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Short communication

Chromosome numbers of South African Umbelliferae (Apiaceae)

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Abstract

Chromosome numbers are reported for 12 species from nine genera of South African Umbelliferae, of which seven species and one genus (*Itasina*) are recorded for the first time. A detailed list of all published chromosome counts for southern African species is also presented, together with a review of the literature. The new data obtained are briefly discussed in the context of the taxonomy and relationships of local Umbelliferae. The counts agree with previous reports except that *Annesorhiza* appears to have 2n=22, with or without one additional B-chromosome, and not 2n=24 as reported in the literature. The number for *Itasina* (2n=24) is of considerable interest and indicates that a detailed chromosome study of the South African genera *Annesorhiza* and *Chamarea* may yield valuable taxonomic information. © 2010 SAAB. Published by Elsevier B.V. All rights reserved.

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1. Introduction

Chromosome numbers are part of a multidisciplinary approach to the characterization of taxa. The South African Umbelliferae include several anomalous and taxonomically isolated genera that are of importance in unravelling the relationships within the family as a whole (Burtt, 1991; Magee et al., 2010a,b; Van Wyk and Tilney, 2004), yet chromosome counts are available only for a limited number of species. Among the approximately 205 species of southern African Umbelliferae (Allison and Van Wyk, 1997; Burtt, 1991; Liu et al., 2007; Magee et al., 2008a,b, 2009a,b; Schubert and Van Wyk, 1997; Tilney and Van Wyk, 2002; Van Wyk and Tilney, 2003, 2004; Winter et al., 2008), chromosome counts based on local material are available for 25 species only, belonging to 19 genera (Table 1). Most of them are South African endemics or they are at least endemic or subendemic to the African continent. The only exceptions are the more widely distributed Apium graveolens L. and Torilis arvensis (Huds.) Link. This means that chromosome numbers of only 23 South African

species (indigenous or naturalised) have been determined up to now (Pimenov et al., 2002).

The most important sources of data include two publications by L. Constance et al. (Constance and Chuang, 1982; Constance et al., 1976). Some additional references on the identity of the species that were counted can be found in Burtt (1991). The only other counts reported are those of Riley and Hoff (1961) for *Steganotaenia araliacea* Hochst., Cauwet (1971) and Cauwet-Marc (1979) for *Bupleurum mundtii* Cham. & Schltdl. and Winter and Van Wyk (1996) for some South African species of *Heteromorpha* Cham. & Schltdl.

The aim of this paper is to (1) report chromosome numbers for an additional 12 South African species from nine genera of Umbelliferae, and (2) to review all available data on southern African species and the associated literature.

2. Materials and methods

Plant material (flower buds and fruits) was collected during two short excursions before and after the 4th International Apiales Symposium in Pretoria, 3 to 19 January 2003 (Table 2). Author citations for the scientific names given in Tables 1 and 2 are not repeated from here on. Fixations were made by M.G. Pimenov and voucher specimens were collected and identified

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by M.G. Pimenov, B.-E. Van Wyk, E.V. Kljuykov and T.A. Ostroumova; they are kept in MW.

Meiotic chromosomes were examined in pollen mother cells (Fig. 1). Flower buds were fixed in the field in 3:1 96% ethanol–glacial acetic acid mixture and then stained with 1% acetocarmine for 3 to 4 days at about 5 °C. After staining, the anthers were squashed and mounted. Somatic chromosomes were examined in meristem cells of root tips from germinating seeds (Fig. 2). After a pretreatment in 0.01% aqueous colchicine solution for 3 h at room temperature, the root tips were fixed in 3:1 96% ethanol–glacial acetic acid mixture and then stained with 1% acetocarmine at 100 °C for 3 to 5 min. Both the anthers and the root tips were squashed on a glass microscope slide in chloral hydrate (Hoyer's solution).

3. Results and discussion

Chromosome numbers were determined for 12 species from nine genera (Table 2). These counts include a first record for the genus *Itasina* Raf. and also first counts for seven other species (*Afrosciadium caffrum, A. magalismontanum, Anginon difforme, A. paniculatum, A. swellendamense, Lichtensteinia lacera* and *Notobubon capense*). In addition, a first correct count for *Annesorhoza macrocarpa* is presented.

The chromosome number of *Itasina filifolia* (n=12) is rather unusual in Apioideae. It is known for only 24 species of the subfamily (representing 1.6% of species investigated) and was found in the traditional tribes Scandiceae (two species), Caucalideae (four species), Smyrnieae (one species), Apieae (14 species), Peucedaneae (one species) and Tordylieae (two species). Itasina traditionally belongs to the tribe Apieae but molecular systematic evidence recently showed that it is related to the genera Annesorhiza Cham. & Schltdl., Astydamia DC., Chamarea Eckl. & Zeyh., Ezosciadium B.L.Burtt and Molopospermum W.J.D. Koch (Magee et al., 2010b) and that these genera should be recognized as a new tribe, the Annesorhizeae Magee et al. (Magee et al., 2010b). These genera (especially Ezosciadium) would be worth sampling in further cytological studies. Counts of n=12 (or 2n=24) occur sporadically in species of unrelated genera, including Ammoselinum Torr. & A. Gray, Apium L., Bunium L., Bupleurum L., Carum L., Cnidium Cusson ex Jussieu, Pimpinella L., Spuriopimpinella (H. Boisseu) Kitag. and Tilingia Regel (Pimenov et al., 2002). The count of n = 12 in Umbelliferae deserves special attention as it is common in Araliaceae, the reputedly related family. Moreover, x=12 or x=6 is regarded as the most plausible basic and ancestral chromosome number in Apiales (Sharma and Chatterji, 1964; Yi et al., 2004). In connection with this putative close affinity, n=12 in the woody African species S. araliacea

Table 1

Previously determined chromosome numbers for 19 genera and 25 species of South African Umbelliferae.

Genera and species	Chromosome number	References
Afroligusticum wilmsianum (H. Wolff.) P.J.D.Winter [as Peucedanum wilmsianum H.Wolff]	n=11	Constance and Chuang (1982)
Afrosciadium platycarpum (Sond.) P.J.D.Winter [as Peucedanum platycarpum E.Mey ex Sond.]	n=22	Constance and Chuang (1982)
	n=11	Burtt (1991) [Eastern Cape specimen]
Agrocharis melanantha Hochst.	n=22	Constance and Chuang (1982);
		Burtt (1991, p. 152; Sani Pass specimen)
Alepidea natalensis J.M.Wood & Evans	n=8	Constance and Chuang (1982)
Anginon rugosum (Thunb.) Raf. [=A. uitenhagense (Eckl. & Zeyh.) B.L.Burtt], as	n=11	Burtt (1991)
Annezorhiza macrocarpa Eckl. & Zeyh.	n=12	Burtt (1991)
Apium graveolens L.	n=11	Constance et al. (1976);
		Constance and Chuang (1982)
Arctopus echinatus L.	n=9	Constance et al. (1976)
Bupleurum mundtii Cham. & Schltdl.	2n=16	Cauwet (1971); Cauwet-Marc (1979)
Conium chaerophylloides (Thunb.) Sond.	n=11	Constance and Chuang (1982)
C. fontaneum Hilliard & B.L.Burtt	n=11	Constance and Chuang (1982)
Cynorhiza typica Eckl. & Zeyh. [as Peucedanum olifantianum (Koso-Pol.) M.Hiroe]	n=11	Constance and Chuang (1982)
Dasispermum capense (Lam.) Magee & BE.Van Wyk [as Stoibrax capense (Lam.) B.L.Burtt]	n=9	Constance et al. (1976)
D. humile (Meisn.) Magee & BE.Van Wyk [as Sonderina humilis (Meisn.) H.Wolff]	n=8	Burtt (1991)
D. suffruticosum (Berg.) B.L.Burtt	n=9	Constance et al. (1976);
		Constance and Chuang (1982)
Glia prolifera (Burm.f.) B.L.Burtt	n=11	Constance et al. (1976)
Hermas villosa (L.) Thunb.	n=7	Constance et al. (1976)
Heteromorpha arborescens (Spreng.) Cham. & Schltdl.	n=11	Constance and Chuang (1982)
H. arborescens var. frutescens P.J.D.Winter	2n=22	Winter and Van Wyk (1996)
H. involucrata Conrath	2n=22	Winter and Van Wyk (1996)
H. pubescens Burtt Davy	2n=22	Winter and Van Wyk (1996)
Lichtensteinia interrupta (Thunb.) Sond. [as L. kolbeana Bolus]	n=11	Constance and Chuang (1982)
Notobubon galbanum (L.) Magee [as Peucedanum galbanum (L.) Benth. & Hook.f.]	n=11	Constance and Chuang (1982)
N. laevigatum (Aiton) Magee [as Peucedanum capense (Thunb.) Sond.]	n=11	
Steganotaenia.araliacea Hochst.	2n=22	Riley and Hoff (1961);
-		Constance and Chuang (1982)
Torilis arvensis (Huds.) Link	n=6	Constance et al. (1976)

Table 2

Newly determined chromosome numbers for nine	genera and 12 species of South	African Umbelliferae (specimens a	are housed in MW).

Species	Origin and voucher specimen		2n
Afrosciadium caffrum (Meisn.) P.J.D.Winter Limpopo Province, slopes of Wolkberg, Haenertsburg, 23°56'S, 29°57'E. 05.01.2003,			22
[=Peucedanum caffrum (Meisn.) Philips]	Pimenov et al. 17		
A. magalismontanum (Sond.) P.J.D.Winter	Gauteng Province, 20 km E of Pretoria, near Bronkhorstspruit, 25°46'S, 28°32'E. 03.01.2003,		22
[=Peucedanum magalismontanum Sond.]	Pimenov et al. 1		
Anginon difforme (L.) B.L.Burtt	Western Cape Province, Kirstenbosch, 34°03'S, 18°23'E. 14.01.2003, Pimenov et al. 73	11	
A. paniculatum (Thunb.) B.L.Burtt	Western Cape Province, near Citrusdal, 32°25′S, 18°57′E. 15.01.2003, Pimenov et al. 82	11	
A. swellendamense (Eckl. & Zeyh.) B.L.Burtt	Western Cape Province, Worcester, 33°12'S, 19°23'E. 15.01.2003, Pimenov et al. 94	11	
Annesorhiza macrocarpa Eckl. & Zeyh.	Western Cape Province, Noordhoek, 34°05'S, 18°21'E. 13.01.2003, Pimenov et al. 59	11, 11+1B	
Cyclospermum leptophyllum (Pers.)	Limpopo Province, slopes of Wolkberg, Haenertsburg, 23°56'S, 29°57'E. 05.01.2003,		14
Sprague ex Britton & P.Wilson	Pimenov et al. 13		
Dasispermum suffruticosum (Berg.) B.L.Burtt	Western Cape Province, Betty's Bay, Stony Point, 34°21'S, 18°55'E. 12.01.2003,	9	18
	Pimenov et al. 47		
Hermas villosa (L.) Thunb.	Western Cape Province, Table Mountain, 33°57′S, 18°24′E.13.01.2003, Pimenov et al. 67	7	
Itasina filifolia (Thunb.) Raf.	Western Cape Province, Cape Peninsula, 34°21′S, 18°22′E. 3.01.2003, Pimenov et al. 61	12	
Lichtensteinia lacera Cham. & Schltdl.	Western Cape Province, near Silver Mine, 34°05′S, 18°25′E. 13.01.2003, Pimenov et al. 49	11	
Notobubon capense (Eckl. & Zeyh.) A.R.Magee	Western Cape Province, Betty's Bay, 34°21'S, 18°55'E. 12.01.2003, Pimenov et al. 41		22
[=Peucedanum multiradiatum Drude]			

(Constance and Chuang, 1982) could be of considerable interest. *Steganotaenia* and *Polemanniopsis* B.L. Burtt (both woody shrubs or trees) have recently been recognized as a new tribe, the Steganotaeniae C.I. Calviño & S.R. Downie (Calviño and Downie, 2007). The count of n=12 in the herbaceous *Itasina* however, seems to be of independent origin, as there is no other evidence of a direct connection with *Steganotaenia* Hochst. or with Araliaceae. It is interesting to note that *Itasina* and *Steganotaenia* both form part of the early diverging and predominantly African lineages of the subfamily Apioideae (Magee et al., 2010b).

Our determination does not confirm the reported chromosome number of n=12 for *A. macrocarpa*, published by Burtt (1991) with a reference to Constance and Chuang, cited as "n=12 (C & C ined. based on *Batten 590* from E. Cape, E. London). Our data showed that the species has n=11, but sometimes its karyotype contains one additional B-chromosome. It is therefore likely that earlier researchers incorrectly counted a B-chromosome as one of the basic set. Since *Annesorhiza*, *Chamarea* and *Itasina* are now considered to be closely related (Magee et al., 2010b; Vessio, 2001), a wider survey of these genera seems desirable and will yield valuable information.

There is no straightforward evidence for the chromosome number of any species of the woody genus *Anginon* Raf. in the karyological literature. However, Burtt (1991) showed n=11 for *A. uitenhagense* on the basis of a re-identification of the *Bayliss 8877* collection studied by Constance and Chuang (1982) and reported as "*Peucedanum zeyheri*". Allison and Van Wyk (1997) reduced *A. uitenhagense* to the synonymy of *A. rugosum* but did not cite *Bayliss 8877* among the material examined. We determined the same count of n=11 for three correctly identified species of *Anginon* (see Table 2). This means that all counts available for the genera of the tribe Heteromorpheae M.F. Watson & S.R. Downie (*Anginon, Glia* Sond. and *Heteromorpha*) are n=11 or 2n=22.

The chromosome number of *Cyclospermum leptophyllum* [=*Apium leptophyllum* (Pers.) F.Muell.], a widely distributed

weed of warm and adjacent temperate regions of the world, has been determined at least 12 times. The determinations for plants from South and North America, Oceania, Europe and China invariably showed a count of n=7 (or 2n=14). This was confirmed by the present determination, the first one for African material.

For Dasispermum suffruticosum and Hermas villosa, our counts confirm previous ones (n=9 and n=7 respectively). In the first case, the chromosome number distinguishes Dasispermum Neck. ex Raf. from the genera Conium L., Athamanta L., Cicuta L., Cnidium, Selinum L., Sium L. and others, to which the species has been referred to at various times during its long taxonomic history. Recently, Magee et al. (2009b) have shown, on the basis of morphological and molecular evidence, that Dasispermum is congeneric with another South African genus Sonderina H.Wolff, and that Stoibrax capensis is also part of this genus. The known counts of n=8 for *Dasispermum humile* (=Sonderina humilis) and n=9 for D. capense (=S. capensis)(Table 1) indicate that the more broadly circumscribed Dasispermum has n=8 or n=9 and that there is a reduction in the chromosome number in this group. More counts will be valuable and interesting, as the genus now comprises seven species (Magee et al., 2009b, 2010a).

Hermas L. is an anomalous genus with affinities in the new subfamily Azorelloideae, where it was moved from its traditional position in the subfamily Hydrocotyloideae. In general, the subfamily Hydrocotyloideae in its traditional circumscription is not monophyletic (Nicolas and Plunkett, 2009). Within the subfamily Azorelloideae, the count of n=7 found in Hermas, is unique. Traditionally Hermas had been included in the tribe of Mulineae, but modern molecular analyses (Nicolas and Plunkett, 2009; Plunkett, 2001) showed an isolated position for the tribe on the border between Umbelliferae and Araliaceae. Morphological analyses by Liu (2004) and Liu et al. (2009), as well as molecular systematic evidence (Plunkett et al., 2004) indicated that the group should be recognized as a separate subfamily, the Azorelloideae.

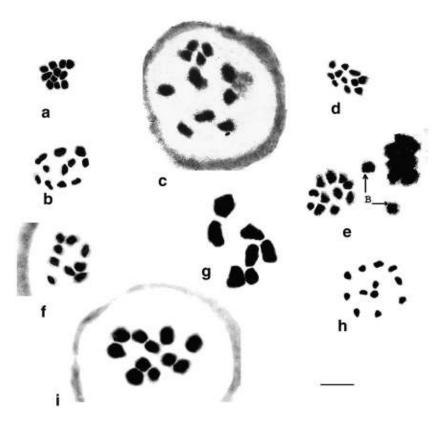


Fig. 1. Microphotographs of meiotic chromosomes of some South African Umbelliferae. (a) Anginon difforme (n=11); (b) A. paniculatum (n=11); (c) A. swellendamense (n=11); (d) Annesorhiza macrocarpa (n=11); (e) A. macrocarpa with B-chromosomes (n=11+1B) [a second set of chromosomes that did not separate is visible to the right, with their corresponding B-chromosome]; (f) Dasispermum suffruticosum (n=9); (g) Hermas villosa (n=7); (h) Itasina filifolia (n=12); and (i) Lichtensteinia lacera (n=11). Voucher specimens are listed in Table 2. Scale bar=10 μ m.

The number of n=11 for *L. lacera* is the second determination for the genus. It corresponds to Constance and Chuang's (1982) count for the eastern Cape form of *L. interrupta*, previously known as *L. kolbeana*. This number is widely distributed in Apioideae, but very rare in Saniculoideae (only the critical South American tetraploid genus *Oligocladus* Chodat & Wilczek has n=11). This confirmation is important because it strongly supports the idea that *Lichtensteinia* is more closely related to the subfamily Apioideae than to the Saniculoideae, despite sharing several morphological characters with the latter. Molecular evidence indicates that *Lichtensteinia* is sister to the cytologically unknown genus *Marlothiella* H. Wolff and that both are sister to all other Apioideae (Calviño et al., 2006, 2008; Magee et al., 2010b).

Finally, according to our data, three South African species that were previously included in the broad concept of *Peucedanum* L. have 2n=22. The two herbaceous species from the northern part of South Africa are now included in the newly described genus *Afrosciadium* P.J.D.Winter, while the shrubby species from the Cape are now placed in the genus *Notobubon* B-E.Van Wyk (Magee et al., 2009a; Winter et al., 2008). These data are unfortunately not very informative to elucidate relationship among the species, because this count is the most frequent in *Peucedanum* sensu lato as well as in related and unrelated genera of Apioideae. The need to subdivide African *Peucedanum* species was also indicated by the studies of Ostroumova and Pimenov (1997), where the considerable diversity in the fruit structure was highlighted for the first time. We were unable to confirm the occurrence of tetraploidy (n=22), reported in *Afrosciadium platycarpum* from the eastern Cape and also *Agrocharis melanantha* from Sani Pass (Table 1).

4. Conclusions

Chromosome numbers of the Umbelliferae of South Africa are insufficiently known and do not yet allow for global comparisons of the family as a whole. We determined chromosome numbers for 12 Umbelliferae species of the South African flora. The genus *Itasina* and seven other species were counted for the first time while the previously reported chromosome numbers of the remaining species were confirmed or sometimes corrected. This study and the review of available data showed that chromosome information can be of value in critical taxonomic revisions of the interesting and anomalous early diverging lineages of southern African Umbelliferae.

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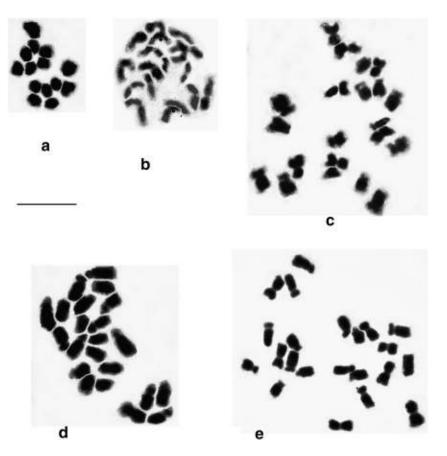


Fig. 2. Microphotographs of somatic chromosomes of some South African Umbelliferae. (a) *Cyclospermum leptophyllum* (2n=14); (b) *Dasispermum suffruticosum* (2n=18); (c) *Afrosciadium caffrum* (2n=22); (d) *A. magalismontanum* (2n=22); and (e) *Notobubon capense* (2n=22). Voucher specimens are listed in Table 2. Scale bar=10 μ m.

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