



The first phylogenetic hypothesis for the southern African endemic genus *Tulbaghia* (Amaryllidaceae, Alliioideae) based on plastid and nuclear DNA sequences

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Southern Africa has three indigenous genera in the Amaryllidaceae subfamily Alliioideae. *Prototulbaghia* is monospecific and *Allium* is represented by a single species, whereas *Tulbaghia* has > 20 described species. *Tulbaghia* spp. are popular in horticulture and used extensively as medicinal plants in southern Africa. Despite their popularity and economic importance, species delimitation is problematic and the infrageneric classification uncertain. The objective of this study was to test the monophyly of *Tulbaghia* and the relationships of *Prototulbaghia* and to produce the first molecular phylogenetic hypothesis for *Tulbaghia* as a basis for a revised infrageneric classification and species concepts. Fifty-four *Tulbaghia* samples covering 17 of the c. 23 accepted species were included in this study. In total, 160 new sequences of nuclear ribosomal internal transcribed spacers (ITS) and plastid encoded *trnL-F* and *ndhF* were produced for this study and were analysed using maximum parsimony and Bayesian inference. *Tulbaghia* including *Prototulbaghia* is strongly supported as monophyletic (BP = 100%, PP = 1.00) and in general clades that are well supported in either or both of the ITS and the combined plastid analysis are also well supported in the total combined analysis. Several major clades within *Tulbaghia* are resolved and ITS and plastid data indicated that *Prototulbaghia* and some *Tulbaghia* spp. are in need of taxonomic re-circumscription. © 2016 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2016, 181, 156–170

ADDITIONAL KEYWORDS: molecular phylogeny – *Prototulbaghia*.

INTRODUCTION

SOCIETY GARLIC (*TULBAGHIA*; AMARYLLIDACEAE), THE ENDEMIC ALLIACEOUS GENUS OF SOUTHERN AFRICA

Southern Africa has representatives of three indigenous genera in Amaryllidaceae subfamily Alliioideae: *Allium* L., *Tulbaghia* L. and *Prototulbaghia* Vosa (Table 1; Fig. 1). The distribution of *Allium* (tribe

Allieae) is almost entirely in North American/Eurasian, but a single species in South Africa, *Allium synnotii* G. Don (synonym *A. dregeanum* Kunth), is the sole known representative of the genus in sub-Saharan Africa (de Wilde-Duyfjes, 1976; de Sarker *et al.*, 1997). The diverse and widespread *Tulbaghia* (20–30 species; tribe Tulbaghieae) and the recently described monospecific genus *Prototulbaghia* (Vosa, 2007b) contain the remaining southern African alliaceous species. *Prototulbaghia* is represented by a single species with a restricted range on the summit of the Leolo Mountain Range, Limpopo Province, South Africa (Vosa, 2007b; Siebert *et al.*, 2008).

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Table 1. Current taxonomic status of the southern African Amaryllidaceae subfamily Allioideae genera, *Allium*, *Prototulbaghia* and *Tulbaghia*

World checklist of selected plant families (Govaerts <i>et al.</i> , 2016)	Plants of southern Africa: an annotated checklist (Archer, 2003)*
<i>Allium synnotii</i> G.Don, Mem. Wern. Nat. Hist. Soc. 6: 19 (1827). Syn: <i>Allium dregeanum</i> Kunth	A. dregeanum Kunth Perennial. Geophyte. Ht 0.3–1.2 m. Alt 120–1525 m. FS, NC, WC, EC
<i>Prototulbaghia siebertii</i> Vosa, Caryologia 60: 277 (2007)	
<i>Tulbaghia acutiloba</i> Harv., Thes. Cap. 2: 51 (1863)	T. acutiloba Harv. Perennial. Herb. Ht 0.12–0.2 m. Alt 100–2530 m. B, LIM, NW, G, M, S, FS, KZN, L, EC
<i>Tulbaghia aequinoctialis</i> Welw. ex Baker, Trans. Linn. Soc. Lond., Bot. 1: 246 (1878). <i>Tulbaghia aequinoctialis</i> subsp. <i>aequinoctialis</i> . <i>Tulbaghia aequinoctialis</i> subsp. <i>monantha</i> (Engl. & Gilg) R.B.Burb., Notes Roy. Bot. Gard. Edinburgh 36: 91 (1978)	T. affinis Link (insufficiently known) [Vosa. 1975. Ann. Bot. (Rome) 34: 47–121.]. Synonym of <i>T. alliacea</i> L.f. T. alliacea L.f. Perennial. Herb, geophyte. Ht 0.26–0.45 m. Alt 50–2250 m. WC T. calcarea Engl. & K. Krause (insufficiently known) [Vosa. 1975. Ann. Bot. (Rome) 34: 47–121.] Perennial. Herb. Ht 0.15–0.4 m. N T. cameronii Baker. Perennial. Herb. Ht 0.15–0.25 m. N
<i>Tulbaghia alliacea</i> L.f., Suppl. Pl.: 193 (1782)	
<i>Tulbaghia calcarea</i> Engl. & Krause, Bot. Jahrb. Syst. 45: 142 (1910)	
<i>Tulbaghia cameronii</i> Baker, J. Bot. 16: 321 (1878)	
<i>Tulbaghia capensis</i> L., Mant. Pl.: 223 (1771)	T. capensis L. <i>T. pulchella</i> Avé-Lall., illegitimate name (Vosa. 1975. Annali di Botanica 34: 47–121.). Perennial. Herb. Ht 0.12–0.35 m. Alt 10–1500 m. WC, EC T. cepacea L.f. var. <i>maritima</i> Vosa. Perennial. Herb. Ht 0.1–0.15 m. EC. Synonym of <i>T. violacea</i> Harv. T. cernua Avé-Lall. Syn: <i>T. campanulata</i> N.E.Br. (Vosa. 1981. J. S. Afr. B. 47: 57–61.). Perennial. Herb. Ht up to 0.25 m. Alt ?–1770 m. B, LIM, NW, M, FS, KZN, L, WC, EC
<i>Tulbaghia cernua</i> Fisch., C.A.Mey. & Avé-Lall., Index Seminum (LE) 9 (Suppl.): 25 (1843)	
<i>Tulbaghia coddii</i> Vosa & R.B.Burb., Ann. Bot. (Rome) 34: 104 (1975 publ. 1977)	T. coddii Vosa & Burb. <i>T. poetica</i> Burb. (Burbidge. 1978. Notes from the Royal Botanic Garden Edinburgh 36: 77–103.). Perennial. Herb. Ht 0.1–0.2 m. Alt ?–2200 m. M T. cominsii Vosa. Perennial. Herb. Ht 0.09–0.2 m. Alt ?–365 m. EC T. dregeana Kunth. Perennial. Herb. Ht 0.15–0.2 m. Alt ?–915 m. NC, WC
<i>Tulbaghia cominsii</i> Vosa, J. S. African Bot. 45: 128 (1979)	
<i>Tulbaghia dregeana</i> Kunth, Enum. Pl. 4: 483 (1843)	
<i>Tulbaghia friesii</i> Suess., Trans. Rhodesia Sci. Assoc. 43: 76 (1951)	
<i>Tulbaghia galpinii</i> Schltr., J. Bot. 35: 282 (1897)	T. galpinii Schltr. Perennial. Herb. Ht up to 0.15 m. Alt 90–2000 m. EC T. hypoxidea Sm. (insufficiently known) [Vosa. 1975. Ann. Bot. (Rome) 34: 47–121.] T. leucantha Baker <i>T. dieterlenii</i> E.Phillips [Vosa. 1975. Ann. Bot. (Rome) 34: 47–121.]. Perennial. Herb. Ht 0.1–0.25 m. Alt 30–2325 m. N, LIM, NW, G, M, S, FS, KZN, L, NC, WC, EC
<i>Tulbaghia leucantha</i> Baker in W.H.Harvey & auct. suc. (eds), Fl. Cap. 6: 404 (1897)	

Table 1. Continued

World checklist of selected plant families (Govaerts <i>et al.</i> , 2016)	Plants of southern Africa: an annotated checklist (Archer, 2003)*
<i>Tulbaghia ludwigiana</i> Harv., Bot. Mag. 64: t. 3547 (1837)	<i>T. ludwigiana</i> Harv. Perennial. Herb. Ht 0.25–0.91 m. Alt 15–2325 m. M, S, KZN, EC
<i>Tulbaghia luebbertiana</i> Engl. & Krause, Bot. Jahrb. Syst. 45: 142 (1910)	<i>T. luebbertiana</i> Engl. & K.Krause (insufficiently known) [Vosa. 1975. Ann. Bot. (Rome) 34: 47–121.]
<i>Tulbaghia macrocarpa</i> Vosa, Ann. Bot. (Rome) 34: 84 (1975 publ. 1977)	
<i>T. maritima</i> Vosa, Herbertia 65: 61 (2011 publ. 2012)	
<i>Tulbaghia montana</i> Vosa, Ann. Bot. (Rome) 34: 84 (1975 publ. 1977)	<i>T. montana</i> Vosa. Perennial. Herb. Ht 0.3–0.4 m. Alt ?–2500 m. KZN, L, EC
<i>Tulbaghia natalensis</i> Baker, Gard. Chron., III, 1891(1): 668 (1891)	<i>T. natalensis</i> Baker. Perennial. Herb. Ht 0.15–0.36 m. Alt 150–1860 m. KZN, EC
<i>Tulbaghia nutans</i> Vosa, Ann. Bot. (Rome) 34: 84 (1975 publ. 1977)	<i>T. nutans</i> Vosa. Perennial. Herb. Ht up to 0.3 m. Alt ?–2400 m. M
	<i>T. pauciflora</i> Baker (insufficiently known) [Vosa. 1975. Ann. Bot. (Rome) 34: 47–121.]
<i>Tulbaghia pretoriensis</i> Vosa & Condy, Caryologia 59: 166 (2006)	
<i>Tulbaghia rhodesica</i> R.E.Fr., Wiss. Erg. Schwed. Rhod.-Kongo Exped. 1: 227 (1916)	
<i>Tulbaghia simmleri</i> Beauverd. Bull. Herb. Boissier, sér. 2, 8:988, 1909	<i>T. simmleri</i> P.Beauv. Syn: <i>T. daviesii</i> Grey, <i>T. fragrans</i> I.Verd., <i>T. pulchella</i> P.E.Barnes, illegitimate name (Vosa. 1980. J. S. Afr. Bot. 46: 109–114.) Perennial. Herb. Ht up to 0.4 m. Alt 960–1120 m. LIM, M
<i>Tulbaghia tenuior</i> K.Krause & Dinter. Bot. Jahrb. 45:141, 1910	<i>T. tenuior</i> K.Krause & Dinter. Syn: <i>T. karasbergensis</i> P.E.Glover (Vosa. 1975. Annali di Botanica 34: 47–121.) Perennial. Herb. Ht up to 0.3 m. Alt ?–1035 m. N, NC
<i>Tulbaghia transvaalensis</i> Vosa. Ann. Bot. (Rome) 34:87, (1975 publ. 1977)	<i>T. transvaalensis</i> Vosa. Perennial. Herb. Ht 0.15–0.25 m. Alt? LIM, KZN
<i>Tulbaghia verdoorniae</i> Vosa & R.B.Burb. Ann. Bot. (Rome) 34:102, (1975 publ. 1977)	<i>T. verdoornia</i> Vosa & Burb. Syn: <i>T. carnosa</i> Burb. (7). Perennial. Herb. Ht 0.15–0.3 m. Alt ± 380 m. EC
<i>Tulbaghia violacea</i> Harv. Bot. Mag. 64: t.3555, 1837.	<i>T. violacea</i> Harv. Syn: <i>Omentaria cepacea</i> Salisb. (Vosa. 1975. Annali di Botanica 34: 47–121.), <i>T. cepacea</i> L.f. (Vosa. 1980. J. S. Afr. Bot. 46: 109–114.) Perennial. Herb. Ht 0.2–0.45 m. Alt 3–1220 m. KZN, WC, EC
<i>Tulbaghia violacea</i> subsp. <i>violacea</i> .	
<i>Tulbaghia violacea</i> subsp. <i>macmasteri</i> Vosa, Herbertia 63: 119 (2009)	

*Countries and current South African (RSA) provinces referred in this section: Namibia (N), Botswana (B), Limpopo Province (LIM), North-West Province (NW), Gauteng Province (G), Mpumalanga Province (M), Swaziland (S), Free State Province (FS), KwaZulu-Natal Province (KZN), Lesotho (L), Northern Cape Province (NC), Western Cape Province (WC), Eastern Cape Province (EC).

Previously these genera have been placed in Liliaceae, Amaryllidaceae (often as tribes Allieae, Gilliesieae and Agapantheae Dumort) and in Alliaceae. Recently, *sensu* APG III (2009), it had been found that they fall into subfamily Allioideae – a widespread group with the majority of the genera found mostly in the New World. The family name Amaryllidaceae has been super-conserved over Alliaceae, the

older name (Meerow *et al.*, 2007; Chase, Reveal & Fay, 2009). A tribal classification of the genera in the Amaryllidaceae subfamily Allioideae according to Fay, Rudall & Chase (2006) and Chase *et al.* (2009) recognized three tribes, the monogeneric Allieae, Gilliesieae (13 genera) and Tulbaghieae including only the southern African genera *Tulbaghia* and *Prototulbaghia*.

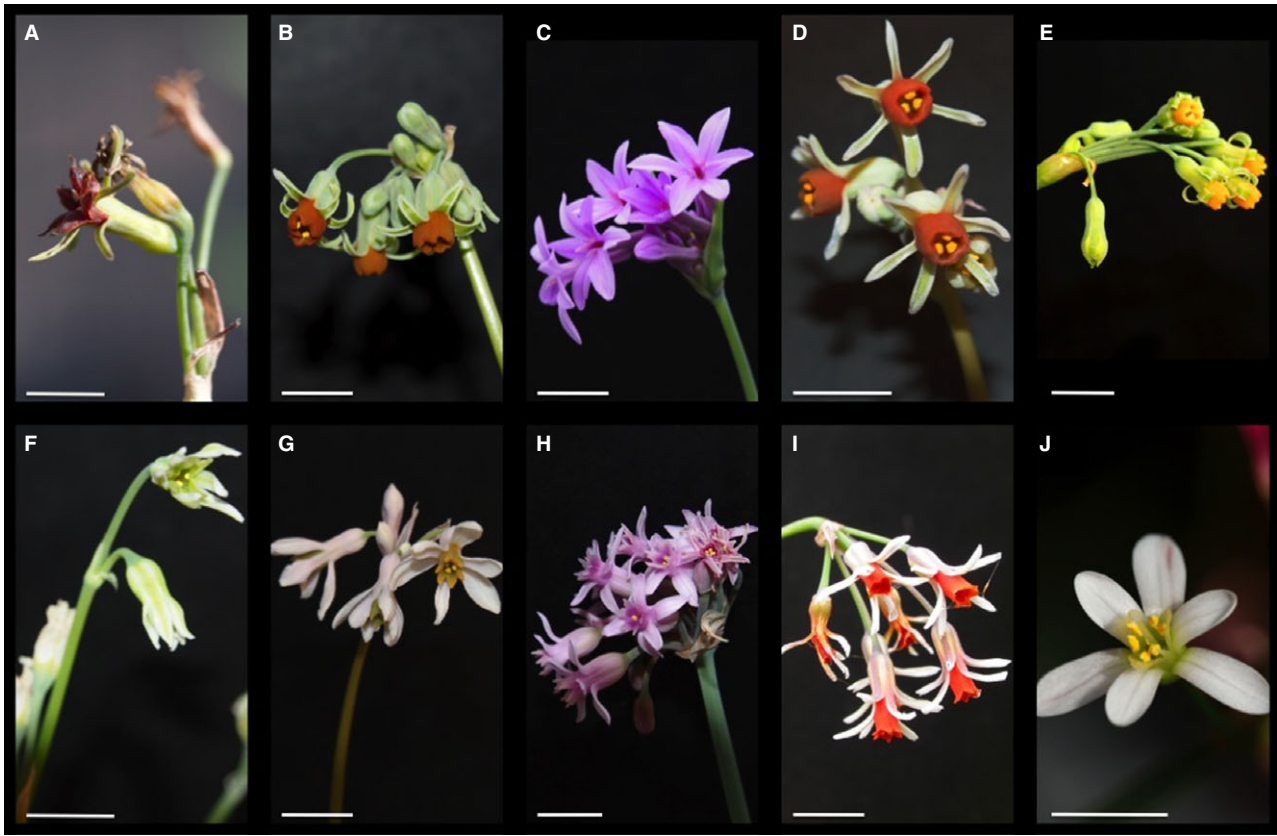


Figure 1. Floral variation among *Tulbaghia* and *Prototulbaghia*: (A) *Tulbaghia capensis*, (B) *T. alliacea*, (C) *T. violacea*, (D) *T. acutiloba*, (E) *T. ludwigiana*, (F) *T. galpinii*, (G) *T. natalensis*, (H) *T. simmleri*, (I) *T. montana*, (J) *Prototulbaghia siebertii* (bar = 10 mm).

TAXONOMIC TREATMENTS

The taxonomy of *Tulbaghia*, since its inception, has been fraught with errors, conflicts and confusion. Linnaeus (*Mant. Pl.* p. 148, 1771) first proposed the genus *Tulbaghia* based on material acquired from Rijk Tulbagh, who was governor of the Dutch-held Cape Colony. Since that time there have been several attempts at revising the genus. In 1844 Avé-Lallemant noted the confusion and attempted to rectify this in a largely neglected paper (Avé-Lallemant, 1844). Baker (1871) recognized seven species in two subgenera, *Eutulbaghia* (= *Tulbaghia*) containing *T. capensis* L. (Fig. 1A), *T. alliacea* L.f. (Fig. 1B), *T. acutiloba* Harv. (Fig. 1D), *T. dregeana* Kunth and *T. hypoxidea* Sm., which have fleshy or scarcely fleshy, fused coronas, and *Omentaria* containing *T. cepacea* L.f. and *T. violacea* Harv. (Fig. 1C) with free, corona lobes. Subsequent revisions were published by Uphof (1943), Vosa (1975) and Burbidge (1978).

Vosa's (1975) revision, with an additional three subsequent revisions (Vosa, 2000, 2007a, 2009), serve as the current taxonomic guide for the genus (Table 1). Both Vosa and Burbidge agreed on the

delimitations of *T. capensis*, *T. alliacea*, *T. ludwigiana* Harv. (Fig. 1E), *T. dregeana*, *T. acutiloba*, *T. galpinii* Schltr. (Fig. 1F), *T. natalensis* Baker (Fig. 1G), *T. tenuior* K.Krause & Dinter, *T. rhodesica* R.E.Fr. and Vosa's new species, *T. macrocarpa* Vosa. However, Vosa also described two new species, *T. coddii* Vosa & R.B.Burb. and *T. verdoorniae* Vosa & R.B.Burb.

Since the publications in the 1970s, a new species, *T. pretoriensis* Vosa & Condy (Vosa & Condy, 2006) and a new related genus *Prototulbaghia* (2007b) that contains one known species, *P. siebertii* Vosa (Fig. 1J), have been described. According to Archer's (2003) annotated checklist and including recently described species (see Table 1) there may be as many as 30 species of which 23 have been formally described (Vosa, 1975, 2000, 2012; Burbidge, 1978; Klopper *et al.*, 2006; Vosa & Condy, 2006), the remainder being insufficiently known or documented species. Vosa (2000) noted that only a few collections of *Tulbaghia* have been made north of the river Limpopo and, with a few exceptions, little information is known about the tropical species of the genus.

MORPHOLOGICAL GROUPINGS IN *TULBAGHIA*

The most discriminatory morphological feature of the genus is the corona. This is a unique feature of the genus, variations of which form much of the basis for subgeneric classifications (Baker, 1871; Vosa, 2009). Preliminary studies by Vosa (2009) on the ontogeny of the flower suggest that the corona is derived from fused dorsal outgrowths of the stamen filaments. These findings support similar assertions by Linnaeus (1771), Salisbury (1866) and Baker (1871), who referred to elements of the corona as staminodia (Vosa, 2009). Baker (1871) first suggested two subgenera for *Tulbaghia*, *Tulbaghia* (as *Eutulbaghia*) and *Omentaria*.

Tulbaghia subgenus *Tulbaghia*, members of which have a fleshy, or scarcely fleshy, fused corona, are divided further into six sections (Vosa, 2009). The first two sections contain one species each, *T. capensis* (section I) and *T. galpinii* (section II), both have a deeply lobed corona with the latter having a less fleshy corona. Section III, those with a fleshy, fused, more or less serrated corona, is the largest section, containing 14 species. This section also includes the problematic, variable species, such as *T. acutiloba*, *T. alliacea*, *T. cameronii* Baker, *T. cernua* Fisch., C.A.Mey. & Avé-Lavall, *T. leucantha* Baker and *T. ludwigiana*, and rare species, such as *T. coddii* Vosa & R.B.Burb., *T. dregeana*, *T. macrocarpa*, *T. nutans* Vosa, *T. pretoriensis*, *T. tenuior*, *T. transvaalensis* Vosa and *T. verdoorniae*, many of which are known from only one or a few localities. Section IV contains only *T. simmleri* Beauv. (Fig. 1H), which has a fused, scarcely fleshy, finely serrate corona. In section V, Vosa included those with a fleshy, fused, but lacinate corona, *T. montana* Vosa (Fig. 1I) and *T. natalensis*. Section VI contains only *T. rhodesica*, which has a scarcely fleshy corona with six short lobes. *Tulbaghia* subgenus *Omentaria* contains two species with free corona lobes, the highly variable *T. violacea* and the less common and smaller *T. cominsii* Vosa. *T. capacea* is currently accepted as a synonym of *T. violacea* (Vosa, 1980). The circumscription of *T. violacea* in particular has been difficult due to populations often showing a kind of uninterrupted variability, in plant size and flower dimensions, including corona length, shape and colour of lobes (Vosa, 1975, 2000, 2007a), which led Burbidge (1978) to establish several varieties. The previously included *T. violacea* var. *maritima* was recently raised to species level as *T. maritima* Vosa (Vosa, 1975) Vosa (2012). *Tulbaghia cominsii* differs from *T. violacea* s.s. in having thin, glaucous leaves (Vosa, 2000).

CONSERVATION AND USE

Tulbaghia is an economically important genus with several species being traded locally in southern

Africa as medicinal plants for various ailments such as colds, fevers, headaches and intestinal worms and as a snake repellent (Hulme, 1954; Watt & Breyer-Brandwijk, 1962; Batten & Bokelmann, 1966; Burton, 1990; Hutchings *et al.*, 1996; Van Wyk, Van Oudtshoorn & Gericke, 1997; Aremu & van Staden, 2013). Internationally, *Tulbaghia* spp. are marketed as horticultural crops. The most common species for medicinal and traditional uses are *T. violacea*, *T. alliacea*, *T. ludwigiana*, *T. natalensis* and *T. capensis*. However, it is the bulbs and rhizomes that are traded and these are often difficult to identify (Jäger & Stafford, 2012). *Tulbaghia alliacea* is considered a scarce, heavily utilized species in South Africa (Marshall, 1998). Dold & Cocks (2002) estimated that an average healer in the Eastern Cape Province of South Africa trades 92.5 kg of *T. alliacea* material per annum at an estimated R83.50 (7.8 USD) per kg. In a recent survey on informal trade in Cape Town, South Africa, two species, *T. capensis* and *T. dregeana*, were identified in use (Petersen *et al.*, 2012). High and increasing usage of especially the underground organs of several *Tulbaghia* spp. and the reliance on wild harvesting, threatens to affect their abundance and possibly make them susceptible to extinction (Aremu & van Staden, 2013).

The objectives of this study were to: (1) test the monophyly of *Tulbaghia* and its relationship to *Prototulbaghia*; and (2) to produce the first molecular phylogenetic hypothesis for *Tulbaghia* to test infra-generic classification and species concepts.

MATERIAL AND METHODS

TAXONOMIC SAMPLING

Most of the silica-dried leaf material used in this study was obtained from the Royal Botanical Garden Edinburgh (E) in Scotland. Additional plants were purchased from specialist growers and propagated at the Botanic Garden of the University of Copenhagen (C) in Denmark (Table 2). Additional taxa were obtained as silica-dried material from Roman Kubec at University of South Bohemia, Czech Republic, or from the living collection in Stellenbosch University Botanic Garden, South Africa. Vouchers of all the material are placed in the official herbaria of the three institutions (Table 2). The material used in this study is of both commercial and natural origin and identified by Gary Stafford, Michael Möller or Roman Kubec, and in some instances confirmed by other experts also listed in Table 2. One hundred and sixty new sequences were produced for this study and 33 sequences were downloaded from GenBank from previous studies primarily by Fay *et al.* (pers. comm. M. Fay, Royal Botanic Gardens,

Table 2. Taxa studied, voucher information and GenBank accessions

Taxon	Voucher	Origin	GenBank accession		
			<i>ndhF</i>	<i>trnL-F</i>	nrITS
<i>Gilliesia graminea</i> Lindl.		GenBank	HQ392923	AF117045	HQ393006
<i>Ipheion sessile</i> (Phil.) Traub		GenBank	HQ392926	HQ392968	HQ393010
<i>Ipheion</i> sp.	<i>Petersen og Seberg C888 (C)</i>	Cult. P1986-5003 (C)	KU692030	KU692085	KU692139
<i>Ipheion</i> cf. <i>uniflorum</i> 'Rolf Fiedler'		GenBank	HQ392928	HQ392969	HQ393012
<i>Leucocoryne coquimbensis</i> F.Phil. ex Phil.		GenBank	HQ392929	HQ392970	HQ393013
<i>Leucocoryne</i> sp.	<i>Petersen og Seberg C924 (C)</i>	Cult. S1994-1407 (C). Collected in Chile	KU692031	KU692086	KU692140
<i>Nothoscordum andicola</i> Kunth		GenBank	HQ392935	HQ392971	HQ393016
<i>Nothoscordum gaudichaudianum</i> Kunth		GenBank	HQ392937	HQ392972	HQ393018
<i>Nothoscordum inodorum</i> (Aiton) G.Nicholson		GenBank	HQ393019	HQ392973	HQ393019
<i>Nothoscordum montevidense</i> Beauverd		GenBank	HQ392939	HQ392974	HQ393020
<i>Nothoscordum nudicaule</i> (Lehm.) Guagl.		GenBank	HQ392940	HQ392975	HQ393021
<i>Nothoscordum texanum</i> M.E.Jones		GenBank	HQ392941	HQ392976	HQ393022
<i>Pabellonia incrassata</i> (Phil.) Quezada & Martic		GenBank	HQ392930	AF117054	HQ393014
<i>Prototulbaghia siebertii</i> Vosa	<i>2013-173 (STEU)</i>	Cult. 2013-173 (STEU)	–	KU692087	KU692141
<i>Prototulbaghia siebertii</i> Vosa	<i>Van Wyk & Siebert 1304 (PRU)</i>	Stephan Siebert, the Leolo Mountains, Limpopo, South Africa, SS001	KU692032	KU692088	–
<i>Tulbaghia acutiloba</i> Harv.	<i>Möller MM012B (E)</i>	David Fenwick 30/07/03. Cult. (E)	KU692033	KU692089	KU692142
<i>Tulbaghia acutiloba</i> Harv.	<i>Möller MM012C (E)</i>	TULB284 H&B 11996. Cult. 2003 0846 (E)	KU692034	KU692090	KU692143
<i>Tulbaghia acutiloba</i> Harv.	<i>Rønsted and Stafford NR529 (C)</i>	Simply Indigenous Nursery, SA	KU692035	KU692091	KU692144
<i>Tulbaghia alliacea</i> L.f.	<i>Rønsted and Stafford NR492 (C)</i>	Hoyland Plants, UK. Cult. (C)	KU692036	KU692092	KU692145
<i>Tulbaghia alliacea</i> L.f.	<i>Rønsted and Stafford NR530 (C)</i>	Simply Indigenous Nursery, SA	KU692037	KU692093	KU692146
<i>Tulbaghia alliacea</i> L.f.	<i>Kubec RK001 (CBFS).</i>	Hoyland Plants, UK. Cult. (CBFS)	KU692038	KU692094	KU692147

Table 2. *Continued*

Taxon	Voucher	Origin	GenBank accession		
			<i>ndhF</i>	<i>trnL-F</i>	nrITS
<i>Tulbaghia capensis</i> L.	Möller MM014 (E)	Cult. 2001 0432 (E)	KU692039	KU692095	KU692148
<i>Tulbaghia capensis</i> L.	1999-784 (STEU)	Cult. 1999-784 (STEU)	KU692040	–	–
<i>Tulbaghia cernua</i> Avé- Lall.	Möller MM001 (E)	David Fenwick CDR199, in trade as <i>T. Ludwigiana</i> . Cult. 2003 0842 (E)	KU692041	KU692096	KU692149
<i>Tulbaghia cernua</i> Avé- Lall.	Kubec RK005 (CBFS)	Liz Powney, Prime Perennials, UK. Cult. (CBFS). www.tulbaghia.com	KU692042	KU692097	KU692150
<i>Tulbaghia coddii</i> Vosa & R.B.Burb.	Möller MM015 (E)	Cult. 2001 0533 (E)	KU692043	KU692098	KU692151
<i>Tulbaghia coddii</i> Vosa & R.B.Burb.	Rønsted and Stafford NR493 (C)	Hoyland Plants, UK. Cult. (C)	KU692044	KU692099	KU692152
<i>Tulbaghia cominsii</i> Vosa	Möller MM010B (E)	C.McMaster A-C 13-03. Cult. 2003 0511 (E)	KU692045	–	–
<i>Tulbaghia cominsii</i> Vosa	Kubec RK007 (CBFS)	B & T Seed Company, France. Cult. (CBFS)	KU692046	KU692100	KU692153
<i>Tulbaghia cominsii</i> Vosa	Möller MM016B (E)	Cult. 2002 1624 (E). First recorded as <i>T. galpinii</i>	KU692047	KU692101	–
<i>Tulbaghia cominsii</i> Vosa	Rønsted and Stafford NR521 (C)	Sue Mann, Priory Plants, UK	KU692048	KU692102	KU692154
<i>Tulbaghia dregeana</i> Kunth	Möller MM002 (E)	David Fenwick – 30.07.03. T-3 DNA only	KU692049	KU692103	KU692155
<i>Tulbaghia dregeana</i> Kunth	Kubec RK009 (CBFS)	Cotswold Gardens Flowers, UK Cult. (CBFS)	KU692050	KU692104	KU692156
<i>Tulbaghia galpinii</i> Schltr.	Möller MM016A (E)	C.McMaster A-C 13-03. Cult. 2003 0512 (E)	KU692051	KU692105	KU692157
<i>Tulbaghia galpinii</i> Schltr.	Rønsted and Stafford NR522 (C)	Liz Powney, UK. Cult. (C). www.tulbaghia.com	KU692052	KU692106	KU692158
<i>Tulbaghia leucantha</i> Baker	Möller MM017B (E)	Canio Vosa 449/38 TULB 271. Cult. 2003 0848 (E)	KU692053	KU692107	KU692159
<i>Tulbaghia leucantha</i> Baker	Rønsted and Stafford NR496 (C)	Hoyland Plants, UK, Sold as <i>T. montana</i> . Cult. (C)	KU692054	KU692108	KU692160
<i>Tulbaghia ludwigiana</i> Harv.	Möller MM004 (E)	C.McMaster A-C 13.03. Cult. 2003 0513 (E)	KU692055	KU692109	KU692161
<i>Tulbaghia ludwigiana</i> Harv.	Möller MM011 (E)	David Fenwick living plant. Cult. 2003 0843 (E). First recorded as <i>T. macrocarpa</i>	KU692056	KU692110	KU692162
<i>Tulbaghia ludwigiana</i> Harv.	Kubec RK008 (CBFS)	Silverhill Seeds, SA. Cult. (CBFS)	KU692057	KU692111	KU692163

Table 2. Continued

Taxon	Voucher	Origin	GenBank accession		
			<i>ndhF</i>	<i>trnL-F</i>	nrITS
<i>Tulbaghia ludwigiana</i> Harv.	Rønsted and Stafford NR531 (C)	Simply Indigenous Nursery, SA	KU692058	KU692112	KU692164
<i>Tulbaghia maritima</i> Vosa	Möller MM009 (E)	A Salmon. Cult. 2001 0532 (E)	KU692059	KU692113	–
<i>Tulbaghia maritima</i> Vosa	Kubec RK006 (CBFS)	USB Liz Powney, UK. Cult. (CBFS). www.tulbaghia.com	KU692060	KU692114	KU692165
<i>Tulbaghia maritima</i> Vosa	Rønsted and Stafford NR594 (C)	Sue Mann, Priory Plants, UK	–	KU692115	KU692166
<i>Tulbaghia montana</i> Vosa	Möller MM020 (E)	David Fenwick 30.7.03. Cult. 2003 0844 (E)	–	KU692116	KU692167
<i>Tulbaghia montana</i> Vosa	Rønsted and Stafford NR420 (C)	Giants Castle Nature Reserve, Drakensberg, SA	KU692061	KU692117	KU692168
<i>Tulbaghia montana</i> Vosa	Möller MM017A (E)	Cult. 2001 0430 (E). First recorded as <i>T. leucantha</i>	KU692062	KU692118	KU692169
<i>Tulbaghia montana</i> Vosa	Möller MM012A (E)	Avon Bulbs Ltd, Somerset, UK, sold as <i>T. acutiloba</i> . Cult. 2001 0526A (E)	KU692063	KU692119	KU692170
<i>Tulbaghia montana</i> Vosa	Rønsted and Stafford NR495 (C)	Hoyland Plants, UK. Cult. (C)	KU692064	KU692120	KU692171
<i>Tulbaghia natalensis</i> Baker	Möller MM018 (E)	A. Salmon. Cult. 2001 0531 (E)	KU692065	KU692121	KU692172
<i>Tulbaghia natalensis</i> Baker	Kubec RK003 (CBFS)	Liz Powney, United Kingdom. Cult. (CBFS). www.tulbaghia.com	KU692066	KU692122	KU692173
<i>Tulbaghia natalensis</i> Baker	Rønsted and Stafford NR497 (C)	Hoyland Plants, UK. Cult. (C)	KU692067	KU692123	KU692174
<i>Tulbaghia natalensis</i> Baker	Rønsted and Stafford NR509 (C)	Greg Petit, Green Goblin Nursery, SA	KU692068	–	KU692175
<i>Tulbaghia natalensis</i> Baker	Rønsted and Stafford NR528 (C)	CDR84 Liz Powney, UK	KU692069	–	KU692176
<i>Tulbaghia simmleri</i> P.Beauv.	Möller MM003 (E)	Dyer (clone 8). Cult. 1999 2213 (E)	KU692070	KU692124	KU692177
<i>Tulbaghia simmleri</i> P.Beauv.	Kubec RK002 (CBFS)	Liz Powney, United Kingdom. Cult. (CBFS). www.tulbaghia.com	KU692071	KU692125	KU692178
<i>Tulbaghia simmleri</i> P.Beauv.	Rønsted and Stafford NR479 (C)	Rare Plants, UK. Cult. (C)	KU692072	KU692126	KU692179
<i>Tulbaghia simmleri</i> P.Beauv.	Rønsted and Stafford NR498 (C)	Hoyland Plants, UK. Cult. (C)	KU692073	KU692127	KU692180
<i>Tulbaghia simmleri</i> P.Beauv.	Rønsted and Stafford NR527 (C)	Greg Petit, Green Goblin Nursery, SA	KU692074	KU692128	KU692181

Table 2. *Continued*

Taxon	Voucher	Origin	GenBank accession		
			<i>ndhF</i>	<i>trnL-F</i>	nrITS
<i>Tulbaghia verdoorniae</i> Vosa & R.B.Burb.	Möller MM019B (E)	RBGE 2003 0850 David Fenwick TULB 220	KU692075	KU692129	KU692182
<i>Tulbaghia violacea</i> Harv. var. <i>robustior</i> (Kunth) R.B.Burb.	Möller MM008 (E)	Dyer A dark-centred small flower. Cult. 1999 2214 €	KU692076	KU692130	KU692183
<i>Tulbaghia violacea</i> Harv. var. <i>robustior</i> (Kunth) R.B.Burb.	Rønsted and Stafford NR525 (C)	Liz Powney, UK. www.tulbaghia.com	KU692077	KU692131	KU692184
<i>Tulbaghia violacea</i> Harv. var. <i>violacea</i>	Möller MM007 (E)	Cult. 2000 1895 (E)	KU692078	KU692132	KU692185
<i>Tulbaghia violacea</i> Harv. var. <i>violacea</i>	Möller MM013 (E)	A-C 13-03 C.McMaster. Cult. 2003 0510 (E). First recorded as <i>T. violacea</i>	KU692079	KU692133	KU692186
<i>Tulbaghia violacea</i> Harv. var. <i>violacea</i>	Rønsted and Stafford NR507 (C)	Greg Petit, Green Goblin Nursery, SA	KU692080	KU692134	-
<i>Tulbaghia violacea</i> Harv. var. <i>violacea</i>	Rønsted and Stafford NR508 (C)	Greg Petit, Green Goblin Nursery, SA	KU692081	KU692135	KU692187
<i>Tulbaghia violacea</i> Harv. var. <i>violacea</i>	Rønsted and Stafford NR510 (C)	Greg Petit, Green Goblin Nursery, SA (variegated)	KU692082	KU692136	KU692188
<i>Tulbaghia violacea</i> Harv. 'Pallida'	Möller MM005 (E)	Cult. 2003 0849 30.7.03 (E)	KU692083	KU692137	KU692189
<i>Tulbaghia violacea</i> Harv. 'Pearl'	Rønsted and Stafford NR526 (C)	Liz Powney, UK. www.tulbaghia.com	KU692084	KU692138	KU692190y

Kew, under submission to *Bot. J. Linn. Soc.*) and some by Meerow *et al.* (1999). These sequences covered 17 species including the monotypic *Prototulbaghia*. Author names are given in Table 1 and details of material examined are presented in Table 2. All included species, except *T. verdoorniae*, are represented by at least two samples. Thirteen representative taxa of tribe Gilliesieae were used as an outgroup (Fay *et al.*, 2006).

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

DNA extractions were conducted using the DNeasy Plant Mini Kit (Qiagen Inc., Copenhagen, Denmark) following the manufacturer's protocol. Amplification and sequencing of the ITS of nuclear ribosomal DNA (nrDNA) (primers ITS4 and ITS5; White *et al.*, 1990) and the plastid *trnL-F* intron-spacer region (primers c and f; Taberlet *et al.*, 1991) followed Larsen *et al.* (2010) and amplification of part of the plastid encoded *ndhF* region (primers 1318F and 2110R; Olmstead & Sweere, 1994) followed Rønsted *et al.*

(2008). PCR amplified products were purified with QIAquick mini columns (Qiagen Inc.) following the manufacturer's protocol before sequencing.

SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSIS

Both strands were sequenced for each region for all samples. Sequences were edited and assembled using Sequencher v.4.8 software (Gene Codes, Ann Arbor, MI, USA), and aligned in Mesquite v.2.7.5 (Maddison & Maddison, 2011). Details of materials, voucher information and GenBank accession numbers are given in Table 2.

Data were analysed combined after individual ITS and plastid DNA (*trnL-F* plus *ndhF*) data, analysed by maximum parsimony (MP) and Bayesian inference (BI), showed no strongly supported topological incongruences (> 80% bootstrap values and/or 0.95 posterior probabilities) between the two datasets (data not shown). Several samples had missing data, six in ITS, two in *trnL-F* and three in *ndhF*. Exclusion of the nine samples with missing data from the

combined analysis resulted in identical species relationships (data not shown). The dataset shown here, which included samples with missing data, contained 69 entities. Phylogenetic analyses implementing MP were conducted using PAUP* v.4.0a146 (Swofford, 2002) on unordered, unweighted characters. The heuristic tree search strategy involved 10 000 random stepwise additions without branch swapping keeping the shortest trees, followed by TBR branch swapping on the trees in memory with MulTrees and Steepest Descent activated. Branch support was obtained as bootstrap analysis (Felsenstein, 1985), with 10 000 replicates of random addition sequence, TBR on but MulTrees off (cf. Möller *et al.*, 2009).

Mr Bayes v.3.2.5 software (Huelsenbeck & Ronquist, 2001; Ronquist *et al.*, 2012) was used for BI analyses with the best fitting models of molecular evolution selected using MrModeltest v.2 software (Nylander, 2004) under the Akaike information criterion. In the light of the expected differences in rate of evolution of the ITS spacers and the 5.8S region and the three codon position in the coding region of *ndhF*, we partitioned the matrices accordingly and selected models separately. These were GR + I + G for the ITS spacers and K80 + G for the 5.8S gene. For the three codon positions of *ndhF*, HKY, GTR + I and GTR + G were selected for the first, second and third positions respectively. For *trnL-F*, GTR + G was selected. Two independent runs of four Monte Carlo Markov chains were sampled every 1000th generation and run until convergence was achieved, implemented as 'stoprule' when the average standard deviation of split frequencies had reached 0.01. The burn-in was set to 0.1 (10%), checked afterwards by plotting likelihoods against generation time, and a 'halfcompat'-tree generated from the remaining trees. With these settings, 470 000 iterations were generated for the combined data.

We considered bootstrap values of < 70% or posterior probabilities < 0.80 to represent low support, 70–85 or 0.80–0.95 as moderate support and > 85 or > 0.95 as strong clade support.

RESULTS

MATRIX CHARACTERISTICS

The combined dataset contained 2515 characters (773 in ITS, 900 in *trnL-F* and 768 in *ndhF*). Of these, 1816 were constant, 178 were variable but parsimony-uninformative and 521 were potentially parsimony-informative.

PHYLOGENETIC ANALYSES

The MP analysis retrieved 187 most-parsimonious trees of 1252 steps length, CI 0.71 and RI 0.92. The

strict consensus tree showed a moderate resolution with the presence of several polytomies across the tree (MP tree not shown, MP bootstrap percentages indicated in the BI tree in Fig. 2). *Tulbaghia* plus *Prototulbaghia* was monophyletic (BS = 100%). In *Tulbaghia*, the position of *Prototulbaghia* received low support. Across the tree, the samples fell in taxon-specific clades in all cases, except for *T. natalensis* where two samples fell in a polytomy. Most taxon-clades received moderate to strong support (except *T. natalensis*, *T. acutiloba*, *T. capensis*); inter-clade support was low.

The BI tree showed a similar topology compared with the MP tree with respect to retrieving taxon-specific clades (including the *T. natalensis* samples). The tree was almost fully resolved, and the branches received much higher support (Fig. 2). A long branch separated the outgroup from the ingroup samples and the monophyly of *Tulbaghia* plus *Prototulbaghia* received maximum support (PP = 1). *Prototulbaghia* was nested in *Tulbaghia* subgenus *Tulbaghia* and was sister to a clade of three species (PP = 0.94), *T. simmleri*, *T. cernua* and *T. ludwigiana*. The remaining samples of subgenus *Tulbaghia* formed a basal polytomy in *Tulbaghia*, including *T. dregeana* and *T. montana*, or derived grades (*T. capensis* and *T. galpinii*) with maximum branch support (PP = 1). The rest of the samples of the six included species of subgenus *Tulbaghia* formed a sister clade (PP = 1) to all samples of the two species, *T. cominsii* and *T. violacea*, in five taxa of subgenus *Omentaria* included (PP = 1). Among the species of subgenus *Tulbaghia*, the *T. natalensis* samples were sister to those of *T. acutiloba* (PP = 0.99), and these in turn fell in a polytomy with two samples of *T. coddii* and a clade comprised of samples of *T. verdoorniae* and *T. leucantha* (PP = 1). All these were sister to the clade of *T. alliacea* samples (PP = 0.99), albeit with low support (PP = 0.77). Among the *Omentaria* samples, the *T. maritima* samples were sister to the remaining samples (PP = 0.76) and those of *T. cominsii* fell among the *T. violacea* samples, which fell in taxon grades starting with *T. violacea* var. *robustior* (Kunth) R.B.Burb. (PP = 1), followed by *T. cominsii* (PP = 1) and samples of *T. violacea* var. *violacea* (PP = 1).

DISCUSSION

PHYLOGENETIC POSITION OF *PROTOTULBAGHIA* AND *TULBAGHIA*

The original generic differentiation between *Prototulbaghia* and *Tulbaghia* was based mainly on gross morphology and geographical distribution. However, the authors did caution that the determination of its true relationships must await comparative molecular

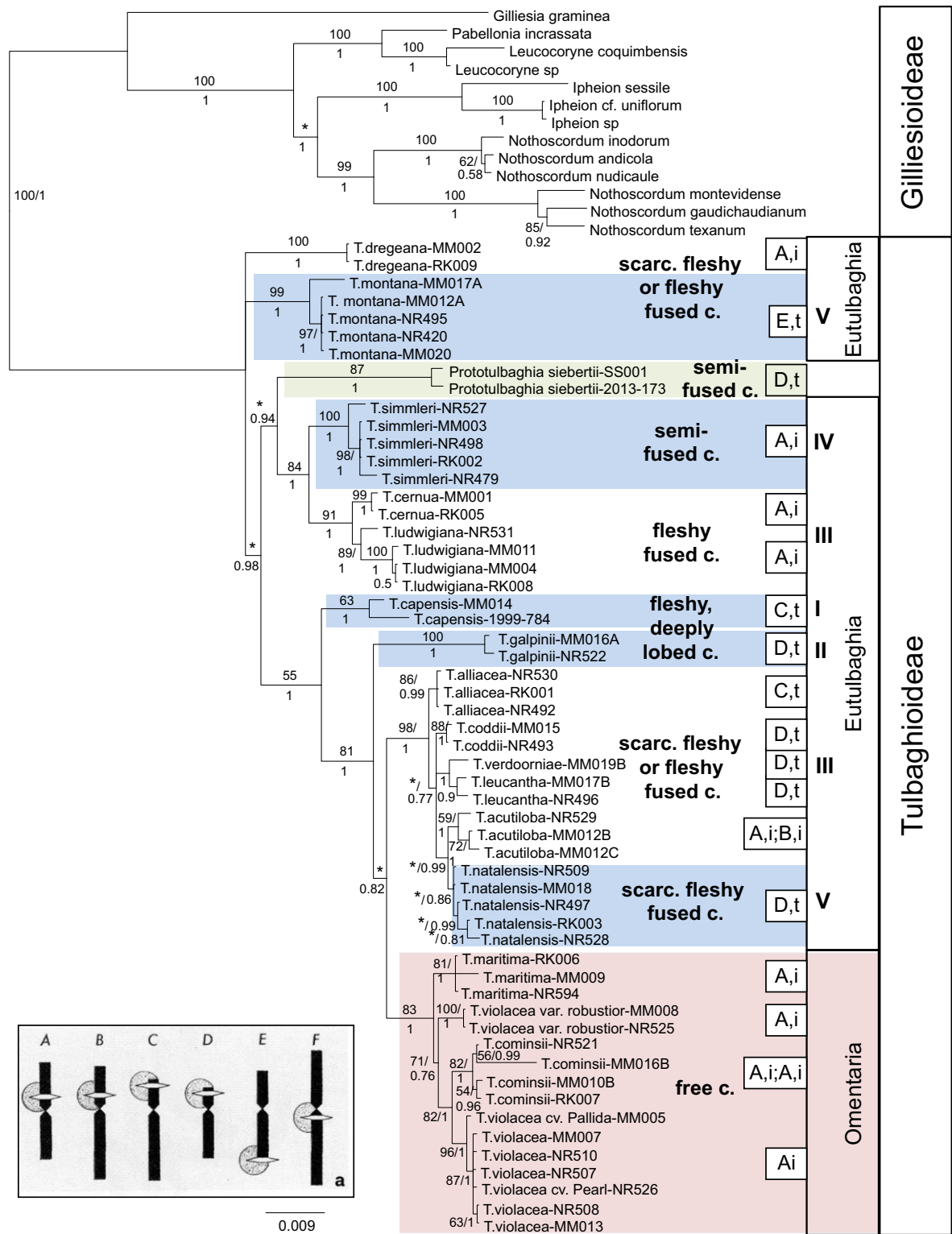


Figure 2. Bayesian inference consensus tree with average branch lengths from analysis of the total combined ITS, *trnL-F* and *ndhF* sequences. Bootstrap percentages and Bayesian posterior probabilities are indicated above and below the branches, respectively. *Indicate support values < 50% BP or PP = 0.5. Infrageneric classification and karyotypes (A–E, i: interstitial, t: terminal) according to Vosa (1975, 2000, 2009) indicated on the right. Illustration of karyotypes on the left-hand side reprinted with permission from Vosa (2000).

studies (Siebert *et al.*, 2008). The inclusion of *Prototulbaghia* in *Tulbaghia* is strongly supported in all our analyses here (BP = 100%; PP = 1.00) and assuming *T. siebertii* in *Tulbaghia* is clearly to be preferred over the presence of a small, monotypic separate genus. Thus *P. siebertii* should therefore be transferred to *Tulbaghia* as *T. siebertii* (Vosa) Mich.Möller & G.Stafford comb. nov. The relationships of *T. siebertii* in *Tulbaghia* are uncertain in the MP analysis, but in the BI analysis (Fig. 2), *T. siebertii* is strongly supported (PP = 0.94) as sister to a clade of *T. cernua*, *T. ludwigiana* and *T. simmleri*. A possible position as sister to the remainder of *Tulbaghia* could be tested in further studies including more species and characters. *Prototulbaghia*, as the name implies, was considered a possible precursor in the evolution of *Tulbaghia* from an *Allium*-like ancestor as is suggested by its intermediate floral character states (Vosa *et al.*, 2011). Vosa *et al.* (2011) suggested that its flowers show some resemblance to those of *Tulbaghia* and *Allium*, but some features are also reminiscent of some genera of the South American tribe, Gillesieae, of which several were used as an outgroup in this study. Sekhukhuneland, particularly the high-elevation parts of the Leolo Mountains, to which *T. siebertii* is confined (Siebert *et al.*, 2008), is widely considered to be a refuge for taxa that were presumably more widespread in southern Africa during previous cooler and wetter climatic conditions. Many such taxa form part of what is known as the Afromontane floristic element, which is proposed to have subsequently evolved independently due to geographical isolation (Siebert & van Wyk, 2005; Vosa *et al.*, 2011).

Nomenclatorial adjustment

Tulbaghia siebertii (Vosa) Mich.Möller & G.Stafford comb. nov.

Basionym: *Prototulbaghia siebertii* Vosa in *Caryologia* 60: 277 (2007b).

Type: Limpopo – 2430 CA (Pilgrim's Rest): Leolo Mountains, Sekhukhuneland, c. 15 km from Schoonoord on mountain road. Growing in seepage areas of grassland in poorly drained soil overlying norite rock, in full sun in a gentle north-facing slope, 4 December 2000, A. E. van Wyk & S. Siebert 1304 (holotype PRU).

PHYLOGENETIC RELATIONSHIPS IN *TULBAGHIA*

Infrageneric classification

The phylogenetic relationships ascertained from this study conflict with the subgeneric relationships proposed by Vosa (2009), which were primarily based on morphology. Vosa's subgenus *Omentaria* (*T. cominsii* and *T. violacea*) is recovered as monophyletic in all

our analyses, but is nested in subgenus *Tulbaghia*, which is therefore paraphyletic.

Vosa's section III with 14 species is not supported, because *T. cernua* and *T. ludwigiana* form a clade with *T. simmleri*, which was a group of its own in Vosa's classification. The remainder of the included species from Vosa's group III, the *alliacea*-complex, here also includes *T. natalensis*, which was placed in group V with *T. montana* in Vosa's classification. Some rare species included in Vosa's group III (*T. macrocarpa*, *T. nutans*, *T. pretoriensis*, *T. tenuior* and *T. transvaalensis*, and *T. rhodesica*, constituting a group (VI) of its own in Vosa's classification, were not included in this study, and their relationships therefore remain uncertain. Vosa's recognition of the monotypic groups I (*T. capensis*) and II (*T. galpinii*) are also supported as separate lineages by the molecular data in this study. Finally, *T. montana* (in Vosa's group V with *T. natalensis*) constitutes a separate lineage with *T. dregeana*. A new infrageneric classification must await inclusion of the remaining species in the phylogenetic analyses.

Tulbaghia spp. possess large and well differentiated chromosomes, which differ mainly in the types or combination of types of the nucleolar chromosomes (Vosa, 2000). On this basis, seven distinct karyotypes have been described, but their taxonomic value is uncertain (Vosa, 2000, 2009). The karyotypes for the different species as indicated in Figure 2 suggest a complex pattern involving two origins of duplication of nucleolar organiser regions (NORs) and independent origins of less common types across the phylogenetic tree. However, the karyotype distribution does not appear to be random across the tree and distinct karyotypes support specific clades such as the splitting of Vosa's section III as specified below. Because of the superficial similarities of karyotypes of some species, a full understanding of the taxonomic value and evolutionary importance of the different karyotypes await further cytological studies such as chromosome *in-situ* hybridization studies.

Major clades and species concepts in Tulbaghia

Tulbaghia dregeana: This is a rather distinct species found around Wupperthal, in the Cederberg, from Worcester northwards to Springbok (western and northern Cape). It has the most common karyotype A (Vosa, 2000).

Tulbaghia montana: Vosa placed *T. montana* with *T. natalensis* in a section of their own because they both possess a scarcely fleshy, fused lacinate corona. *Tulbaghia montana* is a small high-mountain species confined to the south-eastern Drakensberg. It has the unique karyotype E (Vosa, 2000).

The cernua–ludwigiana–simmleri clade: Vosa (1975, 2000) considered *T. cernua* and *T. ludwigiana* to be closely related. However, Burbidge (1978) placed *T. cernua* in the *T. alliacea* complex, a group he admitted was variable and might contain a number of taxonomically ‘good’ entities. Both these species have a wide distribution in the summer-rainfall region of southern Africa concentrated around the Eastern Cape, with *T. cernua* extending into the winter-rainfall region of the Western Cape. *Tulbaghia simmleri* is also from the Eastern Cape, but is confined to a relatively small area in the northern Drakensberg of Mpumalanga and Limpopo. Whereas *T. cernua* and *T. ludwigiana* share an urceolate or barrel-shaped fleshy corona, *T. simmleri* has a peculiar floral morphology with somewhat fleshy perianth segments and it has comparatively small chromosomes (Vosa, 1975). The clade is supported by all three species having the same karyotype A.

Tulbaghia capensis: Easily distinguished by its deeply lobed, fleshy orange-brown corona with contrasting green segments, which become pale towards the margins. It has karyotype C.

Tulbaghia galpinii: Restricted to a few localities in the Eastern Cape, this is a small species resembling a diminutive *T. capensis* with a deeply cut, but not very fleshy corona and thin flaccid leaves (Vosa, 2000; Siebert *et al.*, 2008). It has karyotype D.

The alliacea complex: This includes several highly variable species and a number of rare species, united by a more or less fleshy, complete, more or less serrated corona. In our study, the *alliacea* clade includes at least *T. acutiloba*, *T. alliacea*, *T. coddii*, *T. leucantha*, *T. natalensis* and *T. verdoorniae*. However, several species placed here by Vosa (2000, 2009) are not included and the relationship between species in the complex is unclear and requires additional sampling and support for subclades. *Tulbaghia natalensis* has karyotype D, which is the most common karyotype in the *alliacea* complex based on the species included here.

Tulbaghia cominsii – *T. maritima* – *T. violacea:* This group corresponds to Vosa’s subgenus *Omentaria*. *Tulbaghia violacea* is highly variable, even within populations (Vosa, 1975, 2000). Differences may include, besides the dimension of the plants, the size of the flowers and the length, colour and shape of the lobes of the corona. Vosa (2012) himself segregated *T. maritima* from this species. *Tulbaghia maritima* is quite distinct morphologically, with broader shiny, dark green leaves without a prominent central vein

and small yellow-greenish corona lobes. It is also restricted to rather homogeneous populations in coastal habitats. The phylogenetic tree indicates a paraphyletic *T. violacea*. Taxonomically, either merging *T. cominsii* into *T. violacea* or splitting *T. violacea* is possible. *Tulbaghia cominsii* is an attractive, small species clearly related to *T. violacea*, but it differs significantly in its thin, glaucous leaves, white or rarely pinkish flowers with a pink or purplish perianth tube (Vosa, 2000). This would point to splitting *T. violacea* into separate species rather than merging *T. cominsii* into *T. violacea*. Vosa (2012) already raised *T. violacea* var. *maritima* to *T. maritima*, which is confirmed here, as it formed a strongly supported clade of its own. However, the remaining included samples from several clades related to *T. cominsii*, including *T. violacea* var. *robustior* of Burbidge, but with low support, and we consider it necessary to extend the sampling with more wild collections of *T. violacea* and to obtain stronger support before proposing a revised concept of *T. violacea*. *Tulbaghia cominsii* has a duplication of karyotype A found in *T. violacea*.

CONCLUSIONS

This study, although preliminary, and the confusing historical taxonomic treatments highlight a considerable need for a revision of the genus, guided by molecular analyses. *Tulbaghia siebertii* (synonym *Prototulbaghia siebertii*) is included in *Tulbaghia*, but lack of support for the first-diverging lineages in the phylogenetic tree leaves the infrageneric classification and evolutionary history of the genus uncertain. Several species require closer examination, particularly *T. violacea* and those in the *T. alliacea* complex. Several rare taxa also need to be included in future studies, especially those found north of South Africa and the recently described *T. pretoriensis*. Not only is this genus a desirable horticultural commodity, traded globally, but it is also utilized locally for its purported medicinal properties and, to a lesser extent, as a food additive. Thus, urgency exists for assessing the vulnerability of several taxa, including *T. galpinii* and *T. cominsii*, both only known from a few localities, and *T. coddii*, the habitat of which (bogs and moist acidic soils) is under threat. This action will not be possible without a better understanding of the distribution, biogeography and ecology of the genus. Future studies should firstly focus on testing the present hypothesis by including all species and more characteristics in order to produce a robust infrageneric classification and allow re-evaluation of species concepts. Obtaining a robust and comprehensive phylogenetic hypothesis for *Tulbaghia* will help in the revision of

the genus and will guide conservation and sustainable ornamental and medicinal use of Africa's attractive endemic garlics.

ACKNOWLEDGEMENTS

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REFERENCES

- APG III. 2009.** An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* **161**: 105–121.
- Archer C. 2003.** Alliaceae. In Germishuizen G, Meyer NL. Plants of southern Africa: an annotated checklist. *Strelitzia* **14**: 956–957.
- Aremu AO, van Staden J. 2013.** The genus *Tulbaghia* (Alliaceae) – a review of its ethnobotany, pharmacology, phytochemistry and conservation needs. *Journal of Ethnopharmacology* **149**: 387–400.
- Avé-Lallemant JEL. 1844.** Tulbaghiae speciesquae hucusque innotuerunt expositae. *Bulletin de la Class Physico-Mathématique de l'Académie Impériale des Sciences de Saint-Petersbourg* **3**: 1–11.
- Baker JG. 1871.** A revision of the genera and species of herbaceous capsular gamophyllous Liliaceae. *Botanical Journal of the Linnean Society* **11**: 349–436.
- Batten A, Bokelmann H. 1966.** *Wild flowers of the Eastern Cape Province*. Cape Town: Cape and Transvaal Printers Ltd.
- Burbidge RB. 1978.** A revision of the genus *Tulbaghia* (Liliaceae). *Notes from the Royal Botanic Garden Edinburgh* **36**: 77–103.
- Burton JS. 1990.** A chemical investigation of *Tulbaghia violacea*. MSc Thesis, Department of Chemistry, Rhodes University, Grahamstown, South Africa.
- Chase MW, Reveal JL, Fay MF. 2009.** A subfamilial classification for the expanded asparagalean families Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae. *Botanical Journal of the Linnean Society* **161**: 132–136.
- Dold AP, Cocks ML. 2002.** The trade in medicinal plants in the Eastern Cape Province, South Africa. *South African Journal of Science* **98**: 589–597.
- Fay MF, Rudall PJ, Chase MW. 2006.** Molecular studies of subfamily Gilliesioideae (Alliaceae). *Aliso* **22**: 367–371.
- Felsenstein J. 1985.** Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.
- Govaerts R, Kington S, Friesen N, Fritsch R, Snijman DA, Marcucci R, Silverstone-Sopkin PA, Brullo S. 2016.** *World checklist of Amaryllidaceae*. Facilitated by the Royal Botanic Gardens, Kew. Available at: <http://apps.kew.org/wcps/> Retrieved 2016-01-02
- Huelsenbeck JP, Ronquist F. 2001.** MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.
- Hulme MM. 1954.** *Wild flowers of Natal*. Pietermaritzburg: Shuter and Shooter.
- Hutchings A, Scott AH, Lewis G, Cunningham AB. 1996.** *Zulu medicinal plants: an inventory*. Pietermaritzburg: University of Natal Press.
- Jäger AK, Stafford GI. 2012.** Quality assessment of *Tulbaghia* rhizomes. *South African Journal of Botany* **82**: 92–98.
- Klopper RR, Chatelain C, Bänninger V, Habashi C, Steyn HM, De WH, Arnold TH, Gautier L, Smith GF, Spichiger R. 2006.** Checklist of the flowering plants of sub-Saharan Africa. An index of accepted names and synonyms. Southern African Botanical Diversity Network Report No. 42: 684–685. Pretoria: SABONET.
- Larsen MM, Adersen AA, Davis AP, Lledó MD, Jäger AK, Rønsted N. 2010.** Using a phylogenetic approach to selection of target plants in drug discovery of acetylcholinesterase inhibiting alkaloids in Amaryllidaceae tribe Galantheae. *Biochemical Systematics and Ecology* **38**: 1026–1034.
- Linnaeus C. 1771.** *Mantissa plantarum, altera. Regni animalis, appendix*. Stockholm: Impensis Direct. (Laurentii Salvii).
- Maddison WP, Maddison DR. 2011.** *Mesquite: a modular system for evolutionary analysis. Version 2.75*. Available at: <http://mesquiteproject.org>
- Marshall NT. 1998.** *Searching for a cure: conservation of medicinal wildlife resources in East and southern Africa*. Cambridge: TRAFFIC International.
- Meerow AW, Fay MF, Charles LG, Qin-Bao L, Faridah QZ, Chase MW. 1999.** Systematics of Amaryllidaceae based on cladistic analysis of plastid sequence data. *American Journal of Botany* **86**: 1325–1345.
- Meerow AW, Reveal JL, Snijman DA, Dutilh JH. 2007.** Proposal to conserve the name Amaryllidaceae against Alliaceae, a 'superconservation' proposal. *Taxon* **56**: 1299–1300.
- Möller M, Pfosser M, Jang CG, Mayer V, Clark A, Hollingsworth ML, Barfuss MHJ, Wang YZ, Kiehn M, Weber A. 2009.** A preliminary phylogeny of the 'didymocarpoid Gesneriaceae' based on three molecular data sets: incongruence with available tribal classifications. *American Journal of Botany* **96**: 989–1010.
- Nylander JA. 2004.** *MrModeltest v2*. Program distributed by the author. Uppsala, Sweden: Evolutionary Biology Centre, Uppsala University.

- Olmstead R, Sweere J. 1994. Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. *Systematic Biology* **43**: 467–481.
- Petersen LM, Moll EJ, Collins R, Hockings MT. 2012. Development of a compendium of local, wild-harvested species used in the informal economy trade, Cape Town, South Africa. *Ecology and Society* **17**: 26–57.
- Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539–542.
- Rønsted N, Savolainen V, Mølgaard P, Jäger AK. 2008. Phylogenetic selection of *Narcissus* species for drug discovery. *Biochemical Systematics and Ecology* **36**: 417–422.
- Salisbury RA. 1866. *Genera plantarum*. London: John Van Voorst.
- de Sarker D, Johnson MAT, Reynolds A, Brandham PE. 1997. Cytology of the highly polyploid disjunct species, *Allium dregeanum* (Alliaceae), and of some Eurasian relatives. *Botanical Journal of the Linnean Society* **124**: 361–373.
- Siebert SJ, van Wyk AE. 2005. Scrophulariaceae—*Nemesia zimbabwensis*, a new record for the FSA region with notes on its phytogeographical significance. *Bothalia* **35**: 69–71.
- Siebert SJ, Vosa CG, van Wyk AE, Muller H. 2008. *Prototulbaghia* (Alliaceae), a new monotypic genus from Sekhukhuneland, South Africa. *Herbertia* **62**: 76–84.
- Swofford D. 2002. PAUP*. *Phylogenetic analysis using parsimony (*and other methods)*. Version 4. Sunderland: Sinauer Associates.
- Taberlet P, Gielly L, Pautou G, Bouvet G. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17**: 1105–1109.
- Uphof JT. 1943. A review of *Agapanthus* and *Tulbaghia*. *Herbertia* **10**: 40–51.
- Van Wyk B-E, Van Oudtshoorn B, Gericke N. 1997. *Medicinal plants of South Africa*. Pretoria: Briza Publications.
- Vosa C. 1975. The cytotaxonomy of the genus *Tulbaghia*. *Annali di Botanica (Rome)* **34**: 47–121.
- Vosa C. 2000. A revised cytotaxonomy of the genus *Tulbaghia*. *Caryologia* **53**: 82–112.
- Vosa C. 2007a. The annotated bibliography (1966–2006) of the genus *Tulbaghia* (Alliaceae). *Caryologia* **60**: 69–72.
- Vosa C. 2007b. *Prototulbaghia* (Alliaceae), a new genus of the Alliaceae family from the Leolo mountains in Sekhukhuneland, South Africa. *Caryologia* **60**: 273–278.
- Vosa C, Condry G. 2006. *Tulbaghia pretoriensis*, a new species from the Province of Gauteng (South Africa). *Caryologia* **59**: 164–167.
- Vosa C, van Wyk AE, Siebert SJ, Condry G. 2011. *Prototulbaghia siebertii*. *Flowering plants of Africa* **62**: 22–28.
- Vosa CG. 2009. An updated and illustrated taxonomic synopsis of the genus *Tulbaghia* (Alliaceae). *Herbertia* **63**: 208–219.
- Vosa CG. 2012. *Tulbaghia maritima*: a new species from the southeast Cape Peninsula, South Africa (Alliaceae). *Herbertia* **65**: 60–69.
- Watt JM, Breyer-Brandwijk MG. 1962. *The medicinal and poisonous plants of southern and eastern Africa, 2nd edn*. Edinburgh and London: E. and S. Livingstone.
- White TJ, Bruns TD, Lee SB, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR protocols. A guide to methods and applications*. San Diego: Academic Press, 315–322.
- de Wilde-Duyfjes BEE. 1976. A revision of the genus *Allium* L. (Liliaceae) in Africa. *Mededelingen Landbouwhogeschool Wageningen* **76**: 166–239.