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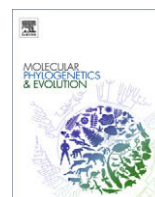
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The polyphyletic genus *Sebaea* (Gentianaceae): A step forward in understanding the morphological and karyological evolution of the Exaceae

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ABSTRACT

Within the Gentianaceae–Exaceae, the most species-rich genus *Sebaea* has received very little attention in terms of phylogenetic or karyological investigations. As a result, the exact number of species remains vague and the relationships with the other members of the Exaceae poorly understood. In this paper, we provide the first comprehensive phylogeny of the Exaceae including most *Sebaea* species known so far based on four cpDNA sequence regions. In addition, morphological and karyological characters were mapped on the inferred phylogenetic trees to detect possible non-molecular synapomorphies. Our results reveal the paraphyly of *Sebaea* and highlight new generic relationships within the Exaceae. *Sebaea pusilla* (lineage S1 – *Lagenias*) forms a highly supported and early diverging clade with *Sebaea s.str.* (clade S2 – *Sebaea*). A third clade of the former *Sebaea* s.l. (clade S3 – *Exochaenium*) contains exclusively tropical African species, and is sister with a large clade containing all the remaining genera of Exaceae. Within the latter, the proposed sister relationships between the recently described *Klackenbergia* and *Ornichia* are highly supported. Optimization of several morphological characters onto the inferred phylogenetic trees reveals several synapomorphies for most highly supported clades. In particular, lineage S1 (*Lagenias*) is supported by medifixed anthers that are inserted at the base of the corolla tube and cubical seeds with polygonal testa cells; clade S2 (*Sebaea*) is supported by both the presence of secondary stigmas along the style and ridged seeds with rectangular testa cells arranged in row; clade S3 (*Exochaenium*) is supported by its particular gynoeceum (stylar polymorphism and clavate, papillose stigma). Finally, karyological reconstructions suggest a basal number of $x = 7$ for the Exaceae and several episodes of dysploidy leading to $x = 8$ and 9.

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1. Introduction

The Exaceae (Gentianaceae) in their current circumscription (Struwe et al., 2002; Klackenberg, 2006) are a small tribe that includes ca. 181 species distributed in the respective genera *Exacum* L. (69 spp. including *Cotylanthera* Blume), *Gentianothamnus* Humbert (1 sp.), *Klackenbergia* Kissling (2 spp.), *Ornichia* Klack. (3 spp.), *Sebaea* Sol. (c. 95 spp.), and *Tachiadenus* Griseb. (11 spp.) (Schinz, 1906; Hill and Prain, 1909; Klackenberg, 1985, 1986, 1987a, 1990, 2002, 2006; Kissling et al., 2009). The Exaceae mainly differ from the five other gentian tribes by the star-shaped testa cells of the seeds and the rounded-convex shape of the epidermis cells of petals (Klackenberg, 1985, 2002; Bouman et al., 2002). A wide range of morphological variation has been found within the tribe (Klackenberg, 2002), including general habit (herb versus subshrub), gynoeceum structure (ovary truly or pseudo-bilocular, style straight or bent downwards), androecium (insertion of filaments, opening mechanism of the stamens), corolla color (white, yellow,

orange, blue, and purple), corolla-merosity (4 or 5 lobes), corolla size (a few mm up to 7 cm in diameter), or length of the corolla tube (a few mm up to 20 cm). To date, important morphological investigations have been carried out on *Exacum*, *Ornichia*, and *Tachiadenus* (Klackenberg, 1983, 1985, 1986, 1987a), while only a few molecular studies have been performed on the Exaceae, most of them focusing on *Exacum* (Yuan et al., 2003, 2005). Surprisingly, systematic studies on *Sebaea*, the most species-rich genus of the tribe, remained anecdotal (Klackenberg, 2002; Yuan et al., 2003).

Sebaea comprises annual to perennial, erect to procumbent herbs, or more rarely achlorophyllous saprophytes (*S. oligantha*). The genus size is still a matter of speculation. Indeed, early estimates ranged from 60 to 159 species (Wielgorskaya, 1995; Schinz, 1906; Boutique, 1972; Paiva and Nogueira, 1990a; Mabblerley, 1997; Dyer, 1975; Adams, 1996), while recent field observations and examination of ca. 3000 herbaria specimens (Jonathan Kissling, pers. obs.), better support ca. 95 species, making *Sebaea* the most species-rich genus within the Exaceae.

Sebaea is widely distributed in the Old World, with two centers of diversity in Tropical Africa and Southern Africa (Schinz, 1906; Hill, 1908; Marais and Verdoorn, 1963; Boutique, 1972; Paiva

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and Nogueira, 1990a, 1990b; Nemomissa, 2002). Tropical species (ca. 25) are mainly distributed on the Katanga plateau at the intersection between the Democratic Republic of Congo, Angola, and Zambia, while the Southern African species (ca. 70 spp.) mostly occur in the Cape Region and the Drakensberg escarpment (Lesotho and South Africa). Four species extend their range eastward to Madagascar, two of them being endemic to the island (Klackenberg, 1987b, 1990). Finally, two species are endemic to the Australia/New Zealand region (Adams, 1996), whereas one species extends its range from Africa to the Indo-Malaysian area (Ho and Pringle, 1995; Suksathan and Sasirat, 2000).

Sebaea species grow from sea level (e.g. *S. minutiflora*, *S. ambigua*) to high elevations (3000–3500 m) in the Drakensberg (*S. marlothii* and *S. thodeana*) or in the East African mountains (*S. brachyphylla* and *S. leiostyla*). They can also be encountered in most parts of Sub-Saharan Africa in a wide variety of habitat including dense tropical forests, grasslands, and savannas, marshes or water-logged areas, alpine meadows, or rocks and sandy riverbanks.

Despite series of systematic works performed on the Exaceae (Brown, 1810; Rafinesque, 1837; Meyer, 1838; Grisebach, 1845; Bentham, 1876; Schinz, 1891, 1903, 1906; Gilg, 1895a, 1898; Hill, 1908), the generic circumscription of *Sebaea* remains controversial. Most of the confusion relies on the frequent use of homoplastic characters (Marais, 1961), including e.g. the form (straight or recurved) and disposition (free or connate) of the anthers, the presence of appendages (glands) on the anthers, and the height of the filament insertion. The circumscription of *Sebaea* is difficult because a large amount of morphological plasticity seems to occur depending on the varying environmental conditions, overall obscuring species boundaries (Hedberg, 1955) and resulting in a high systematic and taxonomic uncertainty (e.g. Chamisso and Schlechtendal, 1826; Rafinesque, 1837; Meyer, 1838; Grisebach, 1849; Bentham, 1876; Schinz, 1891; Knochblauch, 1894; Gilg, 1898; Schinz, 1903, 1906; Hill, 1908; 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. Most molecular studies published so far support the inclusion of the genus in the Exaceae (Struwe et al., 2002; Yuan et al., 2003; Kissling et al., unpublished), and a possible monophyletic status if the newly described *Klackenbergia* is excluded from *Sebaea* s.l. (Kissling et al., 2009; Yuan et al., 2003), but the overall sampling prevents 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 and morphological 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.

2. Materials and methods

2.1. Taxon sampling and outgroup choice

Plant samples were collected in 2004–06 in South Africa and Zambia (*Sebaea*) and Madagascar (*Exacum* and *Tachadenus*), and were determined by the first author using local floras (Marais and Verdoorn, 1963; Taylor, 1963; Boutique, 1972; Klackenberg, 1990; Paiva and Nogueira, 1990a; Nemomissa, 2002). Voucher specimens are deposited in the herbarium of the University of Neuchâtel, Switzerland (NEU). But duplicates can also be found in the following herbaria BOL, BR, PRE, TAN and Mt Makulu Research

Center, Zambia. Material for DNA extraction was obtained from both collected samples and herbarium specimens (BR, C, MO, and PRE).

All genera of Exaceae sensu Struwe et al. (2002) are represented (except *Cotylanthera*, recently transferred to *Exacum*; Klackenberg, 2006). 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, merosity, etc.) variation of the genus.

A total of 165 operational taxonomic units (OTUs) encompassing the six tribes of Gentianaceae were included in this study. Nine additional OTUs, representing the remaining plant families within the Gentianales (Struwe et al., 1994; Backlund et al., 2000; Bremer et al., 2002; APG, 2003), were used as outgroups. They include the Loganiaceae (*Labordia tinifolia* A. Gray and *Mitreola petiolata* (Walt.) Torr. et Gray), the Apocynaceae (*Nerium oleander* L. and *Plumeria obtusa* L.), the Rubiaceae (*Emmenopterys henryi* Oliv., *Gardenia taitensis* DC., *Guettarda boliviana* Standl. and *Mitragyna inermis* (Willd.) K. Schum.) and the Gelsemiaceae (*Gelsemium sempervirens* Ait.).

Four chloroplast regions were used in this study: (1) the *trnL* intron (75 new accessions and 99 retrieved from GenBank); (2) the *trnL-F* intergenic spacer (65 new accessions and 44 retrieved from GenBank); (3) the *atpB-rbcL* intergenic spacer (66 new accessions and 8 retrieved from GenBank); and (4) a part of the *matK* gene (49 new accessions and 2 retrieved from GenBank). Missing data were marked as “?” in the combined dataset. 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 Table 1. New accessions have been submitted to GenBank and sequences directly retrieved from GenBank are indicated with asterisks.

2.2. Molecular methods (DNA extraction, amplification, and sequencing)

Total DNA was extracted from silica gel dried leaves or from herbarium specimens, using the DNeasy Plant Mini Kit (Qiagen, Switzerland). Polymerase Chain Reactions (PCR) were performed on a Biometra thermocycler with 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. The PCR mix (25 µl) contained 2.5 µl 10× PCR buffer (with 1.5 mM MgCl₂), 0.5 µl 10 mM dNTPs, 0.5 µl of 10 mM each forward and reverse primers, 0.2 µl (1 U) HotStar Taq DNA polymerase (Qiagen AG, Basel), 19.8 µl H₂O, and 1 µl (ca. 10–20 ng) genomic DNA. The *trnL* intron and the *trnL-F* region 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), while primer *matK8* and *matK503* (Endress et al., 1996) were used to amplify a part of the *matK* gene. The PCR products were checked on 0.8% agarose gels and purified using the QIAquick PCR purification kit (Qiagen AG, Basel). Cycle sequencing reactions (5 µl) 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 the *atpB-rbcL*, *trnL-F* (UAA) intron and spacer and a part of the *matK* gene. The cycle sequencing products were cleaned using the ethanol/sodium acetate precipitation method and resuspended in 13 µl TSR or Hi-Di Formamide (supplied by Applied Biosystems, Foster City, USA) before electrophoresis on an ABI310 automated sequencer (Applied Biosystem, Foster City, USA). Sequences electropherograms were

Table 1
Origin of plant material, voucher information and EMBL/GenBank accession number of sequence. Sequences retrieved from GenBank are indicated with asterisks.

Species	Voucher specimen and herbarium	Origin	Systematic position	<i>trnL</i> intron	<i>trnL</i> -F spacer	<i>atbp-rbcL</i> spacer	<i>matK</i> partial gene
<i>Anthocleista amplexicaulis</i> Baker	S. Wohlhauser s.n. (PBZT)	Madagascar	Potalieae–Potaliinae	AJ490189*	–	–	–
<i>Anthocleista grandiflora</i> Gilg	M. Callmänder s.n. (NEU)	Madagascar	Potalieae–Potaliinae	AJ490190*	–	–	–
<i>Anthocleista scandens</i> Hook.f.	n/a	n/a	Potalieae–Potaliinae	AF102376*	–	–	–
<i>Aripuana cullmaniorum</i> Struwe, Maas and V.A. Albert	n/a	n/a	Helieae	AJ242603*	–	–	–
<i>Blackstonia imperfoliata</i> (L.f.) Samp.	G. Mansion 010833 (NEU)	Spain	Chironieae–Chironiinae	AY251743*	AY251769*	–	–
<i>Blackstonia perfoliata</i> (L.) Huds.	G. Mansion 98712 (NEU)	France	Chironieae–Chironiinae	AF402198*	AF402254*	–	–
<i>Calolisianthus pendulus</i> (Mart.) Gilg	n/a	n/a	Helieae	AF102387*	–	–	–
<i>Calolisianthus pulcherrimus</i> (Mart.) Gilg	R. M. Harley et al. 15674 (NY)	n/a	Helieae	AF102388*	–	–	–
<i>Canscora alata</i> (Roth) Wallich	J.C. Piso, S. Wohlhauser and L. Zeltner MO24 (NEU)	Madagascar	Chironieae–Canscorinae	AJ490191*	–	–	–
<i>Canscora andrographioides</i> Griff.	P. Chassot 99-234 (NEU)	Thailand	Chironieae–Canscorinae	AJ490192*	–	–	–
<i>Canscora diffusa</i> (Vahl.) Roem. and Schult.	P. Chassot 99-231 (NEU)	Thailand	Chironieae–Canscorinae	AJ490193*	AY251780*	–	–
<i>Centaurium pulchellum</i> (Sw.) Druce	G. Mansion 98505 (NEU)	France	Chironieae–Chironiinae	AY251734*	AY251758*	–	–
<i>Centaurium tenuiflorum</i> (Hoffmgg. and Link) Fritsch	L. and N. Zeltner 1767 (NEU)	Morocco	Chironieae–Chironiinae	AY251735*	AY251759*	–	–
<i>Chelonanthus alatus</i> (Aubl.) Pulle	F. Bretagnolle and J. Piguët C3 (NEU)	Ecuador	Helieae	AJ490194*	AY251775*	–	–
<i>Chelonanthus angustifolius</i> Gilg	F. Bretagnolle and J. Piguët T11 (NEU)	Ecuador	Helieae	AJ490195*	AY251776*	–	–
<i>Chironia baccifera</i> L.	J. Kissling and L. Zeltner 57 (NEU)	South Africa	Chironieae–Chironiinae	FJ014136	FJ013953	FJ014019	–
<i>Chironia lineoides</i> L.	J. Kissling and L. Zeltner 58 (NEU)	South Africa	Chironieae–Chironiinae	FJ014137	FJ013954	–	–
<i>Chironia palustris</i> subsp. <i>transvaalensis</i> (Gilg) Verdoorn	J. Kissling and L. Zeltner 2 (NEU)	South Africa	Chironieae–Chironiinae	FJ014138	FJ013955	FJ014020	–
<i>Chironia purpurascens</i> subsp. <i>humilis</i> (Gilg) Verdoorn	J. Kissling and L. Zeltner 1 (NEU)	South Africa	Chironieae–Chironiinae	FJ014139	FJ013956	FJ014021	–
<i>Cicendia filiformis</i> (L.) Delarbre	M. Thiv 2156 (MJG)	France	Chironieae–Chironiinae	AF102403*	–	–	–
<i>Cicendia quadrangularis</i> (Lam.) Griseb.	P. Maas 8154 (U)	n/a	Chironieae–Chironiinae	AF102404*	AY251765*	–	–
<i>Comastoma pulmonarium</i> (Turcz.) Toyok.	Y.M. Yuan and P. Küpfer 92-279 (NEU)	China, Sichuan	Gentianeae–Swertiinae	AJ315225*	AJ315271*	–	–
<i>Comastoma tenellum</i> (Rottb.) Toyok.	n/a	n/a	Gentianeae–Swertiinae	AJ580518*	–	–	–
<i>Coutoubea ramosa</i> Aubl.	B. Hoffman and C. Capellaro 984 (NY)	n/a	Chironieae–Coutoubeinae	AF102408*	–	–	–
<i>Coutoubea spicata</i> Aubl.	S. Mori 24349	n/a	Chironieae–Coutoubeinae	AY251745*	AY251778*	–	–
<i>Crawfordia delavayi</i> Franch	n/a	n/a	Gentianeae–Gentianinae	AY563391*	–	–	–
<i>Curtia tenuifolia</i> Knobl.	M.J. Jansen–Jacob 2740 (NY)	n/a	Sacchifoliae	AJ242606*	–	–	–
<i>Deianira pallidescens</i> Cham. and Schlecht.	W.A. Anderson 9385 (NY)	n/a	Chironieae–Coutoubeinae	AF102410*	AY251782*	–	–
<i>Djaloniella ypsistylota</i> P. Taylor	Morton SL2442 (K)	n/a	Potalieae–Faroinae	AF102413*	–	–	–
<i>Emmenopterys henryi</i> Oliver	n/a	n/a	Outgroup (Rubiaceae–Ixoroideae)	AF152637*	AF152637*	DQ131728*	–
<i>Enicostema verticillatum</i> (L.) Engl. ex Gilg	J. Pruski and J. Steyermark 1473 (NY)	n/a	Potalieae–Faroinae	AF102414*	–	–	–
<i>Eustoma exaltatum</i> (L.) Salisb.	L. and N. Zeltner 980610-1 (NEU)	Mexico	Chironieae–Chironiinae	AY251752*	AY251789*	–	–
<i>Eustoma grandiflorum</i> (Raf.) Shinners	G. Mansion s.n. (NEU)	Switzerland (cultivated)	Chironieae–Chironiinae	AY251751*	AY251788*	–	–
<i>Exaculum pusillum</i> Caruel	P. Küpfer s.n. (NEU)	Italy	Chironieae–Chironiinae	AY251740*	AY251764*	–	–
<i>Exacum affine</i> I.B. Balf. Ex Regel	Miller and al. 6201 (E)	Oman	Exaceae	AJ490211*	AY251770*	–	FJ014087
<i>Exacum appendiculatum</i> Klack.	S. Buerki SB058 (NEU)	Madagascar	Exaceae	FJ014140	FJ013957	FJ014022	–
<i>Exacum atropurpureum</i> Bedd.	Klackenberg and Lundin 526 (S)	India	Exaceae	AJ490205*	–	–	FJ014088
<i>Exacum caeruleum</i> I.B. Balf.	Miller and al. 11356 (E)	Socotra	Exaceae	AJ490207*	AY251771*	–	FJ014089
<i>Exacum dolichantherum</i> Klack.	LG4291 (NEU)	Madagascar	Exaceae	FJ014141	FJ013959	FJ014025	–
<i>Exacum exigum</i> Klack.	S. Buerki SB040 (NEU)	Madagascar	Exaceae	FJ014142	FJ013960	FJ014026	FJ014090
<i>Exacum hamiltonii</i> G. Don	Wood 7477 (E)	Bhutan	Exaceae	AJ490212*	–	–	–
<i>Exacum humbertii</i> Klack.	Wohlhauser and Pfund M052 (NEU)	Madagascar	Exaceae	AJ490213*	–	FJ014027	–
<i>Exacum linearifolium</i> (Humbert) Klack.	Miller and Randrianasolo 6254 (S)	Madagascar	Exaceae	AJ490215*	–	FJ014028	FJ014091
<i>Exacum macranthum</i> Arn. ex Griseb.	Fagerlind and Klackenberg 767, S	Sri Lanka	Exaceae	AJ490217*	–	–	–
<i>Exacum marojejtense</i> Humbert	Wohlhauser and Pfund M056 (NEU)	Madagascar	Exaceae	AJ490218*	–	FJ014029	–
<i>Exacum nummularifolium</i> Humbert	Wohlhauser and Pfund M058 (NEU)	Madagascar	Exaceae	AJ490221*	–	FJ014030	–
<i>Exacum oldenlandioides</i> (S. Moore) Klack.	Reekmans 9275 (S)	Burundi	Exaceae	AJ490222*	–	FJ014031	–
<i>Exacum pallidum</i> (Trim.) Klack.	Fagerlind and Klackenberg 777 (S)	Sri Lanka	Exaceae	AJ490223*	–	FJ014032	–

Table 1 (continued)

Species	Voucher specimen and herbarium	Origin	Systematic position	trnL intron	trnL-F spacer	atbp-rbcL spacer	matK partial gene
<i>Exacum pedunculatum</i> L.	Bremer, Kerr and Theran 4 (S)	Sri Lanka	Exaceae	AJ490224*	FJ013961	–	–
<i>Exacum quinquevenvium</i> Griseb.	S. Buerki SB052 (NEU)	Madagascar	Exaceae	FJ014143	FJ013962	FJ014033	–
<i>Exacum sessile</i> L.	Klackenberg and Lundin 349 (S)	India	Exaceae	AJ490226*	–	FJ014034	FJ014092
<i>Exacum</i> sp.	CR 2831 (NEU)	Madagascar	Exaceae	FJ014144	FJ013963	FJ014035	–
<i>Exacum stenophyllum</i> Klack.	S. Buerki SB051 (NEU)	Madagascar	Exaceae	FJ014145	FJ013964	FJ014036	FJ014093
<i>Exacum tetragonum</i> Roxb.	Keke 254 (E)	Nepal	Exaceae	AJ490233*	–	–	–
<i>Exacum trinervium</i> (L.) Druce	Zeltner sl001 (NEU)	Sri Lanka	Exaceae	AJ490235*	–	FJ014037	FJ014094
<i>Exacum wightianum</i> Arn.	Klackenberg and Lundin 188 (S)	India	Exaceae	AJ490238*	FJ013965	FJ014038	FJ014095
<i>Exochaenium africanum</i> (Paiva and Nogueira) Kissling	S. Dessein et al. 623 (NEU)	Zambia	Exaceae	FJ014146	FJ013966	FJ014039	FJ014096
<i>Exochaenium africanum</i> (Paiva and Nogueira) Kissling	S. Dessein et al. 604 (NEU)	Zambia	Exaceae	FJ014147	FJ013967	FJ014040	FJ01409
<i>Exochaenium baumianum</i> (Gilg) Schinz	S. Dessein et al. 809 (NEU)	Zambia	Exaceae	FJ014148	FJ013968	FJ014041	FJ014098
<i>Exochaenium clavatum</i> (Paiva and Nogueira) Kissling	S. Dessein et al. 543 (NEU)	Zambia	Exaceae	FJ014149	FJ013969	FJ014042	FJ014099
<i>Exochaenium fernandesianum</i> (Paiva and Nogueira) Kissling	S. Dessein et al. 1011 (NEU)	Zambia	Exaceae	FJ014150	FJ013970	FJ014043	FJ014100
<i>Exochaenium grande</i> (E. Mey.) Griseb.	S. Dessein et al. 657 (NEU)	Zambia	Exaceae	FJ014151	FJ013971	FJ014044	FJ014101
<i>Exochaenium grande</i> (E. Mey.) Griseb.	S. Dessein et al. 752 (NEU)	Zambia	Exaceae	FJ014152	FJ013972	FJ014045	FJ014102
<i>Exochaenium lineariforme</i> (Sileshi) Kissling	S. Bidgood et al. 3630 (C)	Tanzania	Exaceae	FJ014153	FJ013973	FJ014046	FJ014103
<i>Exochaenium macropterum</i> (Sileshi) Kissling	S. Bidgood et al. 4012 (BR)	Tanzania	Exaceae	FJ014154	FJ013974	FJ014047	FJ014104
<i>Exochaenium oliganthum</i> (Gilg) Schinz	S. Dessein et al. 499 (NEU)	Zambia	Exaceae	FJ014155	FJ013975	FJ014048	–
<i>Exochaenium perparvum</i> (Sileshi) Kissling	S. Dessein et al. 827 (NEU)	Zambia	Exaceae	FJ014156	FJ013976	FJ014049	–
<i>Exochaenium platypterum</i> (Baker) Schinz	J. Kissling 75 (NEU)	Zambia	Exaceae	FJ014157	FJ013977	FJ014050	–
<i>Exochaenium</i> sp A	S. Dessein et al. 934 (NEU)	Zambia	Exaceae	FJ014158	FJ013978	FJ014051	FJ014105
<i>Exochaenium</i> sp B	S. Dessein et al. 692 (NEU)	Zambia	Exaceae	FJ014159	FJ013979	FJ014052	FJ014106
<i>Exochaenium</i> sp C	S. Dessein et al. 656 (NEU)	Zambia	Exaceae	FJ014160	FJ013980	FJ014053	FJ014107
<i>Exochaenium</i> sp D	S. Dessein et al. 584 (NEU)	Zambia	Exaceae	FJ014161	FJ013981	FJ014054	FJ014108
<i>Exochaenium teucszii</i> (Schinz) Schinz	S. Dessein et al. 701 (NEU)	Zambia	Exaceae	FJ014162	FJ013982	FJ014055	FJ014109
<i>Fagraea berteriana</i> A.Gray ex Benth	n/a	n/a	Potalieae	AF102419*	–	–	–
<i>Fagraea elliptica</i> Roxb.	n/a	n/a	Potalieae	AF102420*	–	–	–
<i>Fagraea fragrans</i> Roxb.	C.-H. Tsou 207 (NY)	n/a	Potalieae	AF102421*	–	–	–
<i>Faroa axillaris</i> Baker	M. Schaijes 5076 (BR)	n/a	Potalieae	AF102423*	–	–	–
<i>Faroa schaijesiorum</i> Bamps	M. Schaijes 3515 (BR)	n/a	Potalieae	AF102424*	–	–	–
<i>Frasera albomarginata</i> S. Watson	Schweich NEU 00–23 (NEU)	USA, California	Gentianeae–Swertiinae	AJ315187*	–	–	–
<i>Gardenia taitensis</i> DC.	n/a	n/a	Outgroup (Rubiaceae–Ixoroideae)	AF102426*	AF102426*	AJ233988*	–
<i>Gelsemium sempervirens</i> (L.) Aiton	n/a	n/a	Outgroup (Gelsemiaceae)	AJ346932*	AF159696*	AJ233985*	Z70195*
<i>Geniostemon gypsophilum</i> B.L. Turner	G. Nesom et al. 7621 (LL)	Mexico	Chironieae–Chironiinae	AF102429*	AY251766*	–	–
<i>Gentiana acaulis</i> L.	n/a	n/a	Gentianeae–Gentianinae	X77869*	–	–	–
<i>Gentiana brachyphylla</i> Villard	n/a	n/a	Gentianeae–Gentianinae	X77875*	–	–	–
<i>Gentiana cruciata</i> L.	P. Küpfer NEU 2005-G2 (NEU)	Italy	Gentianeae–Gentianinae	DQ398713*	DQ398713*	DQ398600*	–
<i>Gentiana lutea</i> L.	Y. M. Yuan 91-55 (NEU)	Switzerland	Gentianeae–Gentianinae	X75702*	–	–	–
<i>Gentiana purpurea</i> L.	n/a	n/a	Gentianeae–Gentianinae	AJ430909*	AJ430909*	–	–
<i>Gentiana pyrenaica</i> L.	Y. M. Yuan 93-14 (NEU)	Bulgaria	Gentianeae–Gentianinae	X77895*	–	–	–
<i>Gentiana tianschanica</i> Ruprecht	Y. M. Yuan 2004-95 (IBSC)	China, Xinjiang	Gentianeae–Gentianinae	DQ398722*	DQ398722*	DQ398616*	–
<i>Gentianella campestris</i> (L.) Harry Sm.	n/a	n/a	Gentianeae–Swertiinae	AJ580526*	–	–	–
<i>Gentianella engadinensis</i> (Wettst.) Holub	n/a	n/a	Gentianeae–Swertiinae	AJ580526*	–	–	–
<i>Gentianella pilosa</i> (Wettst.) Holub	n/a	n/a	Gentianeae–Swertiinae	AJ580525*	–	–	–
<i>Gentianopsis grandis</i> (Harry Sm.) Ma	Y.M. Yuan and P. Küpfer 92-222 (NEU)	China, Yunnan	Gentianeae–Swertiinae	AJ315227*	–	–	–
<i>Gentianothamnus madagascariensis</i> Humbert	L. Gautier G020 (G)	Madagascar	Exaceae	AJ490240*	–	FJ014056	–
<i>Guettarda boliviana</i> Standl.	n/a	n/a	Outgroup (Rubiaceae–Cinchonoideae)	AF152727*	AF152727*	DQ131738*	–
<i>Gyrandra tenuifolia</i> (Martens and Galeotti) Mansion	G. Mansion, L. and N. Zeltner 990228 (NEU)	Mexico	Chironieae–Chironiinae	AF402186*	AF402242*	–	–

(continued on next page)

<i>Halenia corniculata</i> (L.) Cornaz	Andenberg and Lundin 8	n/a	Gentianeae–Swertiinae	AJ408009*	–	–	–
<i>Halenia elliptica</i> D. Don	Y.M. Yuan and P. Küpfer 93–52 (NEU)	China, Sichuan	Gentianeae–Swertiinae	AJ315193*	AJ315239*	–	–
<i>Halenia weddelliana</i> Gilg.	P. Chassot 00–5 (NEU)	Ecuador	Gentianeae–Swertiinae	AJ315194*	AJ315240*	–	–
<i>Hoppea dichotoma</i> Wild.	C.D.K. Cook RHT307 (MJG)	n/a	Chironieae–Canscorinae	AF102440*	–	–	–
<i>Irlbachia pratensis</i> (H.B.K.) L. Cobb and Maas	n/a	n/a	Helieae	AF102442*	–	–	–
<i>Ixanthus viscosus</i> (Aiton) Griseb.	P. Küpfer s.n. (NEU)	Spain, Tenerife	Chironieae–Chironiinaea	AY251741*	AY251767*	–	–
<i>Klackenbergia stricta</i> (Schinz) Kissling	J.C. Piso, S. Wohlhauser and L. Zeltner M018 (NEU)	Madagascar	Exaceae	FJ014176	FJ013999	FJ014069	–
<i>Labordia tinifolia</i> A. Gray	n/a	n/a	Outgroup (Loganiaceae)	AF102447*	–	–	–
<i>Lagenias pusillus</i> (Eckl. Ex Cham.) E. Mey	J. Kissling and L. Zeltner 64 (NEU)	South Africa	Exaceae	FJ014181	FJ014004	FJ014074	FJ014124
<i>Lisianthus laxiflorus</i> Urban	n/a	n/a	Potalieae–Lisianthiinae	AF102449*	–	–	–
<i>Lisianthus longifolius</i> L.	H. van der Werff 8690 (NY)	n/a	Potalieae–Lisianthiinae	AF102450*	–	–	–
<i>Lomatogonium bellum</i> (Hemsl.) H. Smith	Y.M. Yuan and P. Küpfer 92–236 (NEU)	China, Yunnan	Gentianeae–Swertiinae	AJ315197*	AJ315243*	–	–
<i>Megacodon stylophorus</i> (C.B. Clarke) H. Smith	P. Chassot and Y. M. Yuan 99–36 (NEU)	China, Yunnan	Gentianeae–Swertiinae	AJ315200*	AY251773*	–	–
<i>Metagentiana gentilis</i> (Franch) T.N. Ho and S.W. Liu	n/a	n/a	Gentianeae–Gentianinae	AY563386*	–	–	–
<i>Metagentiana primuliflora</i> (Franch) T.N. Ho and S.W. Liu	n/a	n/a	Gentianeae–Gentianinae	AY563385*	–	–	–
<i>Microphium pubescens</i> C.B. Clarke	P. Chassot 99–243 (NEU)	Thailand	Chironieae–Canscorinae	AJ490241*	AY251781*	–	–
<i>Mitragyna inermis</i> (Willd.) Kuntze	n/a	n/a	Outgroup (Rubiaceae–Cinchonoideae)	AJ346932*	AJ346932*	DQ131751*	–
<i>Mitreola petiolata</i> (Walt.) Torr. and Gray	n/a	n/a	Outgroup (Loganiaceae)	AF102460*	–	DQ131696*	DQ131696*
<i>Nerium oleander</i> L.	n/a	n/a	Outgroup (Apocynaceae–Apocynoideae)	AF214386*	–	–	Z98173*
<i>Obolaria virginica</i> L.	Nicolson 24–IV–00	USA, Virginia	Gentianeae–Swertiinae	AJ315201*	AJ315247*	–	–
<i>Ornichia madagascariensis</i> Klack	S. Wohlhauser M002 (NEU)	Madagascar	Exaceae	AJ490242*	FJ013983	FJ014057	–
<i>Ornichia trinervis</i> (Desrousseaux) Klackenberg	M. Callmänder s.n. (NEU)	Madagascar	Exaceae	AJ490243*	FJ013984	–	–
<i>Orphium frutescens</i> (L.) E. Mey	J. Kissling and L. Zeltner 44 (NEU)	South Africa	Chironieae–Chironiinaea	FJ014163	FJ013985	FJ014058	–
<i>Plumaria plumosa</i> (Hudson) Kuntze	n/a	n/a	Outgroup (Apocynaceae–Rauvolfioideae)	AF214409*	AF214255*	–	–
<i>Potalia amara</i> Aubl.	n/a	n/a	Potalieae–Potaliinae	AF102470*	–	–	–
<i>Potalia resinifera</i> Mart	n/a	n/a	Potalieae–Potaliinae	AF102472*	–	–	–
<i>Pterygocalyx volubilis</i> Maxim.	P. Chassot and Y. M. Yuan 99–100 (NEU)	China, Yunnan	Gentianeae–Swertiinae	AJ315202*	AJ315248*	–	–
<i>Sabatia stellaris</i> Pursh.	J. Grant 97–2871 (NEU)	USA	Chironieae–Chironiinaea	AY255694*	AY255697*	–	–
<i>Saccolobium bandeirae</i> Maguire and Pires	M. Piliackas et al., s.n.	n/a	Saccolobieae	AJ242608*	–	–	–
<i>Schenkia australis</i> (R. Br.) Mansion	L. and N. Zeltner 001216 (NEU)	Australia	Chironieae–Chironiinaea	AY251679*	AY251709*	–	–
<i>Schenkia clementii</i> (Domin.) Mansion	L. and N. Zeltner 001201 (NEU)	Australia	Chironieae–Chironiinaea	AY251739*	AY251763*	–	–
<i>Schenkia spicata</i> (L.) Mansion	G. Mansion 981005 (NEU)	France	Chironieae–Chironiinaea	AF402196*	AF402252*	–	–
<i>Schinziella tetragona</i> (Schinz) Gilg	Malaisse 13852 (BR)	n/a	Chironieae–Canscorinae	AF102479*	–	–	–
<i>Schultesia guianensis</i> (Aubl.) Malmé	C.C. Berg and A.J. Henderson BG661 (NY)	n/a	Chironieae–Coutoubeinae	AF102480*	–	–	–
<i>Sebaea albens</i> (L. f.) Roem. and Schult.	B. Bytebier 2616 (NEU)	South Africa	Exaceae	FJ014164	FJ013986	FJ014059	–
<i>Sebaea ambigua</i> Cham.	J. Kissling and L. Zeltner 45 (NEU)	South Africa	Exaceae	FJ014165	FJ013987	–	FJ014110
<i>Sebaea aurea</i> (L. f.) Roem. and Schult.	B. Bytebier 2620 (NEU)	South Africa	Exaceae	FJ014166	FJ013988	FJ014060	FJ014111
<i>Sebaea aurea</i> (L. f.) Roem. and Schult.	J. Volk, B.E. van Wyk and Schutte A20 (MO)	South Africa	Exaceae	FJ014167	FJ013989	FJ014061	–
<i>Sebaea bojeri</i> Griseb.	L. and N. Zeltner 050302/1b (NEU)	Lesotho	Exaceae	FJ014168	FJ013990	FJ014062	FJ014112
<i>Sebaea brachyphylla</i> Griseb.	J. Raynal 19414	Kenya	Exaceae	AJ490245*	FJ013991	–	–
<i>Sebaea cf. rotundifolia</i> Hill	J. Kissling and L. Zeltner 10 (NEU)	South Africa	Exaceae	FJ014169	FJ013992	FJ014063	FJ014113
<i>Sebaea exacoides</i> (L.) Schinz	B. Bytebier 2610 (NEU)	South Africa	Exaceae	FJ014170	FJ013993	FJ014064	FJ014114
<i>Sebaea filiformis</i> Schinz	L. and N. Zeltner 050304/1a (NEU)	Lesotho	Exaceae	FJ014171	FJ013994	FJ014065	FJ014115
<i>Sebaea junodii</i> Schinz	S. Dessein et al. 577 (NEU)	Zambia	Exaceae	FJ014172	FJ013995	FJ014066	FJ014116
<i>Sebaea leiostyla</i> Gilg	L. and N. Zeltner 050303/2a (NEU)	South Africa	Exaceae	FJ014173	FJ013996	FJ014067	FJ014117
<i>Sebaea longicaulis</i> Schinz	M. Reekmans 8272 (BR)	South Africa	Exaceae	FJ014174	FJ013997	–	FJ014118
<i>Sebaea membranacea</i> Hill	J. Kissling and L. Zeltner 66 (NEU)	South Africa	Exaceae	FJ014175	FJ013998	FJ014068	FJ014119
<i>Sebaea microphylla</i> (Edgew.) Knobl.	J. Kissling 76 (NEU)	Zambia	Exaceae	FJ014177	FJ014000	FJ014070	FJ014120
<i>Sebaea microphylla</i> (Edgew.) Knobl.	S. Dessein et al. 557 (NEU)	Zambia	Exaceae	FJ014178	FJ014001	FJ014071	FJ014121
<i>Sebaea minutiflora</i> Schinz	J. Kissling and L. Zeltner 46 (NEU)	South Africa	Exaceae	FJ014179	FJ014002	FJ014072	FJ014122
<i>Sebaea natalensis</i> Schinz	Mothogoane M.S. 250 (PRE)	South Africa	Exaceae	FJ014180	FJ014003	FJ014073	FJ014123
<i>Sebaea rehmanni</i> Schinz	L. and N. Zeltner 050302/1a (NEU)	South Africa	Exaceae	FJ014182	FJ014005	FJ014075	FJ014125
<i>Sebaea repens</i> Schinz	J. Kissling and L. Zeltner 17 (NEU)	Lesotho	Exaceae	FJ014183	FJ014006	FJ014076	FJ014126

Table 1 (continued)

Species	Voucher specimen and herbarium	Origin	Systematic position	<i>trnL</i> intron	<i>trnL</i> -F spacer	<i>atbp-rbcL</i> spacer	<i>matK</i> partial gene
<i>Sebaea schlechteri</i> Schinz	J. Kissling and L. Zeltner 50 (NEU)	South Africa	Exaceae	FJ014184	FJ014007	–	–
<i>Sebaea sedoides</i> Gilg	L. and N. Zeltner 050303/2b (NEU)	South Africa	Exaceae	FJ014185	FJ014008	–	FJ014127
<i>Sebaea</i> sp1	J. Kissling and L. Zeltner 34 (NEU)	South Africa	Exaceae	FJ014186	FJ014009	FJ014077	FJ014128
<i>Sebaea</i> sp2	J. Kissling and L. Zeltner 31 (NEU)	Lesotho	Exaceae	FJ014187	FJ014010	FJ014078	FJ014129
<i>Sebaea</i> sp3	J. Kissling and L. Zeltner 5 (NEU)	South Africa	Exaceae	FJ014191	FJ014014	FJ014082	FJ014130
<i>Sebaea</i> sp4	J. Kissling and L. Zeltner 35 (NEU)	South Africa	Exaceae	FJ014192	FJ014015	FJ014083	FJ014131
<i>Sebaea spathulata</i> (E. Mey.) Steud.	J. Kissling and L. Zeltner 40 (NEU)	South Africa	Exaceae	FJ014188	FJ014011	FJ014079	FJ014132
<i>Sebaea thodeana</i> Gilg.	J. Kissling and L. Zeltner 16 (NEU)	Lesotho	Exaceae	FJ014189	FJ014012	FJ014080	FJ014133
<i>Sebaea thomasii</i> (S.Moore) Schinz	J. Kissling and L. Zeltner 29 (NEU)	Lesotho	Exaceae	FJ014190	FJ014013	FJ014081	FJ014134
<i>Swertia cordata</i> (Wall. Ex D. Don) C.B. Clarke	P. Chassot 97–17 (NEU)	Nepal	Gentianeae–Swertiinae	AJ408015*	AJ315255*	–	–
<i>Swertia perennis</i> L.	P. K�pfer s.n. (NEU)	Switzerland	Gentianeae–Swertiinae	AY255695*	AY251774*	–	–
<i>Symbolanthus australis</i> Struwe	n/a	n/a	Helieae	AF102489*	–	–	–
<i>Symbolanthus frigidus</i> (Sw.) Struwe and K. Gould	n/a	n/a	Helieae	AF102498*	–	–	–
<i>Symphylophyton caprifolioides</i> Gilg	Ratter 6742 (E)	Brasil	Chironieae–Coutoubeinae	AF102490*	–	–	–
<i>Tachia lorentensis</i> Maguire and Weaver	n/a	n/a	Helieae	AF102492*	–	–	–
<i>Tachiadenus carinatus</i> (Desr.) Griseb.	S. Wohlhauser M059 (NEU)	Madagascar	Exaceae	FJ014193	FJ014016	FJ014084	–
<i>Tachiadenus gracilis</i> Griseb.	n/a (NEU)	Madagascar	Exaceae	FJ014194	FJ014017	FJ014085	–
<i>Tachiadenus longiflorus</i> Bojer ex Griseb.	S. Wohlhauser M006 (NEU)	Madagascar	Exaceae	FJ014195	FJ014018	FJ014086	FJ014135
<i>Tripterosperrum cordatum</i> (Marquand) Harry Sm.	n/a	n/a	Gentianeae–Gentianinae	AY563392*	–	–	–
<i>Tripterosperrum filicaule</i> (Hemsl.) Harry Sm.	n/a	n/a	Gentianeae–Gentianinae	AY858683*	–	–	–
<i>Urogenia ulugurensis</i> Gilg and Gilg-ben	D. J. Mabberley 1432 (K)	n/a	Potalieae–Faroinae	AF102495*	–	–	–
<i>Voyriella parviflora</i> (Miq.) Miq.	G. Cremers 14891	n/a	Sacchifolieae	AJ242607*	–	–	–
<i>Xestaea listanthoides</i> Griseb.	n/a	n/a	Chironieae–Coutoubeinae	AF102499*	–	–	–
<i>Zeltnera martinii</i> (Broome) Mansion	G. Mansion, L. and N. Zeltner 990215 (NEU)	Mexico	Chironieae–Chironiinae	AF400268*	AF402224*	–	–
<i>Zeltnera setacea</i> (Benth.) Mansion	G. Mansion, L. and N. Zeltner 990232 (NEU)	Mexico	Chironieae–Chironiinae	AF402182*	AF402238*	–	–

checked using the software ChromasPro Version 1.33 (Technelysium Pty Ltd.).

2.3. Sequence alignment, congruence test, and phylogenetic analysis

Alignment was performed using the program Clustal W (Thompson et al., 1994) implemented in BioEdit 7.0.1 with subsequent manual improvement. For the *trnL* 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 *trnL*-F spacer. The *atpB-rbcL* spacer and the partial *matK* gene were aligned without ambiguities.

A total of 117 informative and unambiguously assessable indels were scored as binary characters (36 for the *trnL* intron, 36 for the *trnL*-F spacer, 42 for the *atpB-rbcL* spacer and 3 for the partial *matK* gene) following the “simple indel method” coding of Simmons and Ochoterena (2000) and added at the end of the data matrix.

The congruence between the four independent cpDNA datasets was tested by performing an incongruence length difference test (ILD test; Farris et al., 1995) implemented in PAUP* 4.0b10 (Swofford, 2002) as the partition–homogeneity test. The respective data sets were congruent with each other ($P = 0.82$), thus a combined matrix containing 174 accessions was built.

Phylogenetic analyses were conducted using the maximum parsimony (MP) criterion 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 thousand best trees per replicate. Branch lengths were derived under ACCTRAN optimization. Clade support was assessed by a bootstrap analysis (Felsenstein, 1985) with 1000 replicates of heuristic searches applying the same search parameters as above. The complete analysis was limited to 100 trees saved per replicate due to memory overrun. All clades with at least 70% bootstrap values were 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, determined using MrModeltest version 2.2. (Posada and Crandall, 1998; Nylander, 2004; Posada and Buckley, 2004), was the general time reversible model (GTR+I+G) for the *trnL*-F spacer, and the GTR+R model for all the remaining datasets. Default priors 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 ten million generations with trees sampled every 1000 generations. Trees generated prior to the four Markov chains reaching stationarity (4000 trees) 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 $\geq 95\%$ were considered statistically significant.

2.4. Character state optimization

A total of 24 characters (23 morphological and one karyological) were surveyed. The morphological characters (Table 2) 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* were deduced from direct observations on living and herbarium material. For the remaining genera, literature resources were used (Klackenberg, 1985, 1986, 1987a, 2002).

Karyological data were obtained from a recent survey of the tribe (Kissling et al., 2008). This study suggests $x = 7, 8, 9$, and 17

Table 2
Morphological characters and character states used in this study.

1	Seed form: 0 = oval, ridged with a more bilateral symmetry (Fig. 4b); 1 = polyhedral to angled (Fig. 4a,c, and d)
2	Testa cells: 0 = polygonal (Fig. 4a); 1 = \pm rectangular (Fig. 4b); 2 = star-shaped or \pm isodiametric (Fig. 4c and d)
3	Testa cells arrangement: 0 = not in row; 1 = in row (Fig. 4b)
4	Life form: 0 = herbs; 1 = suffrutescent plant; 2 = shrubby plant; 3 = crassulescent herb
5	Leaves: 0 = glabrous; 1 = hairy
6	Bracteoles: 0 = shorter than the whole flower; 1 = much longer than the whole flower
7	Inflorescence: 0 = dichasium; 1 = sub-sessile flowers arranged in axillary fascicles at each node (“spike-like”); 2 = solitary flowers; 3 = panicle; 4 = terminal or axillary umbel-shaped (in fact condensed cymes)
8	Corolla color: 0 = bright yellow; 1 = white; 2 = blue-violet; 3 = salmon–orange; 4 = yellow to orange with at least the lobes red
9	Corolla merosity: 0 = pentamerous; 1 = tetramerous
10	Corolla tube: 0 = long (0.3–2 cm); 1 = short (nearly absent); 2 = extremely long (<3–20 cm)
11	Enantiostyly (declinate style mirroring the stamens): 0 = absent; 1 = present
12	Anthers opening mechanism: 0 = by longitudinal slits; 1 = by apical pores; 2 = by apical pores that do finally widen to slits
13	Anthers appendix: 0 = gland or papilla absent; 1 = with a conspicuous apical gland (\pm = anthers size); 2 = with a small apical gland (<<anthers size); 3 = with a small papilla near the apex
14	Anthers furnished by two basal glands: 0 = absent; 1 = present
15	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
16	Anthers fixation: 0 = medifix; 1 = basifix
17	Ovary: 0 = truly bilocular, at least towards the base; 1 = ovary pseudo-bilocular
18	Style: 0 = straight; 1 = bent downwards below the anthers, or markedly curved
19	Stigma division: 0 = bilobed; 1 = slightly bilobed; 2 = apparently entire
20	Stigma form: 0 = capitate; 1 = capitate to slightly clavate; 2 = linear to clavate; 3 = “flat” to capitate
21	Stigma texture: 0 = smooth; 1 = papillate
22	Secondary stigma on the style: 0 = absent; 1 = present
23	Stylar polymorphism (short-styled versus long-styled flowers): 0 = absent; 1 = present
24	Five lobe disk below the ovary: 0 = absent; 1 = present
25	Chromosomes base numbers: 0 = 7; 1 = 8; 2 = 9; 3 = 17

Table 3

Characteristics of the four cpDNA data sets.

Data set	Aligned positions	No. excluded characters	No. constant sites	No. informative sites	No. uninformative sites	Indels	Number of most parsimonious trees	Tree length	CI	RI
<i>trnL</i> intron	738	65	335	223	115	36	1412	733	0.638	0.913
<i>trnL</i> -F spacer	679	111	240	233	95	36	708	713	0.654	0.917
<i>atpB-rbcl</i> spacer	1006	0	554	330	122	42	1996	759	0.761	0.902
Partial <i>matK</i> gene	469	0	331	84	54	3	30	185	0.865	0.955
Combined dataset	2892	176	1460	870	386	117	926	2414	0.693	0.912

as possible base numbers, which were here coded as four different unordered states implying that no distinctions were made between polyploid species with the same basal number.

Character state evolution was reconstructed with MESQUITE 1.12 (Maddison and Maddison, 2005) using (i) a 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). 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 reported proportional likelihood (P.L.) values of states scaled so that the sum of all states is 1, and used a decision threshold of 2.0 (Maddison and Maddison, 2005). Maximum parsimony optimizations were performed using unordered character state transformations. The Bayesian topology inferred on the combined data was chosen for each character state reconstruction.

3. Results

3.1. Sequence and alignment characteristics

The *trnL* intron sequences ranged from 334 to 539 bp in length. Within Gentianaceae, the Exaceae, and the Saccifoliaeae are characterized by longer sequences (from 449 to 539 bp), resulting in an insertion of 122 bp in the aligned matrix. The *trnL*-F spacer sequences ranged from 335 to 478 bp in length, with 36 indels in the aligned matrix, the longest one being a deletion of 93 bp occurring in some accessions of *Sebaea*. The *atpB-rbcl* spacer sequences ranged from 710 to 781 bp in length, and the partial *matK* gene sequences from 449 to 465 bp.

Finally, the combined dataset contained 2892 bp: 176 bp (6.1%), ambiguously aligned *trnL* and *trnL*-F region, were excluded from phylogenetic analyses and 117 binary coded characters were added at the end of the matrix. Overall, the combined dataset contained 1432 variable characters (49.5%), of which 870 (30.1%) were parsimony informative.

3.2. Phylogenetic analyses (Tables 3 and 4)

Heuristic searches on the combined data set resulted in 926 equally most parsimonious trees of 2414 steps (CI = 0.693, excluding autapomorphic sites, RI = 0.912). The strict consensus tree (Supplementary materials S1 and S2) was well resolved for the

deep nodes, supporting the monophyly of all described tribes and subtribes (BS 71–100%), but received less significant support at the tips. The majority rule consensus tree obtained from the Bayesian analyses (Fig. 1, Table 4) was similar in topology with the strict consensus tree of the MP analysis, the posterior probabilities being in accordance with the bootstrap values.

Within the Exaceae (Fig. 1), *Sebaea* was shown to be polyphyletic and split into two clades and a basal lineage hereafter named S1, S2, and S3. Lineage S1 contains only *S. pusilla* and is sister to S2, which formed a highly supported clade (BS 99%). Clade S1 + S2 are sister to all the remaining genera of the tribe. Clade S3 (BS 100%) comprises most tropical species of *Sebaea*. *Exacum* forms a moderately-supported clade (BS 68%) sister to the *Ornichia*–*Klackenbergia* clade (BS 88%). Finally, the sister relationship between the Madagascan genera *Gentianothamnus* and *Tachiadenus* is strongly supported (99%).

3.3. Character optimization

Among the 23 morphological characters investigated (Table 2), 16 were synapomorphies supporting different clades within the Exaceae (Fig. 2). Both ML and MP ancestral reconstructions gave 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 and homostylous flowers (P.L. = 0.99). Flowers had a yellow corolla (P.L. = 0.57) and 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 stigma (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). The seeds were cubical (P.L. = 0.99) and the testa cells star-shaped (P.L. = 0.59).

The optimization of karyological data supported the base number $x = 7$ as ancestral for the whole tribe as well as for *Exochaenium*, *Lagenias*, *Ornichia* and *Sebaea*, and $x = 8$ for *Tachiadenus*. Reconstructions at the other nodes remain ambiguous. (Fig 3).

4. Discussion

Our phylogenetic analyses of a large dataset, including most species ascribed to Exaceae (71 out of c. 181) and particularly 45% of the diversity encountered in *Sebaea* (43 out of c. 95 species),

Table 4

Summary of the Bayesian inferences.

Data set	Base frequencies				Substitution rate matrix						Invariable site	Gamma distribution parameter	
	A	C	G	T	A–C	A–G	A–T	C–G	C–T	G–T		Alpha	m
<i>trnL</i> intron	0.3922	0.1537	0.1734	0.2805	0.1308	0.2119	0.0475	0.1097	0.3139	0.1859		0.9331	0.0558
<i>trnL</i> -F spacer	0.3222	0.1941	0.1419	0.3416	0.1452	0.2293	0.0758	0.1152	0.2449	0.1893	0.146	3.8912	0.0916
<i>atpB-rbcl</i> spacer	0.3233	0.1399	0.1566	0.38	0.1724	0.2123	0.0407	0.1627	0.2458	0.1658		1.8035	0.0759
Partial <i>matK</i> gene	0.2935	0.1874	0.1459	0.3731	0.2033	0.289	0.0418	0.0654	0.2581	0.1422		0.06856	5.49

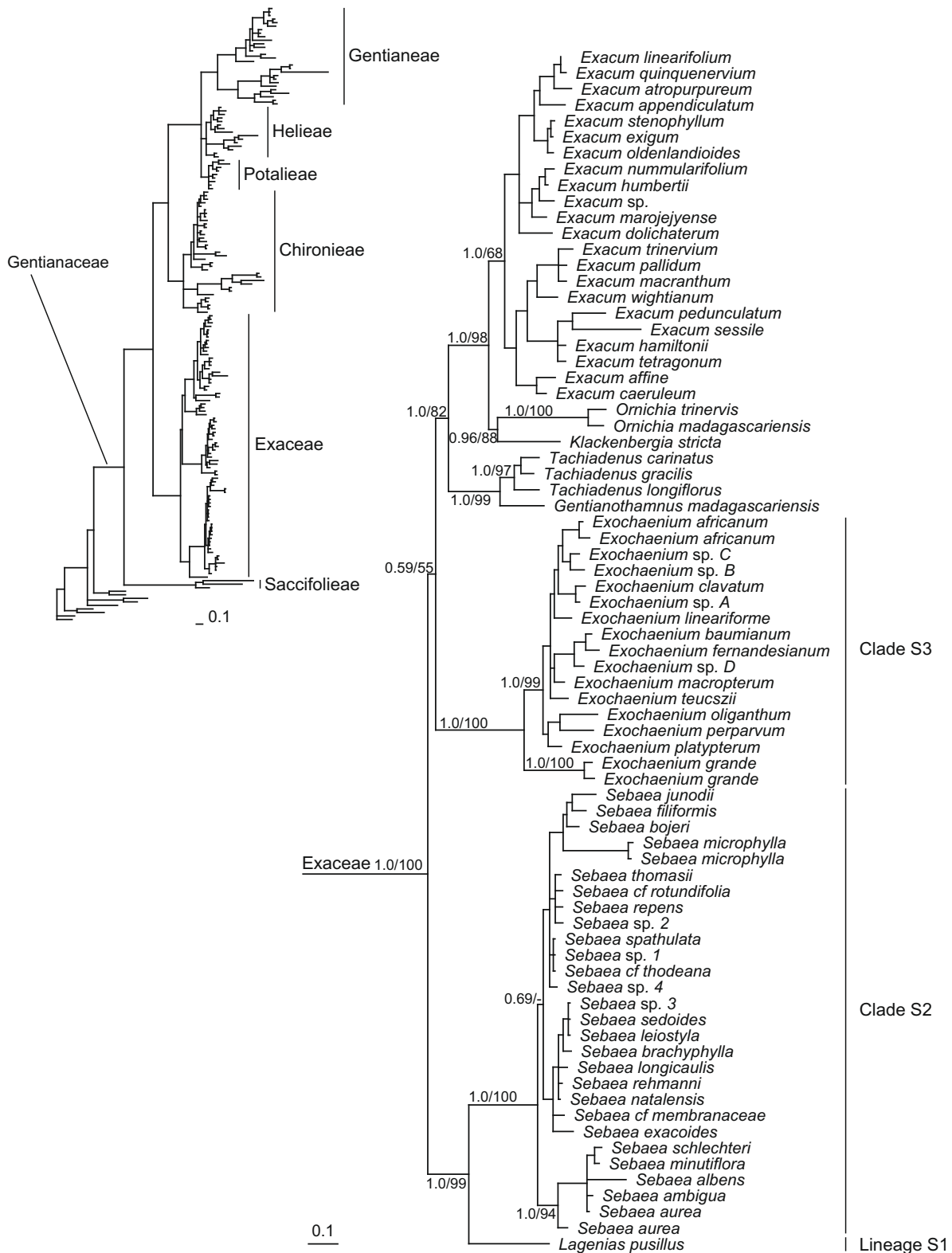


Fig. 1. The 50% majority rule consensus trees from Bayesian inference analyses of the combined data set. Branch support values are posterior probabilities derived from the Bayesian inference analysis and bootstrap values, reported from the MP analysis.

support the monophyly of the tribe (BS 100) and the parphyly of *Sebaea*, currently split into three separate “clades” (S1–S3; Fig. 1). The remaining five genera form a strongly supported (BS 82) clade, sister to S3 (BS 55), while S1 + S2 clade (BS 99), are sister to the rest of the tribe.

4.1. Generic delimitation of *Sebaea* s.l. and taxonomic consequences

The parphyly of *Sebaea* is somewhat surprising for a group that has been regarded as a morphologically well-delimited assemblage by most recent authors (e.g. Marais and Verdoorn,

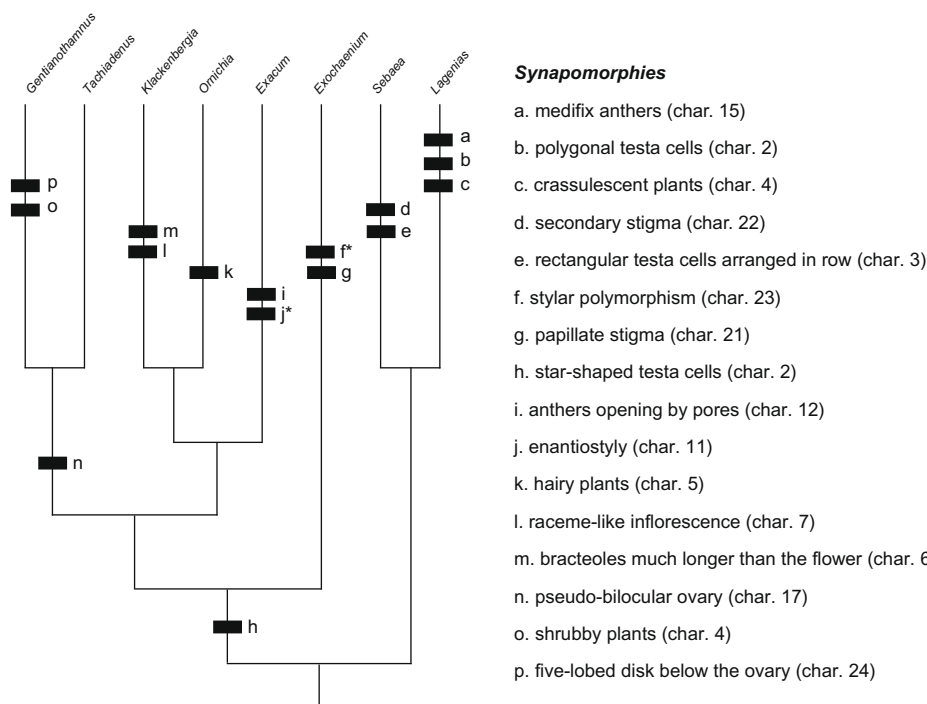


Fig. 2. Distribution of synapomorphic characters in Exaceae. An asterisk indicates that the characters were not observed in all the species. “char.” refers to the character number found in Table 2.

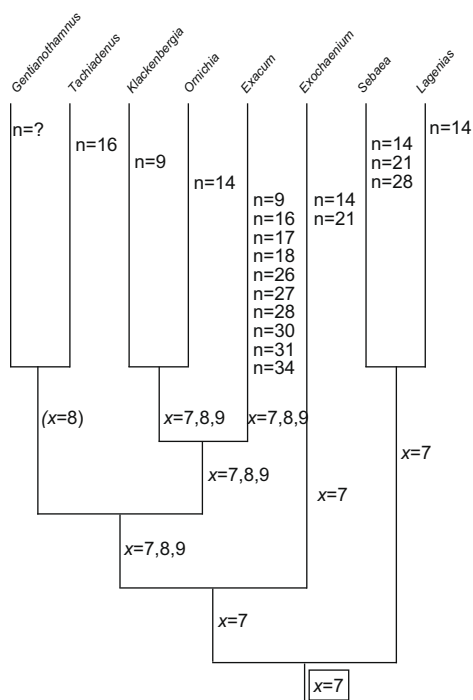


Fig. 3. Summary of the maximum parsimony reconstruction of the ancestral basic chromosome numbers of Exaceae.

1963; Taylor, 1963; Boutique, 1972; Klackenberg, 1990; Paiva and Nogueira, 1990a; Nemomissa, 2002). Nonetheless, in earlier works (Brown, 1810; Rafinesque, 1837; Meyer, 1838; Grisebach, 1845), some genera were described as segregates of *Sebaea* s.l. including *Belmontia* E.Mey., *Exochaenium* Griesb., *Lagenias* E. Mey., and *Parasia* Raf.

Based on the respective branch supports and lengths of the molecular clades and the number of morphological synapomorphies, we deemed necessary to separate S1 and S2. In our study, S1 contains only *Sebaea pusilla*, a species characterized by several morphological apomorphies such as medifix anthers, polygonal testa cells, and a crassulescent habit (Fig. 2). The basal lineage S1 fits the monotypic genus *Lagenias*, described by Meyer (1838) on the basis of *S. pusilla*.

Clade S2 (BS 100) is supported by two morphological apomorphies (the arrangement of seed testa cells and the presence of a secondary stigma on the style; Fig. 2) and contains mainly South African species, some tropical African and Malagasy species, the Australian species and the paleotropical *S. microphylla*. This clade is defined as *Sebaea* s.str. (*Sebaea* Sol Ex. Brown).

Clade S3 (BS 100) comprises mostly tropical African species and is characterized by the papillate texture of the stigma, and the presence of a stylar polymorphism (short-styled versus long-styled flowers). This clade corresponds to *Exochaenium*, first described by Grisebach (1845) on the basis of *Sebaea grandis*. The taxonomic treatment suggested above is followed in the subsequent discussion. Systematic treatments of the segregated genera will be published in Systematic Botany (Kissling, unpublished).

4.2. Phylogenetic relationship within Exaceae

The increase in taxon sampling, with *Lagenias* and *Exochaenium* collected for the first time in our study, reveals the paraphyly of *Sebaea* s.l. but does not strongly disrupt the intergeneric relationships hitherto inferred within the Exaceae (Yuan et al., 2003). The main differences consist in the early diverging position of the *Sebaea*–*Lagenias* clade and the sister position of *Exochaenium* with the remaining five genera of the tribe.

Lagenias and *Sebaea* occur in the Cape region and share some morphological characters such as the shape of the stigma (bilobed) and the color of the corolla (yellow). The sister relationship between *Exochaenium* and the Malagasy genera *Exacum*, *Gentianothamnus*,

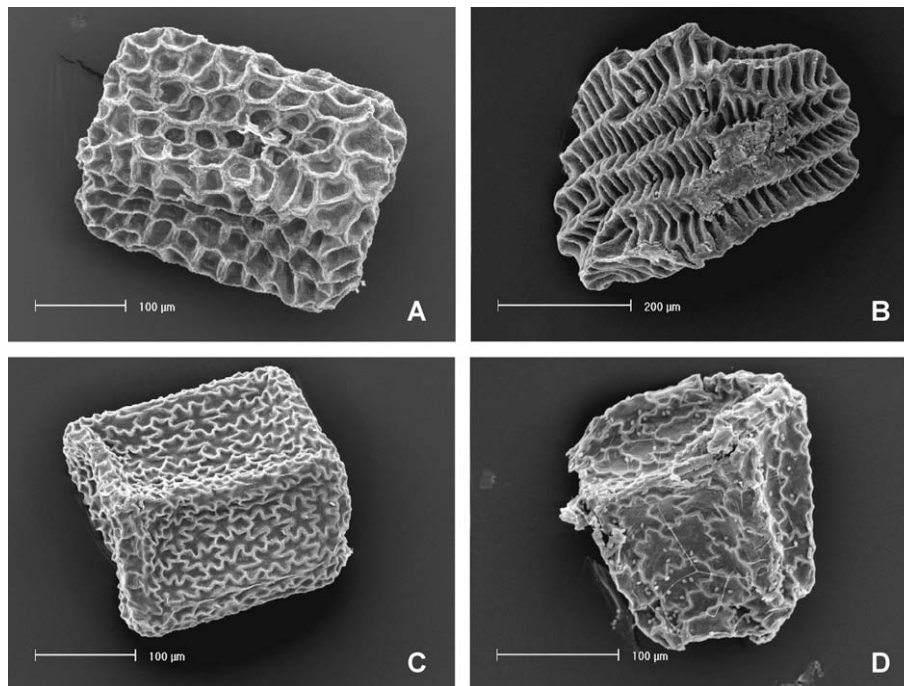


Fig. 4. Seeds of representative species from the three “clades” depicted in *Sebaea* s.l. and of the genus *Klackenbergia* recently described from two *Sebaea* species: (A) *L. pusillus* (S1) showing cubical seeds with a reticulate pattern of polygonal cells; (B) *S. aurea* (clade S2) showing ridged seeds with a bilateral symmetry and rectangular testa cells arranged in longitudinal rows; (C) *E. macropterum* (clade S3); (D) *Klackenbergia stricta*. C and D shows cubical seeds with star-shaped testa cells.

Klackenbergia, *Ornichia*, and *Tachiadenus* is weakly supported (BS 55) by the molecular data but consistent with the seed morphology (see Section 4 below) (Figs. 2 and 4). Within the Malagasy clade, our molecular phylogeny is congruent with previous works performed on these genera (Klackenberg, 2002; Yuan et al., 2003). *Tachiadenus* is closely related to *Gentianothamnus* (BS 99) and forms a sister clade with *Exacum*, *Klackenbergia*, and *Ornichia*. Klackenberg (2002) early suggested a close relationship between *Tachiadenus* and *Gentianothamnus* based on morphological and anatomical characters (e.g. pseudo-bilocular ovary and pollen morphology). Our study support a sister relationships between *Klackenbergia* and *Ornichia* (BS 88), two genera easily separated by e.g. the pubescence of the leaves (*Ornichia*), the color of the corolla (blue-violet in *Ornichia* vs. white in *Klackenbergia*), or the stigma shape (capitate to bilobed in *Ornichia* vs. linear to clavate in *Klackenbergia*) (Klackenberg, 1986; Kissling et al., 2009). Recent karyological investigations (Kissling et al., 2008) also emphasizes the difference between *Ornichia* ($2n = 14$) and *Klackenbergia* ($2n = 18$). Finally, the solitary flowers or lax inflorescence of *Ornichia* contrast with the “spike-like” inflorescence (see Table 2, character 7) of *Klackenbergia*. In this study, we were not able to find morphological or karyological synapomorphies supporting the sister relationship of the clade formed by *Klackenbergia*–*Ornichia* with *Exacum* (BS 98).

4.3. Morphological character evolution

4.3.1. Seeds (Character 1–3, Table 2)

As already observed for some tribes of the Gentianeae (Bouman et al., 2002), characters such as the seed shape, testa cell form and arrangement are phylogenetically informative and further support the segregation between *Exochaenium*, *Lagenias*, and *Sebaea*. Seed structure of the Malagasy Exaceae has been extensively studied by Klackenberg (1983, 1985, 1986, 1987b), and more recently by Bouman et al. (2002) who included some species of *Sebaea*. Our present study, encompassing most species of Exaceae (both from Africa and Madagascar), shows that the seeds of *Sebaea*,

unlike the other genera of the tribe, are often ridged, with a bilateral symmetry, and the testa cells are more or less rectangular and arranged in regular longitudinal rows (Fig. 4). The sister genus *Lagenias* has cubical seeds, similar to other Exaceae, with a distinct reticulate pattern of polygonal cells with straight cell walls (Fig. 4), reminiscent of the Chironieae (Bouman et al., 2002). The seeds of *Exochaenium* (Fig. 4) resemble more those of the Malagasy genera (*Exacum*, *Gentianothamnus*, *Klackenbergia*, *Ornichia*, and *Tachiadenus*), and support the weak sister relationships (BS 55) on the phylogenetic tree (Fig. 1). Star-shaped testa cells are here identified as an apomorphy for the *Exochaenium* – Malagasy genera clade (Fig. 2), but not for the whole Exaceae as suggested by Klackenberg (2002).

4.3.2. Habit (character 4–5, Table 2)

Most Exaceae are annual herbs (character 4) and the suffrutescent habit that developed in the *Tachiadenus*–*Gentianothamnus* clade may be a consequence of their perennial life cycle or environmental pressure. Indeed, most species of *Tachiadenus* and *Gentianothamnus* are perennial, basally woody herbs that occur in the wet places of Madagascar such as forest, marsh, shore or temporary wet savannah (Klackenberg, 1990, 2002). The woodiness can be viewed as a synapomorphy for the *Tachiadenus*–*Gentianothamnus* clade, but outside the Exaceae, this character is known to have evolved several times (e.g. *Anthocleista*, *Fagraea*, *Ixanthus*, *Macrocarpaea*, and *Symphyllophyton*), probably as a result of adaptation to specific environment. The sister relationships between Mediterranean annual *Blackstonia* and the perennial woody *Ixanthus*, endemic to the laurel forests of the Canary Islands, is a good example of such adaptation (Thiv et al., 1999; Mansion and Struwe, 2004).

The hairs (Character 5) characteristic of *Ornichia* (simple and one-celled trichomes; Klackenberg, 1986) are also encountered in some genera or species outside the Exaceae, including e.g. *Orphium* (Chironieae), *Macrocarpaea* (Helieae) or *Swertia racemosa*, and *Metagentiana souliei* (Gentianeae). Trichomes are known to have different functions, like insulation from heat or sunlight, salt

removal, defense against herbivores or water absorption (Callow, 2000), but our current knowledge of *Ornichia* does not allow us to further speculate on their specific role.

4.3.3. Flowers and inflorescence (character 6–12, Table 2)

The variation in the inflorescence (character 7) of the Exaceae (dichasium, panicle, “spike-like” or axillary umbel-shaped) or the size of the bracteoles (character 6) are of limited used at the generic level and does not support any clade phylogenetically, except for the “spike-like” inflorescence and the long bracteoles (twice as long as the flowers) which characterized the genus *Klackenbergia*.

The color of the corolla (character 8) varies greatly within the Exaceae (Supplementary material S3). Yellow corollas appear to be a plesiomorphic state for the tribe (present in *Lagenias*, *Sebaea*, and *Exochaenium*), while blue to lilac occurred only in Malagasy genera (*Exacum*, *Ornichia*, and *Tachiadenus*). It is interesting to note that early diverging genera or species within the Gentianaceae–Chironiinae (e.g. *Blackstonia*, *Centaurium maritimum*, *Cicendia*, or *Ixanthus*) also share yellow corollas (Mansion and Struwe, 2004; Mansion et al., 2005). This pattern somewhat parallels the metabolic pathway of the flavonoids, starting with the synthesis of yellow pigments such as chalcones and flavones and ending with blue or red anthocyanidins (Crozier et al., 2006). More detailed phytochemical surveys are necessary before further conclusions.

Optimization of the corolla-merosity (character 9) onto the phylogenetic reconstruction supports pentamery as a plesiomorphic state for the Exaceae. This pattern is well demonstrated for the Chironieae (Mansion and Struwe, 2004) and seems to be the rule for most tribes of the Gentianaceae (Struwe et al., 2002; Chassot, 2003). Nevertheless, some lineages of *Sebaea* and *Exacum* appear to be characterized by the presence of tetramerous corollas. Within the Exaceae, tetramery occurs apparently only within small short-lived annuals, like in the Chironiinae (e.g. *Cicendia* and *Exaculum*).

The size of the corolla tube (character 10) 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 species of *Exochaenium* and *Sebaea thomasi* show extremely long corolla tubes (3–20 cm.). Our results suggest a mid-size corolla tube length (0.3–2 cm) 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. those with a long proboscis (Faegri and van der Pijl, 1966; Goldblatt and Manning, 2000; Johnson and Steiner, 2000; Fenster et al., 2004), and has no taxonomic value at the generic rank within the Exaceae. All species of the Madagascan *Tachiadenus* show long corolla tubes, but it is still unclear whether or not this adaptation has contributed to the important diversification of the genus on this island.

Within Exaceae, most species of *Exacum* have enantiostylous flowers (character 11), the gynoeceum mirroring the stamen cluster. This pollination syndrome, known to involve bee promote cross-pollination in bee-pollinated plants (Endress, 1994), is also encountered in other Gentianaceae genera like *Chironia*, a primarily South African genus. *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 style direction) flowers. Our data suggest that the loss of enantiostyly has only occurred in a clade from Madagascar (*E. stenophyllum*, *E. oldenlandioides*, and *E. exiguum*).

4.3.4. Androeceum (character 12–16, Table 2)

The androeceum 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*-medifixed), this character (character 16) being the ancestral state for the tribe. Tribes Saccifolieae (Thiv et al., 2002), Chironieae (Mansion and Struwe, 2004), and Helieae (Jason Grant, personal communication) have also basifixed anthers, while in other tribes, such as Gentianeae, medifix anthers are more abundant (Ho and Lui, 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 and outgroups suggests it as a plesiomorphic state. Our study shows that filament insertion (character 15) inside the corolla tube (versus at the corolla sinus) 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 (character 12) either by pores (*Exacum*), or by slits (all other genera). Opening by pores is an apomorphy for the genus *Exacum*, while opening by slits is the ancestral state for the tribe. Several modifications in androeceum, including porandrous anther, are plausible evolutionary responses to a shift to buzz pollination (Hardy et al., 2000), and supernumerary endothelial layers and/or the proliferation and persistence of middle layers in the anthers of buzz-pollinated flowers is not uncommon (e.g. Subramanyam, 1948; Renner, 1990; Venkatesh, 1957; Hardy et al., 2000). In *Exacum*, Sankara Rao and Chinnappa (1983) found three persistent middle layers, while they only found a single ephemeral middle layer in the other gentianaceous genera they investigated, all of which possess longitudinally dehiscent anthers. This together with the presence of several enantiostylous species in *Exacum* strongly suggests a specific adaptation to bee pollination for the genus.

The striking presence of apical or/and basal glands on the anthers of e.g. *Exochaenium* and some *Sebaea* (character 13 and 14) do not show any taxonomic information at the generic level. The sugar solution contained in these appendices (Schinz, 1903) likely attracts potential pollinators such as thrips (Marloth, 1909). Anther glands are supposed to facilitate pollination in certain taxa (Chaudhry and Vijayaraghavan, 1992; Endress, 1994) but there is no demonstrated evidence to support this assertion.

4.3.5. Gynoeceum (character 17–24, Table 2)

The gynoeceum of the Exaceae is bicarpellate, with a superior bilocular ovary with axile placentation, surmounted by a style of variable length. In the clade form by *Gentianothamnus* and *Tachiadenus*, the “pseudo-bilocular” ovary (character 17) is considered as an apomorphy for this clade.

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 (character 20, 21) has proved to be of importance in the systematics of Gentianaceae (e.g. Marais and Verdoorn, 1963; Mansion, 2004; Mansion and Struwe, 2004) and this feature is particularly useful to discriminate between *Exochaenium* and *Sebaea* species. Our reconstruction suggests a smooth stigma of 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 (character 23) (long-styled, versus short-styled flowers) is only present in the genus *Exochaenium*. This stylar polymorphism is in some cases also asso-

ciated with a reciprocal position of the anthers, like in heterostylous flowers, as in *E. grande* (Welwitsch, 1869; Schinz, 1906; Hill, 1908) 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. Leege and Wolfe, 2002; Ornelas et al., 2004; Faivre and McDade, 2001; Naiki and Nagamasu, 2004; Pailler and Thompson, 1997). It is therefore difficult to settle whether the stylar polymorphism is a symplesiomorphy or an apomorphy for *Exochaenium*.

The presence of the striking secondary stigma (character 22) on the style (diplostigmaty; Marloth, 1909) of *Sebaea* s.str. species has already been used as a diagnostic character for this genus (Hill, 1908; Hill and Prain, 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 (Kissling et al., in press). Secondary stigmas might serve to ensure seed set when outcrossed pollen or pollinators are scarce, while at the same time the apical stigma can serve to promote opportunities for outcrossing during the maturation of the anthers (Hill, 1913; Kissling et al., in press).

4.4. Karyotype evolution (Figs. 4 and 5)

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 species with $x = 9$, while section *Exacum* has a wide range of basic chromosome numbers, i.e. $x = 7$, $x = 8$, $x = 9$, and $x = 17$ (Kissling et al., 2008).

We previously proposed (Kissling et al., 2008) 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 (Fig. 5) that minimizes the number of dysploidy 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 (Yuan et al., 2005; Kissling et al., 2008); (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 and Zeltner, 2004; Mansion et al., 2005).

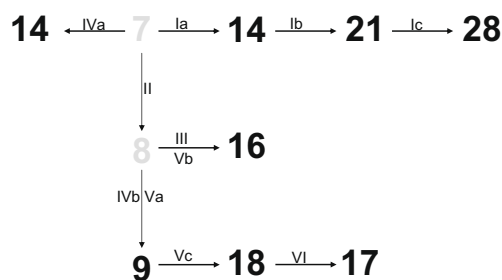


Fig. 5. Scheme of possible pathway of base chromosome number evolution in Exaceae. Black numbers are chromosome numbers presently found in the tribe. Gray numbers are hypothesized ancestral numbers. Bold arrows indicate possible episodes of polyploidization; thin arrows dysploidy. See Section 4 for details (numbers I–V).

Based on $x = 7$ as the base chromosome number for Exaceae, polyploidization of $x = 7$ would lead to $2x = 14$ (*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**, and **Ic**). In the common ancestors of the clade comprising *Exacum*, *Gentianothamnus*, *Klackenbergia*, *Ornichia*, and *Tachiadenus*, a first dysploidy event might have occurred ($x = 7 \rightarrow x = 8$), 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 ($x = 8 \rightarrow x = 9$), 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 results is proposed here for this genus. *Exacum* sect. *Africana* occurs in Madagascar and contains species with $n = 16$ only, with a single exception *E. quinquerivium*, 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 ($x = 8 \rightarrow x = 9$) (**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 both Madagascar and Sri-Lanka/South India (**Vb** and **Vc**). The base chromosome number, $x = 17$, found in several *Exacum* species from Southern India–Sri Lanka could be explained by one more dysploid event $n = 2x = 18$ giving $x = 17$ (**VI**).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ymp.2009.07.025.

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