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Biogeographic origins of southern African *Silene* (Caryophyllaceae)

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ABSTRACT

Silene (Caryophyllaceae) is distributed predominantly in the northern Hemisphere, where it is most diverse around the Mediterranean Basin. The genus is also well represented in North Africa, extending into tropical, sub-Saharan and southern Africa. Eight native species are recognized in southern Africa, taxonomically placed in two sections: *Elisanthe* and *Silene* s.l. Although the taxonomy of the southern African taxa has recently been revised, their phylogenetic relationships and biogeographic history remain unclear. This study aims to infer the phylogenetic position and geographic origins of the southern African taxa. We generated DNA sequences of nuclear and plastid loci from several individuals belonging to all eight species of *Silene* recognized from southern Africa, and combined our DNA sequences with existing data representing species from major clades (i.e. sections) based on the recently revised *Silene* infrageneric taxonomy. We used a Bayesian coalescent species tree continuous diffusion approach to co-estimate the species tree and the ancestral areas of representative members of the genus. Our results show that the perennial southern African members of section *Elisanthe* form a strongly-supported clade with the Eurasian annual *S. noctiflora* and the Central Asian perennial *S. turkestanica*. The rest of the perennial species form a strongly-supported clade together with the annual *S. aethiopica*, which is nested in a larger Mediterranean clade comprising mostly annual species classified in section *Silene* s.l. Estimates of ancestral areas indicate a late Pleistocene dispersal to southern Africa from central and East Africa for the sub-Saharan members of section *Silene* s.l. The *Elisanthe* clade is inferred to have colonized southern Africa through long-distance dispersal from Eurasia during the late Pleistocene. Our findings support the hypothesis of a relatively recent colonization into southern Africa resulting from two independent dispersal events during the Pleistocene.

1. Introduction

Silene L. (Caryophyllaceae) comprises about 850 species of annuals, biennials, or perennials (Oxelman et al., 2013; Naciri et al., 2017; Jafari et al., 2020) distributed predominantly in temperate and alpine habitats of the northern Hemisphere. The genus is most diverse in the eastern parts of the Mediterranean Basin and in southwestern Asia (Oxelman and Lidén 1995; Greuter et al., 1997; Đurović et al., 2017). Most species are endemic to Eurasia, where the bulk of the genus is distributed (e.g.,

Coode and Cullen, 1967; Davis, 1971; Meikle, 1977; Greuter, 1995; Greuter, 1997; Trigas et al., 2007; Yildiz and Çirpici, 2013). Taxonomic revisions of the genus on a global scale have been attempted by several authors including Otth (1824), Rohrbach (1868), Williams (1896), Chowdhuri (1957) and recently Jafari et al. (2020). Life history strategies and morphological traits, including the number of stamens and styles, petal morphology, carpel number, seed morphology, indumentum type, and length of anthophore (refers to the internode between the calyx and corolla) have been emphasized as important features for

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classification (Naciri et al., 2017). Several of these key morphological traits, however, exhibit high levels of homoplasy, both at generic and infrageneric levels. A recent phylogenetic analysis by Jafari et al. (2020) identified three major clades [*S.* subg. *Lychnis* (L.) Greuter, *S.* subg. *Behenantha* (Otth) Torr. & A.Gray, and *S.* subg. *Silene*] comprising 33 sections. This circumscription of the genus provides a well-supported sectional classification but relationships between and within some sections remain unresolved.

Recent molecular studies have resolved several ambiguities at both subgeneric and sectional levels. In particular, the proposed infrageneric division of the genus (Jafari et al., 2020) is well-supported by molecular data from several studies (Oxelman et al., 2001; Popp and Oxelman, 2004; Frajman et al., 2009; Rautenberg et al., 2010). Other studies have used molecular data to resolve the circumscription of some of the sections proposed by Chowdhuri (1957), e.g. *S.* sect. *Physolychnis* (Benth.) Bocquet (Petri and Oxelman, 2011), *S.* sect. *Melandrium* (Röhl.) Rabeler (Rautenberg et al., 2010), *S.* sect. *Siphonomorpha* Otth (Naciri et al., 2017), while others have focused on disentangling lower-level relationships within species complexes (Toprak et al., 2016; Đurović et al., 2017; Du Pasquier et al., 2017).

The bulk of phylogenetic studies on *Silene* have focused on areas where the genus is most diverse (i.e. the Mediterranean Basin extending across Eurasia), with few comprehensive studies on native African taxa. The majority of the African taxa occur in North Africa, with several recorded in sub-Saharan Africa radiating further into southern Africa (Manning and Goldblatt, 2012a). Comprehensive taxonomic classifications of the African taxa are still largely based on regional floristic treatments e.g. Zambia (Bingham et al., 2020), North Africa (Maire, 1963; Dobignard and Chatelain, 2011) and southern Africa (Manning and Goldblatt, 2012a). The southern African taxa were misunderstood by earlier authors such as Burman (1768) and Thunberg (1794), who assigned several of the species to known European taxa. The first comprehensive taxonomic treatment of the southern African taxa was that of Sonder (1860), followed by several advancements by Rohrbach (1868), Bocquet (1977), Masson (1989) and Goldblatt and Manning (2000). Most recently, Manning and Goldblatt (2012a) provided a comprehensive taxonomic treatment of the southern African native and naturalized taxa.

In their taxonomic revision of the southern African taxa, Manning and Goldblatt (2012a) recognized eight native taxa assigned to three sections, i.e. *S.* sect. *Elisanthe* (Fenzl ex Endl.) Ledeb., *S.* sect. *Dipterospermae* (Rohrb.) Chowdhuri and *S.* sect. *Fruticulosae* (Willk.) Chowdhuri. The taxa belonging to section *Elisanthe* are perennials characterized by a dichasial inflorescence, lanceolate leaves, a glandular-pubescent and a reticulately-veined calyx, and globose seeds lacking peripheral wings (Manning and Goldblatt, 2012a, b). Within section *Elisanthe*, *S. undulata* Ait., is the most widespread species, occurring across temperate southern Africa (Manning and Goldblatt, 2012a) extending into tropical Africa (Manning and Goldblatt, 2012b). The rest of the taxa in the section include *S. saldanhensis* J.C.Manning & Goldblatt, *S. ornata* Ait., and *S. rigens* J.C.Manning & Goldblatt, all of which are native to the Core-Cape subregion (CCR) of the Greater Cape Floristic Region (GCFR; Manning and Goldblatt, 2012b). However, the placement of *S. saldanhensis*, *S. ornata* and *S. rigens* within section *Elisanthe* by Manning and Goldblatt (2012a) was primarily based on shared morphological characters, and the phylogenetic position of these taxa remain unknown. Section *Dipterospermae* includes the single species *S. aethiopica* Burm., an erect annual characterized by a monochasial inflorescence, linear to narrowly obovate leaves, a glandular-pubescent calyx lacking reticulate venation, and reniform seeds with peripheral wings (Manning and Goldblatt, 2012a). *Silene aethiopica* is native to the Greater Cape Floristic Region, with a distribution extending from Namaqualand in the west to Gqeberha (formerly Port Elizabeth) in the southeast (Manning and Goldblatt, 2012b).

Among the southern African members of section *Fruticulosae*, *S. burchellii* is the most widespread species with a distribution extending from temperate southern Africa to eastern and northern sub-Saharan Africa and as far as Arabia (Manning and Goldblatt, 2012a). The remaining members of the southern African section *Fruticulosae* include *S. mundiana* Eckl. & Zeyh., a CCR endemic localized to the south-western Cape, and *S. crassifolia* L., distributed along the coast extending from Saldanha Bay in the west to the southeast Natal (Manning and Goldblatt, 2012a). The phylogenetic position of *S. mundiana* and *S. crassifolia* remains unknown, and Manning and Goldblatt (2012a) primarily placed these taxa based on morphological characters in the previously recognized section *Fruticulosae*. Section *Fruticulosae* shares morphological features (e.g. monochasial inflorescence and reniform seeds with peripheral wings) with section *Dipterospermae*, and the two sections are primarily distinguished from one other by a perennial (*Fruticulosae*) versus annual (*Dipterospermae*) habit (Chowdhuri, 1957; Manning and Goldblatt, 2012a, b). Although Manning and Goldblatt (2012a) applied the section names *Dipterospermae* and *Fruticulosae*, in this study we follow the current phylogenetic circumscription of Jafari et al. (2020) which places the sections *Dipterospermae* and *Fruticulosae* into synonymy with the broadly circumscribed section *Silene*. Manning and Goldblatt (2012a) hypothesized that ancestors of section *Elisanthe* colonized southern Africa via long distance dispersal, while the *Dipterospermae* and *Fruticulosae* [hereafter referred to as section *Silene* s.l. following Jafari et al. (2020)] colonized southern Africa via long distance dispersal as well as migration from North Africa through the East African rift mountain system, respectively.

The taxonomic review by Manning and Goldblatt (2012a) has improved the classification of the southern African species, and here we use Bayesian phylogenetic inference to determine their evolutionary history. Several plant groups have disjunct distributions between southern Africa and Eurasia (Goldblatt, 1978), which have commonly been hypothesized to be the result of dispersal from southern Africa northwards (e.g Caujapé-Castells et al., 2001; Goldblatt et al., 2002; Coleman et al., 2003; Galley et al., 2007; del Hoyo et al., 2009; Sanmartín et al., 2010; Désamoré et al., 2011), or vice versa (e.g. Migliore et al., 2012). Disjunct distributions of plant lineages between Africa-Eurasia are suggested to have resulted from fragmentation of a Cenozoic flora that was once widespread (Quézel, 1978; Andrus et al., 2004). In the case of southern African *Silene*, Manning and Goldblatt (2012a) presented two hypotheses for the biogeographic origins of southern African *Silene* (above). Our first aim is to determine the phylogenetic relationships and monophyly of the southern African taxa. Secondly, we infer the historical biogeography of southern African *Silene*. To address these aims, we jointly infer the species tree and ancestral area of *Silene* based on DNA sequence data from the nuclear internal transcribed spacer (ITS) and plastid (*rps16* and *matK*) loci, as well as data representing the geographical location where tissue samples for each sequence in our study was collected.

2. Materials and methods

2.1. Taxon sampling and DNA extraction

Our study included a comprehensive dataset representative of sections recognized in *Silene* following the circumscription of Jafari et al. (2020) and covers the geographic distribution of the taxa. We generated 132 novel DNA sequences from the nuclear ribosomal internal transcribed spacer (ITS, 54 accessions) and two plastid regions, the *rps16* intron (47 accessions) and *matK* (31 accessions). Our study included a total of 55 accessions mostly representing the eight southern African species recognized by Manning and Goldblatt (2012a), and the rest representing Eurasian and other African taxa (Table 1). Additionally, we included previously published sequences for ITS and *rps16* (245 and 243, respectively; Table A.1), representative of phylogenetically close and distantly related species belonging to *S.* subg. *Silene* and *S.* subg. *Behenantha* (see Oxelman et al., 2013; Jafari et al., 2020). We also included 41 sequences of *matK* for those accessions which had

Table 1

Details of voucher specimens sequenced as part of this study. Missing sequences are denoted with –. A "?" denotes unknown herbaria where specimens are deposited. An * denotes an existing sequence obtained from GenBank for the same voucher specimen. Numbers in parentheses in Taxon name column are unique specimen identification numbers in the Sileneae BoxTax database (http://www.sileneae.info).

Taxon name	Voucher	Herbarium	Country	Locality coordinates	Loci		
					ITS	*rps16*	*matK*
S. aethiopica Burm.f. (19153)	B. Oxelman 2710	GB	South Africa	34.3872°S 20.4215°E	MT036623	MT062367	–
S. aethiopica Burm.f. (20305)	P. Goldblatt, J. Manning & L. Porter 10,719	NBG	South Africa	32.9979°S 17.942°E	MT036621	MT062365	MT136996
S. aethiopica Burm.f. (19154)	B. Oxelman 2710	GB	South Africa	34.4568°S 20.3998°E	MT036617	MT062362	–
S. aethiopica Burm.f. (20313)	J. Manning *s.n.*	NBG	South Africa	33.2278°S 21.8569°E	MT036618	MT062363	MT136995
S. arabica Boiss. (17769)	K. U. Kramer 7146	Z	Israel	31.06°N 35.18°E	MT036607	–	–
S. arabica Boiss. (17993)	Iranshahr & Termé 33,684	W	Iran	28.8826°N 51.2746°E	MT036589	–	–
S. atlantica Coss. & Durieu (18053)	Dubuis 15,980	G	Algeria	36.46035°N 4.153761°E	MT036599	MT062347	
S. bellidifolia Jacq. (2255)	B. Oxelman 2165	GB	Greece	35.18°N 24.4°E	MT036610	MT062355	–
S. burchellii Otth. (14830)	M. Popp *s.n.*	GB	Ethiopia	13.290°N 38.118°E	MT036615	MT062360	–
S. biafrae Hook.f. (2614)	A. Rautenberg 3	UPS	Cameroon	4.1527°N 9.241°E	MT036624	MT062368	–
S. burchellii Otth. (20321)	A. Strid 4311	GB	Kenya	1.34637°S 36.578392°E	MT036627	MT062371	–
S. burchellii Otth. (20329)	O. Hedberg *s.n.*	S	Tanzania	3.75°S 37.75°E	MT036616	MT062361	–
S. burchellii Otth. (20330)	Unknown	S	Kenya	1.408117°S 36.58385°E	MT036625	MT062369	–
S. burchellii Otth. (20331)	B. Nordenstam 2804	S	Namibia	21.13333°S 14.58333°E	MT036619	MT062364	–
S. burchellii subsp. *modesta* J.C.Manning & Goldblatt (20311)	Johnson & Nanni *s.n.*	NBG	South Africa	29.63301°S 29.36886°E	MT036614	MT062359	MT136994
S. burchellii subsp. *pilosellifolia* (Cham. & Schltdl.) J.C.Manning & Goldblatt (20309)	P. Goldblatt & L. Porter 13,527	NBG	South Africa	31.37152°S 19.01622°E	MT036613	MT062358	MT136993
S. burchellii subsp. *pilosellifolia* (Cham. & Schltdl.) J.C.Manning & Goldblatt (20332)	B. Nordenstam, J. Lundgren *s.n.*	S	Namibia	26.64131°S 15.17646°E	MT036620	–	–
S. crassifolia L. (19347)	B. Oxelman 2711	GB	South Africa	34.05911°S 22.38191°E	MT036628	MT062372	–
S. crassifolia L. (19351)	B. Oxelman 2715	GB	South Africa		MT036622	MT062366	–
S. crassifolia L. (20308)	P. Goldblatt & L. Porter 13,548	NBG	South Africa	33.98863°S 22.5727°E	MT036626	MT062370	MT136997
S. crassifolia L. (20316)	P. Goldblatt, J. Manning & L. Porter 13,447	NBG	South Africa	32.97051°S 17.89252°E	MT036612	MT062357	MT136992
S. discolor Sm. (17682)	B. Oxelman 2581	GB	Turkey	36.29153°N 29.27537°E	MT036590	MT062339	–
S. ghiarensis Batt. (1312)	B. Oxelman 1824	GB	Algerian	35.25°N 1.15°W	MT036603	LC423982*	–
S. gracilis DC. (1285)	B. Oxelman 1734	GB	Morocco	35.78°N 5.93°W	MT036595	MT062344	–
S. heldreichii Boiss. (1182)	B. Oxelman 1696	GB	Turkey	36.54374°N 31.99981°E	–	MT062374	–
S. heterodonta F.N.Williams (18054)	Charteums s.n	?	Morocco	31.31°N 7.91°W	MT036630	–	–
S. ibosii Emb. & Maire (4722)	M. Lidén 286	?	Morocco	35.032°N 5.023°W	MT036592	MT062341	–
S. imbricata Desf. (1331)	B. Oxelman 1848	GB	Algeria	36.15°N 2.75°E	MT036629	MT062373	–
S. legionensis Lag. (6356)	A. Rautenberg 50	UPS	Spain	43.26409°N 3.68120°W	MT036587	MT062337	–
S. lynesii Norman (19336)	D. Podlech 36,992	M	Algeria	24.55°N 9.483333°E	MT036601	MT062348	–
S. micropetala Lag. (20320)	S. Holmdahl 1396	GB	Spain	36.25438°N 5.9626°W	MT036586	–	–
S. mundiana Eckl. & Zeyh. (19155)	B. Oxelman 2710	GB	South Africa	34.45517°S 20.39639°E	MT036611	MT062356	–
S. niceensis All. (2632)	A. Rautenberg 21	UPS	Tunisia	36.456058°N 10.7376°E	MT036608	MT062353	–
S. obtusifolia Willd. (1287)	B. Oxelman 1730	GB	Morocco	35.78°N 5.93°W	MT036597	–	–
S. obtusifolia Willd. (18383)	P. Escobar Garcia & R. Grasi 191/08	GB	Morocco	35.77651°N 5.35903°W	MT036598	MT062346	–
S. ornata Ait. (20318)	P. Goldblatt & L. Porter 13,254	NBG	South Africa	32.9695°S 17.98788°E	MT036584	MT062335	MT136989
S. palaestina Boiss. (12266)	Eggers 90–1	?	Israel	31.77276°N 35.18951°E	MT036591	MT062340	–
S. reverchonii Batt. (20323)	DSBG	GB	Algeria	36.50°N 5.46°E	MT036594	MT062343	–
S. rigens Goldblatt & J.C.Manning (20314)	P. Goldblatt, J. Manning & L. Porter 13409B	NBG	South Africa	33.1221°S 18.12143°E	MT036580	MT062331	MT136985
S. saldanhensis Goldblatt & J.C.Manning (20317)	P. Goldblatt & J. Manning 13,646	NBG	South Africa	33.01651°S 18.03243°E	MT036582	MT062333	MT136987
S. secundiflora Otth. (18370)	B. Frajman 12,660	GB	Spain	36.73305°N 2.14805°W	MT036609	MT062354	–

Table 1 (*continued*)

Taxon name	Voucher	Herbarium	Country	Locality coordinates	Loci		
					ITS	*rps16*	*matK*
S. sericea All. (18371)	B. Frajman & P. Schönswetter 12,375	GB	France	41.381388°N 9.17305°E	MT036606	MT062352	–
S. spp. (1342)	B. Oxelman 1879	GB	Algeria	36.83°N 5.65°E	MT036593	MT062342	–
S. succulenta Forssk. (18373)	B. Frajman & P. Schönswetter 12,213	GB	France	42.66083°N 9.061388°E	MT036604	MT062350	–
S. tunetana Murb. (5471)	Leippert 7347	B	Tunisia	36.32°N 10.23°E	MT036600	–	–
S. undulata Ait. (19350)	B. Oxelman 2714	GB	South Africa	34.059116°S 22.3819°E	MT036579	MT062330	–
S. undulata Ait. (19354)	B. Oxelman 2718	GB	South Africa	33.59078°S 21.00382°E	MT036577	MT062328	–
S. undulata Ait. (20306)	P. Goldblatt & J. Manning 13,499	NBG	South Africa	33.95048°S 18.40802°E	MT036585	MT062336	MT136990
S. undulata Ait. (20307)	P. Goldblatt & L. Porter 13,523	NBG	South Africa	32.2331°S 18.8449°E	MT036581	MT062332	MT136986
S. undulata Ait. (20310)	P. Goldblatt & L. Porter 13,549	NBG	South Africa	34.00689°S 25.34743°E	MT036583	MT062334	MT136988
S. undulata Ait. (20315)	P. Goldblatt & L. Porter 135,000	NBG	South Africa	32.88132°S 17.89029°E	MT036578	MT062329	MT136984
S. villosa Forssk. (19269)	K. Tielborger *s.n.*	M	Israel	30.96667°N 34.36667°E	MT036602	MT062349	–
S. virescens Coss. (20325)	Skottsberg *s.n.*	GB	Morocco	30.871°N 8.376°W	MT036588	MT062338	–
S. vivianii Steud. (19333)	F. Schuhwerk 90/830	M	Morocco	30.45°N 6.41°W	MT036596	MT062345	–
S. volubilitana Braun-Blanq. & Maire (1302)	Bengt Oxelman 1766	GB	Morocco	34.08°N 5.45°W	MT036605	MT062351	–

complementary sequence data for the other two loci (Table A.1). The individual matrices comprised 299, 290, and 72 sequences for ITS, *rps16* and *matK*, respectively.

Genomic DNA was extracted from silica-dried or herbarium material using the Nucleospin® Plant II extraction kit (Macherey-Nagel, Duren, Germany) following the manufacturer's protocol. For poorly preserved herbarium material (including some more than 30 years old), we used a modified cetyltrimethylammonium bromide (CTAB) method as described by Yoon et al. (1991). Modifications to the protocol involved incubating the plant material in a Carlson lysis buffer containing CTAB/beta-mercaptoethanol (40:1) solution at 65 °C for 30 min. One volume of chloroform/isoamyl-alcohol (24:1) was added to the lysate, mixed by inversion and incubated at 4 °C overnight. The tubes were centrifuged for 10 min at 9,000 rpm. The supernatant was transferred to a new tube, and the extraction step repeated three times (i.e. addition of one volume of chloroform/isoamyl-alcohol to the supernatant, followed by centrifugation for 5 min at 9,000 rpm). A 0.1 vol of sodium acetate (NaOAc) and 2 volumes of ice-cold 95% ethanol was added to the lysate and incubated at −20 °C overnight. The tubes were centrifuged at 14,680 rpm for 10 min, and the supernatant discarded. The resulting pellet was washed with 70% ethanol and centrifuged at 14,680 rpm for 2 min before discarding the ethanol. The pellet was eluted in 20–100 μL of 0.1x tris-EDTA (TE). The extracted DNA obtained using both kit and CTAB methods was purified using the GeneClean™ Turbo Kit (MP Biomedicals™, Fisher Scientific, Ontario, Canada) according to the manufacturer's protocol.

2.2. Amplification and sequencing

Polymerase chain reaction (PCR) amplifications were performed in 25 μL reactions comprising: 1 μL DNA template, 0.6 μL of 20 mM F/R primer, 0.2 μL of 100 mM dNTP mix, 0.2 μL of 2 U/μL Taq polymerase, 5 μL of 5X Thermo Scientific Phusion HF buffer (containing MgCl₂) and 18 μL distilled H₂O. The reactions were run on a Bio-Rad MyCycler™ thermocycler (Bio-Rad, Hercules, CA, USA) with the following conditions: initial denaturation at 98 °C for 2 min, followed by 35 cycles of denaturation at 98 °C for 20 s, annealing at 62–71 °C for 30 s, extension at 72 °C for 1 min; and a final extension at 72 °C for 8 min and a final hold at 4 °C. The three markers (ITS, *rps16* and *matK*) were amplified and sequenced using primer combinations as indicated in Table A.2. Sequencing was performed by Eurofins (Eurofins, Uppsala, Sweden)

following the Applied BioSystems protocols using the BigDye v3.1 with internal primers (Table A.2). Maturase K sequences for this study were generated at Royal Botanic Gardens Kew using primer combinations XF and 5R (Hollingsworth et al., 2009). PCR amplifications for were performed in 25 μL volumes comprising 12.5 μL DreamTaq PCR Master Mix (2×) (4 mM MgCl2; Thermo Fisher Scientific, Waltham, MA, USA), 0.5 μL of each primer (100 ng μL), and 1 μL DNA template. Amplicons were purified using the Nucleospin Extract II kit (Machery-Nagel, Düren, Germany), following the manufacturers' protocols. Sequencing was performed using the BigDye Termination sequencing chemistry (v3.1; ABI) with the same primer combination used for PCR.

2.3. Species tree and probabilistic diffusion analysis

Sequences were assembled and manually edited using Geneious Prime 2020.1.2 (https://www.geneious.com). The International Union of Pure and Applied Chemistry (IUPAC) ambiguity codes were used to highlight nucleotide ambiguities (cpDNA) and polymorphisms (nrDNA). We performed Multiple Sequence Alignment (MSA) using MAFFT v7 online service (https://mafft.cbrc.jp/alignment/server/) using default settings (Katoh et al., 2019). Newly generated sequences were added to existing alignments using the MAFFT-add online tool under default settings. Alignments of each locus were visually inspected using Geneious v11.1.5 and dubiously aligned segments were locally re-aligned with MAFFT using the same settings as previously. Taxa that could not be sequenced for one of the loci were coded as missing.

There are several methods available to study historical biogeography (reviewed in Ronquist and Sanmartín, 2011). The past decade has seen the development of diffusion methods which estimate ancestral nodes based on continuous diffusion processes along a given 2D or 3D landscape (Lemmon and Lemmon, 2008; Lemey et al., 2010; Bouckaert et al., 2012; Bouckaert, 2016). Such methods have largely been utilized under a phylogenetic Bayesian framework to study viral epidemiology (e.g. Lemey et al., 2010; Bouckaert, 2016; Dellicour et al., 2019), languages (e.g. Bouckaert et al., 2012; Bouckaert et al., 2018), reptiles (Leaché et al., 2017) and plants (e.g. Nylinder et al., 2014; Bengtson et al., 2015; Nylinder et al., 2016; Đurović et al., 2017; Min Choo et al., 2020). Species tree diffusion studies on plants have mainly inferred sampling locations from species distribution ranges encoded as Keyhole Markup Language (KML) polygons (Nylinder et al., 2014). These methods have the advantage that they do not require the discretization of areas a priori

and thus do not make explicit assumptions on cladogenetic processes (Johansson et al., 2018). For species migration modelled over wide geographic areas, the development of relaxed random walk (RRW) on a sphere (Bouckaert, 2016) aids in avoiding the tendency of diffusion models to overestimate movement rates towards the poles due to cartographic projection distortions.

We followed a single-tier approach where species tree inference and ancestral areas were jointly estimated through a relaxed random walk diffusion process implemented in BEAST v2.5 (Bouckaert et al., 2019). To achieve this we used two packages, StarBEAST2 (Ogilvie et al., 2017) and GEO_SPHERE (Bouckaert, 2016). Our dataset comprised three unlinked partitions where the first two consisted of DNA sequence data from the nuclear (ITS) and plastid (*rps16* and *matK*) genomes. The third

partition was made up of geographical coordinates of the source localities of the taxa in our study. Previous studies have utilized species distribution ranges shaped as polygons to represent the geographic distribution of taxa (e.g. Nylinder et al., 2014; Bengtson et al., 2015; Nylinder et al., 2016; Johansson et al., 2018). Here we used point coordinates representing sampling/source locations for each taxon. Point coordinates were chosen firstly due to deficiencies in comprehensive distribution range information for many of the taxa. Secondly, using point coordinates, we were able to consider the uncertainties in all the model parameters in an integrated species tree and biogeographic inference. Both partitions were assigned the HKY + gamma substitution model with a mean mutation rate of 1. In addition, the two molecular partitions were assigned a strict clock with a mean rate set to 1 and
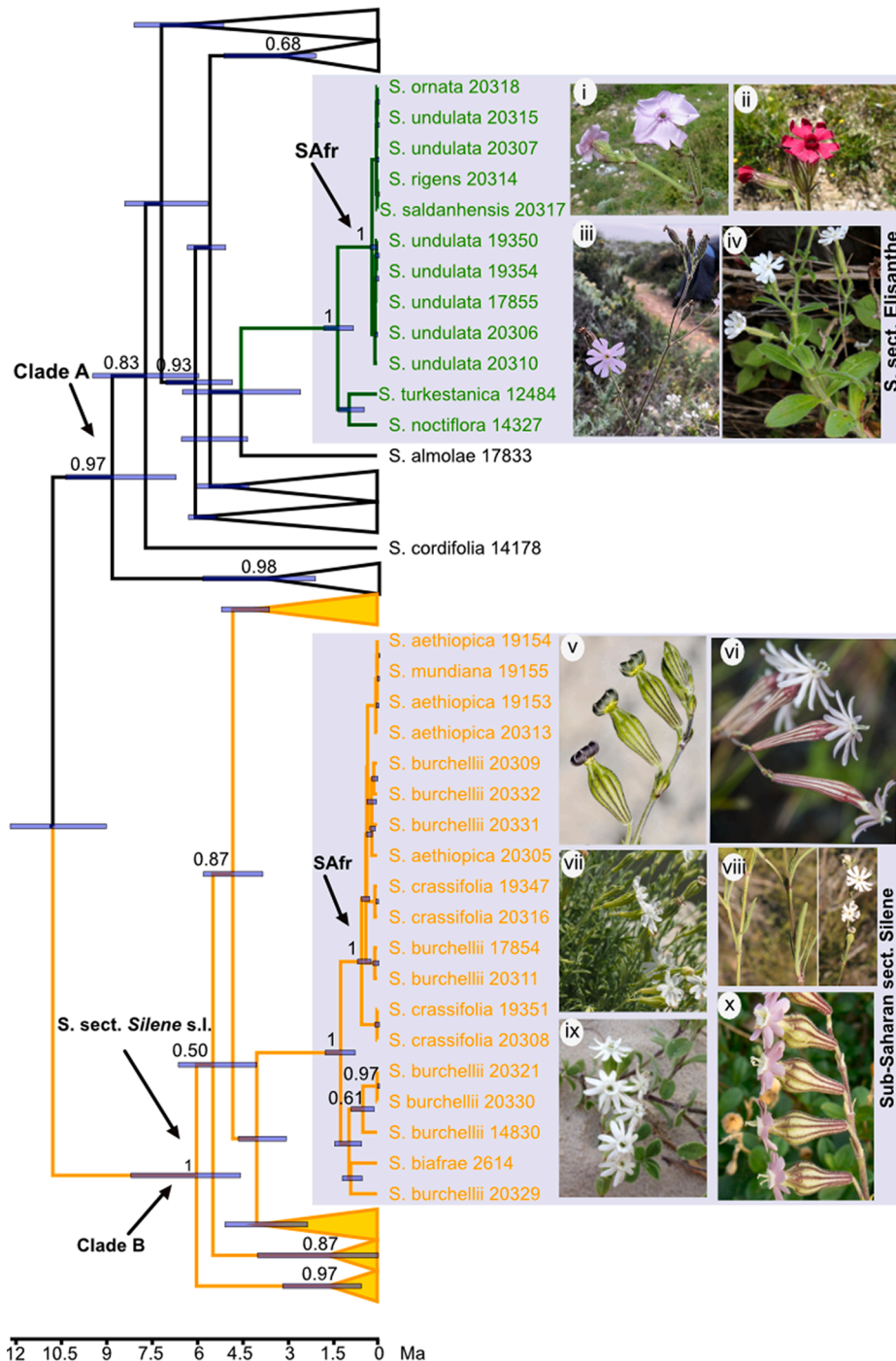

Fig. 1. Summary of the Maximum Clade Credibility species tree estimated using StarBEAST2 in BEAST2. Numbers above the branches are posterior probabilities (only PP > 0.50 are shown). Blue horizontal bars represent 95% highest posterior densities (HPD) of the node heights. Numbers next to the taxon names are unique specimen identification numbers in the Sileneae BoxTax database (http://www.sileneae.info). Taxonomic names of the collapsed clades are provided in supplementary information S.1. SAfr denotes southern African clades. **Images**: Inflorescence morphology of section *Elisanthe*: i– *S. saldanhensis*; ii– *S. ornata* (photo credit = iNaturalist kooscl). iii. *S. rigens* (Bengt Oxelman); iv– *S. undulata* (photo credit = Tony Rebelo); and section *Silene*: v– *S. aethiopica* (photo credit = Richard Adcock); vi– *S. burchellii* (photo credit = Magriet Brink); vii– *S. mundiana* (photo credit = Felix Riegel); viii– *S. aethiopica*– (photo credit = Gerhard Malan); ix– *S. crassifolia* subsp. *crassifolia* (photo credit = Micky Orrey); x– *S. crassifolia* subsp. *primuliflora* (photo credit = Kathy Immelman). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

estimated for both partitions. The geographic partition was assigned an uncorrelated exponential clock with mean rate set to 1 and estimated. Thus, the rates are scaled approximately in substitutions per site.

The prior age of the most recent common ancestor (MRCA) of *Silene-Lychnis* clade was set to a mean of 11.6 million years (Ma; Frajman et al., 2009; Rautenberg et al., 2010; Rautenberg et al., 2012), with a normal distribution and a standard deviation of 0.8. We specified a Yule species tree prior and a lognormal prior (mean = 5, standard deviation = 2) on the speciation rate on the species tree (speciationRate.t:Species). A lognormal prior (mean = −6, Standard deviation = 2) was specified for the popMean.Species (mean population size on the species tree). Four independent analyses were run for 500 million generations each, sampling every 100,000th generation and discarding the first 25% as burn-in. We assessed mixing and convergence for parameters of each independent run using Tracer v1.7.1 (Rambaut et al., 2018). A maximum clade credibility tree with annotated diffusion estimates was generated using TreeAnnotator v2.6.0 (part of the BEAST2 program), after discarding 25% of the trees as burn-in and visualized in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). We used SPREAD3 (Bielejec et al., 2016) to extract 80% high posterior density (HPD) ancestral areas derived from the posterior probability distribution, which were visualized as KML polygons (i.e. representing ancestral areas) using Quantum GIS v3.10.1 (https://qgis.org/en/site/).

3. Results

3.1. Estimated ages and phylogenetic relationships

The root age of *Silene* is estimated to 10.7 Ma with a 95% HPD between 9.02 and 12.19 Ma (Fig. 1). Our analysis recovered several strongly-supported clades representing currently recognized sections in the genus (Fig. 1, S.1, S.2, S.3). We considered strong support to have a posterior probability (PP) of PP ≥ 0.95 and moderate support to have 0.60 ≤ PP < 0.94, while values below 0.60 were considered as weak support. We identified two distinct clades, Clade A and B, which comprise several strongly-supported internal clades corresponding to the various sections in the genus (Fig. 1, S.1). Although the sections are strongly-supported, there was little resolution in relationships within

the sections. Clade A includes several moderately to strongly-supported internal clades comprising members of *S.* subg. *Lychnis*, *S.* subg. *Behenantha*, and sect. *Atocion* Otth (Fig. S.1), corresponding to the sectional classification of Jafari et al. (2020). In addition, Clade A also includes section *Elisanthe*, which is strongly-supported as monophyletic (PP = 1; Fig. 1, S.1, S.2, S.3). Section *Elisanthe*, with an estimated crown age of 1.37 Ma (95% HPD: 0.83–1.83 Ma), comprises four of the eight southern African taxa together with the Eurasian *S. noctiflora* L. and the Central Asian *S. turkestanica* Regel (Fig. 1, S.1). The root age of the southern African members of section *Elisanthe* is estimated to 0.24 Ma (95% HPD: 0.06–0.30 Ma; Fig. 1, S.1). Clade B, with an estimated crown age of 6.04 Ma (95% HPD: 4.59–8.2 Ma; Fig. 1, S.1), comprises several moderately to strongly-supported internal clades corresponding to the broadly circumscribed section *Silene* (Fig. 1, S.1, S.2, S.3). Clade B also includes a strongly-supported internal clade (PP = 1) comprising four of the eight southern African taxa, as well as three accessions of *S. burchellii* from central-eastern Africa, and a single accession of *S. biafrae* Hook.f., collected from central-western Africa (Fig. 1, S.1, S.2, S.3). The root age of the southern African members of section *Silene* is estimated to be 0.59 Ma (95% HPD: 0.27–0.72 Ma; Fig. 1, S.1).

3.2. Species tree diffusion

The diffusion analyses estimated the ancestral area of *Silene* to be in the Eurasian parts of the Mediterranean Basin where the earliest radiation occurred 9.02–12.19 Ma (95% HPD; Fig. 2a). The ancestral area of section *Silene* is uncertain and broadly estimated around the Mediterranean Basin extending into parts of North Africa (6.04 Ma; 95% HPD: 4.59–8.20 Ma) (Fig. 2b). Central and East Africa were colonized as part of a single event where ancestors of extant members of sub-Saharan African section *Silene* dispersed from North Africa to colonize Central and East Africa during the Pleistocene (1.28 Ma; 95% HPD: 0.8–1.79 Ma; Fig. 2b). Subsequently, ancestral lineages of the extant southern African members of section *Silene* (Clade B) colonized southern Africa during the Pleistocene (0.56 Ma; 95% HPD: 0.27–0.72 Ma; Fig. 2b). On the other hand, within Clade A, ancestors of extant members of southern African section *Elisanthe* radiated from Eurasia (1.37 Ma; 95% HPD: 0.86–1.83) and colonized southern Africa during the Pleistocene (0.24 Ma; 95%
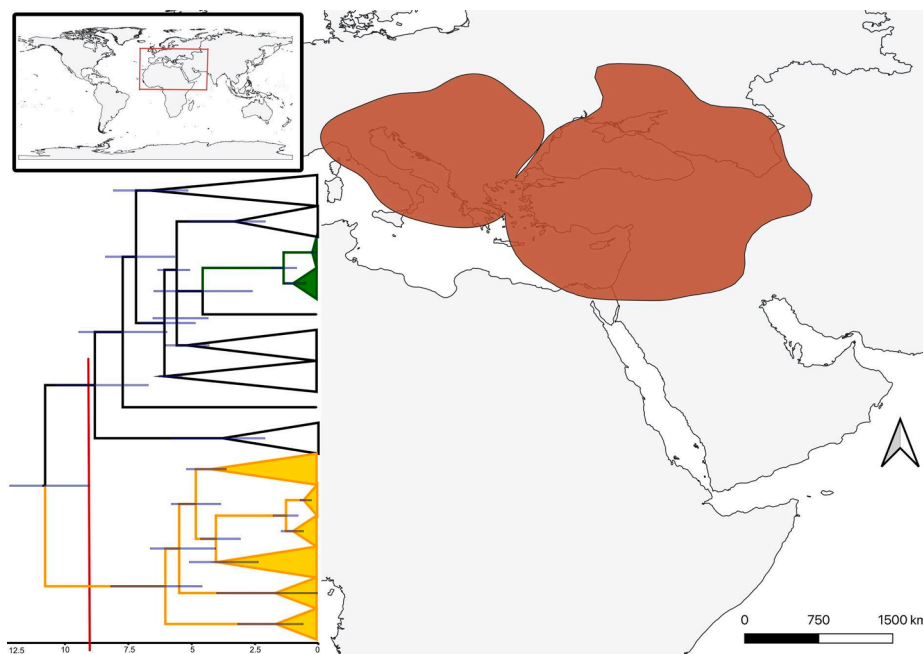

Fig. 2a. Estimated root ancestral area of *Silene* ca 9 Ma (shown by red line on inset phylogeny). Brown polygon represents inferred ancestral area at the time-slice shown by red line on inset phylogeny. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
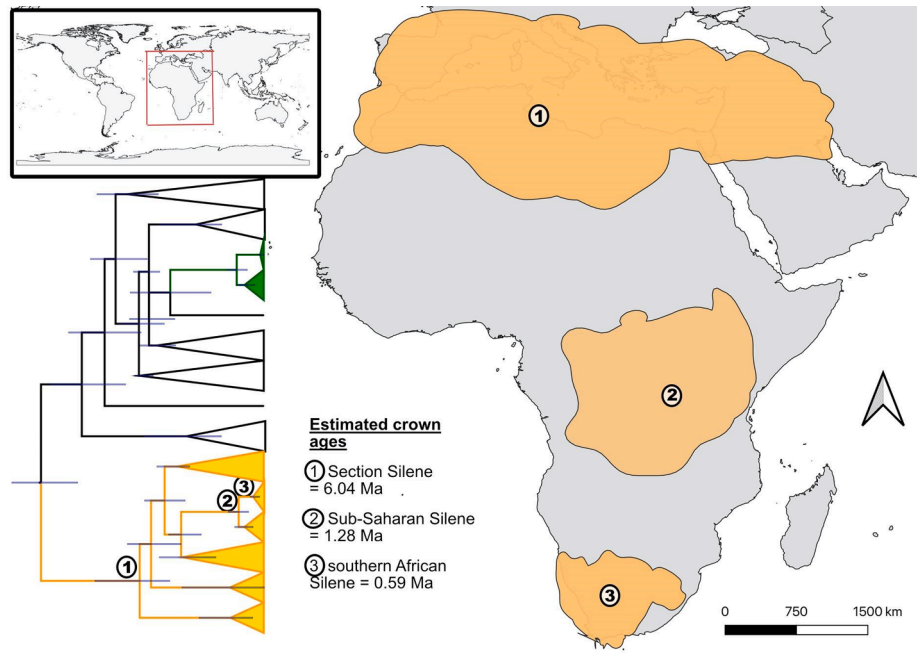
Fig. 2b. Radiation of section *Silene* in Eurasia and Africa. 1 – Estimated ancestral area of *Silene* in the Mediterranean Basin. 2 – Estimated ancestral area of section *Silene* in Central and East Africa. 3 – Estimated ancestral area members of section *Silene* in southern Africa. Orange polygons represent 80% HPD inferred ancestral areas for section *Silene*.

HPD: 0.06–0.3 Ma; Fig. 2c) through long-distance dispersal, followed by a subsequent diversification locally (Fig. 2c).

4. Discussion

4.1. Phylogenetic relationships of southern African Silene

Our results demonstrate that southern African *Silene* belong to two distantly related clades (sections *Silene* s.l. and *Elisanthe*; Fig. 1, S.1, S.2, S3). The placement of four of the eight southern African species (i.e. *S.*

undulata, S. rigens, S. saldanhensis and *S. ornata*) in section *Elisanthe* by Manning and Goldblatt (2012a) is supported by our results, with the species forming a well-supported monophyletic clade (Fig. 1, S.1, S.2, S.3), together with the Central Asian *S. turkestanica* and the Eurasian *S. noctiflora* (type for the section). Previous studies with less comprehensive sampling of the southern African *Silene* have reported a close relationship among *S. noctiflora, S. undulata* and *S. turkestanica* (e.g. Rautenburg et al., 2010: Jafari et al., 2020). Our study is the first to include a complete sampling of the southern African species, resolving the monophyly and composition of section *Elisanthe*. The
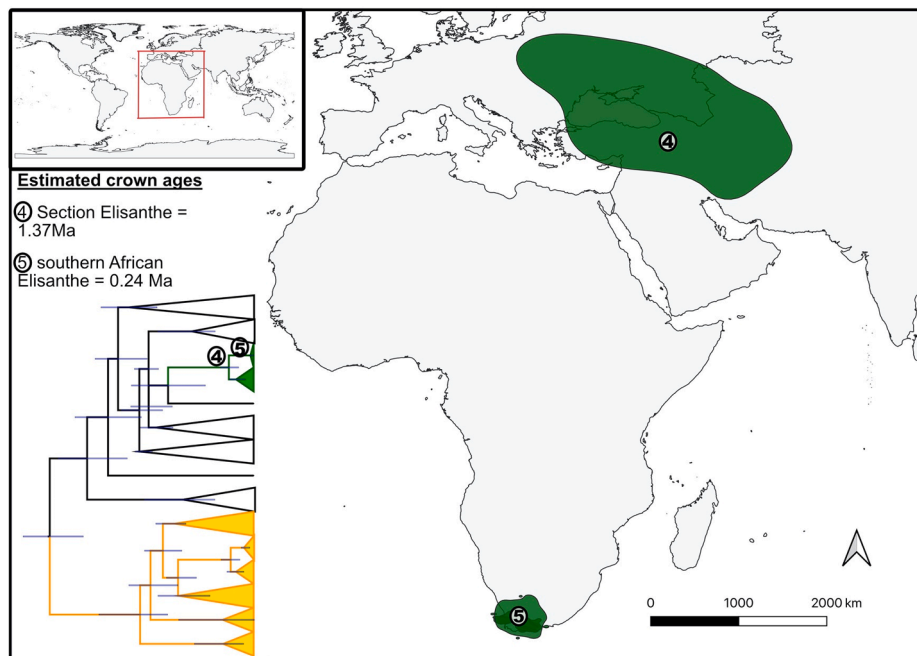

Fig. 2c. 4– Estimated ancestral area of section *Elisanthe* in the Mediterranean Basin. 5– Colonization and diversification of southern African members section *Elisanthe* in southern Africa. Green polygons represent 80% HPD inferred ancestral areas for section *Elisanthe*.

circumscription of section *Elisanthe* is also supported by diagnostic morphological characters which include globose seeds lacking peripheral wings, dichasial inflorescences, and a clavate or conical, pubescent calyx having 10 veins with reticulate connections (Manning and Goldblatt, 2012a). Our study recovered unresolved internal relationships within section *Elisanthe*, and resolving the internal relationships form part of an ongoing study on species delimitation within this group (Moiloa *et al.*, in prep).

Our results also indicate a close relationship between the perennials *S. burchellii*, *S. crassifolia* and *S. mundiana* and the annual *S. aethiopica*, which together form a strongly-supported clade with the Central African *S. biafrae* (Fig. 1, S.1, S.2, S.3). This clade is nested within a Mediterranean clade, the broadly circumscribed section *Silene,* which includes mostly annual species previously assigned to sections *Dipterospermae*, *Fruticulosae*, *Rubellae* (Batt.) Oxelman & Greuter, and *Nicaeenses* (Rohrb.) Talavera. Here, we follow the current phylogenetic circumscription of Jafari et al. (2020) which places all these sections (i.e. *Dipterospermae*, *Fruticulosae*, *Rubellae*, and *Nicaeenses*) into synonymy with the broadly circumscribed section *Silene*. According to the taxonomic classification by Chowdhuri (1957), the type species for *Dipterospermae* and *Fruticulosae* are *S. colorata* and *S. ciliata*, respectively. Our results demonstrate that *S. ciliata* and *S. colorata* do not form part of the sub-Saharan and southern African clade (comprising *S. aethiopica*, *S. burchellii*, *S. crassifolia*, *S. mundiana*, *S. biafrae*), thus highlighting that the sub-Saharan clade could in subsequent studies be described and assigned to a sub-sectional rank. The close relationship between *S. burchellii*, *S. crassifolia* and *S. mundiana* and the annual *S. aethiopica* is also supported by several morphological traits shared by all four species viz. the monochasial inflorescence and reniform seeds with a deep hilar notch and undulate peripheral wings (Fig. 1, S.1, S.2, S.3). A close relationship between *S. burchellii* and *S. aethiopica* was previously reported by Jafari et al. (2020) but their analyses did not include the rest of the southern African taxa. As currently circumscribed by Jafari et al. (2020), section *Silene* s.l. is strongly-supported as monophyletic, but internal relationships remain unresolved, as has been shown in our study. This also applies to the southern African members of section *Silene*, and resolving the internal relationships form part of an ongoing study on species delimitation within this group (Moiloa *et al.*, in prep).

4.2. Origin and diversification of Silene

There are many uncertainties to estimating the root age of *Silene*. Previous studies, based on a calibration with a 34–45 Ma old caryophyllaceous fossil (Jordan and Macphail, 2003) have estimated the root age of *Silene* to be *ca.* 11.6 Ma (95% HPD: 9.177–12.17; Frajman et al., 2009; Rautenberg et al., 2010; Rautenberg et al., 2012). However, in these studies, including ours, the phylogenetic resolution is uncertain. The origin of *Silene* is inferred in the Eurasian parts of the Mediterranean Basin (Fig. 2a) during the mid-late Miocene (95% HPD: 9.02–12.19 Ma). The Mediterranean Basin and adjoining areas have been identified as biodiversity hotspots for several angiosperm groups (Thompson, 2005). This biodiversity has been suggested to be as a result of several factors that include a complex climatic and paleo-geographical history of the region (Meulenkamp and Sissingh, 2003; Blondel et al., 2010). We therefore hypothesize that, similar to other angiosperm groups that originated in the Mediterranean Basin, ancestral *Silene* lineages diversified during the mid–Miocene as a result of microplate tectonic movement, which created geographic barriers between parts of the Mediterranean Basin (Meulenkamp and Sissingh, 2003; Sanmartín, 2003; Mico et al., 2009; Inda et al., 2014). Particularly, the dispersal and colonization of *Silene* in the southwestern parts of the Mediterranean Basin largely coincides with the onset of the Messinian salinity crisis which occurred towards the late Miocene (Meseguer et al., 2013). The Messinian salinity crisis is a paleo-environmental event during which the Mediterranean Sea underwent increased salinity and subsequent desiccation (Krijgsman et al., 1999; Krijgsman, 2002), thus leading to

the emergence of ephemeral land bridges connecting land masses promoting range expansion (Carlson et al., 2012). Similar to our results, studies focusing on other plant groups (e.g. Sanmartín, 2003; Thompson, 2005; Rodríguez-Sánchez et al., 2008; Migliore et al., 2012) have highlighted the role that the Messinian salinity crisis played on promoting range expansion and the diversification of plant lineages across the Mediterranean Basin.

4.3. Diversification of Silene in Africa

In Africa, *Silene* is most diverse in North Africa, particularly within the broadly circumscribed section *Silene* (Jafari et al., 2020). Our results infer the ancestral area for the North African members of section *Silene* clade to be in the northern parts of Africa, where most lineages colonized and diversified across North Africa (Fig. 2b). The current sampling however is not sufficiently complete (taxonomically) to conclusively infer the ancestral area as northwestern Africa, considering the representation of the members of section *Silene* in North Africa, most of which form part of an ongoing phylogenetic circumscription (Mesbah *et al.*, in prep). Our results are interpreted to suggest a northern African origin for African members of section *Silene* during the mid to late-Miocene, coinciding with the aridification event which began in Africa during the mid-Miocene (Coetzee, 1993; Caujapé-Castells et al., 2001; Pokorny et al., 2015). Our results show that during aridification of the Sahara region and other parts of Africa during the mid to late-Miocene (Senut et al., 2009), the geographical range of ancestral taxa of section *Silene* expanded southwards into sub-Saharan Africa (Central and Eastern Africa; Fig. 2b). This temporal framework suggests that range expansion may have been promoted by the emergence of suitable niches during the formation of the East African rift mountains (late Miocene to early Pliocene; Grove, 1983; Chorowicz, 2005; Sepulchre et al., 2006). Similar to our study, McGuire and Kron (2005) found that the formation of East African rift mountains and the establishment of the modern climatic conditions during the Miocene explain the dispersal and range expansion of *Erica* L. (Ericaceae) towards southern Africa (but see Pirie et al., 2019).

In sub-Saharan Africa, section *Silene* is represented by several species mainly occurring in southern Africa, as well as three species in central-western [*Silene cobalticola* P.A.Duvign. & Plancke (not included in this study) and *S. biafrae*] and eastern Africa (*S. burchellii*) (Figs. 1 & 2b). Our results suggest that the most recent common ancestor of section *Silene* underwent a southward radiation resulting in the subsequent colonization of southern Africa (Fig. 2b). Dispersal and radiation events from southern towards northern Africa have commonly been hypothesized to best explain the biogeographical patterns of several plant groups in Africa e.g. *Androcymbium* Willd., (Caujapé-Castells et al., 2001; del Hoyo et al., 2009), *Moraea* Mill. Ex L. (Goldblatt et al., 2002), *Senecio* L. (Coleman et al., 2003), *Disa* P.J.Bergius, the *Pentaschistis* (Nees) Stapf clade, and Restionaceae R.Br. (Galley et al., 2007). However, our results on the radiation of section *Silene* are not congruent with such a hypothesis, but rather support an alternative scenario (i.e. North to South radiation) similar to the findings of studies on *Erica* (McGuire and Kron, 2005), *Dianthus* L. (Valente et al., 2010), *Scabiosa* L. (Carlson et al., 2012) and subfamily Scilloideae Burnett (Asparagaceae) (Buerki et al., 2012).

A complementary and similarly likely explanation for the distribution patterns observed in section *Silene* and the sub-Saharan *Silene* in particular, is that these taxa exhibit characteristics of the 'Rand flora' disjunct distribution pattern (Sanmartín et al., 2010). The Rand flora pattern has been suggested to best explain the distribution patterns of several plant lineages in the Afro-Mediterranean region, where a southward dispersal was promoted by the formation of new niches resulting due to tectonic plate activity in Eastern Africa (Pokorny et al., 2015). A Rand flora pattern is congruent with our results for members of southern African section *Silene* (Figs. 1 & 2b), suggesting an origin in the Mediterranean Basin during the Miocene (Fig. 2b). Following

colonization of northwestern Africa, the lineages dispersed southwards towards sub-Saharan Africa and further southward towards southern Africa (Fig. 2b). This distribution is supported by the widespread distribution of *S. burchellii* (Fig. 2b), which extends throughout southern Africa to Ethiopia and Arabia (Manning and Goldblatt, 2012a). The southern African members of section *Silene* also include the GCFR endemic *S. aethiopica* (Fig. 1), which was placed in section *Dipterospermae* [now placed in synonymy with section *Silene* s.l. following Jafari et al. (2020)] by Manning and Goldblatt (2012a) who accordingly proposed that the species colonized southern Africa via long-distance dispersal. Our results do not support this hypothesis given that *S. aethiopica* is closely related to, and has a shared biogeographical history with several other southern African section *Silene* species as well as Central (*S. biafrae*) and East African (*S. burchellii*) species (Fig. 2b).

The results from the diffusion model show the most recent common ancestor of the African members of section *Elisanthe* colonized southern Africa via long-distance dispersal from Eurasia during the Pleistocene (0.24 Ma; 95% HPD: 0.06–0.30 Ma; Fig. 2c). Long-distance dispersal of lineages via transoceanic channels has been documented for a number of plant groups e.g. *Aeonium* Webb & Berthel. (Kim et al., 2008), *Geranium* L. (Fiz et al., 2008) as well as *Silene* (Eggens et al., 2007). Although less documented, long-distance dispersal within a continental landmass has been reported for *Senecio* (Coleman et al., 2003; Pelser et al., 2012). However, similar to Eggens et al. (2007) and Sochor et al. (2020), our results support a long-distance dispersal event within section *Elisanthe*. Our results suggest that it is less probable that numerous extinctions occurred within section *Elisanthe* because as observed on our phylogeny, the branch lengths are shorter (Figs. 1 & 2c) and also that the temporal gap between the stem and crown is not large, refuting possibilities of numerous extinctions linked to the dispersal of the genus towards southern Africa (Figs. 1 & 2c). A Eurasian origin of ancestors of southern African members of section *Elisanthe* is interesting because the genus is most represented in southern Africa and less in Eurasia (2 species in Eurasia). With no close relatives elsewhere in Africa, southern African members of section *Elisanthe* are derived from a long-distance dispersal event originating in the Mediterranean Basin (Fig. 2c). The disjunction pattern in section *Elisanthe* has also been observed by studies on other plant groups e.g *Erica* (McGuire and Kron, 2005), *Dianthus* (Valente et al., 2010), *Scabiosa* (Carlson et al., 2012), and *Echium* L. (Chacón et al., 2019).

The ability of plants to disperse over long distances has been tightly linked to specific diaspore traits which promote long-distance dispersal [e.g. anemochory (wind dispersed), thalassochory (sea dispersed), endozoochory (ingestion by animals then dispersed), epizoochory (externally attaching to and dispersed by animals)], mostly referred to in the literature as indirect evidence of dispersal syndromes (Vargas et al., 2014; Arjona et al., 2018). Most *Silene* (including members of section *Elisanthe*) do not exhibit any common dispersal traits which can be directly linked to a long-distance dispersal vector. All *Silene* species have rather small (1–2 mm) reniform to globose seeds enclosed in a capsule which ruptures at maturity and seeds are dispersed passively over a short distance as they have no obvious features allowing long-distance dispersal. However, there are studies which have reported how plant lineages that do not possess specialized diaspores have nevertheless been able to colonize new areas putatively via long-distance dispersal (e. g. Nogales et al., 2012; Vargas et al., 2014; Heleno and Vargas, 2015). Given that *Silene* seeds are quite small, with sculptured testa (particularly in sect. *Elisanthe*), it seems most likely that endozoochory, epizoochory or thalassochory best explain the long-distance dispersal of ancestral lineages of section *Elisanthe* which dispersed to southern Africa during the Pleistocene (Figs. 1 & 2c).

4.4. Species-tree diffusion model performance

In this study we followed a novel approach of using multilocus DNA sequence data and locality point coordinates to jointly estimate the species-tree and ancestral areas for the most recent common ancestors (MRCAs) of the extant species of *Silene*. The use of continuous diffusion models takes into account uncertainty in tree estimation and model parameters, allowing to average over all plausible trees for our data. The use of point coordinates allows to account for distribution heterogeneity and is a more direct way of using locality information related to each of the specimens included in our study. Previous implementations of the diffusion model utilized species distribution range encoded as KML polygons assumed a uniform probability (but see Dellicour et al., 2019) of occurrence of the specimens within the specified polygons (known as the uniform prior approach). Such an approach has been criticized due to the potential of overemphasizing sampling particularly on the margins of the species distributions, and also such an approach does not take into consideration erroneous taxonomic assignments. Within *Silene*, there is often an overlap between species distributions and niche preferences which makes the identification of discrete areas for the terminal taxa included in our study extremely difficult. In this study, we observed that where molecular data offered low resolution (i.e. not variable), spatial data contributed to the reconstruction. This is particularly evident at sectional level where several clades with low sequence variability were incorrectly placed in subg. *Behenantha* instead of the correct placement in subg. *Silene* (Fig. S.1). To determine the extent of this artefact we compared a species tree inferred without spatial data (tree not shown; but see Jafari et al., 2020) to our current species tree which includes spatial data (Fig. 1, S.1). In the species tree without spatial data, subg. *Silene* is monophyletic, however, the species tree with spatial data recovered a polyphyletic subg. *Silene*. Given that the loci used in this study had some invariable regions, we concluded that the spatial data may have strongly contributed and influenced the incorrect placement of several clades in subg. *Behenantha* instead of subg. *Silene* (Fig. 1, S.1, S.2, S.3). In addition, Kalkauskas et al. (2021) has demonstrated that continuous diffusion models are prone to negative impacts of sampling bias (e.g. oversampling in certain areas and less sampling in others).

In the context of our study, *Silene* is most taxonomically diverse in the Mediterranean Basin and is in most instances less represented in other areas (e.g. less represented in the Southern Hemisphere). Considering such a geographical distribution representation where most of the taxonomic samples in our study were mostly concentrated in the Mediterranean Basin, with few samples from other regions (i.e. sub-Saharan Africa, North America, Russia and Asia), we considered the impact of utilizing such a geographical representation for *Silene*. For taxonomic consistency, we follow the circumscription of Jafari et al. (2020), which identified several strongly-supported sections within the genus, most of which are represented in the Mediterranean Basin with few represented in the southern Hemisphere (e.g. southern Africa *Silene*). To address or detect the challenges related to sampling biases, Kalkauskas et al. (2021) tested how the inclusion of sequence-free samples is able to account for representation in cases where under-sampled areas are known. We did not include sequence-free samples here, and this approach would be interesting to explore, but we assumed that including sequence-free samples would have not impacted the main results of the clades of interest (*Elisanthe* and section *Silene*). Thus, for this study, spatial sampling bias (i.e. differences in sampling densities) seems to have affected inference less, compared to the instance where genetic loci provided low resolution resulting in a spatial contribution to the reconstruction.

5. Conclusion

Our study presents a phylogenetic reconstruction including all native southern African *Silene* species. We demonstrate that the native southern African taxa belong to two distantly-related groups (sections *Elisanthe* and *Silene* s.l.). Section *Elisanthe* is supported as a clade comprising southern African, and Eurasian taxa. The perennials *S. burchellii*, *S. mundiana* and *S. crassifolia* and the annual *S. aethiopica* comprise a monophyletic African group with the central African perennial *S. biafrae* and are subsequently nested in a currently broadly-circumscribed,

unresolved section *Silene*. Among members of sub-Saharan section *Silene*, the annual *S. aethiopica* is hypothetically derived from a perennial ancestor after the arrival of the lineage in southern Africa. Morphological traits emphasized in traditional classifications of *Silene* remain controversial and our study has demonstrated that the southern African taxa are not exceptional when it comes to this. Life history strategies (annual versus perennial) emphasized in *Silene* classification are unsuitable for use in delineating sections. Our study demonstrates that southern Africa was independently colonized on two different occasions. The *S. burchellii* group was the first to colonize southern Africa in the late early-Pleistocene from central to eastern Africa. Section *Elisanthe* presents an interesting floristic anomaly in Africa, having a disjunct distribution from southern Africa to the Mediterranean Basin, shaped via long-distance dispersal during the late Pleistocene. The biogeographic distribution of *Silene* in Africa has been influenced by the climatic and aridification events which occurred during the late Miocene as well as the Pliocene-Pleistocene.

CRediT authorship contribution statement

Ntwai A. Moiloa: Conceptualization, Investigation, Formal analysis, Data curation, Methodology, Validation, Writing - original draft, Visualization. **Melilia Mesbah:** Investigation, Writing - review & editing. **Stephan Nylinder:** Conceptualization, Methodology, Validation, Visualization, Writing - review & editing. **John Manning:** Resources, Writing - review & editing. **Félix Forest:** Resources, Writing - review & editing. **Hugo J. Boer:** Writing - review & editing, Supervision. **Christine D. Bacon:** Conceptualization, Writing - review & editing, Supervision. **Bengt Oxelman:** Conceptualization, Resources, Data curation, Methodology, Validation, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2021.107199.

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