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Cytogeography of 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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Unlike other tribes of Gentianaceae, Exaceae have so far received little attention regarding their karyological evolution. Indeed, only 35 chromosome number counts (19 species) have been referenced to date, representing only a negligible fraction of the tribal diversity. In this paper, we performed an intensive chromosome count on material collected in the field (South and central Africa, plus Madagascar), encompassing 155 populations and *c*. 60 species from four genera of Exaceae, including *Exacum*, *Ornichia*, *Sebaea* and *Tachiadenus*. Fifty nine species (14 *Exacum*, one *Ornichia*, 42 *Sebaea* and two *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 possible base chromosome numbers for 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 or geographical traits, suggesting new systematic combinations and likely active speciation patterns in the group.

ADDITIONAL KEYWORDS: chromosome numbers – *Exacum* – karyology – *Ornichia* – *Tachiadenus*.

INTRODUCTION

The particular knowledge of chromosome number in plants is critical in detecting processes enabling abrupt speciation such as polyploidy or aneuploidy/dysploidy (Briggs & Walters, 1997). Polyploidy is an extremely important phenomenon in plants and occurs in, for example, 97% of ferns and *c*. 70% of angiosperms (Averett, 1980; Grant, 1981). 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 another before episodes of polyploidization) and 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, for example, *Borago*, *Nonea* (Boraginaceae; Selvi, Coppi & Bigazzi, 2006), *Hypochaeris* (Asteraceae; Cerbah *et al.*, 1998), *Centaurium*, *Gentiana* or *Zeltnera* (Gentianaceae; Yuan, Küpfer & Zeltner, 1998; Mansion, Zeltner & Bretagnolle, 2005; Mansion & Zeltner, 2004).

Despite the importance of karyological studies for understanding evolutionary processes in Gentianaceae or establishing systematic treatments (Favarger, 1949; Rork, 1949; Favarger, 1952), especially within Gentianeae (Shigenobu, 1983; Yuan,

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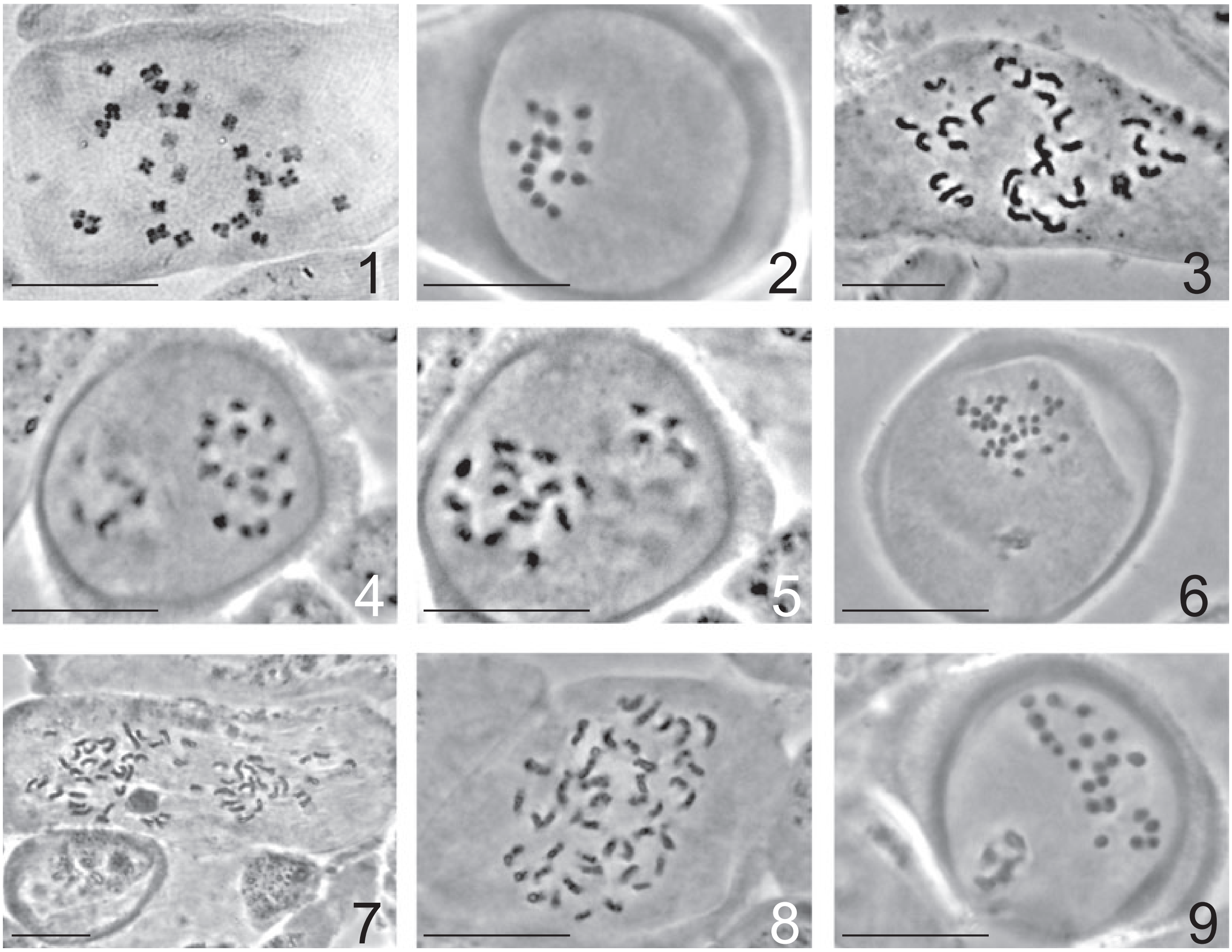

Figure 1. Chromosome of *Sebaea*. Pictures were taken at a magnification of ×1000 and the scale bars on each image correspond to 10 μm. Fig. 1. *S. thomasii*, mitotic metaphase of root tip, $2n = 28$. Fig. 2. *S. bojeri*, meiotic anaphase I, $n = 14$. Fig. 3. *S. filiformis*, mitotic metaphase, $2n = 28$. Fig. 4. *S. leiostyla*, meiotic metaphase II, $n = 14$. Fig. 5. *S. repens*, meiotic metaphase II, $n = 14$. Fig. 6. *S. micrantha*, meiotic anaphase I, $n = 28$. Fig. 7. *S. macrophylla*, mitotic metaphase, $2n = 56$. Fig. 8. *S. sedoides*, mitotic metaphase, $2n = 42$. Fig. 9. *S. rehmanii*, meiotic anaphase I, $n = 21$.

1993; Yuan & Küpfer, 1993a, b; Küpfer & Yuan, 1996; Yuan, Küpfer & Zeltner, 1998; Liu, Ho & Chen, 2002; Chassot, 2003) and Chironieae (Favarger, 1960; Zeltner, 1970; Zeltner & Mansion, 2003; Mansion & Zeltner, 2004), only a few chromosome counts have been performed so far on tropical Exaceae (Table 1).

Exaceae is a small tribe of Gentianaceae, with *c*. 180 species and five genera (Struwe *et al.*, 2002; Klackenberg, 2006). *Exacum* L. (including the saprophytic *Cotylanthera* Blume) comprises 65 species, distributed in tropical Africa, Madagascar and Asia (Klackenberg, 1985, 1990). *Gentianothamnus* Humbert is a monotypic genus occurring in Madagascar (Klackenberg, 1990). *Ornichia* Klack. contains three species endemic to Madagascar (Klackenberg, 1986). *Sebaea* Sol. is the most species-rich genus with *c*. 90–150 species in South Africa, tropical Africa and Madagascar (Schinz, 1906; Boutique, 1972; Paiva & Nogueira, 1990). Finally, *Tachiadenus* Griseb. comprises 11 species endemic to Madagascar (Klackenberg, 1987).

To date, most karyological studies have focused on *Exacum* (Riseman, Sumanasinghe & Craig, 2006), but not including African or Malagasian taxa. Early work on *Exacum tenuis* (under *Cotylanthera tenuis*; Oehler, 1927) failed to establish unambiguously a definite number ($2n = 32$–36). Several species from India (Borgmann, 1964; Subramanian, 1980; Mallikarjuna, Scheriff & Krishnappa, 1987; Riseman *et al.*, 2006) and Socotra (Sugiura, 1936a, b; Post, 1967) have also been examined, showing an extensive range in

Table 1. Chromosome numbers documented for the tribe Exaceae

Taxon	Chromosome number *n*	Chromosome number 2*n*	References
Exacum affine Balf.f.	$n = 18$		Sugiura, 1936a, b; Post, 1967
Exacum affine Balf.f.		$2n = 36$	Rork, 1949; Darlington & Wylie, 1955
Exacum affine Balf.f.		$2n = 36$	Riseman *et al.*, 2006
Exacum affine Balf.f.		$2n = 36$	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 Balf.f.		$2n = 20$	Villemoes, 2000
Exacum grande Klack. Under *E. perrotteti* Griseb.		$2n = 68$	Mallikarjuna *et al.*, 1987
Exacum lawii C.B.Clarke		$2n = 56$	Mallikarjuna *et al.*, 1987
Exacum macranthum Arn.		$2n = 54$	Sumanasinghe, 1986
Exacum pallidum (Trimen) Klack.		$2n = 52$	Sumanasinghe, 1986
Exacum pedunculatum L.		$2n = 62$	Mallikarjuna *et al.*, 1987
Exacum pedunculatum L.		$2n = (30), 54, (56)$	Subramanian, 1980
Exacum pedunculatum L.		$2n = 56$	Riseman *et al.*, 2006
Exacum pedunculatum L.	$n = 28$		Sumanasinghe, 1986
Exacum petiolare Griseb.		$2n = 62$	Mallikarjuna *et al.*, 1987
Exacum pumilum Griseb.	$n = 31$		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* Griseb.		$2n = 68$	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.) L.H.Cramer		$2n = 54$	Riseman *et al.*, 2006
Exacum trinervium subsp. *pallidum* (Trimen) L.H.Cramer		$2n = 52$	Riseman *et al.*, 2006
Exacum trinervium subsp. *ritigalensis* (Willis) L.H.Cramer		$2n = 60$	Riseman *et al.*, 2006
Exacum trinervium subsp. *ritigalensis* (Willis) L.H.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 Arn.		$2n = 68$	Mallikarjuna *et al.*, 1987
Exacum wightianum Arn. Under *E. foliosum* Griseb.		$2n = 68$	Mallikarjuna *et al.*, 1987
Sebaea brachyphylla Griseb.		$2n = 22$	Thulin, 1970
Sebaea ovata (Labill.) R.Br.	$n = c.\ 27$		Beuzenberg & Hair, 1983

chromosome number range, e.g. $2n = 18$, 30, 34, 36, 52, 54, 56, 62, 68. More recently, Riseman *et al.* (2006) reported karyological data in the *Exacum trinervium* complex, showing a large difference in chromosome number. 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 & Hair, 1983).

In this context, the main goals of the present paper are: (1) to determine and confirm chromosome numbers for taxa within Exaceae, with a special focus on the genus *Sebaea*; (2) to check the systematic relevance of the chromosome groups obtained; and (3) to infer patterns of chromosome evolution and speciation mechanisms within the tribe.

MATERIAL AND METHODS

Most samples come from wild populations collected in the field in 1995 and 2003–2006 (Table 2). All voucher

Table 2. Accession information for taxa including name, collector, voucher code, origin and chromosome number. Chromosome numbers not reported for the first time are marked with an asterisk (*) while chromosome counts that are different from previous reports are marked with an exclamation mark (!). 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) and Louis Zeltner (LZ)

Taxon	Collector	Voucher code	Origin	Chromosome numbers	
Exacum affine Balf.f.	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 Schinz	JP, SW & LZ	2 ème arrêt	Madagascar		$2n = 32$
Exacum hoffmannii 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 Griseb.	JP, SW & LZ	–	Madagascar		$2n = 36$
Exacum quinquenervium Griseb.	JP, SW & LZ	–	Madagascar	$n = 16$	
Exacum quinquenervium Griseb.	JP, SW & LZ	–	Madagascar		$2n = 36$
Exacum quinquenervium Griseb.	JP, SW & LZ	2 ème arrêt	Madagascar		$2n = 36$
Exacum quinquenervium Griseb.	JP, SW & LZ	4 ème arrêt	Madagascar		$2n = 36$
Exacum quinquenervium Griseb.	JP, SW & LZ	M007	Madagascar		$2n = 36$
Exacum quinquenervium Griseb.	JP, SW & LZ	M012	Madagascar		$2n = 36$
Exacum quinquenervium Griseb.	JP, SW & LZ	M021	Madagascar		$2n = 36$
Exacum quinquenervium Griseb.	JP, SW & LZ	M025	Madagascar	$n = 16$	
Exacum quinquenervium Griseb.	JP, SW & LZ	M038	Madagascar		$2n = 36$
Exacum quinquenervium Griseb.	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 africana J.Paiva & I.Nogueria	SD, RA, PD, JK, BL & ET	603	Zambia	$n = 21$	
Sebaea albens (L.f.) Roem. & Schult. *aff.*	JK	93	South Africa	$n = 14$	$2n = 28$
Sebaea ambigua Cham.	JK	94	South Africa	$n = 14$	$2n = 28$

Table 2. *Continued*

Taxon	Collector	Voucher code	Origin	Chromosome numbers	
Sebaea ambigua Cham.	JK & LZ	45	South Africa		$2n = 28$
Sebaea aurea (L.f.) Roem. & Schult	JK	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 Griseb.	LZ	05.03.01 2a; I11_Jtr.X	South Africa		$2n = 28$
Sebaea bojeri Griseb.	LZ	05.03.02 1b; I11_Jtr_4	South Africa		$2n = 28$
Sebaea bojeri Griseb.	LZ	05.03.02 2b; H14_Jfr_2	South Africa		$2n = 28$
Sebaea brachyphylla Griseb.	JP, SW & LZ	M044	South Africa		$2n = c.$ 56!
Sebaea brachyphylla Griseb.	LZ	05.03.04 1b; I11_Lpr.3	South Africa		$2n = 42$!
Sebaea gracilis (Welw.) Paiva & Nogueira *aff*.	SD, RA, PD, JK, BL & ET	656	Zambia	$n = 21$	$2n = 42$
Sebaea gracilis (Welw.) Paiva & Nogueira *aff*.	SD, RA, PD, JK, BL & ET	692	Zambia	$n = 21$	$2n = 42$
Sebaea rehmannii Schinz *aff*.	LZ	05.03.02 1; K09_Tal.3	South Africa	$n = 21$	
Sebaea rehmannii Schinz *aff*.	LZ	05.03.02 1a; K03_Jtr.4	South Africa	$n = 21$	
Sebaea scabra Schinz *aff*.	JK, BG & MP	109	South Africa		$2n = 28$
Sebaea clavata J.Paiva & I.Nogueria	SD, RA, PD, JK, BL & ET	543	Zambia	$n = 21$	$2n = 42$
Sebaea exacoides Schinz	JK & LZ	81	South Africa	$n = 14$	$2n = 28$
Sebaea exacoides Schinz	JK & LZ	86	South Africa		$2n = 28$
Sebaea exacoides Schinz	JK & LZ	87	South Africa		$2n = 28$
Sebaea exacoides Schinz	JK & LZ	88a	South Africa		$2n = 28$
Sebaea exacoides Schinz	JK & LZ	88b	South Africa	$n = 14$	
Sebaea exacoides Schinz *aff*.	JK, BG & MP	104	South Africa		$2n = 28$
Sebaea fernandesiana J.Paiva & I.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 Steud.	SD, RA, PD, JK, BL & ET	657	Zambia	$n = 21$	
Sebaea grandis Steud.	SD, RA, PD, JK, BL & ET	752	Zambia		$2n = 42$
Sebaea grandis Steud.	SD, RA, PD, JK, BL & ET	764	Zambia	$n = 21$	
Sebaea grandis 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 hymenosepala Gilg *aff*.	JK & LZ	3	South Africa		$2n = 28$
Sebaea hymenosepala Gilg *aff*.	JK & LZ	4	South Africa	$n = 14$	$2n = 28$

Table 2. *Continued*

Taxon	Collector	Voucher code	Origin	Chromosome numbers	
Sebaea hymenosepala Gilg *aff.*	JK & LZ	5	South Africa		$2n = 28$
Sebaea hymenosepala Gilg *aff.*	JK & LZ	73	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 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 J.Paiva & I.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 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 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.	JK, BG & MP	106a	South Africa		$2n = 28$
Sebaea sp.	JK, BG & MP	107	South Africa	$n = 14$	$2n = 28$
Sebaea sp.	JK, BG & MP	108	South Africa	$n = 14$	
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. C	JK, BG & MP	117	South Africa		$2n = 28$
Sebaea sp. (Undeterminable, only very young buds present)	JK & LZ	30	Leshoto		$2n = 28$
Sebaea sp. (Undeterminable, only very young buds present)	JK & LZ	69	South Africa		$2n = 28$

Table 2. *Continued*

Taxon	Collector	Voucher code	Origin	Chromosome numbers	
Sebaea sp. (Undeterminable, only very young buds present)	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 (Desr.) Griseb.	JP, SW & LZ	M039	Madagascar		$2n = 32$
Tachiadenus longiflorus Bojer ex. Griseb.	JP, SW & LZ	M006	Madagascar		$2n = 32$
Tachiadenus longiflorus Bojer ex. Griseb.	JP, SW & LZ	M016	Madagascar		$2n = 32$
Tachiadenus longiflorus Bojer ex. Griseb.	JP, SW & LZ	–	Madagascar		$2n = 32$

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 Carnoys solution (1/3 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 pretreated with a saturated aqueous solution of α-bromonaphthalene for 1 h 20 min, fixed in Carnoys for 2 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 mitotic plates from young cells of the ovary wall (fixed buds) or root tips (Fig. 1). In a few cases, chromosomes were counted from the second pollen mitosis. All counts were made with a Leica light microscope either with or without phase contrast and a 100× oil immersion objective, given a total magnification of ×1000.

Microphotographs were taken and drawings were made using a camera lucida. Each chromosome number was determined from at least four different preparations by two of the authors (JK and LZ).

RESULTS AND DISCUSSION

A total of 155 accessions representing 61 species and four genera (*Exacum*, *Ornichia*, *Sebaea* and *Tachiadenus*) collected in Lesotho, Madagascar, South Africa and Zambia were analysed (Table 2). Chromosome number 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 59 taxa are reported for the first time, including species of *Exacum* (14 out of 69), *Ornichia* (one out of three), *Sebaea* (*c*. 40 out of *c*. 95; Schinz, 1906) and *Tachiadenus* (two out of 11 species). In the following, we provide an enumeration of the chromosome numbers hitherto found in Exaceae and interpret them in the light of the present results.

Karyological review of exaceae

Based on new chromosome numbers evidenced in this study, several base numbers can be proposed for 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, 28, 30, 31 and 34, reported in the literature

(Table 1), may be interpreted as the result of dysploidy and/or allopolyploidy, two processes frequently occurring in some genera of Gentianaceae, including *Centaurium* (Zeltner, 1970; Mansion *et al.*, 2005), *Exacum* (Darlington & Wylie, 1955; Riseman *et al.*, 2006) or *Gentiana* section *Chondrophyllae* (Yuan *et al.*, 1998).

Base chromosome number x = *7*
The chromosome number $n = 14$ occurs frequently within *Sebaea* (26 species investigated) but also in *Ornichia* (1 species), for which it is the first published report. Fourteen species of *Sebaea* from Zambia and South Africa share the haploid number $n = 21$, whereas 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). Two other species, *S. minutiflora* ($n = 21$ or 28) and *S. aurea* ($n = 14$ or 21) were found to have different chromosome numbers. Finally, $n = 28$ is specific to two species of *Sebaea* collected in South Africa (*S. macrophylla* and *S. aff. micrantha*). 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.

Base chromosome number x = *8*
This number, new for 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 (Table 1; under *Cotylanthera tenuis*). Two species of the Malagasian endemic *Tachiadenus* are reported here with a haploid number of $n = 16$.

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* (Table 1). 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, b; Post, 1967; Riseman *et al.*, 2006).

Other chromosome numbers
The haploid number $n = 17$, reported for *Exacum atropurpureum* from India (Mallikarjuna *et al.*, 1987), has not been found in the present study in which 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 *et al.*, 2006), underlies the great chromosome instability of this taxon. This report might also support $x = 10$ and $x = 13$ as a possible base number for Exaceae. Finally, $n = 31$ and $n = 34$ have been reported for eight species of *Exacum* from India (Mallikarjuna *et al.*, 1987) supporting x = 17 as another possible base number for the tribe.

Chromosome number and systematics of Exaceae

Exacum
In the last monograph of the genus (Klackenberg, 1985), *Exacum* has been divided in two sections based on phenotypic and biogeographic evidence. Section *Exacum* (21 species, including four saprophytes previously included in *Cotylanthera*) is restricted to Sri Lanka, India and the Himalayas. This section comprises biennial robust plants with large flowers (*c.* 2–7 cm in diameter; Klackenberg, 1985) and is characterized by a wide range of published chromosome numbers inferred as $n = 9$, 16, 17, 18, 26, 27, 28, 30, 31 and 34. If we exclude the approximate $n = 16$, reported with caution for *Exacum tenuis* (under *Cotylanthera*), of which confirmation is needed, and the intraspecific variation ($n = 26$, 27, 28, 30) observed in the *E. trinervium* complex, which might be the result of allopolyploidy/dysploidy events (Riseman *et al.*, 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 species) is distributed in Madagascar, Socotra and the African mainland. All the investigated members of this section, generally small annual plants with a tiny corolla (*c.* 0.8–1.5 cm in diameter; Klackenberg, 1985), show either $n = 16$ (14 species, Madagascar) or $n = 18$ (1 species, 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 group. Some systematic support can be drawn if the respective Madacascan and Socotran groups are determined 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 for 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 Asia.

Sebaea

Although the morphologically variable *Sebaea* represents the most important genus in term of species number (*c*. 95 species; Schinz, 1906), no convincing taxonomic treatment has been proposed so far, mainly because of a lack of global studies integrating phylogenetic hypotheses, biogeographical data and cytological evidence. The present karyological investigation and the range of chromosome variation detected ($n = 9$, 14, 21, 28) help elucidate the possible evolutionary history of the genus.

The lowest chromosome number detected so far in *Sebaea* ($n = 9$), indeed in Exaceae, occurs in *S. madagascariensis*. This species, endemic to the north-western part of Madagascar, differs from all other *Sebaea* by having a '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 indicate affinities with *Ornichia* and *Exacum*. As also evidenced by our karyological data, *S. madagascariensis* probably deserves generic ranking.

The most common chromosome number in *Sebaea* ($n = 14$, $2n = 28$; Table 2) 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 mainly between September and December. In addition, members of this group formed a well-supported clade in a phylogenetic analysis of the tribe based on plastid DNA 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 to linear stigma and come into flower in March–April (Paiva & Nogueira, 1990).

Finally, *S. brachyphylla* shows intraspecific variation, with $n = 21$ found in African populations and $n = 28$ in the Malagasian ones (Table 2), adding to the confusing taxonomy reported within this taxon (Hedberg, 1955). Hence, at least four different taxa are referred to as *S. brachyphylla* (Boutique, 1972; Paiva & Nogueira, 1990), with the type species described from Madagascar. It would be interesting to survey additional taxa for chromosome counts from both tropical Africa and Madagascar, including the type locality, to confirm or discuss the observed karyological pattern. Finally, the report from Thulin (1970) for *S. brachyphylla* ($2n = 22$) is not confirmed by our study, either in *Sebaea* or in other Exaceae and might be taken with caution.

Overall, if we exclude *S. madagascariensis* from *Sebaea* (Yuan *et al.*, 2003), we can postulate a base chromosome number of $x = 7$ for *Sebaea*, with a group of tetraploid species ($n = 14$) centered in South Africa and a group of tetra/hexaploid 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 Exaceae, the following haploid chromosome numbers have been detected: $n = 9$ (*Sebaea*, 1 sp.; *Exacum*, 1 sp.), $n = 14$ (*Sebaea*, *c*. 29 spp.; *Ornichia*, 1 sp.), $n = 16$ (*Exacum*, 14 spp.; *Tachiadenus*, 2 spp.), $n = 17$ (*Exacum*, 1 sp.), $n = 18$ (*Exacum*, 2 spp.), $n = 21$ (*Sebaea*, 13 spp.), $n = 26$ (*Exacum*, 2 spp.), $n = 27$ (*Exacum*, 3 spp.), $n = 28$ (*Sebaea*, 4 spp.; *Exacum*, 2 spp.), $n = 30$ (*Exacum*, 1 sp.), $n = 31$ (*Exacum*, 5 spp.), $n = 34$ (*Exacum*, 5 spp.). These numbers confirm the polybasic and dysploid nature of both *Exacum* and *Sebaea s.l.* karyotypes. Hence, several intraspecific cytotypes have been detected for, e.g., *Sebaea brachyphylla*, *S. minutiflora*, *Exacum trinervium* and *E. pedunculatum*, which may indicate cryptic speciation in the absence of detectable morphological variation. Our current sampling does not allow 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 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* (section *Africana*), we can infer a primarily dysploid series $x = 7$, 8 and 9 for 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 Exaceae and thus an ascending dysploid series for the tribe: (1) the haploid numbers $n = 14$, 21 and 28 ($x = 7$) occur in most of the species investigated in this study (44 of 75), followed by $n = 16$ ($x = 8$; 16 of 75) and $n = 9$, 18 ($x = 9$; 5 of 75); (2) Exaceae species based on $x = 7$ generally show a wide distribution; and (3) $x = 7$ occurs in morphologically 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 phylogenetic analysis of the tribe (Yuan *et al.*, 2003).

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