Disentangling the Aizooideae: New generic concepts and a new subfamily in Aizoaceae

Cornelia Klak,[1] Pavel Hanáček[2] & Peter V. Bruyns[1]

1 *Bolus Herbarium, Department of Biological Sciences, University of Cape Town, 7707 Rhodes Gift, South Africa*

2 *Department of Plant Biology, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic*

Author for correspondence: *Cornelia Klak, Cornelia.Klak@uct.ac.za*

Abstract Recent phylogenetic reconstruction of the Aizooideae (Aizoaceae) revealed that it and several of its genera are polyphyletic. Whereas the previous phylogeny was only based on chloroplast markers, we utilize here both nuclear and chloroplast markers. The four chloroplast markers together produce a well-resolved and well-supported phylogenetic hypothesis, while many branches in the tree derived from the nuclear internal transcribed spacer (ITS) are unsupported. The position of *Acrosanthes* as sister to Mesembryanthemoideae+Ruschioideae and the close relationship of *Tetragonia schenckii* to *Tribulocarpus* (Sesuvioideae) is highly supported by both nuclear and chloroplast markers. We revise the classification of the Aizoaceae, redefining subfamilial and generic boundaries to maintain monophyly. A new fifth subfamily, the Acrosanthoideae subfam. nov., is created for *Acrosanthes*, an isolated sister-lineage to the Mesembryanthemoideae+Ruschioideae. It is the only subfamily endemic to mesic *fynbos* and all other subfamilies are mainly associated with the more arid karroid vegetation. Basal, shortly stipitate ovules and a xerochastic, parchment-like capsule are synapomorphies for the Acrosanthoideae. The Eurasian endemic annual, *Aizoanthemum hispanicum*, is not related to the southern African species of *Aizoanthemum* and is placed in a new genus, *Aizoanthemopsis* gen. nov. The southern African genera *Galenia* and *Plinthus* are embedded within *Aizoon*. These genera were separated by differences in the size of their flowers. Since these features are highly homoplasious, we place all three under an enlarged *Aizoon*, which can be recognized by capsules with reduced expanding tissue as well as leaves and/or stems that are covered with silvery hairs or hair-like or rounded papillae. The Aizooideae now consist of the genera *Aizoanthemopsis*, *Aizoanthemum*, *Aizoon*, *Gunniopsis* and *Tetragonia*, with a total of 116 species. Our results also have consequences in the Sesuvioideae, where the generic name *Anisostigma* is resurrected for *Tetragonia schenckii*. The two species of *Tribulocarpus* and the monotypic *Anisostigma* are sister to the other Sesuvioideae and are characterized by tubular flowers. Since we have synapomorphies for the clade consisting of *Anisostigma*+*Tribulocarpus* and for the remainder of the Sesuvioideae (i.e., circumscissile capsules and seeds completely enveloped by an aril), we recognize two tribes, Sesuvieae and Anisostigmateae tr. nov., within the Sesuvioideae. For the expanded concept of *Aizoon* and for *Tetragonia*, we propose a new subgeneric classification to reflect the major clades retrieved. In *Aizoon*, four of the six subgenera are new and for *Tetragonia*, two of the four subgenera are new. We provide keys to the subfamilies of the Aizoaceae, to the genera and subgenera of the Aizooideae and also to the tribes of the Sesuvioideae. Distribution maps are provided for the Aizooideae, Acrosanthoideae and for the little-known *Anisostigma schenckii*.

■ INTRODUCTION

The Aizooideae (Aizoaceae) consists of seven genera and 123 species. Over 90 of these are endemic to southern Africa (Klak & al., 2015), but there are also 30 species restricted to other regions: South America (10 spp.), Eurasia (1 sp.) and Australasia (19 spp.) and one species is cosmopolitan (Fig. 1). Regional revisions exist for the South African species (Adamson, 1955, 1956, 1959a, b, 1961), for the Namibian species of *Aizoanthemum* Dinter ex Friedrich (Friedrich, 1957), for the South American species of *Tetragonia* L. (Taylor, 1994)

and for *Gunniopsis* Pax (Chinnock, 1983), which is endemic to Australia. Hartmann (2001) synthesized all these revisions and transferred the Eurasian endemic *Aizoon hispanicum* L. to *Aizoanthemum*. The subfamily includes annuals and perennials, prostrate to erect herbs and shrubs, as well as geophytes, but the lattermost are only found in southern Africa (Fig. 2).

Characteristics of the Aizooideae that are shared with the Sesuvioideae, are a base chromosome number of $x = 8$, only slightly succulent leaves and flowers made up of a perianth, where the tepals are petaloid (generally brightly coloured) inside and sepaloid (green) outside (Bittrich & Hartmann, 1988).

The presence of epidermal bladder-like hairs on the leaves or stems may be a synapomorphy for the Aizooideae (Bittrich, 1990a; Klak & al., 2003b).

The first molecular study of the Aizoaceae included 9 of the 123 species of the Aizooideae and showed that the subfamily fell into two major clades: one consisted of *Gunniopsis* and *Tetragonia* and the other included *Acrosanthes* Eckl. & Zeyh., *Aizoon* L., *Aizoanthemum*, *Galenia* L. and *Plinthus* Fenzl (Klak & al., 2003b). The circumscription of the Aizooideae was subsequently amended, placing the Tetragonioideae (including *Tetragonia*) into the Aizooideae and transferring *Tribulocarpus* S.Moore (previously in the Tetragonioideae) to the Sesuvioideae (Klak & al., 2003b). The type of *Tribulocarpus*, *T. dimorphanthus* (Pax) S.Moore, was first described as a species of *Tetragonia* (Pax, 1889). On account of the compound fruits, Moore (1921) placed this species in a new genus, *Tribulocarpus*, which was later reduced to a subgenus of *Tetragonia* (Adamson, 1955: 148).

In the only other molecular study which included members of the Aizooideae, Thulin & al. (2012) showed that *Tetragonia retusa* Thulin, endemic to East Africa, belonged in *Tribulocarpus*, thus adding a second species to the previously monotypic *Tribulocarpus*.

The sampling in Klak & al. (2003b) was too limited to assess the monophyly of the genera of the Aizooideae. This was investigated in Klak & al. (2017), where a robust phylogenetic hypothesis based on analyses of sequences of four chloroplast DNA markers with a dense sampling across the full taxonomic and geographical range of the subfamily was put forward.

An important new finding of Klak & al. (2017) is that *Acrosanthes* does not belong to the Aizooideae. The conflict of this result with the conclusions of Klak & al. (2003b), where the Aizooideae was monophyletic, was traced back to a misidentified specimen. *Acrosanthes* is a small genus of six species endemic to the *fynbos* biome, which is sister to Mesembryanthemoideae+Ruschioideae. Despite its few species, the genus represents a genetically isolated and distinct morphological lineage within the Aizoaceae.

In addition, several genera in the Aizooideae are not monophyletic: (1) *Plinthus* and *Galenia* are nested in *Aizoon*; (2) *Aizoanthemum* is polyphyletic: the Eurasian endemic *A. hispanicum* is sister to *Gunniopsis* and *Tetragonia*, whereas the southern African members (*Aizoanthemum* s.str.) are sister to *Aizoon*+*Plinthus*+*Galenia*; (3) the Namibian endemic *Tetragonia schenckii* Schinz is not related to *Tetragonia*, but is sister to the two species of *Tribulocarpus* of the Sesuvioideae (Klak & al., 2017).

Nuclear gene-regions were previously shown to have high levels of paralogy in the Ruschieae (Klak & al., 2013), but not in the Dorotheantheae (Klak & Bruyns, 2012) or in the Sesuvioideae (Hassan & al., 2005; Bohley & al., 2015). Since Klak & al. (2017) was only based on chloroplast markers, our aim here was to produce a phylogeny based on nuclear data and compare these to the results obtained from chloroplast sequences. In addition, we included three previously unsampled species.

A further aim here is to draw taxonomic conclusions from the phylogenetic analyses. We provide morphological characters for the new clades of which some are recognized for the first time. In addition, we also briefly discuss the evolution of flower-size, one of the main features distinguishing genera in the Aizooideae.
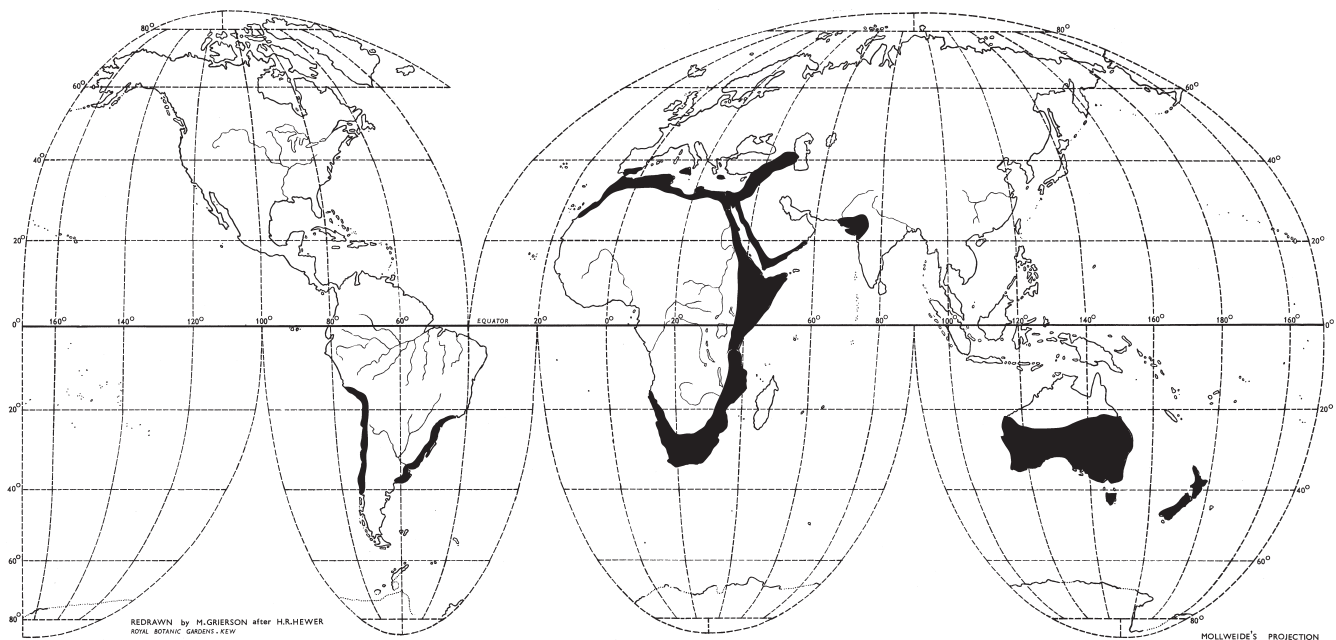

Fig. 1. Distribution of subfamily Aizooideae.

■ MATERIALS AND METHODS

Sampling of taxa. — Ninety-eight accessions of the Aizooideae from 65 out of 123 species (i.e., 53% of the known species) were sampled, with more than one accession for several of the widespread and variable species (Appendix 1). Representatives were chosen to cover both the morphological diversity and the geographical range of the Aizooideae. The majority of sequences for the chloroplast markers were taken from Klak & al. (2017) and we added three accessions from the Western Cape, from two species which had not been sampled previously: two accessions of *Aizoon paniculatum* L. and one of *Tetragonia hirsuta* L.f., as well as two accessions from Australia (*Tetragonia coronata* Rye & Trudgen, *T. cristata* Prescott). For the nuclear data, we included a further accession of *Plinthus sericeus* Pax. Outgroups were included from the subfamilies
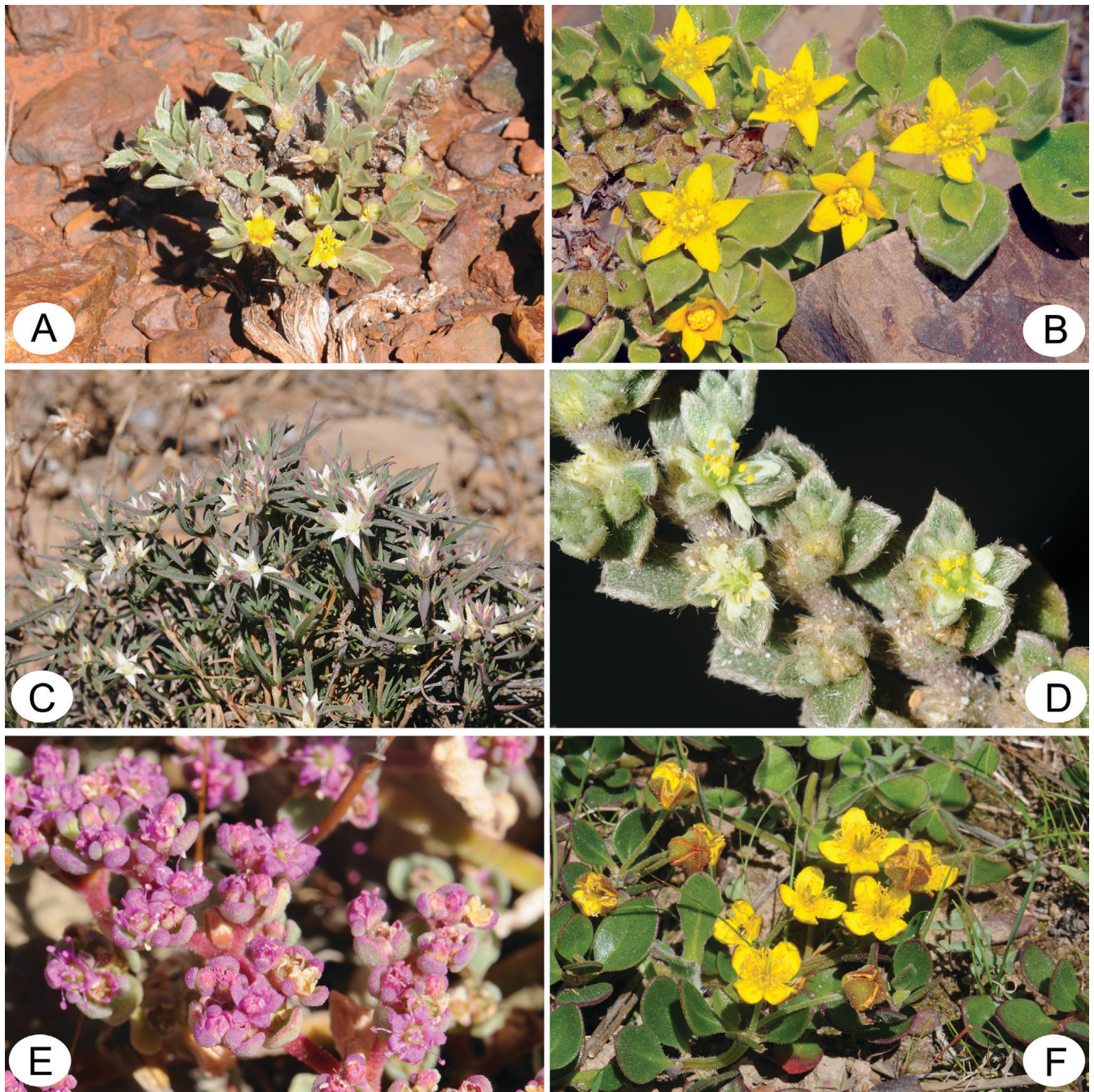
Fig. 2. Representative members of the Aizooideae. **A,** *Aizoon asbestinum*, South Africa, Northern Cape, *Klak 2510* (BOL); **B,** *Aizoon glinoides*, South Africa, Eastern Cape, *Bruyns 12982* (BOL); **C,** *Aizoon sarmentosum*, South Africa, Western Cape, *Klak 2448* (BOL); **D,** *Galenia secunda*, South Africa, Western Cape, *Klak 2447* (BOL); **E,** *Galenia glandulifera*, South Africa, Western Cape, *Bruyns 12955* (BOL); **F,** *Tetragonia herbacea*, one of the geophytic species, South Africa, Western Cape, *Klak 2467* (BOL).

Mesembryanthemoideae, Ruschioideae and Sesuvioideae to show the position of members currently included in the Aizooideae (Klak & al., 2003b). As further outgroups we chose members of the Nyctaginaceae and Phytolaccaceae which are possibly closely related to the Aizoaceae (Hernández-Ledesma & al., 2015) and rooted the tree with the Phytolaccaceae (Klak & al., 2003b). The material sampled with GenBank accession numbers is listed in Appendix 1, where details of the vouchers are also provided. Most of the samples were collected by the authors between 2013 and 2016. We generated a total of 112 new sequences: 16 new chloroplast sequences and 96 new nuclear sequences,

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, Birkenfeld, Germany). For optimal homogenization of tissue samples the FastPrep-24 instrument (MP Biomedicals, LLC, Santa Ana, California, U.S.A.) was used. For the Aizooideae amplification of the ITS1 and ITS2 spacers with universal primers ITS4 and ITS5 was unsuccessful for many samples (Klak & al., 2017). Therefore we used primers P17 (CTACCGATTGAATGGTCCGGTGAA) and 26S-82R (TCCCGGTTCGCTCGCCGTTACTA) (Popp & Oxelman, 2001) in this study. In addition, we sampled four chloroplast gene-regions, which were amplified from total DNA by PCR. Primers used for amplification of *rps16* were rpsF and rpsR2 (Oxelman & al., 1997). The *trnL-F* region (consisting of the adjacent *trnL* intron and *trnL-F* intergenic spacer) was amplified using primers c and f (Taberlet & al., 1991). Samples from herbarium material were amplified using internal primers d and e (Taberlet & 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 & al., 1996) and rpl16 1516R (Kelchner & Clark, 1997). PCRs were performed using 0.75 units of MyTaq Red DNA polymerase (Bioline, London, U.K.) in 15 µl volumes in original buffer containing MgCl₂ and dNTPs, with 0.3 µM of each primer and 1 µl of unquantified DNA template. Thermocycling was carried out on a TProfessional Basic Thermocycler (Biometra, Göttingen, Germany) with 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/µl of DNA), the PCR cycling conditions described in Shaw & 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 a clean-up reaction with exonuclease I and alkaline phosphatase (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.). 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 v.2.6.002 (Nicholas & Nicholas, 1997) and Chromas v.1.43 (McCarthy, 1996–1997). Sequences were aligned by eye.

Phylogenetic analyses. — The combined chloroplast data and the ITS data were each analysed with RAxML (Stamatakis, 2006) using the CIPRES Portal v.2.2 (Miller & al., 2010), which delivered a most likely tree in which support for nodes was measured by bootstrap percentages (>70% considered significant). In addition, we performed analyses (again on the combined chloroplast data and the ITS data separately) using Bayesian inference (BI), also with the CIPRES Portal v.2.2 (Miller & al., 2010) and with the tool MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003). The chloroplast data were partitioned into the four regions, with parameters (statfreq, revmat, shape, pinvar) all unlinked between the partitions but the ITS data was not partitioned. Following the recommendation of Huelsenbeck & 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 that burn-in was set to 2500. From the 7501 trees remaining after the burn-in was discarded, a 50% majority-rule consensus tree and posterior probabilities (PP) were calculated (giving support for the nodes in the tree). Clades with PP greater than 0.95 were considered significantly supported (Alfaro & Holder, 2006).

Geographical, morphological and ecological data. — Distribution maps were drawn for selected taxa. For *Acrosanthes* maps were drawn showing its occurrence per quarter-degree square (Leistner & Morris, 1976), with data from 101 collections at BOL, NBG and SAM as well as collections listed in Adamson (1959a). The distribution of *Tetragonia schenckii* was based on 52 specimens at BOL, K, PRE and WIND.

From morphological information from the literature and from material at B, BOL, KMG, NBG, PRE, SALA, SAM and WIND, we establish the new classification. For species that were not sampled in Klak & al. (2017) we infer their membership by means of morphological features. We also provide brief descriptions, lists of included species, keys to and distributions of each of the genera or subfamilies that we re-circumscribe or recognize for the first time.

Ancestral character-reconstruction of perianth length. — 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 v.3.1 (Maddison & Maddison, 2009). Characters were treated as unordered. We reconstructed the evolution of perianth length, since this character was used in the circumscription of several genera in the Aizooideae. We only coded taxa with homologous perianth structures, as in the Aizooideae, Sesuvioideae and *Acrosanthes* (Bittrich & Hartmann, 1988). Taxa with a different perianth structure (as in the Mesembryanthemoideae

and Ruschioideae and the outgroups) were coded with a "?". We recognized two states: Perianth long, i.e., 3–20 mm long (0) or short, i.e., 1–2.5(–3) mm long (1). Most species could be assigned to either of these two states. Species which were intermediate were coded with a "?".

■ RESULTS

Phylogenetic reconstruction. — For the chloroplast dataset, 21 sequences were missing for the Aizoaceae, through problems in amplification of DNA that had been extracted from herbarium specimens or lack of DNA (Appendix 1). For the nuclear marker, we were not able to obtain readable sequences for several species (Appendix 1), most likely as a result of dissimilar copies of the ITS region. All other sequences were readable and contained no evidence of divergent copies of the ITS region. However, most species of *Plinthus* and *Tetragonia* were found to have one polymorphic site in the ITS1 spacer region, which we coded as "N".

In total, we included 435 chloroplast sequences, of which 16 are new (Appendix 1). We included 107 nuclear sequences, of which 96 are new (Appendix 1).

Maximum likelihood (Electr. Suppl.: Figs. S1, S2) and Bayesian analyses for each of the chloroplast and nuclear data produced very similar trees. Minor differences between the two analyses for the same dataset were not significantly supported. We therefore only display the Bayesian trees for the chloroplast (Fig. 3) and nuclear analysis (Fig. 4). Due to conflict between the results of the nuclear and chloroplast analyses as well as gene paralogy, we did not combine the data.

The chloroplast analysis confirmed the relationships among the major clades in the Aizooideae as reported previously by Klak & al. (2017). New here is the position of *Aizoon paniculatum* as sister to *Aizoon sarmentosum* L.f. In addition, *Tetragonia coronata* and *T. cristata* (accession 2) group with the other *Tetragonia* endemic to Australia.

The nuclear trees are generally less supported than the chloroplast trees. Thus, in the nuclear trees the monophyly of the Aizooideae (excluding *Acrosanthes*), *Tetragonia* and *Aizoanthemum* (excluding *A. hispanicum*) remained unsupported as did the position of *Aizoanthemum hispanicum* (L.) H.E.K.Hartmann and *Gunniopsis* within the Aizooideae.

On the other hand, both the nuclear and chloroplast analyses retrieved *Acrosanthes* as sister to Mesembryanthemoideae + Ruschioideae (PP = 1.00). In addition, the position of *Tetragonia schenckii* as sister to *Tribulocarpus* (Sesuvioideae) is highly supported in both (PP = 1.00).

Conflict was only observed in *Tetragonia*: The nuclear data placed *T. implexicoma* (Miq.) Hook.f. closest to the African and Australasian species of *Tetragonia* (PP = 1.00), whereas the chloroplast data placed this species with high support (PP = 1.00) as sister to the South American species of *Tetragonia*. In addition, the four accessions of *T. implexicoma* are not monophyletic in the nuclear analysis, where accession 2 of this species is within a polytomy of African and Australasian species of *Tetragonia*, whereas the other three accessions are sister to two

African endemics (*T. acanthocarpa* Adamson and *T. echinata* Aiton, accession 3).

A revised classification. — Due to presence of gene-paralogy in the nuclear dataset, we used the well-supported and well-resolved Bayesian tree of the chloroplast analysis to indicate our new classification (Fig. 3). In Table 1 the distribution, number of species for each subfamily of Aizoaceae and for each genus (except genera in the Ruschioideae) is also given.

Repeated reduction of flower-size in the Aizooideae, Sesuvioideae and Acrosanthoideae subfam. nov. — The minimum number of changes inferred for the two states for the perianth (long versus short) is 16 steps (Fig. 5). A long perianth is reconstructed as the ancestral state within the Aizoaceae after which a short perianth evolved several times. In addition, there is evidence of at least two independent reversals to a long perianth: at least once in *Tetragonia* and once in *Galenia*. The frequent reduction of perianth-length and several subsequent reversals to a long perianth, even within currently recognized genera, suggests that the length of the perianth is of limited use for circumscribing taxonomic groups.

■ DISCUSSION

Nuclear data. — Very few nuclear markers have been utilized in the Aizoaceae. In the Sesuvioideae only the ITS region was used (Hassan & al., 2005; Bohley & al., 2015) and divergence among the sequences was low (Hassan & al., 2005). The same was found in the Mesembryanthemoideae (Klak & al., 2007) and the Ruschioideae (Klak & al., 2003a, b; Thiede, 2004), resulting in poorly resolved and weakly supported phylogenies. In addition, amplification of this region was difficult. In several studies only ITS 1 was successfully sequenced and also only for some of the species (Klak & al., 2004, 2007). For the Aizooideae the primer-pairs P17S and P26S (Popp & Oxelmann, 2001) produced the best results for the entire ITS 1+ ITS 2 region (cf. Hassan & al., 2005; Bohley & al., 2015). In the absence of strongly supported conflict, the ITS and chloroplast data have always been combined (Klak & al., 2003a, 2004, 2007, 2015; Klak & Bruyns, 2012; Bohley & al., 2015). In the present much more extensively sampled study an area of conflict has been detected between results from nuclear and chloroplast data. This conflict revolves around the four accession of *T. implexicoma*: using nuclear data three group most closely with two accessions of *Tetragonia* (*T. acanthocarpa* Adamson, *T. echinata* Aiton) from Africa, whereas in the chloroplast tree the four accessions of *T. implexicoma* are monophyletic and sister to South American *Tetragonia*. It is possible that the relationships retrieved by the ITS data are spurious and that additional markers may change the current arrangement and its support.

We also detected gene-paralogy in several species (i.e., those which could not be successfully sequenced) and this may have been undetected in others. The existence of gene-paralogy was first documented in the Ruschieae, where cloned sequences of "chloroplast-expressed glutamine synthetase" (ncpGS) for the same sample appeared in different parts of the tree. This

Fig. 3. Consensus tree from Bayesian analysis of four chloroplast markers with the new classification indicated; Bayesian posterior probabilities (≥0.50) and maximum likelihood bootstrap values (≥50%) are indicated above the branches, separated by a slash mark. *Tetragonia implexicoma* is highlighted to emphasize its different position from that in the nuclear analysis (Fig. 4).
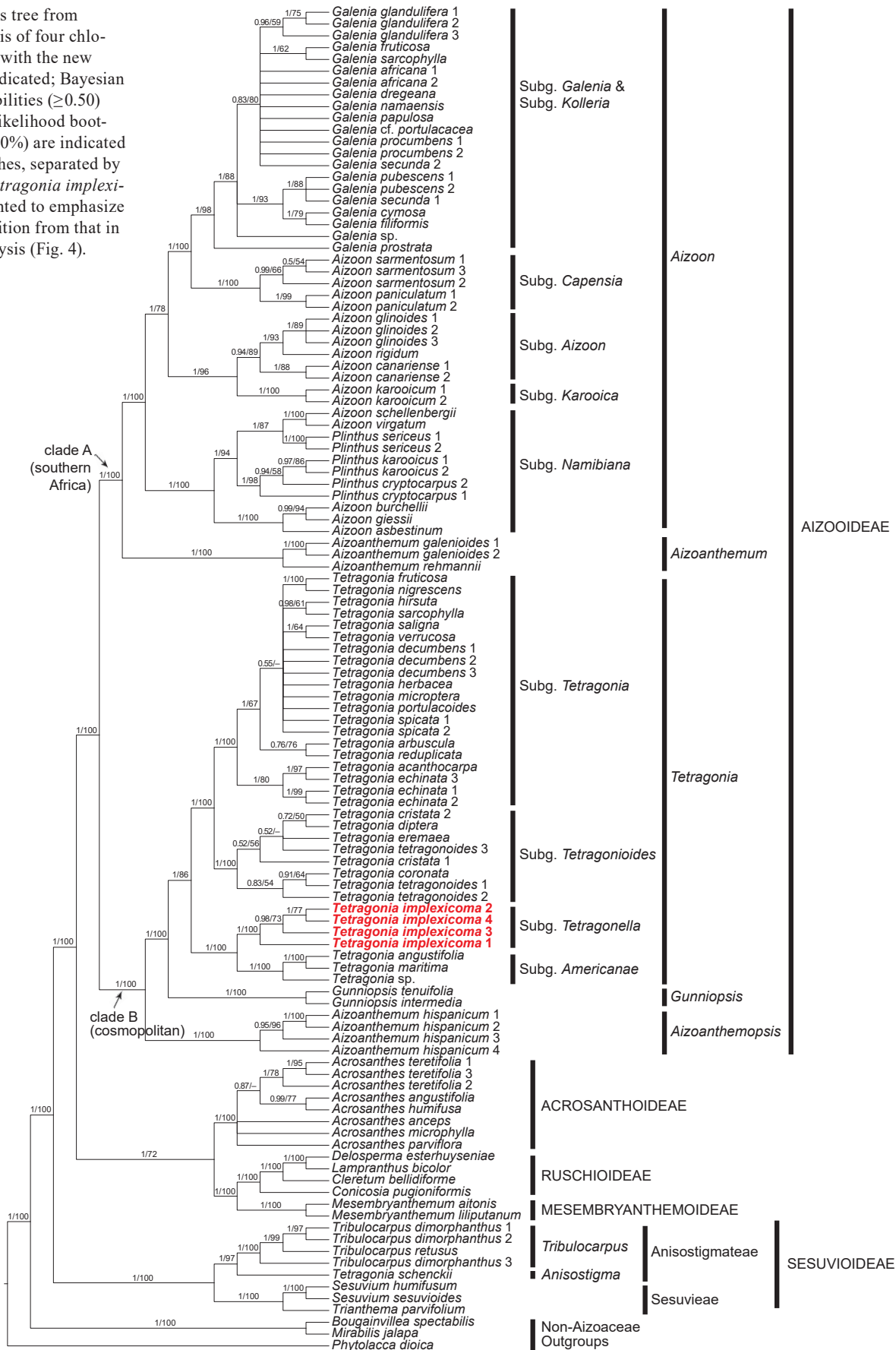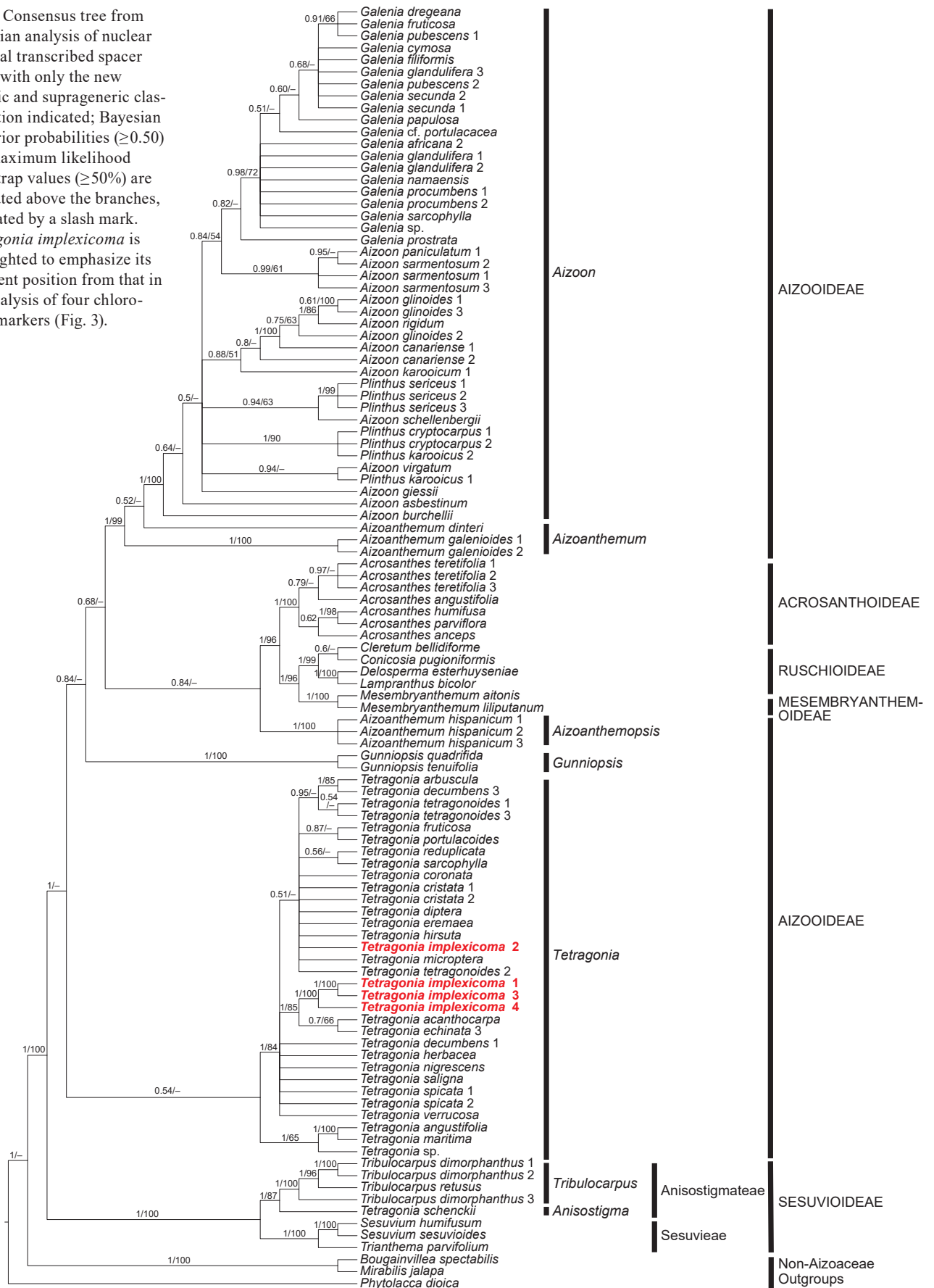
Fig. 4. Consensus tree from Bayesian analysis of nuclear internal transcribed spacer (ITS) with only the new generic and suprageneric classification indicated; Bayesian posterior probabilities (≥0.50) and maximum likelihood bootstrap values (≥50%) are indicated above the branches, separated by a slash mark. *Tetragonia implexicoma* is highlighted to emphasize its different position from that in the analysis of four chloroplast markers (Fig. 3).

Tree tip labels (top to bottom):

Galenia dregeana (0.91/66)
Galenia fruticosa
Galenia pubescens 1
Galenia cymosa
Galenia filiformis (0.68/–)
Galenia glandulifera 3
Galenia pubescens 2 (0.60/–)
Galenia secunda 2
Galenia secunda 1 (0.51/–)
Galenia papulosa
Galenia cf. *portulacacea*
Galenia africana 2
Galenia glandulifera 1 (0.98/72)
Galenia glandulifera 2
Galenia namaensis
Galenia procumbens 1 (0.82/–)
Galenia procumbens 2
Galenia sarcophylla
Galenia sp.
Galenia prostrata (0.84/54)
Aizoon paniculatum 1 (0.95/–)
Aizoon sarmentosum 2 (0.99/61)
Aizoon sarmentosum 1
Aizoon sarmentosum 3
Aizoon glinoides 1 (0.61/100)
Aizoon glinoides 3 (1/86)
Aizoon rigidum (0.75/63)
Aizoon glinoides 2 (1/100)
Aizoon canariense 1 (0.8/–)
Aizoon canariense 2 (0.88/51)
Aizoon karooicum 1
Plinthus sericeus 1 (1/99)
Plinthus sericeus 2
Plinthus sericeus 3 (0.94/63)
Aizoon schellenbergii (0.5/–)
Plinthus cryptocarpus 1 (1/90)
Plinthus cryptocarpus 2
Plinthus karooicus 2 (0.64/–)
Aizoon virgatum (0.94/–)
Plinthus karooicus 1
Aizoon giessii
Aizoon asbestinum (0.52/–)
Aizoon burchellii (1/99)

} *Aizoon*

Aizoanthemum dinteri
Aizoanthemum galenioides 1 (1/100)
Aizoanthemum galenioides 2

} *Aizoanthemum*

Acrosanthes teretifolia 1 (0.97/–)
Acrosanthes teretifolia 2 (0.79/–)
Acrosanthes teretifolia 3
Acrosanthes angustifolia (1/100)
Acrosanthes humifusa
Acrosanthes parviflora (0.62/1/98)
Acrosanthes anceps (1/96)

ACROSANTHOIDEAE

Cleretum bellidiforme (0.6/–)
Conicosia pugioniformis (1/99)
Delosperma esterhuyseniae (1/100)
Lampranthus bicolor (1/96)
Mesembryanthemum aitonis (1/100)
Mesembryanthemum liliputanum

RUSCHIOIDEAE
MESEMBRYANTHEMOIDEAE

Aizoanthemum hispanicum 1 (1/100)
Aizoanthemum hispanicum 2
Aizoanthemum hispanicum 3

} *Aizoanthemopsis*

Gunniopsis quadrifida (1/100)
Gunniopsis tenuifolia

} *Gunniopsis*

Tetragonia arbuscula (1/85)
Tetragonia decumbens 3 (0.95/–)
Tetragonia tetragonoides 1 (0.54/–)
Tetragonia tetragonoides 3
Tetragonia fruticosa (0.87/–)
Tetragonia portulacoides
Tetragonia reduplicata (0.56/–)
Tetragonia sarcophylla
Tetragonia coronata
Tetragonia cristata 1 (0.51/–)
Tetragonia cristata 2
Tetragonia diptera
Tetragonia eremaea
Tetragonia hirsuta
Tetragonia implexicoma 2
Tetragonia microptera
Tetragonia tetragonoides 2
Tetragonia implexicoma 1 (1/100)
Tetragonia implexicoma 3 (1/100)
Tetragonia implexicoma 4 (1/85)
Tetragonia acanthocarpa (0.7/66)
Tetragonia echinata 3
Tetragonia decumbens 1 (1/84)
Tetragonia herbacea
Tetragonia nigrescens
Tetragonia saligna
Tetragonia spicata 1
Tetragonia spicata 2
Tetragonia verrucosa
Tetragonia angustifolia (1/100)
Tetragonia maritima (1/65)
Tetragonia sp.

} *Tetragonia*

Tribulocarpus dimorphanthus 1 (1/100)
Tribulocarpus dimorphanthus 2 (1/96)
Tribulocarpus retusus (1/100)
Tribulocarpus dimorphanthus 3 (1/87)

} *Tribulocarpus*

Tetragonia schenckii (1/100)

} *Anisostigma*

Sesuvium humifusum (1/100)
Sesuvium sesuvioides (1/100)
Trianthema parvifolium

Anisostigmateae
Sesuvieae
SESUVIOIDEAE

Bougainvillea spectabilis (1/100)
Mirabilis jalapa
Phytolacca dioica

Non-Aizoaceae Outgroups

Other internal node supports: 1/– , 0.68/–, 0.84/–, 1/100, 0.84/–, 1/–, 1/100, 0.54/–, 1/–

AIZOOIDEAE

implied that ncpGS was unsuitable for phylogenetic inference here (Klak & al., 2013). Cloning of ITS in the Aizooideae to investigate the extent of paralogy has not been undertaken and so the results presented here for the ITS analysis need to be interpreted with caution.

It becomes evident that, for an in-depth understanding of the relative histories of nuclear versus chloroplast genomes in the Aizoaceae, it is essential to sample additional nuclear markers. So far, the three nuclear markers utilized for systematic questions in the Aizoaceae (i.e., ITS, 5S NTS, ncpGS) showed low levels of genetic divergence (Klak & al., 2003a) as well as gene-paralogy.

The availability of several recently developed high-throughput sequencing technologies, such as genome skimming (Dodsworth, 2015), hold promise for future phylogenetic studies in the Aizoaceae, where many of the crown clades show little genetic divergence, possibly as a consequence of their recent diversification (Klak & al., 2004, 2017; Valente & al., 2014).

Revised circumscription of the Aizooideae. — Bittrich & Hartmann (1988) provided synapomorphies for the major groups in the Aizoaceae. Petaloid staminodes characterize the highly succulent subfamilies Mesembryanthemoideae and Ruschioideae, whereas in the remaining subfamilies the flower has a perianth, where the tepals are sepaloid and green outside and petaloid and differently coloured inside (Bittrich & Hartmann, 1988). Potential synapomorphies were suggested for four of the five subfamilies, except for the Aizooideae (Bittrich & Hartmann, 1988).

The Aizooideae as well as *Acrosanthes* are characterized by leafy inflorescences (Bittrich & Hartmann, 1988), termed anthoclades by Hofmann (1973), which are shoot-systems that form alternately terminal flowers and foliose leaves. The inflorescence is very similar in the Sesuvioideae, but differs in that two membranous or scale-like bracts are formed above the leaves and before the shoot is terminated by a flower (Hofmann, 1973). These bracts also allow clear distinction of the inflorescence from the shoot in the Sesuvioideae, which is not possible in the Aizooideae (Hofmann, 1973) (Table 2), and Bittrich & Hartmann (1988) selected their presence as a synapomorphy for the Sesuvioideae.

Bittrich (1990a) later suggested that the presence of epidermal bladder-like hairs on leaves or stems characterized Aizooideae+Tetragonioideae. These bladder-like hairs consist of a large terminal cell subtended by a multi-cellular socle or stalk. The terminal cell is variable in size and shape and may characterize the subgroups (Bittrich, 1990a). Though the presence of such hairs was established for *Gunniopsis papillata* Chinnock and *Tetragonia tetragonoides* (Pall.) Kuntze, Bittrich (1990a) did not reveal for how many species or genera in the Aizooideae they had been observed, and a comprehensive study of the epidermis and anatomy of the leaves across the Aizooideae and the other subfamilies is still needed.

A new subfamily to accommodate *Acrosanthes*. — The six species of *Acrosanthes* are endemic to the south-western parts of the Western Cape, South Africa (Fig. 6), occurring exclusively among mesic *fynbos* on acidic, nutrient-poor soils derived from

Table 1. Revised classification for the Aizoaceae.

| Subfamily	Genus	Species	Distribution
Acrosanthoideae subfam. nov.	*Acrosanthes* Eckl. & Zeyh.	6	Western Cape, South Africa
Aizooideae	*Aizoanthemopsis* gen. nov.	5	Eurasia
	Aizoanthemum Dinter ex Friedrich	1	Angola and Namibia
	Gunniopsis Pax	14	Australia
	Aizoon L. = *Galenia* L. = *Plinthus* Fenzl	45	endemic to southern Africa, except for *A. canariense* which is also distributed across Eurasia
	Tetragonia L.	35 5 (endemic) 10 (endemic) 1	southern Africa Australia and New Zealand South America worldwide (*T. tetragonoides*)
Mesembryanthemoideae	*Mesembryanthemum* L. (Klak & Bruyns, 2013)	105	mainly southern Africa, with no endemics outside southern Africa
Ruschioideae	110 genera (Hernández-Ledesma & al., 2015)	±1585	mainly southern Africa, with few endemic species outside southern Africa
Sesuvioideae	*Anisostigma* Schinz	1	Namibia
	Sesuvium L.	25	worldwide in the tropics
	Trianthema L.	28	between 40° N and S of the equator
	Tribulocarpus S.Moore	2	Namibia, East Africa
	Zaleya Burm.f.	9	between 30° N and S of the equator
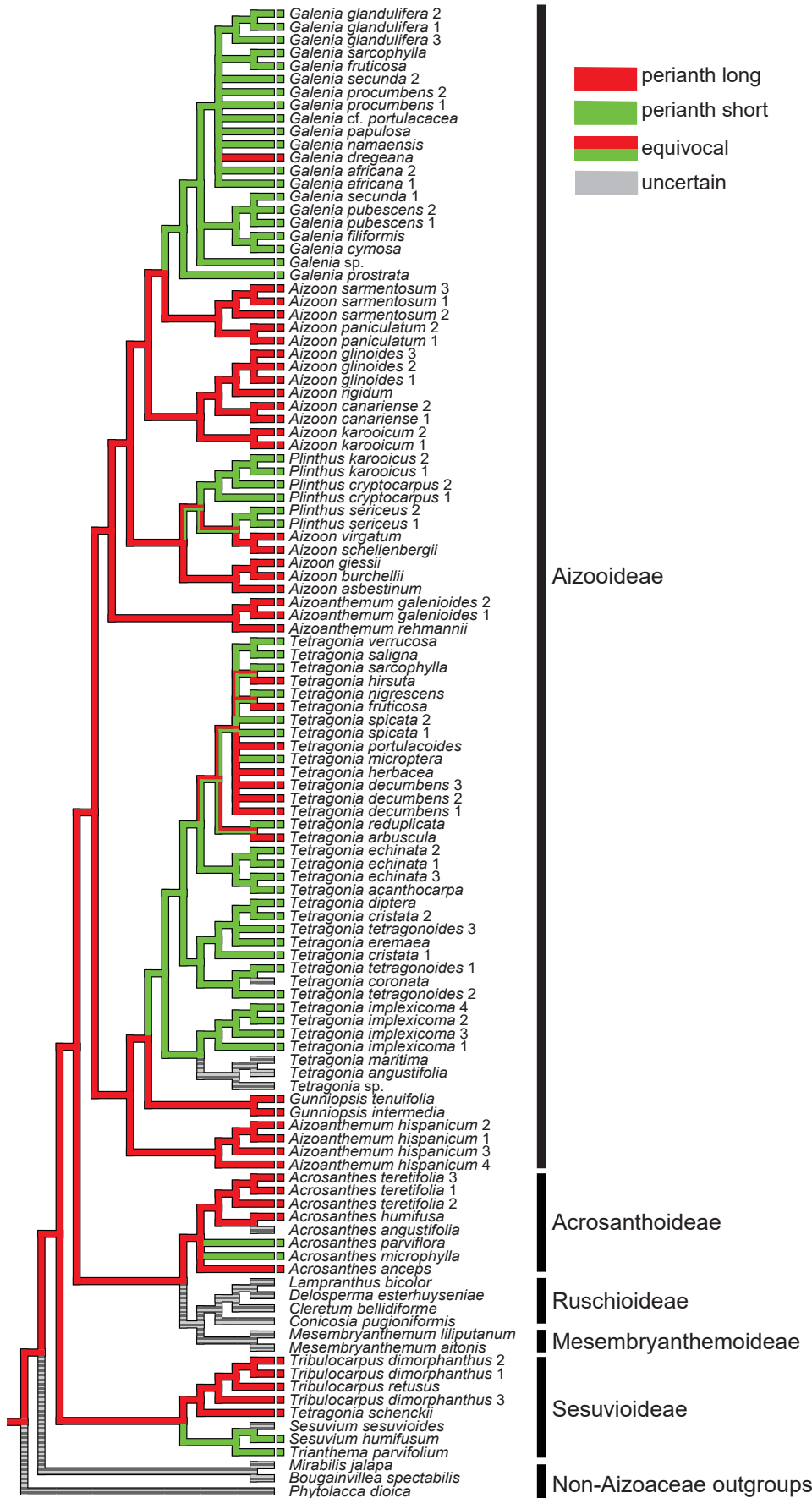
Fig. 5. Tree from Fig. 3 with characters states "long/short" for perianth length in flowers of Aizooideae (including *Acrosanthes*) and Sesuvioideae.

Legend:
- perianth long (red)
- perianth short (green)
- equivocal (red)
- uncertain (grey)

sandstones. The genus was established already in 1837, at which time three species were included (Ecklon & Zeyher, 1837). More than a century later, Adamson (1959a) revised the genus and described *A. microphylla* Adamson. Both *A. microphylla* and the recently described *A. parviflora* J.C.Manning & Goldblatt originate from high, montane areas of the Western Cape.

Like many plants of the *fynbos*, *Acrosanthes* has needle-shaped leaves with much flattened, bladder-like idioblasts on their surface giving the leaf a smooth appearance (Fig. 7A). Such idioblasts are thought to be a synapomorphy for the Aizoaceae (Bittrich & Hartmann, 1988), but they may be greatly reduced in size and number and are largely absent in

Table 2. Major characters distinguishing the little-succulent subfamilies in Aizoaceae.

	Aizooideae	Acrosanthoideae subfam. nov.	Sesuvioideae
Inflorescence	leafy, not clearly distinct from the shoot	leafy, not clearly distinct from shoot system	clearly distinct from shoot system
Bracts	absent	absent	small, i.e., membranous or scale-like
Perianth segments	fused to about ⅓ of their length	fused to about ⅓ of their length	fused to about ⅓ of their length or fused to about ½ of their length into a cylindrical tube
Position of ovules	pendulous along the axis	shortly stipitate and basal	pendulous along the axis
Number of ovules per locule	1 to many	1	1 to many
Fruit	xerochastic or hygrochastic	xerochastic	xerochastic
Type of fruit	dry, leathery, a winged or horned nut, rarely a drupe or loculicidal or rarely septicidal capsule	capsule with thin parchment-like walls opening by a longitudinal split	circumscissile capsule opening by a transverse split or fruit woody with papery wings or spiny
Number of locules	2–10	2, but often incomplete subdivision	1–5
Seeds	aril absent	aril absent	enclosed by an aril or aril absent

Fig. 6. Distribution of *Acrosanthes*, the only genus of the newly recognized subfamily Acrosanthoideae, which is endemic to the Western Cape, South Africa.
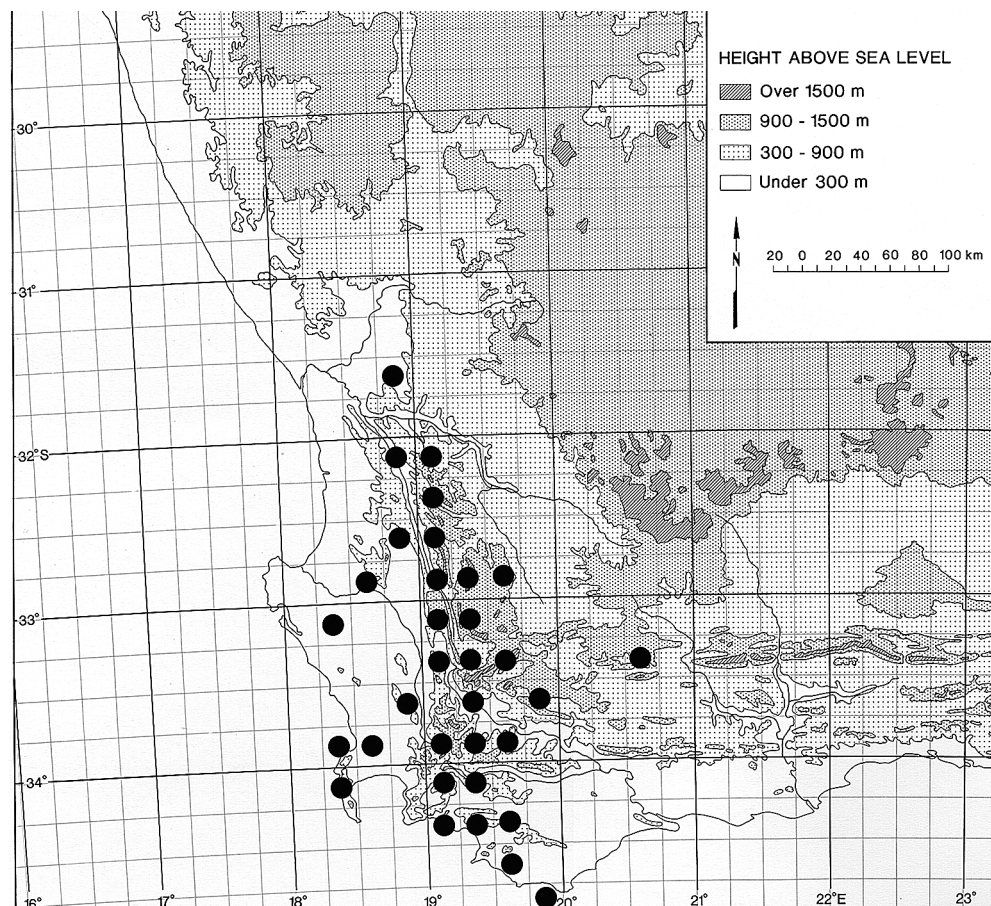
the highly succulent genera of the Ruschioideae (Ihlenfeldt & Hartmann, 1982).

The fruits of *Acrosanthes* lack expanding tissue (Adamson, 1959a; Hartmann, 2001), and this is absent also in the Sesuvioideae as well as in many species in the Aizooideae (Hartmann, 2001). The fruits of *Acrosanthes* are unique in that the capsules have a parchment-like wall. A further unique feature are the basal, shortly stipitate ovules (Fig. 8C, D) (Adamson, 1959a), which are pendulous in all other Aizoaceae (Fig. 9B, E) (Adamson, 1955; Hartmann, 2001). Hofmann (1973) described the orientation of the ovules for taxa with many ovules per locule (*Aizoanthemum*, *Aizoon* s.str., *Gunniopsis*) as anacampylotropous, whereas they are apotropous for the genera with one ovule per locule (*Galenia*, *Plinthus*, *Tetragonia*). In *Acrosanthes* they are also seemingly apotropous, since they are not typically positioned in the middle, but slightly laterally as in one of the two series in the many-ovular carpels (Hofmann, 1973). In addition, the upper (symplicate) part of the ovary is unilocular, with the septum (sometimes incomplete) between the two locules only formed in the lower synascidiate part (Hofmann, 1973). These features further distinguish *Acrosanthes* from the Aizooideae.

Acrosanthes was believed to be closely allied to *Aizoon* and *Galenia* (Adamson, 1959a), whereas Müller (1908) placed it near *Limeum*, largely on account of the solitary basal ovules. Klak & al. (2017) showed conclusively that *Acrosanthes* is sister to subfamilies Mesembryanthemoideae+Ruschioideae. Thus, the characteristic perianth as well as the base chromosome number ($x = 8$), which *Acrosanthes* shares with both the Aizooideae and the Sesuvioideae, are plesiomorphic characters (cf. Bittrich, 1990a). On the other hand, expanding tissue – a possible synapomorphy for Aizooideae (+Tetragonioideae) and Mesembryanthemoideae+Ruschioideae (Bittrich, 1990a) – has been lost in *Acrosanthes* (Klak & al., 2017). Although the split of *Acrosanthes* from the Mesembryanthemoideae+ Ruschioideae is relatively ancient at ±36 million years (Klak & al., 2017), there are no morphological features known to support the sister relationship between *Acrosanthes* and Mesembryanthemoideae+Ruschioideae.

Here we create a new subfamily for *Acrosanthes*. The major characteristics separating this new subfamily from the Aizooideae and Sesuvioideae are summarized in Table 2.

Aizoon, Galenia and Plinthus as one genus, Aizoon s.l. — The revisions for *Aizoon*, *Galenia* and *Plinthus* (Adamson, 1955, 1956, 1959a, b, 1961) are limited by their lack of details of the fruits, flowers and seeds as well as illustrations. Careful consideration of the characteristics used by Adamson
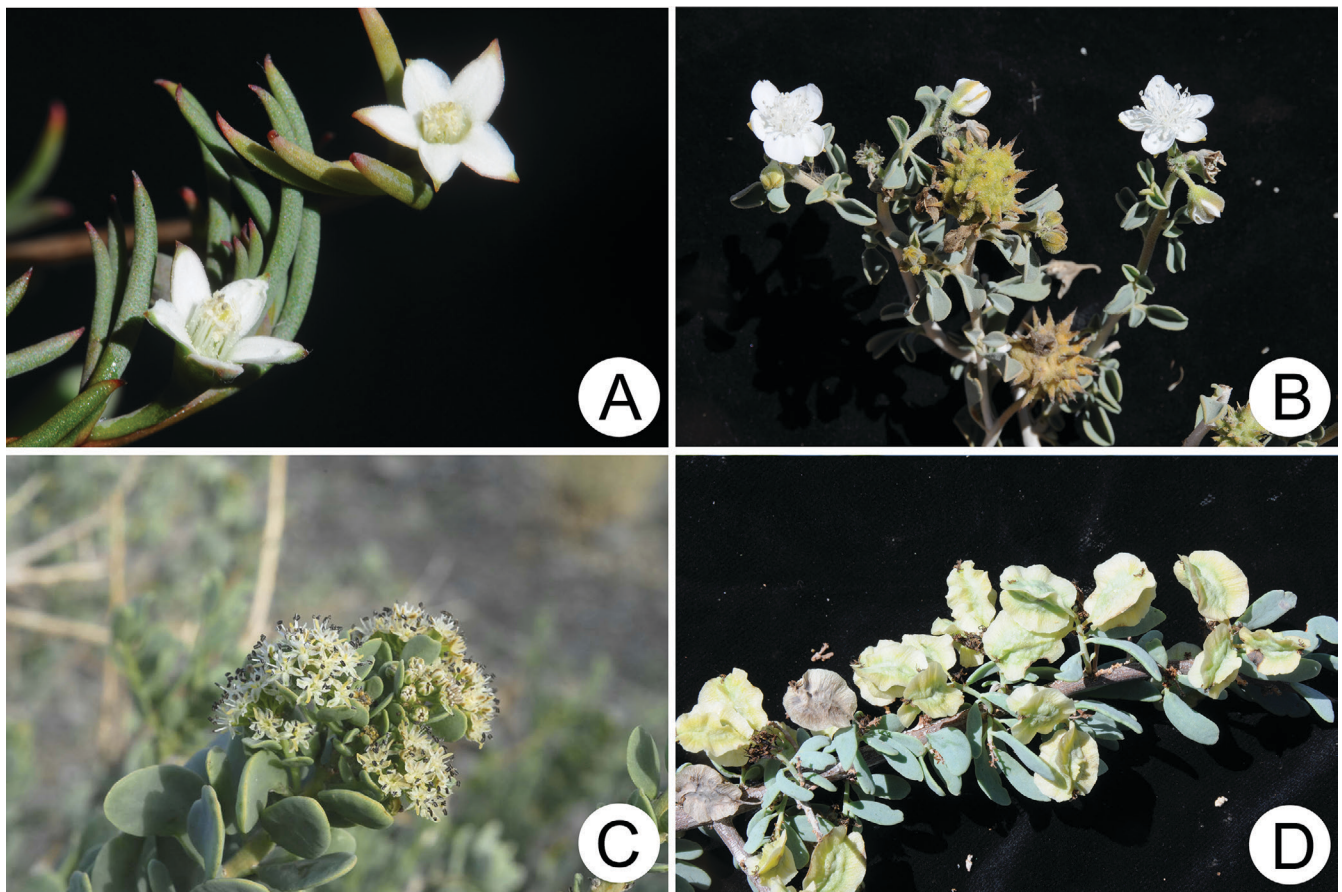

Fig. 7. Taxa excluded from the Aizooideae. **A,** *Acrosanthes teretifolia*, South Africa, Western Cape, *Klak 2464* (BOL); **B,** *Tribulocarpus dimorphanthus*, with terminal bracteate flowers arranged in groups and compound, spiny fruits, Namibia, *Klak 2441* (BOL); **C,** *Tetragonia schenckii*, numerous small flowers arranged in a cymose panicle at the tips of the branches, Namibia; **D,** *Tetragonia schenckii*, with winged fruits, Namibia. — Photo 7C by courtesy of C. Mannheimer.

to separate these genera shows that there is considerable overlap between them (Table 3). For example, *Aizoon* was considered to be well circumscribed by its apically depressed capsules, as opposed to their convex apex in *Aizoanthemum*, *Galenia* and *Plinthus* (Chinnock, 1983; Hartmann, 2001). However, in *Galenia glandulifera* Bittrich and *G. secunda* (L.f.) Sond. the capsules are apically depressed as in *Aizoon* (Adamson, 1956; Bittrich, 1990b), whereas they are convex in *Aizoon schellenbergii* Adamson (Hartmann, 2001).

Bittrich (1990b) also noted that several androecial and gynoecial features used by Adamson (1956) to separate *Aizoon*

from *Galenia* are shared between these two genera (Table 3). Whereas small flowers distinguish most species of *Galenia* from *Aizoon* (except *G. dregeana* Fenzl ex Sond., Electr. Suppl.: Table S1), flowers are also small in *Plinthus* (Table 3). Both *Galenia* and *Plinthus* are nested within *Aizoon*, suggesting that floral size decreased at least twice within this clade (Fig. 5). Even the small southern African clade of *Acrosanthes* has both large- (e.g., *A. teretifolia* Eckl. & Zeyh., Fig. 8) and small-flowered species (e.g., *A. parviflora*, Fig. 5; Electr. Suppl.: Table S1).

All species of *Aizoon* and *Plinthus* as well as many species of *Galenia* have hairs or hair-like papillae on the leaves and

Fig. 8. Flowers of *Acrosanthes teretifolia*. **A,** Side view of flower; **B,** Side view of stigmas and ovary with tepals removed; **C,** Vertical section of flower; **D,** Vertical section through stigmas and ovary; **E,** Horizontal section above ovules. — Drawn from *Klak 2469* (BOL). Scale bars: A & B = 2 mm; C–E = 1 mm (at C).
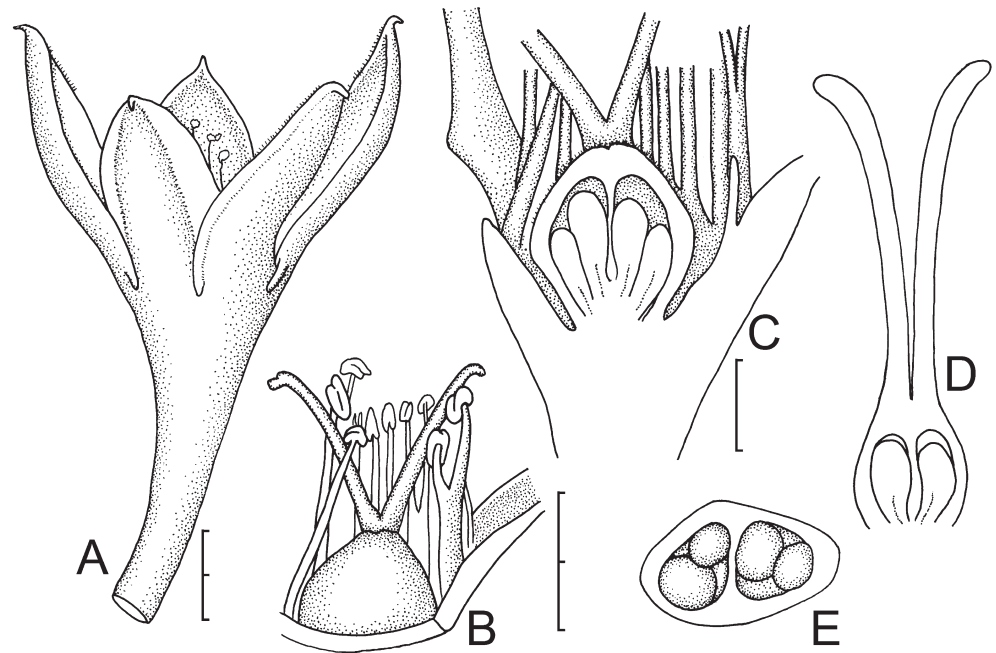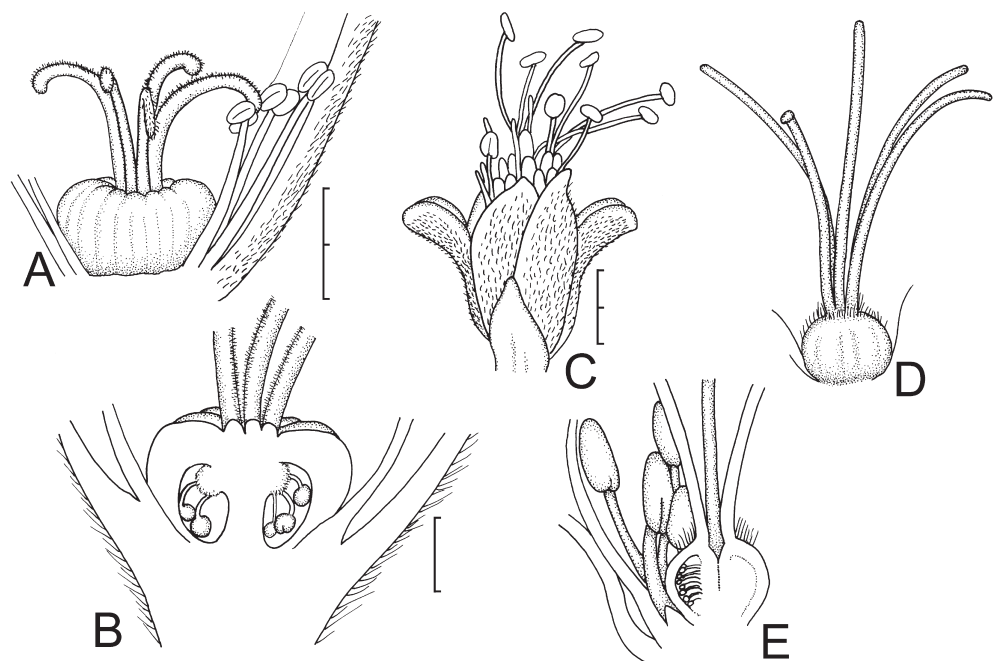

Fig. 9. Flowers of *Aizoon*. **A & B,** *Aizoon sarmentosum*: **A,** Side view of stigmas and ovary with tepals removed; **B,** Vertical section of flower. **C–E,** *Aizoon schellenbergii*: **C,** Side view of flower; **D,** Side view of ovary and stigmas; **E,** Vertical section through stigmas and ovary. — A & B drawn from *Klak 2448* (BOL); C–E drawn from *Klak 2509* (BOL). Scale bars: A & D = 2 mm (at A); B & E = 1 mm (at B); C = 2 mm.

stems, giving them a silvery or grey appearance (Adamson 1959a, 1961). As yet, differences in epidermal features, such as the shapes of papillae and hairs across the Aizooideae, have not been considered. Thus, the "hair-like" structures found in *Galenia* were called "hairs" by Adamson (1956) and "papillae" by Hartmann (2001). Several species of *Galenia* lack hair-like papillae on the leaves (e.g., *G. africana* L., *G. dregeana* and *G. glandulifera*), although very short appressed hairs may be present on the stems (e.g., *G. africana*; Adamson, 1956). In contrast, in the southern African species of *Aizoanthemum* (clade A, Fig. 3) as well as *A. hispanicum* (clade B) the epidermis is papulose (with rounded globose papillae), similar to those found in *Galenia dregeana*, *G. glandulifera* and *G. papulosa* (Eckl. & Zeyh.) Sond. Thus, the rounded, globose papillae found in some species of *Galenia* may constitute a reversal.

Capsules with reduced expanding tissue are common to *Aizoon*, *Galenia* and *Plinthus* and this provides a synapomorphy for this lineage. The expanding keels are much shortened in *Aizoon* and are even further reduced in *Galenia* and *Plinthus*, where the fruits open at most very slightly (Bittrich, 1990b). This feature distinguishes them from *Aizoanthemum* and *Gunniopsis*, which have fully functioning expanding keels. It also distinguishes them from *Tetragonia*, where the fruits are winged or horned nuts, rarely drupes without locular structure and therefore without expanding keels. Expanding tissue was lost several times across the Aizoaceae and different types of xerochastic fruits evolved: xerochastic capsules versus nut-like fruits in the Aizooideae, or the schizocarpous fruits in the Apatesieae (Ruschioideae). On account of overlaps in the characters distinguishing *Aizoon*, *Galenia* and *Plinthus* (Table 3), we chose to enlarge the concept of *Aizoon*, rather than breaking it up into several genera. Since we have a synapomorphy holding them all together, this is a further argument for a much expanded *Aizoon* s.l.

Subgeneric classification for *Aizoon* s.l. — Hartmann (2001) followed Adamson (1956) and Friedrich (1970), who recognized two subgenera in *Galenia*, namely subg. *Galenia*

and subg. *Kolleria* Fenzl emend. Adamson. However, Bittrich (1990b) pointed out that members of subg. *Kolleria* agree in several features with those of *Aizoon* and that their monophyly had never been established. In the phylogeny for the Aizooideae (Fig. 3), *Galenia* is monophyletic, albeit nested within *Aizoon*, but with no members of *Aizoon* nested within *Galenia*. Since relationships among species of *Galenia* remained largely unresolved and unsupported (Fig. 3), the monophyly of subgenera *Kolleria* and *Galenia* remains undecided. Consequently, we maintain the two subgenera until further data become available.

We propose below four new subgenera for *Aizoon*, which are based on the clades retrieved in the phylogeny (Fig. 3). The key has been adapted from characters used previously to distinguish among groups of species within *Aizoon* (Adamson, 1959a). Due to convergence, in some cases the distinction between the subgenera is only slight. Therefore, our key also includes the geographical area in which the clades occur.

***Aizoanthemum hispanicum* in a new monotypic genus. —** *Aizoanthemum* s.str. was distinguished from *Aizoon* by differences in the fruits (Friedrich, 1957; Chinnock, 1983; Hartmann, 2001). *Aizoon hispanicum* was transferred to *Aizoanthemum* by Hartmann (2001), possibly on account of the relatively long expanding keels in its capsules, similar to those of the southern African species but different from the much reduced expanding keels of *Aizoon*. However, the two lineages of *Aizoanthemum* (clades A & B, Fig. 3) differ markedly in the shape of their capsules: from above they are prominently 5-angled in *A. hispanicum* (clade B, Fig. 3), whereas they are globose without angles in the southern African species (clade A) (Friedrich, 1957; Hartmann, 2001). Hofmann (1973) noted that the ovules in *A. hispanicum* are positioned in four indistinct instead of two distinct series, which is an additional difference to other members of the Aizooideae with multi-ovular carpels (*Aizoanthemum*, *Aizoon* s.str., *Gunniopsis*).

There are also several floral characters which indicate that *A. hispanicum* is closer to *Gunniopsis* (clade B), rather than to members of clade A.

Table 3. Characters used to separate *Aizoanthemum*, *Aizoon*, *Galenia*, and *Plinthus* according to Adamson (1956, 1959b, 1961).

	Aizoon L.	*Galenia* L.	*Plinthus* Fenzl.	*Aizoanthemum* Dinter ex Friedrich p.p. (excl. *A. hispanicum*)
Life form	annual herbs or perennial shrubs	perennial herbs or shrubs	perennial shrubs	annual herbs
Perianth size	large, perianth 3–8 mm long	small, perianth 1–2.5(–3) mm long	small, perianth 1.75–2.5 mm long	large, perianth 5–12 mm long or rarely small, perianth 2–3 mm long
Flowers	1–3 or in rich cymes	in rich cymes	solitary or in groups of 2–3	in rich cymes
Ovules	2 to many pendulous ovules per locule	single, pendulous ovule per locule	single, pendulous ovule per locule	2 to many pendulous ovules per locule
Stamens	numerous	twice as many as perianth lobes	as many as perianth lobes	numerous
Apex	indented, rarely rounded	rounded, rarely indented	rounded	rounded
Locules	5	2–5	2–5	5–10
Expanding tissue	reduced	much reduced to absent	much reduced to absent	fully developed

(1) All members of clade A have superior ovaries, whereas in clade B the ovaries are semi-inferior in *A. hispanicum* and *Gunniopsis* (Chinnock, 1983). In *Tetragonia* (clade B) ovaries are mostly inferior, but in several species they are incompletely inferior, in that they bulge above the insertion of the perianth (e.g., *T.* subg. *Tetragonoides*; Adamson, 1955; Taylor, 1994).

(2) The ovary is prominently 4-angled in *Gunniopsis* (Chinnock, 1983) and 5-angled in *A. hispanicum* (Hartmann, 2001), whereas it is rounded and without angles in *Aizoanthemum* s.str. (Friedrich, 1957). *Aizoanthemum hispanicum* and *Gunniopsis* are the only lineages in clade B with long expanding keels. However, in *Gunniopsis* the expanding keels are situated along the rims of the valves, which is unique within the Aizoaceae.

Geographically *A. hispanicum* is also isolated within clade B, being the only species endemic to Eurasia. Other species of clade B, apart from the cosmopolitan *T. tetragonoides*, are restricted to the Southern Hemisphere. Although there are only plesiomorphic characters defining *A. hispanicum*, we erect a new genus for it, based on its phylogenetic position and geographical isolation.

Subgeneric classification of *Tetragonia*. — Adamson (1955) subdivided the southern African species of *Tetragonia* into four subgenera: subg. *Tetragonia*, subg. *Tetragonoides* DC., subg. *Anisostigma* (Schinz) Adamson and subg. *Tribulocarpus* (S.Moore) Adamson. The last two are not related to the rest of *Tetragonia* but belong to the Sesuvioideae (Klak & al., 2003b, 2017; Thulin & al., 2012). The number of stamens and the position of the ovary with respect to the insertion of the perianth distinguished *Tetragonia* subg. *Tetragonia* from *T.* subg. *Tetragonoides*. The latter included species from Africa and the cosmopolitan *T. tetragonoides*. Taylor (1994) tentatively placed the endemic South American species of *Tetragonia* in *T.* subg. *Tetragonia*. Our results show that Adamson's subdivision of *Tetragonia* based on floral differences does not correspond to monophyletic lineages.
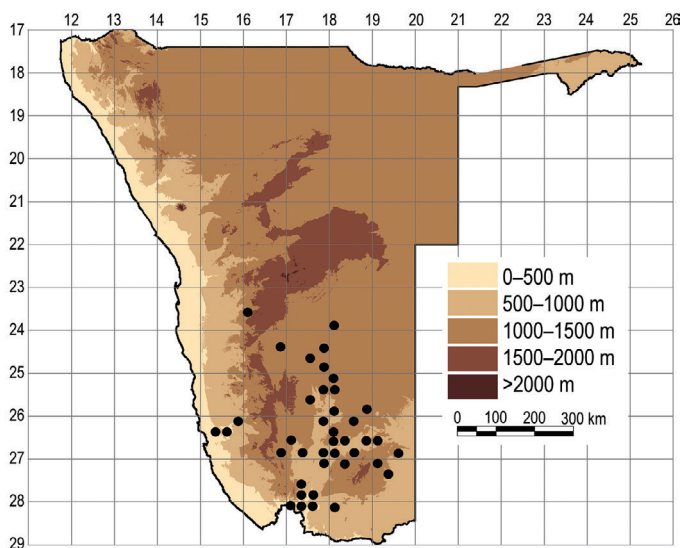

Fig. 10. Distribution of *Tetragonia schenckii* (= *Anisostigma schenckii*). The species is endemic to Namibia.

Here we propose a new subgeneric treatment for *Tetragonia*, which is based on the fruits: *T.* subg. *Tetragonia* for the southern African species with dry woody fruits, mostly with broad, membranous wings or wingless with ridges or papillae, rarely with rigid spines; *T.* subg. *Tetragonoides* for the Australasian species and the cosmopolitan *T. tetragonoides*, with dry fruits, horned or with broad woody wings or wings membranous and extending above the fruit; *T.* subg. *Tetragonella* for *T. implexicoma* from Australasia, at some stage in its own genus, *Tetragonella* Miq., the only species of *Tetragonia* with succulent fruits; and *T.* subg. *Americanae* for the South American endemic species with dry, (3–)4-angled fruits, where the angles are sharp or rounded and sometimes have narrow longitudinal wings.

The molecular markers used in our study did not resolve relationships within *Tetragonia* subg. *Tetragonia*, suggesting that the series erected by Adamson (1955) should be maintained.

***Tetragonia schenckii* and *Tribulocarpus* as sister to Sesuvioideae. —** Prior to the inclusion of *Tribulocarpus* (Fig. 7B), the Sesuvioideae were well characterized by their circumscissile capsules and arillate seeds (Bittrich & Hartmann, 1988; Hartmann, 2001). After *Tribulocarpus* was included, these features no longer defined the subfamily, since in *Tribulocarpus* the seeds lack an aril and the fruits are woody nuts with 3–5 broad, papery wings or compound, woody, subglobose structures with a spiny exterior. Bittrich & Hartmann (1988: table 1) suggested that inflorescences with small, membranous or scale-like bracts is a synapomorphy for the Sesuvioideae, a feature also present in *Tetragonia schenckii* (Schinz, 1894) and widespread in the Ruschioideae, but absent in the Aizooideae (Hofmann, 1973). A special case is *Tribulocarpus dimorphanthus*, where the bracts become enlarged and lignified when they develop into spines, which later cover the compound fruit (Moore, 1921). Whether the bracts have a scale-like or membranous consistency in fresh flowers of *Tribulocarpus* remains unclear (Thulin, 1993; Thulin & al., 2012). Despite this uncertainty, the membranous bracts in *T. schenckii* support the inclusion of the *T. schenckii*–*Tribulocarpus* lineage into the Sesuvioideae. Furthermore, Thulin & al. (2012) suggested that perianth segments with a subapical, dorsal mucro is possibly a synapomorphy for Sesuvioideae including *Tribulocarpus*. Both species of *Tribulocarpus* possess this feature, but it is unknown whether it is present in *T. schenckii*. The question arises whether the differences in the fruits across the Sesuvioideae–*T. schenckii*–*Tribulocarpus* lineage should favour the re-establishment of the Sesuvioideae s.str. and the recognition of the *T. schenckii*–*Tribulocarpus* lineage as a new subfamily. However, we are not in support of this narrow subfamilial concept, since also in the Aizooideae the fruits are variable, ranging from hygrochastic fruits with expanding keels, which are reduced in many species to dry, nut-like and winged fruits or even drupes. The diversity of fruits in the Aizooideae would therefore be consistent with the current expanded concept of the Sesuvioideae.

According to Adamson (1955) and Hartmann (2001), *Tetragonia schenckii* belongs to *Tetragonia*, but we showed that it is not related to *Tetragonia* and is sister to *Tribulocarpus*

(Klak & al., 2017) (Fig. 3). *Tetragonia schenckii* is endemic to Namibia (Fig. 10), forming woody shrubs to 2 m tall, with slightly succulent leaves and 3- to 4-winged fruits (Fig. 7C, D). The unusual feature of four styles, of which two are much shorter than the others, led Schinz (1894) to place *Tetragonia schenckii* tentatively in *Tetragonia*, but he later moved it to a monotypic *Anisostigma* (Schinz, 1897).

Tribulocarpus consists of the two species, *T. dimorphanthus* and *T. retusus* (Thulin) Thulin & Liede (Thulin, 1993; Thulin & al., 2012). It is found in northeast Africa and in Namibia and has been extensively discussed on account of its disjunct distribution within Africa. The two species differ markedly in their fruits, with winged, simple fruits in *T. retusus* and compound, spiny fruits in *T. dimorphanthus* (Fig. 7B). Since *T. schenckii* also has winged fruits (Fig. 7D), winged fruits are now known from both Namibia and Somalia in the *T. schenckii–Tribulocarpus* lineage.

Thulin & al. (2012) gave "flowers with an elongated perianth tube" and "1-ovulate locules" as potential synapomorphies for *Tribulocarpus*. Both of these features are present in *T. schenckii* (Adamson, 1955) and so the floral characters support the monophyly of the *T. schenckii–Tribulocarpus* lineage.

If one were to combine *Tribulocarpus* and *T. schenckii* into one genus, the older generic name, *Anisostigma* would have to be used. Whereas it could be argued that the few species included in the *T. schenckii–Tribulocarpus* lineage should be recognized as a single genus, we maintain *Tribulocarpus* and *Anisostigma* for the following reasons: (1) the split of *Tribulocarpus* from *Anisostigma* was estimated at 18.3 million years (i.e., since the Miocene, Klak & al., 2017), suggesting that they are relatively old lineages with little recent diversification; (2) there are several floral characters which separate these two genera (Table 4). Consequently, we reinstate *T. schenckii* as *Anisostigma schenckii* (Schinz) Schinz.

We have argued above that inflorescences with small bracts (with bracts possibly modified in *Tribulocarpus*) and perianth segments with a subapical, dorsal mucro are possible synapomorphies for the Sesuvioideae, including the *T. schenckii–Tribulocarpus* lineage. By recognizing the two

lineages as one subfamily, this classification will reflect the sister relationship between the Sesuvioideae s.str. and the *T. schenckii–Tribulocarpus* lineage. Nevertheless, since both lineages are distinctive and have easy-to-recognize synapomorphies, we recognize them each as tribes.

■ TAXONOMIC CONCLUSIONS

Key to the subfamilies of Aizoaceae (cf. Table 2)

1. Petals present and of staminodial origin ……………… 2
1. Petals absent; tepals brightly coloured and petaloid inside, green and sepaloid outside …………………………… 3
2. Placentation central, nectaries shell-shaped to tubular, petals and stamens mostly united into a tube ……………… ………………………………… **Mesembryanthemoideae**
2. Placentation basal or parietal, very rarely central, nectaries crest-shaped, petals mostly free ………… **Ruschioideae**
3. Fruit a xerochastic capsule with 2 locules; ovules basal and 1–2 per locule, shortly stipitate to sessile ………….. ………………………………………… **Acrosanthoideae**
3. Fruit xerochastic or hygrochastic with 1–10 locules; ovules axile, pendulous and 1-many per locule ……………… 4
4. Fruit xerochastic, without expanding tissue, capsule circumscissile and aril present on seeds or fruit woody, either winged or spiky, aril absent ……………… **Sesuvioideae**
4. Fruit xerochastic or hygrochastic, expanding tissue reduced to absent, dry, a winged or horned nut, rarely a drupe or a hygrochastic, loculicidal or rarely septicidal capsule; aril absent …………………………………… **Aizooideae**

1. **Acrosanthoideae** Klak, **subfam. nov.** – Type: *Acrosanthes* Eckl. & Zeyh.

Mat-forming or sprawling shrublet with long branches, internodes woody towards their bases. Leaves opposite, exstipulate, usually connate at the bases, terete to flat, glabrous, dark green or yellowish. Inflorescences leafy (as anthocladia). Flowers solitary, seemingly lateral, but instead terminal and

Table 4. Differences between *Tribulocarpus* and *Anisostigma*.

	Tribulocarpus S.Moore	*Anisostigma* Schinz
Height	herbaceous shrubs, woody at base, 0.5–1(–1.5) m high	erect woody shrubs, 0.5–2.0 m high
Leaves	subsessile or petiolate	shortly petiolate
Leaf shape	flat, obovate, obtuse	flat, oblong, obovate or spatulate
Leaf epidermis	covered by globose papillae	leathery, with low round papillae
Flowers	pedicellate and solitary or sessile in pedunculate heads	numerous, pedicellate, in cymose panicles at the tips of short branches
Ovary	2-locular	1-locular
Style	2 stigmatic lobes fused into a style	4 styles with two much longer than other two
Stamens	Numerous (i.e., more than 20)	5
Fruits	fruit 5-winged or compound, subglobose and spiny	fruit 3- to 4-winged
Ecology	in dry grassland	in sand of dry riverbeds, often brackish soils
Distribution	East Africa and Namibia	Namibia

overtopped by one of the side branches, pedicellate, perianth 5-lobed, internally petaloid, white, externally sepaloid, green, united at the base into a short tube, the lobes longer than the tube, stamens 8 to numerous, inserted at the lower part of the perianth, sometimes in groups, ovary superior and 2-locular, with 1–2 basal ovules per locule, stigmas 2 (Fig. 8). Fruit a xerochastic capsule, thin-walled, splitting along the sides when mature, 2-chambered, but septum incomplete, with 1–4 seeds. Seeds compressed, reniform or orbicular, rugose, black.

Ecology. – Among *fynbos*, with winter-rainfall >300 mm mean annual precipitation (MAP), in sandstone soils. *Acrosanthes teretifolia* Eckl. & Zeyh. is restricted to relatively low-lying areas from 160 to 600 m, whereas the other five species are found between 410 and 2240 m. Two species, *A. teretifolia* and *A. anceps* Sond., are frequent after fires. Since all species occur in *fynbos*, their increase in abundance after fires may hold true for the entire genus as well.

Distribution. – The six species of this subfamily are endemic to a small area within the Western Cape Province of South Africa, from the Clanwilliam District in the north to the Cape Peninsula and eastwards as far as the districts of Worcester and Caledon (Fig. 6).

Genera (1). – *Acrosanthes* Eckl. & Zeyh.

Recircumscription of Aizooideae

After the exclusion of *Acrosanthes* from the Aizooideae, we recognize five lineages at generic level:

(1) *Gunniopsis* is retained as before (Chinnock, 1983);

(2) *Aizoanthemum* only includes species from southern Africa;

(3) *Aizoanthemum hispanicum*, endemic to Eurasia, forms a new genus, *Aizoanthemopsis* gen. nov.;

(4) *Aizoon* was described at the same time as *Galenia* by Linnaeus in 1753 and therefore both generic names have equal priority. However, since the family as well as subfamily name is based on *Aizoon*, and with *A. canariense* also occurring outside of southern Africa, it is better known than *Galenia*. For these reasons we maintain the name *Aizoon* instead of *Galenia* and transfer all its species as well as those included in *Plinthus* to *Aizoon*. We divide *Aizoon* into six subgenera;

(5) *Tetragonia schenckii* is excluded from *Tetragonia*. We divide *Tetragonia* into four subgenera.

2. **Aizooideae** Spreng. ex Arn., Botany: 112. 1832 [preprint from Napier, M. (ed.), Encyclopaedia Britannica, ed. 7, vol. 5] – Type: *Aizoon* L.
= Tetragonioideae Lindl., Veg. Kingd.: 527. 1846 – Type: *Tetragonia* L.

Annuals to perennials, with herbaceous or woody branches, rarely geophytes, dioecious or rarely monoecious. Leaves slightly succulent, usually alternate or occasionally opposite, flat or more rarely sub-cylindrical, epidermis of stems and leaves often hairy or with rounded papillae. Inflorescences leafy (as anthocladia), without bracts. Flowers perigynous, epigynous or rarely hypogynous, in *Tetragonia* bisexual, male and transitional on the same plant; perigone lobes fused up to ⅓

of their length, sepaloid and green outside, petaloid and white, yellow or greenish inside; ovules 1 to many, axile, pendulous. Fruit a loculicidal or more rarely septicidal capsule, hygrochastic to xerochastic or nut-like, woody, either winged or spiky, rarely a drupe, locules 2–10 with 1 to many seeds per locule; base chromosome number $x = 8$.

Ecology. – Mostly in Mediterranean and subtropical climates in Africa, South America, Europe, Asia, Australia and New Zealand.

Distribution. – The majority of species is found in southern Africa, from southern Angola to South Africa. Relatively few species occur outside of this area: Macaronesia, northern Africa along the Mediterranean coast, also in Spain and S Italy, Socotra, N Kenya to India, in southern Africa also in Zimbabwe, Australia, New Zealand, South America (Fig. 1).

Genera (5): *Aizoanthemopsis* Klak, *Aizoanthemum* Dinter ex Friedrich, *Aizoon* L. (= *Galenia* L., = *Plinthus* Fenzl), *Gunniopsis* Pax, *Tetragonia* L.

Key to the genera of Aizooideae

1. Fruit hygrochastic, with long (i.e., valves opening to 180 degrees) expanding keels ……………………………… 2
1. Fruit hygrochastic or xerochastic, if hygrochastic then with short expanding keels or expanding keels much reduced to absent (valves opening at most slightly), fruit leathery and dry, nut-like, winged, ridged or tuberculate, rarely a drupe ……………………………………………… 4
2. Fruit with expanding keels along the outer margins of the valves ………………………………… ***Gunniopsis***
2. Fruit with expanding keels in a medial position on the valves ……………………………………………… 3
3. Fruits globose, locules 5–10, tepals yellowish inside ….. ………………………………… ***Aizoanthemum***
3. Fruits 5–angled, locules 5, tepals white inside ………… ………………………………… ***Aizoanthemopsis***
4. Ovary superior, fruit a capsule, at most slightly hygrochastic (expanding tissue reduced to absent), leaves often grey from pale hairs or hair-like papillae on the epidermis or ± low, rounded papillae ………………………… ***Aizoon***
4. Ovary inferior, fruit xerochastic, nut-like, winged, ridged or tuberculate, rarely a drupe, leaves mostly green, with papillae of various shapes ………………… ***Tetragonia***

2.1 ***Aizoon*** L., Sp. Pl.: 488. 1753 – Type (designated by M.L. Green in Sprague, Nom. Prop. Brit. Bot.: 159. 1929): *Aizoon canariense* L.
= *Galenia* L., Sp. Pl.: 359. 1753, **syn. nov.** – Type: *Galenia africana* L.
= *Plinthus* Fenzl in Endlicher & Fenzl, Nov. Stirp. Dec.: 51. 1839, **syn. nov.** – Type: *Plinthus cryptocarpus* Fenzl.

Annual slightly succulent herbs to perennial shrubs, procumbent to erect, flowering branches often dying back, often secund, internodes hairy or papillate to smooth. Leaves opposite or alternate, petiolate (rarely sessile), often deciduous (at least partly during the dry season), lanceolate to linear, terete to spathulate and oval, with white hairs besides much smaller

bladder cells, or epidermis with low papillae, glistening in the fresh state, turning white when dry. Flowers solitary, in groups of 2–3 or in ± many-flowered cymes, sessile or shortly stalked, perianth hairy or smooth outside, tepals (3)4 or 5, fused to ⅓ of their length, yellow, rarely pink or white inside, stamens numerous, isomerous or twice as many as the perianth segments, alternating with the segments, in groups or in a ring, filaments filiform, ovary superior, carpels 2–5, ovules pendulous, 1 to many in each locule, stigmas free, as many as carpels (Fig. 9). Fruit a capsule with 2–5 locules, distinct expanding keels present or rudimentary to absent, opening only a little near the centre of the fruit or fruit dry, leathery, indehiscent or dehiscent, splitting at the top. Seeds with concentric ridges, pear to kidney-shaped, mostly slightly flattened, brown to black.

Ecology. – In arid areas, often in sand or fine gravel in disturbed, overgrazed areas.

Distribution. – The majority of species is found in southern Africa, from southern Angola to South Africa. Only one species, *Aizoon canariense* is very widespread and is also found in Macaronesia, northern Africa along the Mediterranean coast, also in Spain and S Italy, Socotra, N Kenya to India, in southern Africa also in Zimbabwe.

Aizoon acutifolium (Adamson) Klak, **comb. nov.** ≡ *Galenia acutifolia* Adamson in J. S. African Bot. 22: 112. 1956.

Aizoon affine (Sond.) Klak, **comb. nov.** ≡ *Galenia affinis* Sond. in Harvey & Sonder, Fl. Cap. 2: 476. 1862.

Aizoon africanum (L.) Klak, **comb. nov.** ≡ *Galenia africana* L., Sp. Pl.: 359. 1753.

Aizoon arenarium (Adamson) Klak, **comb. nov.** ≡ *Plinthus arenarius* Adamson in J. S. African Bot. 27: 149. 1961.

Aizoon collinum (Eckl. & Zeyh.) Klak, **comb. nov.** ≡ *Kolleria collina* Eckl. & Zeyh., Enum. Pl. Afric. Austral. 3: 327. 1837.

Aizoon cryptocarpum (Fenzl) Klak, **comb. nov.** ≡ *Plinthus cryptocarpus* Fenzl in Endlicher & Fenzl, Nov. Stirp. Dec.: 52. 1839

Aizoon cymosum (Adamson) Klak, **comb. nov.** ≡ *Galenia cymosa* Adamson in J. S. African Bot. 22: 122. 1956.

Aizoon dregeanum (Fenzl ex Sond.) Klak, **comb. nov.** ≡ *Galenia dregeana* Fenzl ex Harv. & Sond., Fl. Cap. 2: 477. 1862.

Aizoon ecklonis (Walp.) Klak, **comb. nov.** ≡ *Galenia ecklonis* Walp., Repert. Bot. Syst. 2: 232. 1843.

Aizoon exiguum (Adamson) Klak, **comb. nov.** ≡ *Galenia exigua* Adamson in J. S. African Bot. 22: 101. 1956.

Aizoon filiforme (Thunb.) Klak, **comb. nov.** ≡ *Mesembryanthemum filiforme* Thunb., Prodr. Pl. Cap. 2: 89. 1800.

Aizoon glanduliferum (Bittrich) Klak, **comb. nov.** ≡ *Galenia glandulifera* Bittrich in Bothalia 20: 217. 1990.

Aizoon hemisphaericum (Adamson) Klak, **comb. nov.** ≡ *Galenia hemisphaerica* Adamson in J. S. African Bot. 22: 122. 1956.

Aizoon herniariifolium (C.Presl.) Klak, **comb. nov.** ≡ *Kolleria herniariifolia* C.Presl., Symb. Bot. 1: 24, t. 14. 1831.

Aizoon hispidissimum (Fenzl ex Sond.) Klak, **comb. nov.** ≡ *Galenia hispidissima* Fenzl ex Sond. in Harvey & Sonder, Fl. Cap. 2: 478. 1862.

Aizoon karooicum Klak, **comb. nov.** ≡ *Plinthus karooicus* I.Verd. in Bothalia 4: 177. 1941.

Aizoon mezianum (K.Müll.) Klak, **comb. nov.** ≡ *Galenia meziana* K.Müll. in Beibl. Bot. Jahrb. Syst. 97: 68. 1908.

Aizoon namaense (Schinz) Klak, **comb. nov.** ≡ *Galenia namaensis* Schinz in Bull. Herb. Boissier 5(App. 3): 76. 1897.

Aizoon neorigidum Klak, **nom. nov.** ≡ *Galenia rigida* Adamson in J. S. African Bot. 22: 94. 1956, pro. sp.

Aizoon pallens (Eckl. & Zeyh.) Klak, **comb. nov.** ≡ *Kolleria pallens* Eckl. & Zeyh., Enum. Pl. Afric. Austral. 3: 328. 1837.

Aizoon portulacaceum (Fenzl ex Sond.) Klak, **comb. nov.** ≡ *Galenia portulacacea* Fenzl ex Sond. in Harvey & Sonder, Fl. Cap. 2: 475. 1862.

Aizoon procumbens (L.f.) Klak, **comb. nov.** ≡ *Galenia procumbens* L.f., Suppl. Pl.: 227. 1782.

Aizoon prostratum (G.Schellenb.) Klak, **comb. nov.** ≡ *Galenia prostrata* G.Schellenb. in Bot. Jahrb. Syst. 48: 501. 1912.

Aizoon pruinosum (Sond.) Klak, **comb. nov.** ≡ *Galenia pruinosa* Sond. in Harvey & Sonder, Fl. Cap. 2: 477. 1862.

Aizoon rehmannii (Schellenb.) Klak, **comb. nov.** ≡ *Plinthus rehmannii* Schellenb. in Bot. Jahrb. Syst. 48: 499. 1912.

Aizoon sarcophyllum (Fenzl ex Sond.) Klak, **comb. nov.** ≡ *Galenia sarcophylla* Fenzl ex Sond. in Harvey & Sonder, Fl. Cap. 2: 475. 1862.

Aizoon sericeum (Pax) Klak, **comb. nov.** ≡ *Plinthus sericeus* Pax in Bot. Jahrb. Syst. 48: 499. 1912.
Note. – The name *Aizoon sericeum* was only used by Fenzl on a herbarium-specimen and was not validly published, although it was cited by Sonder (1862).

Aizoon subcarnosum (Adamson) Klak, **comb. nov.** ≡ *Galenia subcarnosa* Adamson in J. S. African Bot. 22: 103. 1956.

Insufficiently known species

Aizoon camforosma Rchb. ex Spreng., Syst. Veg., ed. 16, 4(2): 195. 1827.

Note. – No type could be linked to this name. The glabrous leaves mentioned in the protologue excludes this species from *Galenia*. However, glabrous, slender leaves and axillary and pedunculate flowers agree with most features of *Acrosanthes*. All species of *Acrosanthes* have opposite leaves (Adamson, 1959a), which is in conflict with the protologue, where the leaves were said to be alternate. There is thus doubt whether this species even belongs to the Aizoaceae.

Key to the subgenera of *Aizoon*

1. Leaves with ± low rounded or hair-like papillae; flowers in many-flowered cymes, small with perianth 1–2.5(–3) mm long, capsules split open at the top, rarely valves slightly opening outwards after wetting, apex of capsule rounded, rarely depressed centrally ……………………………… 2
1. Leaves with white hairs, flowers 1–3 or in many-flowered cymes, perianth (1.75–)3–8 mm long, valves at most slightly opening outwards after wetting, apex of capsule depressed centrally, rarely rounded (then perianth 1.75–2.5 mm long) or flat ……………………………… 3
2. Plants green or yellow, suberect or erect, leaves linear or linear-oblong, usually glabrous, flowers in terminal cymes, usually paniculate not secund ….. subg. *Galenia*
2. Plants prostrate or diffuse shrublets, leaves usually secund, often gray or white, flowers in secund many-flowered cymes, fruit dehiscent …………………… subg. *Kolleria*
3. Flowers in terminal cymes, inside of perianth pink or whitish ……………………………………… subg. *Capensia*
3. Flowers among the leaves apparently lateral or axillary, inside of perianth yellow or whitish …………………… 4
4. Annual or perennial prostrate herbs ……… subg. *Aizoon*
4. Plants perennials, stout woody shrubs, or at least woody at base, with erect or decumbent, rarely scrambling branches ……………………………………………… 5
5. Stems short, young stems and leaves thinly covered with short appressed hairs, Western Cape …. subg. *Karooica*
5. Stems short or elongated, young stems and leaves thinly or thickly covered with short appressed hairs, Namibia, Northern Cape, Eastern Cape and Free State ………….. ……………………………………… subg. *Namibiana*

Aizoon subg. *Aizoon* – Type: *Aizoon canariense* L.

Woody or herbaceous annuals or perennials, prostrate, some with ascending branches. Leaves opposite or alternate, covered with short appressed or spreading white hairs. Flowers in many-flowered cymes or solitary, sessile, dull yellow-green to bright yellow, tepals 4–5, 2–8 mm long, stamens 12–15 in clusters. Fruit woody, pentagonal, persistent on the older stems, depressed on top, locules 5, valves slightly opening outwards after wetting.

Species (4). – *Aizoon canariense* L., *A. glinoides* L.f., *A. rigidum* L.f., *A. zeyheri* Sond.

Aizoon subg. *Capensia* Klak, **subg. nov.** – Type: *Aizoon paniculatum* L.

Prostrate to erect herbaceous perennials. Leaves alternate, covered thinly with short appressed white hairs. Flowers solitary, sessile, tepals 4–5, 4–8 mm long, red or white to cream inside, stamens in clusters of 3–10. Fruit woody, persistent on the older stems, flat-topped, locules 5, valves slightly opening outwards after wetting.

Species (2). – *Aizoon paniculatum* L., *A. sarmentosum* L.f.

Aizoon subg. *Galenia* (L.) Klak, **comb. & stat. nov.** ≡ *Galenia* L., Sp. Pl.: 359. 1753 – Type: *Galenia africana* L. = *Sialodes* Eckl. & Zeyh., Enum. Pl. Afric. Austral. 3: 329. 1837 – Type: *Sialodes glauca* Eckl. & Zeyh. (= *Aizoon ecklonis* (Walp.) Klak).

Erect or suberect shrubby perennials. Leaves opposite, linear or linear-oblong, slightly papillate, green or yellow, not gray or white. Flowers in terminal cymes, usually paniculate, not secund, small, tepals 4, 1–2.5(–4) mm long, white, yellow or pink inside, stamens 8. Fruit leathery, not or tardily dehiscent, with 2–5 locules, expanding tissue much reduced to absent, capsules split open at the top, rarely valves slightly opening outwards after wetting, apex of capsule rounded, rarely depressed centrally.

Species (6). – *Aizoon africanum* (L.) Klak, *A. ecklonis* (Walp.) Klak, *A. hispidissimum* (Fenzl ex Sond.) Klak, *A. namaense* (Schinz) Klak, *A. neorigidum* Klak, *A. procumbens* (L.f.) Klak.

Aizoon subg. *Karooica* Klak, **subg. nov.** – Type: *Aizoon karooicum* Compton.

Woody, erect perennials. Leaves alternate, covered thinly with short appressed white hairs. Flowers solitary, sessile, tepals 5, 4–5 mm long, stamens about 20 in clusters. Fruit woody, flattened, surrounded by the perianth, locules 5, valves opening slightly outwards after wetting.

Species (1). – *Aizoon karooicum* Compton.

Aizoon subg. *Kolleria* (C.Presl) Klak, **comb. nov.** ≡ *Kolleria* Presl, Symb. Bot. 1: 23. 1831 ≡ *Galenia* subg. *Kolleria* (C.Presl) Fenzl in Ann. Wiener Mus. Naturgesch. 2: 288. 1839 – Type: *Kolleria herniariifolia* C.Presl (≡ *Galenia herniariifolia* (C.Presl) Walp).

Woody or herbaceous, prostrate or diffuse annuals or perennials. Leaves usually secund, opposite or alternate, mostly obovate or spatulate, very often gray or white. Flowers in secund cymes, small, tepals 4–5, 1–2.5(–4) mm long, white, yellow or pink inside, stamens 8–10. Fruit leathery, dehiscent, with 2–5 locules, expanding tissue much reduced to absent, capsules split open at the top, rarely valves slightly opening outwards after wetting, apex of capsule rounded, rarely depressed centrally.

Species (24). – *Aizoon acutifolium* (Adamson) Klak, *A. affine* (Sond.) Klak, *A. collinum* (Eckl. & Zeyh.) Klak, *A. crystallinum* Eckl. & Zeyh., *A. cymosum* (Adamson) Klak, *A. dregeanum* (Fenzl ex Sond.) Sond., *A. ecklonis* (Walp.) Klak, *A. exiguum* (Adamson) Klak, *A. filiforme* (Thunb.) Klak, *A. fruticosum* L.f., *A. glanduliferum* (Bittrich) Klak,

A. hemisphaericum (Adamson) Klak, *A. herniariifolium* (C.Presl) Klak, *A. mezianum* (K.Müll.) Klak, *A. pallens* (Eckl. & Zeyh.) Klak, *A. papulosum* Eckl. & Zeyh., *A. portulacaceum* (Fenzl ex Sond.) Klak, *A. prostratum* (G.Schellenb.) Klak, *A. pruinosum* (Sond.) Klak, *A. pubescens* Eckl. & Zeyh., *A. sarcophyllum* (Fenzl ex Sond.) Klak, *A. secundum* L.f., *A. squamulosum* Eckl. & Zeyh., *A. subcarnosum* (Adamson) Klak.

Aizoon subg. ***Namibiana*** Klak, **subg. nov.** – Type: *Aizoon virgatum* Welw. ex Oliv.

Woody, prostrate or erect perennials. Leaves opposite or alternate, densely covered by appressed white hairs. Flowers sessile, solitary or in groups of 2–3, tepals 4–5, small (1.75–2.3(–3) mm long) or large (3–8 mm long), bright to pale yellow, stamens as many as tepals or more. Fruit woody, rounded at the top or centrally depressed, locules 2–5, valves opening at mostly slightly outwards after wetting.

Species (7). – *Aizoon asbestinum* Schltr., *A. burchellii* N.E. Br., *A. cryptocarpum* (Fenzl) Klak, *A. giessii* Friedrich, *A. laxifolium* (Pax) Klak, *A. schellenbergii* Adamson, *A. virgatum* Welw. ex Oliv.

2.2 ***Aizoanthemopsis*** Klak, **gen. nov.** – Type: *Aizoanthemopsis hispanicum* (L.) Klak (≡ *Aizoon hispanicum* L.).

Diagnosis. – Differs from *Aizoon* by the long expanding keels, which are reduced to absent in *Aizoon*; from *Aizoanthemum* by having 5-angled capsules and tepals coloured white inside, whereas capsules are globose and tepals are yellowish inside in *Aizoanthemum*.

Annual, decumbent to erect succulent herb to 25 cm high, consisting of a highly branched terminal cyme and 2–3 basal additional cymes, internodes covered with dense, rounded papillae. Leaves flat, lanceolate, apex rounded, with a prominent middle vein, sessile, opposite and alternate within the same plant, exstipulate, margins entire, epidermis with glistening, low, rounded bladder-cells, 5–40 × 1–9 mm. Flowers in many-flowered cymes, ± sessile, tepals green and papillate outside, white inside, prominently keeled, 4–15 mm long, stamens numerous, arranged in five groups, ovary semi-inferior, 5-ribbed. Fruit a hygrochastic 5-locular capsule, valves opening completely by parallel, long expanding keels reaching from the centre of the fruit to the tip of the valve, 5–6 mm diam. Seeds black, 0.75–1 mm long, with concentric ridges. Chromosome number $2n = 32$ (Bittrich, 1986; Peruzzi & Cesca, 2004), $n = 9$ (Malallah & al., 2001).

Ecology. – In sandy, often saline soils in Mediterranean climates.

Distribution. – Algeria, Armenia, Egypt, Iran, Iraq, Israel, Italy, Libya, Morocco, Portugal (Madeira), Spain (Gran Canaria and the mainland), Syria, Tunisia.

Species (1). – *Aizoanthemopsis hispanicum* (L.) Klak.

Aizoanthemopsis hispanicum (L.) Klak, **comb. nov.** ≡ *Aizoon hispanicum* L., Sp. Pl.: 488. 1753.

2.3 ***Tetragonia*** L., Sp. Pl.: 480. 1753 – Type (designated by Britton, Fl. Bermuda: 125. 1918): *Tetragonia fruticosa* L.

Annual to perennial shrublets or herbs, with herbaceous or woody branches, sometimes geophytes, stems mostly covered by papillae to different degrees. Leaves usually petiolate, rarely sessile, flat, entire, alternate, exstipulate, slightly fleshy, epidermis with papillae of various shapes. Inflorescence often spike-like. Flowers bisexual or unisexual and the plants monoecious or dioecious, axillary or terminal, pedicellate or sessile, in cymose groups (rarely solitary), cymes often with a peduncle from which 1 to more pedicels arise as fascicles, [peduncle and pedicel also named "stipe" for the South American species, when it adheres to the fruit after ripening], tepals 3–5, ovary inferior or bulging above the insertion of the tepals, (1–)3–9 chambered, styles free and equal in number to chambers in the ovary, rarely more numerous, each chamber with one pendulous ovule, stamens mostly twice the number of tepals. Fruit dry, a woody nut, either winged or variously sculptured or rarely fleshy.

Ecology. – In sandy or rocky, dry areas.

Distribution. – Southern Africa, arid Chile and Peru, Australasia. One species, *T. tetragonoides*, is found worldwide in tropical and temperate climates.

Key to the subgenera of *Tetragonia*

1. Fruit succulent .... **T.** subg. ***Tetragonella***
1. Fruit dry 2
2. Fruit not winged or with narrow longitudinal wings, often stipitate, sharply (3–)4-angled, angles rarely rounded ... . **T.** subg. ***Americanae***
2. Fruit with broad wings or wingless with either ridges or spines, not stipitate and not sharply angled 3
3. Fruit either horned or with woody wings or wings membranous and extending above the fruit .... **T.** subg. ***Tetragonoides***
3. Fruit not horned, with broad membranous wings not overtopping the fruit or wingless with ridges, papillae or rigid spines .... **T.** subg. ***Tetragonia***

Tetragonia subg. ***Tetragonia***
= *Tetragonia* subg. *Tetragonocarpus* Rchb., Consp. Regn. Veg.: 166. 1828 – Type: *Tetragonia fruticosa* L.

Annuals to perennials with herbaceous or woody branches or geophytes. Leaves alternate, petiolate or sessile, epidermis papillate. Flowers bisexual, male and transitional on the same plant, axillary or terminal, solitary or more often in cymose groups, pedicellate or more rarely sessile, tepals usually 4, yellow or rarely reddish inside. Fruits dry, mostly with broad, membranous wings or wingless with ridges or papillae, rarely with rigid spines.

Ecology. – Many species in sand, but also found in rocky habitats.

Distribution. – Southern Africa. *Tetragonia decumbens* introduced to Australia and New Zealand, *T. acanthocarpa* introduced to East Africa.

Species (34). – *Tetragonia acanthocarpa* Adamson, *T. arbuscula* Fenzl ex Harv. & Sond., *T. arbusculoides* Engl., *T. caesia* Adamson, *T. calycina* Fenzl, *T. chenopodioides* Eckl. & Zeyh., *T. decumbens* Mill., *T. distorta* Fenzl, *T. echinata* Ait., *T. erecta* Adamson, *T. fruticosa* L., *T. galenioides* Fenzl,

T. glauca Fenzl, *T. halimoides* Fenzl, *T. haworthii* Fenzl, *T. herbacea* L., *T. hirsuta* L.f., *T. lasiantha* Adamson, *T. macrocarpa* Phil., *T. microptera* Fenzl, *T. namaquensis* Schltr., *T. nigrescens* Eckl. & Zeyh., *T. pillansii* Adamson, *T. portulacoides* Fenzl, *T. rangeana* Engl., *T. reduplicata* Welw. ex Oliv., *T. robusta* Fenzl, *T. rosea* Schltr., *T. saligna* Fenzl, *T. sarcophylla* Fenzl, *T. sphaerocarpa* Adamson, *T. spicata* L.f., *T. verrucosa* Fenzl, *T. virgata* Schltr.

Tetragonia subg. **Tetragonella** (Miq.) Klak, **comb. & stat. nov.**
≡ *Tetragonella* Miq. in Lehmann, Pl. Preiss. 1: 245. 1845 – Type: *Tetragonia implexicoma* (Miq.) Hook.f.
Prostrate, scrambling or clambering herb, branches densely papillate. Leaves alternate, clustered or scattered, petiolate, epidermis rough, later glabrescent. Flowers bisexual, solitary or in pairs, pedicels 5–30 mm long, tepals 4, yellow inside. Fruit succulent, red, globular.
Ecology. – On limestone and sand dunes.
Distribution. – Southern coast of Australia and New Zealand.
Species (1). – *Tetragonia implexicoma* (Miq.) Hook.f.

Tetragonia subg. **Tetragonoides** DC., Prodr. 3: 452. 1828 – Type: *Tetragonia tetragonoides* (Pall.) Kuntze.
Prostrate to semi-erect mostly annual or perennial herbs. Leaves alternate, petiolate, epidermis papillate. Flowers bisexual, solitary or occasionally paired, sessile or very shortly pedicellate, tepals 4–5, yellow or green inside. Fruit dry, horned or with broad woody wings or wings membranous and extending above the fruit.
Ecology. – Often near the sea and also inland, one species also in dry central interior, in red sand or loam or rocky habitats.
Distribution. – All species are endemic to Australasia, except for *T. tetragonoides* found worldwide in tropical to mild climates.
Species (5). – *Tetragonia coronata* Rye & Trudgen, *T. cristata* C.Gardner ex A.M.Prescott, *T. diptera* F.Muell., *T. eremaea* Ostenf., *T. tetragonoides* (Pall.) Kuntze (= *T. borealis* Batt. & Trab. – Type: Morocco, Casablanca, 2 Nov 1917, *L. Ducellier s.n.* (MPU barcode MPU006541)).
Note. – We followed Maire (1962: 288) in treating *T. borealis* Batt. & Trab. from Morocco as a synonym of *T. tetragonoides*. This contrasts with Hartmann (2001: 318) who re-instated *T. borealis* because of "the greyish-green colour of the leaves due to papillae". However, the papillae on leaves of the type of *T. borealis* are very similar to those found in other collections of *T. tetragonoides* and this collection also agrees in all other critical features with *T. tetragonoides*.
Hartmann (2001) had not seen the type of *T. borealis* and tentatively indicated that it might be at P. A specimen in "Herbier Battandier" (now at MPU), collected by L. Ducellier in 1917 in Casablanca, Morocco, determined by Battandier and labelled "Type" is understood to be the type.

Tetragonia subg. **Americanae** Klak, **subg. nov.** – Type: *T. maritima* Barnéoud.

Monoecious or dioecious, erect to weak annual or perennial herbs or low shrubs. Leaves alternate or sometimes the basal ones opposite, sessile to shortly petiolate. Flowers axillary, solitary to fascicled or cymose, subsessile to stipitate or pedunculate, tepals 4–5, yellow or yellow-green inside. Fruit dry, (3–)4-angled, angles sharp or rounded, sometimes with narrow longitudinal wings.
Ecology. – In sandy or rocky substrates with very little soil.
Distribution. – Arid south-western part of South America along the Pacific coast, from Peru to Chile.
Species (10): *Tetragonia angustifolia* Barnéoud, *T. crystallina* L'Hér., *T. copiapina* Phil., *T. espinosae* Muñoz, *T. macrocarpa* Phil., *T. maritima* Barnéoud, *T. microcarpa* Phil., *T. ovata* Phil., *T. pedunculata* Phil., *T. vestita* I.M. Johnst.

3. **Sesuvioideae** Lindl., Veg. Kingd.: 527. 1846 ≡ Sesuviaceae Horan., Prim. Lin. Syst. Nat.: 83. 1834 – Type: *Sesuvium* L.
Prostrate to erect herbaceous annuals or perennials or woody shrubs. Leaves alternate or opposite, flat to terete, with or without stipules, epidermis papillate, bladder cells rounded or hairy or much reduced and appearing glabrous. Inflorescences distinct, terminal, connected sympodially, bracteate, usually bracts small and membranous. Flowers solitary or in clusters, epigynous or perigynous, perianth petaloid inside, sepaloid outside, tepals 5, free or fused into a tube, with a subapical dorsal mucro or rarely obtuse, styles 1–5, ovules 1 to many, pendulous, locules 1–5. Fruit a circumscissile capsule or if woody, either winged or compound and spiny. Seed enclosed by an aril or aril absent.

Key to the tribes of Sesuvioideae

1. Tepals fused to about ½ of their length into a cylindrical tube, fruit a woody nut with 3–5 broad, papery wings or compound, woody, subglobose, spiny, xerochastic, with 1 or 2 locules each with 1 ovule, aril absent ……………… ………………………………………… **Anisostigmateae**
1. Tepals fused to about ⅓ of their length, but never forming a tube; capsule xerochastic, circumscissile, with 1–5 locules each with 2 to many ovules, aril present …… **Sesuvieae**

Sesuvieae Fenzl in Ann. Wiener Mus. Naturgesch. 2: 289. 1839 – Type: *Sesuvium* L.
Prostrate to erect herbaceous annuals or perennials. Leaves flat to terete, slightly unequal, with or without stipules, often with membranous sheaths at the base of the petiole, epidermis with raised bladder cells, hairy or smooth. Inflorescence terminal but apparently axillary through sympodial branching. Flowers bisexual, solitary or in clusters, rarely in loose dichasia, tepals not fused, with subapical appendages with unifacial tips, rarely without, perigynous, 1–5 styles, ovules 2 to many per locule, pendulous. Fruit a circumscissile capsule with 1–5 locules. Seed completely enveloped by an aril. Base chromosome number $x = 8$.
Distribution and ecology. – Worldwide in the tropics and subtropics. The species are often found in saline soils, usually in dry places.

Genera (3). – *Sesuvium* L., *Trianthema* L., *Zaleya* Burm.f.

Note. – Bohley & al. (2015) found that the small genus *Cypselea* Turpin (incl. 3 spp.), an endemic to North and Central America as well as islands in the Atlantic off Central America, is nested within *Sesuvium*. The recent revision of *Sesuvium* therefore also included *Cypselea* in *Sesuvium* (Bohley & al., 2017).

Anisostigmateae Klak, **tr. nov.** – Type: *Anisostigma* Schinz.

Shrubs 0.5–2.0 m tall; stems stout and woody or herbaceous but woody at base; epidermis of stems, leaves, and outside of perianth densely covered by bladder-cells and appearing papillate. Leaves alternate, petiolate or subsessile, exstipulate, oblong-obovate or spatulate, obtuse or retuse, 1–4 cm long, 0.3–1.5 cm wide. Flowers bisexual, pedicellate or sessile, solitary or numerous in umbel-like groups or in cymose panicles, epigynous. Perianth with an upper deciduous part with 5 segments united for about half their lengths into a cylindrical tube and a lower part enclosing the ovary that is winged in solitary flowers and develops into the fruit, when flowers in head the lower part of the perianths, bracts and ovaries form a subglobose structure developing into a compound spiny fruit; tepals obtuse with a subapical ± distinct mucro on the back, with inner face white or cream, or white-edged. Stamens 5 or numerous, inserted at mouth of perianth-tube; filaments filiform, glabrous. Ovary of 1–2 carpels, inferior, one pendulous ovule per locule. Styles one, with two stigmatic lobes, or 4 with two as long as the perianth and apparently sterile, other two very short, fertile. Fruit a woody nut with 3–5 broad, papery wings or compound, woody, subglobose, spiny, 1–2-locular. Seeds ovate in outline, with brittle testa, without aril.

Distribution and ecology. – The tribe includes three species, with disjunct distributions. *Anisostigma schenckii* is endemic to Namibia (Fig. 10). *Tribulocarpus dimorphanthus* is a paraphyletic species with disjunct distribution in northeastern Africa and Namibia (Thulin & al., 2012). The presence of *T. dimorphanthus* in South Africa is based on a single collection by Scully (K) from Okiep near Springbok in Namaqualand (Adamson, 1955). This appears to be an error, since it has never been recollected in this area and is otherwise only known from collections in Namibia, with its most southern extension in the Rehoboth district some 700 km north of Okiep (Thulin & al., 2012). The north-east African populations of *T. dimorphanthus* are most closely related to *T. retusus*, an endemic to Somalia (Thulin & al., 2012). The species are found in open bushland, woodland or dry river beds in sandy, silty or gravelly soils.

Genera (2). – *Anisostigma* Schinz, *Tribulocarpus* S.Moore.

Key to the genera of Anisostigmateae

1. Erect woody shrubs, 0.5–2.0 m high; stamens 5, 4 styles, ovary 1-locular; fruit 3–4-winged ………. ***Anisostigma***
1. Shrubs herbaceous, woody at base, 0.5–1(–1.5) m high; stamens numerous, 1 style with 2 stigmatic lobes, ovary 2-locular, fruit 5-winged or compound, subglobose and spiny …………………………………… ***Tribulocarpus***

■ LITERATURE CITED

Alfaro, M.E. & Holder, M.T. 2006. The posterior and the prior in Bayesian phylogenetics. *Annual Rev. Ecol. Syst.* 37: 19–42. https://doi.org/10.1146/annurev.ecolsys.37.091305.110021

Adamson, R.S. 1955. The South African species of Aizoaceae. II. *Tetragonia. J. S. African Bot.* 21: 109–154.

Adamson, R.S. 1956. The South African species of Aizoaceae. III. *Galenia. J. S. African Bot.* 22: 87–127.

Adamson, R.S. 1959a. The South African species of Aizoaceae. VI. *Acrosanthes. J. S. African Bot.* 25: 23–28.

Adamson, R.S. 1959b. The South African species of Aizoaceae. VII. *Aizoon. J. S. African Bot.* 25: 29–51.

Adamson, R.S. 1961. The South African species of Aizoaceae. XI. *Plinthus. J. S. African Bot.* 27: 147–151.

Bittrich, V. 1986. Untersuchungen zu Merkmalsbestand, Gliederung und Abgrenzung der Unterfamilie Mesembryanthemoideae (Mesembryanthemaceae Fenzl). *Mitt. Inst. Allg. Bot. Hamburg* 21: 5–116.

Bittrich, V. 1990a. Systematic studies in Aizoaceae. *Mitt. Inst. Allg. Bot. Hamburg* 23b: 491–507.

Bittrich, V. 1990b. Aizoaceae: A new species of *Galenia* from the Great Karoo. *Bothalia* 20: 217–219. https://doi.org/10.4102/abc.v20i2.924

Bittrich, V. & Hartmann, H.E.K. 1988. The Aizoaceae – A new approach. *Bot. J. Linn. Soc.* 97: 239–254. https://doi.org/10.1111/j.1095-8339.1988.tb01581.x

Bohley, K., Joos, O., Hartmann, H., Sage, R., Liede-Schumann, S. & Kadereit, G. 2015. Phylogeny of Sesuvioideae (Aizoaceae) – Biogeography, leaf anatomy and the evolution of C$_4$ photosynthesis. *Perspect. Ecol. Evol. Syst.* 17: 116–130. https://doi.org/10.1016/j.ppees.2014.12.003

Bohley, K., Winter, P.J.D. & Kadereit, G. 2017. A revision of *Sesuvium* (Aizoaceae, Sesuvioideae). *Syst. Bot.* 42: 124–147. https://doi.org/10.1600/036364417X694575

Chinnock, R. 1983. The Australian genus *Gunniopsis* Pax (Aizoaceae). *J. Adelaide Bot. Gard.* 6: 133–179.

Dodsworth, S. 2015. Genome skimming for next-generation biodiversity analysis. *Trends Pl. Sci.* 20: 525–527. https://doi.org/10.1016/j.tplants.2015.06.012

Ecklon, C.F. & Zeyher, C. 1837. *Enumeratio plantarum Africae australis extratrioucae*, fasc. 3. Hamburgi [Hamburg]: sumtibus auctorum. https://doi.org/10.5962/bhl.title.48

Friedrich, H.-C. 1957. *Aizoanthemum* Dinter ex Friedr., eine wenig beachtete Gattung der Ficoideae aus Südwestafrika. *Mitt. Bot. Staatssamml. München* 2: 339–347.

Friedrich, H.-C. 1970. 27. Aizoaceae. Pp. 1–135 in: Merxmüller, H. (ed.), *Prodromus einer Flora von Südwestafrika*, Lfg. 31. Lehre: Cramer.

Hartmann, H.E.K. 2001. *Illustrated handbook of succulent plants*, 2 vols. Berlin: Springer.

Hassan, N.S., Thiede, J. & Liede-Schumann, S. 2005. Phylogenetic analysis of Sesuvioideae (Aizoaceae) inferred from nrDNA internal transcribed spacer (ITS) sequences and morphological data. *Pl. Syst. Evol.* 255: 121–143. https://doi.org/10.1007/s00606-005-0357-x

Hernández-Ledesma, P., Berendsohn, W.G., Borsch, T., Mering, S. von, Akhani, H., Arias, S., Castañeda-Noa, I., Eggli, U., Eriksson, R., Flores-Olvera, H., Fuentes-Bazán, S., Kadereit, G., Klak, C., Korotkova, N., Nyffeler, R., Ocampo, G., Ochoterena, H., Oxelman, B., Rabeler, R.K., Sanchez, A., Schlumpberger, B.O. & Uotila, P. 2015. A taxonomic backbone for the global synthesis of species diversity in the angiosperm order Caryophyllales. *Willdenowia* 45: 281–383. https://doi.org/10.3372/wi.45.45301

Hofmann, U. 1973. Morphologische Untersuchungen zur Umgrenzung und Gliederung der Aizoaceen. *Bot. Jahrb. Syst.* 93: 247–324.

Huelsenbeck, J.P. & Rannala, B. 2004. Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. *Syst. Biol.* 53: 904–913. https://doi.org/10.1080/10635150490522629

Ihlenfeldt, H.-D. & Hartmann, H.E.K. 1982. Leaf surfaces in Mesembryanthemaceae. Pp. 397–423 in: Cutler, D.F., Alvin, F.L. & Price, C.E. (eds.), *The plant cuticle*. London: Academic Press.

Jordan, W.C., Courtney, M.W. & Neigel, J.E. 1996. Low levels of intraspecific genetic variation at a rapidly evolving chloroplast DNA locus in North American duckweeds (Lemnaceae). *Amer. J. Bot.* 83: 430–439. https://doi.org/10.2307/2446212

Kelchner, S.A. & Clark, L.G. 1997. Molecular evolution and phylogenetic utility of the chloroplast *rpl16* intron in *Chusquea* and the Bambusoideae (Poaceae). *Molec. Phylogen. Evol.* 8: 385–397. https://doi.org/10.1006/mpev.1997.0432

Klak, C. & Bruyns, P.V. 2012. Phylogeny of the Dorotheantheae (Aizoaceae), a tribe of succulent annuals. *Taxon* 61: 293–307.

Klak, C. & Bruyns, P.V. 2013. A new infrageneric classification for *Mesembryanthemum* L. (Aizoaceae: Mesembryanthemoideae). *Bothalia* 43: 197–206. https://doi.org/10.4102/abc.v43i2.95

Klak, C., Hedderson, T. & Linder, H.P. 2003a. A molecular systematic study of the *Lampranthus* Group (Aizoaceae) based on the chloroplast *trnL-trnF* and nuclear ITS and *5S* NTS sequence data. *Syst. Bot.* 28: 70–85.

Klak, C., Khunou, A., Reeves, G. & Hedderson, T.A.J. 2003b. A phylogenetic hypothesis for the Aizoaceae (Caryophyllales) based on four plastid DNA regions. *Amer. J. Bot.* 90: 1433–1445. https://doi.org/10.3732/ajb.90.10.1433

Klak, C., Reeves, G. & Hedderson, T. 2004. Unmatched tempo of evolution in southern African semi-desert ice plants. *Nature* 427: 63–65. https://doi.org/10.1038/nature02243

Klak, C., Bruyns, P.V. & Hedderson, T.A.J. 2007. A phylogeny and new classification for Mesembryanthemoideae (Aizoaceae). *Taxon* 56: 737–756. https://doi.org/10.2307/25065858

Klak, C., Bruyns, P.V. & Hanáček, P. 2013. A phylogenetic hypothesis for the recently diversified Ruschieae (Aizoaceae) in southern Africa. *Molec. Phylogen. Evol.* 69: 1005–1020. https://doi.org/10.1016/j.ympev.2013.05.030

Klak, C., Hanáček, P. & Bruyns, P.V. 2015. A phylogeny and revised classification for the Apatesieae (Aizoaceae: Ruschioideae) with a comparison of centres of diversity. *Taxon* 64: 507–522. https://doi.org/10.12705/643.7

Klak, C., Hanáček, P. & Bruyns, P.V. 2017. Out of southern Africa: Origin, biogeography and age of the Aizooideae (Aizoaceae). *Molec. Phylogen. Evol.* 109: 203–216. https://doi.org/10.1016/j.ympev.2016.12.016

Leistner, O.A. & Morris, J.W. 1976. Southern African place names. *Ann. Cape Prov. Mus., Nat. Hist.* 12: 1–565.

Maddison, W.P. & Maddison, D.R. 2009. Mesquite: A modular system for evolutionary analysis, version 3.1. http://mesquiteproject.org

Maire, R. 1962. *Flore de l'Afrique du Nord*, vol. 8. Paris: Lechevalier.

Malallah, G.A., Masood, M. & Al-Dosari, M. 2001. Chromosome numbers of the Kuwaiti flora, III. *Willdenowia* 31: 411–418. https://doi.org/10.3372/wi.31.31208

McCarthy, C. 1996–1997. Chromas, version 1.43. School of Biomolecular and Biomedical Science, Brisbane, Australia.

Miller, M.A., Pfeiffer, W. & Schwartz, T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pp. 45–52 in: *Proceedings of the Gateway Computing Environments Workshop (GCE)*, New Orleans, Louisiana, 14 Nov 2010. Piscataway: IEEE. https://doi.org/10.1109/GCE.2010.5676129

Moore, S. 1921. Alabastra diversa 34. 1. Plantae Rogersianae – VI. *J. Bot.* 59: 226–232.

Müller, K. 1908. Beiträge zur Systematik der Aizoaceen. *Beibl. Bot. Jahrb. Syst.* 97: 54–98.

Nicholas, K.B. & Nicholas, H.B., Jr. 1997. GeneDoc, version 2.6.002. A tool for editing and annotating multiple sequence alignments. http://www.nrbsc.org/gfx/genedoc/index.html

Oxelman, B., Lidén, M. & Berglund, D. 1997. Chloroplast *rps16* intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Pl. Syst. Evol.* 206: 393–410. https://doi.org/10.1007/BF00987959

Pax, F. 1889. Aizoaceae (Ficoideae). Pp. 10–13 in: Engler, A., Plantae Marlothianae: Ein Beitrag zur Kenntnis der Flora Südafrikas: I. Teil: Monokotyledoneae und Dikotyledoneae archichlamydeae. *Bot. Jahrb. Syst.* 10: 1–50.

Peruzzi, L. & Cesca, G. 2004. Chromosome numbers of flowering plants from Calabria, S Italy, II. *Willdenowia* 34: 353–360. https://doi.org/10.3372/wi.34.34203

Popp, M. & Oxelman, B. 2001. Inferring the history of the polyploid *Silene aegaea* (Caryophyllaceae) using plastid and homoeologous nuclear DNA sequences. *Molec. Phylogen. Evol.* 20: 474–481. https://doi.org/10.1006/mpev.2001.0977

Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Schinz, H. 1894. Ficoideae. Pp. 204–205 in: Schinz, H. (ed.), Beiträge zur Kenntnis der afrikanischen Flora (Neue Folge). *Bull. Herb. Boissier* 2: 180–228.

Schinz, H. 1897. Die Pflanzenwelt Deutsch-Südwest-Afrikas (mit Einschluß der westlichen Calahari). *Bull. Herb. Boissier* 5(App. 3): 59–101.

Shaw, J., Lickey, E.B., Schilling, E.E. & Small, R.L. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. *Amer. J. Bot.* 94: 275–288. https://doi.org/10.3732/ajb.94.3.275

Sonder, O.W. 1862 ("1861–1862"). Mesembryaceae. Pp. 386–479 in: Harvey, W.H. & Sonder O.W., *Flora Capensis*, vol. 2 Dublin: Hodges, Smith, and Co.; Capetown: A. S. Robertson. https://doi.org/10.5962/bhl.title.821

Stamatakis, A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analysis with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1109. https://doi.org/10.1007/BF00037152

Taylor, C.M. 1994. Revision of *Tetragonia* (Aizoaceae) in South America. *Syst. Bot.* 19: 575–589. https://doi.org/10.2307/2419778

Thiede, J. 2004. Phylogeny, systematics and classification of the Aizoaceae: A reconsideration based on molecular data. *Schumannia* 4: 51–58.

Thulin, M. 1993. Notes on *Tetragonia* (Aizoaceae-Tetragonioideae) in Somalia. *Nordic J. Bot.* 13: 165–167. https://doi.org/10.1111/j.1756-1051.1993.tb00029.x

Thulin, M., Thiede, J. & Liede-Schumann, S. 2012. Phylogeny and taxonomy of *Tribulocarpus* (Aizoaceae): A paraphyletic species and an adaptive shift from zoochorous trample burrs to anemochorous nuts. *Taxon* 61: 55–66.

Valente, L.M., Britton, A.W., Powell, M.P., Papadopulos, A.S.T., Burgoyne, P.M. & Savolainen, V. 2014. Correlates of hyperdiversity in southern African ice plants (Aizoaceae). *Bot. J. Linn. Soc.* 174: 110–129. https://doi.org/10.1111/boj.12117

Appendix 1. Taxa with voucher information, herbarium where voucher is lodged, GenBank accession numbers for *trnL-F*, *rps16*, *rpl16*, *matK* and ITS. New sequences are indicated with an asterix. See Electr. Suppl.: Table S1 for a tabular version of this Appendix, with additional information on perianth length. For origin of newly added and sequenced specimens, see Materials and Methods.

OUTGROUPS: Nyctaginaceae: *Bougainvillea spectabilis* Willd., *Klak 987* (BOL), AJ558036, AJ532732, –, JN114741, KJ161170; *Mirabilis jalapa* L., *Klak 986* (BOL), AJ558035, AJ532731, –, JQ412267, EF079461; **Phytolaccaceae:** *Phytolacca dioica* L., *Klak 988* (BOL), AJ558037, AJ532733, –, AY042631, KY657356*; — **AIZOACEAE: AIZOOIDEAE:** *Acrosanthes anceps* (Thunb.) Sond., *I. Jardine 2260* (BOL), KX197542, KX197800, KX197716, KX197631, KY657364*; *Acrosanthes angustifolia* Eckl. & Zeyh., *M.N. Britton 180* (BOL), KX197543, KX197801, KX197717, KX197632, KY657365*; *Acrosanthes humifusa* (Thunb.) Sond., *C.H. Stirton 14165* (BOL), KX197544, KX197802, KX197718, KX197633, KY657366*; *Acrosanthes microphylla* Adamson, *Jardine 2341* (BOL), KX197545, KX197803, KX197719, KX197634, –; *Acrosanthes parviflora* J.C.Manning & Goldblatt, *C.H. Stirton 14133* (BOL), KX197546, KX197804, KX197720, KX197635, KY657367*; *Acrosanthes teretifolia* Eckl. & Zeyh. 1, *Klak 2458* (BOL), KX197547, KX197805, KX197721, KX197636, KY657368*; *Acrosanthes teretifolia* 2, *Klak 2464* (BOL), KX197548, KX197806, KX197722, KX197637, KY657369*; *Acrosanthes teretifolia* 3, *Klak 2469* (BOL), KX197549, KX197807, KX197723, KX197638, KY657370*; *Aizoanthemum dinteri* (Schinz) Friedrich, *Hachfeld s.n.* (HBG), –, –, –, –, AJ577768; *Aizoanthemum galenioides* (Fenzl ex Sond.) Friedrich 1, *Mannheimer 1728* (WIND), AJ558041, AJ532737, KX197724, –, KY657371*; *Aizoanthemum galenioides* 2, *Klak 2436* (BOL), KX197550, KX197808, KX197725, KX197639, KY657372*; *Aizoanthemum hispanicum* (L.) H.E.K.Hartmann 1, *A. Juan & M. Juan AJ104-2* (SALA), KX422628, KX422613, KX422606, KX422621, KY657373*; *Aizoanthemum hispanicum* 2, *Gutiérrez-Larruscain & E. Rico DG704-1* (SALA), KX422629, KX422614, KX422607, KX422622, KY657374*; *Aizoanthemum hispanicum* 3, *E. Rico & al. MS-511* (SALA [123319]), KX422630, KX422615, KX422608, –, KY657375*; *Aizoanthemum hispanicum* 4, *R.Hand 2912* (B [B 10 0163219]), KX422631, KX422616, KX422609, KX422623, –; *Aizoanthemum rehmannii* (Schinz) H.E.K.Hartmann, *Erb s.n.* (BOL), –, KX197811, KX197727, KX197640, –; *Aizoon asbestinum* Schltr., *Klak 2510* (BOL), KX197551, KX197812, KX197728, KX197641, KY657376*; *Aizoon burchellii* N.E.Br., *Mannheimer 4918* (WIND), KX197552, KX197813, KX197729, KX197642, KY657377*; *Aizoon canariense* L. 1, *Klak 1793* (BOL), KX197553, KX197814, KX197730, –, KY657378*; *Aizoon canariense* 2, *Barnick 1* (HBG), HE585091, HE585060, –, –, AJ577767; *Aizoon giessii* Friedrich, *Mannheimer 4921* (WIND), KX197554, KX197815, KX197731, KX197643, KY657379*; *Aizoon glinoides* L.f. 1, *Dold 15008* (BOL), KX197555, KX197816, KX197732, KX197644, KY657380*; *Aizoon glinoides* 2, *Dold 15009* (BOL), KX197556, KX197817, KX197733, KX197645, KY657381*; *Aizoon glinoides* 3, *Dold 15004* (BOL), KX197557, KX197818, KX197734, KX197646, KY657382*; *Aizoon karooicum* Compton 1, *Klak 1971* (BOL), KX197558, KX197819, KX197735, KX197647, KY657383*; *Aizoon karooicum* 2, *Klak 2454* (BOL), KX197559, KX197820, KX197736, KX197648, –; *Aizoon paniculatum* L. 1, *Bruyns 13188* (BOL), KY115204*, KY115207*, KY115210*, KY115213*, KY657384*; *Aizoon paniculatum* 2, *Manning s.n.* (BOL), KY115205*, KY115208*, KY115211*, KY115214*, –; *Aizoon rigidum* L.f., *Bruyns 12989* (BOL), KX197560, KX197821, KX197737, KX197649, KY657385*; *Aizoon sarmentosum* L.f. 1, *Klak 2448* (BOL), KX197561, KX197822, KX197738, KX197650, KY657386*; *Aizoon sarmentosum* 2, *Klak 2466* (BOL), KX197562, KX197823, KX197739, KX197651, KY657387*; *Aizoon sarmentosum* 3, *Bruyns 13003* (BOL), KX197563, KX197824, KX197740, KX197652, KY657388*; *Aizoon schellenbergii* Adamson, *Klak 2509* (BOL), KX197564, KX197825, KX197741, KX197653, KY657389*; *Aizoon virgatum* Welw. ex Oliv., *Mannheimer 4913* (WIND), KX197565, KX197826, KX197742, KX197654, KY657390*; *Galenia africana* L. 1, *Klak 713* (BOL), AJ558043, AJ532740, KX197743, KX197655, –; *Galenia africana* 2, *Bruyns 13051* (BOL), KX197566, KX197827, KX197744, KX197656, KY657391*; *Galenia cf portulacacea* Fenzl ex Sond., *A.B. Low 14201* (NBG [0277784-0*]), KX197567, KX197828, –, KX197657, KY657392*; *Galenia cymosa* Adamson, *Klak 1969* (BOL), KX197568, KX197829, KX197745, KX197658, KY657393*; *Galenia dregeana* Fenzl ex Sond., *Klak 2012* (BOL), KX197569, KX197830, KX197746, KX197659, KY657394*; *Galenia filiformis* (Thunb.) N.E.Br., *Bruyns 13054* (BOL), KX197570, KX197831, KX197747, KX197660, KY657395*; *Galenia fruticosa* (L.f.) Sond., *Klak 2432* (BOL), KX197571, KX197832, KX197748, KX197661, KY657396*; *Galenia glandulifera* Bittrich 1, *Klak 2490* (BOL), KX197572, KX197833, KX197749, KX197662, KY657397*; *Galenia glandulifera* 2, *Bruyns 12955* (BOL), KX197573, KX197834, KX197750, KX197663, KY657398*; *Galenia glandulifera* 3, *Klak 2506* (BOL), KX197574, KX197835, KX197751, KX197664, KY657399*; *Galenia namaensis* Schinz, *Klak 2437* (BOL), KX197575, KX197836, KX197752, KX197665, KY657400*; *Galenia papulosa* (Eckl. & Zeyh.) Sond., *Klak 2439* (BOL), KX197576, KX197837, KX197753, KX197666, KY657401*; *Galenia procumbens* L.f. 1, *Klak 2485* (BOL), KX197577, KX197838, KX197754, KX197667, KY657402*; *Galenia procumbens* 2, *Klak 2504* (BOL), KX197578, KX197839, KX197755, KX197668, KY657403*; *Galenia prostrata* G.Schellenb., *Klak 2505* (BOL), KX197579, KX197840, KX197756, KX197669, KY657404*; *Galenia pubescens* (Eckl. & Zeyh.) Druce 1, *Klak 2446* (BOL), KX197580, KX197841, KX197757, KX197670, KY657405*; *Galenia pubescens* 2, *Bruyns 12978* (BOL), KX197581, KX197842, KX197758, KX197671, KY657406*; *Galenia sarcophylla* Fenzl ex Sond., *Klak 2428* (BOL), KX197582, KX197843, KX197759, KX197672, KY657407*; *Galenia secunda* Fenzl ex Sond. 1, *Klak 2447* (BOL), KX197583, KX197844, KX197760, KX197673, KY657408*; *Galenia secunda* 2, *Klak 2488* (BOL), KX197584, KX197845, KX197761, KX197674, KY657409*; *Galenia sp.*, *Bruyns 10590* (BOL), KX197585, KX197846, KX197762, –, KY657410*; *Gunniopsis intermedia* Diels, *R.J.C. Chinnock s.n.* (BOL), AJ558045, AJ532741, KX197763, KX197675, –; *Gunniopsis quadrifida* (F.Muell.) Pax, *R.J.C. Chinnock 5506* (HBG), –, –, –, –, AJ582914; *Gunniopsis tenuifolia* Chinnock, *R.J.C. Chinnock 9532* (BOL), AJ558046, AJ532742, KX197764, KX197676, KY657411*; *Plinthus cryptocarpus* Fenzl 1, *A.A. Gubb 2108* (KMG), KX197586, KX197847, –, KX197677, KY657412*; *Plinthus cryptocarpus* 2, *Klak 2522* (BOL), KX422632, KX422617, KX422610, KX422624, KY657413*; *Plinthus karooicus* I.Verd. 1, *T. Anderson & Van Heerden s.n.* (BOL), KX197587, KX197848, KX197765, KX197678, KY657414*; *Plinthus karooicus* 2, *P. Bester 6131* (NBG [0210479-0*]), KX197588, KX197849, KX197766, KX197679, KY657415*; *Plinthus sericeus* Pax 1, *Glen 1447* (BOL), KX197589, KX197850, KX197767, KX197680, KY657416*; *Plinthus sericeus* 2, *Klak 2527* (BOL), KX422633, KX422618, KX422611, KX422625, KY657417*; *Plinthus sericeus* 3, *Smook 10476* (KMG), –, –, –, –, KY657418*; *Tetragonia acanthocarpa* Adamson, *Thulin & al. 3427* (UPS), KX197590, KX197851, KX197768, KX197681, KY657419*; *Tetragonia angustifolia* Barnéoud, *C.M. Taylor & A. Pool 11621* (MO), KX197591, KX197852, KX197769, KX197682, KY657420*; *Tetragonia arbuscula* Fenzl, *Klak 2486* (BOL), KX197592, KX197853, KX197770, KX197683, KY657421*; *Tetragonia coronata* Rye & Trudgen, *Mitchell 7615* (PERTH), KY657452*, KY657354*, –, –, KY657422*; *Tetragonia cristata* Prescott 1, *R. Davies 12553* (BOL), KX197593, –, –, –, KY657423*; *Tetragonia cristata* 2, *Byrne 361* (PERTH), KY657453*, KY657355*, –, –, KY657424*; *Tetragonia decumbens* Miller 1, *M. Hislop 4425* (BOL), KX197594, KX197854, KX197771, KX197684, KY657425*; *Tetragonia decumbens* 2, *Dold 15005* (BOL), KX197595, KX197855, KX197772, KX197685, –; *Tetragonia decumbens* 3, *Klak 2445* (BOL), KX197596, KX197856, KX197773, KX197686, KY657426*; *Tetragonia diptera* F.Muell., *Brummitt & al. 21277* (BOL), KX197597, KX197857, KX197774, KX197687, KY657427*; *Tetragonia echinata* Aiton 1, *Klak 2455* (BOL), KX197598, KX197858, KX197775, KX197688, –; *Tetragonia echinata* 2, *Klak 2460a* (BOL), KX197599, KX197859, KX197776, KX197689, –; *Tetragonia echinata* 3, *Bruyns 12983* (BOL), KX197600, KX197860, KX197777, KX197690, KY657428*; *Tetragonia eremaea* Ostenf., *Gibson & Langley 5328* (BOL), KX197601, KX197861, KX197778, KX197691, KY657429*; *Tetragonia fruticosa* L., *Klak 2451* (BOL), KX197602, KX197862, KX197779, KX197692, KY657430*; *Tetragonia herbacea* L., *Klak 2467* (BOL), KX197603, KX197863, KX197780, KX197693, KY657431*; *Tetragonia hirsuta* L.f., *Bruyns 13187* (BOL), KY115206*, KY115209*, KY115212*, KY115215*, KY657432*; *Tetragonia implexicoma* (Miq.) Hook.f. 1, *R. Davis 12538* (BOL), KX197605, KX197865, KX197782, KX197695, KY657433*; *Tetragonia implexicoma* 2, *M. Buys 1588* (NZFRI), KX197604, KX197864, KX197781, KX197694, KY657434*; *Tetragonia implexicoma* 3, *R.J.C. Chinnock 10513* (BOL), KX422634, KX422619, KX422612, KX422626,

Appendix 1. Continued.

KY657435*; ***Tetragonia implexicoma*** 4, *R.J.C. Chinnock 10496* (BOL), KX422635, KX422620, –, KX422627, KY657436*; ***Tetragonia maritima*** Barnéoud, *C.M. Taylor & A Pool 11587* (MO), KX197606, KX197866, KX197783, KX197696, KY657437*; ***Tetragonia microptera*** Fenzl, *Klak 2430* (BOL), KX197607, KX197867, KX197784, KX197697, KY657438*; ***Tetragonia nigrescens*** Eckl. & Zeyh., *Klak 2456* (BOL), KX197608, KX197868, KX197785, KX197698, KY657439*; ***Tetragonia portulacoides*** Fenzl, *Klak 2468* (BOL), KX197609, KX197869, KX197786, –, KY657440*; ***Tetragonia reduplicata*** Welw. ex Oliv., *Klak 2438* (BOL), KX197610, KX197870, KX197787, KX197699, KY657441*; ***Tetragonia saligna*** Fenzl, *Klak 2460* (BOL), KX197611, KX197871, KX197788, KX197700, KY657442*; ***Tetragonia sarcophylla*** Fenzl, *Klak 1970* (BOL), KX197612, KX197872, KX197789, KX197701, KY657443*; ***Tetragonia schenckii*** Schinz, *Mannheimer 4920* (WIND), KX197613, KX197873, KX197790, KX197702, KY657444*; ***Tetragonia*** sp., *C.M. Taylor & M. Richardson 12432* (MO), KX197614, KX197874, KX197791, KX197703, KY657445*; ***Tetragonia spicata*** L.f. 1, *Klak 2325* (BOL), KX197615, KX197875, KX197792, KX197704, KY657446*; ***Tetragonia spicata*** 2, *Klak 2465* (BOL), KX197616, KX197876, KX197793, KX197705, KY657447*; ***Tetragonia tetragonoides*** (Pall.) Kuntze 1, *Buys 1586* (NZFRI), KX197617, KX197877, KX197794, KX197706, KY657448*; ***Tetragonia tetragonoides*** 2, *R. Davis 12537* (BOL), KX197618, KX197878, KX197795, KX197707, KY657449*; ***Tetragonia tetragonoides*** 3, *Klak 2501* (BOL), KX197619, KX197879, KX197796, KX197708, KY657450*; ***Tetragonia verrucosa*** Fenzl, *Klak 2452* (BOL), KX197620, KX197880, KX197797, KX197709, KY657451*; — **MESEMBRYANTHEMOIDEAE: *Mesembryanthemum aitonis*** Jacq., *Bruyns 9563* (BOL), KM272631, AM161197, KX197714, KX197629, KM272631; ***Mesembryanthemum liliputanum*** Klak, *Bruyns 9930* (BOL), AM161395, AM161212, KX197715, KX197630, AM162368; — **RUSCHIOIDEAE: *Cleretum bellidiforme*** (Burm.f.) G.D.Rowley, *Klak 627* (BOL), AJ439000, AJ532769, KF132145, KF132629, AJ438211; ***Conicosia pugioniformis*** subsp. ***muirii*** (N.E.Br.) Ihlenf. & Gerbaulet, *Klak 1570* (BOL), KF132776, KF132433, KF132144, KF132628, JN896508; ***Delosperma esterhuyseniae*** L.Bolus, *Bruyns 7141* (BOL), AJ439002, AJ532776, KF132187, KF132671, AJ438213; ***Lampranthus bicolor*** (L.) N.E.Br., *Klak 543* (BOL), AJ439042, AJ532807, KF132221, KF132709, AJ438250; — **SESUVIOIDEAE: *Sesuvium humifusum*** (Turpin) Bohley & G.Kadereit, *L. Ahart 9922* (BOL), AM161379, AM161196, –, KX197623, KY657357*; ***Sesuvium sesuvioides*** (Fenzl) Verdc., *Klak 2431* (BOL), KX197539, AJ532734, –, KX197624, KY657358*; ***Trianthema parvifolium*** E.Mey. ex Sond., *Klak 2429* (BOL), KX197540, AJ532735, –, KX197625, KY657359*; ***Tribulocarpus dimorphanthus*** (Pax) S.Moore 1, *Thulin & al. 11226* (UPS), HE585101, HE585080, KX197711, KX197626, KY657360*; ***Tribulocarpus dimorphanthus*** 2, *Thulin & Warfa 5343* (UPS), HE585100, HE585079, KX197712, KX197627, KY657361*; ***Tribulocarpus dimorphanthus*** 3, *Klak 2441* (BOL), KX197541, AJ532745, KX197713, KX197628, KY657362*; ***Tribulocarpus retusus*** (Thulin) Thulin & Liede, *Thulin & al. 10511* (UPS), HE585102, HE585081, –, –, KY657363*