Out of southern Africa: Origin, biogeography and age of the Aizooideae (Aizoaceae)

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A R T I C L E   I N F O

A B S T R A C T

The Aizooideae is an early-diverging lineage within the Aizoaceae. It is most diverse in southern Africa, but also has endemic species in Australasia, Eurasia and South America. We derived a phylogenetic hypothesis from Bayesian and Maximum Likelihood analyses of plastid DNA-sequences. We find that one of the seven genera, the *fynbos*-endemic *Acrosanthes*, does not belong to the Aizooideae, but is an ancient sister-lineage to the subfamilies Mesembryanthemoideae & Ruschioideae. *Galenia* and *Plinthus* are embedded inside *Aizoon* and *Aizoanthemum* is polyphyletic. The Namibian endemic *Tetragonia schenckii* is sister to *Tribulocarpus* of the Sesuvioideae. For the Aizooideae, we explored their possible age by means of relaxed Bayesian dating and used Bayesian Binary MCMC reconstruction of ancestral areas to investigate their area of origin. Early diversification occurred in southern Africa in the Eocene-Oligocene, with a split into a mainly African lineage and an Eurasian-Australasian-African-South American lineage. These subsequently radiated in the early Miocene. For *Tetragonia*, colonisation of Australasia via long-distance dispersal from Eurasia gave rise to the Australasian lineage from which there were subsequent dispersals to South America and Southern Africa. Despite the relatively old age of the Aizooideae, more than half the species have radiated since the Pleiocene, coinciding with the large and rapid diversification of the Ruschioideae. The lineage made up of *Tetragonia schenckii* & *Tribulocarpus* split from the remainder of the Sesuvioideae already in the mid Oligocene and its disjunct distribution between Namibia and north-east Africa may be the result of a previously wider distribution within an early Arid African flora. Our reconstruction of ancestral character-states indicates that the expanding keels giving rise to hygrochastic fruits originated only once, i.e. after the split of the Sesuvioideae from the remainder of the Aizoaceae and that they were subsequently lost many times. Variously winged and spiky fruits, adapted to dispersal by wind and animals, have evolved independently in the Aizooideae and the Sesuvioideae. There is then a greater diversity of dispersal systems in the earlier lineages than in the Mesembryanthemoideae and Ruschioideae, where dispersal is mainly achieved by rain.

1. Introduction

Earlier phylogenetic studies of the Aizoaceae using chloroplast (Klak et al., 2003) and nuclear markers (Thiede, 2004) showed that each of the four subfamilies (Aizooideae, Mesembryanthemoideae, Ruschioideae and Sesuvioideae) is monophyletic. For three of the subfamilies, the Mesembryanthemoideae (Klak et al., 2007), the Ruschioideae (Klak and Bruyns, 2012; Klak et al., 2013; Klak et al., 2015) and the Sesuvioideae (Hassan et al., 2005; Thulin et al., 2012; Bohley et al., 2015), much denser sampling led to a better understanding of relationships within them. For the

Aizooideae, Klak et al. (2003) sampled all currently recognized genera, but only nine of the 125 species and so phylogenetic relationships within the Aizooideae are the least understood in the family.

Klak et al. (2003) showed that the Aizooideae consisted of two major clades: one containing *Tetragonia* and *Gunniopsis* and one made up of *Acrosanthes*, *Aizoon*, *Aizoanthemum*, *Galenia* and *Plinthus*. The small genus *Tribulocarpus* S.Moore, formerly placed with *Tetragonia* in the subfamily Tetragonioideae, was found to be a close relative of the Sesuvioideae. Thulin et al. (2012) later confirmed this position and transferred a second species, *Tetragonia retusa* Thulin to *Tribulocarpus*. The two species of *Tribulocarpus* differ remarkably in their fruits: *T. retusus* (Thulin) Thulin & Liede has winged, simple fruits (as in many *Tetragonia* from southern Africa), while *T. dimorphanthus* has a compound, spiny fruit.

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Seven genera and 125 species make up the Aizooideae. Over 70% of them occur only in southern Africa (Table 1, Klak et al., 2015), but 30 are endemic to Australasia, Eurasia and South America and one is cosmopolitan (Table 2). Revisions for the Southern African species of *Acrosanthes*, *Aizoon*, *Galenia*, *Plinthus* and *Tetragonia* were compiled by Adamson (1955, 1956, 1959a&b, 1961), for the Namibian species of *Aizoanthemum* by Friedrich (1957), for *Tetragonia* in South America by Taylor (1994) and for the endemic Australian *Gunniopsis* by Chinnock (1983). The subfamily is diverse, with annuals, perennials and geophytes and its members vary in size from small, prostrate or erect herbs to large shrubs. Characteristics of the Aizooideae, as well as the Sesuvioideae, are a basic chromosome number of n = 8, only slightly succulent leaves and flowers consisting of tepals, which are petaloid inside and sepaloid outside (Bittrich and Hartmann, 1988). The presence of epidermal bladder hairs has been suggested as a possible synapomorphy for the Aizooideae (Bittrich, 1990a; Klak et al., 2003).

In the Aizooideae xerochastic or hygrochastic capsules as well as woody or rarely drupaceous fruits are found. Hygrochastic capsules (which open when moistened and close again when dried out) divided into locules are typical for the Mesembryanthemoideae and Ruschioideae and are also found in all genera of the Aizooideae (except *Acrosanthes* and *Tetragonia*), but are not present in the Sesuvioideae (Bittrich, 1990a). The advantages of hygrochasy for dispersal of seeds, especially where rainfall is erratic, has been dealt with extensively (e.g. Ihlenfeldt, 1983; Hartmann, 1988; Parolin 2001, 2006). Opening of the capsule is achieved by 'expanding tissue' (present in the 'expanding keels'), which swells when it becomes wet and pushes the valves of the capsules open. Seeds are then splashed out of the open capsule by rain. As the capsules dry out, the expanding tissue contracts again and pulls the valves closed. In this way the seeds are dispersed over longer periods (Ihlenfeldt, 1983; Parolin 2001). Only a few species in the Ruschioideae (e.g. the 11 species of the Apatesieae, *Stoeberia gigas* Dinter & Schwantes) and Mesembryanthemoideae (e.g. *Mesembryanthemum nucifer* (Ihlenf. & Bittrich) Klak) lack expanding tissue (and have xerochastic rather than hygrochastic capsules). In the Aizooideae the formation of expanding tissue is variable: in *Gunniopis* and *Aizoanthemum* the fruits are hygrochastic with fully functioning expanding keels; in *Aizoon*, *Galenia* and *Plinthus* the expanding tissue is much reduced; in *Tetragonia* and *Acrosanthes* the expanding tissue is absent (Hartmann, 2001). Whereas the fruits of *Acrosanthes* are capsules, those of *Tetragonia* are woody and nut-like and may be winged or variously sculptured (Hartmann, 2001). Morphologically and anatomically the expanding keels are so similar among the subfamilies that they are thought to have evolved just once in the Aizoaceae (Bittrich, 1990a).

In the Aizoaceae, age has been estimated only for the hyperdiverse Ruschieae (Klak et al., 2004; Arakaki et al., 2011; Valente et al., 2014). Two of these give a recent age of c. 8 Ma, whereas the third is slightly older at 17 Ma (Arakaki et al., 2011). The two younger estimates support the hypothesis that the radiation post-dates the establishment of an arid environment in the Greater Cape Floristic Region (of southern Africa) at 10–15 Ma (Cowling et al., 2009). The younger dates are further corroborated by a major increase in fossilized Aizoaceae pollen after 8 Ma (Dupont et al., 2011). Much less is known about the ages of other subfamilies in the Aizoaceae. It is possible that the species-rich *Aizoon*, *Galenia* and *Tetragonia* diversified rapidly and relatively recently, as did the Ruschieae. Alternatively, the diversity may be the result of a gradual accumulation of species from old lineages (Linder, 2008; Verboom et al., 2009; Valente et al., 2010, 2011).

The origins of the endemic species of *Tetragonia* in South America and Australasia are similarly uncertain. So far, these disjunctions have been interpreted as examples of long distance dispersals from Africa (Bittrich, 1990a; Taylor, 1994).

Here we sampled both the morphological diversity and the geographical distribution of the genera better than before for the Aizooideae. This allows us to investigate generic monophyly and relationships between the species. In addition, we ask what the phylogeny reveals about the biogeographical history of the subfamily. In particular, since the subfamily is so widely distributed (relative to the other subfamilies), we aim to estimate both the timing of the disjunctions and the direction in which the events of trans-oceanic dispersal took place. We also briefly discuss morphological attributes of the fruits to reveal patterns underlying the radiation of the Aizooideae.

2. Materials and methods

2.1. Sampling of taxa

93 accessions of the Aizooideae from 62 out of 125 species (i.e. 50% of the species) were sampled, with more than one accession for several of the widespread and variable species (Table 3). Representatives were chosen to cover both the morphological diversity and the geographical range of the Aizooideae and therefore included accessions from Australia and New Zealand, Chile (representing South America), the Mediterranean (southern Europe and North Africa –representing Eurasia), as well as Namibia and South Africa in southern Africa as well as East Africa. The greatest number of species is found in southern Africa (Table 2) and consequently the most species were sampled from here. Outgroups were chosen from the subfamilies Mesembryanthemoideae, Ruschioideae and Sesuvioideae to test the monophyly of the Aizooideae (Klak et al., 2003). As further outgroups we chose members of the Amaranthaceae, Anacampserotaceae, Didiereaceae, Gisekiaceae, Kewaceae, Nyctaginaceae and Phytolaccaceae, which are possibly closely related to the Aizoaceae (Hernández-Ledesma et al., 2015) and we rooted the tree with *Plumbago europaea* L. (Plumbaginaceae) (Hernández-Ledesma et al., 2015). The material sampled is listed with GenBank accession numbers in Table 3, where details of the vouchers are also provided. Most of the samples were collected by the authors between 2013 and 2015.

2.2. Plant material, DNA extraction, PCR, sequencing and alignment

Total DNA was isolated (from fresh leaves or from material dried in silica) using the Invisorb Spin Plant Mini Kit (STRATEC Biomedical AG, Birkenfeld, Germany). For optimal homogenization of tissue samples the FastPrep®-24 instrument (MP Biomedicals, LLC, Santa Ana, CA, USA) was used. We sampled four chloroplast gene-regions, which were amplified from total DNA by PCR. Primers used for amplification of *rps*16 were rpsF and rpsR2 (Oxelman et al., 1997). The *trn*L-F region (consisting of the adjacent *trn*L intron and *trn*L-F intergenic spacer) was amplified using pri-

Table 1
Number of genera and species per subfamily in Aizoaceae and their distribution (after Hartmann, 2001a,b; Klak et al., 2003, Klak and Bruyns, 2013).

Subfamily	No. of genera	No. of species	Distribution
Sesuvioideae	5	64	Worldwide in the tropics and subtropics
Aizooideae	7	124	Mediterranean and subtropical climates in Africa, Eurasia, Australasia and South America
Mesembryanthemoideae	1	105	Mainly southern Africa
Ruschioideae	111	±1585	Mainly southern Africa

Table 2

Distribution and number of genera and species of Aizooideae: number of species is shown for each genus/subgenus as well as species and accessions sampled for this study.

Genus	Subgenus	Species	Species (accessions) included	Distribution
Galenia	*Kolleria*	23	14 (16)	Southern Africa
	Galenia	6	3 (5)	Southern Africa
Acrosanthes		6	6 (8)	Western Cape, South Africa
Tetragonia	*Tetragonia*	30	11 (13)	Southern Africa
	Tetragonoides	5	4 (6)	Southern Africa, *T. tetragonoides* worldwide
	Anisostigma	1	1 (1)	Namibia
	Unplaced	1[a]	0	Morocco
	Unplaced	5 (endemic)	6 (10) (incl. 1 introduced + 1 worldwide)	Australia & New Zealand
	Unplaced	10 (endemic)	3 (3)	South America
Gunniopsis		14	2 (2)	Australia
Plinthus		5	3 (6)	Southern Africa
Aizoon		13	10 (16)	Southern Africa, *A. canariense* also found in Eurasia
Aizoanthemum		5	3 (7)	Angola and Namibia, *A. hispanicum* endemic to Eurasia (from Mediterranean to Iran)
Total		125	62 (93)	

[a] Note: Maire (1962: 288) placed *T. borealis* Battandier & Trabut from Morocco as a synonym of the cosmopolitan *Tetragonia tetragonoides*, whereas Hartmann (2001: 318) kept *T. borealis* as distinct.

mers c and f (Taberlet et al., 1991). Samples from herbarium material were amplified using internal primers d and e (Taberlet et al., 1991). A portion of the *matK* gene was amplified using DNA barcoding primers *matK* 2.1f and *matK* 5r (http,//www.kew.org/barcoding/protocols.html). The *rpl16* intron was amplified using primers *rpl16* 71F (Jordan et al., 1996) and *rpl16* 1516R (Kelchner and Clark, 1997). PCRs were performed using 0.75 units of MyTaq Red DNA polymerase (Bioline, London, UK) in 15 L volumes in original buffer containing $MgCl_2$ and dNTPs, with 0.3 M of each primer and 1 L of unquantified DNA template. Thermocycling was carried out on a TProfessional Basic Thermocycler (Biometra GmbH, Göttingen, Germany) to the following thermal conditions, initial denaturation at 97 °C for 2 min., followed by 30 cycles of 97 °C for 1 min., 52 °C for 1 min., 72 °C for 2 min., with a final extension step of 72 °C for 7 min. Quality of PCR products was checked by agarose electrophoresis. For samples with weak PCR products (less than 20 ng/l of DNA), the PCR cycling conditions described in Shaw et al. (2007) were used: initial denaturation at 80 °C for 5 min.; 30 cycles of 95 °C for 1 min, 50 °C for 1 min, a ramp of 0.3 °C/s to 65 °C and kept at 65 °C for 4 min., with a final extension step of 65 °C for 5 min. For removing of unincorporated primers and nucleotides before sequencing we used clean-up reaction with exonuclease I and alkaline phosphatase (Thermo Fisher Scientific, Waltham, MA, USA). The PCR products were sent to Macrogen Europe (Netherlands) for automated sequencing. The primers used for amplification were also used for the sequencing reactions.

Data files were assembled and edited using GeneDoc version 2.6.002 (Nicholas and Nicholas, 1997) and Chromas version 1.43 (McCarthy, 1996–1997). Sequences were aligned by eye.

2.3. Phylogenetic analyses

The combined chloroplast data was analysed with RAxML (Stamatakis, 2006) using the CIPRES Portal version 2.2 (Miller et al., 2010), which delivered a maximum likelihood tree with bootstrap node support. In addition, we performed analyses using Bayesian Inference (BI), also with the CIPRES Portal version 2.2 (Miller et al., 2010) and with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). The data were partitioned into the four regions, with parameters (statfreq, revmat, shape, pinvar) all unlinked between the partitions. Following the recommendation by Huelsenbeck and Rannala (2004) the most complex model GTR + G + I was implemented for each partition. Several analyses were conducted with four Markov Chains in each of two independent runs with the following settings, 10^7 generations, with trees sampled every 1000th generation. After 10^7 generations it was found that the standard deviation of split frequencies was below 0.01 and the analysis was discontinued. In each analysis, by examining the decrease in the standard deviation of split frequencies, it was found that chains had achieved stability within the first quarter of the samples so burnin was set to 2500. The most likely tree was selected from among those found. From the 7501 trees remaining after the burnin was discarded, a 50% majority rule consensus tree and posterior probabilities (PP) were calculated. Supports for the nodes in the tree selected were taken from these PP values. Clades with posterior probabilities greater than 0.95 were considered significantly supported (Alfaro and Holder, 2006).

In addition, we repeated the above BI analysis, where we included the *trnL-F* (AJ558040) and *rps16* (AJ532736) accessions of "*Acrosanthes teretifolia*" (Klak et al., 2003) in order to verify its phylogenetic placement.

2.4. Divergence time estimation

Previous estimates of divergence times of the core Ruschioideae (the tribes Ruschieae & Drosanthemeae) (Klak et al., 2004; Valente et al., 2014) used a calibration age from Wikström et al. (2001), where ages were estimated from an angiosperm-wide tree using non-parametric rate smoothing (NPRS). Arakaki et al. (2011), on the other hand, used the estimated age of pollen from the fossil *Chenopodipollis multiplex* (believed to belong to the Amaranthaceae, Nichols and Traverse, 1971), as a minimum age for nodes within the Caryophyllales. *Plumbago* was used to root the tree and we dated the split of Amaranthaceae from the remainder of the Caryophyllales at 65–56.5 Mya (as in Arakaki et al., 2011).

Divergence times were estimated using a lognormal, relaxed (uncorrelated) molecular clock as implemented in BEAST v 1.8.0 (Drummond et al., 2012) by analysing the combined plastid data without gaps. For Sites we used: substitution model GTR with base frequencies 'Estimated' and the site heterogeneity model set to 'Gamma + Invariant sites'. For Trees we used Tree prior speciation with 'Yule Process'. Ucld mean was set to Normal prior with standard deviation of 10.

We ran the Markov Chain twice for 10^7 generations on unpartitioned data, sampling every 1000th generation. The first 2500 samples were discarded as burn-in as suggested by an examination of the parameters with Tracer v. 1.5 (Rambaut and Drummond, 2003). From the remaining 7501 samples the most

Table 3

Taxa with voucher information, herbarium where voucher is lodged, coding of distribution area from which taxon originated (A = Southern Africa, B = Australasia (Australia & New Zealand), C = North and South America, D = Eurasia (including the Mediterranean region), E = East Africa), Genbank accession numbers for *trnL-F*, *rps16*, *rpl16* and *matK*.

Species	Voucher	Herbarium	Distribution area	trnL-F	rps16	rpl16	matK
Outgroups							
Plumbaginaceae							
Plumbago europaea L.				AJ392334	–	–	AY042634
Amaranthaceae							
Manochlamys albicans (Soland in Ait.) Aellen	*I Jardine 2232*	BOL	A	KX197537	KX197798	–	KX197621
Amaranthus spinosus L.				EF688743	–	–	GQ248076
Gisekiaceae							
Gisekia africana Kuntze			ADE	HE585085	HE585062	–	AY042591
Nyctaginaceae							
Bougainvillea spectabilis Willd.	*Klak 987*	BOL	C	AJ558036	AJ532732	–	JN114741
Mirabilis jalapa L.	*Klak 986*	BOL	C	AJ558035	AJ532731	–	JQ412267
Phytolaccaceae							
Phytolacca dioica L.	*Klak 988*	BOL	C	AJ558037	AJ532733	–	AY042631
Kewaceae							
Kewa salsoloides (Burch.) Christenh.	*Bruyns 13053*	BOL	A	KX197538	KX197799	KX197710	KX197622
Anacampserotaceae							
Anacampseros albissima Marloth	*Bruyns 10699*	BOL	A	KM261952	KM261927	–	DQ855856
Didiereaceae							
Portulacaria afra Jacq.	*Bruyns 11348a*	BOL	A	KM261950	KM261925	KM261901	JQ412280
Aizoaceae							
Sesuvioideae							
Cypselea humifusa Turpin	*L Ahart 9922*	BOL	C	AM161379	AM161196	–	KX197623
Sesuvium sesuvioides (Fenzl) Verdc.	*Klak 2431*	BOL	A	KX197539	AJ532734	–	KX197624
Trianthema parvifolium E.Mey.	*Klak 2429*	BOL	A	KX197540	AJ532735	–	KX197625
Tribulocarpus dimorphanthus (Pax) S.Moore 1	*Thulin et al. 11226*	UPS	E	HE585101	HE585080	KX197711	KX197626
Tribulocarpus dimorphanthus 2	*Thulin & Warfa 5343*	UPS	E	HE585100	HE585079	KX197712	KX197627
Tribulocarpus dimorphanthus 3	*Klak 2441*	BOL	A	KX197541	AJ532745	KX197713	KX197628
Tribulocarpus retusus (Thulin) Thulin & Liede	*Thulin et al. 10511*	UPS	E	HE585102	HE585081	–	–
Mesembryanthemoideae							
Mesembryanthemum aitonis Jacq.	*Bruyns 9563*	BOL	A	KM272631	AM161197	KX197714	KX197629
Mesembryanthemum liliputanum Klak	*Bruyns 9930*	BOL	A	AM161395	AM161212	KX197715	KX197630
Ruschioideae							
Conicosia pugioniformis N.E.Br. ssp. *muirii* (N.E. Br.) Ihlenf. & Gerbaulet	*Klak 1570*	BOL	A	KF132776	KF132433	KF132144	KF132628
Cleretum bellidiforme (Burm.f.) G.D.Rowley	*Klak 627*	BOL	A	AJ439000	AJ532769	KF132145	KF132629
Delosperma esterhuyseniae L.Bolus	*Bruyns 7141*	BOL	A	AJ439002	AJ532776	KF132187	KF132671
Drosanthemum zygophylloides (L.Bolus) L.Bolus	*Klak 830*	BOL	A	AJ558081	AJ532783	KF132156	KF132641
Lampranthus bicolor (L.) N.E.Br.	*Klak 543*	BOL	A	AJ439042	AJ532807	KF132221	KF132709
Ruschia sandbergensis L.Bolus	*Klak 471*	BOL	A	KF132855	KF132516	KF132263	KF132755
Aizooideae:							
Acrosanthes anceps (Thunb.) Sond.	*I Jardine 2260*	BOL	A	KX197542	KX197800	KX197716	KX197631
Acrosanthes angustifolia Eckl. & Zeyh.	*MN Britton 180*	BOL	A	KX197543	KX197801	KX197717	KX197632
Acrosanthes humifusa (Thunb.) Sond.	*CH Stirton 14165*	BOL	A	KX197544	KX197802	KX197718	KX197633
Acrosanthes microphylla Adamson	*Jardine 2341*	BOL	A	KX197545	KX197803	KX197719	KX197634
Acrosanthes parviflora J.C. Manning & Goldblatt	*CH Stirton 14133*	BOL	A	KX197546	KX197804	KX197720	KX197635
Acrosanthes teretifolia Eckl. & Zeyh. 1	*Klak 2458*	BOL	A	KX197547	KX197805	KX197721	KX197636
Acrosanthes teretifolia 2	*Klak 2464*	BOL	A	KX197548	KX197806	KX197722	KX197637
Acrosanthes teretifolia 3	*Klak 2469*	BOL	A	KX197549	KX197807	KX197723	KX197638
Aizoanthemum galenioides (Fenzl. ex Sond.) Friedrich 1	*Mannheimer 1728*	WIND	A	AJ558041	AJ532737	KX197724	–
Aizoanthemum galenioides 2	*Klak 2436*	BOL	A	KX197550	KX197808	KX197725	KX197639
Aizoanthemum hispanicum (L.) H.E.K.Hartmann 1	*A.Juan & M.Juan AJ104-2*	BOL	D	KX422628	KX422613	KX422606	KX422621
Aizoanthemum hispanicum 2	*Gutiérrez-Larruscain & E. Rico DG704-1*	BOL	D	KX422629	KX422614	KX422607	KX422622
Aizoanthemum hispanicum 3	*E Rico et al., MS-511 (barcode SALA 123319)*	SALA	D	KX422630	KX422615	KX422608	–
Aizoanthemum hispanicum 4	*R.Hand 2912 (barcode B100163219)*	B	D	KX422631	KX422616	KX422609	KX422623
Aizoanthemum rehmannii (Schinz) Friedrich	*Erb s.n.*	BOL	A	–	KX197811	KX197727	KX197640
Aizoon asbestinum Schlechter	*Klak 2510*	BOL	A	KX197551	KX197812	KX197728	KX197641
Aizoon burchellii N.E.Br.	*Mannheimer 4918*	WIND	A	KX197552	KX197813	KX197729	KX197642
Aizoon canariense L. 1	*Klak 1793*	BOL	A	KX197553	KX197814	KX197730	–
Aizoon canariense 2	*Barnick 1*	HBG	AD	HE585091	HE585060	–	–
Aizoon giessii Friedrich	*Mannheimer 4921*	WIND	A	KX197554	KX197815	KX197731	KX197643
Aizoon glinoides L.f. 1	*Dold 15008*	BOL	A	KX197555	KX197816	KX197732	KX197644
Aizoon glinoides 2	*Dold 15009*	BOL	A	KX197556	KX197817	KX197733	KX197645
Aizoon glinoides 3	*Dold 15004*	BOL	A	KX197557	KX197818	KX197734	KX197646
Aizoon karooicum Compton 1	*Klak 1971*	BOL	A	KX197558	KX197819	KX197735	KX197647
Aizoon karooicum 2	*Klak 2454*	BOL	A	KX197559	KX197820	KX197736	KX197648
Aizoon rigidum L.f.	*Bruyns 12989*	BOL	A	KX197560	KX197821	KX197737	KX197649
Aizoon sarmentosum L.f. 1	*Klak 2448*	BOL	A	KX197561	KX197822	KX197738	KX197650
Aizoon sarmentosum 2	*Klak 2466*	BOL	A	KX197562	KX197823	KX197739	KX197651

Table 3 (continued)

Species	Voucher	Herbarium	Distribution area	trnL-F	rps16	rpl16	matK
Aizoon sarmentosum 3	*Bruyns 13003*	BOL	A	KX197563	KX197824	KX197740	KX197652
Aizoon schellenbergii Adamson	*Klak 2509*	BOL	A	KX197564	KX197825	KX197741	KX197653
Aizoon virgatum Welw. ex Oliver	*Mannheimer 4913*	WIND	A	KX197565	KX197826	KX197742	KX197654
Galenia africana L. 1	*Klak 713*	BOL	A	AJ558043	AJ532740	KX197743	KX197655
Galenia africana 2	*Bruyns 13051*	BOL	A	KX197566	KX197827	KX197744	KX197656
Galenia cf portulacacea Fenzl ex Sond.	*AB Low 14201*(barcode 0277784-0*)	NBG	A	KX197567	KX197828	–	KX197657
Galenia cymosa Adamson	*Klak 1969*	BOL	A	KX197568	KX197829	KX197745	KX197658
Galenia dregeana Fenzl ex Sond.	*Klak 2012*	BOL	A	KX197569	KX197830	KX197746	KX197659
Galenia filiformis (Thunb.) N.E.Br.	*Bruyns 13054*	BOL	A	KX197570	KX197831	KX197747	KX197660
Galenia fruticosa (L.f.) Sond.	*Klak 2432*	BOL	A	KX197571	KX197832	KX197748	KX197661
Galenia glandulifera Bittrich 1	*Klak 2490*	BOL	A	KX197572	KX197833	KX197749	KX197662
Galenia glandulifera 2	*Bruyns 12955*	BOL	A	KX197573	KX197834	KX197750	KX197663
Galenia glandulifera 3	*Klak 2506*	BOL	A	KX197574	KX197835	KX197751	KX197664
Galenia namaensis Schinz	*Klak 2437*	BOL	A	KX197575	KX197836	KX197752	KX197665
Galenia papulosa (Eckl. & Zeyh.) Sond.	*Klak 2439*	BOL	A	KX197576	KX197837	KX197753	KX197666
Galenia procumbens L.f. 1	*Klak 2485*	BOL	A	KX197577	KX197838	KX197754	KX197667
Galenia procumbens 2	*Klak 2504*	BOL	A	KX197578	KX197839	KX197755	KX197668
Galenia prostrata Schellenberg	*Klak 2505*	BOL	A	KX197579	KX197840	KX197756	KX197669
Galenia pubescens (Eckl. & Zeyh.) Druce 1	*Klak 2446*	BOL	A	KX197580	KX197841	KX197757	KX197670
Galenia pubescens 2	*Bruyns 12978*	BOL	A	KX197581	KX197842	KX197758	KX197671
Galenia sarcophylla Fenzl ex Sond.	*Klak 2428*	BOL	A	KX197582	KX197843	KX197759	KX197672
Galenia secunda Fenzl ex Sond. 1	*Klak 2447*	BOL	A	KX197583	KX197844	KX197760	KX197673
Galenia secunda 2	*Klak 2488*	BOL	A	KX197584	KX197845	KX197761	KX197674
Galenia sp.	*Bruyns 10590*	BOL	A	KX197585	KX197846	KX197762	–
Gunniopsis intermedia Diels	*RJC Chinnock s.n.*	BOL	B	AJ558045	AJ532741	KX197763	KX197675
Gunniopsis tenuifolia Chinnock	*RJC Chinnock 9532*	BOL	B	AJ558046	AJ532742	KX197764	KX197676
Plinthus cryptocarpus Fenzl 1	*AA Gubb 2108*	KMG	A	KX197586	KX197847	–	KX197677
Plinthus cryptocarpus 2	*Klak 2522*	BOL	A	KX422632	KX422617	KX422610	KX422624
Plinthus karooicus I.Verdoorn 1	*T. Anderson & Van Heerden s.n.*	BOL	A	KX197587	KX197848	KX197765	KX197678
Plinthus karooicus 2	*P Bester 6131* (barcode 0210479-0∗)	NBG	A	KX197588	KX197849	KX197766	KX197679
Plinthus sericeus Pax 1	*Glen 1447*	BOL	A	KX197589	KX197850	KX197767	KX197680
Plinthus sericeus 2	*Klak 2527*	BOL	A	KX422633	KX422618	KX422611	KX422625
Tetragonia acanthocarpa Adamson	*Thulin et al. 3427*	UPS	E	KX197590	KX197851	KX197768	KX197681
Tetragonia angustifolia Barnéoud	*C.M. Taylor & A. Pool 11621*	MO	C	KX197591	KX197852	KX197769	KX197682
Tetragonia arbuscula Fenzl	*Klak 2486*	BOL	A	KX197592	KX197853	KX197770	KX197683
Tetragonia cristata Prescott	*R Davies 12553*	BOL	B	KX197593	–	–	–
Tetragonia decumbens Miller 1	*M Hislop 4425*	BOL	B	KX197594	KX197854	KX197771	KX197684
Tetragonia decumbens 2	*Dold 15005*	BOL	A	KX197595	KX197855	KX197772	KX197685
Tetragonia decumbens 3	*Klak 2445*	BOL	A	KX197596	KX197856	KX197773	KX197686
Tetragonia diptera F.Mueller	*Brummitt et al. 21277*	BOL	B	KX197597	KX197857	KX197774	KX197687
Tetragonia echinata Aiton 1	*Klak 2455*	BOL	A	KX197598	KX197858	KX197775	KX197688
Tetragonia echinata 2	*Klak 2460a*	BOL	A	KX197599	KX197859	KX197776	KX197689
Tetragonia echinata 3	*Bruyns 12983*	BOL	A	KX197600	KX197860	KX197777	KX197690
Tetragonia eremaea Ostenfeld	*Gibson & Langley 5328*	BOL	B	KX197601	KX197861	KX197778	KX197691
Tetragonia fruticosa L.	*Klak 2451*	BOL	A	KX197602	KX197862	KX197779	KX197692
Tetragonia herbacea L.	*Klak 2467*	BOL	A	KX197603	KX197863	KX197780	KX197693
Tetragonia implexicoma (Miquel) Hook.f. 1	*R Davis 12538*	BOL	B	KX197605	KX197865	KX197782	KX197695
Tetragonia implexicoma 2	*M Buys 1588*	NZFRI	B	KX197604	KX197864	KX197781	KX197694
Tetragonia implexicoma 3	*RJC Chinnock 10513*	BOL	B	KX422634	KX422619	KX422612	KX422626
Tetragonia implexicoma 4	*RJC Chinnock 10496*	BOL	B	KX422635	KX422620	–	KX422627
Tetragonia maritima Barnéoud	*CM Taylor & A Pool 11587*	MO	C	KX197606	KX197866	KX197783	KX197696
Tetragonia microptera Fenzl	*Klak 2430*	BOL	A	KX197607	KX197867	KX197784	KX197697
Tetragonia nigrescens Eckl. & Zeyh.	*Klak 2456*	BOL	A	KX197608	KX197868	KX197785	KX197698
Tetragonia portulacoides Fenzl	*Klak 2468*	BOL	A	KX197609	KX197869	KX197786	–
Tetragonia reduplicata Welw. ex Oliver	*Klak 2438*	BOL	A	KX197610	KX197870	KX197787	KX197699
Tetragonia saligna Fenzl	*Klak 2460*	BOL	A	KX197611	KX197871	KX197788	KX197700
Tetragonia sarcophylla Fenzl	*Klak 1970*	BOL	A	KX197612	KX197872	KX197789	KX197701
Tetragonia schenckii Schinz	*Mannheimer 4920*	WIND	A	KX197613	KX197873	KX197790	KX197702
Tetragonia sp.	*CM Taylor & M Richardson 12432*	MO	C	KX197614	KX197874	KX197791	KX197703
Tetragonia spicata L.f. 1	*Klak 2325*	BOL	A	KX197615	KX197875	KX197792	KX197704
Tetragonia spicata 2	*Klak 2465*	BOL	A	KX197616	KX197876	KX197793	KX197705
Tetragonia tetragonoides (Pallas) O.Kuntze 1	*Buys 1586*	NZFRI	B	KX197617	KX197877	KX197794	KX197706
Tetragonia tetragonoides 2	*R Davis 12537*	BOL	B	KX197618	KX197878	KX197795	KX197707
Tetragonia tetragonoides 3	*Klak 2501*	BOL	A	KX197619	KX197879	KX197796	KX197708
Tetragonia verrucosa Fenzl	*Klak 2452*	BOL	A	KX197620	KX197880	KX197797	KX197709

credible tree with posterior probabilities, mean ages, substitution rates and standard deviations was compiled with TreeAnnotator v.1.4.8 (Drummond and Rambaut, 2007).

2.5. Biogeographical analysis with RASP (reconstruct ancestral state in phylogenies)

The biogeographical analysis was performed with RASP 3.1 (Yu et al., 2015) using a condensed tree from the Bayesian analysis. Distributions of species were coded with A = Southern Africa, B = Australasia (Australia and New Zealand), C = North and South America, D = Eurasia, including the Mediterranean coast of North Africa and Spain to Iran, E = East Africa (Table 3). For all species we coded the entire distribution area of the species for the terminals included in the tree. In case of the several accessions of widespread species, the actual place of origin of the sample was used. For the downloaded *Aizoon canariense* (2) and *Amaranthus spinosus*, the origin was unknown and therefore the entire known distribution for the species was coded for these accessions. A Bayesian Binary MCMC analysis with standard "Gamma" variation was conducted with a maximum distribution of four regions from the five coded areas allowed per node. All other settings were in the default mode.

2.6. Ancestral character reconstruction of expanding keels

Morphological information was extracted from the literature (Hartmann, 1988, 2001), from herbarium specimens at BOL and NBG as well as from field records made over several seasons. We used the consensus tree from BI for the ancestral reconstruction of characters, with Fitch parsimony as implemented in the Ancestral State Reconstruction Package in Mesquite Version 2.72 (Maddison and Maddison, 2009). Characters were treated as unordered. We reconstructed the evolution of expanding keels in the fruits, since this feature is instrumental in pushing open the valves of the fruits after rain in hygrochastic capsules. We recognized three states: Expanding keels absent (0) (*Acrosanthes*, *Tetragonia*; Hartmann, 2001); Expanding keels reduced (1), so that the valves open at most slightly (*Aizoon*, *Galenia* and *Plinthus*; Bittrich, 1990b); Expanding keels present (2), so that the valves open fully, i.e. at ±180° (*Aizoanthemum*, *Gunniopsis*; Hartmann, 2001; Chinnock, 1983).

3. Results

3.1. Phylogenetic reconstruction

Altogether, 19 sequences were missing from the Aizoaceae, through problems in amplification of DNA from herbarium specimens or unavailability of DNA material (Table 3). In total we included 445 sequences, of which 70 were downloaded from Genbank and 375 were new (Table 3).

Maximum likelihood (Fig. S1) and Bayesian analyses (Fig. 2) produced very similar trees. Minor differences between the two analyses involved relationships between closely related species and were not strongly supported. We therefore only display the Bayesian tree (Fig. 2).

3.1.1. Polyphyly of Aizooideae

Acrosanthes is not part of the Aizooideae, but is sister to the Mesembryanthemoideae + Ruschioideae (PP = 0.99, Fig. 2). All eight accessions of the six species of *Acrosanthes* form a highly supported clade (PP = 1.00), within which the three collections of *Acrosanthes teretifolia* resolve in a subclade. In an additional BI analysis, the one accession of "*Acrosanthes teretifolia*" from Klak

et al. (2003) was included and was sister to *Aizoon sarmentosum* (results not shown). This extraction (LHMS 214 of SANBI) was said to have *Goldblatt & Manning 11664* (MO) as a voucher. However, this collection belongs to *Tetragonia* (http://www.tropicos.org/Specimen/1587195). Thus, we believe that details of the voucher and the extraction were mixed up.

3.1.2. Monophyly and relationships among genera of Aizooideae

The Aizooideae (excluding *Acrosanthes*) consists of major clades A and B (Fig. 1). Relationships among genera of the Aizooideae are similar to those found previously (Klak et al., 2003), where *Tetragonia* & *Gunniopsis* (clade B) are sister to *Aizoon*, *Plinthus* and *Galenia* (clade A). A novel finding is that *Aizoanthemum hispanicum* (L.) H.E. K. Hartmann, which occurs in Eurasia, is sister to *Tetragonia* + *Gunniopsis* (clade B), whereas the African species of *Aizoanthemum* are sister to *Aizoon*, *Plinthus* and *Galenia* (clade A).

In addition, the increased sampling reveals that several other genera are not monophyletic.

Plinthus is nested within one of several clades of *Aizoon* and *P. sericeus* is more closely related to members of *Aizoon* than to the other *Plinthus*.

All species of *Galenia* form a clade (PP = 1.00) that is nested within a grade of well-supported clades along with members of *Aizoon* and *Plinthus*.

Tetragonia is monophyletic, except for *T. schenckii*, which is sister to the two species of *Tribulocarpus* of the Sesuvioideae (PP = 1.00).

3.2. Estimation of ages

Ages for the major clades in the Aizoaceae are given in Table 4 (chronogram Fig. S2). The Aizoaceae have a mean age of 41.5 My, which falls within the Eocene (38–55 My).

The major evolutionary lineages within the Aizoaceae diverged in the Oligocene:

(a) Diversification of the Aizooideae began in the early Oligocene ± 35.0 My. The Eurasian - southern hemisphere Aizooideae (clade B) diverged since the late Oligocene (±27.5 My), followed closely by the African clade (A) of the Aizooideae, which diversified since the early Miocene (±22.2 My).
(b) The split between *Acrosanthes* and subfamilies Mesembryanthemoideae & Ruschioideae dates to the early Oligocene (± 36.4 My).
(c) In the Sesuvioideae, *Tribulocarpus* & *Tetragonia schenckii* diverged from the other Sesuvioideae in the mid Oligocene (± 29.6 My).

The disjunctions of *Tetragonia* are more recent: The node subtending *Tetragonia implexicoma* from Australasia and the South American species of *Tetragonia* dates to the late Miocene (± 10.0 My). The split between the Australasian species and the African species of *Tetragonia* is slightly earlier in the mid Miocene (± 17.1 My).

Similarly, for *Tribulocarpus*, the disjunction between the northern and southern populations of *T. dimorphanthus* is estimated at 7.4 My and is thus fairly recent (between late Miocene and start of Pliocene).

However, most species of *Tetragonia* and *Galenia*, which are restricted to southern Africa and make up about half of the Aizooideae (Table 2), have diverged only within the last ±5 My, i.e. since the Pliocene.

Also the six species of *Acrosanthes* only diversified since the Pliocene, i.e. in the last ±5.3 My.
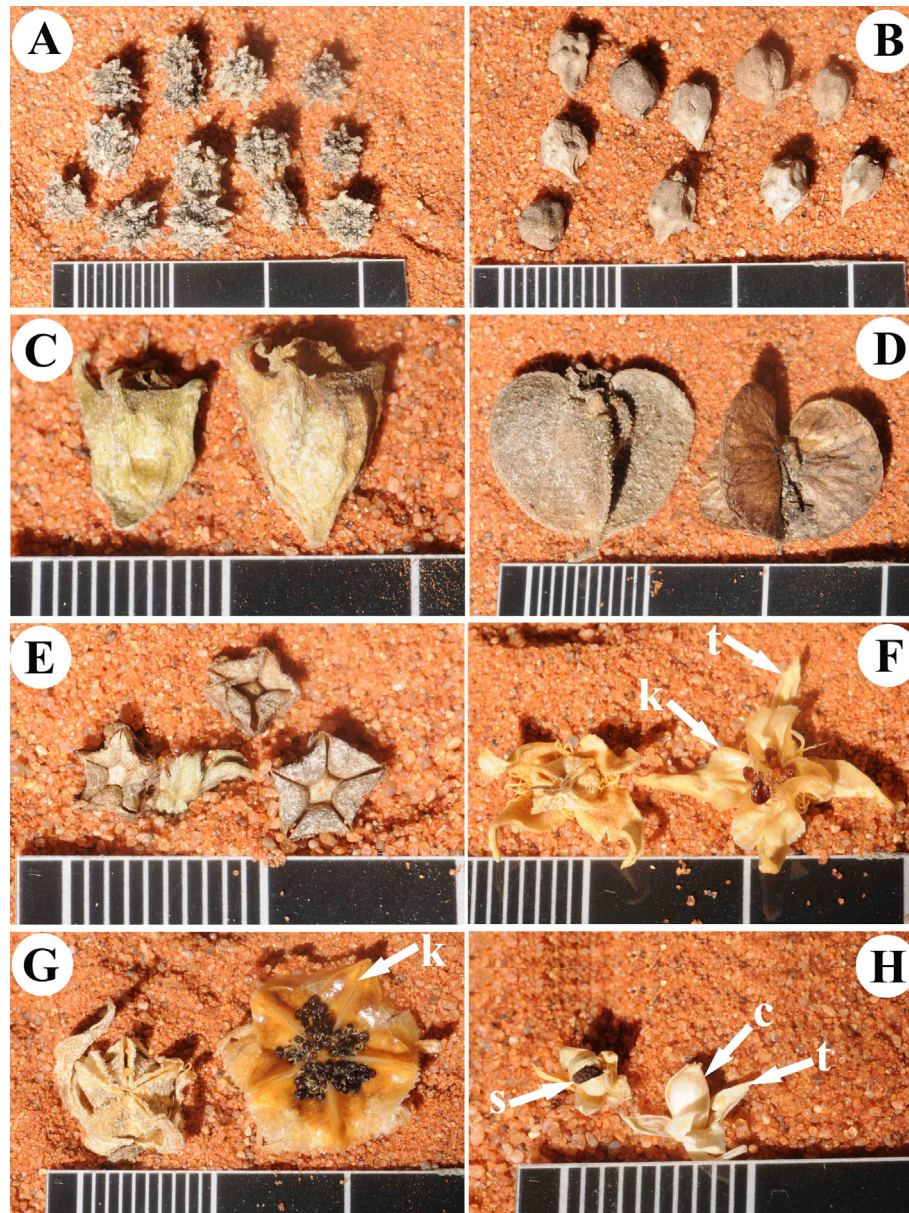
Fig. 1. Diversity of fruits in Aizooideae. **A–D**. Side view of xerochastic fruits of *Tetragonia*: **A**. *T. echinata*, *Klak 2480* (BOL), wingless, with spikes; **B**.*T. saligna*, *Klak 2460* (BOL), wingless with smooth surface; **C**. *T. tetragonoides*, *Klak 2501* (BOL); **D**. winged seeds of *T. decumbens*, *Klak 2445* (BOL) (left) and *T. spicata*, *Klak* 2325 (BOL) (right), note that the surface of *T. decumbens* is papillate, whereas it is smooth in *T. spicata*; **E**. Top view of closed (left) and open (right) loculicidal, hygrochastic capsules of *Aizoon glinoides*, *Dold 15004* (BOL), showing the depressed centre of the fruits; expanding keels are reduced, so that the valves open only slightly in the two capsules on the right; **F**. Top view of closed (left) and open (right) septicidal, hygrochastic capsules of *Gunniopsis tenuifolia*, *Chinnock 9532* (BOL), arrows indicating position of the expanding keel (k) along the margins of the valve and one of the four tepals (t); **G**. Top view of closed (left) and open (right) loculicidal, hygrochastic capsule of *Aizoanthemum hispanicum*, SALA 46080 (SALA), arrow indicates median position of the long expanding keel (k); **H**. Parchement-like xerochastic capsules of *Acrosanthes anceps*, *Jardine 2260* (BOL): top view of open (left) capsule, which splits open along a longitudinal split releasing the single seed (s); side view of closed capsule (right) with arrows indicating the capsule (c) and one of the five tepals (t). Scale: Distances between white lines indicate 1 mm.

3.3. Biogeographical analysis

(i) Clade A (A1-A4) is endemic to southern Africa, except for *Aizoon canariense*, (from southern Africa, as well as Eurasia). In addition, subclades A1-A3 occupy distinct regions within southern Africa:

Clade A2 includes species of *Aizoon* and *Plinthus* from the northern Cape (South Africa) and Namibia, whereas the species of *Aizoon* from the Little Karoo and the Eastern Cape constitute a separate clade (clade A3).

Clade A1 consists of those species of *Aizoanthemum* which are endemic to the hyper-arid coastal plain of the Namib Desert, from Namibia into Angola.

(ii) Clade B, apart from *Aizoanthemum hispanicum* (B1, from Eurasia), is confined to the southern hemisphere (except for the cosmopolitan *Tetragonia tetragonoides* (Pallas) Kuntze), in South America, Australasia and southern Africa.

It consists of five major lineages (B1-B5) where the African clade (B5) is nested within clades B2-4, which are endemic to Australasia and South America (Fig. 1).

Tetragonia forms three major clades (B3-B5): the southern African clade (B5), an Australasian clade (B4) and a South American/Australasian clade (B3), where the South American species are sister to four accessions of *T. implexicoma*, from New Zealand and Australia.

Table 4
Node ages of lineages in Aizoaceae. Capital letters refer to selected nodes in supplementary Figure S2.

Group	Node age (median)	95% confidence interval (Ma)
A: Aizoaceae	41.5	38.7–56.4
B: Aizooideae	35.0	26.1–43.6
C: Aizooideae: clade A (mainly southern Africa, except *A. canariense*)	22.2	14.0–31.4
D:Aizooideae: clade B (Eurasia-Australasia-Africa-America)	27.5	19.1–35.1
E: *Acrosanthes*-Mesembryanthemoideae & Ruschioideae	36.4	23.8–45.4
F: *Acrosanthes*	5.3	1.9–10.7
G: "core" Ruschioideae (= tribes Ruschieae & Drosanthemeae)	7.0	3.4–12.6
H: Mesembryanthemoideae-Ruschioideae	29.0	19.4–37.2
I: Sesuvioideae	29.6	17.9–40.7
J: *Tribulocarpus* (incl.*Tetragonia schenckii*)	18.3	8.8–29.2
K: *Tribulocarpus. dimorphanthus* (Namibia) - *T. dimorphanthus* & *T. retusus* (E Africa)	7.4	2.2–15.6
L: *Gunniopsis-Tetragonia*	20.4	13.4–28.5
M: *Tetragonia*	17.1	10.9–25.2
N: *Tetragonia implexicoma* Australia/New Zealand – *Tetragonia* South America	10.0	5.3–16.8
O: *Tetragonia* Australasia – *Tetragonia* Africa	11.3	6.7–17.7
P: Southern African *Tetragonia*	5.7	2.9–10.2
Q: *Aizoon & Plinthus & Galenia*	16.7	9.5–24.9
R: *Galenia*	5.3	2.9–9.6

All three accessions of *Tetragonia decumbens*, from three distant localities (Cape Town, Eastern Cape (both in South Africa) and Australia), fall in the southern African clade (B5).

The cosmopolitan *T. tetragonoides* or "New Zealand spinach" is in the Australasian clade (B2), with later dispersals to South Africa (accession 3).

Our reconstruction of the distribution shows southern Africa to be the most likely ancestral area for the Aizooideae *s.str.* (excluding *Acrosanthes*) (Fig. 2). Dispersal from Southern Africa to Eurasia occurred twice, once in *Aizoanthemum hispanicum* and (at least) once in *Aizoon canariense*.

Dispersal from Eurasia (46.38%) seems to have given rise to the lineages in the southern hemisphere (clade B), rather than a dispersal event from Africa to Australasia.

The ancestral area for *Tetragonia* is Australasia and not southern Africa, as might have been inferred from the large number of endemic species in southern Africa (Table 2). Transoceanic dispersal of *Tetragonia* from Australasia occurred three times: one gave rise to the South American lineage of *Tetragonia*, while two separate dispersal-events occurred from Australasia to southern Africa. One involved the widely distributed *Tetragonia tetragonoides* and the second gave rise to the large southern African clade of *Tetragonia*, of which the coastal *T. decumbens* subsequently made its way back to Australasia.

3.4. Repeated shifts in fruit morphology

The minimum number of changes of the three character state, present/reduced/absent, inferred for the expanding keels in hygrochastic capsules is 5 steps (Fig. 3).

4. Discussion

4.1. Phylogeny

4.1.1. Acrosanthes – an ancient lineage endemic to the fynbos-biome

The Aizoaceae is generally associated with Karoo vegetation, where it forms an important element in the arid parts of the Greater Cape Floristic Region (GCFR) (Manning and Goldblatt, 2012; Snijman, 2013). Among the four subfamilies of the Aizoaceae all, except the Sesuvioideae, have endemic species in the *fynbos* biome (Manning and Goldblatt, 2012; Snijman, 2013). It is therefore remarkable that *Acrosanthes* represents a distinct lineage within the Aizoaceae, which is endemic to *fynbos* with no representatives in the Karoo. *Acrosanthes* is found from low-lying areas at 160 m to over 2200 m in the mountains of the south-western Cape (Adamson, 1959a; Manning and Goldblatt, 2012). It lacks any expanding tissue in the fruits (Adamson, 1959a), which is absent also in all Sesuvioideae as well as in many species in the Aizooideae (Hartmann, 2001). Nevertheless, the fruits of *Acrosanthes* are unique, in that the capsules consist of a parchment-like wall (Fig. 1H), which persists in the drying perigone. Once the capsules are mature, they drop off readily. The parchment-like walls are fragile and probably decompose quickly, so that the 1–2 seeds in the capsule may be dispersed further by seed-collecting insects or by rain. A further apomorphic character is the possession of erect, basally attached but almost sessile ovules (Adamson, 1959a), which are pendulous on longer funicles in all other Aizoaceae (e.g. Adamson, 1955; Hartmann, 2001). Like many plants of the *fynbos*, *Acrosanthes* has needle-shaped leaves, which are covered with inconspicuous, flattened bladder-like idioblasts. Such idioblasts are thought to be synapomorphic for the Aizoaceae (Bittrich and Hartmann, 1988). They can also be greatly reduced in size and number in the Ruschioideae (Ihlenfeldt and Hartmann, 1982).

Bittrich (1990a) suggested the presence of epidermal bladder-hairs as a synapomorphy for the Aizooideae. These bladder-hairs consist of a large terminal cell and a multi-cellular stalk (Bittrich, 1990b). He recorded them in *Gunniopsis papillata* Chinnock, *Tetragonia tetragonoides* (Bittrich, 1990a), though not in *Acrosanthes*. *Acrosanthes* shares with the Sesuvioideae and the Aizooideae the characteristic perigone, where the tepals are sepaloid outside and petaloid inside, whereas its sisters, the Mesembryanthemoideae and Ruschioideae, have petals of staminodial origin (Bittrich and Hartmann, 1988).

Although *Acrosanthes* split from its sisters, the Mesembryanthemoideae and Ruschioideae, ±36 My, the species diversified relatively recently (since the Pliocene, ± 5 My, Table 4) and this coincides with the radiation of the Ruschieae, where numerous lineages expanded into the *fynbos* (including *Erepsia* N.E.Br., *Esterhuysenia* L.Bolus, *Lampranthus* N.E.Br., *Machairophyllum* Schwantes, *Oscularia* Schwantes, *Phiambolia* Klak and *Vlokia* S.A. Hammer).

#### 4.1.2. Phylogenetic relationships within genera

Several genera in the Aizooideae are not monophyletic. The southern African endemics *Galenia* and *Plinthus* are nested within *Aizoon*. *Aizoon* was considered to be well circumscribed, on account of its apically depressed capsules, as opposed to a convex apex in *Aizoanthemum* (Chinnock, 1983; Hartmann, 2001). However, Bittrich (1990a) noted similar capsules also for most species of *Galenia* and *Plinthus* and pointed out (Bittrich, 1990b) that there are no clear characters distinguishing the subgenera of *Galenia* or delimiting it from *Aizoon*. Thus, the androecial and gynoecial features used by Adamson (1956) to distinguish *Galenia* from *Aizoon* are actually shared by these two genera (Bittrich, 1990b). Characteristic for most species in the *Aizoon*-clade (incl. *Galenia* and *Plinthus*), are the long, often silvery hairs on the leaves, which give their leaves a silvery sheen. Although several species of *Galenia* (such as *G. glandulifera* and *G. africana*) do not possess "hairs", these are possibly reduced or modified (Bittrich, 1990b).

Our phylogeny demonstrates for the first time that *Aizoanthemum hispanicum* forms a geographically isolated lineage which is distinct from the southern African species of *Aizoanthemum* as well as from *Aizoon*, where it was originally placed. The southern
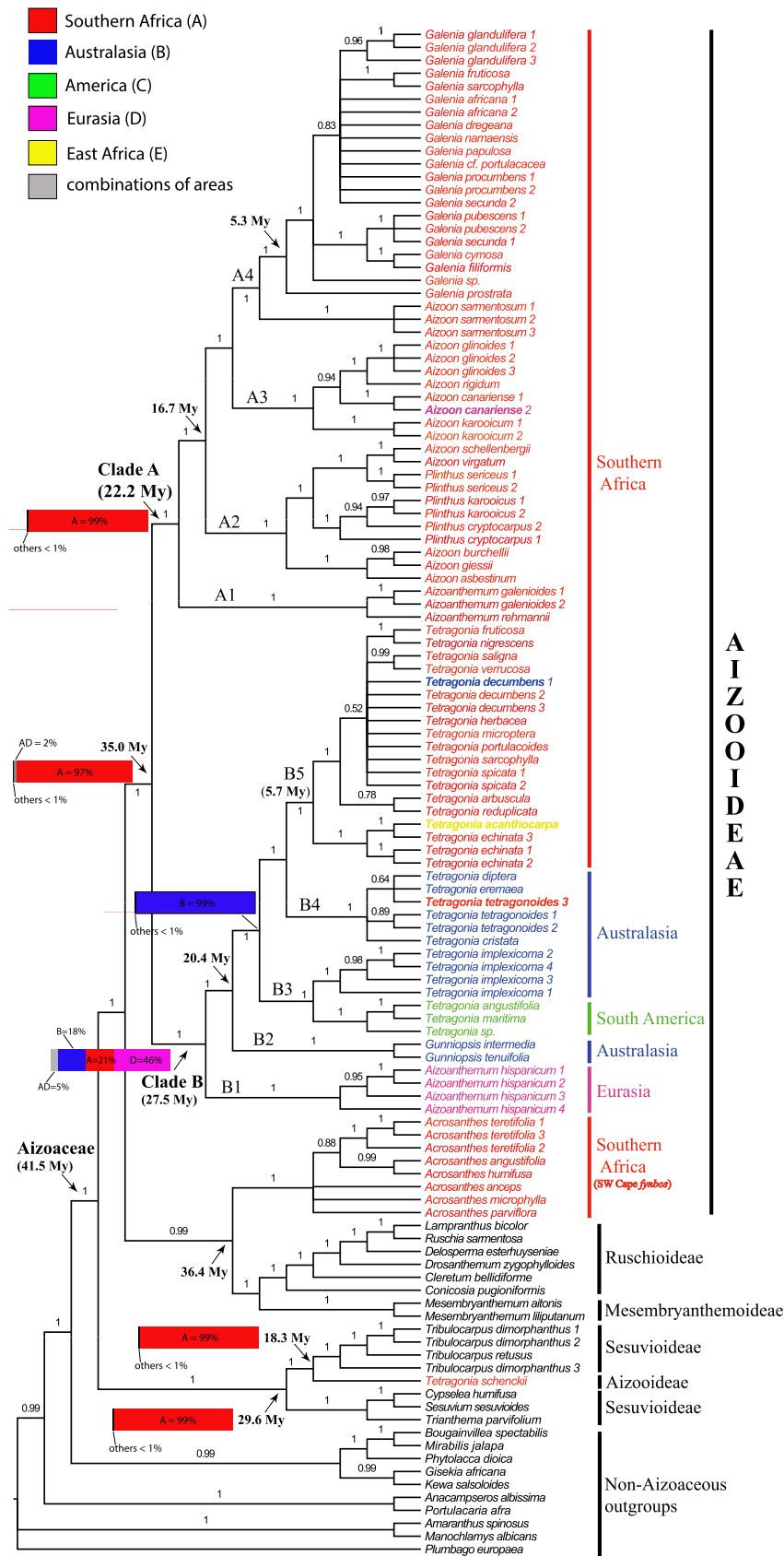
Fig. 2. Consensus tree from Bayesian analysis of four chloroplast markers; posterior probability (PP) values of 0.70 or above are indicated on the branches. Distributions for members of the Aizooideae are indicated in brackets. Taxa in bold indicate species with an extended distribution: *Aizoon canariense* present in southern Africa, East Africa and Eurasia; *Tetragonia decumbens* introduced/dispersed to Australasia; *Tetragonia acanthocarpa* introduced to East Africa; *Tetragonia tetragonoides* (New Zealand spinach) introduced/dispersed to South Africa and found worldwide in suitable climates. Results of a biogeographical analysis are indicated by horizontal bars which illustrate the frequency of reconstructed ancestral areas resulting from a Bayesian analysis at the respective nodes (see materials and methods).
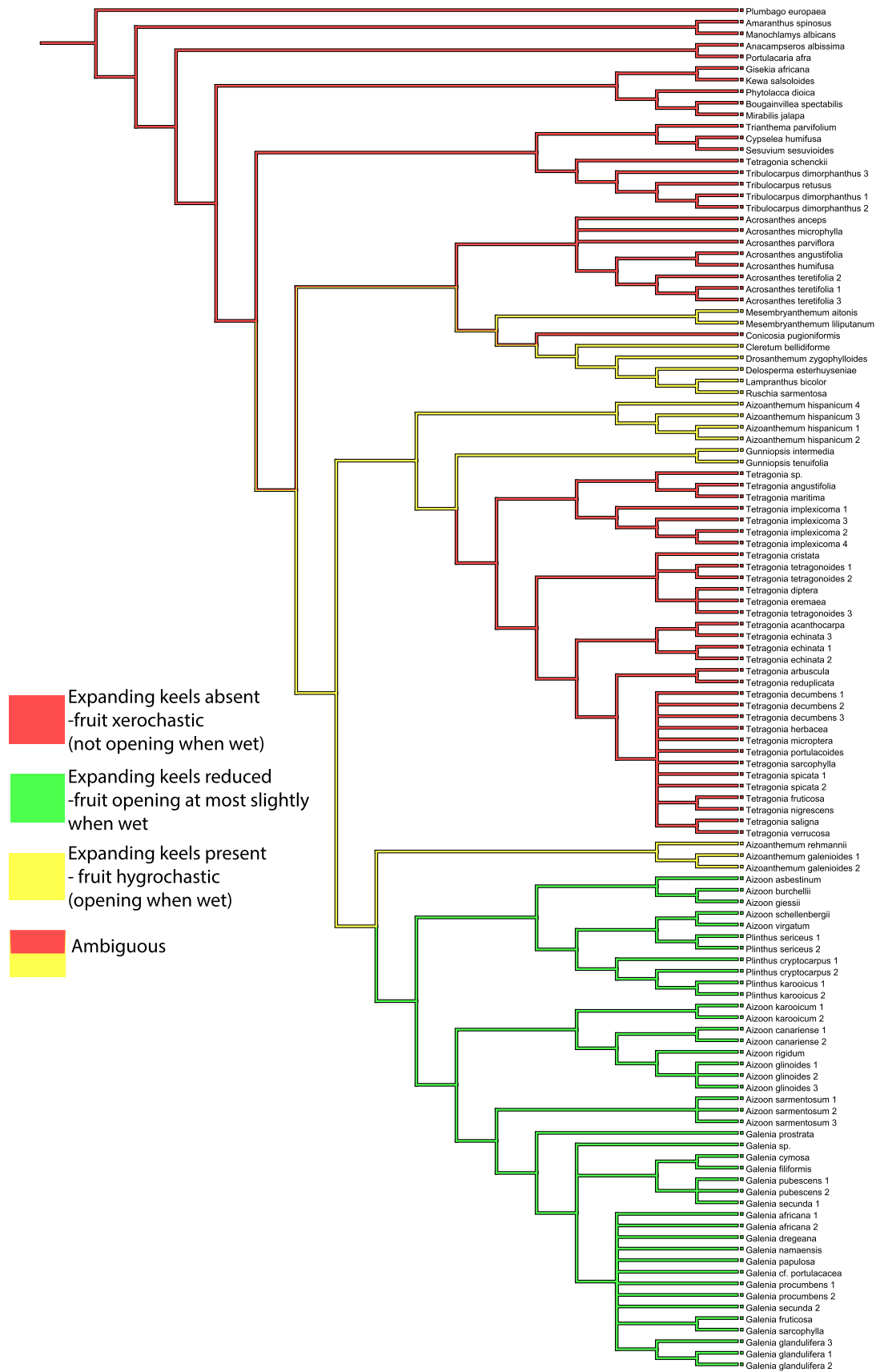
Fig. 3. Phylogenetic tree from Fig. 2 on which characters states "present/reduced/absent" of expanding keels in fruits of Aizoaceae and outgroups has been mapped. If expanding keels are present, fruits are hygrochastic and open when wet (see Fig. 1F–G); with reduced expanding keels, the fruits open at most slightly (see Fig. 1E); without expanding keels, the fruits are xerochastic and do not open when wet (see Fig. 1A–D, H).

African species of *Aizoanthemum* were distinguished from *Aizoon* by differences in fruits (Friedrich, 1957; Chinnock, 1983; Hartmann, 2001). The transfer of *Aizoon hispanicum* L. to *Aizoanthemum* was based on "literature and some material only" (Hartmann, 2001). Capsules of the two lineages of *Aizoanthemum* differ markedly: 5-angled in *A. hispanicum*; globose with 5–10 fine incisions in the southern African species (Friedrich, 1957; Hartmann, 2001). The fruits of *A. hispanicum* also differ in the formation and position of expanding keels from those in *Gunniopsis* and *Tetragonia* (clade B; see Section 4.3.1).

Tetragonia is monophyletic, except for *T. schenckii*. Schinz (1894) discussed the unusual morphology of *Tetragonia schenckii* and tentatively placed it in *Tetragonia*. Later he moved it to the monotypic *Anisostigma* (Schinz, 1897), currently recognized as a subgenus of *Tetragonia* (Adamson, 1955; Hartmann, 2001). *Tetragonia schenckii* is endemic to Namibia and forms woody shrubs to 2 m tall, with slightly succulent leaves and 3- to 4-winged fruits. According to our results it has no close relationship to the other species of *Tetragonia*, but is sister to *Tribulocarpus*, which was also treated as a subgenus of *Tetragonia* by Adamson (1955). However, most authors recognized *Tribulocarpus* as a distinct genus on account of its flowers in terminal capitula, perianth with a distinct tube, single 2-lobed style, and spiny compound fruits (Bittrich and Hartmann, 1988; Thulin, 1993).

Tribulocarpus S.Moore was recently revised and consists of two species: *T. dimorphanthus* (Pax) S. Moore and *T. retusus* (Thulin) Thulin & Liede (Thulin et al., 2012). The authors gave "flowers with an elongated perianth tube" and "1-ovulate locules" as potential synapomorphies and both are present in *T. schenckii* (Adamson, 1955). These characters therefore support the monophyly of the lineage consisting of *T. schenckii* and *Tribulocarpus*.

Clearly there is a need for extensive realignment within the subfamily and this will follow in a subsequent paper.

4.2. Divergence times and biogeography

4.2.1. Age of the Aizooideae

The Aizoaceae dates back to the Eocene at 41.5 My, with its major lineages originating between the end of the Eocene and the Oligocene (Table 4). This agrees with estimates by Arakaki et al. (2011). These clades arose after the peak in global temperatures around 50 My (Zachos et al., 2001) and coincide with the presence of many new open habitats and the development of an arid-adapted vegetation since the early Eocene (Bobe, 2006).

The initial split of the Aizooideae into two clades, the African clade (clade A) and the Eurasian-Southern hemisphere clade (clade B), dates to the Oligocene. Although the Aizooideae have endemic species on each of the continents where they occur (Table 2), the largest radiations took place in southern Africa. Here, two of the crown clades, *Galenia* (27 spp.) and the southern African *Tetragonia* (35 spp.) make up half of the Aizooideae and are fairly young lineages that diversified since the Pliocene (last 5 My). Although the radiations of *Tetragonia* in South America (10 spp.) and Australasia (14 spp.) are of a similar age (±10 My), they are markedly smaller. Specialization to seasonal aridity subsequent to climate change, is thought to have led to the Late Miocene radiation of clades in the succulent Karoo (Ihlenfeldt, 1994; Klak et al., 2004; Verboom et al., 2009; Linder, 2014). Further reasons, include the unique climatic regime and special properties of the soils of the succulent Karoo (southern Africa), where the highest diversity in the Aizooideae occurs (Klak et al., 2015). This area is characterized by mild, oceanic- and fog-ameliorated conditions, combined with low but predictable rainfall mainly received in winter with periodic droughts. This combination allows for regular germination and space for seedling recruitment and is thought to contribute to the exceptionally high diversity in this area (Cowling and Hilton-Taylor, 1999). In addition, soils in the northern part of the succulent Karoo show an exceptionally high diversity and spatial variability in both physical and chemical properties, possessing special features, which influence the availability of water and may be important in governing biodiversity (Francis et al., 2007).

The radiations of *Tetragonia* and *Galenia* in southern Africa are contemporaneous with the diversification of the "core" Ruschioideae and with several other major succulent lineages such as the cacti (Arakaki et al., 2011), succulent *Euphorbia* (Bruyns et al., 2011), the stapeliads (Apocynaceae; Bruyns et al., 2014a) and *Ceropegia* (incl. *Brachystelma*; Apocynaceae; Bruyns et al., 2015). Within southern Africa, the much smaller radiation of the Aizooideae compared to the ±1500 species of the "core" Ruschioideae (that arose over a similar time interval), supports the hypothesis that certain "key innovations", such as the shift from a flat to a trigonous leaf (in cross-section), the evolution of wide band tracheids and of highly specialized hygrochastic capsules, facilitated the spectacular radiation of the "core" Ruschioideae (Klak et al., 2004) and that these were absent in the Aizooideae.

4.2.2. Disjunct distributions in Africa

Tribulocarpus is of considerable interest in the history of African arid regions, since it shows a disjunct distribution between Namibia and north-eastern Africa (e.g. Verdcourt, 1969; Thulin, 1994), with *T. dimorphanthus* (Pax) S.Moore, currently recognized as a paraphyletic species occurring in both areas (Thulin et al., 2012). On account of the genetic difference between the populations, this disjunction is thought to be comparatively old (Thulin et al., 2012), possibly dating back to the early Miocene (22 My) at which time an "arid corridor" may have linked the two areas (Caujapé-Castells et al., 2001).

The lineage made up of *Tetragonia schenckii* & *Tribulocarpus* is relatively ancient. With the incorporation of *T. schenckii* into *Tribulocarpus* (based on the shared synapomorphies, see Section 4.1.2. above), *Tribulocarpus* would have one endemic species each in Namibia (*T. schenckii*) and Somalia (*T. retusus*; Thulin et al., 2012). Such disjunct distributions may be the remnants of a wider distribution for this lineage. Jürgens (1997) suggested that an arid African flora may exist, which is centred in the Horn of Africa with a secondary centre in south-western Africa and that an arid corridor may have linked the two regions during the Miocene (Caujapé-Castells et al., 2001), as well as during the Pleistocene (Bellstedt et al., 2008; Schrire et al., 2009). The existence of an ancient "Arid Flora" is supported by patterns of disjunction in other succulent lineages such as *Sesamothamnus* (Pedaliaceae, Ihlenfeldt, 1967), the Didiereaceae (Arakaki et al., 2011; Bruyns et al., 2014b) and in certain subgenera of *Euphorbia* (Bruyns et al., 2011), but this was not detected in a recent analysis of African floras (Linder, 2014).

Such disjunct distributions were regarded by Thulin (1994) either as Pleistocene relicts or as arising from fairly recent, long-distance dispersals. The disjunction of the north-east African populations and the southern African populations of *T. dimorphanthus* dates to the late Miocene. This relatively old age is supported by the genetic differences between the southern and northern populations, though there is surprisingly little morphological divergence between them (Thulin et al., 2012). The fruits of this species are spiny and may be adapted to dispersal by animals, as found for many spiny fruits of Pedaliaceae (Van der Pijl, 1969; Ihlenfeldt, 2010). Animals involved in the dispersal of spiny fruits, which may explain such long-distance dispersal, could also include already extinct animals (Midgley and Illing, 2009).

4.2.3. Long distance and trans-oceanic dispersal

The major centres for the Aizoaceae are the arid regions of southern Africa, with minor centres in arid Australia and the arid

Pacific side (west coast) of South America (Bittrich, 1990a; Taylor, 1994). With the exception of the North American endemic *Cypselea* Turpin (Sesuvioideae) and the Australian endemics *Sarcozona* (Ruschioideae) and *Gunniopsis*, all other genera occur in southern Africa (Hartmann, 2001). We identified Southern Africa as the ancestral area for the Aizooideae and this is also where most endemic species and the greatest diversity occur (Klak et al., 2015). In order to explain the observed disjunctions in the Aizoaceae, Bittrich (1990b) rejected the possibility that such distributions indicate a Gondwanan connection, but favoured the idea that some widespread species had adapted to long-distance dispersal by seabirds and this led to the wide distribution of the subfamily. Since Africa had separated from the other Gondwanan land-masses already by 100 My (Scotese et al., 2008), our estimate substantiates the hypothesis of long-distance dispersal, which was also found for the Sesuvioideae (Bohley et al., 2015). For the first time our biogeographical analysis lends support to the hypothesis of a transoceanic dispersal from Eurasia to Australasia initiating the *Gunniopsis* & *Tetragonia* clade, rather than a dispersal from southern Africa to Australasia (Fig. 2). The two independent dispersals from Australasia (one to South America and one to southern Africa) after the diversification of *Tetragonia* began in the early Miocene, must also have taken place by long-distance dispersal.

There are only two widespread, salt-adapted, coastal species of *Tetragonia* and both also have vast, trans-oceanic distributions: *Tetragonia tetragonoides* (worldwide) and *T. decumbens* (southern Africa--Australasia). Although dispersal by seabirds or seawater may be possible, the introduction of these species by humans is also conceivable. The latter scenario may apply to the edible *T. tetragonoides*, which could have been spread from Australasia to other continents by the early explorers and is now a cosmopolitan weed (Holm et al., 1979: 360). Intercontinental dispersal of salt-adapted species by rafting has occurred frequently in other angiosperm families (Asteraceae, Bergh and Linder, 2009; Amaranthaceae, Kadereit et al., 2005, 2006) and may have happened here too.

4.3. Evolution of fruits

4.3.1. Hygrochastic capsules with expanding tissue

In the Aizoaceae most fruits are capsules and many of them are hygrochastic. In addition, many have capsules without expanding tissue (xerochastic capsules) or the fruits are nut-like, stiffly winged or horned nuts and occasionally even drupes (Fig. 1). Expanding keels, which are responsible for the hygrochastic function, are absent in the Sesuvioideae, but are present in all other subfamilies. If we assume a single origin for expanding keels (supported by their similar structure, Bittrich, 1990a), these would have originated after the split of the Sesuvioideae from the remainder of the Aizoaceae and were then reduced or lost repeatedly in the family.

Capsules with fully functional expanding keels are present in both Clades A and B of the Aizooideae (Fig. 1). In Clade A, only *Aizoanthemum* has fully functional expanding keels, whereas they are much shortened in *Aizoon* and even further reduced in *Galenia* and *Plinthus*, whose fruits open at most slightly (Bittrich, 1990b). In Clade B, *Aizoanthemum hispanicum* and *Gunniopis* have fully functional expanding keels, whereas these are absent in *Tetragonia*. Unique for *Gunniopsis* is that the expanding keels are septicidal, which means that the expanding tissue is lateral on the valves (Chinnock, 1983), whereas it is in a medial position in all other Aizoaceae with expanding keels. The different position of the expanding keels in *Gunniopsis* may not necessarily indicate a separate origin, since expanding tissue generally can be found on different parts of the capsule also in the Ruschioideae, where it can extend beyond the keels (e.g. expanding sheets, Hartmann, 1988).

Reduction in or loss of expanding tissue occasionally occurred also in the Ruschioideae (e.g. most species in the Apatesieae, *Stoeberia gigas* Dinter & Schwantes) and Mesembryanthemoideae (e.g. *Mesembryanthemum nucifer* (Ihlenf. & Bittrich) Klak). Although it remains ambiguous how often expanding tissue has evolved (Fig. 3), it has been lost several times independently in different lineages in the Aizooideae and in the Aizoaceae as a whole. It is noteworthy that this tissue is not regained once loss or reduction has occurred (Fig. 3). We therefore favour Bittrich's hypothesis of a single origin for the evolution of expanding tissue.

4.3.2. Capsules and fruits without expanding tissue

Acrosanthes and *Tetragonia* are the two genera in the Aizooideae, which have fruits without any expanding tissue. We discussed above (see Section 4.1.1.) the unique, thin-walled, parchment-like capsules of *Acrosanthes*. In *Tetragonia* (Fig. 1A–D) and *Tribulocarpus* (Sesuvioideae) the fruits are woody, winged or horned nuts, compound and spiny or sometimes drupes. This led to the notion that the two genera were closely allied and, consequently, to them being placed together in the Tetragonioideae (Hartmann, 2001). Other species with winged nuts were also placed in *Tetragonia* (Schinz, 1894; Adamson, 1955; Thulin, 1993). Later this feature was found not to indicate a close relationship (Klak et al., 2003; Thulin et al., 2012) and winged nuts had developed independently in both the Aizooideae and the Sesuvioideae. The position of *Tetragonia schenckii* (also with winged nuts) as sister to *Tribulocarpus* now contributes a further species to this clade with such fruit.

The early diverging lineages, the Sesuvioideae and Aizooideae, include a wide range of different fruit types: circumscissile capsules, winged or variously sculptured nuts, compound, spiny fruits, hygrochastic and xerochastic loculicidal capsules or rarely drupes. This contrasts with the Mesembryanthemoideae and Ruschioideae where most species (see exceptions Section 4.3.1 above) have hygrochastic, loculicidal capsules.

Different types of fruits imply different strategies of dispersal. In hygrochastic capsules seeds are mainly dispersed by rain, rarely by animals or by wind (Hartmann, 1991). On the other hand, the winged or spiky fruits of *Tetragonia* and *Tribulocarpus* are adapted to wind- or animal-disperal (Van der Pijl, 1969; Thulin et al., 2012). The higher diversity of fruits in the early-diverging lineages of the Aizoaceae, suggests more strategies of dispersal. This contrasts with the Mesembryanthemoideae and Ruschioideae, where the most extensive specialization and diversification occurred with a single strategy of dispersal, achieved mainly by rain (Parolin, 2006).

5. Conclusions

Our phylogeny is derived from four chloroplast markers used on a sampling of the Aizooideae that is broad in terms of their distributions, their morphological diversity and taxonomic groupings. The Aizooideae are relatively ancient (having been around since the Oligocene), whereas several of its crown clades diversified only since the Pliocene. The major radiations in the Aizooideae coincide with those of the hyperdiverse Ruschieae (Ruschioideae) as well as other African succulent lineages in *Euphorbia* (Euphorbiaceae) and the Ceropegieae (Apocynaceae). Most of the species belong to two major clades: one endemic to southern Africa (except for *Aizoon canariense* also found in Eurasia); a second endemic to Eurasia (1 sp), Australasia, southern Africa and South America (as well as the cosmopolitan *Tetragonia tetragonoides*). Southern Africa is most likely the ancestral area for the Aizooideae, with an early event of long-distance dispersal to Eurasia. The Australasian lineages of Aizooideae were likely founded by trans-oceanic dispersal from Eurasia, with subsequent dispersals from Australasia giving rise to the South American and African *Tetragonia*.

The Aizooideae and several of its genera are not monophyletic. Our results form the basis for re-evaluating the taxonomic value of various morphological features used in the past and have identified new clades for which synapomorphies are needed.

Acknowledgments

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2016.12.016.

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