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**Phylogeny, biogeography and ecological diversification of *Sarcocornia*
(Salicornioideae, Amaranthaceae)**

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• **Background and Aims** *Sarcocornia* comprises about 28 species of perennial succulent halophytes distributed worldwide, mainly in saline environments of warm-temperate and subtropical regions. The genus is characterized by strongly reduced leaves and flowers, which cause taxonomic difficulties; however, species in the genus show high diversity in growth form, with a mat-forming habit found in coastal salt marshes of all continents. *Sarcocornia* forms a monophyletic lineage with *Salicornia* whose species are all annual, yet the relationship between the two genera is poorly understood. This study is aimed at clarifying the phylogenetic relationship between *Sarcocornia* and *Salicornia*, interpreting biogeographical and ecological patterns in *Sarcocornia*, and gaining insights into putative parallel evolution of habit as an adaptation to environmental factors.

• **Methods** A comprehensively sampled and dated phylogeny of *Sarcocornia* is presented based on nuclear ribosomal DNA (external transcribed spacer) and chloroplast DNA (*atpB-rbcL*, *rpl32-trnL*) sequences; representative samples of *Salicornia* were also included in the analyses. To infer biogeographical patterns, an ancestral area reconstruction was conducted.

• **Key Results** The *Sarcocornia*/*Salicornia* lineage arose during the Mid-Miocene from Eurasian ancestors and diversified into four subclades: the *Salicornia* clade, the American *Sarcocornia* clade, the Eurasian *Sarcocornia* clade and the South African/Australian *Sarcocornia* clade. *Sarcocornia* is supported as paraphyletic, with *Salicornia* nested within *Sarcocornia* being sister to the American/Eurasian *Sarcocornia* clade. The American and the South African/Australian *Sarcocornia* clade as well as the *Salicornia* clade were reconstructed to be of Eurasian origin. The prostrate, mat-forming habit arose multiple times in *Sarcocornia*.

• **Conclusions** *Sarcocornia* diversified in salt-laden environments worldwide, repeatedly evolving superficially similar prostrate, mat-forming habits that seem advantageous in stressed environments with prolonged flooding, high tidal movement and frost. Some of these prostrate-habit types might be considered as ecotypes (e.g. *S. pacifica* or *S. pillansii*) while others represent good ecospecies (e.g. *S. perennis*, *S. decumbens*, *S. capensis*), hence representing different stages of speciation.

Key words: Africa, Australia, Chenopodiaceae, ecotypes, ecospecies, flooding tolerance, halophytes, growth form plasticity, Mediterranean, *Sarcocornia*, *Salicornia*, salinity, South America.

INTRODUCTION

Sarcocornia A.J. Scott (glasswort, samphire) is one of 16 genera in the Salicornioideae (Kadereit *et al.*, 2006) and comprises about 28 succulent species which can be found in saline vegetation worldwide, predominantly in warm-temperate and to a lesser extent in subtropical regions (Davy *et al.*, 2006; Kadereit *et al.*, 2006; Alonso and Crespo, 2008; Steffen *et al.*, 2010). These perennial herbs, subshrubs and shrubs are characterized by strongly simplified morphology giving a particular appearance to the plants (Fig. 1A–F). The genus *Sarcocornia* was established by Scott (1977) who separated it from *Salicornia* L. and *Arthrocnemum* Moq. on the basis of morphological characters. *Salicornia* and *Sarcocornia* show strong morphological similarity and can be distinguished only by inflorescence characters and life form. The individual flowers of *Sarcocornia*, which is always perennial, are more or less equal

in size and arranged in a horizontal row, while in *Salicornia*, which is always annual, the distinctly unequal sized flowers show a triangular arrangement and the two lateral flowers are in contact with each other beneath the central flower (Kadereit *et al.*, 2007). Both genera can be distinguished from *Arthrocnemum* and other Salicornioideae by their membranous testa, covered with papillae or hairs, a horseshoe-shaped embryo and the lack of perisperm (Scott, 1977).

A molecular phylogeny of the subfamily based on internal transcribed spacer (ITS) and *atpB-rbcL* spacer sequences revealed *Sarcocornia* formed a monophyletic lineage together with *Salicornia* L., which is clearly separated from *Arthrocnemum* (Kadereit *et al.*, 2006). The *Salicornia*/*Sarcocornia* clade is sister to a clade comprising *Halosarcia* P.G. Wilson and the exclusively Australian genera *Pachycornia* Hook.f., *Sclerostegia* P.G. Wilson, *Tecticornia* Hook.f. and



FIG. 1. Morphological characteristics and growth forms of *Sarcocornia*: the genus comprises perennial herbs, subshrubs and shrubs of strongly deviating growth form. The articulated, decumbent to erect stems are composed of fleshy, cylindrical, barrel- or club-shaped internodes (segments) and more or less constricted nodes that often bear adventitious roots (C, D, F–H). The segments emerge from the opposite connate leaves that are strongly reduced and only visible as a small rim at the upper edge of each segment, all forming a vascular cylinder enclosed by an inner water storage tissue and an outer photosynthetically active chlorenchyma (cortex). The spike-like inflorescence is a thyse (A, B, D) with 3–12 strongly reduced flowers per cyme (for detailed descriptions see: [Alonso and Crespo, 2008](#); [Steffen et al., 2010](#); [de la Fuente et al., 2013](#)). (A) *Sarcocornia blackiana*, Yalgorup National Park, Western Australia; (B) *S. pacifica*, Tomales Bay, California; with a *Cuscuta* growing on it; (C) *S. littorea*, Pearly Beach, Western Cape, South Africa; (D) *S. xerophila*, Knersvlakte, Western Cape, South Africa (A–D all erect, broomy or spreading shrubs); (E) dense cushions forming clonal *S. pulvinata*, Moquegua, Peru; (F) loose carpet-forming clonal *S. tegetaria*, Langebaan Lagoon, Western Cape; (G) short-lived prostrate herb *S. natalensis* subsp. *affinis*, West Coast, Western Cape, South Africa; (H) loose spreading spider-like clonal *S. capensis*, West Coast, Western Cape, South Africa. Photo credits: A, C, D, F, G: L. Mucina; B, F: G. Kadereit; E: D. Montesinos Tubée.

Tegicornia P.G. Wilson (now all included in *Tecticornia* according to [Shepherd and Wilson, 2007](#)). The *Salicornia*/*Sarcocornia* lineage diversified during the Middle Miocene (14.2–9.4 Ma) and split into three primary clades: (1)

American/Eurasian *Sarcocornia*, (2) *Salicornia* and (3) South African/Australian *Sarcocornia*. These findings are congruent with a detailed molecular phylogeny of *Salicornia* based on external transcribed spacer (ETS) sequence data

(Kadereit *et al.*, 2007). Relationships among these three clades remain unresolved. In the ITS tree, *Salicornia* arises from within the South African/Australian *Sarcocornia*, while in the *atpB-rbcL* spacer tree it originates from within the American/Eurasian *Sarcocornia*. In the ETS tree, *Salicornia* is sister to a clade comprising American and Eurasian *Sarcocornia*. However, none of these topologies receives sufficient statistical support and it remains unclear if *Sarcocornia* is paraphyletic with respect to *Salicornia*.

Ecologically *Sarcocornia* and *Salicornia* are very similar. Both genera contain predominantly typical halophytes found in coastal salt marshes, tidal mud flats, coastal cliffs, inland salt pans and edges of saline lakes throughout the world (Davy *et al.*, 2006; Alonso and Crespo, 2008; Steffen *et al.*, 2010; de la Fuente *et al.*, 2013; Fig. 2). Here, the high ambient salinity (exacerbated by seasonal lack of precipitation) and frequent and often prolonged flooding are the driving ecological forces in the environments occupied by *Sarcocornia* (Fig. 2C, F). Only rarely do they occur in habitats experiencing low salinity levels such as semi-desert quartz patches [*S. xerophila* (Tölken) A.J. Scott] in South Africa (Steffen *et al.*, 2010; Fig. 2A). Some members of *Sarcocornia* also inhabit continental areas of high mountain ranges but always associated with saline habitats (e.g. Montesinos Tubée, 2012; Fig. 2D).

Sarcocornia occurs in the Mediterranean and winter-mild Atlantic coasts of Europe, the Americas, southern Africa and Australia (compare Table 1). In *Flora Europaea* (Ball, 1964) only two species are accepted, the type species *S. perennis* (Miller) A.J. Scott [as *Arthrocnemum perenne* (Miller) Moss] and *S. fruticosa* (L.) A.J. Scott [as *A. fruticosum* (L.) Moq.]. However, more recent work concentrating on the Western Mediterranean added *Sarcocornia alpini* (Lag.) Rivas-Martínez (Castroviejo, 1990), *S. hispanica* Fuente, Rufo & Sánchez-Mata (de la Fuente *et al.*, 2011) and *S. pruinosa* Fuente, Rufo & Sánchez-Mata (de la Fuente *et al.*, 2013) showing that the diversity of the genus in the Mediterranean was probably underestimated. A molecular study based on ITS sequence data revealed that *S. hispanica* is sister to all other Eurasian *Sarcocornia* species that form a large polytomy (de la Fuente *et al.*, 2013). *Sarcocornia hispanica* is also morphologically distinct by a partial fusion of seed and pericarp and by conical seeds papillae (de la Fuente *et al.*, 2011). Although lacking unique mutations in ITS, the remaining species are either ecologically and/or morphologically distinct (compare Table 1).

Seven species of *Sarcocornia* are native to the Americas (Ball, 2003; Alonso and Crespo, 2008; Table 1), three in North America and four in South America. Four of the American species are predominantly coastal species (Figs 1B and 2E) while three occur exclusively or predominantly inland, two of these being endemic to the high plateaus of Argentina, Bolivia, Chile and Peru. *Sarcocornia andina* (Phil.) Freitag, M.A. Alonso & M.B. Crespo grows on dry high-latitude flanks (3100–3500 m) of the Andes and *S. pulvinata* (R.E. Fr.) A.J. Scott over 3500 m on the Altiplano Andino (Figs 1E and 2D; Table 1).

From Australia, three species have been described (Wilson, 1980, 1984; Figs 1A and 2B). *Sarcocornia quinqueflora* (Ung.-Sternb.) A.J. Scott and *Sarcocornia blackiana* (Ulbr.) A.J. Scott show a wide distribution along Australian, Tasmanian and New Caledonian coasts as well as a few inland

localities in New Zealand and New Caledonia (<http://herbier-noumea.plantnet-project.org/intro?strLangue=fr>; Connor, 1984). *Sarcocornia blackiana* is found in rarely flooded saline habitats, while *S. quinqueflora* prefers intertidal habitats, but also occurs on supratidal flats and along the edges of inland salt lakes and pans (Fig. 2B). The third species, *S. globosa*, is restricted to inland areas and occurs on elevated edges of saline lakes and alluvia of Western Australia (Wheatbelt; Datson, 2002).

The centre of diversity of the genus is clearly located in southern Africa where 12 species are recognized (Steffen *et al.*, 2010). The widespread *Sarcocornia tegetaria* S. Steffen, Mucina & G. Kadereit and the narrow endemic *S. mossambicensis* Brenan occupy the lower zone of estuarine salt marshes where they are subject to tidal flooding (Figs 1F and 2G). Also, *S. dunensis* (Moss) S. Steffen, Mucina & G. Kadereit is a coastal taxon endemic to sabkhas of the Namibian Desert coast. Other species can be found in salt marshes and tidal flats along the southern African coastline, although they are not necessarily restricted to coastal habitats but also occur in inland localities such as salt pans, brackish lakes and saline bottoms of intermittent rivers (Fig. 2G). Of these *S. natalensis* (Bunge ex Ung.-Sternb.) A.J. Scott and *Sarcocornia pillansii* (Moss) A.J. Scott are the most widespread while *S. capensis* (Moss) A.J. Scott and *S. decumbens* (Tölken) A.J. Scott show a more restricted distribution (Table 1). *Sarcocornia littorea* (Moss) A.J. Scott is ecologically unique as it occurs exclusively on surf-splashed coastal cliffs. In the Western Cape of South Africa there are five strictly inland taxa. *Sarcocornia xerophila* is endemic to the quartz patches of the Knervslakte in southern Namaqualand (Fig. 2A), while *S. decussata* S. Steffen, Mucina & G. Kadereit occurs on quartz patches and adjacent saline alluvia in the Piketberg region. *Sarcocornia freitagii* S. Steffen, Mucina & G. Kadereit is a local endemic of the surroundings of Veldrif, where it occurs in salt pans and the alluvia of intermittent saline streams. *Sarcocornia terminalis* (Tölken) A.J. Scott has been described from banks of intermittent saline rivers in Namaqualand. Finally *Sarcocornia mossiana* is the most abundant inland species, occurring in saline depressions and on alluvia of rivers draining the West Coast and the Overberg region of the Western Cape. There are also some deep inland populations (limited to semi-desert scrub on quartz patches) that might be assigned to *S. mossiana* in the broad sense although these are in need of further enquiry.

The strongly reduced morphology of *Sarcocornia* results in a paucity of characters useful for species delimitations. Seed micromorphology proved to be valuable (Wilson, 1980, 1984; Ball, 2003; Davy *et al.*, 2006; Alonso and Crespo, 2008; Steffen *et al.*, 2010; Guilló *et al.*, 2013) and probably stomatal position might become a valuable character in the future (Guilló *et al.*, 2013). Other morphological features such as growth form, shape and size of segments, and inflorescence characters have also proved useful taxonomically. However, these traits must be considered critically and preferably in combination with each other to allow unambiguous species identification. It is, for instance, unwise to rely solely on growth form as the shape of branching and form is more than often not preserved on herbarium specimens, the dry tissues change their shape in dramatic manner (O'Callaghan, 1992; Datson, 2002) and reliable habit notes are a rarity.

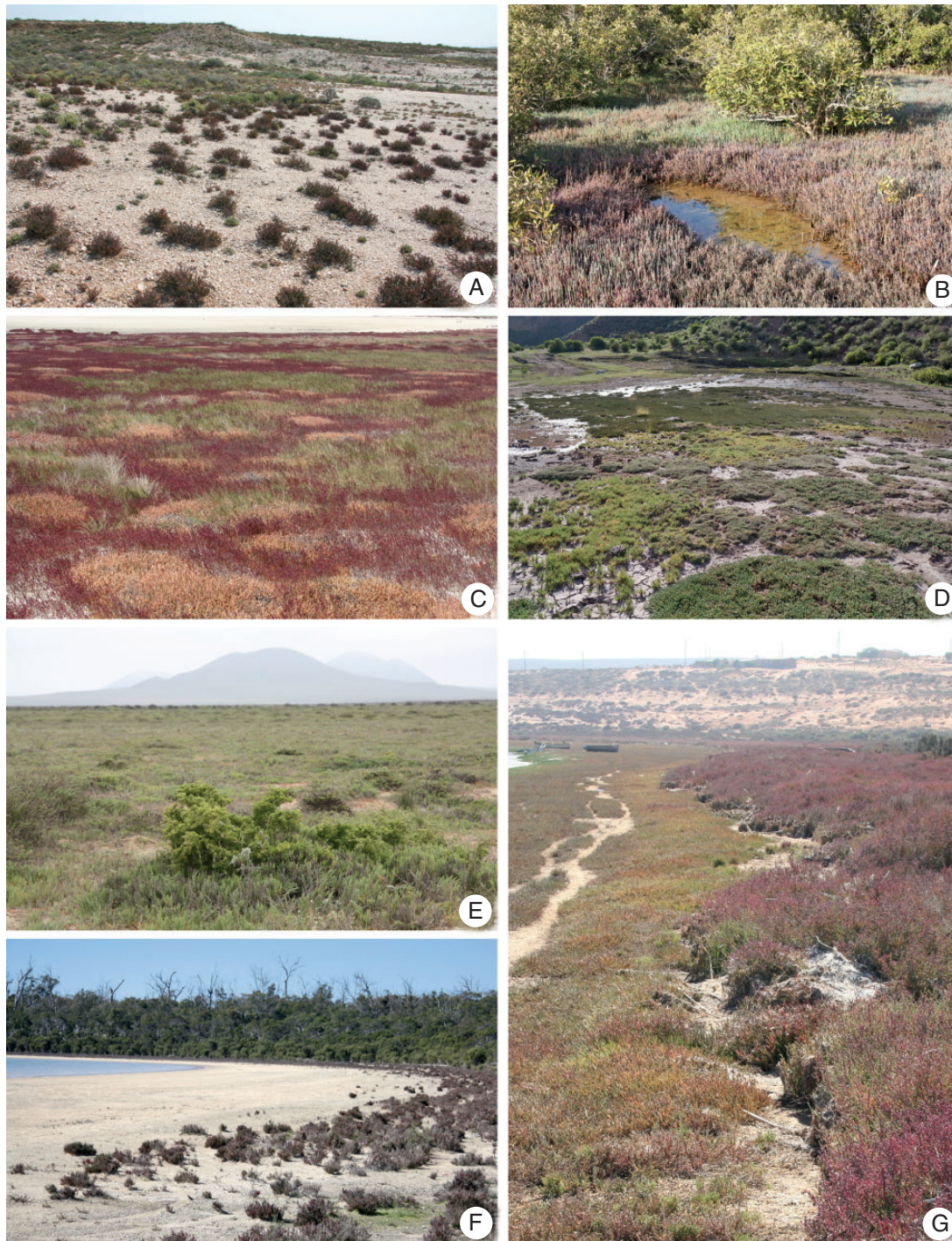


FIG. 2. Habitats of *Sarcocornia*. (A) Inland quartz patch supporting stone-plant-rich succulent scrub, habitat of *S. xerophila*, Knersvlakte, Western Cape, South Africa; (B) edge of the subtropical *Avicennia marina* mangrove fringed by dense carpet of low-growth carpet-forming *S. quinqueflora*, Little Lagoon, Denham, Western Australia; (C) colourful mosaic of light-coloured patches of non-clonal patch-forming short-lived herbaceous *S. natalensis* subsp. *affinis* and deep-purple, dense populations of the west Coast ecotype of *S. meyeriana*, Jakkalsrivier Lagoon near Lambert's Bay, West Coast, South Africa; (D) high-altitude salt pan at Moquegua, Peru – habitat of *S. pulvinata*; (E) supratidal terrace of the coastal salinas near San Quintin, Baja California, Mexico, with *S. pacifica*, *Arthrocnemum terminale* and *Sporobolus virginicus* (*Lycium* sp. in foreground); (F) bank of coastal lagoon (Harvey Lake) cut of the sea (Indian Ocean) near Preston in Western Australia – the fringes of the lake are habitats of *S. blackiana*; (G) strandline separating the low-tidal microterrace with *S. tegetaria* and slightly elevated terrestrial (supratidal terrace) with *S. pillansii* (West Coast arid ecotype) in the semi-arid estuary of the Olifants River Mouth near Vredendal, Namaqualand, South Africa. All photos: L. Mucina (except for D: D. Montesinos Tubée).

TABLE 1. Currently accepted species of *Sarcocornia* (*Salicornioideae*, *Amaranthaceae*), their distribution, habitat, growth form and chromosome number

Species*	Distribution	Habitat	Growth form	Chromosome number (2n)	No of accessions included in this study†	Reference(s)
Eurasian & North African <i>Sarcocornia</i>						
<i>S. alpini</i> (Lag.) Rivas-Martínez	Iberian Peninsula	Middle to high intertidal zones; inland salt pans	Prostrate to erect shrub, sometimes rooting at the nodes	18 (diploid)	1	de la Fuente <i>et al.</i> (2013)
<i>S. fruticososa</i> (L.) A.J. Scott	Mediterranean coasts, Atlantic coast of France	Upper tidal and supra-tidal coastal terraces	Erect shrub	36, 54, 72 (tetraploid to octoploid)	6	Castraviejo (1990), Greuter <i>et al.</i> (1984) de la Fuente <i>et al.</i> (2011)
<i>S. hispanica</i> Fuente, Rufo & Sánchez-Mata	SE Iberian Peninsula	Saline depressions outside of tidal influence, endorheic lakes, canals of salt works	Erect shrub	54 (hexaploid)	2	
<i>S. perennis</i> (Miller) A.J. Scott	Atlantic coasts of W and S Europe and N Africa, Mediterranean coasts	Intertidal zone of salt marshes	Mat-forming, decumbent sub-shrub, rooting at the nodes	18 (diploid)	7	Davy <i>et al.</i> (2006)
<i>S. pruinosa</i> Fuente, Rufo & Sánchez-Mata	Atlantic coasts of France, Spain and Portugal	Intertidal zone of salt marshes	Erect sub-shrub, occasionally rooting at the nodes	72 (octoploid)	–	de la Fuente <i>et al.</i> (2013)
American <i>Sarcocornia</i>						
<i>S. ambigua</i> (Michx.) M.A. Alonso & M.B. Crespo	Atlantic coasts of North America, Caribbean coasts	Coastal salt marshes	Prostrate to erect shrub sometimes rooting at the base	–	10	Ball (2003), Alonso and Crespo (2008)
<i>S. andina</i> (Phil.) Freitag, M.A. Alonso & M.B. Crespo	Atacama Desert in Chile	Saline soils of the high plateaus	Sub-caespitose, cushion-like dwarf shrub, rooting at the nodes	–	1	Alonso and Crespo (2008)
<i>S. magellanica</i> (Phil.) M.A. Alonso & M.B. Crespo	Patagonia and Tierra del Fuego in Argentina	Coastal salt marshes	Mat-forming, decumbent sub-shrub, rooting at the nodes	–	3	Alonso & Crespo (2008)
<i>S. neei</i> (Lag.) M.A. Alonso & M.B. Crespo	Pacific Coast of Chile and Peru, through the northern half of Argentina, to the Atlantic coast of Argentina	Saline deserts	Erect shrub	–	8	Alonso and Crespo (2008)
<i>S. pacifica</i> (Standl.) A.J. Scott	Pacific coast of North America from Alaska to Baja California, inland in southern California and Death Valley bordering the Panamint Lakes	Coastal salt marshes	Prostrate to erect shrub, sometimes rooting at the nodes	–	22	Ball (2003)
<i>S. pulvinata</i> (R.E. Fr.) A.J. Scott	Altiplano Andino in Peru and Bolivia	Strongly saline soils on the edges of temporary, endorheic salt pans	Pulvinate, in dense tufts	–	1	Alonso and Crespo (2008)

(Continued)

TABLE 1. *Continued*

Species*	Distribution	Habitat	Growth form	Chromosome number (2n)	No of accessions included in this study†	Reference(s)
<i>S. utahensis</i> (Tidestr.) A.J. Scott	Utah south to Texas and northern Mexico; shores of the Gulf of Mexico from north-eastern Mexico eastwards to Louisiana, with a disjunct location in the Bahamas	Coastal salt marshes and inland saline habitats	Erect to decumbent shrub	–	3	Ball (2003)
South African <i>Sarcocornia</i>						
<i>S. capensis</i> (Moss) A.J. Scott	Western and Eastern Cape of South Africa	Inland salt pans, supratidal terraces in estuarine salt marshes, depressions	Decumbent creeping shrub, rooting at the nodes	–	8	Steffen et al. (2010)
<i>S. decumbens</i> (Tölken) A.J. Scott	Southern and eastern coasts of South Africa and Mozambique	Coastal and inland salt marshes	Decumbent, often mat-forming shrub, rooting at the nodes	–	6	Steffen et al. (2010)
<i>S. decussata</i> S. Steffen, Mucina & G. Kadereit	Western Cape of South Africa	Inland quartz patches and adjacent saline alluvia	Erect or spreading shrub	–	3	Steffen et al. (2010)
<i>S. dunensis</i> (Moss) S. Steffen, Mucina & G. Kadereit	Lüderitz, Namibia	Sandy edges of arid-region saline flats	Decumbent creeping sub-shrub, rooting at the nodes	–	1	Steffen et al. (2010)
<i>S. freitagii</i> S. Steffen, Mucina & G. Kadereit	West coast of South Africa	Inland salt pans and saline alluvia	Prostrate to decumbent short-lived perennial herb	–	2	Steffen et al. (2010)
<i>S. littorea</i> (Moss) A.J. Scott	West and South Coasts of South Africa	Coastal cliffs	Erect shrub	–	5	Steffen et al. (2010)
<i>S. mossambicensis</i> Brenan	Southern Mozambique	Estuarine flats and edges of mangroves	Prostrate to decumbent sub-shrub, mat-forming, rooting at the nodes	–	–	Steffen et al. (2010)
<i>S. mossiana</i> (Tölken) A.J. Scott	Western Cape of South Africa	Inland salt pans and saline alluvia	Spreading shrub	–	4	Steffen et al. (2010)
<i>S. natalensis</i> (Bunge ex Ung.-Stemb.) A.J. Scott	Ocean coasts of Namibia, South Africa and Mozambique	Wetlands, coastal and inland salt pans and salt marshes	Prostrate to decumbent herb or sub-shrub	–	16	Steffen et al. (2010)
<i>S. pillansii</i> (Moss) A.J. Scott	South African coasts	Supratidal terraces of coastal salt marshes, inland salt pans and saline alluvia	Erect (sometimes decumbent) shrub	–	24 (incl. putative hybrids)	Steffen et al. (2010)
<i>S. tegetaria</i> S. Steffen, Mucina & G. Kadereit	Atlantic and Indian Ocean coasts of Namibia, South Africa and Mozambique	Intertidal zones of estuaries	Prostrate to decumbent sub-shrub, mat-forming, rooting at the nodes	–	10 (incl. putative hybrids)	Steffen et al. (2010)

(Continued)

TABLE 1. *Continued*

Species*	Distribution	Habitat	Growth form	Chromosome number (2n)	No of accessions included in this study†	Reference(s)
<i>S. terminalis</i> (Tölken) A.J. Scott‡	Namaqualand in South Africa	Elevated, saline river alluvia	Erect shrub	–	4	Steffen <i>et al.</i> (2010)
<i>S. xerophila</i> (Tölken) A.J. Scott	Namaqualand in South Africa	Inland quartz patches	Erect shrub	–	1	Steffen <i>et al.</i> (2010)
Australian <i>Sarcocornia</i> <i>S. blackiana</i> (Ulbr.) A.J. Scott	Southern and western coasts of Australia, coasts of Tasmania, coasts and inland localities of New Zealand and New Caledonia	Rarely flooded saline habitats	Erect shrub	–	2	Wilson (1980, 1984)
<i>S. globosa</i> P.G. Wilson	Western Australia	Elevated edges of saline lakes and alluvia	Erect shrub	18 (diploid)	1	Shepherd and Yan (2003), Wilson (1980, 1984)
<i>S. quinqueflora</i> (Bunge ex Ung.-Stemb.) A.J. Scott	Eastern, southern and western coasts of Australia, coasts of Tasmania, coasts and inland localities of New Zealand and New Caledonia	Intertidal habitats, supratidal flats, edges of inland salt lakes and pans	Mat-forming, decumbent subshrub	36 (tetraploid)	2	Shepherd and Yan (2003), Wilson (1980, 1984)

*This compilation does not include subspecies.

†Our assessment for the sampling in this study was based on previous molecular studies, and biogeographical and morphological diversity. The first was only available for Eurasian and North African *Sarcocornia* (de la Fuente *et al.*, 2013). Detailed molecular studies were not available for the American, South African and Australian species. Therefore, we used more accessions for these regions. In the Americas we had to cover a very large distribution area for *Sarcocornia* (Alonso and Crespo, 2008) and in South Africa we had to cover very high morphological diversity (Steffen *et al.*, 2010).

‡The systematic status of *S. terminalis* remains unclear. Morphologically it is most similar to *S. pillansii* (Steffen *et al.*, 2010), although Tölken (1967) states in his original description strong similarities to *S. mossiana*.

In *Sarcocornia*, there are mat-forming, decumbent subshrubs, often showing clonal growth patterns (*S. capensis*, *S. decumbens*, *S. dunensis*, *S. magellanica*, *S. mossambicensis*, *S. perennis*, *S. quinqueflora*, *S. tegetaria*), prostrate to decumbent herbaceous perennials (*S. freitagii*, *S. natalensis*) and more or less erect shrubs (*S. ambigua*, *S. blackiana*, *S. decussata*, *S. fruticosa*, *S. globosa*, *S. hispanica*, *S. littorea*, *S. mossiana*, *S. neei*, *S. pacifica*, *S. pillansii*, *S. utahensis*, *S. xerophila*; Fig. 1; Table 1). In high altitudes of the Andes, two peculiar, cushion-forming species can be found (*S. andina* and *S. pulvinata*). The clonal habit, involving above-ground creeping stems rooting at nodes, found in the intertidal zone of estuaries and salt-marshes on various continents (e.g. *S. magellanica*, *S. mossambicensis*, *S. perennis* and *S. tegetaria*) is suggestive of a filtering pressure of the local stress factors on selecting traits producing putative ecological adaptations. Interestingly, prostrate or mat-forming ecomorphotypes of widespread species such as *S. pillansii* might have originated multiple times and display local adaptation, a pattern also found in *Salicornia* (Teege *et al.*, 2011).

Due to their high salt tolerance, high nutritional value and long tradition of consumption by humans, *Salicornia* and *Sarcocornia* are among the most promising halophyte crops (Ventura and Sagi, 2013, and references therein). They serve as model plants to develop cultivation systems for halophyte food production using saline irrigation. Equally important might be their role in rehabilitation of salinized environments (Barrett-Lennard, 2003). To assess genetic diversity and to select the most promising candidate species within the two genera, phylogenetic, genetic and cytological background studies are essential. For *Salicornia*, known to be a taxonomically notoriously difficult genus, several phylogenetic and genetic studies have been conducted (Kadereit *et al.*, 2007, 2012; Vanderpoorten *et al.*, 2010; Teege *et al.*, 2011; Slenzka *et al.*, 2013) yielding important insights regarding breeding system, polyploidization, phenotypic plasticity, local adaptation, dispersal and biogeography. For *Sarcocornia*, such data are still scarce and a worldwide phylogenetic treatment of the genus is still missing.

Using molecular-phylogenetic analyses based on ETS, *atpB-rbcL* and *rpl32-trnL* sequences and a broad sampling of *Sarcocornia* the present study is aimed at (1) clarifying the phylogeny of the genus *Sarcocornia* and its relationship to *Salicornia*, (2) interpreting the biogeographical and ecological

patterns in *Sarcocornia* and (3) contributing to understanding of putative parallel evolution of habit types in response to environmental factors.

MATERIAL AND METHODS

Plant material and sampling

We used herbarium specimens from Aarhus University (AAU), Ankara University (ANK), Botanical Garden and Botanical Museum Berlin-Dahlem (B), California Academy of Sciences (CAS), Field Museum of Natural History (F), Johannes Gutenberg-University Mainz (MJG), University of Michigan (MICH), Moscow State University (MW), Royal Botanic Gardens and Domain Trust Sydney (NSW), Kassel University (KAS), Herbario Nacional de Bolivia, Universidad Mayor de San Andrés (LPB), Leipzig University (LZ), Osnabrück University (OSBU), Royal Ontario Museum (TRT), Erindale College, University of Toronto (TRTE), and University of California (UC) for this study (Supplementary Data Table S1). Sampling of currently recognized *Sarcocornia* species is nearly complete according to recent taxonomic treatments (Wilson, 1984; Ball, 2003; Davy *et al.*, 2006; Alonso and Crespo, 2008; Steffen *et al.*, 2010). We included multiple accessions for most species to test for species monophyly. Representative species of *Salicornia* were included as informed by the studies of Kadereit *et al.* (2007, 2012). Representatives of *Microcnemum*, *Arthrocnemum* and *Tecticornia* were included as outgroups (Kadereit *et al.*, 2006). Altogether 167 accessions were included in the phylogenetic analyses representing approx. 28 species of *Sarcocornia*, approx. 10 species of *Salicornia* and four outgroups (Supplementary Data Table S1).

Sequencing and phylogenetic inference

Total DNA was extracted from 20 mg of dried leaf-material using the Dneasy Plant Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's specifications. PCR was carried out in a T-Professional or T-Gradient Thermocycler (Biometra, Göttingen, Germany). Table 2 gives the details of primer sequences, PCR recipe and cyler programme for each marker. We

TABLE 2. PCR conditions and primer sequences for the three markers sequenced in this study.

Marker	Primer sequences and references	PCR recipe (μL); 25-μL aliquots	Cycler programme
ETS	ETS <i>Salicornia</i> -F 5'-GTCCCTATTGT GTAGATTTCAT-3'; 18S-II R 5'-CTCTAACTGATTTAATGAGCC ATTCGCA-3' (Kadereit <i>et al.</i> , 2007)	ddH ₂ O 16.3, MgCl ₂ (50 mg mL ⁻¹) 2.25, buffer 2.5, dNTPs (10 μM) 0.25, <i>Taq</i> polymerase 0.2, F primer (10 μM) 1, R primer (10 μM) 1, DNA template 1.5	95 °C for 4 min, 30 cycles of [95 °C for 30 s, 50.5 °C for 45 s, 72 °C for 4 min], 50.5 °C for 72 s, 72 °C for 8 min or 97 °C for 90 s, 35 cycles of [97 °C for 20 s, 69 °C for 90 s, 72 °C for 90 s], 72 °C for 7 min
<i>rpl32-trnL</i> spacer	trnL (UAG): 5'-CTGCTTCCTAAGAG CAGCGT-3'; rpl 32-F: 5'-CAGTTCCA AAAAACGTACTTC-3' (Shaw <i>et al.</i> , 2007)	ddH ₂ O 19.45, MgCl ₂ (50 mg mL ⁻¹) 0.6, buffer 2.5, dNTPs (10 μM) 0.25, <i>Taq</i> poly- merase 0.2, F primer (10 μM) 0.5, R primer (10 μM) 0.5, DNA template 1.0	94 °C for 60 s, 35 cycles of [94 °C for 30 s, 52 °C for 50 s, 72 °C for 60 s], 94 °C for 30 s, 52 °C for 72 s, 72 °C for 8 min
<i>atpB-rbcL</i> spacer	<i>atpB-rbcL</i> -spacer F 5'-GAAGTAGT AGGATTGATTCTC-3'; <i>atpB</i> - <i>rbcL</i> -spacer R 5'-CAACACTTGCTTT AGTCTCTG-3' (Kadereit <i>et al.</i> , 2006)	ddH ₂ O 19.25, MgCl ₂ (50 mg mL ⁻¹) 1, buffer 2.5, BSA 0.25, dNTPs (10 μM) 0.25, <i>Taq</i> polymerase 0.25, F primer (10 μM) 0.5, R primer (10 μM) 0.5, DNA template 0.5	94 °C for 1 min, 35 cycles of [94 °C for 20 s, 56 °C for 30 s, 72 °C for 60 s], 94 °C for 20 s, 56 °C for 80 s, 72 °C for 8 min

selected two variable chloroplast (cp) markers, *atpB-rbcL* spacer and *rplL32-trnL* spacer, and ETS as the most variable nuclear marker known for Salicornioideae (Kadereit *et al.*, 2007, 2012). We did not use ITS because of its limited variability in *Sarcocornia* (Kadereit *et al.*, 2006; de la Fuente *et al.*, 2013). PCR products were checked on 1 % agarose gels and purified subsequently using the NucleoSpin Gel and PCR clean-up-Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's manual. DNA sequences were obtained using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) in combination with the primers mentioned above following a purification step using an Illustra Sephadex™ G-50 Fine DNA Grade (GE Healthcare, Little Chalfont, UK). DNA fragments were sequenced using an automatic capillary sequencer GA3130XL (Applied Biosystems) following the Sanger method. Forward and reverse sequences were edited and merged to consensus sequences, which then were aligned using Sequencher 4.1.4 (Gene Codes Corp., Ann Arbor, MI, USA). All alignments were checked and corrected manually.

At first the chloroplast data set consisting of the *atpB-rbcL* spacer and *rplL32-trnL* spacer sequences and the nuclear data set consisting of ETS sequences were analysed separately. For all accessions included in both partitions (cp and nuclear) a combined analysis was then conducted. Maximum-likelihood (ML) phylogenetic analyses including bootstrapping were performed using RaxML for all three matrices (cp, nuclear, combined; Stamatakis, 2006; Stamatakis *et al.*, 2008). The cp data set contained a high number of informative indels. Therefore, in a parsimony analysis 27 informative indels were coded in a 0/1 matrix in the cp data set. Parsimony analyses were performed for the cp and nuclear data set using PAUP* (Swofford, 2003) with 1000 replicates of random addition of sequences and TBR branch swapping. A maximum of 20 best trees were stored for each replicate. Bootstrap support was calculated using ten random additions of taxa in 100 bootstrap replicates. Otherwise the same settings as in the heuristic search were used.

Additionally, trees and node ages were generated using BEAST v1.5.4 (Rambaut and Drummond, 2003; Drummond and Rambaut, 2007). The BEAST xml input files were generated with BEAUti v1.5.4 (Drummond and Rambaut, 2007). Monophyly of the ingroup (all except *Arthrocnemum*) was constrained to root the tree. The following settings were chosen: substitution model parameters GTR + G with four categories for G, relaxed clock model with exponential distribution, and a birth and death demographic model were assumed (Drummond *et al.*, 2006). We used published node ages to constrain the stem node age of the *Sarcocornia/Salicornia* lineage (Kadereit *et al.*, 2006). The corresponding prior age constraints used in the analyses were 14.6–19.6 Ma for the stem of the *Sarcocornia/Salicornia* clade. The Markov chain Monte Carlo (MCMC) method was initiated twice independently on a random starting tree with 30 000 000 iterations and sampling frequency of 1000. Convergence of model parameters was confirmed using TRACER (Rambaut and Drummond, 2003). Burn-in values were determined empirically from the likelihood values and posterior probability (PP) clade support was calculated together with the medians and 95 % confidence limits for ages of the nodes. The post-burn-in tree sample was sub-sampled to obtain approx. 1000 trees for use in character optimizations.

Ancestral area analysis

The ancestral area analysis was conducted using RASP software (Yu *et al.*, 2010, 2011). Five broad areas based on the extant distribution of the *Sarcocornia/Salicornia* lineage were coded: A = Eurasia/North Africa, B = North America, C = South America, D = southern Africa and E = Australia. Outgroups were coded according to their distribution, *Microcnemum* = A, *Tecticornia* = E and *Arthrocnemum* = A. For the coding for *Arthrocnemum* we choose not to consider the North American *A. subterminale* because it seems not to be closely related to *A. macrostachyum* (G. Kadereit, unpubl. res.). One thousand random post-burn-in trees from the BEAST analysis of the ETS data set (see above) were loaded in RASP and a Bayesian MCMC analysis was conducted with 50 000 generations, ten runs and a sample frequency of 100. The maximum number of ancestral areas was in three independent runs set to two, three and four allowing widespread ancestors. Relative frequencies of ancestral areas were plotted on a condensed tree.

RESULTS

Phylogenetic inference

The combined data set included 2284 aligned positions and 44 accessions (Bayesian tree in Fig. 3). The cp data included 1769 aligned positions, 205 of which were variable (ML tree in Supplementary Data Fig. S1), and the ETS data set included 515 aligned positions, 250 of which were variable (dated Bayesian tree in Fig. 4). The cp data set is lacking accessions of *S. alpini*, *S. dunensis*, *S. magellanica* and *S. hispanica*. Supported branches did not differ in the Bayesian, ML and parsimony approaches. Incongruency between cp and nuclear data were found only in terminal branches mainly among the South African accessions. Basal branches show no topological conflict in the gene trees and the combined matrix revealed a well-supported tree with increased support values along the basal branches. As the cp data were mostly uninformative among closely related species we did not compile a data set that was strictly congruent with the ETS data set but we aimed to represent all major clades and subclades found in the much larger ETS data set.

All data sets revealed a strongly supported *Sarcocornia/Salicornia* clade with three congruently well-supported sub-lineages, the *Salicornia* clade, American *Sarcocornia* clade and Eurasian *Sarcocornia* clade (Figs 3 and 4; Fig. S1). The combined analysis additionally shows good support for the monophyly of *Salicornia* plus Eurasian/American *Sarcocornia* and these are sister to a highly supported South African *Sarcocornia* (Fig. 3). In contrast, the gene trees fail to resolve the monophyly of South African *Sarcocornia* and their sister group relationship to *Salicornia* plus Eurasian/American *Sarcocornia*. The South African (including Australian) accessions of *Sarcocornia* formed a monophyletic group with weak support only in the ETS tree (Fig. 4). In the cp tree two clades of South African accessions form a polytomy with a clade comprising the *Salicornia* clade/American *Sarcocornia* clade/Eurasian *Sarcocornia* clade. Relationships among the *Salicornia* clade, American *Sarcocornia* clade and Eurasian

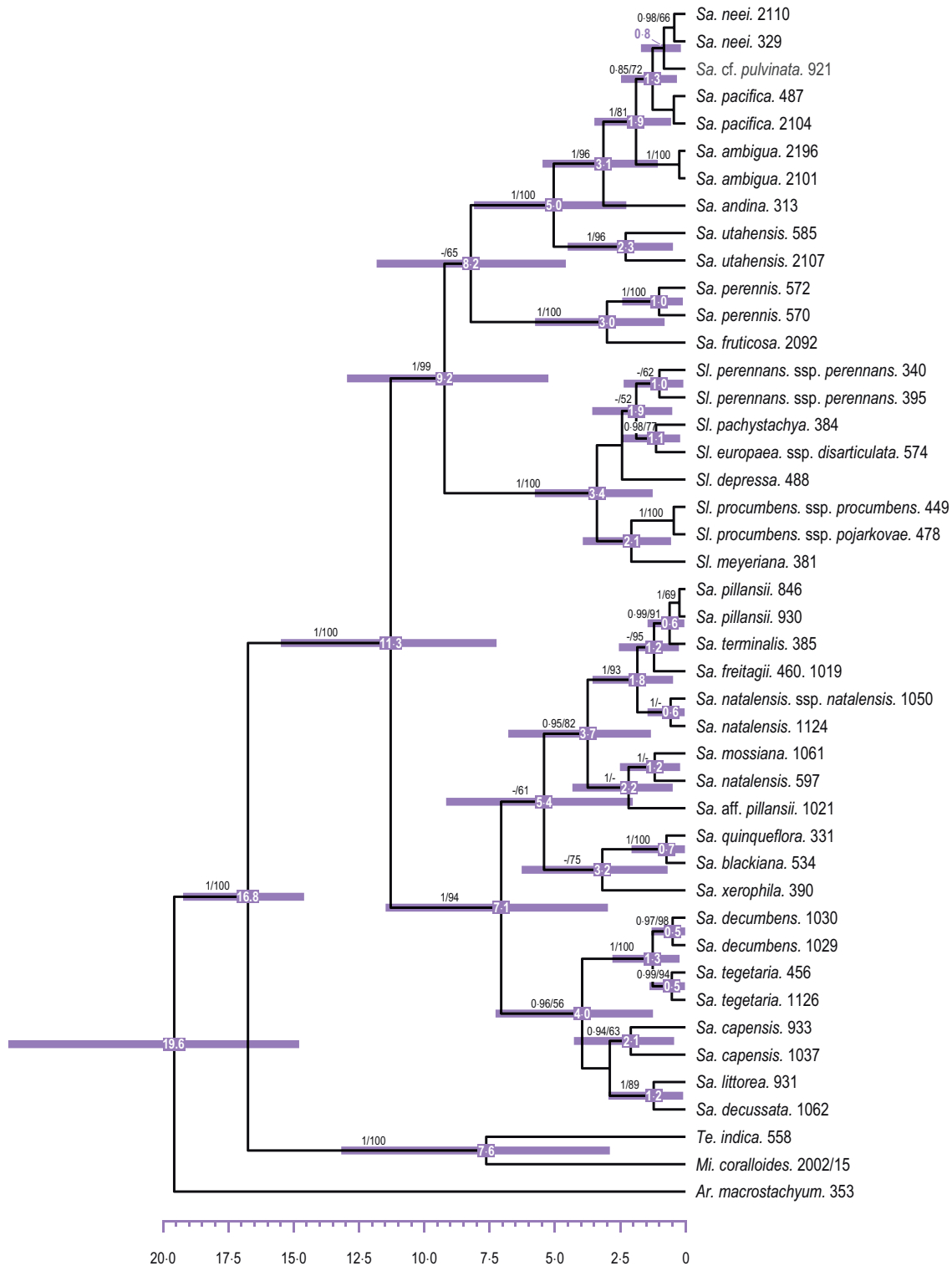


FIG. 3. Dated Bayesian tree based on sequences of two chloroplast markers (*atpB-rbcL* spacer and *rpL32-trnL* spacer) combined with ETS sequences of 41 accessions of *Sarcocornia* (*Sa.*) and *Salicornia* (*Sl.*). The tree was rooted with *Arthrocnemum* (= *Ar.*); other abbreviations: *Mi.* = *Microcnemum*, *Te.* = *Tecticornia*. Numbers above branches are posterior probabilities (>0.95)/ML bootstrap values (>50), respectively. Numbers at nodes refer to mean node ages and the respective bars to the 95 % confidence intervals of the age estimate. Numbers at the end of each taxon name refer to the lab code (Supplementary Data Table S1).

Sarcocornia clade are not resolved in the cp tree (Fig. S1) while there is weak support for a sister group relationship of *Salicornia* and American/Eurasian *Sarcocornia* in the ETS tree (Fig. 4).

Ancestral area analysis

Relative frequencies of ancestral areas did not change significantly in the independent runs with the maximum number of areas per node set to two, three or four. The full reconstruction is available from the corresponding author upon request. The ancestral area reconstructions of the most interesting nodes are mapped on the tree shown in Fig. 4.

DISCUSSION

Phylogenetic relationship of *Sarcocornia* and *Salicornia* and biogeographical considerations

Our broadly sampled molecular trees of the *Sarcocornia*/*Salicornia* lineage revealed that both genera form a well-supported clade (Figs 3 and 4) that originated probably during the Mid-Miocene from ancestors probably distributed in Eurasia (Fig. 4). This supports previous findings (Kadereit et al., 2006) but on the basis of a much more extensive data set. Both gene trees and the combined tree support a paraphyletic *Sarcocornia* with *Salicornia* more closely related to the Eurasian/American clade of *Sarcocornia*. Statistical support for a nested *Salicornia* within *Sarcocornia* is low in the ETS tree [ML bootstrap support (BS) 65, parsimony BS < 50, PP < 0.95; Fig. 4], high in the cp tree (ML BS 97, parsimony BS < 94; Supplementary Data Fig. S1) and high in the combined analysis (ML BS 99, PP = 1; Fig. 3). Monophyly of the Eurasian plus American *Sarcocornia* is only weakly supported in the ETS tree (ML BS 63, parsimony BS < 50, PP < 0.95; Fig. 4). Monophyly of the South African/Australian *Sarcocornia* is also only weakly supported by the ETS data (ML BS 65, parsimony BS < 50, PP = 0.99; Fig. 4) but highly supported in the combined analysis (ML BS 94, PP = 1; Fig. 3). A paraphyletic *Sarcocornia* raises the question of taxonomic adjustments in the lineages, an issue that is dealt with later in this Discussion.

The South African/Australian *Sarcocornia* clade probably originated from the Eurasian ancestors and started to diversify during the Late Miocene (Fig. 4). As the ETS data set provides better resolution for the South African/Australian lineage than the cpDNA and combined data sets our discussion is based mainly on the ETS tree. *Sarcocornia capensis* is not monophyletic but forms a polytomy together with a clade containing the remaining South African/Australian species (PP = 0.99). However, this species is morphologically distinct from the other taxa and can be easily recognized (Steffen et al., 2010). The clade with the remaining taxa consists of four groups in a polytomy that started to diversify during the Pliocene. The Australian accessions included in our analyses form a monophyletic group well nested within this Southern African clade (PP = 1). Therefore, *Sarcocornia* can be considered a rather 'recent' addition to the Australian flora, probably reaching Australia during the Pliocene (see also Kadereit et al., 2005). This claim is also well supported by the fact that the ETS data showed hardly any variation in the Australian accessions of

Sarcocornia, despite considerable morphological variation of this genus in Australia (G. Kadereit and L. Mucina, unpubl. res.). The shrub-forming species *S. decussata*, *S. littorea* and *S. mossiana* are well supported as monophyletic (PP = 1). However, two accessions putatively identified as *S. natalensis* and *S. pillansii* are also grouped in this clade. *Sarcocornia xerophila* (also a shrub) is ecologically distinct from all other species as it grows solely on quartz patches in southern Namaqualand (Steffen et al., 2010). Relationships of this taxon remain unresolved in the ETS tree, while it is weakly supported as sister to the Australian species in the cp tree (ML BS 63, parsimony BS 57; Fig. S1). The clade containing the remaining South African species is well supported (PP = 1) and splits into two subclades. *Sarcocornia decumbens* and *S. tegetaria* are well supported as sister to each other (PP = 1). Both mat-forming species are characterized by adventitious roots, yet *S. decumbens* can easily be identified as it is the only South African species with more than three flowers per cyme (Steffen et al., 2010). Together these two species are sister to a clade comprising the prostrate, rosette-shaped *S. Freitagii* and *S. natalensis* as well as the shrub-forming *S. pillansii* and *S. terminalis* (PP = 0.97). This clade diversified rather recently during the Quaternary, and relationships within this group remain largely unresolved. However, it is obvious that accessions assigned to a certain taxon appear in different lineages in this clade. *Sarcocornia natalensis*, for example, can be found in two different subclades that are statistically moderately supported. Furthermore, accessions identified as *S. capensis* and *S. tegetaria* or interspecific hybrids also appear in this clade. Approximately 14 % of the sequences generated from South African material show additional peaks indicating hybridization. In southern Africa, often two or more species (in some places up to six!) can be found in the same locality. Here they occupy different positions along salinity and inundation gradients, thus showing strong ecological differentiation. Hybrid sequences are mainly found in the *S. natalensis/pillansii* clade as well as in *S. capensis*. Furthermore, hybrids between South African *Sarcocornia* species, based on morphological data, have been described (Tölken, 1967; O'Callaghan, 1992; Steffen et al., 2010). South African *Sarcocornia* also shows different ploidy levels, even within species (L. Mucina, unpubl. res.). Hybridization has been shown in Eurasian *Sarcocornia* (Castroviejo and Lago, 1992; Figueroa et al., 2003), and the morphological, karyological and molecular data point towards hybridization in South African *Sarcocornia*.

The clade consisting of American and Eurasian *Sarcocornia* plus *Salicornia* was reconstructed to be of Eurasian origin (Fig. 4). For *Salicornia*, an eastern Mediterranean or Asian origin was proposed and the South African species of *Salicornia* clearly form two derived lineages in *Salicornia* that reached South Africa during the Pleistocene independently (Kadereit et al., 2007; Slenzka et al., 2013). This supports the closer relatedness of *Salicornia* to the originally Northern Hemisphere (Eurasian and American) *Sarcocornia*.

American *Sarcocornia* is clearly monophyletic (Figs 3 and 4) and most likely derived from Eurasian ancestors. The ancestral area of the crown node of the American clade was reconstructed to be located in the Northern Hemisphere (either North America or Eurasia or both). The distribution of the American accessions (Supplementary Data Fig. S2) studied

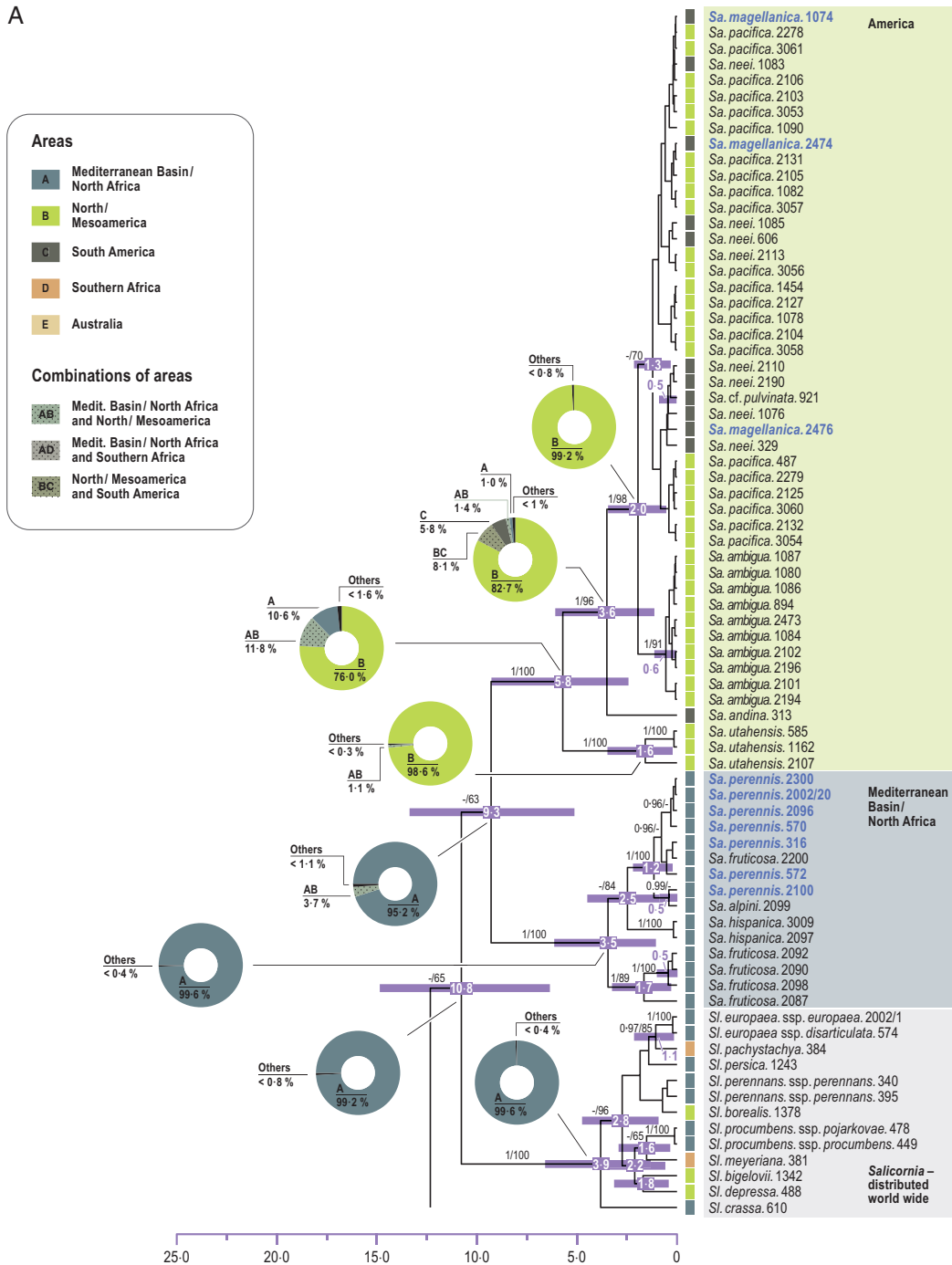


Fig. 4. Chronogram of the *Sarcocornia/Salicornia* lineage based on the ETS sequence data and 164 accessions of *Sarcocornia* and *Salicornia*. The tree was rooted with *Arthrocnemum* (= *Ar.*); other abbreviations: *Mi.* = *Microcnemum*, *Te.* = *Tecticornia*. Numbers above branches are posterior probabilities (>0.95)/ML bootstrap (>50) values, respectively. Numbers at nodes are the mean node ages and bars refer to the 95 % confidence intervals of the respective node age. The numbers at the end of each taxon name refer to the lab code (Supplementary Data Table S1). Accessions with identical ETS sequences were summarized under one terminal. Terminals written in blue refer to species that show a true mat-forming growth form. The tree was calibrated using the published node ages for the stem of the *Sarcocornia/Salicornia* lineage (see Kadereit *et al.*, 2006). Crucial results of the ancestral area analysis are plotted on the tree (see inset legend for colour coding of the areas). Numbers in the respective area colour refer to relative frequencies of ancestral areas at the respective node.

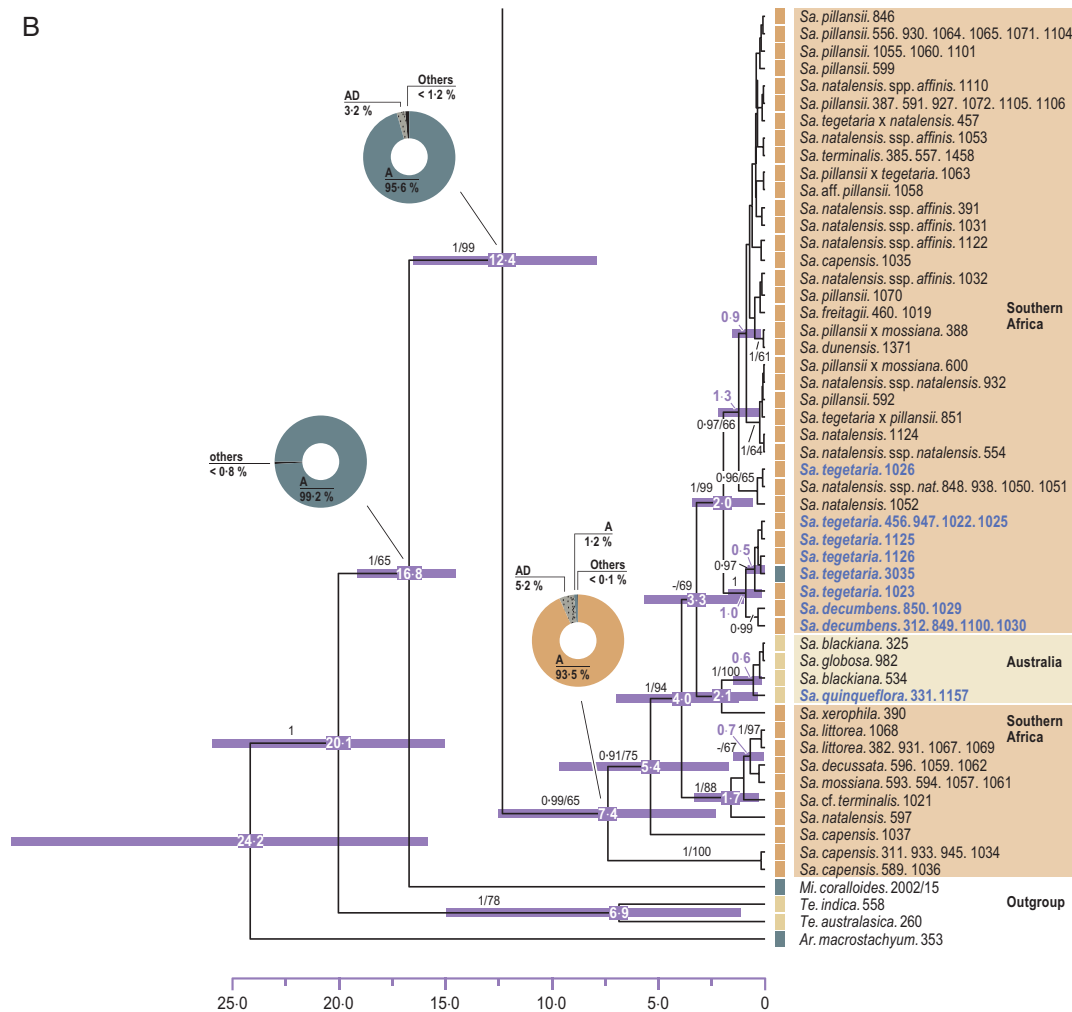


Fig. 4. (Continued)

shows a clear geographical signal in the phylogenetic relationships of the American species. With high statistical support, the North American *S. utahensis* is sister to the remaining American species. This first split within American *Sarcocornia* dates back to the Late Miocene (approx. 5–6 Ma; Figs 3 and 4). *Sarcocornia utahensis* is mainly found at inland localities, rarely on the coast. The South American inland species *Sarcocornia andina* is sister to a clade consisting of two widely distributed coastal clades, the East coast clade with *S. ambigua* and the West coast clade with *S. pacifica*, *S. neei* and *S. magellanica* (including also the Andean *S. pulvinata*; Figs 3 and 4). The two clades are comparatively young, having diversified in the Quaternary, and as they show no further resolution, the relationships among *S. pacifica*, *S. neei*, *S. magellanica* and *S. pulvinata* remain uncertain. While *S. magellanica* and *S. pulvinata* are clearly differentiated by morphological characters from *S. neei* and *S. pacifica* (Alonso and Crespo, 2008) the separation of *S. pacifica* and *S. neei* seems to be merely based on geographical separation (North vs. South America).

Resolving relationships among Eurasian species of *Sarcocornia* seems difficult. De la Fuente et al. (2013) using

ITS sequence data and a sampling that was largely restricted to the Iberian Peninsula found *S. hispanica* to be sister to the remaining species with good support, although relationships among *S. perennis*, *S. alpini*, *S. fruticosa* and *S. pruinosa* remained unclear in their analyses. In contrast to de la Fuente et al. (2013), our ETS data (Fig. 4) support a sister group relationship of *S. fruticosa* and the remaining species. The latter branch splits into two supported clades, one containing samples that were mostly identified as *S. perennis* and *S. alpini* (one sample) and the other clade containing samples of *S. hispanica*. Although the sampling included here is limited, the *S. fruticosa* clade and the *S. perennis* clade seem to be widely distributed, occurring in the Western and Eastern Mediterranean, *S. perennis* even reaching the surroundings of the Dead Sea and the coast of Senegal (Supplementary Data Table S1). According to the results of Guilló et al. (2013) seed and stomatal characters are reliable for the identification of *Sarcocornia* species in the Mediterranean region. Therefore, the three clades found in this study are well separated by molecular and morphological characters. The *S. perennis*/*S. alpini*/*S. pruinosa* clade shows

seeds with hairs while *S. fruticosa* and *S. hispanica* show seeds with papillae (de la Fuente *et al.*, 2013; Guilló *et al.*, 2013). Additionally, *S. perennis* and *S. alpini* show prominent stomata while *S. fruticosa* and *S. hispanica* have sunken stomata (Guilló *et al.*, 2013; our pers. obs.). This latter character is not known for *S. pruinosa*. To resolve relationships within the *S. perennis/S. alpini/S. pruinosa* clade, more variable markers than ITS and ETS are needed (de la Fuente *et al.*, 2013; Fig. 4). However, *S. alpini* and *S. pruinosa* are distinguished by further morphological and ecological characters (de la Fuente *et al.*, 2013). Interestingly, *S. hispanica*, a species with a predominantly inland distribution, is sister to a widely distributed and mostly coastal clade, similar to patterns found in the American *Sarcocornia* clade.

On growth forms and ecological diversification in *Sarcocornia*

Salicornioideae is a subfamily that comprises roughly 100 species and consists entirely of leaf- or stem-succulent halophytes (Ulbrich, 1934; Kühn *et al.*, 1993; Kadereit *et al.*, 2006). The most diverse lineages within Salicornioideae are the Australian *Tecticornia sensu lato* (Shepherd *et al.*, 2004) and the *Sarcocornia/Salicornia* lineage. Both represent highly specialized hygro-halophyte lineages that started to diversify during the Mid-Miocene (Kadereit *et al.*, 2006). In contrast to other Salicornioideae, *Tecticornia* and the *Sarcocornia/Salicornia* lineage show the fusion of opposite bracts and the inflorescence axis forms rather compact, tightly packed, club-shaped spikes with flowers hidden and protected in cavities. In many species the tepals are fused, leaving only a small opening through which stigmata and anthers are presented. These changes in inflorescence and flower morphology might have favoured increased cleistogamy which can, for example, be observed in *Salicornia* (Kadereit *et al.*, 2007, and references therein; Vanderpoorten *et al.*, 2010). High self-fertility might be of profound advantage during colonization processes along coasts as well as in remote inland localities. In *Salicornia*, diversification is promoted by the rapid dispersal of inbreeding lines while hybridization seems to be rare (Kadereit *et al.*, 2007; Vanderpoorten *et al.*, 2010; Teege *et al.*, 2011). In contrast, molecular and morphological evidence indicates that hybridization might be common in *Sarcocornia* (Castroviejo and Lago, 1992; Figueroa *et al.*, 2003; Steffen *et al.*, 2010).

The low growth, most commonly manifested in prostrate, creeping forms, and sometimes forming cushion (pulvinate) structures, appears to be a characteristic feature of stressed habitats. Here the impeded access to nutrients (high salinity, low available water) and in consequence impeded nutrient uptake impose natural constraints on biomass production (Grime's concept of 'ecological stress': Grime, 2002, and an ecophysiological understanding as discussed by Schultze *et al.*, 2005). Low (creeping or cushion-forming) status is also of advantage in climatically exposed environments, such as at high altitudes or high latitudes (Billings and Mooney, 1968; Körner, 1999). From their positions in the tree (Fig. 4), it is clear that the prostrate and mat-forming growth form in *Sarcocornia* arose multiple times; however, these superficially similar growth-form types are most probably a result of a plethora of

environmental filters fostering traits responsive to stress (e.g. high salinity, high-frequency and prolonged flooding, high drag from tidal movement, occurrence of frost and ice formation). The evolutionary processes underpinning the trait selection leading to the prostrate/cushion growth forms is an intriguing topic for 'evo-devo' research. Furthermore, the different prostrate growth forms found within the different lineages of *Sarcocornia* might represent different stages of speciation (Lowry, 2012).

Sarcocornia pacifica, for example, typically grow as more or less erect shrubs possessing a well-developed primary central root system and few or no adventitious roots (P. Ball, pers. obs.). Towards its northern distribution limit, the species tends to a low growth-form, creeping on the surface, with main branches spreading horizontally in all directions with short erect vegetative and flowering shoots, and hence producing what appears to be mats, but these do not necessarily root. This apparently mat-forming type occurs where the species reaches areas where tall erect stems are damaged by frost. These forms of *S. pacifica* were previously called '*S. perennis*' (see Davy *et al.*, 2006, and references therein) and might represent locally adapted ecotypes. They can be found, for example, in the vicinity of Victoria (B.C., Canada). In Florida *S. ambigua* occurs in extensive tidal flats where the plants are subjected to frequent tidal movement. Here a creeping ecotype of the species but with only few roots at the nodes has also evolved, albeit without the influence of frost. The creeping habit of what is known today as *S. tegetaria* (Steffen *et al.*, 2010) misled European taxonomists visiting South Africa who 'recognized' the local taxon as '*Salicornia perennis*' (Moss, 1954; Davy *et al.*, 2006). It is interesting that the mat-forming (clonal) taxa in southern Africa (*S. tegetaria*, *S. decumbens*, *S. natalensis*) are associated with increased flooding stress/disturbance (either regular tidal influence of less-predictable yet seasonal fluctuating water levels of inland salt lakes). The most complicated creeping/clonal growth form characterized by thin, intertwined branches that may be several metres in length, profusely rooting at nodes, is found in *S. capensis* which grows on sandy supratidal terraces. Due to their morphological and ecological distinctness we consider the South African mat-forming types as clearly separate ecospecies (Steffen *et al.*, 2010). Interesting ecotypes are common in the extremely variable species *S. pil-lansii*; among the various morphotypes of this species are also decumbent forms (Steffen *et al.*, 2010). In Australia, although the genetic distinction between *S. quinqueflora* and *S. blackiana* has not yet been established, the growth forms tell an interesting story worth investigating in more detail: the creeping forms (*S. quinqueflora*) are limited to low-tidal and more frequently flooded environments, while *S. blackiana* occurs on high ground along the flooding gradients.

In Europe the low-tidal creeping and clonal *S. perennis* and upper tidal or non-tidal erect-shrub species *S. alpini* and *S. fruticosa* follow a similar ecological pattern to that described for *S. quinqueflora/S. blackiana*. While *S. fruticosa* is genetically clearly distinct and phylogenetically separated (Fig. 4; Davy *et al.*, 2006), *S. perennis* and *S. alpini* are not differentiated by ITS (de la Fuente *et al.*, 2013) and ETS sequence data (this analysis). However, these two have been carefully investigated by a reciprocal transplantation experiment showing clear niche differentiation of the two taxa along a saltmarsh elevation

gradient with very limited overlap of their fundamental niches (Redondo-Gómez *et al.*, 2007). Niche differentiation in these two taxa was attributed to intolerance to low redox potential in *S. alpini* and intolerance to hypersalinity in *S. perennis*. Finally, *S. magellanica*, which belongs to the American clade, is in contrast to the creeping forms of *S. pacifica* (see above) a truly mat-forming species with roots at the nodes and (again) strongly resembles mat-forming forms of the Eurasian *S. perennis* (Alonso and Crespo, 2008). It occurs in saltmarshes where it is subjected to tidal movement and winter frost.

In summary, we suggest that the sub-cosmopolitan distribution of *Sarcocornia* is a result of a combination of effective long-distance dispersal (possibly the same drivers as we have hypothesized to disperse *Salicornia*, including birds and sea currents; Kadereit *et al.*, 2007, 2012) and the ability to remain (by means of clonal growth ensuring population stability under steadily changing environmental conditions) in newly colonized habitats. These new and changing habitats posed similar ecological gradients such as salinity, frequency and duration of flooding and its impact on the redox potential of the sediment, drag and disturbance from tidal movement, and occurrence of frost and ice formation to *Sarcocornia*, causing the evolution of locally adapted ecotypes and eventually ecospecies that appear superficially similar but evolved independently.

Taxonomic issues of the *Salicornia/Sarcocornia* clade

Salicornia is clearly nested within *Sarcocornia*, which means that *Sarcocornia* is a paraphyletic taxon. Furthermore, a critical assessment shows that traditional characters used to separate the two genera are inconsistent. Scott's (1977) main morphological arguments for separating *Sarcocornia* were the perennial habit and the equal-sized flowers. However, *Sarcocornia* contains short-lived, herbaceous perennials such as *S. natalensis* and *S. freitagii*. Within the Salicornioideae there are other genera that contain annuals and perennials (e.g. *Halopeplis*, *Tecticornia*). Distinctly smaller lateral flowers and their composition in a triangle might be more convincing. However, also in *Sarcocornia* there are many species with three-flowered cymes in which the lateral flowers are smaller than the central one (e.g. *S. freitagii*). In *Salicornia* there are species with a tendency to reduce the number of flowers to one (*S. meyeriana*, *S. europaea* subsp. *disarticulata*) and there are species in which the outline of the flowers is no longer visible (*S. heterantha*). Hence, with the discovery of more and more overlapping variation in both genera, Scott's (1977) concept seems outdated.

There are several clades within *Sarcocornia* in which species delimitation is particularly difficult due to either the lack of molecular resolution or morphological distinctness or both. These are, for example, in America the clade containing *S. pacifica*, *S. neei*, *S. pulvinata* and *S. magellanica*, in South Africa the clade containing *S. pillansii*, *S. natalensis* and *S. freitagii* (Fig. 4), the Australian species of *Sarcocornia* and the *Sarcocornia perennis* clade of the Eurasian species. These groups need further attention using more variable molecular markers. Taxonomic adjustments in the *Sarcocornia/Salicornia* clade should await the detailed analyses of these subclades.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxford-journals.org and consist of the following. Table S1: species and accessions included in the molecular analysis. Figure S1: ML tree based on the two chloroplast markers (*atpB-rbcL* spacer and *rpL32-trnL* spacer) and 42 accessions of *Sarcocornia* and *Salicornia*. Figure S2: distribution of American accessions of *Sarcocornia* included in the phylogenetic analysis.

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