL* intron and the *trnL-trnF* intergenic spacer were amplified as a single PCR fragment using the two universal primers „„c‟‟ and „„f‟‟ of Taberlet et al. (1991). The *atpB-rbcL* intergenic spacer was amplified using universal primers „„Oligo 2‟‟ and „„Oligo 5,‟‟ (Manen et al., 1994). Primers *mat*K8 and *mat*K503 (Endress et al., 1996) were used to amplify a part of the *mat*K gene, while the universal primers “ITS4” and “ITS5” (White et al., 1990) were used to amplify the nrDNA Internal Transcribed Spacer (ITS) region. ChromasPro Version 1.33 (Technelysium Pty Ltd) was used to check the quality of the electropherograms and compile the contiguous sequences (contigs) for each amplicon. Alignment was performed using the program

ClustalW (Thompson et al., 1994) as implemented in the program BioEdit 7.0.1 with subsequent manual improvement.

Phylogenetic analyses

Because the chloroplast genome is inherited as a single unit without recombination, combining sequences from multiple cpDNA regions seems to be justified (Soltis and Soltis, 1998). Moreover, improved phylogenetic resolution in analyses of the combined cpDNA dataset, compared to separate analyses of the individual regions, is often reported in the literature (e.g. Asmussen and Chase, 2001). However, before combining the cpDNA dataset, we separately analyzed each cpDNA dataset (*trn*L intron, *trn*L-F spacer, *atp*B-*rbc*L spacer, and partial matK gene) under maximum parsimony (MP) optimization, in order to see whatever they present similar topologies with similar well supported clades (Bootstrap > 70). Three data sets were analysed: the combined cpDNA data set, the ITS data set, and a total evidence approach (Kluge, 1989; Nixon and Carpenter, 1996) with the combined cpDNA and ITS data sets. Congruence in the phylogenetic signal of the cpDNA and ITS data sets was examined through visual comparison of the tree topologies and branch support, and conducting a partition homogeneity (ILD) test (Farris et al., 1995).

Heuristic searches were performed with character states weighted equally, gaps treated as missing data and the following parameters: TBR branch swapping, Mulpars ON and Collapse branch option ON for branches with a minimum length of zero. One thousand searches were performed under these conditions, and five hundred trees were saved per replicate. Branch support evaluation was performed using the bootstrap method (Felsenstein, 1985) with 1000 heuristic search pseudoreplicates using the same search parameters as those in the parsimony analysis. The overall analysis was limited to 100 trees saved per replicate due to memory overrun.

Sequences of the chloroplast genome of *Nicotiana tabacum* L. from GenBank (Http://www.ncbi.nlm.nih.gov) were used as guides to partition sequences into coding, intron and intergenic spacer regions prior to Bayesian inference (BI) analyses. Six partitions (5 cpDNA and 3 nrDNA) were identified. The cpDNA comprises the *trn*L intron, flanking *trn*L 5'' and 3'' exons, the *trn*L-F spacer, the *atp*B-*rbc*L spacer, and partial *mat*K gene, the ITS region comprising the 5.8S gene and the flanking internal transcribed spacers ITS1 and ITS2. The *trn*L exon was deleted from the dataset due to the lack of variation (zero variable site). The most appropriate model of sequence evolution for each partition was determined using MrModeltest version 2.2. (Nylander, 2004) to be GTR + G model for the *trn*L-F spacer, ITS-1 and ITS-2 spacer, GTR + I model for the *atp*B-*rbc*L spacer, GTR model for the partial *mat*K gene, HKY +

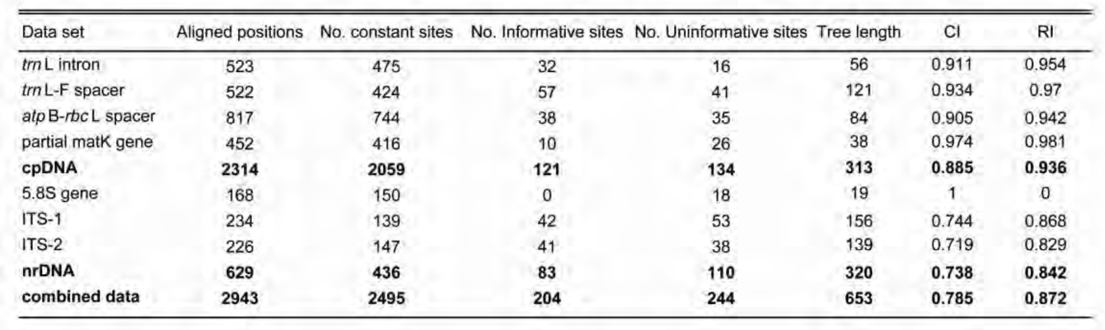
G model for the *trn*L intron, and finally the SYM model for the 5.8S gene dataset. Two independent analyses each with four Markov chains, three heated and one cold, starting from a random tree were run simultaneously for five million generations with trees sampled every 100 generations. Trees generated prior to the four Markov chains reaching stationarity (the burn-in) were discarded. The remaining trees were used to construct a 50% majority rule consensus tree.

RESULTS

Chloroplast DNA data set - The main characteristics of each DNA region used for the present study and the combined dataset are summarized in Table 2. Parsimony analyses performed on the combined cpDNA dataset produced 1722 MP trees of length = 313, consistency index (CI) = 0.885, retention index (RI) = 0.936, rescaled consistency index (RC) = 0.829; not shown.

Internal transcribed spacer data set - Parsimony analyses of the ITS data set yielded 1300 MP trees of length = 236, consistency index (CI) = 0.738, retention index (RI) = 0.842, rescaled consistency index (RC) = 0.621; not shown.

Table 2 Characteristics of the data sets.



Data set	Aligned positions	No. constant sites	No. Informative sites	No. Uninformative sites	Tree length	CI	RI
*trn*L intron	523	475	32	16	56	0.911	0.954
*trn*L-F spacer	522	424	57	41	121	0.934	0.97
*atp*B-*rbc*L spacer	817	744	38	35	84	0.905	0.942
partial matK gene	452	416	10	26	38	0.974	0.981
cpDNA	**2314**	**2059**	**121**	**134**	**313**	**0.885**	**0.936**
5.8S gene	168	150	0	18	19	1	0
ITS-1	234	139	42	53	156	0.744	0.868
ITS-2	226	147	41	38	139	0.719	0.829
nrDNA	**629**	**436**	**83**	**110**	**320**	**0.738**	**0.842**
combined data	**2943**	**2495**	**204**	**244**	**653**	**0.785**	**0.872**

Congruence assessment – Inspection of tree topologies revealed no incongruence between the cpDNA and nrDNA phylogenies. An ILD test (α = 0.05) found significant heterogeneity between the cpDNA and nrDNA data sets (P = 0.07). Sequential exclusion of all the *Sebaea* species and groups of species, does not improve the ILD test. Recent studies and simulations suggested that the ILD test could fail to detect congruence due to different noise levels of the data sets (Dolphin et al., 2000; Yoder et al., 2001) or incongruence (Darlu and Lecointre, 2002; Dowton and Austin, 2002) due to large difference of the size and evolutionary condition of the data partitions. Our data sets varies in size (2314 characters in the cpDNA data set vs 629 characters in the ITS) and in the number of informative characters (5.2 % for the cpDNA data set vs 13.2% for the ITS one). Since the topologies of the different dataset is not incongruent, we thus combined both for a total evidence approach (Kluge, 1989; Nixon and Carpenter, 1996).

Total evidence– Parsimony analysis yielded 146 MP trees of length = 653, consistency index (CI) = 0.786, rentention index (RI) = 0.872, rescaled consistency index (RC) = 0.685. The strict MP consensus tree leads to a fully resolveed tree similar to the 50% majority rule Bayesian tree (result not shown).

The 50 % Majority rule Bayesian tree generated from the global data set produces a well resolved topology and strong support for five clades (Fig. 1), and a basal position for *S. sulphurea*. Clade A (0.94 posterior probability (PP)), comprises annual, pentamerous species from the Cape region. Clade B (0.96 PP) contains perennial species endemic to the Drakensberg mountains. Clade C forms a well-supported clade (1.0 PP), of tetramerous species, endemic of the Cape region. Clade D and E form a well-supported super-clade (1.0 PP) and are both composed of pentamerous species distributed in the Drakensberg Mountains, and extending their range to tropical Africa, and to Asia for *S. microphylla*. Clade D (1.0 PP) consists of annual plants, while clade E (1.0 PP) is most probably composed of biennial plants.

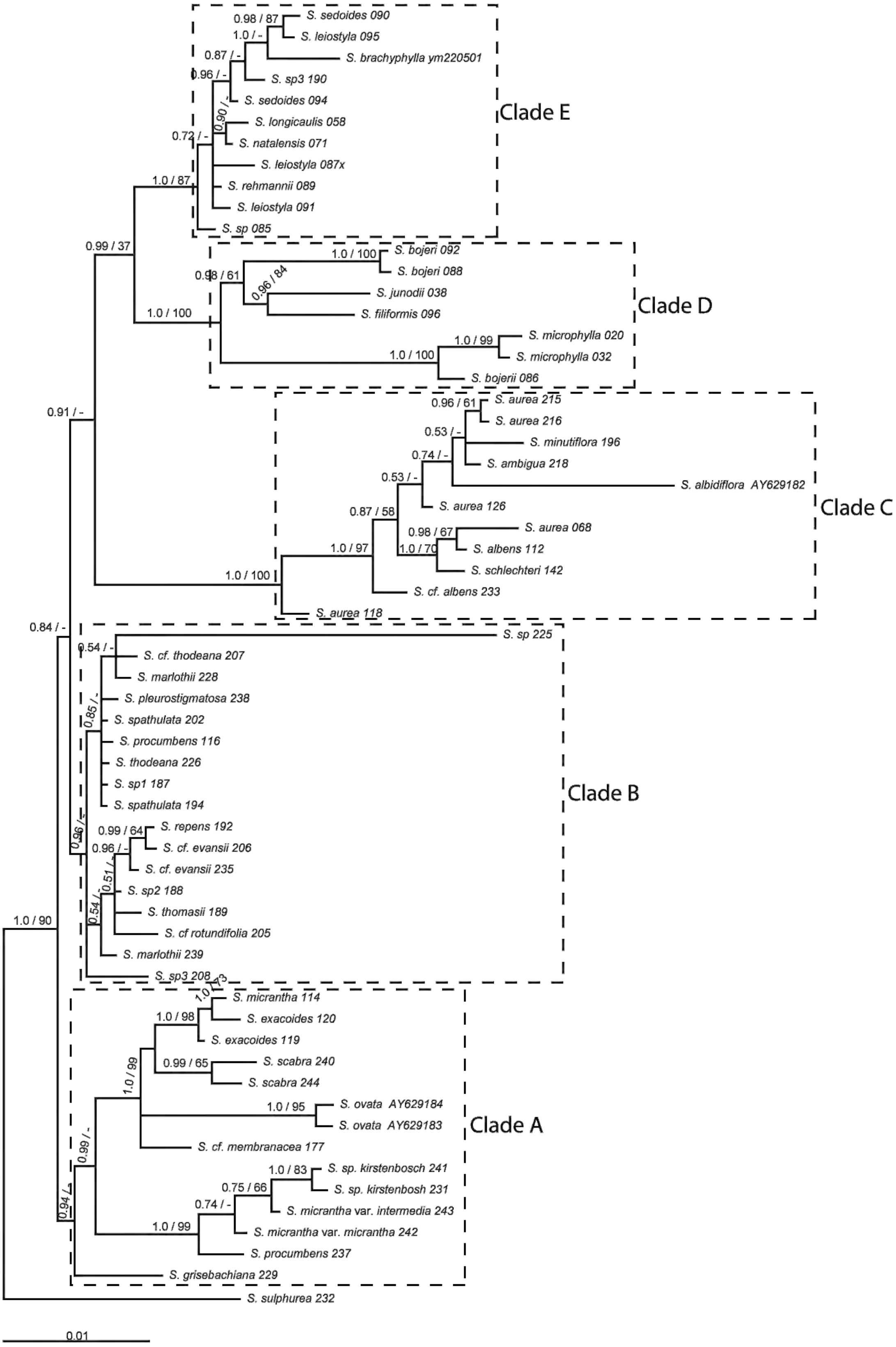


Figure 1 The 50% majority rule consensus tree from Bayesian inference analyses of the combined data set. Branch support values are posterior probabilities from a 50% majority rule consensus tree derived from a Bayesian inference analysis. Bootstrap values are indicated just after the posterior probabilities.

DISCUSSION

The potential difficulties inherent to the distinction between a gene tree and species phylogeny were previously explored in detail (e.g. Doyle, 1992). Combining data sets increases the phylogenetic signal, which in turn increases support for the "correct" phylogenetic tree (e.g. Bremer et al., 1999; Hardig et al., 2000; Hagen and Kadereit, 2001). The tree derived from the Bayesian analysis of the combined data sets was thus used as final phylogenetic hypothesis.

Phylogenetic relationships within Sebaea

With the exception of *S. sulphurea*, sister to the rest of the genus, *Sebaea* is divided into five well supported clades. Clade A is morphologically homogenous and contains pentamerous annual species growing in the Cape region, except *S. ovata* (Australia and New Zealand). Clade B, only weakly supported by the bootstrap values, contains perennial pentamerous species, all endemic of the Drakensberg and growing at high altitude (usually above 2000 m). Despite the short branches of the species, this clade contains most of the morphological variation of *Sebaea*. It contains species with small yellow solitary flowers (*S. repens*, *S. pleurostigamtosa*, *S. marlothii*) or rather big plants with white flowers and paniculate inflorescences (*S. spathulata*, *S. thodeana*). Species of this clade also show variation in the level of insertion of the stamen filaments (inside the corolla tube, e.g. *S. thomasii*, or in the sinus of the corolla lobes, e.g. *S. procumbens*). Many species form roots at the nodes (e.g. *S. marlothii*, *S. repens*), but others possess a rhizome (e.g. *S. spathulata*). However, this clade is ecologically homogenous (high elevation species) and contains species exclusively endemic to the Drakensberg.

Clade C is composed of annual tetramerous species, endemic to the Cape, (except *S. albidiflora*, growing in Australia). This clade matches the section *Tetrandria* described by Schinz (1906).

Clade D and E contain pentamerous species occurring in the Drakensberg up to tropical Africa. Clade D is composed of small slender annuals, not exceeding 10 cm in height and growing in tropical Africa (*S. junodii*, *S. microphylla*), or in Madagascar (*S. bojeri*. *S. microphylla*) extends its range to India, China and Thailand. Clade E is composed of more robust, most probably biennial, species with a size often exceeding 20 cm, up to 70 cm for some of them. Many species of this group also extend their distribution range to tropical Africa, (e.g. *S. leiostyla*, *S. sedoides*, etc.), with one species occurring in Yemen, and a single species, *S. brachyphylla*, growing in Madagascar.

The inferred molecular phylogeny of *Sebaea* is not congruent with previous infrageneric classifications (Schinz, 1906; Hill, 1908), except for clade C, which corresponds to subsection *Tetrandria* of Schinz (1906) or section *Tetrandria* of Hill (1908). Either the sections *Eusebaea* and *Belmontia* (Schinz, 1906) or *Pentandria* (Hill, 1908) are paraphyletic. In his treatment of the genus, Hill (1908) divided his section *Pentandria* into subsections and “groups”. These groups correspond more or less to the clades obtained here, and will be used to establish a systematic treatment of the genus (Kissling, unpublished data).

Branch length and statistical support

The sequenced regions (e.g. *atp*B-*rbc*L spacer, or the ITS region) are known to evolve relatively rapidly (Shaw et al., 2005; Mort et al., 2007; Shaw et al., 2007). Branch lengths in the overall tree are very short (notice the scale of substitution = 0.01), indicating few genetic differences among clades and furthermore among species (e.g. less than 2% of the sequenced nrDNA differs between “*S. aurea 215*” and “*S. aurea 118*”). Moreover, within each clade the statistical support (both posterior probabilities and bootstrap values) are generally weak, suggesting, e.g., a high level of hybridization, or a recent radiation of the genus. But before discussing any other (or additional) hypothesis, sequences evolving more rapidly are needed for an improved resolution of the phylogenetic relationship among species.

The few “long-branched species” (e.g. *S. albidiflora*, *S. ovata*, *S. brachyphylla*, *S. aurea 68*, *S. sp 255*) are due to missing data in the combined data set. (e.g. the cpDNA were not obtainable for *S. albidiflora* and *S. ovata*), while species of clade D, especially *S. microphylla* (for which all the sequenced region were available), show an accelerated evolution, as indicated by its extraordinary long branch in the phylogram.

Morphological evolution

Using the molecular cladogram (Fig. 1) as a framework, I traced the pattern of variation and some general tendencies in morphological characters by manually mapping character states so that transition was minimized (i.e., application of parsimony). Some examples are summarized below, e.g. insertion of the anthers, anther gland colours, secondary stigma, presence of coloured line on the petals, length of the corolla tube, perennial versus annual habit (Fig. 2). Although homoplasious, several characters are potential synapomorphies for certain clades. In addition, we acknowledge that the limited number of specimens available for several species, as well as missing species, may eventually affect the validity of the following discussion.

Floral morphology

A yellow pentamerous corolla is a symplesiomorphy for both Exaceae and *Sebaea* (CHAPTER 2). As shown on figure 3, tetramery has evolved only once within *Sebaea* and is considered as a synapormophy for clade C, while white corollas have evolved several times from the ancestral yellow condition in both clade B and C. Red-orange coloured lines on the petals indicating the center of the corolla (Fig 2) are quite common in clade A, and this feature is a potential synapomorphy for this clade with reversal to unlined petals in a few species (e.g. *S. ovata*).

Figure 2 Photographs of *Sebaea* species, illustrating variation in colour and size of the apical glands on the anthers, the presence and absence of coloured line on the petals, and colour variation of the flowers. From left to right, on the top: *S. marlothii*, *S. minutiflora*, *S. repens*, on the bottom: *S. exacoides*, *S. sp.*, *S. spathulatha*.

The anthers in *Sebaea* are inserted at the sinus of the corolla lobe. In a few species (*S. exacoides*, *S. micrantha*, *S. pleurostigmatosa*, *S. thomasii*), the stamens are inserted within the corolla tube. This feature is regarded as an autapomorphy for these species.

The anthers of *Sebaea* are furnished with an apical gland of varying size and colour. The gland is usually small (1-3 mm) and yellow, but in clade B, several species present huge apical glands (up to 1-1.2 cm), often black, brown, red or orange. This character is associated with a large inflorescence (dichasium or panicle), while species with a solitary flower of this clade, have usually small yellow glands (exception: *S. marlothii*). The coloured glands are supposed to act as guides for pollinators, as they indicate the flower centre by contrasting with petal colour (Fig 2). Thus they may have a similar function as the coloured line on the petals found in clade A.

The gynoecium of *Sebaea* is morphologically relatively constant, with a bilocular ovary and the presence of a secondary stigma characterizing the genus. However in *S. thomasii*, the secondary stigma is seemingly absent, because it is placed immediately under the normal stigma, due to a reduction of the style length. This reduction of the style length is regarded as an autapomorphy of *S. thomasii*.

Vegetative characters

Plants belonging to clade B and E are generally of large size, ranging from 10 cm to c. 70 cm (except for: *S. marlothii*, *S. repens*, and *S. pleurostigmatosa* which are only 3-4 cm high and form large mats), with all the species in clade E higher than 20 cm.

In clade B, most of the species possess a tap root indicating their perennial nature, or are rooting at the node, permitting the colonization of large areas (e.g. *S. thomasii* which covers huge rock surfaces in the Drakensberg). Greenhouse experiments on representatives of clade B proved the perennial nature of these species (personal observations). In clade E the roots are fleshy but numerous, in the greenhouse the cultivated specimens of this clade appear to be at least biennials. Clade A, C, and D have very few fleshy small roots, clearly indicating their annual nature.

Pollination syndromes

Within *Sebaea*, like in most other Exaceae genera (Chapter 2), pollination might have played an important role in its present success. The presence of coloured line on the corolla lobes, or similarly exserted coloured glands, indicating the centre of the flower, the specific variation of odours, or the “gland-sugar-reward” for insects (Marloth, 1909), suggest that pollinators play an important role in the pollination of *Sebaea*. A good example of pollinator adaptation is *S. thomasii*. Its bright yellow flowers have a particularly long corolla tube, with included anthers, and a reduced style; they do not close at night in contrast to most *Sebaea* (personal observation) or Gentianaceae (Ph. Küpfer, Y.-M. Yuan and L. Zeltner, personal communication; Bynum and Smith, 2001; He et al., 2005); and are very strongly scented, especially at night when it is usually first detected by scent (personal observation). All this features suggest strongly an adaptation to nocturnal long-proboscis pollinators. Conversely the presence of a secondary stigma which is supposed to favour self-fertilization (Marloth, 1909; Hill, 1913) is striking.

Figure 4 Photographs of *Sebaea thomasii*. Notice the particularly long and thin corolla tube, and the included stamens.

The flower morphology varies from clade to clade. Clade A and B are especially variable, and show the most evident syndromes of pollination adaptation, while clade C, D, and E are morphologically homogenous. In clade A and B, the secondary stigma is sometimes reduced, or merged with the apical one, while in clade C, D, and E it is obviously present and functional. Furthermore, clade C, D, and E apparently contain several polyphyletic species.

This suggests that when clades are well adapted to particular visitors or groups of visitors, the role of the secondary stigmas becomes less important. Nonetheless, and in order to better understand the morphological variation, and the speciation process within *Sebaea*, the functional role of the secondary stigma, as well as the specificity of flower visitors should be further studied.

New insight into the biogeography of Sebaea s.str.

Clade A and C are strictly restricted to the Western and Southern Cape each with one representative in Australia and/or New Zealand (*S. ovata* and *S. albidiflora* respectively). Species of clade A and C usually grow at an altitude between 0-1000 m, and flower mainly in September-October. Clade B is confined to the Drakensberg and its representatives are generally found above 2000 m altitude. Species of clade B are mainly flowering in October-December. All species of clade E are found in the Drakensberg, even though some extend their range into tropical Africa (*S. junodii*, *S. filiformis*) and as far as to Indo-Malaysia (*S. microphylla*). They are growing at an altitude between 1000 and 2000 m and are flowering in March-April. Clade F representatives are also found in the Drakensberg, with a few species extending their range in tropical Africa (*S. leiostyla*, *S. sedoides*, *S. brachyphylla*) and Madagascar (*S. brachyphylla*). They are usually found at an elevation between 1000 and 2000

m. However, in the eastern African mountains, they occur up to 3000 m (*S. leiostyla* and *S. brachyphylla*). The flowering time of these species varies with latitude and altitude, but is mainly in March-April.

The biogeographical scenario developed in CHAPTER 3 suggests a Cape origin for *Sebaea* followed by dispersal to the Drakensberg, and from there to Tropical Africa, Madagascar and Asia. It seems likely that the two Australian-New Zealand species have been long dispersed from the Cape. The current phylogeny supports this hypothesis, and suggests moreover an origin of the genus at a "low" altitude (0-1000 m), followed by a shift for several clades (B, E and F) to a higher attitude (1000-2000 m), thereby coinciding with the dispersal to the Drakensberg. A single clade (B) adapted to high altitude regions (2000-3000 m). The common ancestor of *Sebaea* was probably flowering between September and December, while a shift to a flowering time in March-April occurs once for the common ancestor of clade E and F. This temporal segregation of clade A-B-C and clade E-F not only reproductively isolates these two groups, but also permits a wider use of possible ecological niches, by favouring the colonization of tropical Africa. Indeed the flowering time of clade E and F corresponds to the end of the raining season of the Zambesian region, a favourable time for *Sebaea* to grow there.

Polyphyly / paraphyly of some species: the case of clade C

Our phylogeny suggests that *Sebaea* comprises highly paraphyletic or polyphyletic species, mainly found within three clades (C, D, and E). As already discussed, the statistical support for these para-polyphyletic species is generally weak (Fig. 1). However in clade C some branches received strong statistical support for the polyphyly of *S. aurea*, and in clade D for *S. bojeri*. In order to discuss these para-polyphyletic species, and to illustrate one of the apparently recurrent problem within *Sebaea*, I will only focus on clade C, which comprise species with tetramerous flowers. However the situation is similar with the two other clades.

Clade C is currently composed of eight species, all endemic from the Cape, one of them, *S. aurea*, being polyphyletic.

As currently defined, *S aurea* includes at least 6 entities put in synonymy by different authors (*S. cymosa*, *S. minima*, *S. pallida*, *S. gibbosa*, *S. paludosa*, and *S. glauca*). Slight morphological differences exist between these "micro-species". The paraphyly of *S. aurea* is quite surprising, because the samples added in this study were morphologically very similar. Only geography appears to be a good criterion. Despite the low number of sampled species, geographical pattern can be distinguished from the tree topology; e.g the basal species (*S. aurea*, and *S. cf. albens*) comes from an area located around the table mountain, while the clade formed of *S. aurea*, *S. albens* and *S. schlechterii* comes from an area located around Villiersdrop. More samples are needed to test whether biogeography can support the main nodes obtained within this clade.

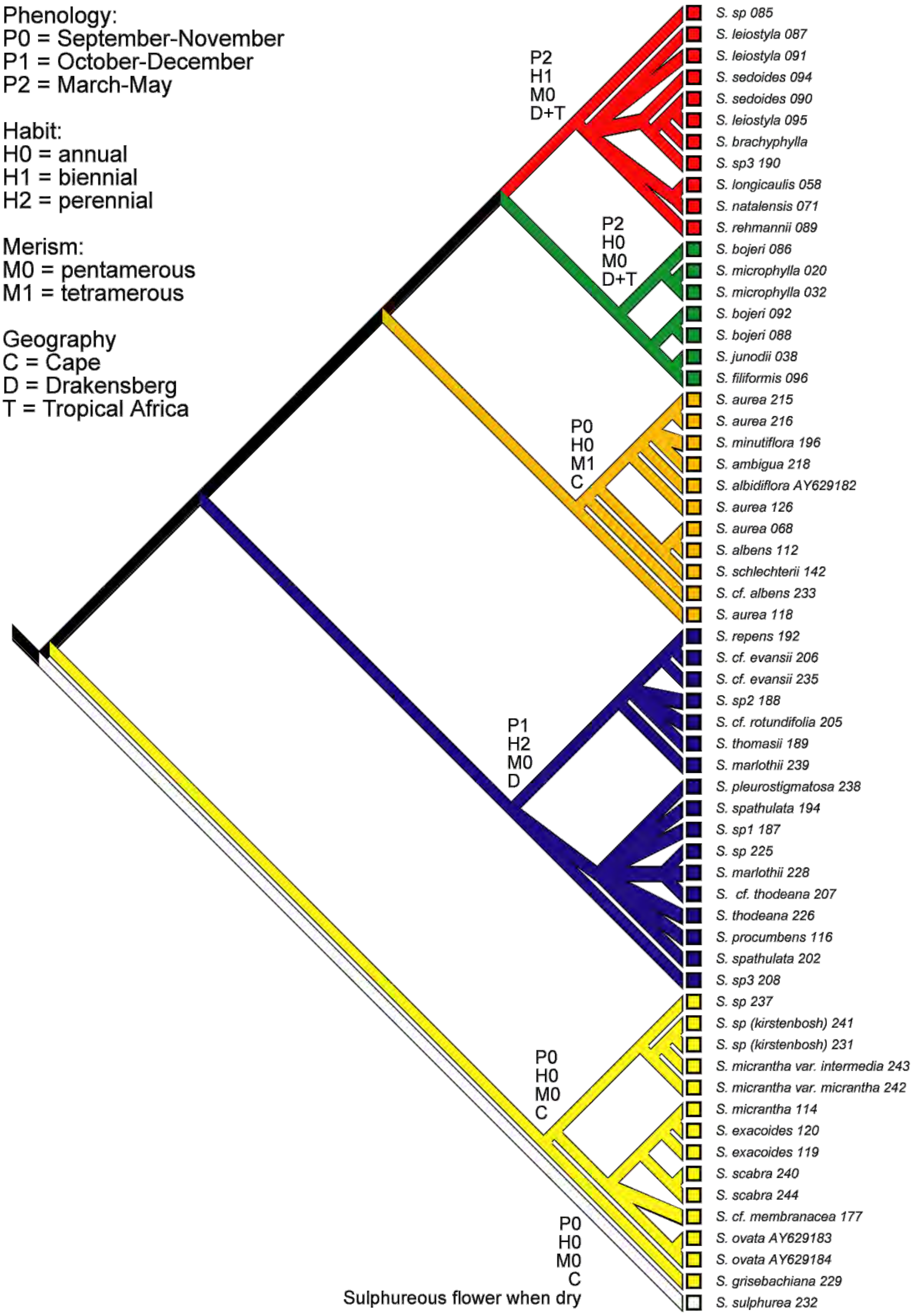


Figure 3 the 50% majority rule consensus tree from Bayesian inference analyses of the combined data set. The different clades are coloured, yellow for clade A, blue for clade B, orange for clade C, green for clade D, and red for clade E. Important characters (phenology, geography, habit and merism) supporting each clades are also indicated.

The current sampling of *S. aurea* and relatives in our phylogenetic analyses as well as the low statistical support obtained for most of the branches of the tree, only permit hypothesis concerning this polyphyly. Regarding the few genetic differences between species (less than 2% between e.g. *S. aurea* 118 and *S. aurea* 215), it will not be surprising if hybridization occurs. If the cpDNA and nrDNA tree are congruent in our analysis, they are unresolved at the species level and therefore e.g. introgression or chloroplast capture can not be excluded. Further sampling and sequencing of more variable cp and nrDNA region are needed to better understand the polyphyletic nature of *S. aurea*.

Taxonomy of Sebaea. s.str..

Due to the presence of apparently polyphyletic species, only a preliminary attempt of classification is proposed here.

Key to the groups:

1. Flowers tetramerous …………………………………………………………… *Tetrandria*
1. Flowers pentamerous …………………………………………...………….……….……..2.

2. Corolla becoming green-yellowish when dry……….……...……...………………*Sulphurea*
2. Corolla remaining yellow when dry …………………………………….…………………3.

3. Creeping or rosette-forming perennials with numerous spathulate or ovate leaves; usually with a long tap root (e.g. *S. spathulata*) or forming roots at the nodes (e.g. *S. thomasii*); flowers more or less sessile at the end of branches or borne on definite erect inflorescences (Drakensberg)………………………………………………………………………… *Repentes*
3. Erect annuals or biennials, rarely perennials with linear to cordate leaves along the stems, flowers always yellow, on a dichasium inflorescence……………………………………..….4.

4. Perennials or biennials, with more than 6 pairs of leaves; stem usually branching the base or at leaf axils; usually taller than 25 cm …………………………………………..……*Perennes*
4. Annuals, with a few pairs of leaves; usually shorter than 25 cm ……………………………5.

5. Leaves minute, more or less filiform (Drakensberg, Tropical Africa, Madagascar and Asia)……..……………………………………..…………………...……… *Filiformes*
5. Leaves ovate or cordate, usually conspicuous (South and western Cape)…………………………………………………………………...…………. *Ovatae*

group *Tetrandria*

S. aurea (L.f.) Roem. & Schult.
S. albens (L.f.) Roem. & Schult.
S. schlechteri Schinz
S. ambigua Cham.
S. minutiflora Schinz
S. albidiflora F. Muell.
S. capitata Cham. & Schlechtdl.
    var. *capitata* Cham. & Schlechtdl.
    var. *sclerosepala* (Schinz) Marais
S. amicorum I.M. Oliv. & Beyers
S. laxa N.E.Br.

group *Sulphureae*

S. sulphurea Cham & Schlechtdl.

group *Repentes*

S. marlothii Gilg
S. repens Schinz
S. pleurostigmatosa Hilliard & B.L.Burtt
S. thomasii (S. Moore) Schinz
S. thodeana Gilg
S. spathulata (E. Mey.) Steud.
S. procumbens Hill
S. pentandra E. Mey.
    var. *pentandra*
    var. *burchellii* (Gilg) Marais

group *Filiformes*

S. filiformis Schinz
S. bojerii Griseb.
S. microphylla (Edgew.) Knobl.
S. junodii Schinz

group *Perennes*

S. longicaulis Schinz
S. sedoides Gilg.
    var. *sedoides*
    var. *schoenlandii* (schinz) Marais
    var. *confertiflora* (Schinz) Marais
S. rehmanii Schinz
S. brachyphylla Griseb.
S. leiostyla Gilg
S. macrophylla Gilg
S. natalensis Schinz
S. hymenosepala Gilg
S. stricta (E. Mey.) Gilg

group *Ovatae*

S. exacoides (L.) Schinz
S. ovata (Labill.) R. Br.
S. membranacea Hill
S. micrantha (Cham. & Schlechtdl.) Schinz
    var. *micrantha*
    var. *intermedia* (Cham. & Schlechtdl.) Marais
S. grisebchiana Schinz
S. scabra Schinz
S. exigua (Oliv.) Schinz
S. zeyheri Schinz
    subsp. *zeyheri*
    subsp. *acutiloba* (Schinz) Marais
    subsp. *cleistantha* (R.A.Dyer) Marais
S. fourcadei Marais
S. ramosissima Gilg

Incertae sedis

S. rara W. Dod
S. erosa Schinz
S. elongata E. Mey.
S. radiata Hilliard & B.L. Burtt

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PRELIMINARY NOTE ON THE SECONDARY STIGMA PRESENT IN *SEBAEA*

PRELIMIARY NOTE ON THE SECONDARY STIGMA: A UNIQUE FEATURE PRESENT IN *SEBAEA* (GENTIANACEAE)

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Abstract – The gynoecium, the female organ, is the central and most complicated structure of the angiosperm flower. It consists mostly of several structural units, the carpels. In the majority of taxa the carpels are more or less united from the beginning of the development (congenitally fused or continuous) to form a unified construction (syncarpous gynoecium; probably more than 80% of the angiosperm species). In some case the carpels are free (apocarpous or choricarpous gynoecium; c. 10% of Angiosperms), and in a few groups the gynoecium consist of a single carpel (unicarpellate gynoecium; c. 10% of the angiosperm). Rarely, free carpels are postgenitally united to form a gynoecium that is functionally syncarpous (some Gentianales; some Liliales). This particularity has permitted the development of a second pair of functional stigmas in the genus *Sebaea* (Gentianaceae). This is the only known case within angiosperms. Descriptions of the secondary stigmas, as well as a hypothesis on their evolution are given.

Keywords – Africa; Exaceae; Gentianaceae; Gynoecium; *Sebaea*; Stigma

INTRODUCTION

As implied by their name, angiosperms (from the Greek, “seeds within a vessel”) were originally defined by the nature of their female reproductive structures in which the seeds are enclosed within an ovary. The angiosperms are both the dominant group of land plants and by far the most important plants for human use. Although numerous specific adaptations were involved in the rise of the angiosperms to their current dominance, it is not surprising that several of these adaptations relate specifically to the female parts of the flower (the gynoecium). The unique morphology of angiosperm female reproductive structures has facilitated the evolution of highly diverse and sometimes complex mechanisms to ensure appropriate pollination (Gasser and Robinson-Beers, 1993).

Sebaea (Gentianaceae–Exaceae) is composed of c. 70 species and subspecies, essentially small herbs, with attractive yellow, rarely white flowers. *Sebaea* is characterised by some particular floral features including the presence of apical gland on the stamens, the bilobed stigma shape and in most species, the presence of striking protuberances on the style. In the early history of the genus *Sebaea*, this protuberance has been designated by various names: “Haarwulsts” by Schinz (1891), “tubercular thickening” (Baker and Brown, 1903), “style muni d’un bourrelet de poils vers son milieu” (Perrot, 1897). Marloth (1909), while studying *S. exacoides*, first suggested that those protuberances are of stigmatic nature: “When, years ago, I examined the swollen part of the style for the first time under the microscope, I could not help noticing, that it possessed exactly the same structure as the receptive surface of the knob-shaped stigma, and that numerous pollen grains were adhering to the papillose surface.”. He also found, by staining the style that pollen grains had germinated on those two “protuberances”, and therefore suggested that it was a secondary stigma. He proposed to name this condition diplostigmatic. Only a few years later, Hill (1913) published his own observations on the secondary stigma. He performed, during two years, a series of greenhouse experiments, were he removed the apical stigma or/and the anthers (to prevent self-fertilization). He demonstrated that when removing the upper stigma, secondary stigmas enlarge considerably, and that the plant produced seeds normally and thus that the secondary stigma is functional. Both Hill (1913) and Marloth (1909) suggested that the functional role of the secondary stigma was to promote self-pollination, but did not exclude cross pollination, either by the apical stigma or by the secondary stigma. The fact of having two stigmas is only known in *Sebaea* and nowhere else. The aim of this note is to understand the morphological structure, the role, and evolutionary origin of this striking feature.

MATERIAL & METHODS

Flowering material of *Sebaea* was either collected in the field, in South Africa, and conserved in 70% alcohol or grown in a greenhouse at the Botanical Garden of Neuchâtel, Switzerland. Gynoecia of *S. ambigua*, were investigated at various developmental stages, using Scanning Electron Microscopy (SEM). Semi-thin microtom sections of *S. macrophylla* were examined with a light microscope.

For SEM studies specimens fixed in ethanol were critical-point dried and sputter-coated with gold.

Gynoecia of *S. macrophylla*, were removed from fresh plants growing in the greenhouse. And prepared according to Karnovsky (1965), using a 0.1 mol l^{-1} cacodylate buffer (pH 7.4) with 4% sucrose added. Specimens were washed four times in the same buffer and postfixed for 1 h at room temperature in 1% buffered OsO_4. After three washes with cacodylate buffer, specimens were dehydrated for 10 min in 6 solutions of increasing ethanol concentration (30-100%). Finally, the samples were embedded in LR White resin (Craig and Miller, 1984) and polymerised for 24 h at 60°C. Semi-thin sections (500 nm) were obtained on a Reichert Ultracut S microtome and were stained with Richardson Blue.

RESULTS

General Floral Morphology

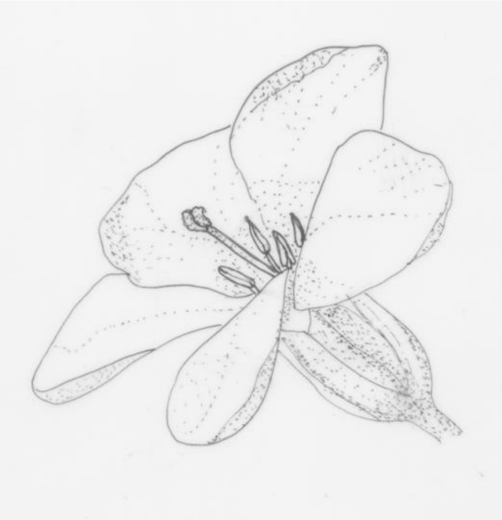

Figure 1: Flower of *S. macrophylla*

The actinomorphic flower has five sepals, a corolla of five petals basally united into a long tube but forming five free lobes. Five basifixed stamens attached by a long filament to the sinus of the corolla lobes. Two carpels that are congenitally united ovaries, and a postgenitally united in the style and stigma. A particularity of *Sebaea* species is the presence of secondary stigmas on the style (Fig. 3). As our aim is to understand how the secondary stigma develops, only the ontogeny of the gynoecium will be further considered in this study.

Gynoecial development

At initiation the floral apex develops into two carpels, which are apparently entirely separated from one another and consequently apocarpous at the time of origin (not shown, illustration in prep). Later on, the two carpels possibly touch on each other by their adaxial face, and begin to fuse postgenitally in the apical part (not shown, illustration in prep; Fig. 2A). The fusion of the two carpels appears slightly before the gynoecium is differentiated into style head, style and ovary. In the mature postgenitally fused ovary (Fig. 2C), a suture line remains visible externally. Moreover the epidermal layers of the style (Fig CB) are still discernible in the region of fusion. The very bases of the two carpels remain separated (not shown, illustration in prep).

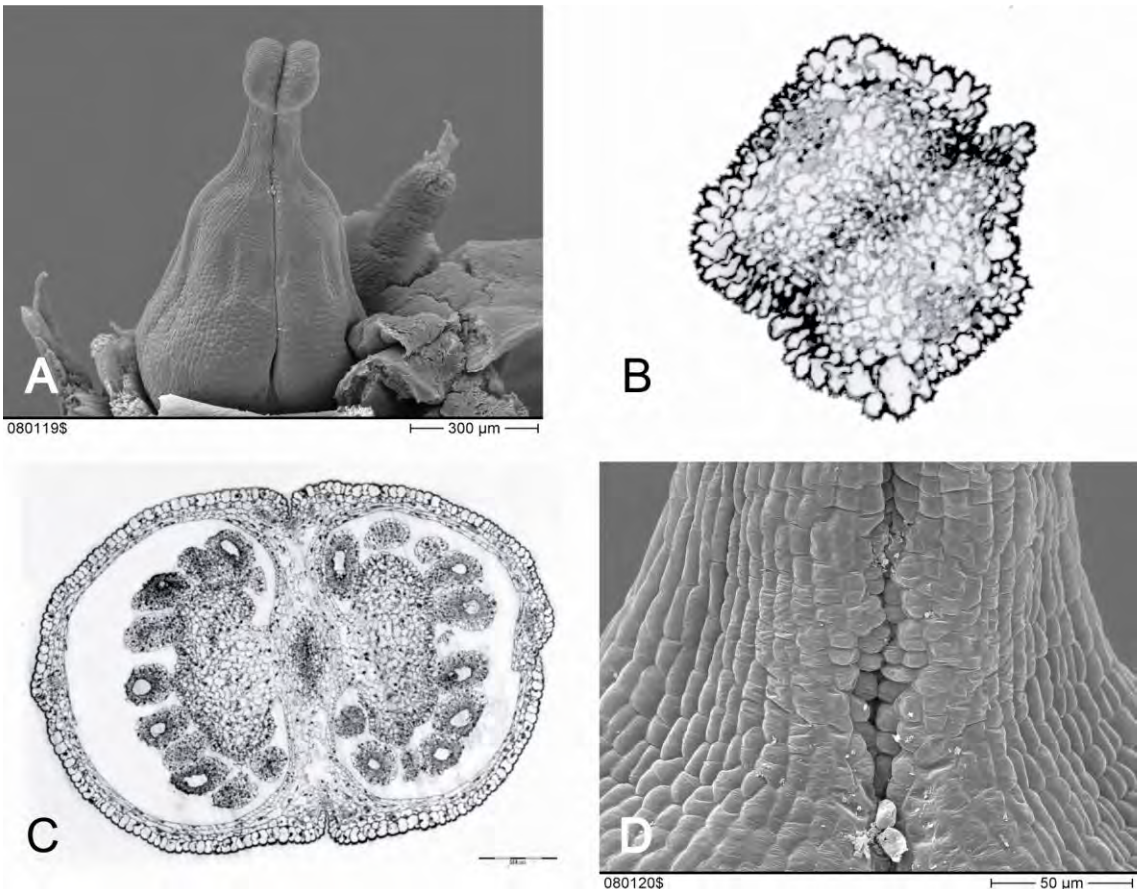

Figure 2: **A**: SEM of the gynoecium at an early stage, the carpels has already fused, the suture line is visible; **B**: transversal section of the style. the epidermal layers are still discernible in the region of fusion, indicated by arrows; **C**: transversal section of a mature ovary; **D**: SEM of the suture of postgenital fusion, where the secondary stigmas will develop, appears a little bit expanded and epidermal papillae of the secondary stigma become visible.

Before anthesis, the style head of the gynoecium is situated slightly below the level of the exserted stamen, while the secondary stigmas are appearing on the style, along the suture line.

The suture of postgenital fusion appears somewhat expanded and epidermal papillae become visible (Fig. 2D). When anthers start their dehiscence, the upper stigma is situated more or less at the same level as the anthers, and the secondary stigmas continue their development. The style continues its elongation and simultaneously the style head start to “open” into a bilobed stigma, and is mature when situated slightly above the anthers. The secondary stigmas continue to grow and are fully developed only much after the upper stigma reached its maturity, sometime one, two or three days later (Fig 3).

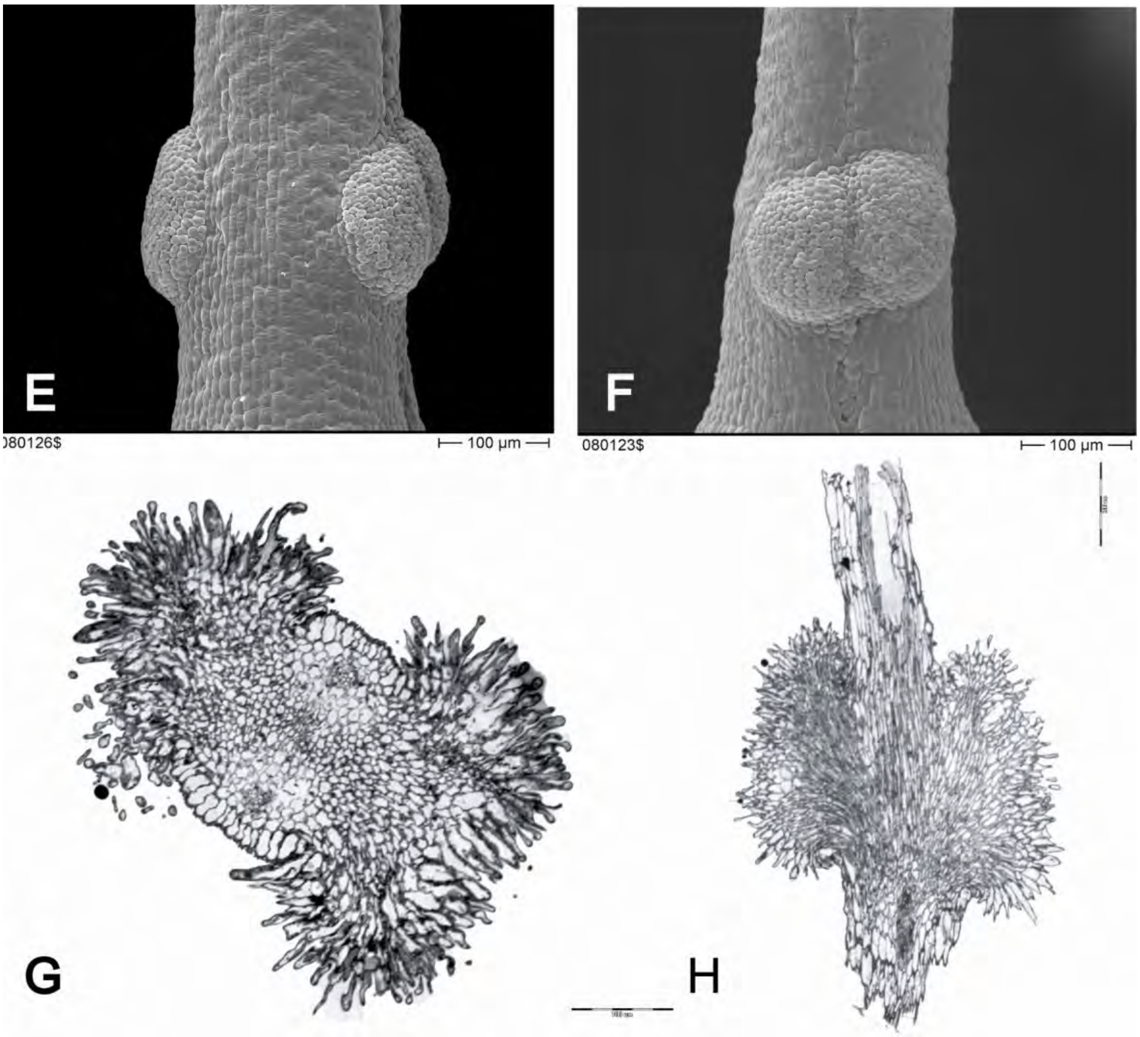

Figure 3: **E** & **F**: Lateral and ventral views of the developing secondary stigmas. **G**: transversal section of a mature secondary stigma, **H**. longitudinal view of mature secondary stigma.

DISCUSSION

The development of the gynoecium in *Sebaea macrophylla* reveals that the gynoecium is initiated as two separate carpels that partially fuse postgenitally[1] during development. In the majority of Angiosperm the carpels are congenitally fused to from a unified construction (syncarpous gynoecium; probably more than 80% of the angiosperm species) (Endress, 1994). In some cases the carpels are free (apocarpous gynoecium; c. 10% of Angiosperms), and in a few groups the gynoecium consist of a single carpel (unicarpellate gynoecium; c. 10% of the angiosperm) (Endress, 1994). Rarely, free carpels are postgenitally united to form a gynoecium that is functionally syncarpous, like in *Sebaea*. This type of gynoecial development appears to be the commonest, state in the Gentianaceae (Perrot, 1897; McCoy, 1940; Baum, 1948; Leinfellner, 1951; Vijayaraghavan and Padmanaban, 1968). Although such postgenital fusion, leading to a functional syncarpous ovary, has been reported for other Gentianales (Walker, 1975; Fallen, 1985), it is not known elsewhere in the angiosperms. Even though, some monocots present partial postgenital fusions, it never leads to complete syncarpy (Stauffer et al., 2002; Rudall et al., 2003; Remizowa et al., 2006).

Within *Sebaea*, the secondary stigma can be found at different levels on the style. It can be found either at the base of the style, close to the ovary, like in e.g. *S. pleurostigmatosa* or *S. macrophylla*, or nearly confluent with the upper stigma, like in *S. microphylla* or *S. ovata*, but in most species the secondary stigma is found around the middle of the style. Moreover the secondary stigma develops in all species, always along the postgenital suture line, implying that the primordial cells of the stigmatic region have to be found all along this line. This suggests that the development of a secondary stigma has been possible because of the particular ontogeny of the pistil.

Therefore similar stigmatic “patches” should be found in the other groups (Gentianales) presenting this pistil ontogeny. Hence, a similar case is found in two other Gentianaceae genus: *Lomatogonium* A. Braun. and *Bartonia* Mühl ex Willd. In these genera, the species have “bands” of stigmatic tissue on the side of the ovary itself, partly (*Bartonia*) and all along (*Lomatogonium*) the suture line (not shown, illustration in prep.).

As suggested by Hill (1913), it seems reasonable that the secondary stigma has evolved from a prolongation of the apical stigma, along the suture line, followed by the individualisation of stigmatic patches (Fig 5).

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[1] Postgenital fusion is likely to be confused with the better-known process of congenital fusion. Both types of union can produce a homogenous tissue, but the origin of the tissue is drastically different for each type. In a congenitally union the “fused” tissues arise already confluent from a basal meristematic zone that produces an expanse of tissue in the position where, presumably, separate organs once existed. In contrast, postgenital fusion involves the uniting of tissues that arise as separate and discrete primordial; epidermal surfaces actually contact and adhere; definition from WALKER, D. B. 1975. Postgenital Carpel Fusion in *Catharanthus roseus* (Apocynaceae). 1. Light and scanning electron-microscopic study of gynoecial ontogeny. *American Journal of Botany* 62: 457-467..

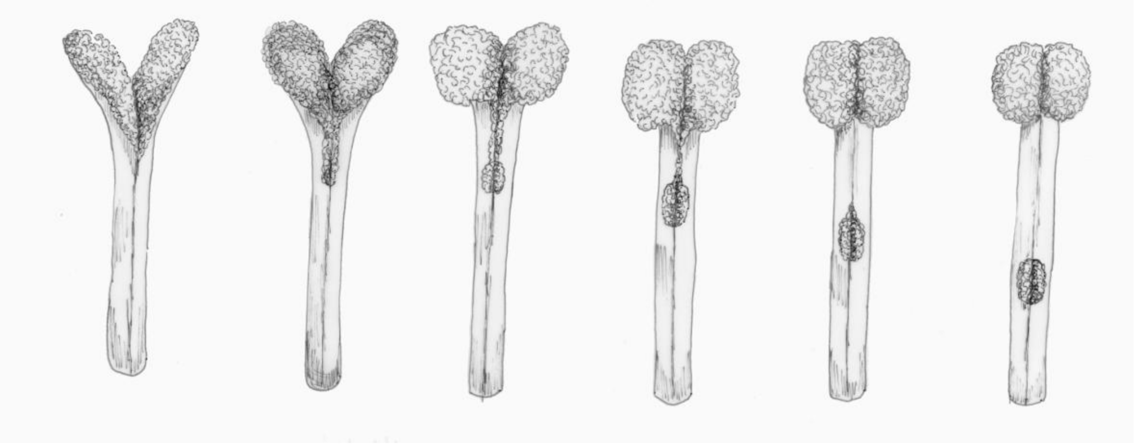

Figure 5: scheme illustrating how the secondary stigma might have developed (the supposed ancestral state is on the left, while the more derived one on the right).

Particularly, some similar structures are likely to be found in related species, presenting a syncarpous gynoecium issue from a postgenital fusion of carpels. It seem reasonable to assume that this particular ontogeny of the gynoecium (postgenital fusion of carpels) permits the development of stigmatic region all along the postgenital suture line, like the stigmatic “bands” found on the ovary of *Lomatogonium* and *Bartonia* or the secondary stigma in *Sebaea*.

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SOME PERSPECTIVES …

SOME PERSPECTIVES …

Some perspectives emerging from the present work can be drawn. Those proposed here are focusing on the evolution of “saprophytism” in Gentianaceae, the evolution of the pollination systems within Exaceae, and the reticulate evolution of *Exochaenium*.

Mixotrophy in Gentianaceae: the case of Exochaenium oliganthum

In contrast to green plants that fix atmospheric carbon dioxide photosynthetically (phototrophy), a variety of angiosperm, pteridophytes and liverworts, get access to carbon, by assimilation of organic matter (heterotrophy). Mixotrophy is an intermediate strategy that simultaneously combines both carbon sources. Among land plants, mixotrophy has been long reported for green hemiparasitic plants, such as some Loranthaceae (Bannister and Strong, 2001), while recently some terrestrial orchids were suggested to achieve mixotrophy by using C from their mycorrhizal fungi (Gebauer and Meyer, 2003; Bidartondo et al., 2004; Selosse et al., 2004; Julou et al., 2005). The type of heterotrophic metabolism that access to carbon, partially or even exclusively, by “digestion” of root colonizing fungi (Leake, 1994), is described as “myco-heterotrophy”.

It is very likely that, within Gentianaceae, myco-heterotrophy has evolved several times independently (Oehler, 1927; Raynal, 1967; Leake, 1994). The genus *Exochaenium* shows a transition from comparatively large-leaved phototrophic species, such as *Exochaenium grande*, to species with reduced leaves and size, like *E. perparvum* and to myco-heterotrophic species such as *E. oliganthum*.

E. oliganthum is an annual herb of c. 1-5 cm high. The distribution of the species follows mainly the Guineo-Congolian region, as defined by White (1986). Its ecology is of interest, because it grows mainly in the litter of forests, while the other *Exochaenium* species grow usually in savannahs. The biology of this species is remarkable, and was studied by (Raynal, 1967). It is usually described as a saprophyte, because of the lack of chlorophyll. However within a population, both green and achlorophyllous specimens are to be found (Raynal, 1967; Kissling J., pers. obs.). Nonetheless, it has been observed that this species can be a root parasite, especially of Cyperaceae or grasses (Nemomissa, 2002); Kissling J., pers. obs.). The phyllotaxy tends to be alternate, while the stem is often reduced and the flowers are brought above the ground by rather long pedicels. Most interestingly, it happens that the inflorescence is composed of two parts. The first part, found within the littery of the forest, presents cleistogamous flowers, while the second part is normally aerial and bears chasmogamous flowers. Chasmogamous flowers, like in other *Exochaenium* species, are heterostylous (reciprocal position of the anthers and the stigma). The plants are mycorrhizal (Raynal, 1967), and associated mycorrhizal fungi have been recently found to belong to the *Glomus*–group A lineage (Glomerales; Glomeromycota) (Franke, 2002)

In order to document the mixotrophy of *E. oliganthum*, and to better understand the transition from phototrophy to myco-heterotrophy and finally to parasitism, a two step study should be conducted.

The first part (which could be done by a master student) concerns the study of the morphology, anatomy and the mycorrhiza of *Exochaenium oliganthum*. A morphological and anatomical comparison of the roots as well as the associated fungus of the different "forms" (mixotrophy, complete myco-heterotrophy, and parasitism) of *E. oliganthum* should help to understand the factors implied in the transition from chlorophyllous to parasitic plants. Thus a simple quantitative comparison of the contents of chlorophyll a and b could also be undertaken.

The second part of such a study could focus on the identification of mycorrhizal associates of related species (*E. perparvum*, *E. platypterum* and *E. grande*) which might provide further clues of how extreme mycorrhizal specificity could be correlated to the evolution of myco-heterotrophic mode of life.

Contacts have been made with M.A. Selosse and T. Franke, in order to further investigate the mixotrophy of *E. oliganthum*, and its possible parasitism.

Reticulate evolution and biogeography of Exochaenium

Preliminary phylogenies of the genus, based on cpDNA (*trn*L intron, *trn*L-F spacer, *atp*B-*rbc*L spacer) and nr DNA (ITS) were obtained from a dataset comprising 29 accession representing 14 species (half the genus). Both phylogenies (cpDNA and nrDNA) were compared and found to be discordant. Preliminary hypothesis of species phylogeny is proposed as being the result of hybrid speciation followed by inheritance of cpDNA of one parent and fixation of ITS sequences of another parent. Furthermore, exploratory NPRS dating, using our inferred age (chapter 3) of the genus as calibration point suggest that Pleistocene glaciations may have played an important role in triggering extensive reticulate evolution within *Exochaenium*. Further investigations could be undertaken in order to verify this hypothesis.

Key innovation, mating system and pollinators within Exaceae

A – *The role of the secondary stigma of* Sebaea*: the pollination and breeding system in* Sebaea

Flowering plants are predominantly hermaphroditic, with most species producing flowers that contain both female (carpels) and male (stamens) sexual organs. These sexual structures can be spatially separated in a flower (herkogamy) or can function at different times (dichogamy), so reducing the likelihood of self-pollination (Barrett, 2002). Both strategies are present in most *Sebaea* species; the anthers are mature before the stigma, while the position of the stigma, much above the anthers, avoids any contact with them (pers. obs.). Therefore, the hypothesis that the secondary stigma favours self pollination (Marloth, 1909; Hill, 1913) apparently contradicts other flower "efforts" to avoid self pollination. Thus a simultaneous study of pollination and breeding system within *Sebaea* should be conducted in order to understand the role of these unique secondary stigmas. Preliminary field experiments should be undertaken this autumn (Northern hemisphere) in collaboration with Steven D. Johnson and Craig I. Peter.

***B** – The floral evolution of the genus Exacum (Gentianaceae – Exaceae)*

The genus *Exacum* is characterised by anthers opening by pores (Klackenberg, 1985, 2002, 2006), while anthers opening by longitudinal slits is thougt to be the plesiomorphic state of the tribe Exaceae and probably the family Gentianaceae (Chapter 2). However four *Exacum* species have anthers dehiscing firstly by an apical pore that is elongating into a slits (Klackenberg, 1985), and one species *Exacum wightianum* is opening its anthers by longitudinal slits. These five species occur in the Sri-lankan / India region.

Exacum has its origin in Madagascar, and has dispersed recently to India / Sri-Lanka (Yuan et al., 2005). Currently the transition of the anther opening mechanism from pores to longitudinal slits is not well understood. In order to better understand which factors (e.g. polyploidisation, pollination pression,) favouring the slits aperture in the Sri-Lankan / India *Exacum*, a study should be undertaken. Our recent contact with Andrew Riesmann, working on the Sri-Lankan *Exacum*, and Steven D. Johnson will most probably lead to a future collaboration on this topic.

***C** – Heterostyly in Gentianaceae: the case of* Exochaenium*, from homostyly to heterostyly*

In our morphological investigation of *Exochaenium*, we found that nearly all the species (available in sufficient quantity), except e.g. *E. teucszii*, *E. chionanthum*, *E. clavatum* were heterostylous (reciprocal herkogamy), although it is yet unknown if those plants have a heteromorphic incompatibility system that prevents selfing and intramorph mating. One species *Exochaenium grande* possesses a remarkable range of stylar conditions and diverse types of floral morphology (so far, nothing is known on its pollination biology). Monomorphic style, a stylar height dimorphism with short-styled and long-styled flower, but which have anthers placed at roughly the same position in the middle of the corolla tube, and heterostyly apparently more rare which contain two floral morphs that differ reciprocally in stigma and anther height (reciprocal herkogamy) are both present within this species.

A common feature of the models for the evolution of distyly is the existence of an intermediate stage of stigma height dimorphism (see Richards, 1997). This polymorphism is apparently a very rare condition in flowering plants (Graham and Barrett, 2004), and even more so within a single species.

Investigating the evolutionary history of stylar variation in *E. grande*, and further within the genus, by constructing phylogenetic trees, and using character optimizations of floral traits might help to answer the following questions: (1) Is stylar monomorphism the ancestral condition, and (2) Which stigma variation is ancestral, heterostyly or stigma height dimorphism?

Furthermore, observations of three well defined colour variations (white, bright yellow and salmon) of the corolla have been made, are apparently fixed within populations. This, linked with observations of a striking resemblance with *Thunbergia atriplicifolia* (Acanthaceae, salmon colour) (J. Kissling and L. Zeltner, pers. obs.), suggest an adaptation to similar pollinators.

Exochaenium grande is found in a large tropical African region, from West Africa, to Ethiopia, and down to South Africa, while *Thunbergia atriplicifolia* is apparently restricted to South-eastern Africa.

From these observations several question arise and might also be explored: (1) Where is the origin of *E. grande*, South Africa or tropical Africa? (2) Which flower colour is ancestral to this species? (3) What are the respective pollinators of *E. grande* and *T. atriplicifolia* (5) Are the pollinators of the different colour morphs of *E. grande* the same? (5) is one of those species mimicking the other one?

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SEED ATLAS OF THE AFRICAN EXACEAE (*EXOCHAENIUM*, *KLACKENBERGIA*, *LAGENIAS* AND *SEBAEA*)

Exochaenium

Klackenbergia

Lagenias

Sebaea

—

A Seed Atlas

—

In Exaceae the structure of the seed surface is determined mainly by the pattern of the anticlinal walls and by the thickening of the inner periclinal and anticlinal walls (Bouman et al., 2002). The seed structure of the Malagasy Exaceae (*Exacum*, *Gentianothamnus*, *Ornichia*, and *Tachiadenus*) has been studied by Klackenberg (Klackenberg, 1983, 1985, 1986, 1987). In this "Seed Atlas", we investigate the remaining genera of Exaceae, namely *Exochaenium*, *Klackenbergia*, *Lagenias*, and *Sebaea*. For the description of exotestal characters, I used the terminology proposed by Barthlott (1981).

EXOCHAENIUM

Seeds are numerous, an average of c. 4500 seeds per fruit were counted for *E. grande* (Kissling J.; unpublished data), and small, between 180-250 µm, usually angular. The testal structure is very homogenous within the genus. All species have star-shaped exotestal cells, although star-shape is less pronounced in *E. grande*. The anticlinal walls can be of variable thickness. For example, it is very thin in *E. leariformis* or much thicker in *E. perparvum* (p. 5). Seeds of Exochaenium species resemble to the seeds found in the other "Malagasy" genera (*Exacum*, *Ornichia*, *Klackenbergia*, *Gentianothamnus*, and *Tachiadenus*).

Seed dispersal

There have been no studies on seed dispersal in *Exochaenium*. The fruits are septicidal capsules, standing on erect peduncles that dehisce with two "valves" from the apex. The corolla falls off when the fruit is mature. But the calyx lobes usually furnished with a prominent wing on the dorsal side are persistent. As known for some species of *Gentiana* (e.g. *Gentiana verna*), an elongation of the peduncle after fruit development, which rises the capsule up might occur in *Exochaenium*. Considering also that the majority of *Exochaenium* species grow in open vegetation, wind might be the prevalent dispersal vector of *Exochaenium* seeds.

An interesting exception should be mentioned. *E. oliganthum*, a saprophytic species usually growing within the forest litter, produces two kinds of flowers; chasmogamous flowers on an erect peduncle and underground cleistogamous flowers (Raynal, 1967). The underground cleistogamous underground flowers probably help reseeding in a favourable environment.

SEBAEA

The seeds are often ridged or fringed and somewhat flat, reflecting their anatropous nature (Bouman et al., 2002). Seed size varies from 240 to 815 µm in length and from 100 to 500 µm in width. Testa cells are more or less rectangular, elongate perpendicularly to the length of the seed, and arranged in rectangular longitudinal rows.

The only known exception within the genus is *S. exacoides*. The seeds lack ridges or fringes, and the testa cells are very slightly star-shaped, but still arranged in rows. Within Exaceae, this kind of seed is found only in *Sebaea* species, and the "in row" arrangement of the testa cells is a good diagnostic character of the genus.

Seed dispersal

No studies have been yet performed on seed dispersal within *Sebaea*. Nevertheless, for the same reason as for *Exochaenium* (wings on the calyx, growing in open vegetation), the wind is likely to be the most important disperser.

KLACKENBERGIA [1]

Seeds are numerous, small, angular and polyhedral in shape. The seeds of *K. condensata* (c. 100 X 150 µm) are slightly smaller than those of *K. stricta* (c. 160 X 250 µm). Testa cells are like in most Exaceae star-shaped, and do not significantly differ from genera such as *Exacum*, *Exochaenium*, *Gentianothamnus*, *Ornichia* or *Tachiadenus*.

LAGENIAS [1]

Seeds of *Lagenias* are cubical in shape and measure c. 200-250 X 300-330 µm. Testa cells are polygonal, isodiametric and irregularly oriented. The anticlinal walls are very thick, and the curvature of the outer periclinal wall is distinctly convex. The shape of the testa cells is unique within the tribe Exaceae. Testa cells resemble more to what can be found in the tribe Chironiinae, like, e.g., in the genera *Blackstonia* or *Ixanthus*.

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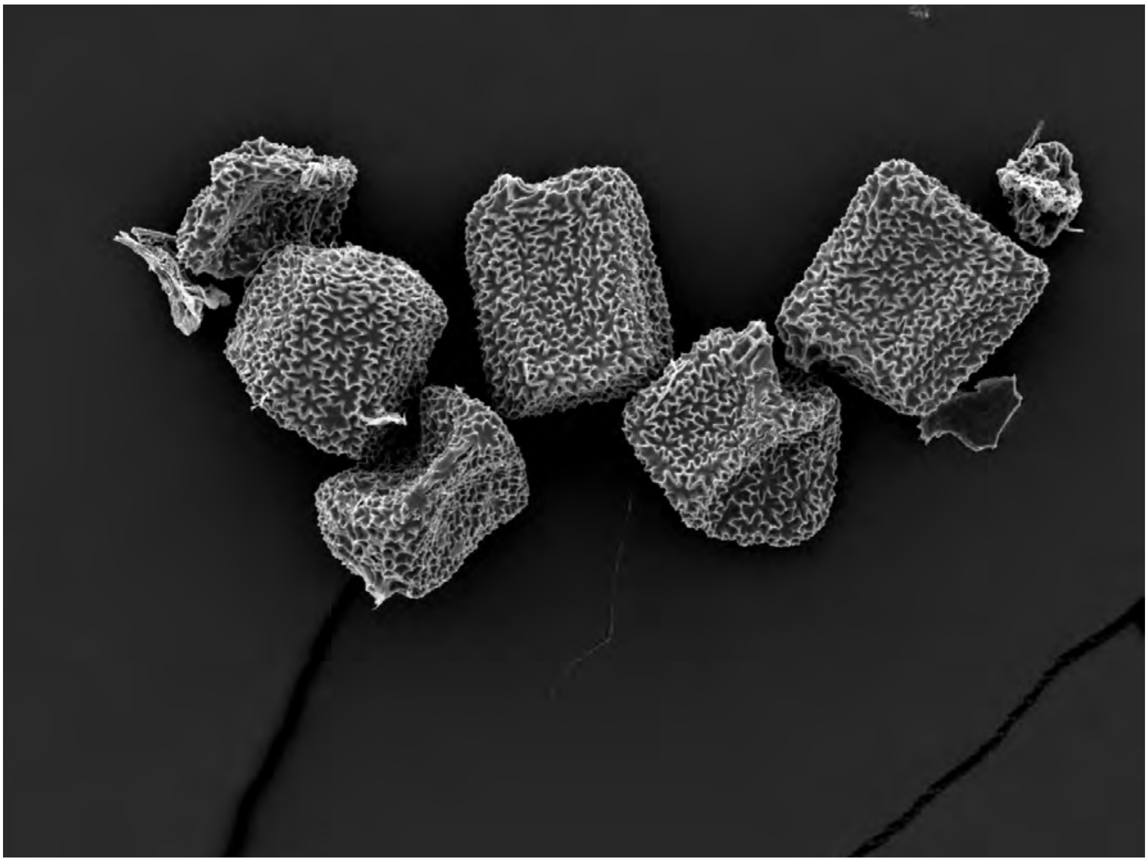
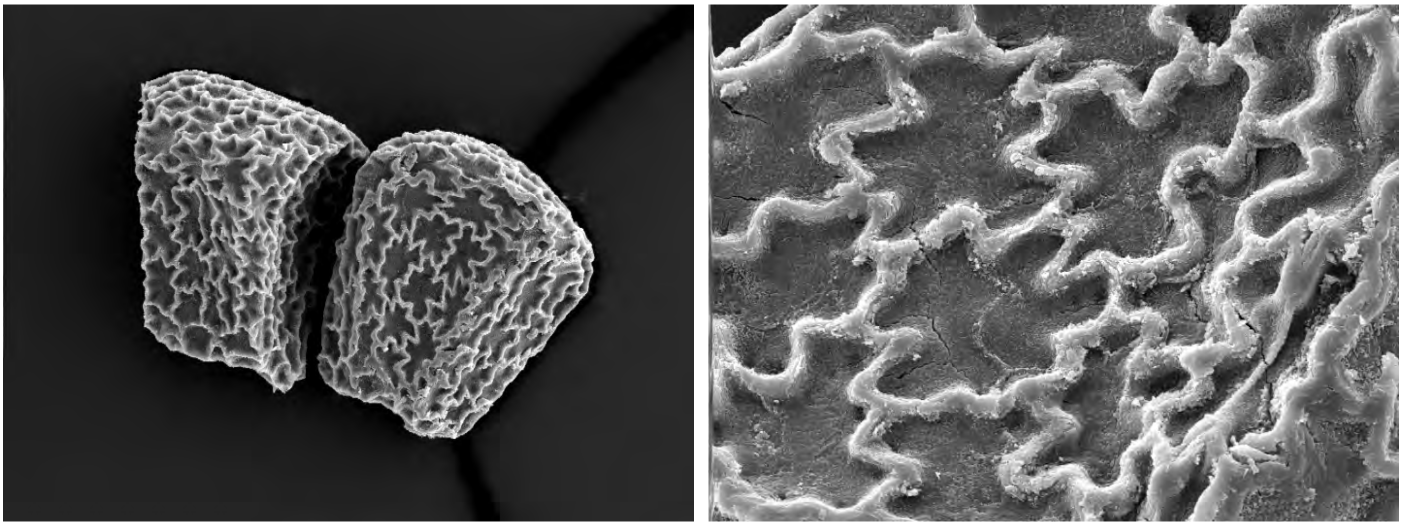
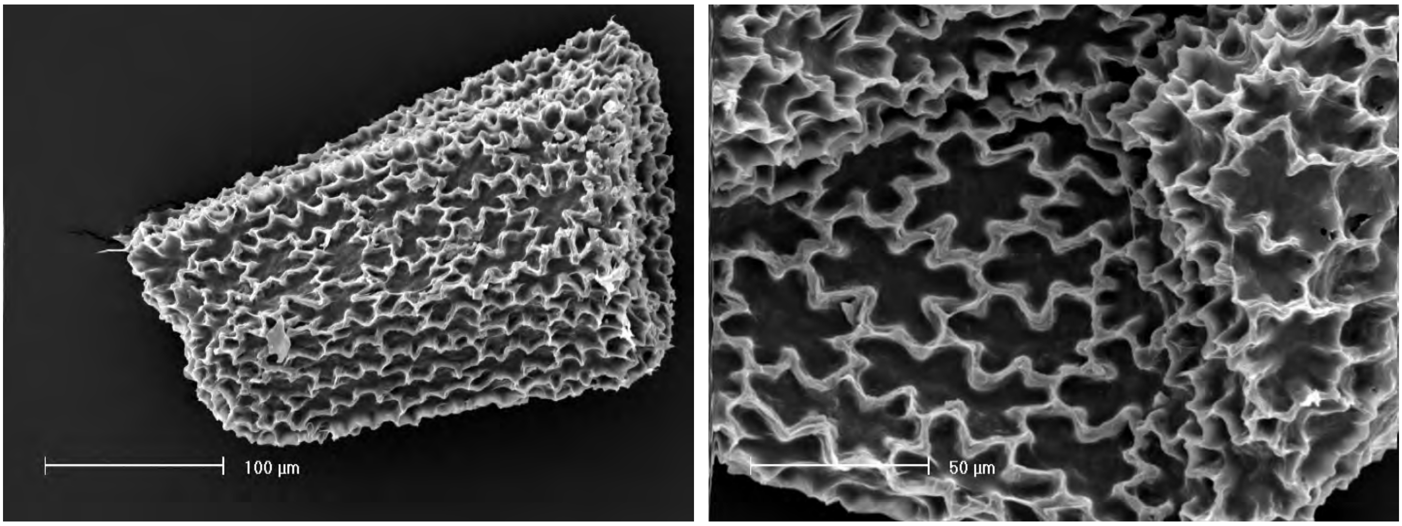
[1] No studies have been performed on seed dispersal on both *Klackenbergia* and *Lagenias*, and no hypothesis could be drawn.

Exochaenium Griseb.

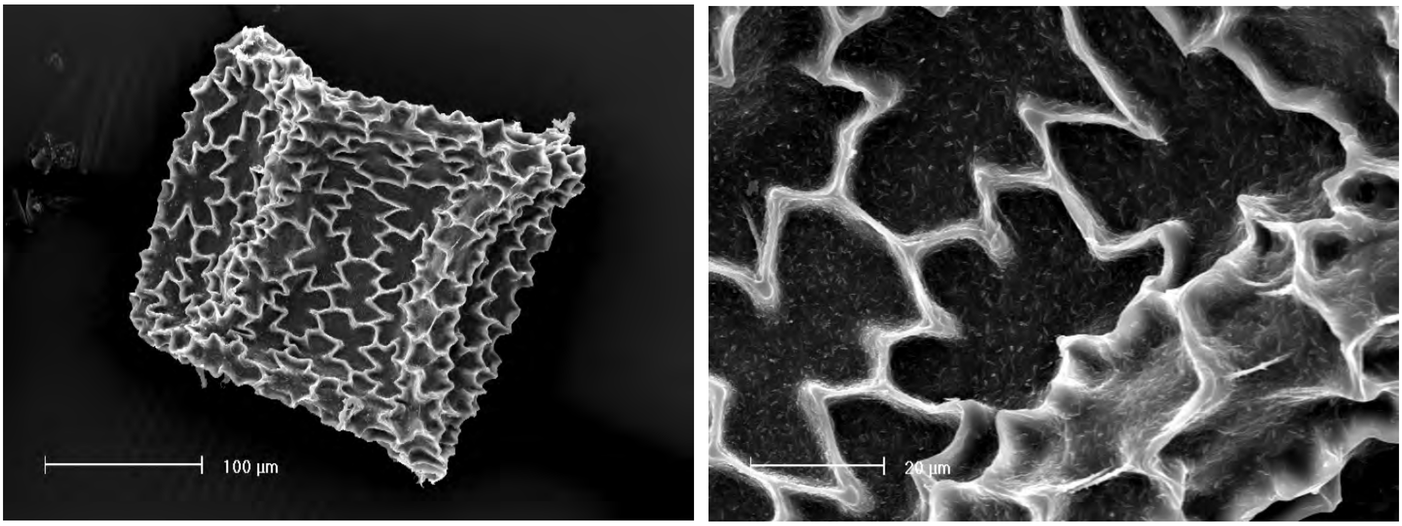




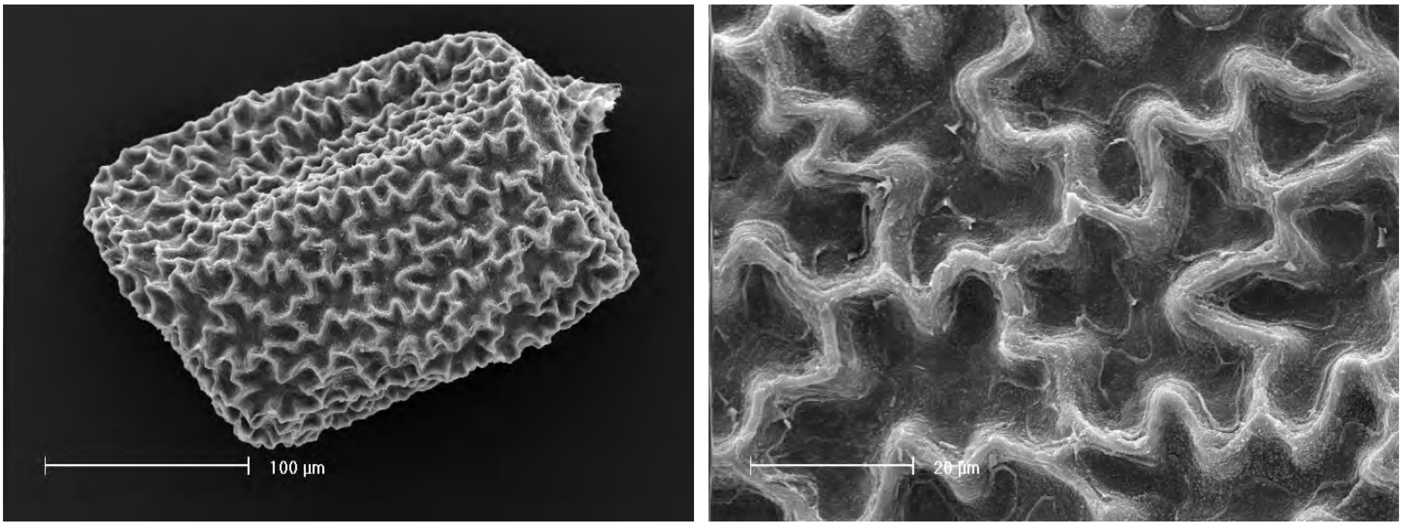
E. baumianum (Gilg) Schinz, *Dessein et al. 970*, NEU



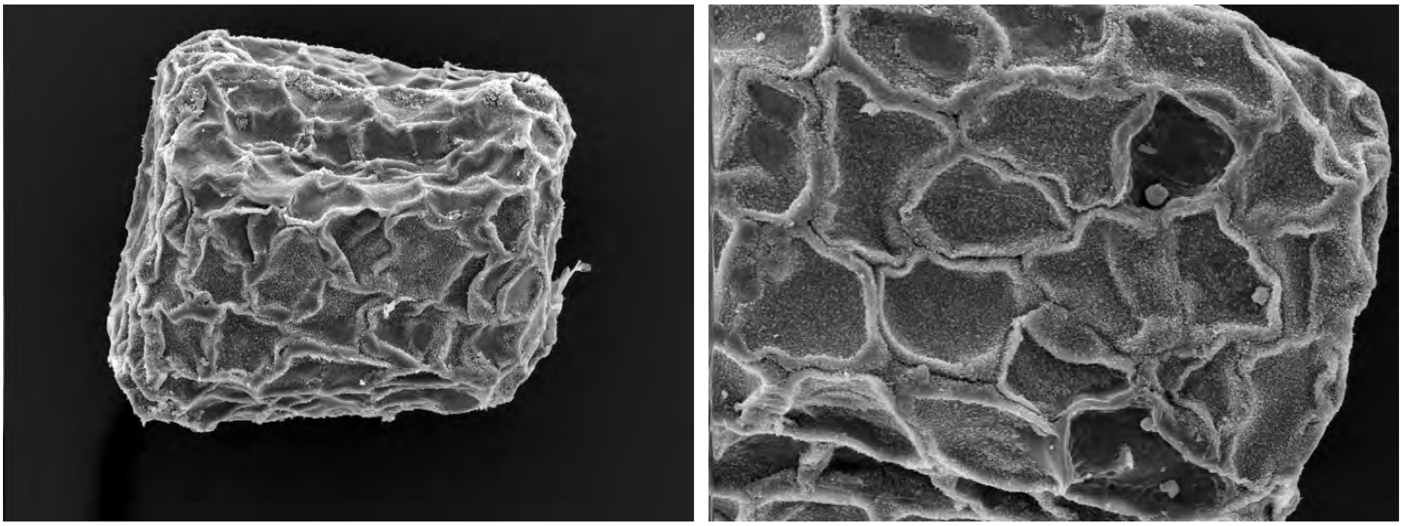
E. clavatum (Paiva & Nogueira) Kissling, *Dessein et al. 543*, NEU



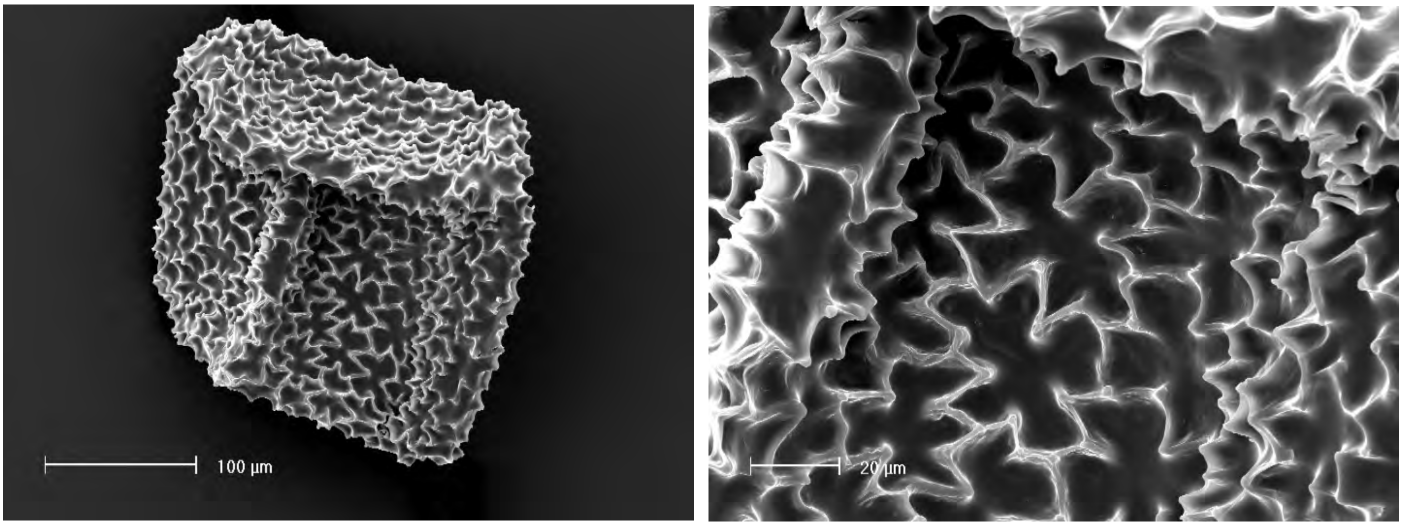
E. dimidiatum (Sileshi) Kissling, *Burtt 3632*, K



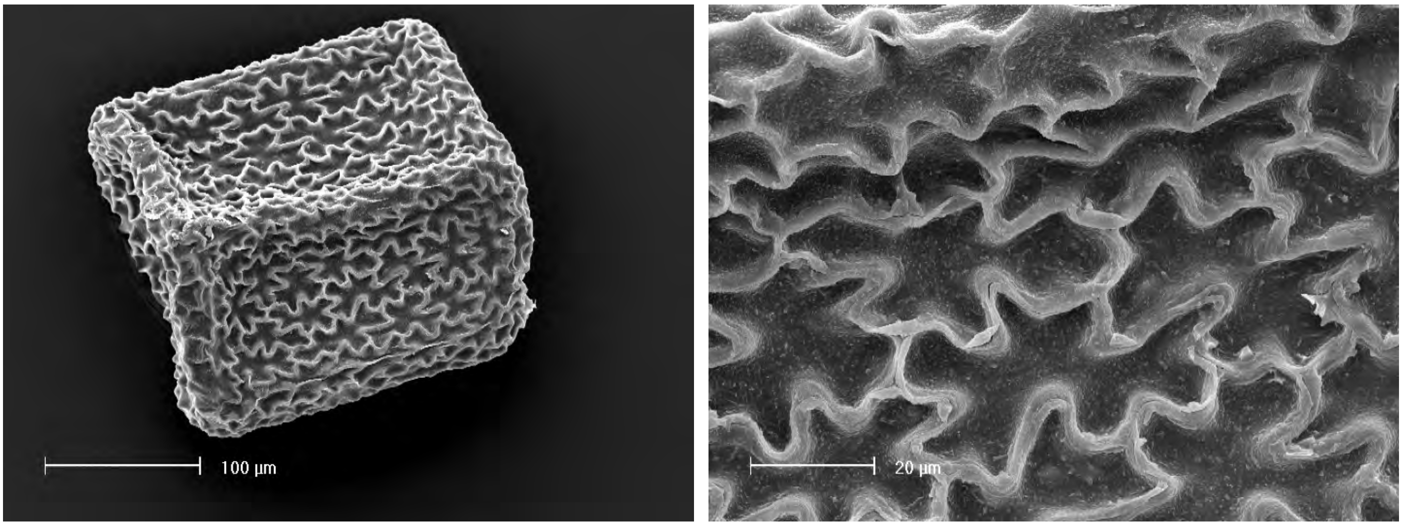
E. fernandesianum (Paiva & Nogueira) Kissling, *Dessein et al. 1011*, NEU



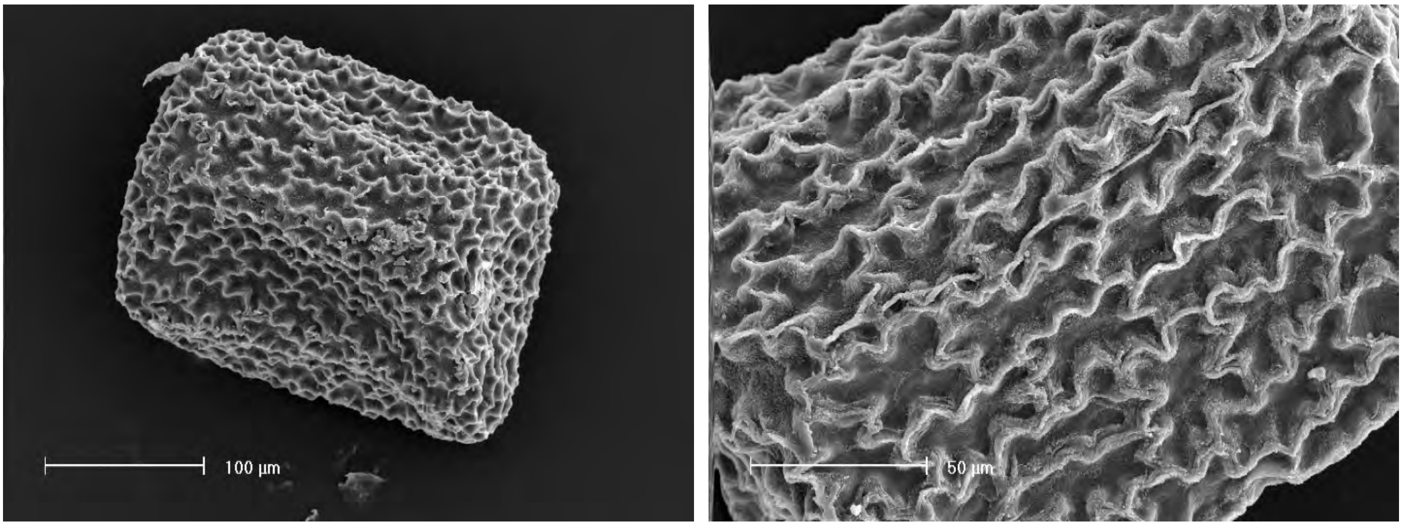
E. grande (E. Mey) Griseb., *Dessein et al. 806*, NEU



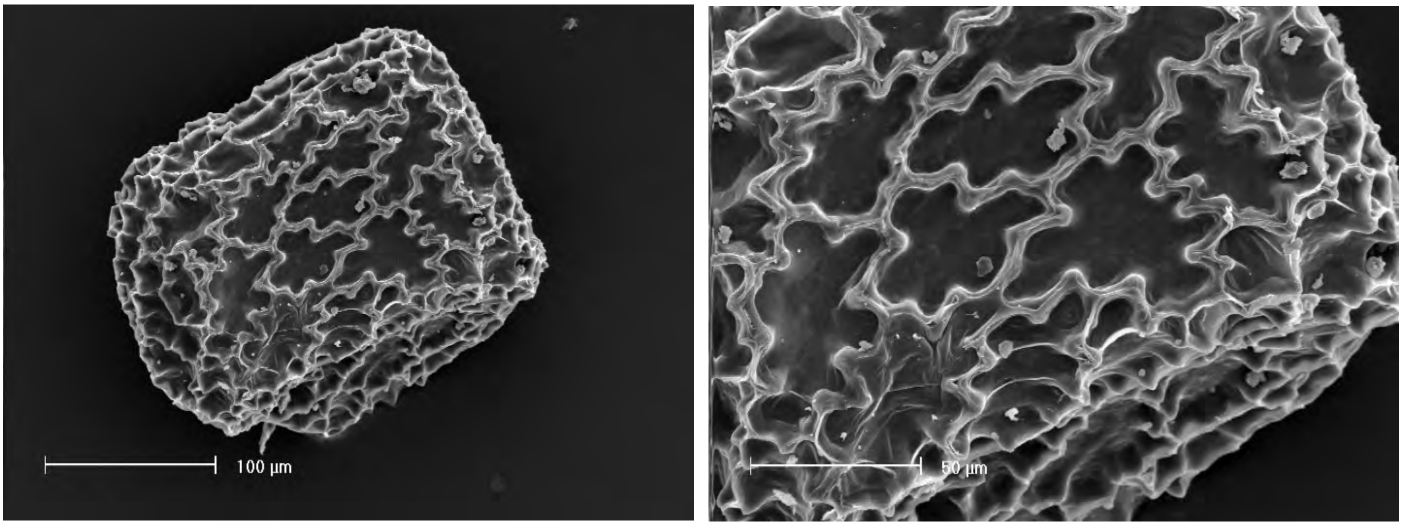
E. lineariforme (Sileshi) Kissling*, Bidgood et al. 3630,* K



E. macropterum (Sileshi) Kissling, *Bidgood et al., 4012*, K



E. exiguum A.W. Hill, *Dessein et al 656.*, NEU



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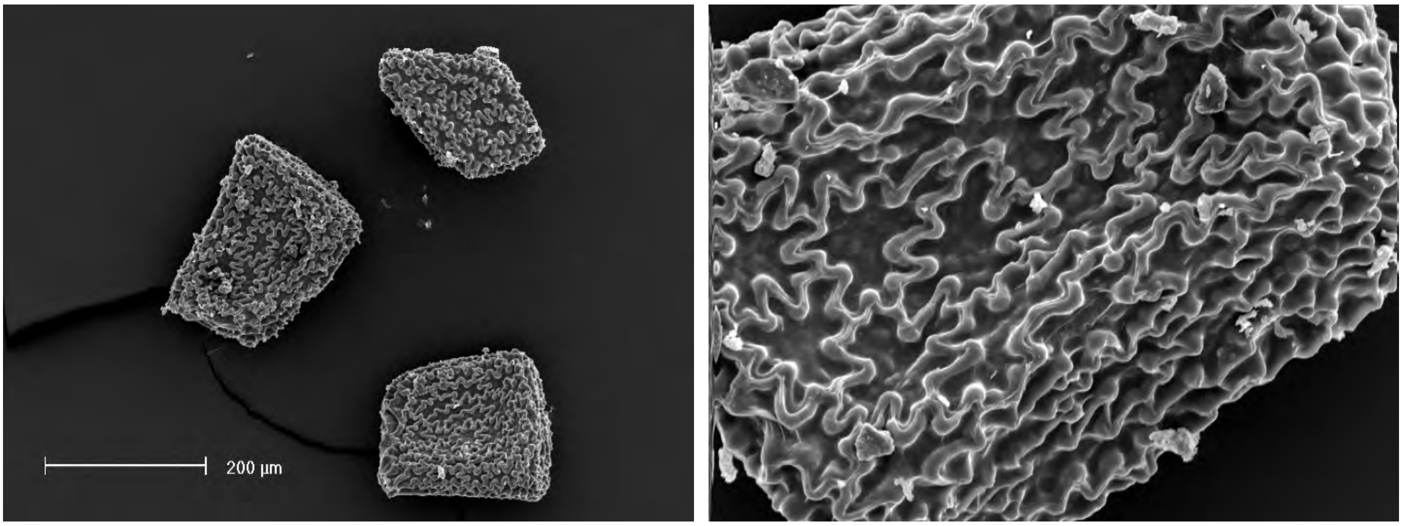

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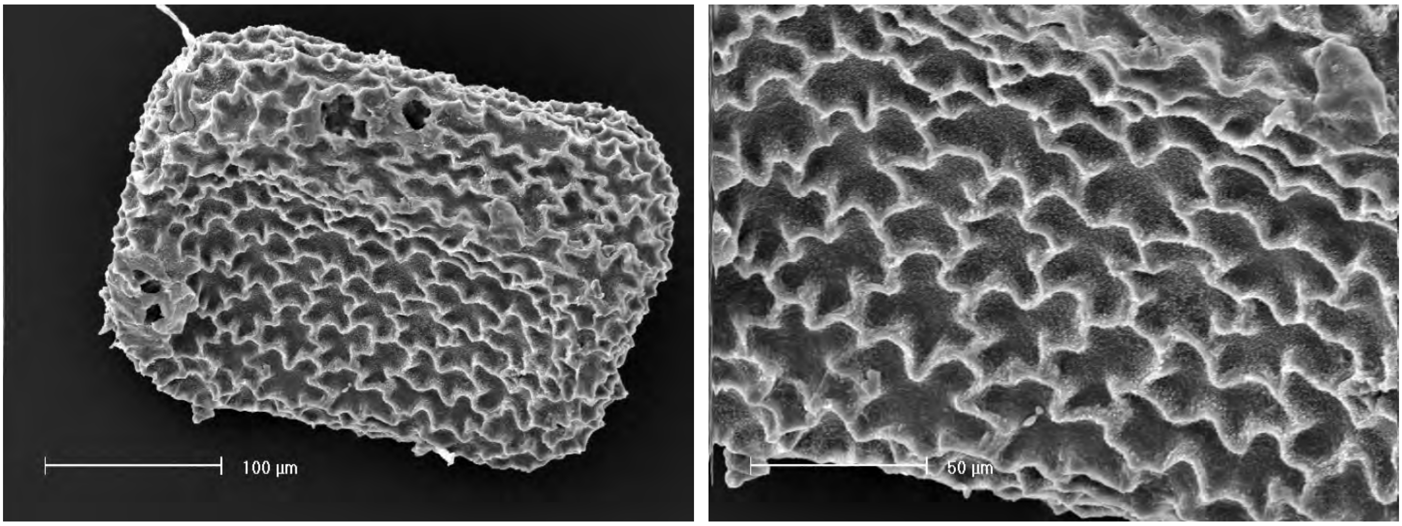
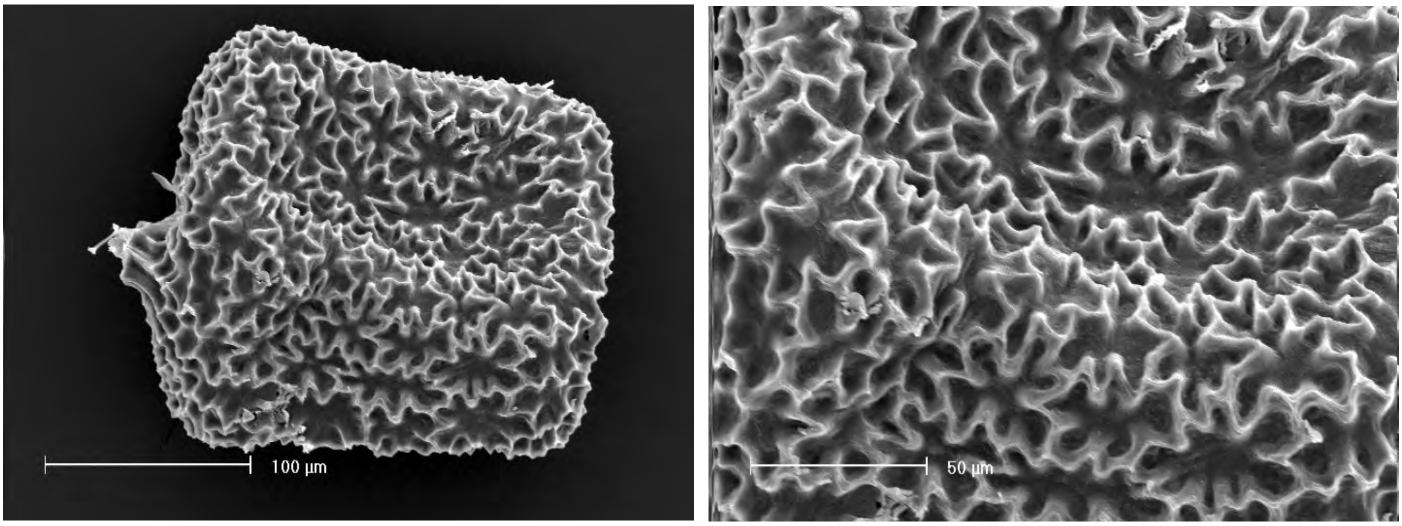
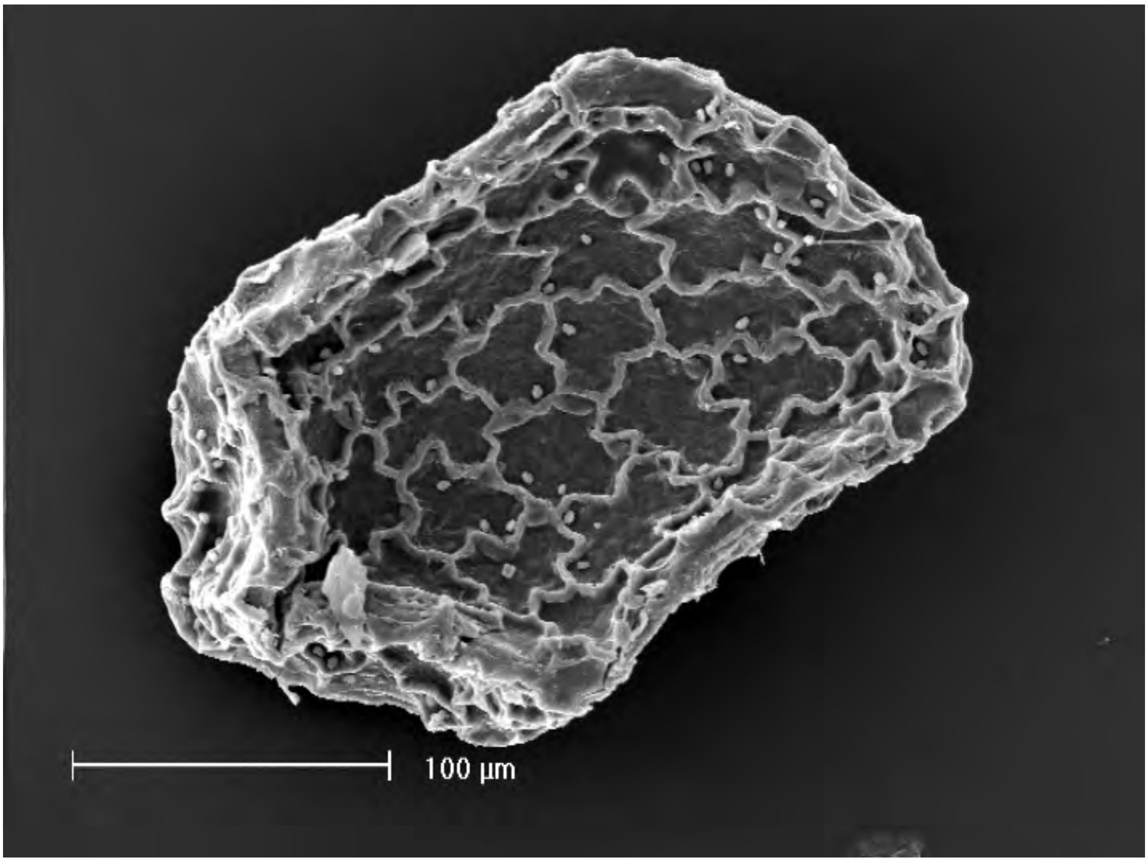


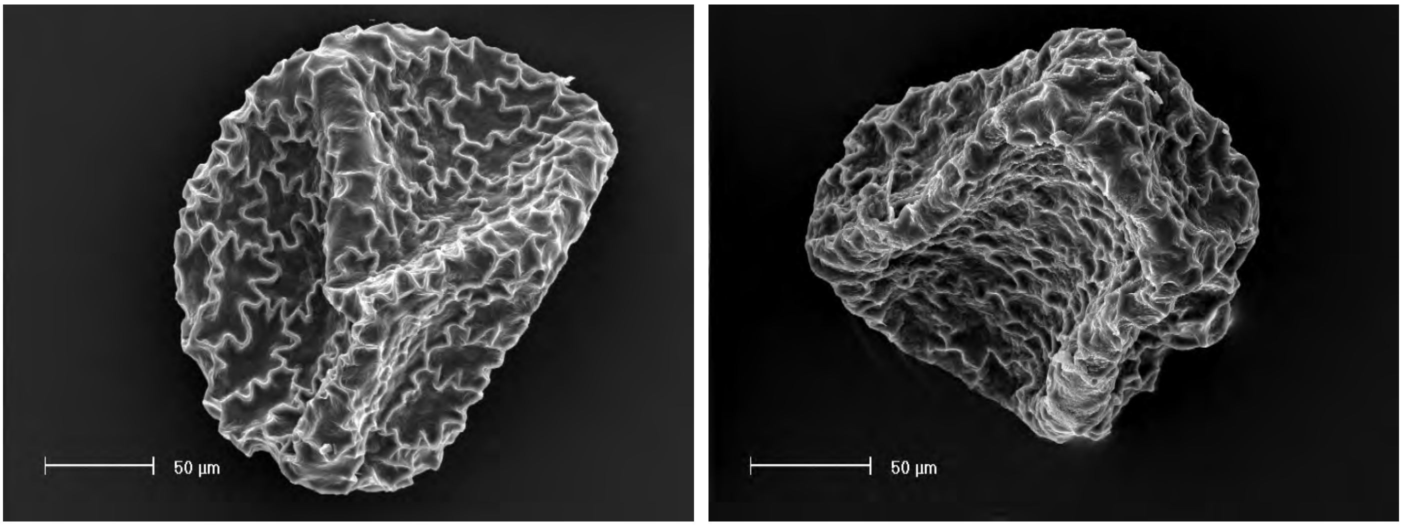
E. platypterum (Baker) Schinz, *Kissling 75*, NEU



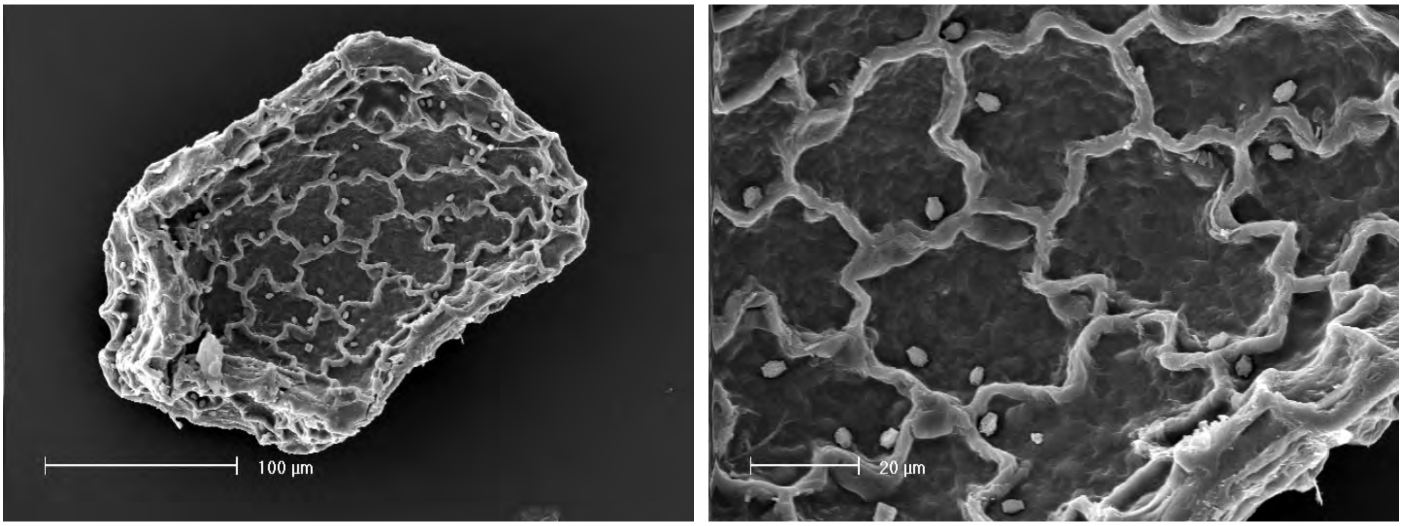
E. teuczsii (Schinz) Schinz, *Dessein et al. 599*, NEU

Klackenbergia Kissling

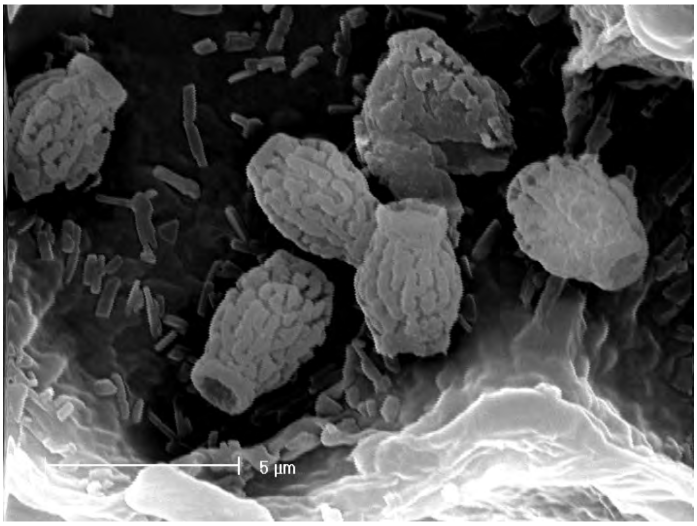




Klackenbergia condensata (Klack.) Kissling, *Croat 32108*, MO

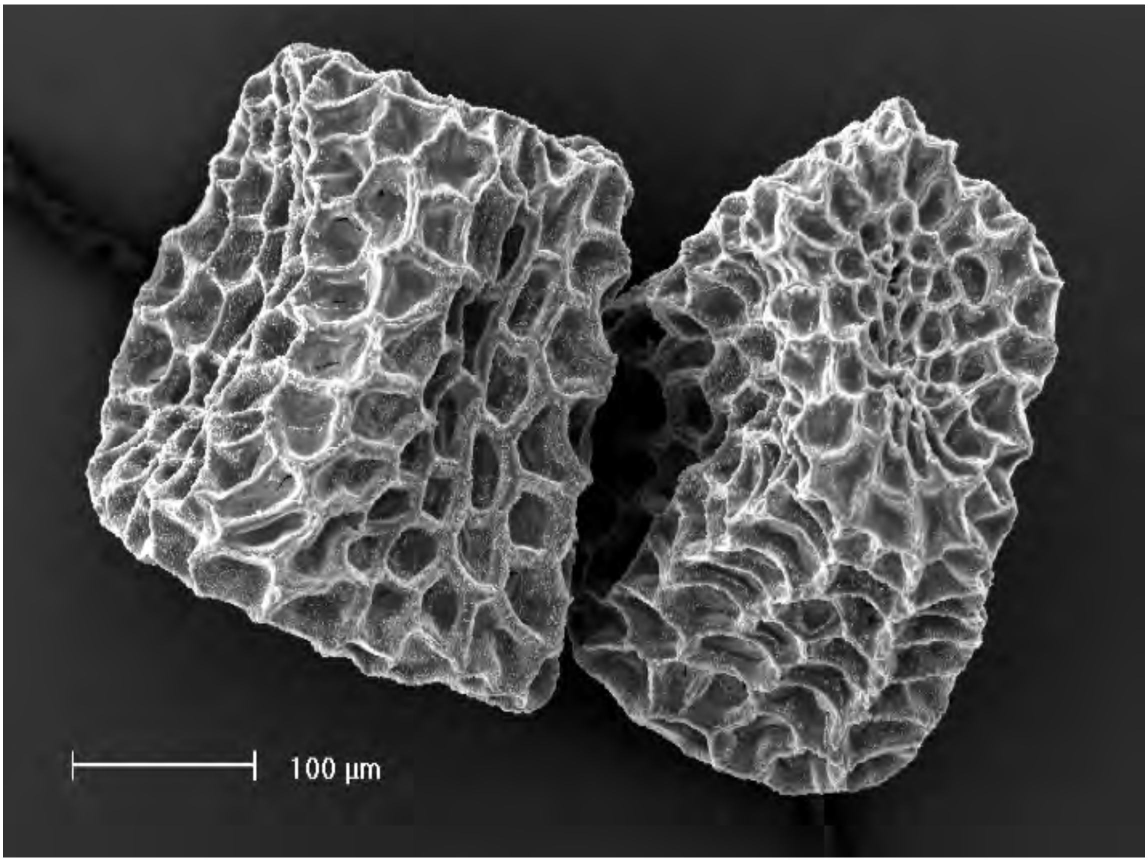


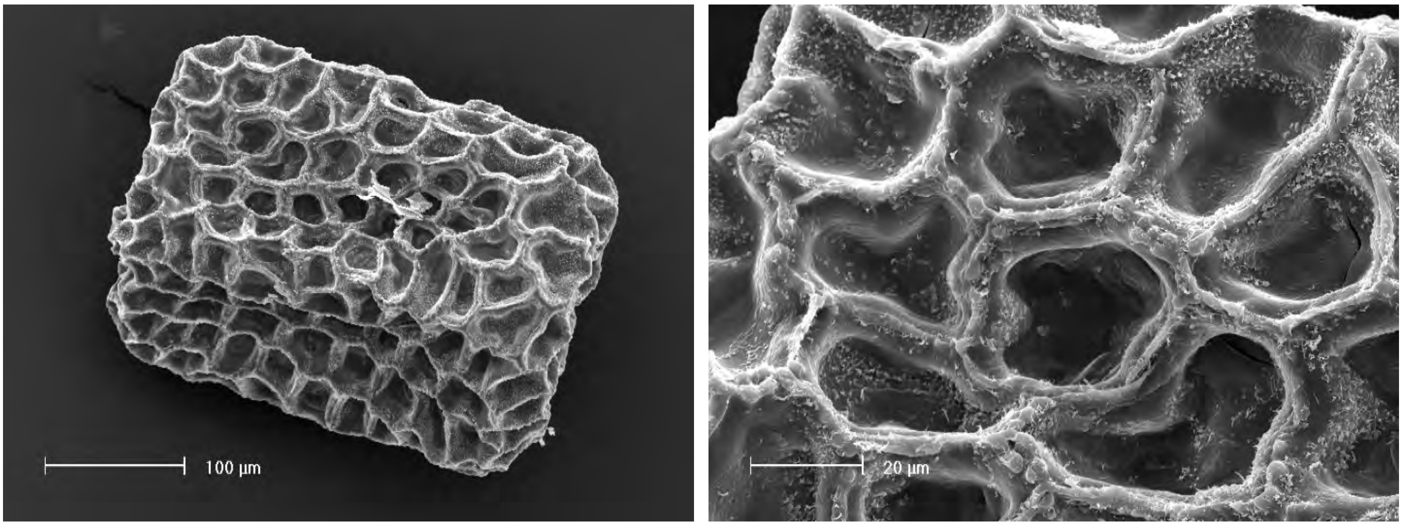
K. stricta (Schinz) Kissling, *Hildebrandt s.n.* , MO



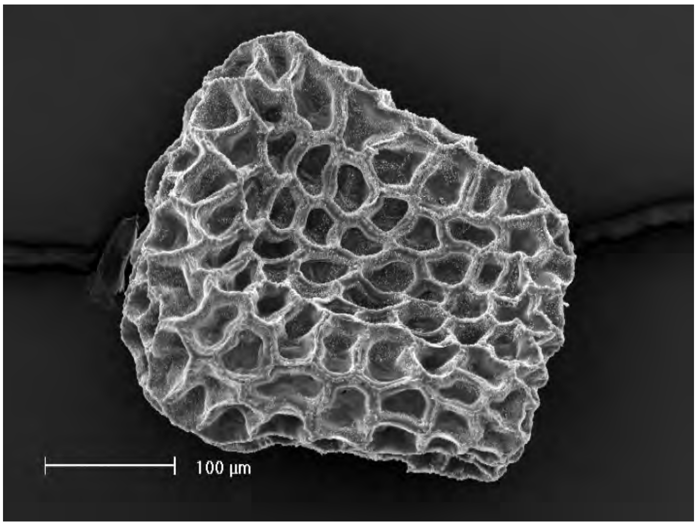
Unidentified artifacts on the seed, of c. 3µm

Lagenias E. Mey.

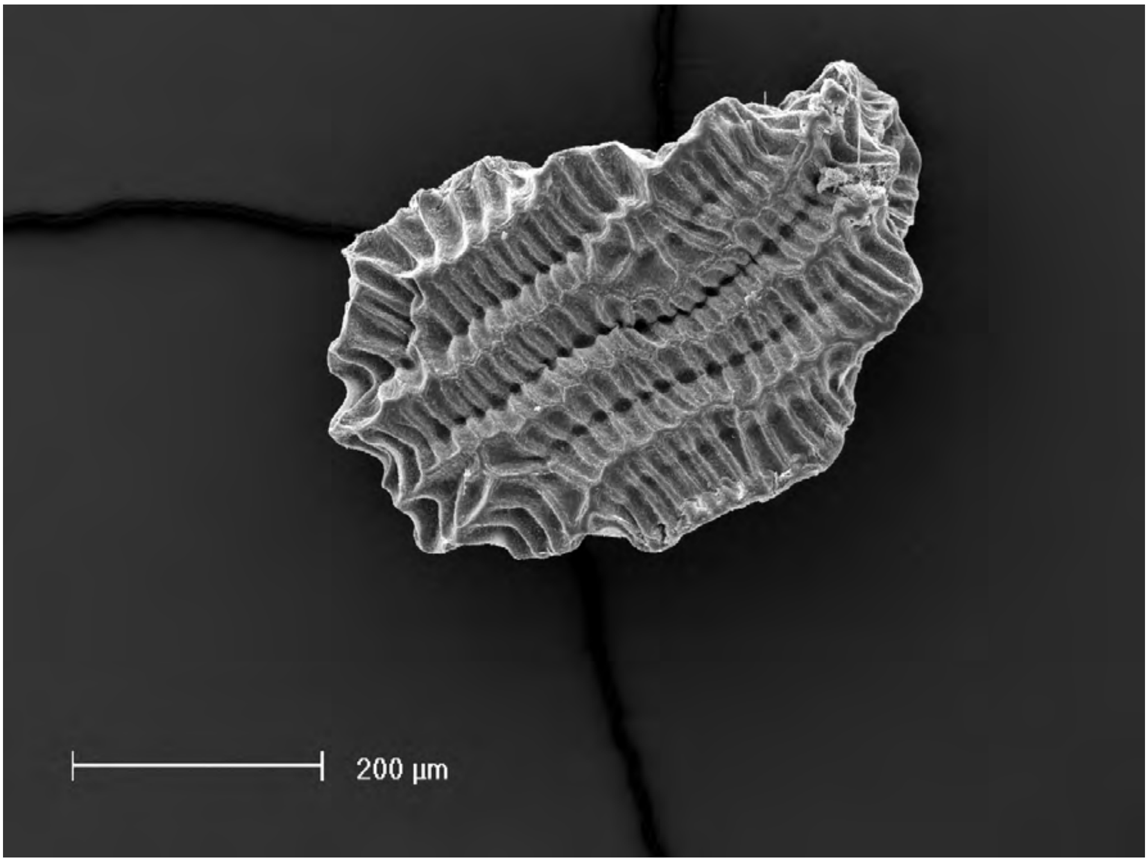


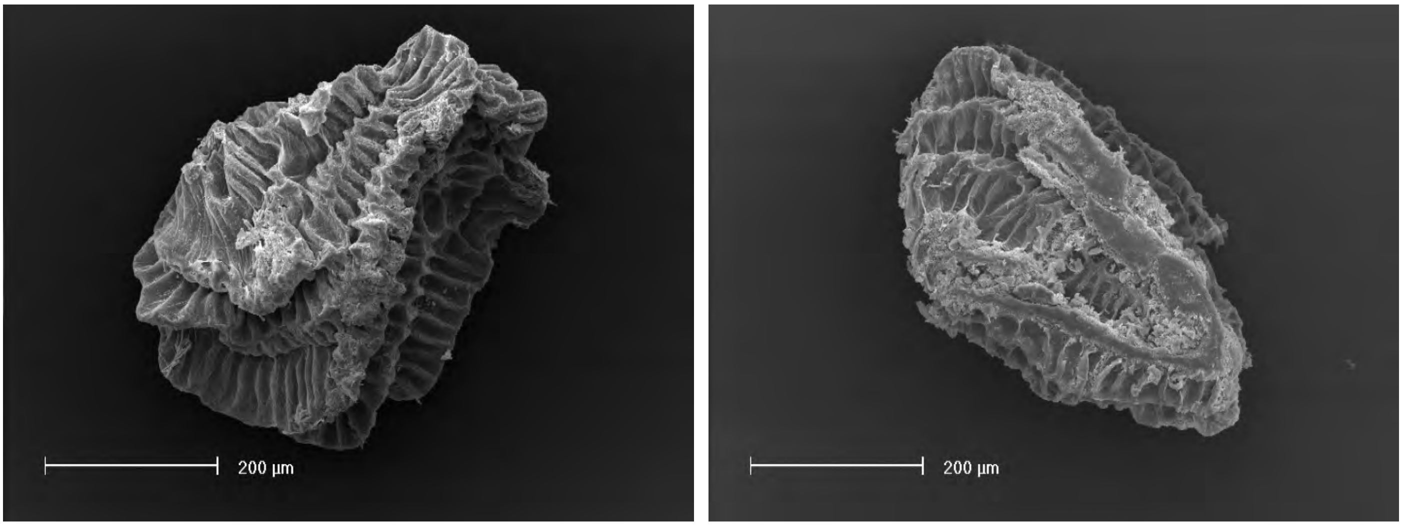


Lagenias pusillus (Eckl. ex Cham.) E. Mey., *Esterhuysen 35319*, MO

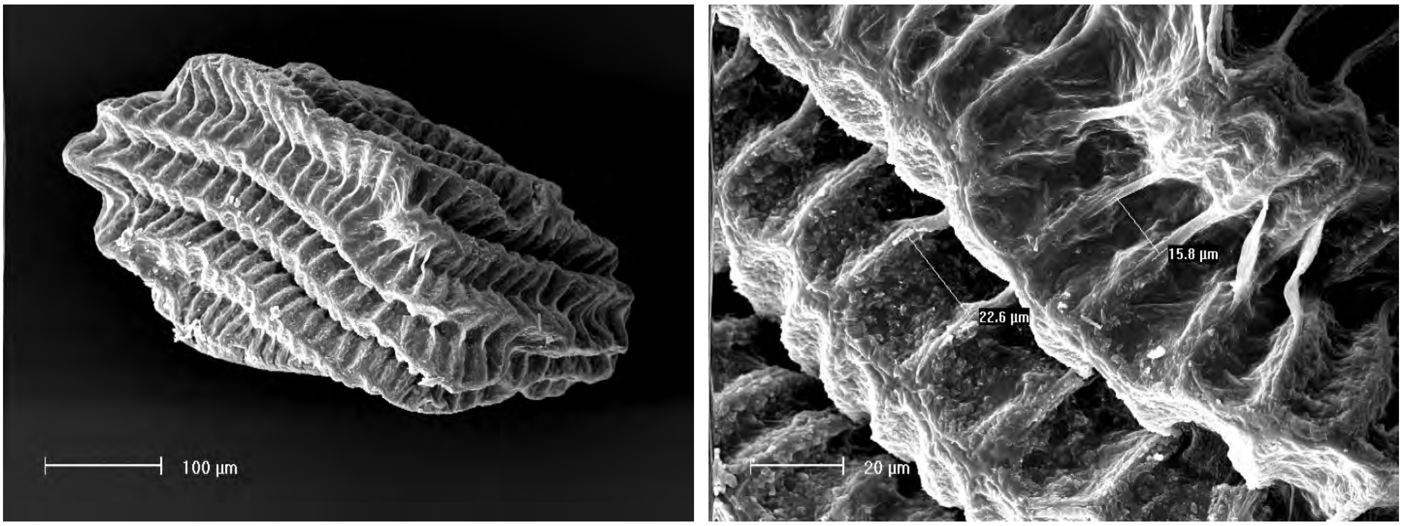


Sebaea Sol. ex R. Br.

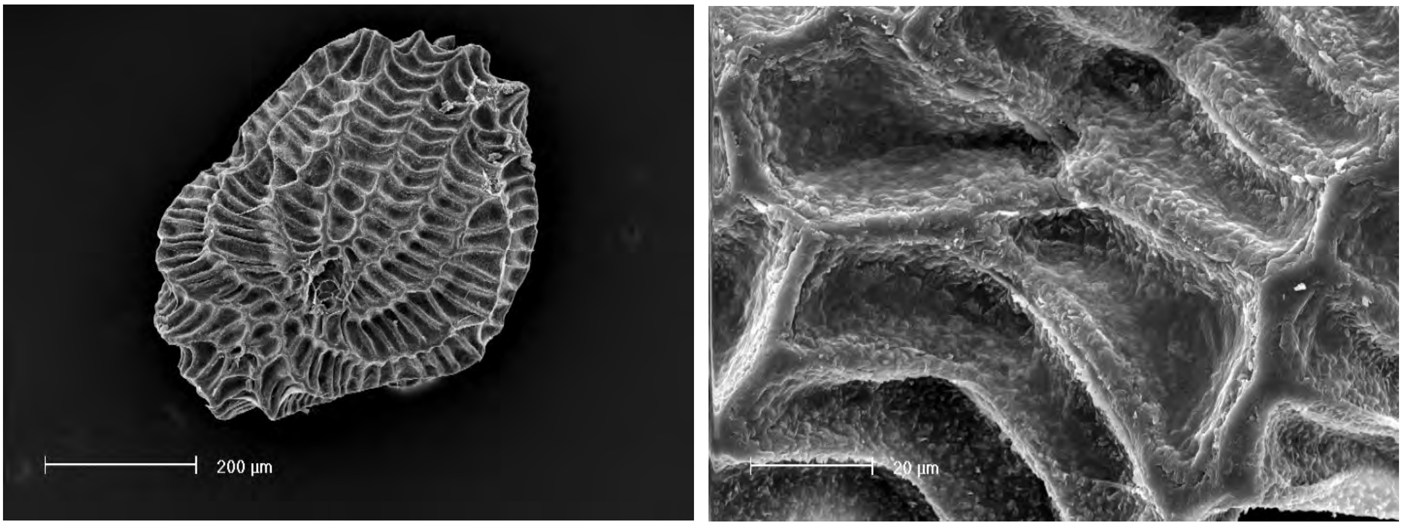




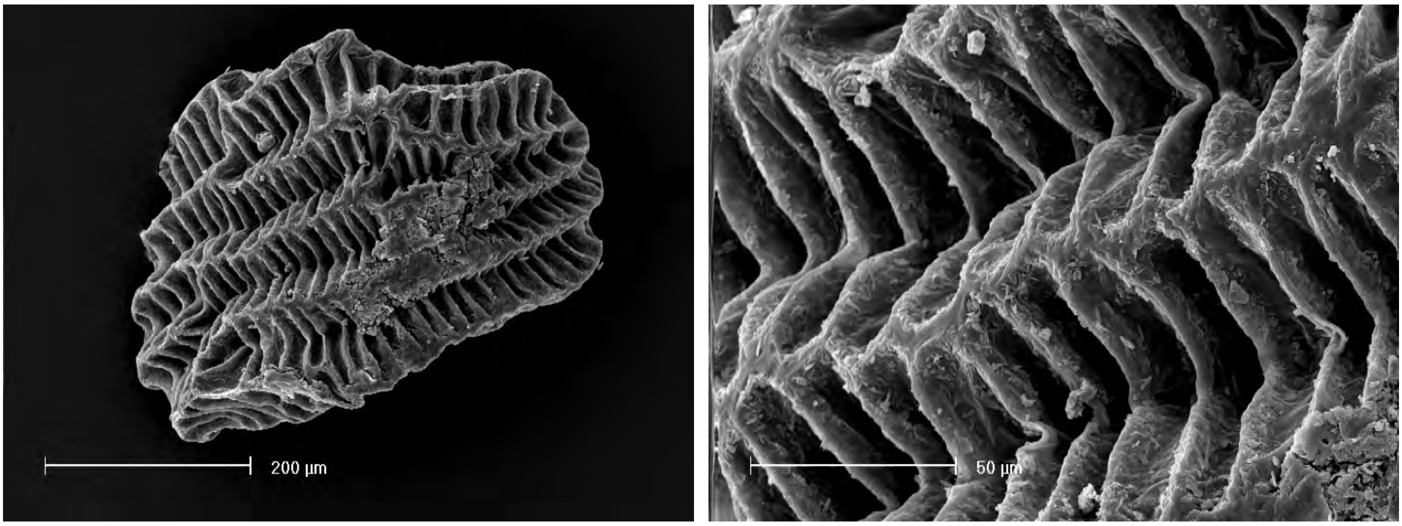
Sebaea albens (L. f.) Roem. & Schult., C



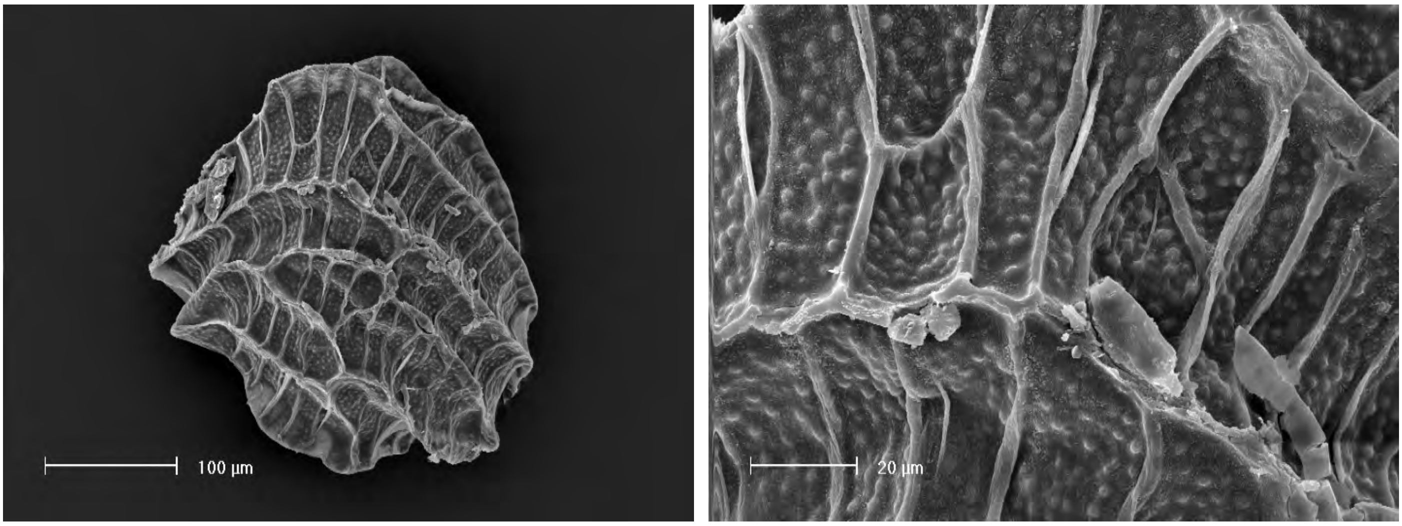
S. albidiflora F. Muell., *Goddlieb s. n.*, C



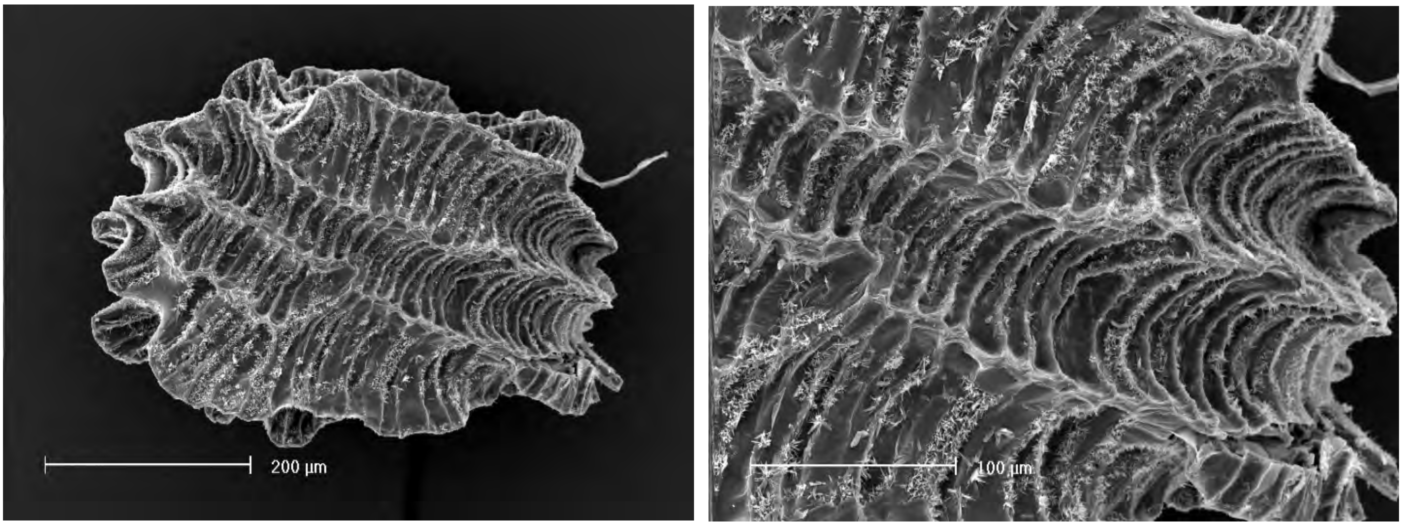
S. ambigua Cham., *Schlechter 7308*, WAG



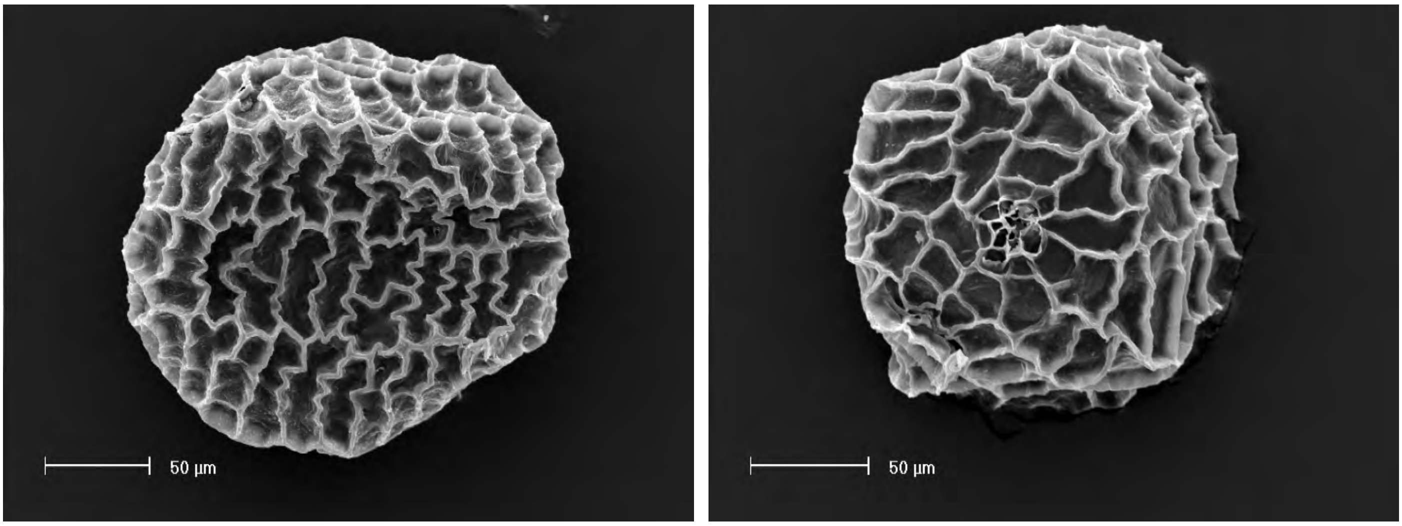
S. aurea (L. f.) Roem. & Schult.*, Lavranos 32108*, MO



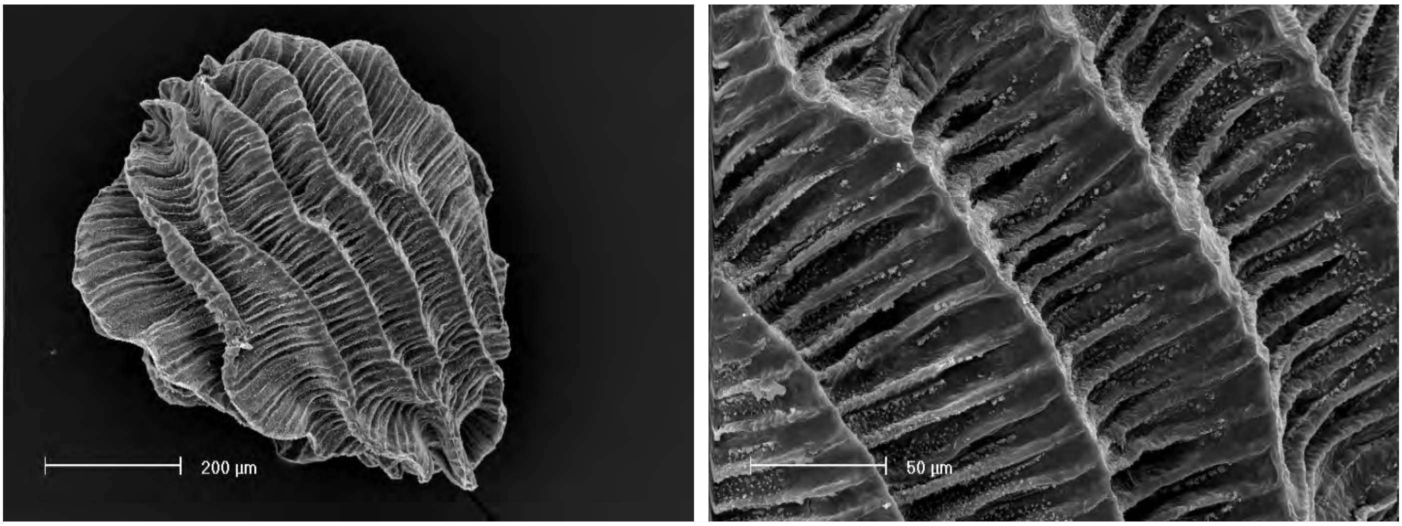
S. brachyphylla Griseb, *Bretler et al*., WAG



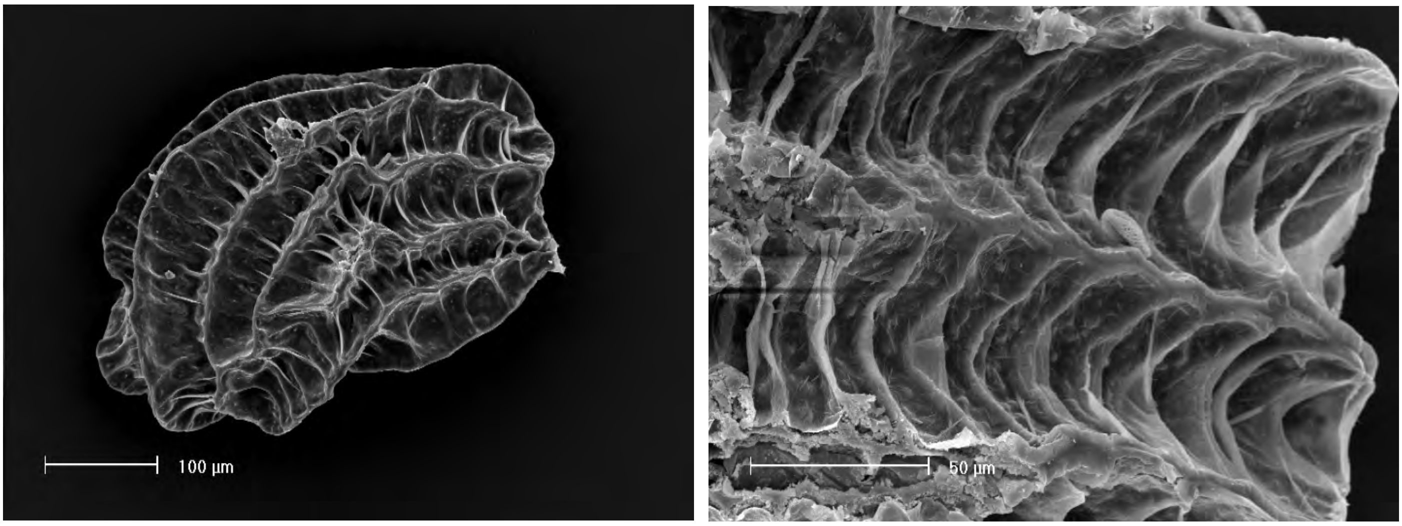
S. capitata Cham. & Schlechtdl. var. *sclerosepala* (Schinz) Marais, MO



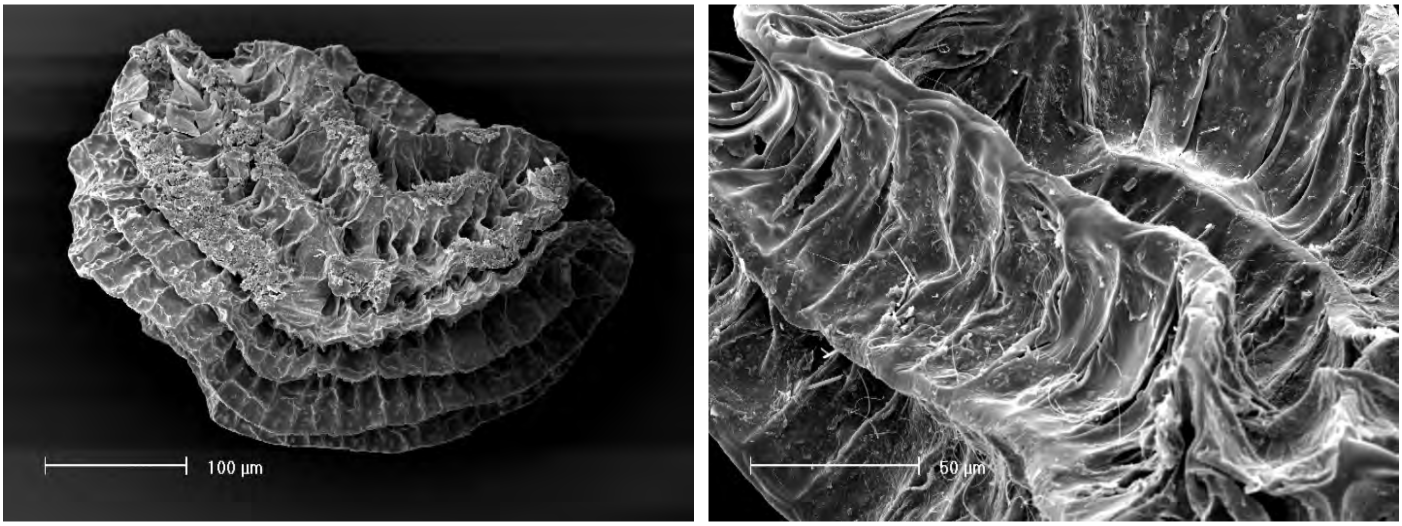
S. exacoides, *Karis & Johansson 753*, S



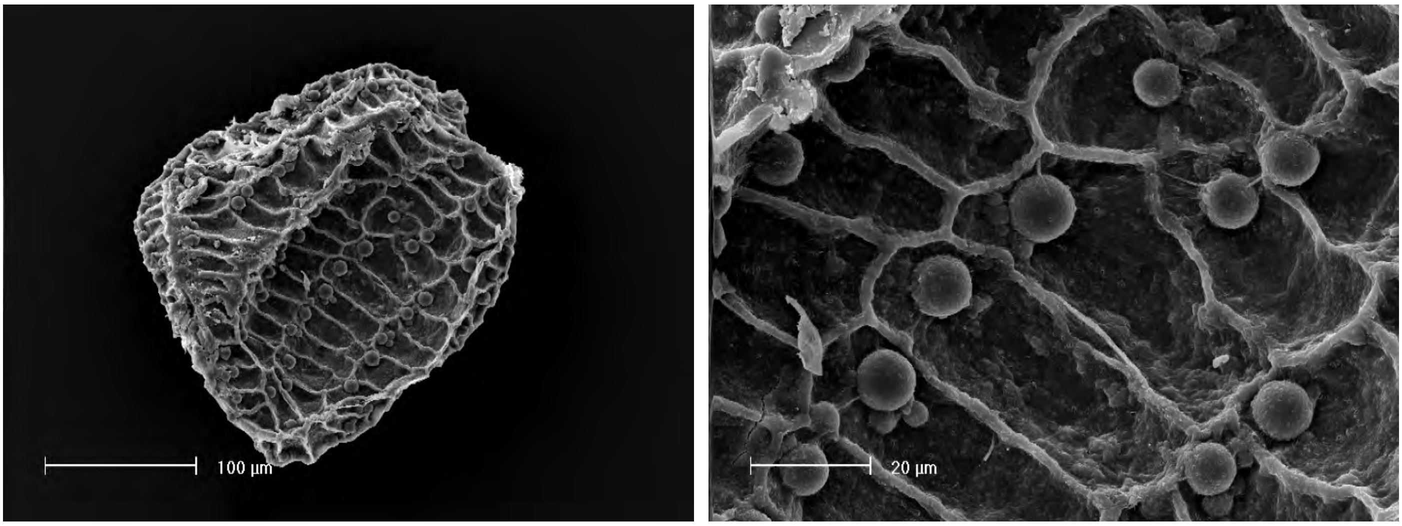
S. laxa N. E. Br., *Esterhuysen 28244*, MO



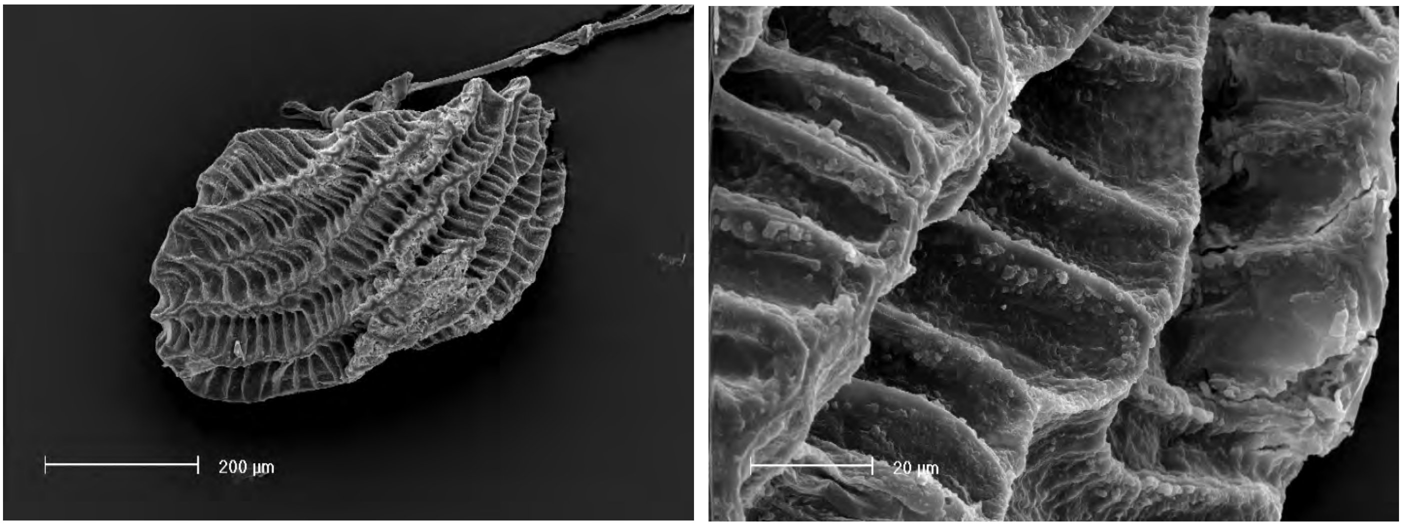
S. leiostyla Gilg, *Burtt 7767*, MO



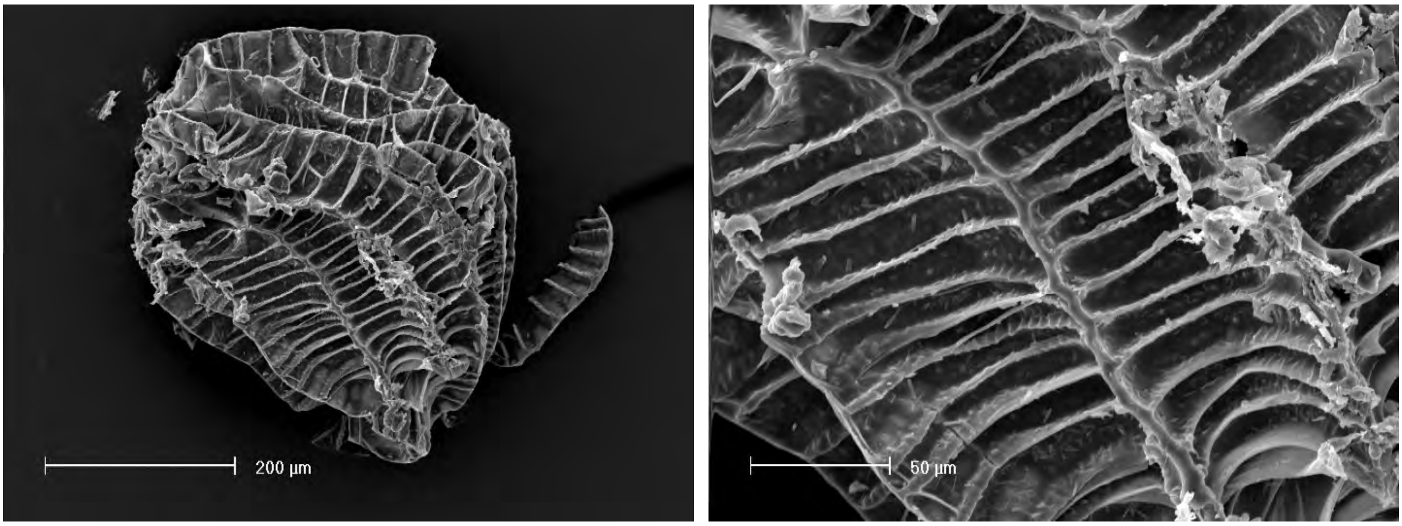
S. macrophylla Gilg, *Fries et al.* 696, MO



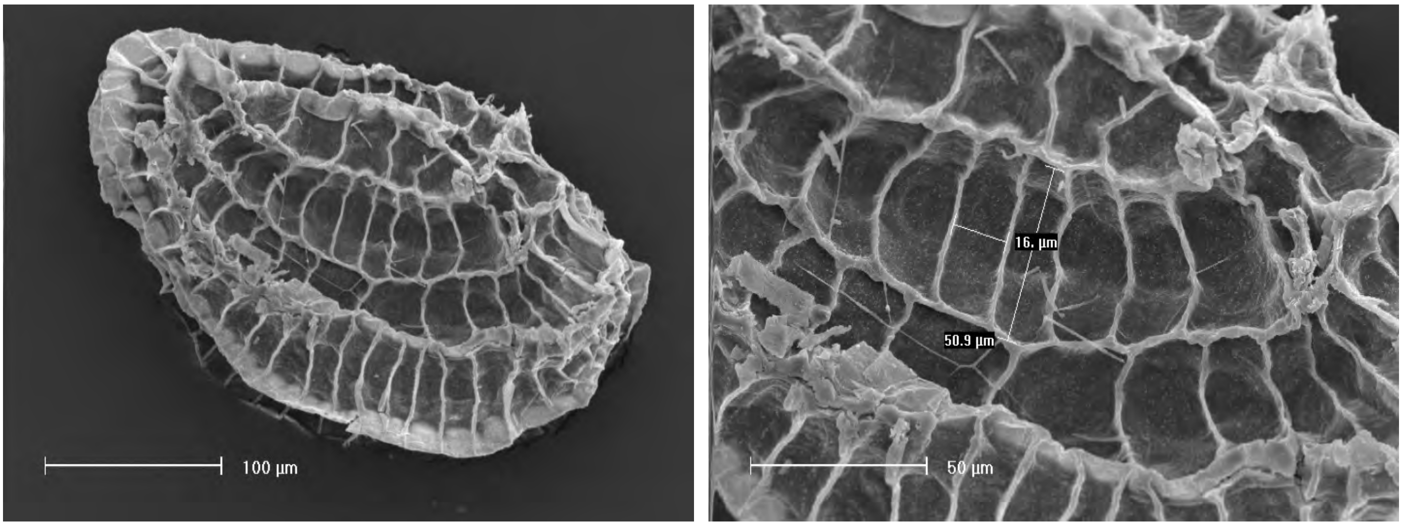
S. micrantha (Cham. & Schlechtdl.) Schinz var. *intermedia* (Cham. & Schlechtdl.) Marais, *Esterhuysen 32964*, S



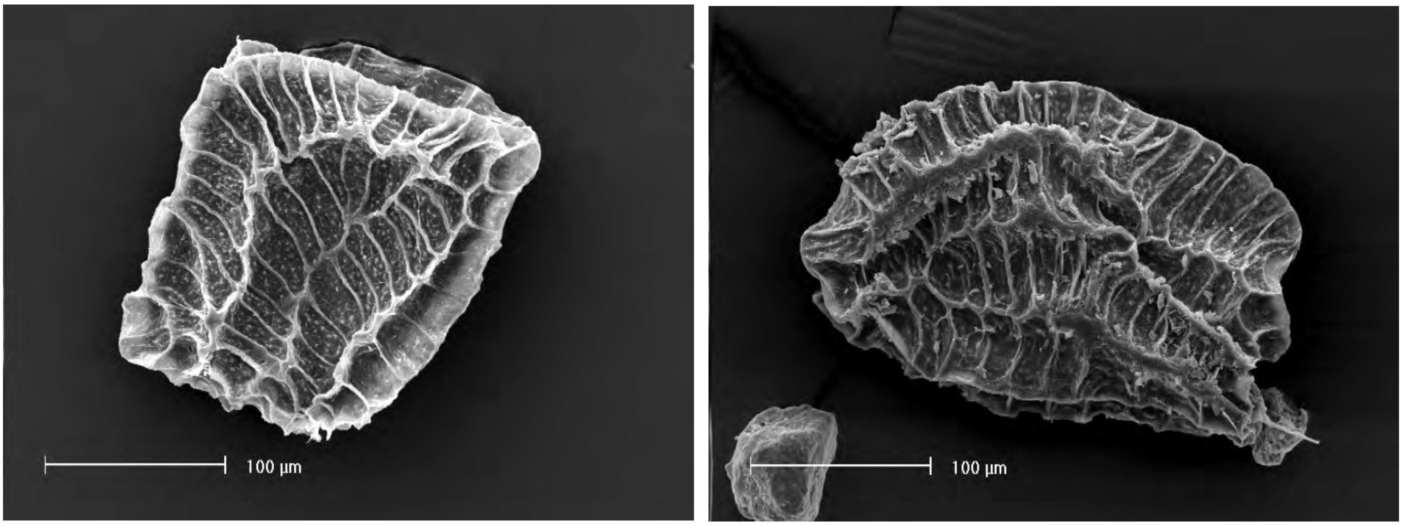
S. minutiflora Schinz, *Wall 3681*, S



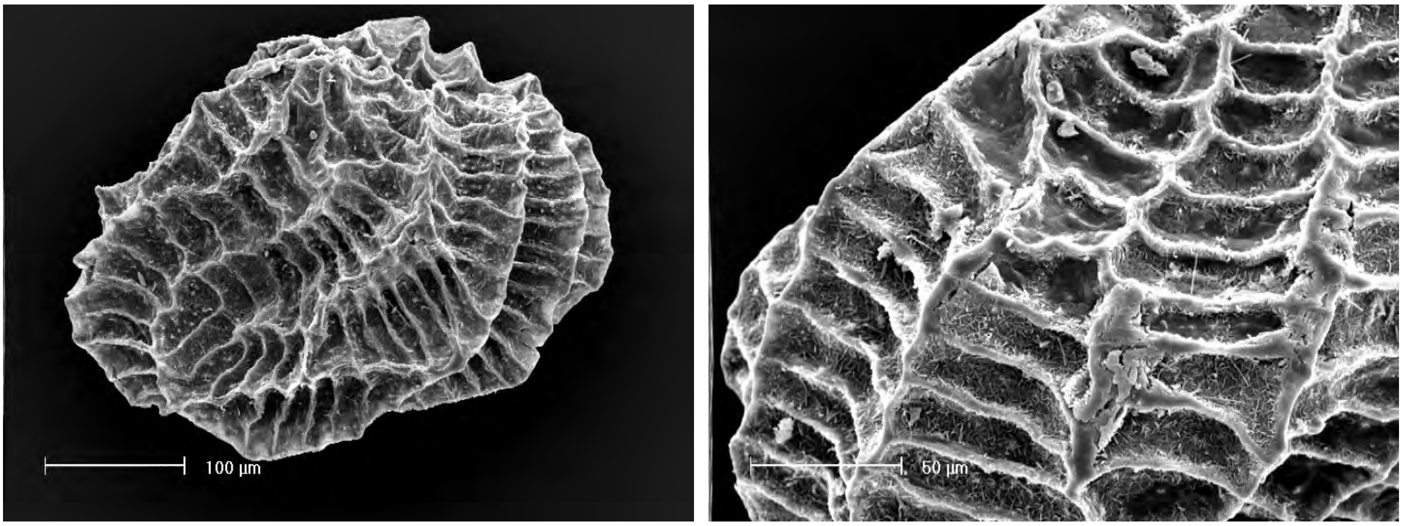
S. natalensis Schinz, B&T*



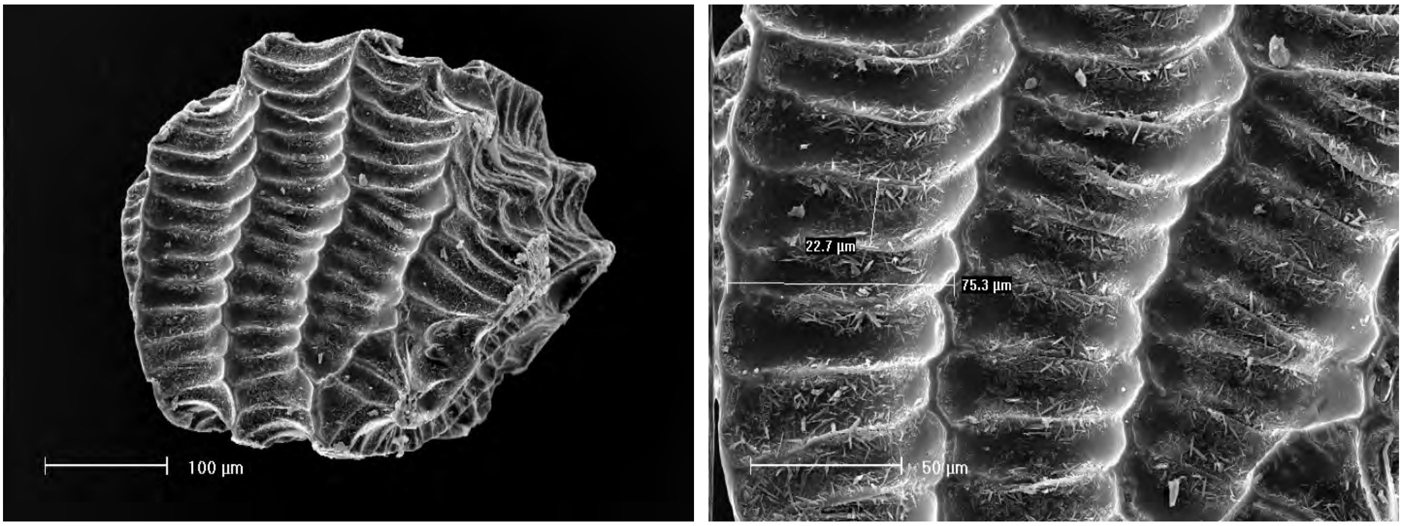
S. ovata (Labill.) R. Br., *Boormann s.n.*, UPS



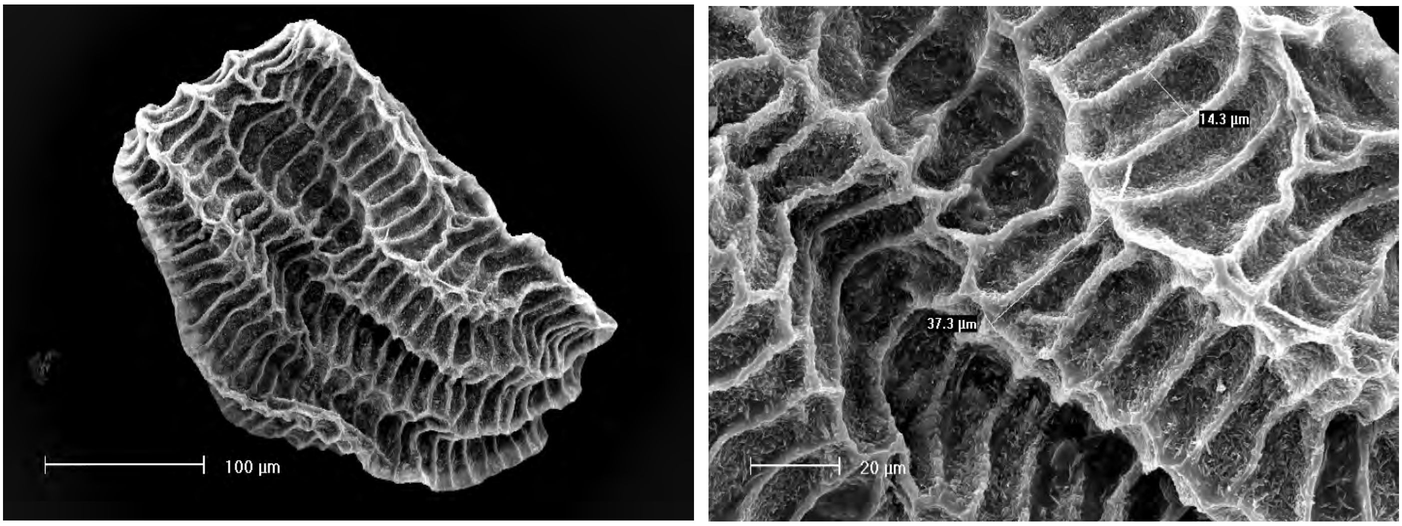
S. pentandra E. Mey, *Robinson 5593*, S



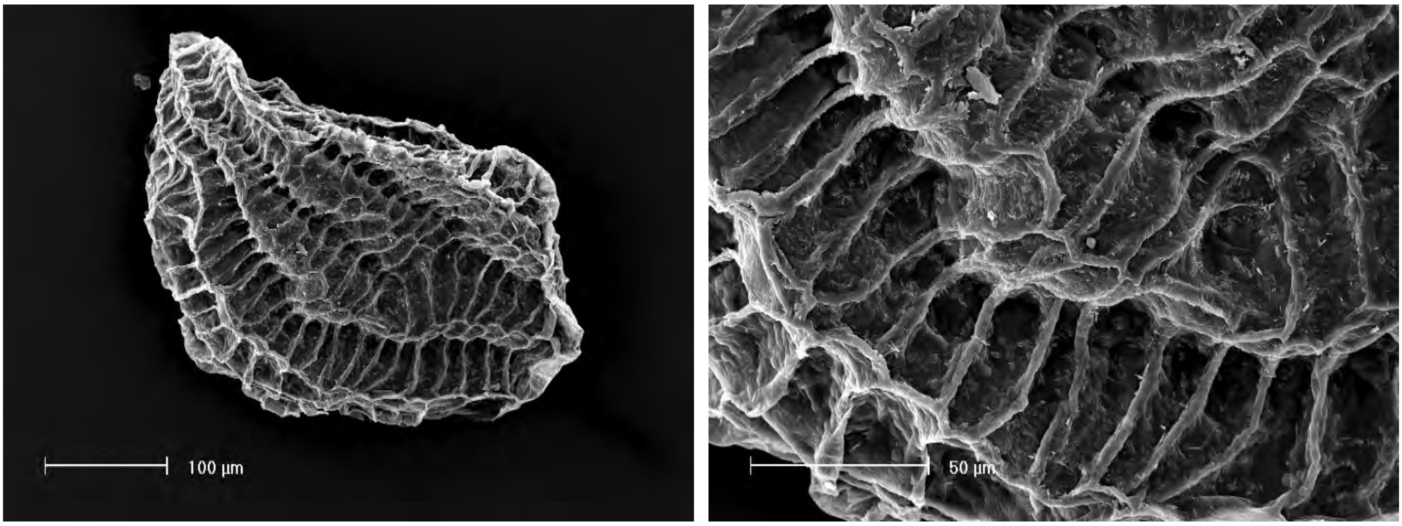
S. ramomissa Gilg, B&T*



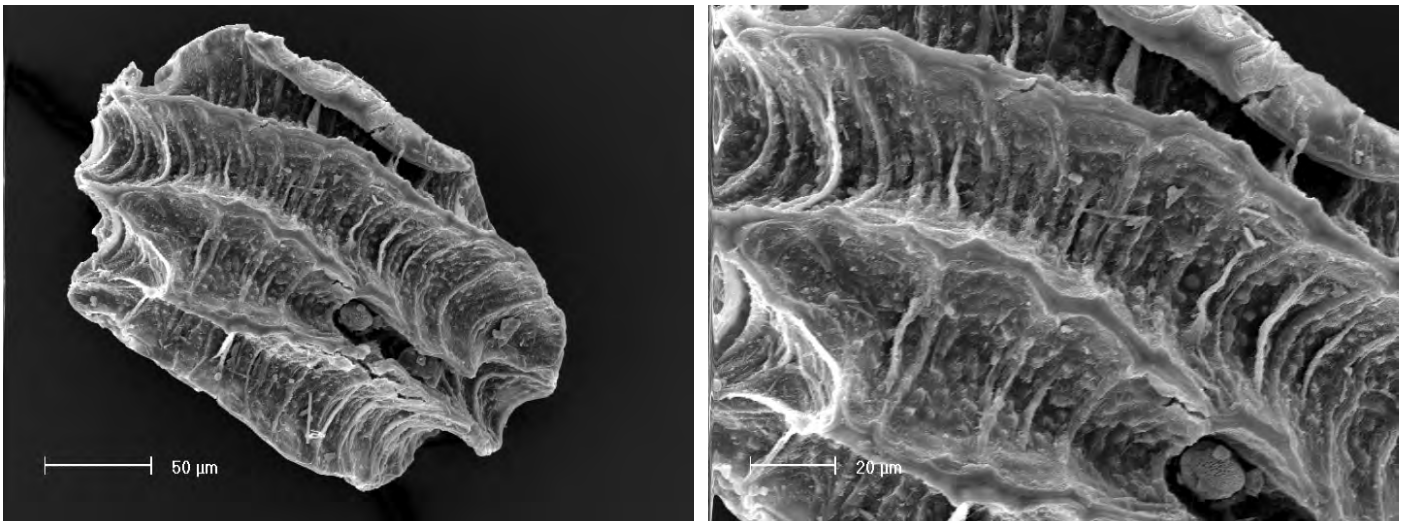
S. rehmanii aff. Schinz, *Hilliard & Burtt 9905*, MO



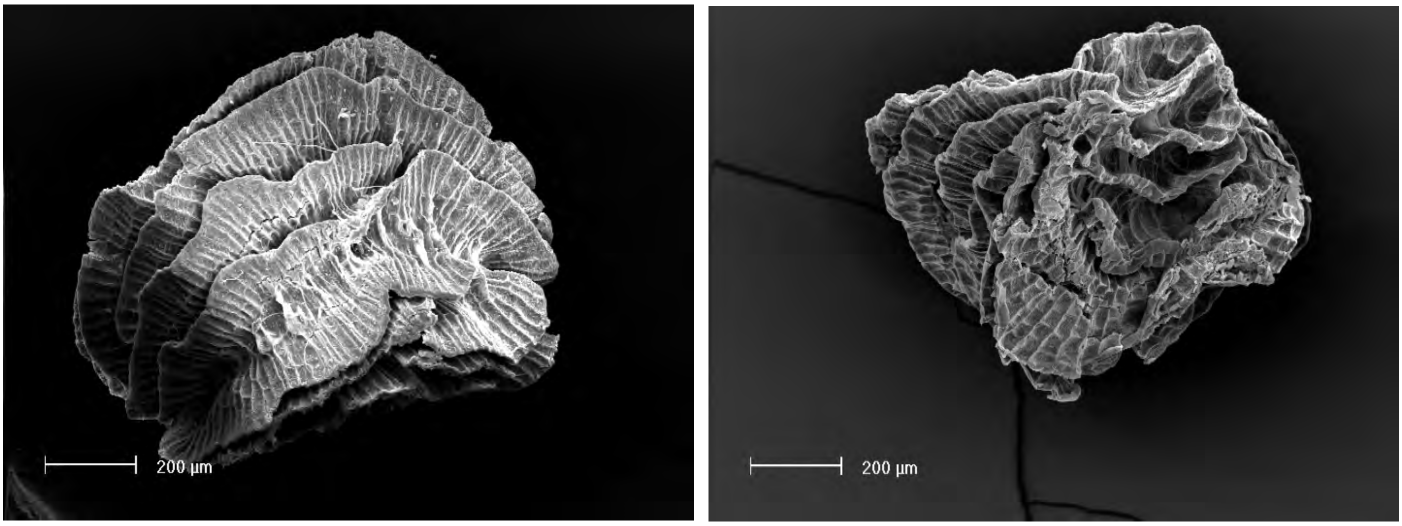
S. scabra Schinz, *Fries et al. 1362*, MO



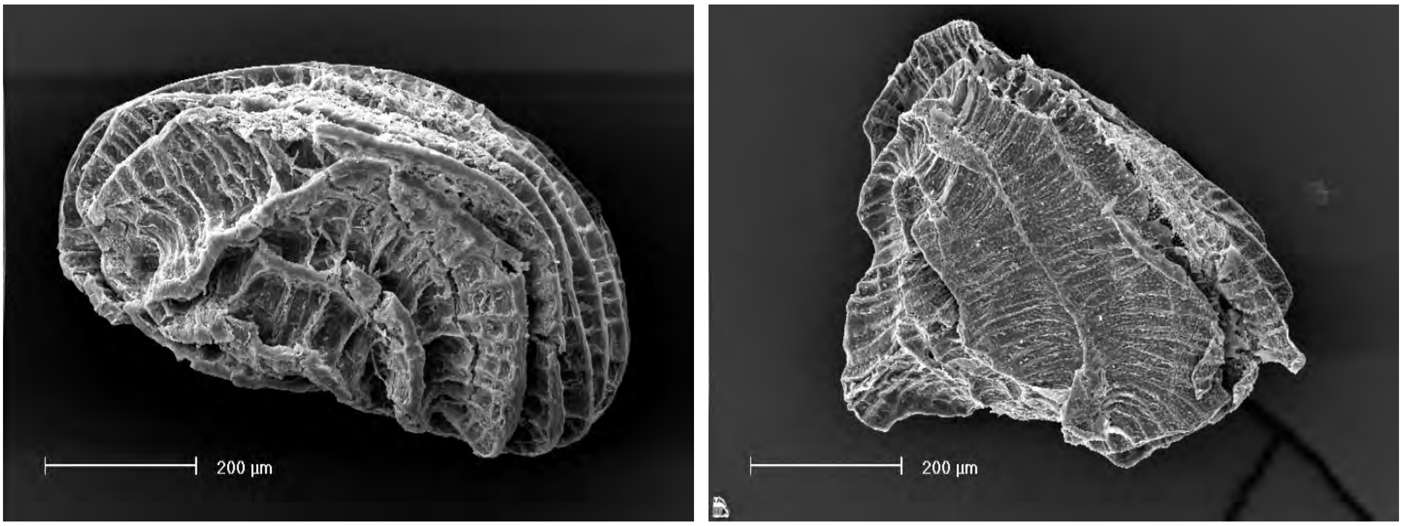
S. schlechterii Schinz, *Burman 1378*, MO



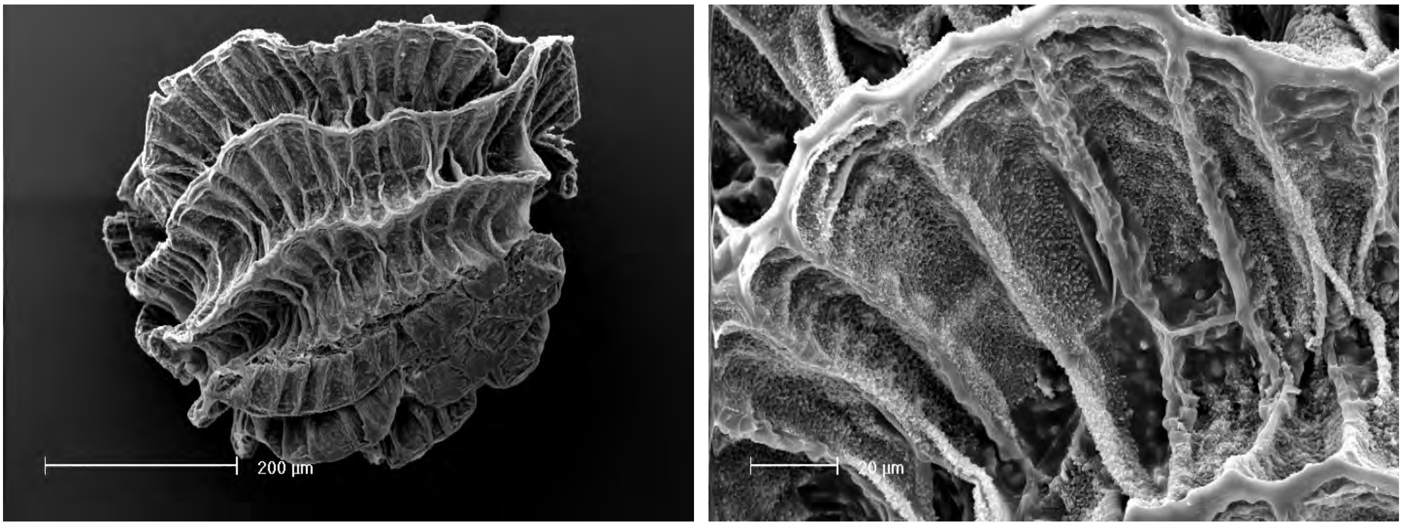
S. sedoides Gilg var. *sedoides* Gilg*,* B&T*



S. spathulata (E. Mey.) Steud.*,* B&T*



S. thodeana Gilg, B&T*



S. thomasii (S. Moore) Schinz, BOL

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* The seeds were purchased at B&T World Seeds. The obtained seeds were planted to obtain some fresh material. Unfortunately, the plants have not flowered. Therefore, no rigorous determination was done by the author. However, the species used are easily recognisable by their leaves only, and this is particularly true for *S. spathulata* and *S. thodeana*. Moreover, the seeds purchased at B&T World Seeds are collected in the field by local Botanists. All the purchased species come from the Drakensberg region (South Africa), and are easily recognisable.

SPECIMENS COLLECTED FOR THIS STUDY AND DEPOSITED AT THE HERBARIUM OF THE UNIVERSITY OF NEUCHÂTEL (NEU)

Collecteurs	**N°**	**Family**	***Genus***	***species***	**author**	**South**	**East**	**elevation**	**Date**	**Country**	**Locality**
Kissling J. & Zeltner L.	1	Gentianaceae	*Chironia*	*palustris var. transvalensis*	(Gilg) Verdoo	25° 46.811'	028° 21.054'		11/12/2005	South Africa	Six fonteins (N4 to Witbank from Pretoria, Close to Trade center)
Kissling J. & Zeltner L.	2	Gentianaceae	*Chironia*	*purpurascens var. humilis*	(Gilg) Verdoo	25° 46.811'	028° 21.054'		11/12/2005	South Africa	Six fonteins (N4 to Witbank from Pretoria, Close to Trade center)
Kissling J. & Zeltner L.	3	Gentianaceae	*Sebaea*	*hymenosepala aff.*	Gilg	28° 17.132'	029° 09.771'	1730	11/13/2005	South Africa	Harrismith, before entrance of the botanical Garden, on the right hand side of the road
Kissling J. & Zeltner L.	4	Gentianaceae	*Sebaea*	*hymenosepala aff.*	Gilg	28°17.041'	029°06.759'	1609	11/13/2005	South Africa	On the road to Bethlem from Harrismith, right hand side of the road, in front of houses
Kissling J. & Zeltner L.	5	Gentianaceae	*Sebaea*	*hymenosepala aff.*	Gilg	28° 25.167'	029° 03.911'	1683	11/13/2005	South Africa	On the road from Harrismith to Bergville (Crossroad to Sterkfonteindam, on the lefthand side of the road
Kissling J. & Zeltner L.	10	Gentianaceae	*Sebaea*	*procumbens*	Hill	29° 35.692'	029° 18.183'	2450	11/14/2005	South Africa	Sur la route du Sani pAss, à la limite inférieure de la prairie "alpine"
Kissling J. & Zeltner L.	11	Gentianaceae	*Sebaea*	*cf marlothii*	Gilg	29° 35.692'	029°18.183'	2470	11/14/2005	South Africa	Route du Sani pass, quelque virage avant le sommet
Kissling J. & Zeltner L.	12	Gentianaceae	*Sebaea*	*spathulata*	(E. Mey.) Ste	29° 35.268'	029° 17.555'	2681	11/14/2005	South Africa	Route du Sani Pass, 200 m de dénivellé avant le sommet
Kissling J. & Zeltner L.	13	Gentianaceae	*Sebaea*	*sp.*		29° 35.268'	029° 17.555'	2681	11/14/2005	South Africa	Route du Sani Pass, 200 m de dénivellé avant le sommet
Kissling J. & Zeltner L.	14	Gentianaceae	*Sebaea*	*cf. evansii*	N.E.Br.	29° 35.268'	029° 17.555'	2681	11/14/2005	South Africa	Route du Sani Pass, 200 m de dénivellé avant le sommet
Kissling J. & Zeltner L.	14	Gentianaceae	*Sebaea*	*procumbens*	Schinz	29° 35.268'	029° 17.555'	2681	11/14/2005	South Africa	Route du Sani Pass, 200 m de dénivellé avant le sommet
Kissling J. & Zeltner L.	15	Gentianaceae	*Sebaea*	*repens*	Schinz	29° 35.077'	029° 17.275'	2874	11/14/2005	Leshoto	Sani Pass
Kissling J. & Zeltner L.	16	Gentianaceae	*Sebaea*	*thodeana*	Gilg	29° 35.077'	029° 17.275'	2874	11/14/2005	Leshoto	Sani Pass
Kissling J. & Zeltner L.	17	Gentianaceae	*Sebaea*	*repens*	Schinz	29° 35.077'	029° 17.275'	2874	11/14/2005	Leshoto	Sani Pass
Kissling J. & Zeltner L.	18	Gentianaceae	*Sebaea*	*repens*	Schinz	29° 35.077'	029° 17.275'	2874	11/14/2005	Leshoto	Sani Pass
Kissling J. & Zeltner L.	19	Gentianaceae	*Sebaea*	*marlothii*	Gilg	29° 31.663'	029° 12.594'	3176	11/15/2005	Leshoto	Kotisephala Pass un virage sous le col)
Kissling J. & Zeltner L.	20	Gentianaceae	*Sebaea*	*thodeana*	Gilg	29° 31.663'	029° 12.594'	3176	11/15/2005	Leshoto	Kotisephala Pass un virage sous le col)
Kissling J. & Zeltner L.	21	Gentianaceae	*Sebaea*	*marlothii*	Gilg	29° 25.951'	028° 57,406'	2984	11/15/2005	Leshoto	route en direction de Tabatseka
Kissling J. & Zeltner L.	22	Gentianaceae	*Sebaea*	*thodeana*	Gilg	29° 25.951'	028° 57,406'	2984	11/15/2005	Leshoto	route en direction de Tabatseka
Kissling J. & Zeltner L.	23	Gentianaceae	*Sebaea*	*repens X thodeana?*		29° 25.951'	028° 57,406'	2984	11/15/2005	Leshoto	route en direction de Tabatseka
Kissling J. & Zeltner L.	24	Gentianaceae	*Sebaea*	*repens*	Schinz	29° 52.369'	029° 04.795'	2456	11/16/2005	Leshoto	route Sehlabathebe lodge, dans la montée depuis le village
Kissling J. & Zeltner L.	25	Gentianaceae	*Sebaea*	*spathulata*	(E. Mey.) Ste	29° 52.369'	029° 04.795'	2456	11/16/2005	Leshoto	route Sehlabathebe lodge, dans la montée depuis le village
Kissling J. & Zeltner L.	26	Gentianaceae	*Sebaea*	*spathulata*	(E. Mey.) Ste	29° 52.692'	029° 05.342'	2599	11/16/2005	Leshoto	route Sehlabathebe lodge, avant de redescendre
Kissling J. & Zeltner L.	29	Gentianaceae	*Sebaea*	*thomasii*	(S. Moore) Sc	29°52.058'	029°06.966'	2461	11/16/2005	Leshoto	Schelabathebe National Park, 1 km de Schelabathebe"lodge", direction du village de Schelabathebe
Kissling J. & Zeltner L.	30	Gentianaceae	*Sebaea*	sp.(Undeterminable, only vers young	bud)	29°52.058'	029°06.966'	2461	11/16/2005	Leshoto	Schelabathebe National Park, 1 km de Schelabathebe"lodge", direction du village de Schelabathebe
Kissling J. & Zeltner L.	33	Gentianaceae	*Sebaea*	*cf. repens*	Schinz	30° 45.765'	028° 12.764'	1841	11/17/2005	South Africa	Elandshigh
Kissling J. & Zeltner L.	34	Gentianaceae	*Sebaea*	*pentendra aff.*	E. Mey.	30° 43.934'	028° 08.442'	2470	11/17/2005	South Africa	Naude's Nek, avant le col depuis Elands high
Kissling J. & Zeltner L.	35	Gentianaceae	*Sebaea*	*sp. A*		30° 43.934'	028° 08.442'	2470	11/17/2005	South Africa	Naude's Nek, avant le col depuis Elands high
Kissling J. & Zeltner L.	36	Gentianaceae	*Sebaea*	*hybride de 34X35?*		30° 43.934'	028° 08.442'	2470	11/17/2005	South Africa	Naude's Nek, avant le col depuis Elands high
Kissling J. & Zeltner L.	37	Gentianaceae	*Sebaea*	*sp. A*		30° 43.859'	028° 08.236'	2497	11/17/2005	South Africa	Naude's Nek summit (co^té Rhode), sur le début de la route en direction du lodge.
Kissling J. & Zeltner L.	38	Gentianaceae	*Sebaea*	*marlothii*	Gilg	30° 43.125'	028° 08.394'	2513	11/17/2005	South Africa	Après Naudes' Nek, dircetion du lodge
Kissling J. & Zeltner L.	39	Gentianaceae	*Sebaea*	*sp.*		30° 45.650'	028° 04.730'	2468	11/17/2005	South Africa	Naud's Nek Lodge (juste avant le lodge, sur le côté droite dela route.
Kissling J. & Zeltner L.	40	Gentianaceae	*Sebaea*	*spathulata*	(E. Mey.) Ste	30° 45.650'	028° 04.730'	2468	11/17/2005	South Africa	route de Naudes Nek à Rhodes, après avoir passé le col.
Kissling J. & Zeltner L.	41	Gentianaceae	*Chironia*	*baccifera*	L.	34° 04.720'	023° 03.755'	91	11/19/2005	South Africa	Viewpoint head's Knysna
Kissling J. & Zeltner L.	42	Gentianaceae	*Chironia*	*tetragona*	L. f.	34° 04.720'	023° 03.755'	91	11/19/2005	South Africa	Viewpoint head's Knysna
Kissling J. & Zeltner L.	43	Gentianaceae	*Chironia*	*tetragona*	L. f.	33° 53.635'	022° 25.269'	508	11/19/2005	South Africa	Montagu Pass (depuis George)
Kissling J. & Zeltner L.	44	Gentianaceae	*Orphium*	*frutescens*	(L.) E. Mey	34° 22.627'	018° 49.814'	4	11/20/2005	South Africa	Pringel Bay
Kissling J. & Zeltner L.	45	Gentianaceae	*Sebaea*	*ambigua*	Cham	34° 22.627'	018° 49.814'	4	11/20/2005	South Africa	Pringel Bay
Kissling J. & Zeltner L.	46	Gentianaceae	*Sebaea*	*minutiflora*	Schinz	34° 22.627'	018° 49.814'	4	11/20/2005	South Africa	Pringel Bay
Kissling J. & Zeltner L.	47	Gentianaceae	*Chironia*	*baccifera*	L.	34° 24.704'	019° 15.442'	21	11/20/2005	South Africa	Hermanus Graal Rock
Kissling J. & Zeltner L.	48	Gentianaceae	*Orphium*	*frutescens*	(L.) E. Mey	34° 22.043'	019° 08.542'	35	11/20/2005	South Africa	bord de route près de Frish haven
Kissling J. & Zeltner L.	49	Gentianaceae	*Sebaea*	*aurea*	(L.f.) Roem. &	34° 22.043'	019° 08.542'	35	11/20/2005	South Africa	bord de route près de Fisher haven
Kissling J. & Zeltner L.	50	Gentianaceae	*Sebaea*	*aurea*	(L.f.) Roem. &	33° 56.274'	019° 09.687'	441	11/22/2005	South Africa	Jan Jorbertsgat Brug, Du toit river, route de Franschoek Pass
Kissling J. & Zeltner L.	51	Gentianaceae	*Chironia*	*baccifera*	L.	33° 54.916'	019° 07.317'	264	11/22/2005	South Africa	Franschoek, Hugenot Cemetry
Kissling J. & Zeltner L.	52	Gentianaceae	*Sebaea*	*micrantha var. intermedia*	(Cham & Schl	34° 08.949'	018° 55.656'	445	11/23/2005	South Africa	Sir Lowry's Pass
Kissling J. & Zeltner L.	52	Gentianaceae	*Sebaea*	*cf. exacoides*	(L.) Schinz	34° 08.949'	018° 55.656'	445	11/23/2005	South Africa	Sir Lowry's Pass
Kissling J. & Zeltner L.	53	Gentianaceae	*Orphium*	*frutescens*	(L.) E. Mey	34° 23.416'	019° 13.734'	65	11/24/2005	South Africa	route Caledon-Hermanus, 15 km après avoir paséé le Mont babylon
Kissling J. & Zeltner L.	54	Gentianaceae	*Chironia*	*baccifera*	L.	34° 03.735'	018° 52.428'	139	11/24/2005	South Africa	Helderberg Nature Reserve
Kissling J. & Zeltner L.	55	Gentianaceae	*Sebaea*	*schlechterii*	Schinz	34° 03.735'	018° 52.428'	139	11/24/2005	South Africa	Helderberg Nature Reserve
Kissling J. & Zeltner L.	56	Gentianaceae	*Sebaea*	*aurea*	(L.f.) Roem. & Schult.				11/24/2005	South Africa	Lion's head
Kissling J. & Zeltner L.	57	Gentianaceae	*Chironia*	*cf.baccifera*	L.	33° 55.308'	019° 29.215'	390	11/25/2005	South Africa	sur la route qui mène à Jonaskop, juste avant la barrière y interdisant l'accès,env. 150m sur la droite, vers un pompe à eau qui fui.
Kissling J. & Zeltner L.	58	Gentianaceae	*Chironia*	*linoides*	L.	33° 55.308'	019° 29.215'	390	11/25/2005	South Africa	sur la route qui mène à Jonaskop, juste avant la barrière y interdisant l'accès,env. 150m sur la droite, vers un pompe à eau qui fui.
Kissling J. & Zeltner L.	59	Gentianaceae	*Sebaea*	*minutiflora*	Schinz	34° 08.513'	018° 51.112'	3	11/25/2005	South Africa	Gordon's Bay, lotissement à batir, le long de la route R44, en face de la station essence BP.
Kissling J. & Zeltner L.	60	Boragninaceae	*Echium?*	*?*		34° 08.513'	018° 51.112'	3	11/25/2005	South Africa	Gordon's Bay, lotissement à batir, le long de la route R44, en face de la station essence BP.
Kissling J. & Zeltner L.	61	Boragninaceae	*Echium?*	*?*					11/25/2005	South Africa	sommet de Joaneskop
Kissling J. & Zeltner L.	62	Gentianaceae	*Sebaea*	*aurea*	(L.f.) Roem. & Schult.				11/25/2005	South Africa	sommet de Joaneskop
Kissling J. & Zeltner L.	63	Boragninaceae	*Echium?*	*?*		33° 21.819'	019° 10.222'	214	11/26/2005	South Africa	route (R46) Stellenbosh-Xeres, au croisement Artois, Malmesbury, Ceres et Welteverde.
Kissling J. & Zeltner L.	64	Gentianaceae	*Sebaea*	*pusilla*	Eckl. Ex Chan	31° 23.346'	019° 02.640'	773	11/26/2005	South Africa	Nieuvodville (8 km avant Nieuvodville, en venant depuis)
Kissling J. & Zeltner L.	65	Gentianaceae	*Chironia*	*baccifera*	L.	31° 22.279'	019° 01.892'	805	11/27/2005	South Africa	avant l'entrée dans la "Nieuvodville Nature Reserve"le long des barrière bordant la route (après l'air de picnic sous les pins)
Kissling J. & Zeltner L.	66	Gentianaceae	*Sebaea*	*membranacea aff.*	Hill	31° 18'	19° 07'	700	11/27/2005	South Africa	les chute d'eau de Nieuvodville (7 km depuis le croisement)
Kissling J. & Zeltner L.	67	Rubiaceae	*Spermacocee*	*sp.*		18° 07.320'	02° 34.982'	1016	12/4/2005	Namibia	Popa Falls (près des rapides)
Kissling J. & Zeltner L.	68	Rubiaceae	*Spermacocee*	*sp.*		17° 47.688'	025° 14.465'	920	12/5/2005	Botswana	Toro Safari Lodge
Kissling J. & Zeltner L.	69	Gentianaceae	*Sebaea*	sp.(Undeterminable, only vers young	bud)	28° 43.472'	028° 53.602'	2478	12/9/2005	South Africa	route Sentinel Peak. 3 km vant le parking
Kissling J. & Zeltner L.	70	Gentianaceae	*Sebaea*	*thomasii*	(S. Moore) Sc	28° 43.148'	028° 53.736'	2415	12/9/2005	South Africa	route Sentinel Peak. 3 km vant le parking
Kissling J. & Zeltner L.	71	Gentianaceae	*Sebaea*	sp.(Undeterminable, only vers young	bud)	28° 41.997'	028° 53.773'	2286	12/9/2005	South Africa	route menant au pic Sentinel
Kissling J. & Zeltner L.	72	Gentianaceae	*Sebaea*	*macrophylla*	Gilg	28° 40.548'	028° 53.588'	2158	12/9/2005	South Africa	route menant au pic Sentinel, avant le loidge, le long de la route pavée.
Kissling J. & Zeltner L.	73	Gentianaceae	*Sebaea*	*hymenosepala aff.*	Gilg	28° 17.132'	029° 09.771'	1730	12/9/2005	South Africa	Harrismith, before entrance of the botanical Garden, on the right hand side of the road
Kissling J. & Zeltner L.	74	Gentianaceae	*Sebaea*	*macrophylla*	Gilg	28°17.041'	029°06.759'	1609	12/9/2005	South Africa	On the road to Bethlem from Harrismith, right hand side of the road, in front of houses
Kissling J.	75	Gentianaceae	*Exochaenium*	*platypterum*	(Baker) Boutic	028°25.22'	015°28.51'	1325	4/18/2004	Zambia	Lusaka East Protected Forest Reserve
Kissling J.	76	Gentianaceae	*Sebaea*	*micropylla*	(Edgew.) Kno	028°25.22'	015°28.51'	1325	4/18/2004	Zambia	Lusaka East Protected Forest Reserve
Kissling J.	77	Gentianaceae	*Sebaea*	*junodii*	Schinz	028°25.22'	015°28.51'	1325	4/18/2004	Zambia	Lusaka East Protected Forest Reserve
Kissling J.	78	Gentianaceae	*Sebaea*	*micrantha var. micrantha*	Cham & Schle	34° 22.627'	018° 49.814'	4	28.09.06	South Africa	Pringle Bay
Kissling J.	79	Gentianaceae	*Sebaea*	*micrantha var. intermedia*	(Cham & Schl	34° 08.949'	018° 55.656'	445	29.09.06	South Africa	Sir Lowry's Pass

Kissling J.	80	Gentianaceae	*Sebaea*	*schlechterii*	Schinz				29.09.06	South Africa	Road from Villersdrop to Franschoek (R45), close to Theewaterskloof Dam, along the road
Kissling J.	81	Gentianaceae	*Sebaea*	*exacoides*	(L.) Schinz	33° 56.274'	019° 09.687'	441	29.09.06	South Africa	between Jan Joubertsberg bridge and Franschoek Pass, road R45
Kissling J.	82	Gentianaceae	*Sebaea*	*schlechterii*	Schinz	33° 56.274'	019° 09.687'	441	29.09.06	South Africa	between Jan Joubertsberg bridge and Franschoek Pass, road R45
Kissling J.	83	Gentianaceae	*Sebaea*	*minutiflora*	Schinz	34° 08.513'	018° 51.112'	3	29.09.06	South Africa	in front of the second BP station on the road from Gordon's Bay to Strand (R44)
Kissling J.	84	Gentianaceae	*Sebaea*	*scabra*	Schinz				30.09.06	South Africa	6 km from stormvlei in direction to Robertson, road R317
Kissling J.	85	Gentianaceae	*Sebaea*	*exacoides*	(L.) Schinz				30.09.06	South Africa	road between Montagu and Koo, Burger Pass, Road R318
Kissling J.	87	Gentianaceae	*Sebaea*	*exacoides*	(L.) Schinz				30.09.06	South Africa	road between Montagu and Koo, Burger Pass, Road R318
Kissling J.	88	Gentianaceae	*Sebaea*	*exacoides*	(L.) Schinz				30.09.06	South Africa	DutoitKloof Pass Road N1
Kissling J.	89	Gentianaceae	*Sebaea*	*aurea*	(L.f.) Roem. & Schult.				01.10.06	South Africa	Swartkop Mts, along road R310 to Cape point, in front Partridge point
Kissling J.	91	Gentianaceae	*Sebaea*	*aurea*	(L.f.) Roem. & Schult.				01.10.06	South Africa	along trail, in front of Vasco Da gamma peak, Table mts National Park
Kissling J.	92	Gentianaceae	*Sebaea*	*exacoides*	(L.) Schinz				01.10.06	South Africa	parking of Cape point view
Kissling J.	93	Gentianaceae	*Sebaea*	*cf. albens*	(L.f.) Roem. & Schult. aff.				01.10.06	South Africa	parking of Cape point view
Kissling J.	94	Gentianaceae	*Sebaea*	*ambigua*	Cham.	34° 22.627'	018° 49.814'	4	02.10.06	South Africa	Pringle Bay
Kissling J.	95	Gentianaceae	*Sebaea*	*cf. micrantha*	(Cham & Schlechdtl.) Schinz				02.10.06	South Africa	Road from Villersdrop to Franschoek (R45), close to Theewaterskloof Dam, along the road
Kissling J.	96	Gentianaceae	*Sebaea*	*exacoides*	(L.) Schinz				02.10.06	South Africa	Road from Villersdrop to Franschoek (R45), close to Theewaterskloof Dam, along the road
Kissling J.	97	Gentianaceae	*Sebaea*	*exacoides?*	(L.) Schinz	34° 08.949'	018° 55.656'	445	02.10.06	South Africa	Sir Lowry's Pass
Kissling J.	98	Gentianaceae	*Sebaea*	*aurea*	(L.f.) Roem. & Schult.				04.10.06	South Africa	Sir Lowry's village, along road…
Kissling J.	99	Gentianaceae	*Sebaea*	*sp. B*					05.10.06	South Africa	Kirstenbosh Botanical garden, on the way to summit mountain, via …
Kissling J., Gehrke B. & F	100	Gentianaceae	*Sebaea*	*sulphurea*	Cham & Schlechtdl.				05.10.06	South Africa	Table mountain, path from … to …
Kissling J., Gehrke B. & F	101	Gentianaceae	*Sebaea*	*exacoides*	(L.) Schinz				05.10.06	South Africa	Table mountain, path from … to …
Kissling J., Gehrke B. & F	102	Gentianaceae	*Sedum*	*sebaoides*					05.10.06	South Africa	Table mountain, path from … to …
Kissling J., Gehrke B. & F	103	Gentianaceae	*Sebaea*	*scabra*	Schinz				06.10.06	South Africa	5 km after Bonnievale, in direction to Montagu, along road
Kissling J., Gehrke B. & F	104	Gentianaceae	*Sebaea*	*exacoides?*	(L.) Schinz	34° 08.949'	018° 55.656'	445	06.10.06	South Africa	Sir Lowry's Pass
Kissling J., Gehrke B. & F	105	Gentianaceae	*Sebaea*	*exacoides?*	(L.) Schinz	33°56' 44.4''	021°10'32.3''		07.10.06	South Africa	
Kissling J., Gehrke B. & F	107	Gentianaceae	*Sebaea*	*zeyheri aff.*	Schinz	34°00'53.6''	021°13'00.1''		07.10.06	South Africa	
Kissling J., Gehrke B. & F	108	Gentianaceae	*Sebaea*	*zeyheri aff.*	Schinz	33°53'31.4''	022°01'26.5''		07.10.06	South Africa	
Kissling J., Gehrke B. & F	109	Gentianaceae	*Sebaea*	*zeyheri aff.*	Schinz	33°53'09.6''	022°26'02.9''	746	07.10.06	South Africa	Montagu Pass
Kissling J., Gehrke B. & F	110	Gentianaceae	*Sebaea*	*zeyheri aff.*	Schinz	33°59'16.0''	022°39'02.3''	79	07.10.06	South Africa	
Kissling J., Gehrke B. & F	111	Gentianaceae	*Chironia*	*baccifera*	L.	34°00'31.1''	022°4'29.2''	100	07.10.06	South Africa	small road from Karatora to Sedgeville, just befor junction with the N2
Kissling J., Gehrke B. & F	112	Gentianaceae	*Sebaea*	*grisebachiana*	Schinz				07.10.06	South Africa	
Kissling J., Gehrke B. & F	113	Gentianaceae	*Sebaea*	*zeyheri aff.*	Schinz				07.10.06	South Africa	
Kissling J., Gehrke B. & F	114	Gentianaceae	*Sebaea*	*grisebachiana*	Schinz	33°16.751'	026°29.359'	646	08.10.06	South Africa	Grahamstown
Kissling J., Gehrke B. & F	115	Gentianaceae	*Sebaea*	*grisebachiana*	Schinz	33°22.566'	027°00.732'	197	08.10.06	South Africa	Shaw Park
Kissling J., Gehrke B. & F	116	Gentianaceae	*Chironia*	*tetragona*	L.f.				08.10.06	South Africa	
Kissling J., Gehrke B. & F	117	Gentianaceae	*Sebaea*	*grisebachiana*	Schinz					South Africa	Grahamstown
Kissling J., Gehrke B. & F	119	Gentianaceae	*Sebaea*	*sp. A*		30°45'41.2''	028°03'45.7''	2109	10.10.06	South Africa	road to Naudes'Nek
Kissling J., Gehrke B. & F	120	Gentianaceae	*Sebaea*	*thomasii*	(S. Moore) Sc	30°45'	028°03'	2050	11.10.06	South Africa	road to Naudes Nek, close to the river
Kissling J., Gehrke B. & F	121	Gentianaceae	*Sebaea*	*repens*	Schinz	30°45'	028°03'	2050	12.10.06	South Africa	road to Naudes Nek, close to the river
Kissling J., Gehrke B. & F	122	Gentianaceae	*Sebaea*	*thomasii*	(S. Moore) Sc	30°44'07.3''	028°08'27.7''	2450	12.10.06	South Africa	
Kissling J., Gehrke B. & F	123	Gentianaceae	*Sebaea*	*repens*	Schinz	30°45'55.2''	028°12'44.8''	1848	12.10.06	South Africa	
Kissling J., Gehrke B. & F	124	Gentianaceae	*Sebaea*	*thomasii*	(S. Moore) Sc	29°50'0.26''	029°08'57.3''	2307	14.10.06	South Africa	on the way to goat's cave (Bushman's nek area)
Kissling J., Gehrke B. & F	125	Gentianaceae	*Sebaea*	*repens*	Schinz	29°49'58.4''	029°08'01.7''	2395	14.10.06	South Africa	down Goat's cave, close to the starting stream
Kissling J., Gehrke B. & F	126	Gentianaceae	*Sebaea*	*spathulata*	(E. Mey) Steu	29°49'59.8''	029°07'47.9''	2565	14.10.06	South Africa	Goat Cave, west face
Kissling J., Gehrke B. & F	127	Gentianaceae	*Sebaea*	*cf. evansii*	N.E.Br.	29° 35.268'	029° 17.555'	2681	16.10.06	South Africa	Sani Pass
Kissling J., Gehrke B. & F	128	Gentianaceae	*Sebaea*	*cf. rotundifolia*	Hill	28°56'12.4''	029°10'46.3''	1772	19.10.06	South Africa	
Kissling J., Gehrke B. & F	129	Gentianaceae	*Sebaea*	*thomasii*	(S. Moore) Sc	28° 43.148'	028° 53.736'	2415	21.10.06	South Africa	Way to sentinel Peak
Kissling J., Gehrke B. & F	130	Gentianaceae	*Sebaea*	*procumbens*	Hill	28°44'01.5''	028°53'33.0''	2481	21.10.06	South Africa	Way to sentinel Peak
Kissling J., Gehrke B. & F	131	Gentianaceae	*Sebaea*	*pleurostigmatosa*	Hilliard & Burtt	28°44'42.4''	028°53'14.8''	2753	21.10.06	South Africa	way to Mont aux Sources
Kissling J., Gehrke B. & F	132	Gentianaceae	*Sebaea*	*spathulata*	(E. Mey) Steu	28°44'48.2''	028°53'16.6''	2774	21.10.06	South Africa	way to Mont aux Sources
Kissling J., Gehrke B. & F	133	Gentianaceae	*Sebaea*	*marlothii*	Gilg	28°45'03.8''	028°52'50.7''	2876	21.10.06	South Africa	Monts aux Sources
Dessein S., Archer R., De	499	Gentianaceae	*Exochaenium*	*oliganthum*	(Gilg) Schinz	15° 25' 12.9	28° 10' 37.7	1259	3/12/2004	Zambia	14 km W of Lusaka
Dessein S., Archer R., De	501	Gentianaceae	*Exochaenium*	*oliganthum*	(Gilg) Schinz	15° 25' 12.9	28° 10' 37.7	1259	3/12/2004	Zambia	14 km W of Lusaka
Dessein S., Archer R., De	516	Gentianaceae	*Faroa*	*pusilla*	Baker	13° 00' 00.8	30° 27' 24.2	1500	3/14/2004	Zambia	Along road towards Samfya, just N of junction with Serenje-Mpika road
Dessein S., Archer R., De	519	Gentianaceae	*Pychnosphaera*	*buchananii*	(Baker) N.E.B	13° 00' 00.8	30° 27' 24.2	1500	3/14/2004	Zambia	Along road towards Samfya, just N of junction with Serenje-Mpika road
Dessein S., Archer R., De	521	Gentianaceae	*Faroa*	*cf. axillaris*	Baker	12° 23' 02.8	30° 10' 34.7	1187	3/14/2004	Zambia	Along road towards Samfya, c. 50 km N of junction with Serenje-Mpika road
Dessein S., Archer R., De	543	Gentianaceae	*Exochaenium*	*clavatum*	(Paiva & Nogu	11° 49' 18.5	29° 36' 32.7	1170	3/14/2004	Zambia	Swamp area near Kalaso Mukuso village
Dessein S., Archer R., De	557	Gentianaceae	*Sebaea*	*microphylla*	(Edgew.) Kno	11° 26' 53.8	29° 33' 55.5	1188	3/15/2004	Zambia	South of Samfya, along road towards Twingi
Dessein S., Archer R., De	558	Gentianaceae	*Exochaenium*	*teucszii*	(Schinz) Schin	11° 28' 31.6	29° 39' 57.9	1171	3/15/2004	Zambia	Along road between Samfya and Twingi
Dessein S., Archer R., De	559	Gentianaceae	*Pychnosphaera*	*buchananii*	(Baker) N.E.B	11° 28' 31.6	29° 39' 57.9	1171	3/15/2004	Zambia	Along road between Samfya and Twingi
Dessein S., Archer R., De	561	Gentianaceae	*Exochaenium*	*teucszii*	(Schinz) Schin	11° 28' 31.6	29° 39' 57.9	1171	3/15/2004	Zambia	Along road between Samfya and Twingi
Dessein S., Archer R., De	562	Gentianaceae	*Exochaenium*	*teucszii*	(Schinz) Schin	11° 29' 21.1	29° 38' 04.5	1133	3/15/2004	Zambia	Along road between Samfya and Twingi
Dessein S., Archer R., De	575	Gentianaceae	*Faroa*	*sp.*		11° 18' 12.6	29° 21' 10.9	1179	3/16/2004	Zambia	West of Samfya
Dessein S., Archer R., De	577	Gentianaceae	*Sebaea*	*junodii*	Schinz	10° 55' 46.4	28° 44' 05.5	1189	3/16/2004	Zambia	Mumbuluma falls
Dessein S., Archer R., De	582	Gentianaceae	*Faroa*	*sp.*		10° 55' 46.4	28° 44' 05.5	1189	3/16/2004	Zambia	Mumbuluma falls
Dessein S., Archer R., De	584	Gentianaceae	*Exochaenium*	*gracile*	A.W. Hill	10° 55' 46.4	28° 44' 05.5	1189	3/16/2004	Zambia	Dambo just E of Mumbuluma falls
Dessein S., Archer R., De	593	Gentianaceae	*Faroa*	*cf. axillaris*	Baker	10° 55' 29.5	28° 45' 59.6	1239	3/16/2004	Zambia	Dambo just E of Mumbuluma falls
Dessein S., Archer R., De	599	Gentianaceae	*Exochaenium*	*teucszii*	(Schinz) Schin	09° 51' 06.2	28° 45' 28.6	930	3/17/2004	Zambia	Between Kasenga and Kazembe, just W of Pembe lagoon
Dessein S., Archer R., De	603	Gentianaceae	*Exochaenium*	*africanum*	(Paiva & Nogu	09° 51' 11.7	28° 56' 42.1	1050	3/17/2004	Zambia	Area around the Ntumbachushi waterfalls
Dessein S., Archer R., De	604	Gentianaceae	*Exochaenium*	*africanum*	(Paiva & Nogu	09° 50' 12.6	28° 57' 13.9	1298	3/18/2004	Zambia	Dambo along Mbereshi-Kawambwa route
Dessein S., Archer R., De	609	Gentianaceae	*Schinziellia*	*tetragona*	(Schinz) Gilg	09° 44' 33.9	29° 04' 46.5	1261	3/18/2004	Zambia	Dambo along Mbereshi-Kawambwa route
Dessein S., Archer R., De	617	Gentianaceae	*Exochaenium*	*africanum*	(Paiva & Nogu	09° 43' 09.8	29° 04' 44.2	1336	3/18/2004	Zambia	Dambo near Ntenke village
Dessein S., Archer R., De	620	Gentianaceae	*Schinziellia*	*tetragona*	(Schinz) Gilg	09° 38' 59.7	29° 03' 41.6	1390	3/18/2004	Zambia	Dambo along the Mbereshi stream
Dessein S., Archer R., De	621	Gentianaceae	*Faroa*	*cf. axillaris*	Baker	09° 38' 59.7	29° 03' 41.6	1390	3/18/2004	Zambia	Dambo along the Mbereshi stream
Dessein S., Archer R., De	623	Gentianaceae	*Exochaenium*	*africanum*	(Paiva & Nogu	09° 32' 06.6	28° 56' 54.1	1255	3/19/2004	Zambia	Shallow dambo along the Kawambwa - Lake Mweru road
Dessein S., Archer R., De	628	Gentianaceae	*Pychnosphaera*	*buchananii*	(Baker) N.E.B	09° 25' 42.2	28° 44' 25.1	938	3/19/2004	Zambia	Dambo near Nchelenge village

Dessein S., Archer R., De	646	Gentianaceae	*Faroa*	*cf. axillaris*	Baker	09° 24' 22.2	30° 04' 32.2	1414	3/20/2004	Zambia	Dambo area along Mporokoso-Kawambwa road, near Mutamba village
Dessein S., Archer R., De	656	Gentianaceae	*Exochaenium*	*exigum*	A.W. Hill	10° 24' 22.2	31° 04' 32.2	1415	3/21/2004	Zambia	Area around Lupupa waterfalls
Dessein S., Archer R., De	657	Gentianaceae	*Exochaenium*	*grande*	(E. Mey.) Gris	11° 24' 22.2	32° 04' 32.2	1416	3/22/2004	Zambia	Area around Lupupa waterfalls
Dessein S., Archer R., De	685	Gentianaceae	*Schinziellia*	*tetragona*	(Schinz) Gilg	09° 26' 02.0	30° 25' 07.2	1470	3/21/2004	Zambia	Dambo along Mporokoso-Kasama road, bordering the Katutwa river
Dessein S., Archer R., De	692	Gentianaceae	*Exochaenium*	*exigum aff.*	A.W. Hill	09° 29' 24.5	30° 34' 37.8	1411	3/21/2004	Zambia	Kaniki dambo along the Mporokoso-Kasama road, bordering the Kasanshi river
Dessein S., Archer R., De	701	Gentianaceae	*Exochaenium*	*teucszii*	(Schinz) Schin	09° 29' 48.7	31° 12' 47.7	1541	3/22/2004	Zambia	Dambo near Mwiluzi village, N of Kasama along the Kasama-Mbala road
Dessein S., Archer R., De	702	Gentianaceae	*Pychnosphaera*	*buchananii*	(Baker) N.E.B	10° 29' 48.7	32° 12' 47.7	1542	3/22/2004	Zambia	Dambo near Mwiluzi village, N of Kasama along the Kasama-Mbala road
Dessein S., Archer R., De	703	Gentianaceae	*Schinziellia*	*tetragona*	(Schinz) Gilg	11° 29' 48.7	33° 12' 47.7	1543	3/22/2004	Zambia	Dambo near Mwiluzi village, N of Kasama along the Kasama-Mbala road
Dessein S., Archer R., De	706	Gentianaceae	*Schinziellia*	*tetragona*	(Schinz) Gilg	09° 12' 57.1	31° 20' 22.0	1542	3/22/2004	Zambia	Dambo bordering the Chambeshi River along the Kasama-Mbala road
Dessein S., Archer R., De	728	Gentianaceae	*Exochaenium*	*perparvum*	(Sileshi) Kissli	08° 56' 49.8	31° 11' 25.7	1524	3/23/2004	Zambia	Dambo near Twatasha village, W of Mbala
Dessein S., Archer R., De	732	Gentianaceae	*Schinziellia*	*tetragona*	(Schinz) Gilg	08° 57' 22.5	31° 10' 22.1	1453	3/23/2004	Zambia	Dambo along Lungzua River, W of Mbala
Dessein S., Archer R., De	752	Gentianaceae	*Exochaenium*	*grande*	(E. Mey.) Gris	08° 49' 57.2	31° 22' 58.1	1626	3/25/2004	Zambia	Dambo along Shila River
Dessein S., Archer R., De	761	Gentianaceae	*Exochaenium*	*grande*	(E. Mey.) Gris	08° 50' 22.2	31° 29' 47.3	1702	3/25/2004	Zambia	E of Mbala, on road towards Tanzanian border
Dessein S., Archer R., De	764	Gentianaceae	*Exochaenium*	*grande*	(E. Mey.) Gris	08° 49' 09.4	31° 33' 33.1	1691	3/25/2004	Zambia	Along Mbala-Tanzanian border road
Dessein S., Archer R., De	767	Gentianaceae	*Swertia*	*cf. welwitschii*	Engl.	08° 48' 23.4	31° 33' 22.3	1650	3/25/2004	Zambia	Wet grassland bordering the Lumi River along the Mbala-Tanzanian border road
Dessein S., Archer R., De	768	Gentianaceae				08° 48' 23.4	31° 33' 22.3	1650	3/25/2004	Zambia	Wet grassland bordering the Lumi River along the Mbala-Tanzanian border road
Dessein S., Archer R., De	770	Gentianaceae	*Pychnosphaera*	*buchananii*	(Baker) N.E.B	08° 56' 09.5	31° 21' 36.7	1674	3/26/2004	Zambia	Uningi dambo, S of Mbala
Dessein S., Archer R., De	771	Gentianaceae	*Exochaenium*	*teucszii*	(Schinz) Schin	08° 56' 09.5	31° 21' 36.7	1674	3/26/2004	Zambia	Uningi dambo, S of Mbala
Dessein S., Archer R., De	783	Gentianaceae	*Pychnosphaera*	*buchananii*	(Baker) N.E.B	09° 31' 29.5	31° 05' 19.7	1506	3/26/2004	Zambia	W of Mbala-Kasama road, c. 20 km on road towards Mporokoso
Dessein S., Archer R., De	784	Gentianaceae	*Schinziellia*	*tetragona*	(Schinz) Gilg	09° 31' 29.5	31° 05' 19.7	1506	3/26/2004	Zambia	W of Mbala-Kasama road, c. 20 km on road towards Mporokoso
Dessein S., Archer R., De	788	Gentianaceae	*Pychnosphaera*	*buchananii*	(Baker) N.E.B	10° 13' 29.0	31° 20' 34.1	1383	3/26/2004	Zambia	Mungwi area, 20 km from Kasama
Dessein S., Archer R., De	789	Gentianaceae	*Schinziellia*	*tetragona*	(Schinz) Gilg	10° 13' 29.0	31° 20' 34.1	1383	3/26/2004	Zambia	Mungwi area, 20 km from Kasama
Dessein S., Archer R., De	801	Gentianaceae	*Pychnosphaera*	*buchananii*	(Baker) N.E.B	10° 11' 50.0	30° 20' 35.2	1384	3/27/2004	Zambia	Dambo area along Kasama-Luwingu road, 114 km W of Kasama
Dessein S., Archer R., De	802	Gentianaceae	*Exochaenium*	*grande*	(E. Mey.) Gris	10° 11' 50.0	30° 20' 35.2	1384	3/27/2004	Zambia	Dambo area along Kasama-Luwingu road, 114 km W of Kasama
Dessein S., Archer R., De	803	Gentianaceae	*Schinziellia*	*tetragona*	(Schinz) Gilg	10° 11' 50.0	30° 20' 35.2	1384	3/27/2004	Zambia	Dambo area along Kasama-Luwingu road, 114 km W of Kasama
Dessein S., Archer R., De	805	Gentianaceae	*Schinziellia*	*tetragona*	(Schinz) Gilg	10° 11' 29.6	29° 42' 20.2	1451	3/28/2004	Zambia	Dambo along Luongo River
Dessein S., Archer R., De	806	Gentianaceae	*Exochaenium*	*grande*	(E. Mey.) Gris	10° 11' 29.6	29° 42' 20.2	1451	3/28/2004	Zambia	Dambo along Luongo River
Dessein S., Archer R., De	808	Gentianaceae	*Pychnosphaera*	*buchananii*	(Baker) N.E.B	10° 11' 29.6	29° 42' 20.2	1451	3/28/2004	Zambia	Dambo along Luongo River
Dessein S., Archer R., De	809	Gentianaceae	*Exochaenium*	*baumiana*	(Gilg) Schinz	10° 11' 29.6	29° 42' 20.2	1451	3/28/2004	Zambia	Dambo along Luongo River
Dessein S., Archer R., De	814	Gentianaceae	*Exochaenium*	*baumiana*	(Gilg) Schinz	10° 06' 05.6	29° 43' 26.2	1562	3/28/2004	Zambia	Dambo complex, Chishinga ranch
Dessein S., Archer R., De	815	Gentianaceae	*Exochaenium*	*grande*	(E. Mey.) Gris	10° 06' 05.6	29° 43' 26.2	1562	3/28/2004	Zambia	Dambo complex, Chishinga ranch
Dessein S., Archer R., De	816	Gentianaceae	*Schinziellia*	*tetragona*	(Schinz) Gilg	10° 06' 05.6	29° 43' 26.2	1562	3/28/2004	Zambia	Dambo complex, Chishinga ranch
Dessein S., Archer R., De	818	Gentianaceae	*Exochaenium*	*exigum*	A.W. Hill	10° 06' 05.6	29° 43' 26.2	1562	3/28/2004	Zambia	Dambo complex, Chishinga ranch
Dessein S., Archer R., De	819	Gentianaceae	*Exochaenium*	*teucszii*	(Schinz) Schin	10° 06' 05.6	29° 43' 26.2	1562	3/28/2004	Zambia	Dambo complex, Chishinga ranch
Dessein S., Archer R., De	823	Gentianaceae	*Exochaenium*	*baumianum*	(Gilg) Schinz	10° 06' 05.6	29° 43' 26.2	1562	3/28/2004	Zambia	Dambo complex, Chishinga ranch
Dessein S., Archer R., De	824	Gentianaceae	*Exochaenium*	*baumianum*	(Gilg) Schinz	10° 06' 05.6	29° 43' 26.2	1562	3/28/2004	Zambia	Dambo complex, Chishinga ranch
Dessein S., Archer R., De	827	Gentianaceae	*Exochaenium*	*perparvum*	(Sileshi) Kissli	10° 13' 16.3	29° 44' 57.7	1548	3/28/2004	Zambia	Dambo 15 km W of Luwingu
Dessein S., Archer R., De	832	Gentianaceae	*Exochaenium*	*teucszii*	(Schinz) Schin	10° 15' 35.2	29° 59' 11.0	1335	3/29/2004	Zambia	Lueno dambo, near Luwingu
Dessein S., Archer R., De	833	Gentianaceae	*Exochaenium*	*teucszii*	(Schinz) Schin	10° 15' 08.5	30° 03' 39.3	1348	3/29/2004	Zambia	Chikoti dambo
Dessein S., Archer R., De	834	Gentianaceae	*Exochaenium*	*grande*	(E. Mey.) Gris	10° 15' 08.5	30° 03' 39.3	13480	3/29/2004	Zambia	Chikoti dambo
Dessein S., Archer R., De	835	Gentianaceae	*Pychnosphaera*	*buchananii*	(Baker) N.E.B	10° 15' 08.5	30° 03' 39.3	13480	3/29/2004	Zambia	Chikoti dambo
Dessein S., Archer R., De	836	Gentianaceae	*Schinziellia*	*tetragona*	(Schinz) Gilg	10° 15' 08.5	30° 03' 39.3	13480	3/29/2004	Zambia	Chikoti dambo
Dessein S., Archer R., De	845	Gentianaceae	*Exochaenium*	*baumianum*	(Gilg) Schinz	11° 12' 41.1	31° 44' 59.4	1461	3/30/2004	Zambia	Grazed dambo along lake Ishiba Ngandu
Dessein S., Archer R., De	851	Gentianaceae				12° 12' 03.5	29° 57' 20.6	1167	3/31/2004	Zambia	Along Mpika-Isoka road, c. 39 km N of Mpika
Dessein S., Archer R., De	871	Gentianaceae				15° 16' 06.6	28° 38' 21.6	1085	4/6/2004	Zambia	Wet grassland along Chongwe road
Dessein S., Archer R., De	906	Gentianaceae	*Exochaenium*	*baumianum*	(Gilg) Schinz	12° 50' 49.6	28° 21' 25.4	1133	4/11/2004	Zambia	Bridge over Kafue River near Kitwe
Dessein S., Archer R., De	907	Gentianaceae	*Congolanthus aff.*			12° 50' 49.6	28° 21' 25.4	1133	4/11/2004	Zambia	Bridge over Kafue River near Kitwe
Dessein S., Archer R., De	926	Gentianaceae	*Faroa*	*cf. axillaris*	Baker	12° 22' 05.9	27° 14' 47.9	1324	4/13/2004	Zambia	Kafwasa dambo, just south from the Chingola-Solwezi road
Dessein S., Archer R., De	933	Gentianaceae	*Exochaenium*	*baumianum*	(Gilg) Schinz	12° 22' 26.3	27° 14' 41.0	1321	4/14/2004	Zambia	Kafwasa dambo, just south from the Chingola-Solwezi road
Dessein S., Archer R., De	934	Gentianaceae	*Exochaenium*	*cf. clavatum*	(Paiva & Nogu	12° 22' 26.3	27° 14' 41.0	1321	4/14/2004	Zambia	Kafwasa dambo, just south from the Chingola-Solwezi road
Dessein S., Archer R., De	937	Gentianaceae				12° 22' 26.3	27° 14' 41.0	1321	4/14/2004	Zambia	Kafwasa dambo, just south from the Chingola-Solwezi road
Dessein S., Archer R., De	938	Gentianaceae				12° 22' 26.3	27° 14' 41.0	1321	4/14/2004	Zambia	Kafwasa dambo, just south from the Chingola-Solwezi road
Dessein S., Archer R., De	939	Gentianaceae	*Chironia*	*purpurascens subsp. humils*	(E.Mey.) bent	12° 22' 26.3	27° 14' 41.0	1321	4/14/2004	Zambia	Kafwasa dambo, just south from the Chingola-Solwezi road
Dessein S., Archer R., De	955	Gentianaceae				11° 31' 41.8	24° 24' 38.7	1320	4/14/2004	Zambia	Luakera rapids along Mwinilunga-Ikelenge road
Dessein S., Archer R., De	969	Gentianaceae	*Exochaenium*	*baumianum*	(Gilg) Schinz	11° 30' 19.0	24° 22' 47.5	1389	4/14/2004	Zambia	Chintunta dambo along the Mwinilunga-Ikelenge road
Dessein S., Archer R., De	970	Gentianaceae	*Exochaenium*	*baumianum*	(Gilg) Schinz	11° 30' 19.0	24° 22' 47.5	1389	4/14/2004	Zambia	Chintunta dambo along the Mwinilunga-Ikelenge road
Dessein S., Archer R., De	971	Gentianaceae	*Exochaenium*	*baumianum*	(Gilg) Schinz	11° 27' 41.9	24° 17' 57.8	1449	4/14/2004	Zambia	Chintunta dambo along the Mwinilunga-Ikelenge road
Dessein S., Archer R., De	974	Gentianaceae	*Exochaenium*	*baumianum*	(Gilg) Schinz	11° 27' 41.9	24° 17' 57.8	1449	4/14/2004	Zambia	Kaseki dambo, S from Mwinilunga-Ikelenge road
Dessein S., Archer R., De	1011	Gentianaceae	*Exochaenium*	*fernandesianum*	(Paiva & Nogu	11° 07' 38.8	24° 11' 21.3	1223	4/15/2004	Zambia	Zambezi rapids near Kalene Hill
Dessein S., Archer R., De	1014	Gentianaceae	*Exochaenium*	*baumianum*	(Gilg) Schinz	11° 07' 38.8	24° 11' 21.3	1223	4/15/2004	Zambia	Zambezi rapids near Kalene Hill
Dessein S., Archer R., De	1015	Gentianaceae	*Exochaenium*	*fernadesianum X baumianum ?*		11° 07' 38.8	24° 11' 21.3	1223	4/15/2004	Zambia	Zambezi rapids near Kalene Hill

ORIGINS OF THE EXACEAE SPECIES EXAMINED AND THEIR CHROMSOME NUMBERS

Annex 1 – The chromosome numbers observed, and the origins of materials and voucher specimens. The chromosome numbers reported for the first time are marked with "*", the number different from previous reports is marked with "!", and the numbers confirmed some of the previous reports but different from other reports are marked with "◊". Collectors' names are abbreviated as follow: Robert Archer (RA), Martin Callmander (MC), Petra DeBlock (PD), Steven Dessein (SD), Berit Gehrke (BG), Jonathan Kissling (JK), Brian Luwingu (BL), Michael Pirie (MP), J.C. Piso (JP), Elias Tembo (ET), Sébastien Wohlhauser (SW), Louis Zeltner (LZ)

Taxon	Collecteur	N°	Origin	Chromosome numbers	
Exacum affine I.B. Balf	*JK*	Cultivar 1	Madagascar	**n=18**	**2n=36**
Exacum appendiculatum Klack.	*JP, SW & LZ*	M028	Madagascar	**n=16**	**2n=32**
Exacum appendiculatum Klack.	*JP, SW & LZ*	M030	Madagascar	**n=16**	**2n=32**
Exacum dolichantherum Klack.	*SW & MC*	M064	Madagascar		**2n=32**
Exacum exiguum Klack.	*JP, SW & LZ*	M008	Madagascar	**n=16**	
Exacum exiguum Klack.	*JP, SW & LZ*	M015	Madagascar	**n=16**	**2n=32**
Exacum exiguum Klack.	*JP, SW & LZ*	M046	Madagascar		**2n=32**
Exacum exiguum Klack.	*JP, SW & LZ*	M048	Madagascar		**2n=32**
Exacum exiguum Klack.	*JP, SW & LZ*	M051	Madagascar	**n=16**	
Exacum exiguum Klack. aff.	*JP, SW & LZ*	M050	Madagascar	**n=16**	**2n=32**
Exacum hoffmannii Vatke ex Schinz	*JP, SW & LZ*	2 ème arrêt	Madagascar		**2n=32**
Exacum hoffmannii Vatke ex Schinz	*JP, SW & LZ*	M026	Madagascar		**2n=32**
Exacum humbertii Klack.	*SW & J.-I. Pfund*	M052	Madagascar		**2n=32**
Exacum marojejyense Humbert	*JP, SW & LZ*	M033	Madagascar	**n=16**	**2n=32**
Exacum marojejyense Humbert	*SW & J.-I. Pfund*	M056	Madagascar		**2n=32**
Exacum microcarpum Klack.	*SW & J.-I. Pfund*	M054	Madagascar		**2n=32**
Exacum microcarpum Klack.	*SW & J.-I. Pfund*	M055	Madagascar		**2n=32**
Exacum millotii Humbert	*JP, SW & LZ*	M032	Madagascar		**2n=32**
Exacum millotii Humbert	*JP, SW & LZ*	M035	Madagascar		**2n=32**
Exacum millotii Humbert	*JP, SW & LZ*	M036	Madagascar		**2n=32**
Exacum millotii Humbert	*SW & J.-I. Pfund*	M057	Madagascar		**2n=32**
Exacum nummularifolium Humbert	*SW & J.-I. Pfund*	M058	Madagascar		**2n=32**
Exacum quinquenervium Griesb.	*JP, SW & LZ*	-	Madagascar		**2n=36**
Exacum quinquenervium Griesb.	*JP, SW & LZ*	-	Madagascar	**n=16**	
Exacum quinquenervium Griesb.	*JP, SW & LZ*	-	Madagascar		**2n=36**

Exacum quinquenervium Griesb.	*JP, SW & LZ*	2 ème arrêt	Madagascar		**2n=36**
Exacum quinquenervium Griesb.	*JP, SW & LZ*	4 ème arrêt	Madagascar		**2n=36**
Exacum quinquenervium Griesb.	*JP, SW & LZ*	M007	Madagascar		**2n=36**
Exacum quinquenervium Griesb.	*JP, SW & LZ*	M012	Madagascar		**2n=36**
Exacum quinquenervium Griesb.	*JP, SW & LZ*	M021	Madagascar		**2n=36**
Exacum quinquenervium Griesb.	*JP, SW & LZ*	M025	Madagascar	**n=16**	
Exacum quinquenervium Griesb.	*JP, SW & LZ*	M038	Madagascar		**2n=36**
Exacum quinquenervium Griesb.	*JP, SW & LZ*	M065	Madagascar		**2n=32**
Exacum spathulatum Baker	*JP, SW & LZ*	M011	Madagascar		**2n =32**
Exacum stenophyllum Klack.	*JP, SW & LZ*	M045	Madagascar	**n=16**	
Exacum stenophyllum Klack.	*JP, SW & LZ*	M049	Madagascar		**2n=36?**
Exacum stenopterum Klack.	*JP, SW & LZ*	Station 3	Madagascar		**2n=32**
Exacum stenopterum Klack.	*JP, SW & LZ*	M019	Madagascar		**2n =32**
Exacum stenopterum Klack.	*JP, SW & LZ*	M020	Madagascar	**n=16**	**2n=32**
Exacum stenopterum Klack.	*JP, SW & LZ*	M027	Madagascar		**2n =32**
Exacum stenopterum Klack.	*JP, SW & LZ*	M029	Madagascar		**2n =32**
Exacum subteres Klack.	*SW & J.-I. Pfund*	M053	Madagascar		**2n =32**
Ornichia madagascariensis Klack.	*SW*	M002	Madagascar		**2n=28**
Sebaea "*pentendra aff.* X 35?"	*JK & LZ*	36	South Africa	**n=14**	**2n=28**
Sebaea "*repens* X *thodeana*?"	*JK & LZ*	23	Leshoto		**2n=28**
Sebaea 28	*JK, BG & MP*	106a	South Africa		**2n=28**
Sebaea 29	*JK, BG & MP*	107	South Africa	**n=14**	**2n=28**
Sebaea 30	*JK, BG & MP*	108	South Africa	**n=14**	
Sebaea 31	*JK, BG & MP*	109	South Africa		**2n=28**
Sebaea 39	*JK, BG & MP*	117	South Africa		**2n=28**
Sebaea africana Paiva & Nogueria	*SD, RA, PD, JK, BL & ET*	603	Zambia	**n=21**	
Sebaea albens (L.f.) Roem. & Schult. *aff.*	*Kissling J.*	93	South Africa	**n=14**	**2n=28**
Sebaea ambigua Cham.	*Kissling J.*	94	South Africa	**n=14**	**2n=28**
Sebaea ambigua Cham.	*JK & LZ*	45	South Africa		**2n=28**
Sebaea aurea (L.f.) Roem. & Schult	*Kissling J.*	91	South Africa		**2n=42**
Sebaea aurea (L.f.) Roem. & Schult	*JK*	98	South Africa		**2n=28**
Sebaea aurea (L.f.) Roem. & Schult	*JK*	89	South Africa	**n=14**	**2n=28**
Sebaea aurea (L.f.) Roem. & Schult.	*JK & LZ*	49	South Africa	**n=14**	
Sebaea baumiana (Gilg) Boutique	*SD, RA, PD, JK, BL & ET*	809	Zambia	**n=21**	**2n=42**

Sebaea baumiana (Gilg) Boutique	*SD, RA, PD, JK, BL & ET*	824	Zambia	**n=21**	
Sebaea baumiana (Gilg) Boutique	*SD, RA, PD, JK, BL & ET*	845	Zambia	**n=21**	**2n=42**
Sebaea baumiana (Gilg) Boutique	*SD, RA, PD, JK, BL & ET*	906	Zambia	**n=21**	
Sebaea baumiana (Gilg) Boutique	*SD, RA, PD, JK, BL & ET*	933	Zambia	**n=21**	**2n=42**
Sebaea baumiana (Gilg) Boutique	*SD, RA, PD, JK, BL & ET*	969	Zambia		**2n=42**
Sebaea baumiana (Gilg) Boutique	*SD, RA, PD, JK, BL & ET*	970	Zambia	**n=21**	**2n=42**
Sebaea baumiana (Gilg) Boutique	*SD, RA, PD, JK, BL & ET*	971	Zambia		**2n=42**
Sebaea baumiana (Gilg) Boutique	*SD, RA, PD, JK, BL & ET*	974	Zambia	**n=21**	**2n=42**
Sebaea bojeri Griesb.	*LZ*	05.03.01 2a; I11_Jtr.X	South Africa		**2n=28**
Sebaea bojeri Griesb.	*LZ*	05.03.02 1b; I11_Jtr_4	South Africa		**2n=28**
Sebaea bojeri Griesb.	*LZ*	05.03.02 2b; H14_Jfr_2	South Africa		**2n=28**
Sebaea brachyphylla Griesb.	*JP, SW & LZ*	M044	South Africa		**2n= *c.* 56**
Sebaea brachyphylla Griesb.	*LZ*	05.03.04 1b; I11_Lpr.3	South Africa		**2n=42**
Sebaea cf. *gracilis* (Welw.) Paiva & Nogueira	*SD, RA, PD, JK, BL & ET*	656	Zambia	**n=21**	**2n=42**
Sebaea cf. *gracilis* (Welw.) Paiva & Nogueira	*SD, RA, PD, JK, BL & ET*	692	Zambia	**n=21**	**2n=42**
Sebaea cf. *rehmannii* Schinz	*LZ*	05.03.02 1; K09_Tal.3	South Africa	**n=21**	
Sebaea cf. *rehmannii* Schinz	*LZ*	05.03.02 1a; K03_Jtr.4	South Africa	**n=21**	
Sebaea clavata Paiva & Nogueria	*SD, RA, PD, JK, BL & ET*	543	Zambia	**n=21**	**2n=42**
Sebaea exacoides (L.) Schinz	*JK & LZ*	81	South Africa	**n=14**	**2n=28**
Sebaea exacoides (L.) Schinz	*JK & LZ*	86	South Africa		**2n=28**
Sebaea exacoides (L.) Schinz	*JK & LZ*	87	South Africa		**2n=28**
Sebaea exacoides (L.) Schinz	*JK & LZ*	88a	South Africa		**2n=28**
Sebaea exacoides (L.) Schinz	*JK & LZ*	88b	South Africa	**n=14**	
Sebaea exacoides aff. (L.) Schinz	*JK, BG & MP*	104	South Africa		**2n=28**
Sebaea fernandesiana Paiva & Nogueria	*SD, RA, PD, JK, BL & ET*	1011	Zambia	**n=21**	**2n=42**
Sebaea filiformis Schinz	*LZ*	05.03.03 1a; I11_Gre1	South Africa		**2n=28**
Sebaea filiformis Schinz	*LZ*	05.03.04 1a; H14_Jtr.1	South Africa		**2n=28**
Sebaea grandis (E.Mey.) Steud.	*SD, RA, PD, JK, BL & ET*	657	Zambia	**n=21**	
Sebaea grandis (E.Mey.) Steud.	*SD, RA, PD, JK, BL & ET*	752	Zambia		**2n=42**
Sebaea grandis (E.Mey.) Steud.	*SD, RA, PD, JK, BL & ET*	764	Zambia	**n=21**	
Sebaea grandis (E.Mey.) Steud.	*SD, RA, PD, JK, BL & ET*	815	Zambia		**2n=42**
Sebaea griesbachiana Schinz *aff.*	*JK, BG & MP*	112	South Africa	**n=14**	
Sebaea griesbachiana Schinz *aff.*	*JK, BG & MP*	115	South Africa		**2n=28**
Sebaea leiostyla Gilg	*LZ*	05.03.01 2b; I11_Aob_4	South Africa		**2n=28**

Sebaea leiostyla Gilg	*LZ*	05.03.02 2a; I11_Jfr_3	South Africa		**2n=28**
Sebaea leiostyla Gilg	*LZ*	05.03.03 2a; H14_Gac_2	South Africa		**2n=28**
Sebaea leiostyla Gilg	*LZ*	05.03.01 3	South Africa		**2n=28**
Sebaea macrophylla Gilg	*JK & LZ*	72	South Africa		**2n=56**
Sebaea macrophylla Gilg	*JK & LZ*	74	South Africa	**n=28**	
Sebaea madagascariensis (Schinz) Klack.	*JP, SW & LZ*	M017	Madagascar	**n=9**	**2n=18**
Sebaea marlothii Gilg	*JK & LZ*	11	South Africa		**2n=28**
Sebaea marlothii Gilg	*JK & LZ*	15	Leshoto	**n=14**	
Sebaea marlothii Gilg	*JK & LZ*	17	Leshoto		**2n=28**
Sebaea marlothii Gilg	*JK & LZ*	18	Leshoto		**2n=28**
Sebaea marlothii Gilg	*JK & LZ*	19	Leshoto		**2n=28**
Sebaea marlothii Gilg	*JK & LZ*	21	Leshoto		**2n=28**
Sebaea marlothii Gilg	*JK & LZ*	38	South Africa		**2n=28**
Sebaea membranaceae Hill. *aff.*	*JK & LZ*	66	South Africa		**2n=28**
Sebaea micrantha aff. (Cham & Schlechdtl.) Schinz	*JK*	95	South Africa	**n=28**	**2n=56**
Sebaea minuta Paiva & Nogueria	*SD, RA, PD, JK, BL & ET*	623	Zambia	**n=21**	**2n=42**
Sebaea minutiflora Schinz	*JK*	83	South Africa		**2n=42**
Sebaea minutiflora Schinz	*JK & LZ*	46	South Africa		**2n=28**
Sebaea oligantha (Gilg.) Schinz	*SD, RA, PD, JK, BL & ET*	499	Zambia	**n=14**	
Sebaea pentendra E.Mey. *aff.*	*JK & LZ*	34	South Africa	**n=14**	
Sebaea perparva Sileshi	*SD, RA, PD, JK, BL & ET*	728	Zambia	**n=21**	**2n=42**
Sebaea procumbens Hill.	*JK & LZ*	10	South Africa		**2n=28**
Sebaea pusilla Eckl. Ex Cham.	*JK & LZ*	64	South Africa		**2n=28**
Sebaea repens Schinz *aff.*	*JK & LZ*	14a	South Africa		**2n=28**
Sebaea repens Schinz *aff.*	*JK & LZ*	14b	South Africa		**2n=28**
Sebaea hymenosepala Gilg *aff.*	*JK & LZ*	3	South Africa		**2n=28**
Sebaea hymenosepala Gilg *aff.*	*JK & LZ*	4	South Africa	**n=14**	**2n=28**
Sebaea hymenosepala Gilg *aff.*	*JK & LZ*	5	South Africa		**2n=28**
Sebaea hymenosepala Gilg *aff.*	*JK & LZ*	73	South Africa		**2n=28**
Sebaea scabra Schinz	*JK*	85	South Africa		**2n=28**
Sebaea scabra Schinz	*JK, BG & MP*	103	South Africa		**2n=28**
Sebaea schlechterii Schinz	*JK & LZ*	50	South Africa	**n=14**	
Sebaea schlechterii Schinz	*JK & LZ*	55	South Africa		**2n=28**
Sebaea sedoides var. *confertiflora* (Schinz) Marais	*LZ*	05.03.03 1b; H14_Lpr_2	South Africa		**2n=42**

Sebaea sedoides var. *confertiflora* (Schinz) Marais	*LZ*	05.03.03 2b; I11_Cun_2	South Africa		**2n=42**
Sebaea sedoides var. *sedoides* Gilg	*LZ*	05.03.03 2b; I11_Cun_2	South Africa		**2n=42**
Sebaea sp. A	*JK & LZ*	35	South Africa	**n=14**	
Sebaea sp. A	*JK & LZ*	37	South Africa	**n=14**	
Sebaea sp. B	*JK*	99	South Africa		**2n=28**
Sebaea sp.(Undeterminable, only vers young bud)	*JK & LZ*	30	Leshoto		**2n=28**
Sebaea sp.(Undeterminable, only vers young bud)	*JK & LZ*	69	South Africa		**2n=28**
Sebaea sp.(Undeterminable, only vers young bud)	*JK & LZ*	71	South Africa		**2n=28**
Sebaea spathulata (E. Mey.) Steud.	*JK & LZ*	12	South Africa		**2n=28**
Sebaea spathulata (E. Mey.) Steud.	*JK & LZ*	25	Leshoto		**2n=28**
Sebaea spathulata (E. Mey.) Steud.	*JK & LZ*	26	Leshoto		**2n=28**
Sebaea spathulata (E. Mey.) Steud.	*JK & LZ*	40	South Africa		**2n=28**
Sebaea sulphurea Cham & Schlechtdl.	*JK, BG & MP*	100	South Africa		**2n=28**
Sebaea teucszii (Schinz) Taylor	*SD, RA, PD, JK, BL & ET*	557	Zambia		**2n=42**
Sebaea teucszii (Schinz) Taylor	*SD, RA, PD, JK, BL & ET*	599	Zambia	**n=21**	
Sebaea teucszii (Schinz) Taylor	*SD, RA, PD, JK, BL & ET*	701	Zambia	**n=21**	
Sebaea teucszii (Schinz) Taylor	*SD, RA, PD, JK, BL & ET*	771	Zambia		**2n=42**
Sebaea thodeana Gilg	*JK & LZ*	20	Leshoto		**2n=28**
Sebaea thodeana Gilg.	*JK & LZ*	16	Leshoto		**2n=28**
Sebaea thodeana Gilg.	*JK & LZ*	22	Leshoto		**2n=28**
Sebaea thodeana Gilg. *aff.*	*JK & LZ*	31a	Leshoto		**2n=28**
Sebaea thodeana Gilg. *aff.*	*JK & LZ*	31b	Leshoto	**n=14**	
Sebaea thomasii (S. Moore) Schinz	*JK & LZ*	29	Leshoto		**2n=28**
Sebaea thomasii (S. Moore) Schinz	*JK & LZ*	70	South Africa		**2n=28**
Tachiadenus carinatus (Desrousseaux) Grisebach	*JP, SW & LZ*	M039	Madagascar		**2n=32**
Tachiadenus longiflorus Bojer ex. Griesb.	*JP, SW & LZ*	M006	Madagascar		**2n=32**
Tachiadenus longiflorus Bojer ex. Griesb.	*JP, SW & LZ*	M016	Madagascar		**2n=32**
Tachiadenus longiflorus Bojer ex. Griesb.	*JP, SW & LZ*	-	Madagascar		**2n=32**

PREVIOUS REPORTS OF CHROMSOME DATA ON EXACEAE

Annex 2 - Chromosome numbers documented for the tribe Exaceae.

Taxon	**chromosome number** **n**	 **2n**	**References**
Exacum affine I.B. Balf	**n=18**		Sugiura, 1936; Post, 1933
Exacum affine I.B. Balf		**2n=36**	Rork, 1949; Darlington and Wylie, 1955
Exacum affine I.B. Balf		**2n=36**	Riseman et al., 2006
Exacum affine I.B. Balf		**2n=36**	Sumanasinghe, 1986
Exacum atropurpureum Bedd.		**2n=34**	Mallikarjuna et al., 1987
Exacum courtallens var *courtallens* Arn.	**n=34**		Mallikarjuna et al., 1987
Exacum courtallens var *laxiflorum* Gamble		**2n=68**	Mallikarjuna et al., 1987
Exacum gracilipes I.B. Balf		**2n=20**	Villemoes, 2000
Exacum grande Klack. Under *E. perrotteti* Griesb.		**2n=68**	Mallikarjuna et al., 1987
Exacum lawii C.B. Clarke		**2n=56**	Mallikarjuna et al., 1987
Exacum macranthum Arn. Ex Griseb.		**2n=54**	Sumanasinghe, 1986
Exacum pallidum (Trim.) Klack.		**2n=52**	Sumanasinghe, 1986
Exacum pedunculatum L.		**2n=62**	Mallikarjuna et al., 1987
Exacum pedunculatum L.		**2n=(30,) 54, (56)**	Subramanian D., 1980
Exacum pedunculatum L.		**2n=56**	Riseman et al., 2006
Exacum pedunculatum L.	**n=28**		Sumanasinghe, 1986
Exacum petiolare Griesb.		**2n=62**	Mallikarjuna et al., 1987
Exacum pumilum Griesb.	**n=31**		Mallikarjuna et al., 1987
Exacum sessile L.	**n=31**		Mallikarjuna et al., 1987
Exacum tenue (Blume) Klack. Under *Cotylanthera tenuis* Blume	**n = 16-18**		Oehler, 1927
Exacum tetragonum Roxb.		**2n=18**	Borgmann, 1964
Exacum tetragonum Roxb. Under *E. bicolor* Roxb.		**2n=62**	Mallikarjuna et al., 1987
Exacum tetragonum Roxb. Under *E. perrottetii* Griesb.		**2n=68**	Mallikarjuna et al., 1987
Exacum travancoricum Bedd.		**2n=68**	Mallikarjuna et al., 1987
Exacum trinervium (L.) Druce		**2n=60**	Sumanasinghe, 1986
Exacum trinervium subsp. *macranthum* (Arn. Ex Griesb.) Cramer		**2n=54**	Riseman et al., 2006
Exacum trinervium subsp. *pallidum* (Trim.) Cramer		**2n=52**	Riseman et al., 2006
Exacum trinervium subsp. *ritigalensis* (Willis) Cramer		**2n=60**	Riseman et al., 2006
Exacum trinervium subsp. *ritigalensis* (Willis) Cramer		**2n=60**	Sumanasinghe, 1986
Exacum trinervium subsp. *trinervium* (L.) Druce		**2n=60**	Riseman et al., 2006
Exacum trinervium subsp. *trinervium* (L.) Druce		**2n=60**	Sumanasinghe, 1986
Exacum wightianum Arnott.		**2n=68**	Mallikarjuna et al., 1987
Exacum wightianum Arnott. Under *E. foliosum* Griesb.		**2n=68**	Mallikarjuna et al., 1987
Sebaea brachyphylla Griesb.		**2n=22**	Thulin, 1970
Sebaea ovata (Labill.) R.Br.	**n=** ***c.*** **27**		Beuzenberg & Hair, 1983

DATA SETS AND ACCESSIONS NUMBERS USED IN THE PRESENT THESIS

Origin of plant material, voucher information and EMBL/GenBank accession number of sequence (Chapter 2).

Species	Voucher specimen and herbarium	Origin	*Trn* L intron	*Trn* L-F spacer	Atbp-Rbcl spacer
Anthocleista amplexicaulis Baker	S. Wohlhauser s.n. (PBZT)	Madagascar	AJ490189*	n/a	n/a
Anthocleista grandiflora Gilg	M. Callmander s.n. (NEU)	Madagascar	AJ490190*	n/a	n/a
Anthocleista scandens Hook.f.			AF102376*	n/a	n/a
Aripuana cullmaniorum Struwe, Maas & V.A. Albert			AJ242603*	n/a	n/a
Blackstonia imperfoliata (L.F.) Samp.	G. Mansion 010833	Spain	AY251743*	AY251769*	n/a
Blackstonia perfoliata (L.) Huds.	G. Mansion 98712 (NEU)	France	AF402198*	AF402254*	n/a
Calolisianthus pendulus (Mart.) gilg			AF102387*	n/a	n/a
Calolisianthus pulcherrimus (Mart.) gilg	R. M. Harley et al. 15674 (NY)	n/a	AF102388*	n/a	n/a
Canscora alata (Roth) Wallich	J. C. Piso, S. Wohlhauser & L. Zeltner MO24 (NEU)	Madagascar	AJ490191*	n/a	n/a
Canscora andrographioides Griff.	P. Chassot 99-234 (NEU)	Thailand	AJ490192*	n/a	n/a
Canscora diffusa (Vahl.) Roem. & Schult.	P. Chassot 99-231 (NEU)	Thailand	AJ490193*	AY251780*	n/a
Centaurium pulchellum (Sw.) Druce	G. Mansion 98505 (NEU)	France	AY251734*	AY251758*	n/a
Centaurium tenuiflorum (Hoffmgg. & Link) Fritsch	L. & N. Zeltner 1767 (NEU)	Morocco	AY251735*	AY251759*	n/a
Chelonanthus alatus (Aubl.) Pulle	F. bretagnolle & J. Piguet C3 (NEU)	Ecuador	AJ490194*	AY251775*	n/a
Chelonanthus angustifolius Gilg	F. bretagnolle & J. Piguet T11 (NEU)	Ecuador	AJ490195*	AY251776*	n/a
Chironia baccifera L.	J. Kissling & L. Zeltner 57 (NEU)	South Africa	present study	present study	present study
Chironia palustris subsp. *transvaalensis* (Gilg) Verdoorn	J. Kissling & L. Zeltner 2 (NEU)	South Africa	present study	present study	present study
Chironia purpurascens subsp. *humilis* (Gilg) Verdoorn	J. Kissling & L. Zeltner 1 (NEU)	South Africa	present study	present study	
Cicendia filiformis (L.) Delarbre	M. Thiv 2156 (MJG)	France	AF102403*	n/a	n/a
Cicendia quadrangularis (Lam.) Griesb.	P. Maas 8154 (U)	n/a	AF102404*	AY251765*	n/a
Comastoma pulmonarium (Turcz.) Toyok.	Yuan & Küpfer 92-279 (NEU)	China, Sichuan	AJ315225*	AJ315271*	n/a
Comastoma tenellum (Rottb.) Toyok.			AJ580518*	n/a	n/a
Coutoubea ramosa Aubl.	B. Hoffman & C. Capellaro 984 (NY)	n/a	AF102408*	n/a	n/a
Coutoubea spicata Aubl.	S. Mori 24349	n/a	AY251745*	AY251778*	n/a
Crawfurdia delavayi Franch	n/a	n/a	AY563391*	n/a	n/a
Curtia tenuifolia Knobl.	M. J. Jansen-Jacob 2740 (NY)	n/a	AJ242606*	n/a	n/a
Deianira pallescens Cham. & Schlecht.	W. A. Anderson 9385 (NY)	n/a	AF102410*	AY251782*	n/a
Djaloniella ypsilostyla P. Taylor	Morton SL2442 (K)	n/a	AF102413*	n/a	n/a
Emmenopterys henryi Oliver			AF152637*	AF152637*	DQ131728*
Enicostema verticillatum (L.) Engl. ex Gilg	J. Pruski & J. Steyermark 1473 (NY)	n/a	AF102414*	n/a	n/a
Eustoma exaltatum (L.) Salisb.	L. & N. Zeltner 980610-1 (NEU)	Mexico	AY251752*	AY251789*	n/a
Eustoma grandiflorum (Raf.) Shinners	G. Mansion s.n. (NEU)	Switzerland (cultivated)	AY251751*	AY251788*	n/a
Exaculum pusillum Caruel	P. Küpfer s.n. (NEU)	Italy	AY251740*	AY251764*	n/a
Exacum affine I.B. Balf. ex Regel	Miller & al. 6201 (E)	Oman	AJ490211*	AY251770	n/a
Exacum appendiculatum Klack.	S. Buerki SB058 (NEU)	Madagascar	present study	present study	present study
Exacum atropurpureum Bedd.	Klackenberg & Lundin 526 (S)	India	AJ490205*	n/a	n/a
Exacum caeruleum I.B. Balf.	Miller & al. 11356 (E)	Socotra	AJ490207*	AY251771	n/a
Exacum dolichanterum Klack.	LG4291 (NEU)	Madagascar	present study	present study	present study
Exacum exiguum Klack.	S. Buerki SB040 (NEU)	Madagascar	present study	present study	present study
Exacum hamiltonii G. Don	Wood 7477 (E)	Bhutan	AJ490212*	n/a	n/a
Exacum humbertii Klack.	Wohlhauser & Pfund M052 (NEU)	Madagascar	AJ490213*	n/a	present study
Exacum linearifolium (Humbert) Klack.	Miller & Randrianasolo 6254 (S)	Madagascar	AJ490215*	n/a	n/a
Exacum macranthum Arn, ex Griesb.	Fagerlind & Klackenberg 767, S	Sri Lanka	AJ490217*	n/a	n/a
Exacum marojejyense Humbert	Wohlhauser & Pfund M056 (NEU)	Madagascar	AJ490218*	n/a	present study
Exacum nummularifolium Humbert	Wohlhauser & Pfund M058 (NEU)	Madagascar	AJ490221*	n/a	present study
Exacum oldenlandioides (S. Moore) Klack.	Reekmans 9275, S	Burundi	AJ490222*	n/a	present study
Exacum pallidum (Trim.) Klack.	Fagerlind & Klackenberg 777, S	Sri Lanka	AJ490223*	n/a	n/a
Exacum pedunculatum L.	Bremer, Kerr & Theran 4 (S)	Sri Lanka	AJ490224*	present study	n/a
Exacum quinquenervium Griesb.	S. Buerki SB052 (NEU)	Madagascar	present study	present study	present study
Exacum sessile L.	Klackenberg & Lundin 349 (S)	India	AJ490226*	n/a	n/a
Exacum sp.	CR 2831	Madagascar	present study	present study	present study
Exacum stenophyllum Klack.	S. Buerki SB051 (NEU)	Madagascar	present study	present study	present study
Exacum tetragonum Roxb.	Keke 254 (E)	Nepal	AJ490233*	n/a	n/a
Exacum trinervium (L.) Druce	Zeltner sl001 (NEU)	Sri Lanka	AJ490235*	n/a	n/a
Exacum wightianum Arn.	Klackenberg & Lundin 188 (S)	India	AJ490238*		n/a
Exochaenium africanum (Paiva & Nogueira) Kissling	S. Dessein et al. 604 (NEU)	Zambia	present study	present study	present study
Exochaenium africanum (Paiva & Nogueira) Kissling	S. Dessein et al. 623 (NEU)	Zambia	present study	present study	present study
Exochaenium baumianum (Gilg) Schinz	S. Dessein et al. 809 (NEU)	Zambia	present study	present study	present study
Exochaenium clavatum (Paiva & Nogueira) Kissling	S. Dessein et al. 543 (NEU)	Zambia	present study	present study	present study
Exochaenium fernandesianum (Paiva & Nogueira) Kissling	S. Dessein et al. 1011 (NEU)	Zambia	present study	present study	present study
Exochaenium grande (E. Mey.) Griesb.	S. Dessein et al. 752 (NEU)	Zambia	present study	present study	present study
Exochaenium leariforme (Sileshi) Kissling	S. Bidgood et al. 3630 (C)	Tanzania	present study	present study	present study
Exochaenium macropterum (Sileshi) Kissling	S. Bidgood et al. 4012 (BR)	Tanzania	present study	present study	present study
Exochaenium oliganthum (Gilg) Schinz	S. Dessein et al. 499 (NEU)	Zambia	present study	present study	present study
Exochaenium perparvum (Sileshi) Kissling	S. Dessein et al. 827 (NEU)	Zambia	present study	present study	present study
Exochaenium platypterum (Baker) Schinz	J. Kissling 75 (NEU)	Zambia	present study	present study	present study
Exochaenium sp	S. Dessein et al. 934 (NEU)	Zambia	present study	present study	present study
Exochaenium sp1	S. Dessein et al. 692 (NEU)	Zambia	present study	present study	present study
Exochaenium sp2	S. Dessein et al. 656 (NEU)	Zambia	present study	present study	present study
Exochaenium sp3	S. Dessein et al. 584 (NEU)	Zambia	present study	present study	present study
Exochaenium teucszii (Schinz) Schinz	S. Dessein et al. 701 (NEU)	Zambia	present study	present study	present study
Fagraea berteroana A.Gray ex Benth			AF102419*	n/a	n/a
Fagraea elliptica Roxb.			AF102420*	n/a	n/a
Fagraea fragans Roxb.	C.-H. Tsou 207 (NY)	n/a	AF102421*	n/a	n/a
Faroa axillaris Baker	M. Schaijes 5076 (BR)	n/a	AF102423*	n/a	n/a
Faroa schaijesiorum Bamps	M. Schaijes 3515 (BR)	n/a	AF102424*	n/a	n/a
Frasera albomarginata S. Watson	Schweich NEU 00-23	USA, California	AJ315187*	n/a	n/a
Gardenia taitensis DC.			Af102426*	Af102426*	AJ233988*
Gelsemium sempervirens (L.) Aiton			AJ346932*	AF159696*	AJ233985*
Geniostemon gypsophilum B.L. Turner	G. Nesom et al. 7621 (LL)	Mexico	AF102429*	AY251766*	n/a
Gentiana acaulis L.	n/a	n/a	X77869*	n/a	n/a
Gentiana brachyphylla Villard	n/a	n/a	X77875*	n/a	n/a

Gentiana cruciata L.	Küpfer NEU 2005-G2 (NEU)	Italy	DQ398713*	DQ398713*	DQ398600*
Gentiana lutea L.	Yuan Y.-M. 91-S5 (NEU)	Switzerland	X75702*	n/a	n/a
Gentiana pupurea L.			AJ430909*	AJ430909*	n/a
Gentiana pyrenaica L.	Yuan Y.-M. 93-14 (NEU)	Bulgaria	X77895*	n/a	n/a
Gentiana tianschanica Ruprecht	Yuan 2004-95 (IBSC)	Xinjiang	DQ398722*	DQ398722*	DQ398616*
Gentianella campestris (L.) Harry Sm.			AJ580526*	n/a	n/a
Gentianella engadinensis (Wettst.) holub			AJ580526*	n/a	n/a
Gentianella pilosa (Wettst.) Holub			AJ580525*	n/a	n/a
Gentianopsis grandis (Harry Sm.) Ma	Yuan & Küpfer 92-222 (NEU)	China, Yunnan	AJ315227*	n/a	n/a
Gentianothamnus madagascariensis Humbert	L. Gautier G020	Madagascar	AJ490240*	n/a	present study
Guettarda boliviana Standl.			AF152727*	AF152727*	
Gyrandra brachycalyx (Standley & L.O. Willams) Mansion	G. Mansion, L. & N. Zeltner 990205 (NEU)	Mexico	AF402184*	AF402240*	n/a
Gyrandra tenuifolia (Martens & Galeotti) Mansion	G. Mansion, L. & N. Zeltner 990228 (NEU)	Mexico	AF402186*	AF402242*	n/a
Halenia corniculata (L.) Cornaz	Anderberg & Lundin 8	n/a	AJ408009*	n/a	n/a
Halenia elliptica D. Don	Yuan & Küpfer 93-52 (NEU)	China, Sichuan	AJ315193*	AJ315239*	n/a
Halenia weddelliana Gilg.	Chassot 00-5 (NEU)	Ecuador	AJ315194*	AJ315240*	n/a
Hoppea dichotoma Wild.	C. D. K. Cook RHT307 (MJG)	n/a	AF102440*	n/a	n/a
Irlbachia pratensis (H. B. K.) L. Cobb & Maas			AF102442*	n/a	n/a
Ixanthus viscosus (Aiton) Griesb.	P. Küpfer s.n. (NEU)	Spain, Tenerife	AY251741*	AY251767*	n/a
Labordia tinifolia A. Gray			AF102447*	n/a	n/a
Lisianthus laxiflorus Urban			AF102449*	n/a	n/a
Lisianthus longifolius L.	H. van der Werff 8690 (NY)	n/a	AF102450*	n/a	n/a
Lomatogonium bellum (Hemsl.) H. Smith	Yuan & Küpfer 92-236 (NEU)	China, Yunnan	AJ315197*	AJ315243*	n/a
Megacodon stylophorus (C.B. Clarke) H. Smith	Chassot & Yuan 99-36 (NEU)	China, Yunnan	AJ315200*	AY251773*	n/a
Metagentiana gentilis (Franch) T.N.Ho & S.W. Liu			AY563386*	n/a	n/a
Metagentiana primuliflora (Franch) T.N.Ho & S.W.Liu			AY563385*	n/a	n/a
Microphium pubescens C.B. Clarke	P. Chassot 99-243 (NEU)	Thailand	AJ490241*	AY251781*	n/a
Mitragyna inermis (Willd.) Kuntze.			AJ346932*	AJ346932*	DQ131751*
Mitreola petiolata (Walt.) Torr. & Gray			AF102460*	n/a	DQ131696*
Nerium oleander L.			AF214386*	n/a	n/a
Obolaria virginica L.	Nicolson 24-IV-00	USA, Virginia	AJ315201*	AJ315247	n/a
Ornichia madagascariensis Klack.	S. Wohlhauser M002	Madagascar	AJ490242*	present study	present study
Ornichia trinervis (Desrousseaux) Klack.	M. Callmander s.n. (NEU)	Madagascar	AJ490243*	present study	n/a
Orphium frutescens (L.) E. Mey.	J. Kissling & L. Zeltner 44 (NEU)	South Africa	present study	present study	present study
Plumaria plumosa (Hudson) Kuntze			AF214409*	AF214255*	n/a
Potalia amara Aubl.			AF102470*	n/a	n/a
Potalia resinifera Mart.			AF102472*	n/a	n/a
Pterygocalyx volubilis Maxim.	Chassot & Yuan 99-100 (NEU)	China, Yunnan	AJ315202*	AJ315248*	n/a
Sabatia dodecandra (L.) Britton	J. Grant 97-2858 (NEU)	USA	AY255693*	AY255697*	n/a
Sabatia stellaris Pursh.	J. Grant 97-2871 (NEU)	USA	AY255694*	AY255697*	n/a
Saccifolium bandeirae Maguire & Pires	M. Piliackas et al., s. n.	n/a	AJ242608*	n/a	n/a
Schenkia australis (R. Br.) Mansion	L. & N. Zeltner 001216 (NEU)	Australia	AY251679*	AY251709*	n/a
Schenkia clementii (Domin.) Mansion	L. & N. Zeltner 001201 (NEU)	Australia	AY251739*	AY251763*	n/a
Schenkia spicata (L.) Mansion	G. Mansion 981005 (NEU)	France	AF402196*	AF402252*	n/a
Schinziella tetragona (Schinz) Gilg	Malaisse 13852 (BR)	n/a	AF102479*	n/a	n/a
Schultesia guianensis (Aubl.) Malme	C. C. Berg & A. J. Henderson BG661 (NY)	n/a	AF102480*	n/a	n/a
Sebaea albens (L.f.) Roem. & Schult.	B. Bytebier 2616 (NEU)	South Africa	present study	present study	present study
Sebaea ambigua Cham.	J. Kissling & L. Zeltner 45 (NEU)	South Africa	present study	present study	present study
Sebaea aurea (L.f.) Roem. & Schult.	B. Bytebier 2620 (NEU)	South Africa	present study	present study	present study
Sebaea bojeri Griseb.	L. & N. Zeltner 050302/1b (NEU)	Lesotho	present study	present study	present study
Sebaea brachyphylla Griseb.	J. Raynal 19414	Kenya	AJ490245*	n/a	n/a
Sebaea cf. rotundifolia Hill.	J. Kissling & L. Zeltner 10 (NEU)	South Africa	present study	present study	present study
Sebaea exacoides (L.) Schinz	B. Bytebier 2610 (NEU)	South Africa	present study	present study	present study
Sebaea filiformis Schinz	L. & N. Zeltner 050304/1a (NEU)	Lesotho	present study	present study	present study
Sebaea junodii Schinz	S. Dessein et al. 577 (NEU)	Zambia	present study	present study	n/a
Sebaea leiostyla Gilg	L. & N. Zeltner 050303/2a (NEU)	South Africa	present study	present study	present study
Sebaea longicaulis Schinz	M. Reekmans 8272 (BR)	South Africa	present study	present study	n/a
Sebaea madagascariensis Klack.	J. C. Piso, S. Wohlhauser & L. Zeltner M018 (NEU)	Madagascar	AJ490247*	present study	present study
Sebaea membranacea Hill	J. Kissling & L. Zeltner 66 (NEU)	South Africa	present study	present study	present study
Sebaea microphylla (Edgew.) Knobl.	J. Kissling 77 (NEU)	Zambia	present study	present study	present study
Sebaea microphylla (Edgew.) Knobl.	S. Dessein et al. 557 (NEU)	Zambia	present study	present study	present study
Sebaea minutiflora Schinz	J. Kissling & L. Zeltner 46 (NEU)	South Africa	present study	present study	present study
Sebaea natalensis Schinz	Mothogoane M.S. 250 (PRE)	South Africa	present study	present study	present study
Sebaea pusilla Eckl. Ex Cham.	J. Kissling & L. Zeltner 64 (NEU)	South Africa	present study	present study	present study
Sebaea rehmannii Schinz	L. & N. Zeltner 050302/1a (NEU)	South Africa	present study	present study	present study
Sebaea repens Schinz	J. Kissling & L. Zeltner 17 (NEU)	Lesotho	present study	present study	present study
Sebaea schlechteri Schinz	J. Kissling & L. Zeltner 50 (NEU)	South Africa	present study	present study	n/a
Sebaea sedoides Gilg.	L. & N. Zeltner 050303/2b (NEU)	South Africa	present study	present study	present study
Sebaea sp1	J. Kissling & L. Zeltner 34 (NEU)	South Africa	present study	present study	present study
Sebaea sp2	J. Kissling & L. Zeltner 31 (NEU)	Lesotho	present study	present study	present study
Sebaea sp3	J. Kissling & L. Zeltner 5 (NEU)	South Africa	present study	present study	present study
Sebaea sp4	J. Kissling & L. Zeltner 35 (NEU)	South Africa	present study	present study	present study
Sebaea spathulata (E. Mey.) Steud.	J. Kissling & L. Zeltner 40 (NEU)	South Africa	present study	present study	present study
Sebaea thodeana	J. Kissling & L. Zeltner 16 (NEU)	Lesotho	present study	present study	present study
Sebaea thomasii (S.Moore) Schinz	J. Kissling & L. Zeltner 29 (NEU)	Lesotho	present study	present study	present study
Swertia cordata (Wall. Ex D. Don) C. B. Clarke	Chassot 97-17 (NEU)	Nepal	AJ408015*	AJ315255*	n/a
Swertia perennis L.	Küpfer s.n. (NEU)	Switzerland	AY255695*	AY251774*	n/a
Symbolanthus australis Struwe			AF102489*	n/a	n/a
Symbolanthus frigidus (Sw.) Struwe & K.Gould			AF102498*	n/a	n/a
Symphyllophyton caprifolioides Gilg	Ratter 6742 (E)	Brasil	AF102490*	n/a	n/a
Tachia loretensis Maguire & Weaver			AF102492*	n/a	n/a
Tachiadenus carinatus (Desr.) Griesb.	S. Wohlhauser M059	Madagascar	AJ490249*	present study	present study
Tachiadenus carinatus (Desr.) Griesb.		Madagascar	present study	present study	present study
Tachiadenus gracilis Griesb.		Madagascar	present study	present study	present study
Tachiadenus longiflorus Bojer ex Grisebach	S. Wohlhauser M006	Madagascar	AJ490250*	present study	present study

Tachiadenus tubiflorus (Roemer & Schultes) Griesb.		Madagascar	present study	present study	present study
Tripterospermum cordatum (Marquand) Harry Sm.			AY563392*	AY563392*	n/a
Tripterospermum filicaule (Hemsl.) Harry Sm.			AY858683*	n/a	n/a
Urogentia ulugurensis Gilg & Gilg-Ben	D. J. Mabberley 1432 (K)	n/a	AF102495*	n/a	n/a
Voyriella parviflora (Miq.) Miq.	G. Cremers 14891	n/a	AJ242607*	n/a	n/a
Xestaea lisianthoides Griesb.	n/a	n/a	AF102499*	n/a	n/a

Origin of Exaceae material, voucher information and EMBL/GenBank accession number of sequence (Chapter 3).

Species	Voucher specimen and herbarium	Origin	GenBank accessions						Reference
			ITS 1	ITS 2	*trn* L intron	*trn* L-F sapcer	*atp* B-*rbc* L	partial matK	
Exacum affine I. B. Balf. ex Regel - M17126	M & al. 17126, E	Socotra	AJ489886	AJ489886	AJ490211	AY251770			Yuan & al. (2005)
Exacum affine I. B. Balf. ex Regel - M6201	M & al. 6201, E	Oman	AJ489879*	AJ489879*	AJ490204*				Yuan & al. (2005)
Exacum affine I. B. Balf. ex Regel - Wcff5	W cff5, NEU	Socotra	AJ489878*	AJ489878*	AJ490203*				Yuan & al. (2005)
Exacum atropurpureum Bedd.	K & L 526, S	India	AJ489880*	AJ489880*	AJ490205*				Yuan & al. (2005)
Exacum bulbilliferum Baker	W & C M070, NEU	Madagascar	AJ489881*	AJ489881*	AJ490206*				Yuan & al. (2005)
Exacum caeruleum I. B. Balf.	M & al. 11356, E	Socotra	AJ489882	AJ489882	AJ490207	AY251771			Yuan & al. (2005)
Exacum dolichanterum Klack.	W & C M064, NEU	Madagascar	AJ489883*	AJ489883*	AJ490208*				Yuan & al. (2005)
Exacum exiguum Klack.	Pi, W & Z M048, NEU	Madagascar	AJ489884*	AJ489884*	AJ490209*				Yuan & al. (2005)
Exacum fruticosum Humbert	W & P M055, NEU	Madagascar	AJ489885	AJ489885	AJ490210				Yuan & al. (2005)
Exacum hamiltonii G. Don	Wo 7477, E	Bhutan	AJ489887	AJ489887	AJ490212				Yuan & al. (2005)
Exacum humbertii Klack.	W & P M052, NEU	Madagascar	AJ489888*	AJ489888*	AJ490213*				Yuan & al. (2005)
Exacum intermedium Klack.	W & La M060, NEU	Madagascar	AJ489889*	AJ489889*	AJ490214*				Yuan & al. (2005)
Exacum linearifolium (Humbert) Klack.	M & R 6254, S	Madagascar	AJ489890*	AJ489890*	AJ490215*				Yuan & al. (2005)
Exacum macranthum Arn. ex Griseb. - FK767	F & K 767, S	Sri Lanka	AJ489892*	AJ489892*	AJ490217*				Yuan & al. (2005)
Exacum macranthum Arn. ex Griseb. - Zsl003	Z sl003, NEU	Sri Lanka	AJ489891*	AJ489891*	AJ490216*				Yuan & al. (2005)
Exacum marojejyense Humbert	W & P M056, NEU	Madagascar	AJ489893	AJ489893	AJ490218				Yuan & al. (2005)
Exacum microcarpum Klack.	W & La M061, NEU	Madagascar	AJ489894*	AJ489894*	AJ490219*				Yuan & al. (2005)
Exacum millotii Humbert	W & P M057, NEU	Madagascar	AJ489895*	AJ489895*	AJ490220*				Yuan & al. (2005)
Exacum nummularifolium Humbert	W & P M058, NEU	Madagascar	AJ489896	AJ489896	AJ490221				Yuan & al. (2005)
Exacum oldenlandioides (S. Moore) Klack.	Re 9275, S	Burundi	AJ489897	AJ489897	AJ490222				Yuan & al. (2005)
Exacum pallidum (Trim.) Klack.	F & K 777, S	Sri Lanka	AJ489898*	AJ489898*	AJ490223*				Yuan & al. (2005)
Exacum pedunculatum L.	B, Ke & T 4, S	Sri Lanka	AJ489899*	AJ489899*	AJ490224*				Yuan & al. (2005)
Exacum quinquenervium Griseb.	W M063, NEU	Madagascar	AJ489900	AJ489900	AJ490225				Yuan & al. (2005)
Exacum sessile L.	K & L 349, S	India	AJ489901*	AJ489901*	AJ490226*				Yuan & al. (2005)
Exacum stenophyllum Klack.	Pi, W & Z M049, NEU	Madagascar	AJ489902	AJ489902	AJ490227				Yuan & al. (2005)
Exacum subacaule Humbert	M 3755, S	Madagascar	AJ489903*	AJ489903*	AJ490228*				Yuan & al. (2005)
Exacum subteres Klack.	W & P M053, NEU	Madagascar	AJ489904*	AJ489904*	AJ490229*				Yuan & al. (2005)
Exacum subverticillatum Humbert	Madagascar, s. n., NEU	Madagascar	AJ489905*	AJ489905*	AJ490230*				Yuan & al. (2005)
Exacum sutaepense Hosseus ex Craib	Ch 99 - 230, NEU	Thailand	AJ489906*	AJ489906*	AJ490231*				Yuan & al. (2005)
Exacum tetragonum Roxb. - K254	Keke 254, E	Nepal	AJ489908*	AJ489908*	AJ490233*				Yuan & al. (2005)
Exacum tetragonum Roxb. - LK332	L & K 332, S	India	AJ489907	AJ489907	AJ490232				Yuan & al. (2005)
Exacum trinervium (L.) Druce - Zsl001	Z sl001, NEU	Sri Lanka	AJ489910*	AJ489910*	AJ490235*				Yuan & al. (2005)
Exacum trinervium (L.) Druce - Zsl002	Z sl002, NEU	Sri Lanka	AJ489909	AJ489909	AJ490234				Yuan & al. (2005)
Exacum walkeri Arn. ex Griseb. - K539	K 539, S	Sri Lanka	AJ489911*	AJ489911*	AJ490236*				Yuan & al. (2005)
Exacum walkeri Arn. ex Griseb. - Zsl004	Z sl004, NEU	Sri Lanka	AJ489912*	AJ489912*	AJ490237*				Yuan & al. (2005)
Exacum wightianum Arn.	K & L 188, S	India	AJ489913	AJ489913	AJ490238				Yuan & al. (2005)
Exacum affine I. B. Balf. ex Regel - M8238a	M & al. 8238a, E	Socotra	AJ489877	AJ489877	AJ490202				Yuan & al. (2005)
Exacum appendiculatum Klack.	SB058	Madagascar							Present study
Exacum exiguum Klack.	SB040	Madagascar							Present study
Exacum quinquenervium Griseb.	SB052	Madagascar							Present study
Exacum stenophyllum Klack.	SB051	Madagascar							Present study
Exacum stenophyllum Klack.	SB053	Madagascar							Present study
Exochaenium africanum 604 23	Dessein et al. 604 (NEU)	Zambia							Present study
Exochaenium africanum 656	Dessein et al. 656 (NEU)	Zambia							Present study
Exochaenium africanum 656b	Dessein et al. 656 (NEU)	Zambia							Present study
Exochaenium africanum 692 13	Dessein et al. 692 (NEU)	Zambia							Present study
Exochaenium africanum 692 84	Dessein et al. 692(NEU)	Zambia							Present study
Exochaenium baumianun 1014	Dessein et al. 1014 (NEU)	Zambia							Present study
Exochaenium baumianun 1014 36	Dessein et al. 1014 (NEU)	Zambia							Present study
Exochaenium baumianun 809	Dessein et al. 809 (NEU)	Zambia							Present study
Exochaenium baumianun 814	Dessein et al. 814 (NEU)	Zambia							Present study
Exochaenium baumianun 906	Dessein et al. 906 (NEU)	Zambia							Present study
Exochaenium baumianun 933	Dessein et al. 933 (NEU)	Zambia							Present study

Exochaenium baumianun 933b	Dessein et al. 933 (NEU)	Zambia				Present study
Exochaenium baumianun 969	Dessein et al. 969 (NEU)	Zambia				Present study
Exochaenium baumianun 971	Dessein et al. 971 (NEU)	Zambia				Present study
Exochaenium baumianun aff 814	Dessein et al. 814 (NEU)	Zambia				Present study
Exochaenium clavatum 543	Dessein et al. 543 (NEU)	Zambia				Present study
Exochaenium fernandesianum 1011	Dessein et al. 1011 (NEU)	Zambia				Present study
Exochaenium fXb 1015	Dessein et al. 1015 (NEU)	Zambia				Present study
Exochaenium gracilis 584	Dessein et al. 584 (NEU)	Zambia				Present study
Exochaenium grandis 657	Dessein et al. 657 (NEU)	Zambia				Present study
Exochaenium grandis 752	Dessein et al. 752 (NEU)	Zambia				Present study
Exochaenium linaeriformis C 75	Bidgood et al. 3630 (C)	Tanzania				Present study
Exochaenium macropterum 31	Bidgood et al. 4012 (BR)	Tanzania				Present study
Exochaenium oliganthum 499 41	Dessein et al. 499 (NEU)	Zambia				Present study
Exochaenium perparvum 728	Dessein et al. 728 (NEU)	Zambia				Present study
Exochaenium perparvum 827	Dessein et al. 827 (NEU)	Zambia				Present study
Exochaenium perparvum 851	Dessein et al. 851 (NEU)	Zambia				Present study
Exochaenium platypterum	Kissling J. 1 (NEU)	Zambia				Present study
Exochaenium sp 584	Dessein et al. 584 (NEU)	Zambia				Present study
Exochaenium sp 934	Dessein et al. 934 (NEU)	Zambia				Present study
Exochaenium teucszii 558	Dessein et al. 558 (NEU)	Zambia				Present study
Exochaenium teucszii 599	Dessein et al. 599 (NEU)	Zambia				Present study
Exochaenium teucszii 701	Dessein et al. 701 (NEU)	Zambia				Present study
Gentianothamnus madagascariensis Humbert	G G020, NEU	Madagascar	AJ489914	AJ489914	AJ490240	Yuan & al. (2005)
O. trinervis (Desrousseaux) Klack.	C s. n., NEU	Madagascar	AJ489918	AJ489918	AJ490243	Yuan & al. (2005)
Ornichia madagascariensis (Baker) Klack.	W M002, NEU	Madagascar	AJ489917	AJ489917	AJ490242	Yuan & al. (2005)
Saccifloium bandeirae Maguire & Pires	Piliackas & al., s.n.	n/a	AJ242611	Aj242612	Aj242608	Thiv & al. (1999)
Sebaea albidiflora F. Muell		Australia	AY629182	AY629182	n/a	Present study
Sebaea amicorum	Linder PRE	South Africa				Present study
Sebaea aurea (L.f.) Roem. & Schult. 68	Volk J.H.J. 1217 PRE	South Africa				Present study
Sebaea aurea ? (L.F.) Roem & Schult	Bytebier B 2616	South Africa				Present study
Sebaea aurea ? (L.F.) Roem & Schult	Bytebier B 2620	South Africa				Present study
Sebaea bojeri Griesb.	050302/1b I11-Jtr-4	South Africa				Present study
Sebaea brachyphylla	Raynald 19414 (P)		AJ489920	AJ489920	AJ490245	Yuan & al. (2003)
Sebaea brachyphylla Grisebach	J. Raynald 19414	Tanzania	AJ489920	AJ489920	Aj490245	Yuan & al. (2003)
Sebaea brachyphylla Grisebach	Phillipson PB 5021 C					Present study
Sebaea cf. *ambigua* Cham.	Kissling J. & Zeltner L. 46	South Africa				Present study
Sebaea cf. macrophylla Gilg	Bayliss 8765	n/a	AJ489920	AJ489920	AJ490245	Yuan & al. (2003)
Sebaea erosa Schinz	Germishuizen G. 843 (PRE)	South Africa				Present study
Sebaea exacoides (L.) Schinz	Bytebier B 2612	South Africa				Present study
Sebaea exacoides (L.) Schinz	Bytebier B 2610	South Africa				Present study
Sebaea exacoides L.	Snijman 1562	n/a	n/a	n/a	AF102481	Struwe & al. (1998)
Sebaea filiformis Schinz	050304/1a H14-Jtr-1	South Africa				Present study
Sebaea hymenosepala Gilg	Vam Wyke B.E. & C.M. PRE					Present study
Sebaea junodii 577	Dessein et al. (NEU)	Zambia				Present study
Sebaea junodii jeff 1	Dessein et al. (NEU)	Zambia				Present study
Sebaea leiostyla Gilg	Germishuizen G. 2961 (PRE)					Present study
Sebaea leiostyla Gilg	050301/2b I11-Aob-4	South Africa				Present study
Sebaea leiostyla Gilg	050302/2a I11-Jtr-3	South Africa				Present study
Sebaea leiostyla Gilg	050303/2a H14-Gac-2	South Africa				Present study
Sebaea longicaulis BR 58	Reekmans M. 8272 (BR)					Present study
Sebaea longicaulis Schinz	Poilecot P. 8000	Zimbabwe	n/a	n/a	AJ490245	Yuan & al. (2003)
Sebaea madagascariensis Klack.	W & Z MO18 (NEU)	Madagascar	AJ489921	AJ489921	AJ490247	Yuan & al. (2003)
Sebaea marlothiii Gilg	Weger M.J.A. 1606 (PRE)	South Africa				Present study
Sebaea microphylla 557	Dessein et al. (NEU)	Zambia				Present study
Sebaea natalensis 71	Mothogoane M.S. 250 (PRE)					Present study
Sebaea nov.sp.	Kissling J. & Zeltner L. 66	South Africa				Present study
Sebaea ovata (Labill.) R.Br.		New Zealand	AY629184	AY629184	n/a	Present study

Sebaea ovata (Labill.) R.Br.		Australia	AY629183	AY629183	n/a	Present study
Sebaea pentendra aff.	Bayliss R.D.A.029 MO	South Africa				Present study
Sebaea pleurostigmatosa Hilliard & B.L.Burtt	Hilliard & Burtt 17874 (PRE)	South Africa				Present study
Sebaea procumbens Hill	Bellstedt DUB 902	South Africa				Present study
Sebaea pusilla Eckl. Ex Cham.	Kissling J. & Zeltner L. 64	South Africa				Present study
Sebaea rehmannii Schinz	050302/1a K09-Jtr-4	South Africa				Present study
Sebaea schlechteri Schinz	Kissling J. & Zeltner L. 50	South Africa				Present study
Sebaea sedoides Gilg	050303/1b H14-Lpr-2	South Africa				Present study
Sebaea sedoides Gilg	050303/2b I11-Cun-2	South Africa				Present study
Sebaea sp.	050301/1 K09-Tal-3	South Africa				Present study
Sebaea spathulata (E. Mey.) Steud	Bellstedt DUB 899	South Africa				Present study
Sebaea thodeana Gilg	Kissling J. & Zeltner L. 20	South Africa				Present study
Sebaea thomasii (S.moore) Schinz	Bester S.P. 2912 PRE	South Africa				Present study
Sebaea thomasii (S.moore) Schinz	Victor J.E. (PRE)	South Africa				Present study
Sebaea thomasii (S.moore) Schinz	Victor J.E. 1580 PRE	South Africa				Present study
Sebaea thomasii (S.moore) Schinz	Bellstedt DUB 903	South Africa				Present study
Tachiadenus carinatus (Desrousseaux) Griseb.	W M059, NEU	Madagascar	AJ489923	AJ489923	AJ490249	Yuan & al. (2005)
Tachiadenus carinatus aff. (Desrousseaux) Grisel	Callmander	Madagascar				Present study
Tachiadenus gracilis	Callmander	Madagascar				Present study
Tachiadenus longiflorus Bojer ex Griseb.	W M006, NEU	Madagascar	AJ489924	AJ489924	AJ490250	Yuan & al. (2005)
Tachiadenus tubiflorus	Callmander	Madagascar				Present study