Phylogenetics and Evolution of Crassulaceae subf. Sempervivoideae

Dissertation
zur Erlangung des Grades
Doktor der Naturwissenschaften

Am Fachbereich Biologie
der Johannes Gutenberg-Universität Mainz
(D77)

Thibaud Felix Edmond Messerschmid
geb. am 26.11.1991 in Starnberg

Mainz, Februar 2022

Dekan:

1. Berichterstatter: Herr Prof. Joachim W. Kadereit, PhD

2. Berichterstatter:

Tag der mündlichen Prüfung: Mittwoch, 09.02.2022

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General introduction

Succulence is a complex of anatomical and morphological traits in plants that enables a "storage of utilizable water in living tissues in one or several plant parts in such a way to allow the plant to be temporarily independent from external water supply but to retain at least some physiological activity" (Eggli and Nyffeler, 2009). This property of succulent plants does not only make them attractive for horticulture but also enables their growth on marginal land (Borland et al., 2015; Grace, 2019) which will increasingly replace arable land in the course of climate change. The above definition of succulence (Eggli and Nyffeler, 2009) is by no means the only one that can be found in the literature (Eggli, 2005; see Males (2017) for review), and depending on the duration of the temporal independence from external water supply, succulence is clearly a feature that can be realized to a greater or lesser degree (Von Willert et al., 1990). Succulence evolved in many different plant lineages independently as an adaptation to arid conditions and can therefore be regarded as a classic case of convergent evolution. However, the organs and tissues in which succulence is developed as well as the coordination of succulence with physiological traits varies widely between succulent plant lineages (Males, 2017). Studying the morphology and systematics of succulent plants has always been a particular challenge because of the inherent difficulty of preparing useful herbarium specimens from succulent plant material, although drying techniques have improved over the past 40 years (Leuenberger, 1982; Eggli and Leuenberger, 1996). Bradley (1716–1727) even characterized succulents as plants that cannot be prepared as herbarium specimens, and instead produced many illustrations of the succulent plants that he studied.

With approximately 1410 species in 35 genera (Thiede and Eggli, 2007; Klein and Kadereit, 2015), the Crassulaceae family of the order Saxifragales is among the five most species-rich succulent plant lineages of the world (Arakaki et al., 2011). The Crassulaceae are leaf-succulent and predominantly herbaceous, rarely woody plants. Their flowers are usually arranged in cymose inflorescences, but flower merism, petal fusion, the number of stamen whorls and presence and shape of hypogynous nectar glands are very variable. Variation in some of these floral characters and in phyllotaxis has largely been the foundation for earlier systematic treatments of Crassulaceae and has formerly led to the recognition of six subfamilies by Berger (1930). This subfamilial classification was followed by many taxonomists who made important contributions to Crassulaceae systematics (e.g., Fröderström, 1930–1935; Uhl, 1948, 1961; Ohba, 1978). However, essentially all morphological characters that were used for Berger's (1930) classification later turned out to be highly homoplastic when the first molecular phylogenies of Crassulaceae were made available (Van Ham, 1995; Van Ham and 't Hart, 1998; Mort et al., 2001). For example, 't Hart et al. (1999) demonstrated in a study of restriction site variation of the plastid genome that sympetaly evolved at least eight times independently in the European Crassulaceae.

The development of molecular phylogenetics has been one of the most fruitful new avenues for biological systematics in the 20th century. One of its most important advantages is its independence from morphological characters which allows researchers to infer phylogenetic relationships among taxa and the evolution of characters without risking circularity (Givnish, 1997). Molecular phylogenetic and phylogenomic methods have been successfully developed and applied to infer relationships among the major lineages (orders and families) of angiosperms (APG IV, 2016), but also among much older (e.g., seed plants; Ran et al., 2018), and much younger taxa of different systematic rank (e.g., Pouchon et al., 2018; Bagley et al., 2020). In addition to resolving evolutionary relationships between taxa, molecular phylogenies of plant lineages have been commonly used for the timing of diversification events and inference of diversification rate shifts (e.g., Sanderson and Donoghue, 1994; Onstein et al., 2014; Ramírez-Barahona et al., 2020) and to address problems concerning biogeography, habitat shifts, and mechanisms of diversification (e.g., Veranso-Libalah et al., 2018; White et al., 2020). However, estimating divergence times on phylogenies of succulent plants is particularly impeded by the lack of succulent plants in the fossil record.

Building on the earliest family-wide molecular phylogenies of Crassulaceae (Van Ham, 1995; Van Ham and 't Hart, 1998; Mort et al., 2001), Thiede and Eggli (2007) introduced a classification in three well-supported subfamilies, i.e., Crassuloideae, Kalanchoideae and Sempervivoideae, and presented possible apomorphies for each of them. Instead of the more evident but homoplastic morphological characters that Berger (1930) had used for classification, seed testa ornamentation is currently regarded as the (micro)morphological character that best reflects the affiliation to subfamilies of Crassulaceae (Thiede and Eggli, 2007). In addition, Crassuloideae, the earliest-branching lineage of Crassulaceae comprising only *Crassula* L., is characterized by its pentamerous and haplostemonous flowers while Kalanchoideae is characterized by tetramerous flowers (only *Kalanchoe* Adans.) and fused petals. Both these subfamilies almost exclusively occur in the southern hemisphere and are important floristic elements of the south African centre of Crassulaceae diversity. Mexico represents another centre of Crassulaceae diversity, and there, only members of the third subfamily, Sempervivoideae, are native (Thiede and Eggli, 2007). However, Sempervivoideae, the most diverse subfamily with approximately 1050 species in 30 genera (Chapter 1), is much more widely distributed throughout the northern hemisphere with additional centres of diversity in the wider Californian winter-rainfall region, the Mediterranean region, Canary Islands, the Himalayas and East Asia (Thiede and Eggli, 2007).

Within Sempervivoideae, Mort et al. (2001) identified five major lineages which they referred to as clades, i.e, the Telephium clade, Sempervivum clade, Aeonium clade, Leucosedum clade and Acre clade. However, few of these "clades" actually received appreciable support in their phylogenetic analysis of the plastid DNA marker *matK*, and their Telephium and Sempervivum clades each consisted of several lineages in a polytomy. Furthermore, as had been the case in an earlier analysis of plastid DNA restriction site variation (Van Ham and 't Hart, 1998), species of *Sedum* L. were recovered in several of these clades, rendering *Sedum* a non-monophyletic genus. *Sedum* (approximately 470 species) is also the largest genus of Sempervivoideae and of Crassulaceae altogether (Chapter 1). It was introduced in Linnaeus's (1753) seminal work "Species Plantarum" where, in general, no genus descriptions were given. However, the name *Sedum* was already used by Linnaeus (1737) in the first edition of "Genera Plantarum", and the generic circumscription given there is highly inclusive and based on floral traits that are plesiomorphic in Crassulaceae, i.e., pentamerous, diplostemonous flowers with free petals. Consequently, many later authors have considered *Sedum* as the least derived lineage of Crassulaceae ('t Hart and Eggli, 1995) lacking morphological synapomorphies, and this caused *Sedum* to become a hold-all taxon for those Crassulaceae that can hardly be defined by unique features (Van Ham and 't Hart, 1998), an issue that has been known as the "*Sedum* problem" (Mort et al., 2010). While there are other genera of Sempervivoideae (e.g., *Echeveria* DC., *Pistorinia* DC., *Thompsonella* Britton & Rose) that are each characterized by a set of unique morphological characters (and that are usually also restricted to a defined geographical region), all of their defining characters that are usually thought of as derived in the evolution of Crassulaceae can also be found in at least some *Sedum* species.

The present dissertation investigates the phylogenetics and evolution of Crassulaceae subf. Sempervivoideae. One of the focal goals is to address the "*Sedum* problem" using, besides nearly all other genera of Sempervivoideae, the most comprehensive sample of *Sedum* in any phylogenetic study yet (nearly one third of the species included). In order to identify well-supported clades in the Sempervivoideae phylogeny that contain *Sedum* species and may be segregated from *Sedum*, as many infrageneric taxa as possible were sampled (80% of the infrageneric taxa of *Sedum* were represented). The working hypothesis for this study was that infrageneric taxa within *Sedum* that had been defined by previous systematic treatments may correspond to monophyletic lineages and could thus be segregated from *Sedum* to arrive at a new generic classification of Sempervivoideae that is based on morphologically characterizable clades. Some of the species that Linneaus (1753) classified in *Sedum*, most notably those flat-leaved species that he listed under "Planifolia", were later transferred into other genera that partly better reflect the phylogenetic relationships among lineages of Sempervivoideae. This illustrates that the definition of *Sedum* has been problematic from its very beginning and that more phylogenetic work is needed upon which a generic classification of Sempervivoideae can be founded (Chapter 1).

As outlined above, several *Sedum* species deviate from the plesiomorphic pentamerous flower structure by having reduced or elevated flower merism ('t Hart and Bleij, 2005). Some of the *Sedum* species with polymerous flowers (usually 6- to 10-merous) have been transferred into *Petrosedum* Grulich (1984), a segregate genus of *Sedum* that is morphologically well characterized. Others that are native to Northwest Africa and some western Mediterranean islands have been recovered in the Aeonium clade (Mort et al., 2001, 2002) as close relatives of the Macaronesian *Aeonium* alliance, i.e., the most species-rich plant radiation of the Macaronesian floristic region (Jorgensen and Olesen, 2001), composed of the genera *Aeonium* Webb & Berthel., *Aichryson* Webb & Berthel. and *Monanthes* Haw. Phylogeny and evolution of *Aeonium* (Chapter 2), the most diverse genus of the Macaronesian *Aeonium* alliance (Liu, 1989; Bañares Baudet, 2015), and of the closely related continental *Sedum* species (Chapter 3) are also in the focus of this dissertation.

Species of *Aeonium* exhibit a high diversity of growth forms, ranging from biennial monocarpic rosette plants to perennial ramified shrubs (Mes and 't Hart, 1996), and high diversity and flexibility in the expression of Crassulacean Acid Metabolism (Lösch, 1990; Mort et al., 2007), a specialized photosynthetic pathway that is commonly associated with succulence and enhanced water-use efficiency (Lüttge, 2004; Males, 2017). This morphological and physiological diversity has been thought to be highly adaptive for different *Aeonium* species to fulfil different ecological roles in the various habitats in which they occur on the Macaronesian islands, and they have therefore been discussed as a case of adaptive radiation (Jorgensen and Olesen, 2001; Givnish, 2010; Thiv et al., 2010). However, the current knowledge of the phylogeny and evolution of *Aeonium* is insufficient for addressing questions about diversification processes and adaptive radiation due to poor phylogenetic resolution and an incomplete taxon sample (Mort et al., 2002). Using a modified ddRADseq protocol that yielded a DNA sequence dataset of 4,280 loci for all 40 species of *Aeonium*, both these shortcomings could be overcome. Furthermore, diversification events in the *Aeonium* phylogeny were dated using a secondary age estimate for calibration of the molecular clock, and an ancestral area reconstruction with subsequent biogeographical stochastic mapping was carried out in order to investigate the consequences of sympatric and allopatric diversification for the morphological and ecological divergence of the respective sister lineages (Chapter 2).

Of the five major lineages of Sempervivoideae identified by Mort et al. (2001), the Sempervivum and Aeonium clades are characterized by polymerous flowers. Results in chapter 1 of this dissertation suggest that taxa of the Sempervivum clade are recovered in two independent well-supported clades, i.e., the Petrosedum clade (comprising *Petrosedum* and *Sedum* ser. *Nana* ’t Hart & Alpınar) and a clade comprising *Sempervivum* L. and *Jovibarba* Opiz. Due to the poor resolution in the backbone of the Sempervivoideae phylogeny, relationships among these clades of species featuring polymerous flowers are unknown, but sequence data of plastid DNA alone support the Petrosedum clade, *Sempervivum*/*Jovibarba* and the Aeonium clade as successive sister lineages to the Leucosedum plus Acre clades (Chapter 1), a relationship that is best explained by one shift towards elevated flower merism in the common ancestor of Sempervivoideae excl. the Telephium clade and one reversal to pentamerous flower merism in the common ancestor of the Leucosedum and Acre clades (i.e., of tribe Sedeae). In the Aeonium clade, those predominantly Northwest African species that have been recovered as close relatives of the Macaronesian *Aeonium* alliance in previous phylogenetic studies (Mes, 1995; Van Ham and 't Hart, 1998; Mort et al., 2001, 2002; Nikulin et al., 2016) belong to *Sedum* sers. *Caerulea* Fröd. (2 spp.), *Monanthoidea* Batt. ex Fröd. (3 spp.) and *Pubescens* T.Mes (3 spp.) and have polymerous flowers and partly other morphological characters in common with representatives of the Macaronesian *Aeonium* alliance. If the proposition of an extended classification of *Sedum* to encompass all genera of tribe Sedeae (Chapter 1) should be followed, species of the above-mentioned *Sedum* series of the Aeonium clade would need to be segregated from *Sedum*. However, phylogenetic relationships among these series are unknown because not all relevant species belonging to these series were sampled in the previous phylogenetic studies. In order to infer monophyletic lineages in the phylogeny of the eight *Sedum* species of the Aeonium clade that may be segregated from *Sedum* and in order to identify the closest relative(s) of the Macaronesian *Aeonium* alliance, a complete sample of the eight relevant *Sedum* species was analysed on the basis of two nuclear and four plastid DNA markers. Candidates for closest relatives of the Macaronesian *Aeonium* alliance and relevance of the Macaronesian Enclave in Morocco (Peltier, 1973; Sunding, 1979) as potential source area for migration to the Canary Islands are discussed (Chapter 3).

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TAXON 69 (5) • October 2020: 892–926

SYSTEMATICS AND PHYLOGENY

Linnaeus's folly – phylogeny, evolution and classification of *Sedum* (Crassulaceae) and Crassulaceae subfamily Sempervivoideae

Thibaud F.E. Messerschmid,[1,2] **Johannes T. Klein,**[3] **Gudrun Kadereit**[2] **& Joachim W. Kadereit**[1]

1 *Institut für Organismische und Molekulare Evolutionsbiologie, Johannes Gutenberg-Universität Mainz, 55099 Mainz, Germany*
2 *Institut für Molekulare Physiologie, Johannes Gutenberg-Universität Mainz, 55099 Mainz, Germany*
3 *Gothenburg Global Biodiversity Centre, Gothenburg, Sweden*
Address for correspondence: *Thibaud Messerschmid, tmessers@uni-mainz.de*

DOI https://doi.org/10.1002/tax.12316

Abstract *Sedum*, containing approximately 470 species, is by far the largest genus of Crassulaceae. Three decades of molecular phylogenetic work have provided evidence for the non-monophyly of *Sedum* and many more of the 30 genera of Crassulaceae subfam. Sempervivoideae. In this study, we present a broadly sampled and dated molecular phylogeny of Sempervivoideae including 80% of all infrageneric taxa described in *Sedum* as well as most other genera of the subfamily. We used sequences of one nuclear (ITS) and three plastid markers (*matK*, *rps16*, *trnL-trnF*). The five major lineages of Sempervivoideae (i.e., Telephium clade, Petrosedum clade, *Sempervivum*/*Jovibarba*, Aeonium clade, Leucosedum plus Acre clades) were resolved as successive sister to each other in the phylogenetic analysis of the plastid markers, while in the ITS phylogeny the Petrosedum clade is the closest relative of the Aeonium clade. Our dating analysis of ITS suggests that Sempervivoideae diversified rapidly throughout the Paleocene and Eocene, possibly in the area of the former Tethys and Paratethys archipelago. A biogeographic pattern emerges in which migration out of this ancestral area is linked to pronounced morphological evolution resulting in several distinct lineages recognized as segregate genera thought to be derived from *Sedum*. These segregate genera, however, have been defined on the basis of strongly homoplasious characters such as degree of petal fusion, petal colouration or flower merism. Moreover, all character states currently used for the delimitation of segregate genera seem to be homoplasious, and each of them can be found in at least one species of *Sedum*. Extensive literature work led to the conclusion that only few of the monophyletic clades found by us can be defined unambiguously by morphological characters. Mainly for these two reasons, we believe that combination of all 14 genera currently recognized in tribe Sedeae (= clades Leucosedum plus Acre) into *Sedum* might be the most stable solution of the "*Sedum* problem". This new *Sedum* s.l. would then comprise approximately 755 species.

Keywords hybridization; large genera; molecular dating; morphological evolution; rapid radiation

Supporting Information may be found online in the Supporting Information section at the end of the article.

INTRODUCTION

Crassulaceae, a family of ca. 1410 species in 34 genera according to the latest family account by Thiede & Eggli (2007), contains three subfamilies, i.e., Crassuloideae, Kalanchoideae and Sempervivoideae. *Sedum* L., by far the largest genus of Sempervivoideae and indeed of Crassulaceae, contains ca. 430 species in the circumscription by Thiede & Eggli (2007), i.e., after treatment at generic rank of several lineages formerly classified as *Sedum* (see below). With the valid publication of 42 new species names since then, *Sedum* now contains ca. 470 species. In the first comprehensive molecular phylogeny of the family, Van Ham & 't Hart (1998) concluded that "*Sedum* is generally considered as a hold-all taxon that encompasses the least derived Crassulaceae as well as homoplastic or transitional phenotypes to nearly every other genus of the family." These authors, based on an RFLP analysis of plastid DNA, and Mort & al. (2001), based on *matK* sequences, identified five major groups within Sempervivoideae, referred to as clades by these authors although partly not supported or comprising two or three lineages of a larger polytomy. All these five "clades" contained species of *Sedum* or species included in *Sedum* in more or less recent accounts but also representatives of other genera. These five "clades" are: (1) Telephium clade (tribes Telephieae and Umbiliceae sensu Thiede & Eggli, 2007; containing *Hylotelephium* H.Ohba, *Kungia* K.T.Fu, *Meterostachys* Nakai, *Orostachys* Fisch., *Phedimus* Raf., *Pseudosedum* (Boiss.) A.Berger, *Rhodiola* L., *Sinocrassula* A.Berger and *Umbilicus* DC.; Mayuzumi & Ohba, 2004; Gontcharova & al., 2006); (2) Sempervivum clade (tribe Semperviveae sensu Thiede & Eggli, 2007; containing *Petrosedum* Grulich, *Sempervivum* L. and *Jovibarba* Opiz; the latter treated as synonym to *Sempervivum* by Thiede & Eggli, 2007, but as a distinct genus by Klein & Kadereit, 2015); (3) Aeonium clade (tribe

Article history: Received: 28 Feb 2020 | returned for (first) revision: 16 Apr 2020 | (last) revision received: 20 May 2020 | accepted: 31 May 2020 | published online: 4 Sep 2020 | **Associate Editor:** Levent Can | © 2020 The Authors.
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Aeonieae sensu Thiede & Eggli, 2007; containing approximately 8 species of *Sedum* [Mes, 1995a] as well as *Aeonium* Webb & Berthel., *Aichryson* Webb & Berthel., *Monanthes* Haw. and *Hypagophytum* A.Berger; Mort & al., 2002; our study); (4) Leucosedum clade (tribe Sedeae/Leucosedum clade sensu Thiede & Eggli, 2007; containing ca. 120 *Sedum* species as well as *Pistorinia* DC., *Rosularia* (DC.) Stapf, *Prometheum* (A.Berger) H.Ohba, *Afrovivella* A.Berger, *Sedella* Britton & Rose and *Dudleya* Britton & Rose); and (5) Acre clade (tribe Sedeae/Acre clade sensu Thiede & Eggli, 2007; containing ca. 345 *Sedum* species as well as *Cremnophila* Rose [included in *Sedum* by Thiede & Eggli, 2007], *Echeveria* DC., *Graptopetalum* Rose, *Lenophyllum* Rose, *Pachyphytum* Link & al., *Thompsonella* Britton & Rose and *Villadia* Rose; Carrillo-Reyes & al., 2008, 2009). The clades Acre and Leucosedum together constitute Sedeae of subfam. Sempervivoideae. These five "clades" form a well-supported monophyletic group (Sempervivoideae sensu Thiede & Eggli, 2007) and, together with the largely African Crassula and Kalanchoe clades (Crassuloideae and Kalanchoideae, respectively, sensu Thiede & Eggli, 2007) as successive sisters (Van Ham & 't Hart, 1998; Mort & al., 2001) to this group, make up Crassulaceae. This classification of Crassulaceae and approximate species numbers in the above-listed groups are summarized in Table 1.

In the above five clades, with the exception of the Sempervivum clade, species of *Sedum* (or former *Sedum*) never form a monophyletic group but either are more or less scattered across the respective phylogenetic trees (e.g., clades Acre and Leucosedum) or form a basal grade (e.g., Aeonium clade). In conclusion, *Sedum* in its current circumscription indeed is a highly polyphyletic (or paraphyletic) taxon. The two comprehensive analyses of Crassulaceae by Van Ham & 't Hart (1998) and Mort & al. (2001) included 23 and 30 species of *Sedum*, respectively, the phylogenetic analysis of the Acre clade alone by Carrillo-Reyes & al. (2009) included 68 species of *Sedum*, and a recent analysis focusing on *Sedum* (but including representatives of other genera) and using only ITS sequences included 114 species of the genus (Nikulin & al., 2016). Considering the size of *Sedum* this still represents only a fairly small number of species, and indeed Mort & al. (2010), when discussing the "*Sedum* problem", pointed out that much of the taxonomic diversity of the genus has not yet been included in phylogenetic studies.

On this background, we here make an effort to represent as many as possible infrageneric taxa recognized in *Sedum* in a phylogenetic analysis based on plastid and nuclear DNA sequences, with our nuclear dataset being substantially larger than the plastid dataset. We present molecular phylogenies derived from a sample of 298 species of Crassulaceae, including 145 *Sedum* species (not counting 7 species segregated as *Petrosedum*) representing 144 of the 186 infrageneric taxa of *Sedum* described in the taxonomic literature (suppl. Table S1). In particular, we (1) aim at showing into which major clades of Sempervivoideae the various infrageneric groups of *Sedum* fall or are likely to fall, (2) will offer an evolutionary interpretation of Sempervivoideae and the "*Sedum* problem" (Mort & al., 2010), and (3) will explore options for the classification of *Sedum*. With respect to the last point we will argue in favour of broadening the circumscription of the genus by including all genera of Sedeae in *Sedum*.

■ MATERIALS AND METHODS

Taxon sampling. — The main aim of our sampling effort was to represent as many infrageneric taxa of *Sedum* as possible and to include all other genera of Sempervivoideae. To this end, we first compiled ITS (internal transcribed spacer) sequences of species of Sempervivoideae available from GenBank. To broaden our sample of Sempervivoideae and particularly of

Table 1. Taxonomic system of the family Crassulaceae following Thiede & Eggli (2007) and Klein & Kadereit (2015) and updated according to results of the present study.

Subfamily	Major clade	Corresponding tribes	Genera	Approximate species number
Crassuloideae	Crassula clade		*Crassula*	200
Kalanchoideae	Kalanchoe clade		*Adromischus*, *Kalanchoe*, *Tylecodon*, *Cotyledon*	240
Sempervivoideae	Telephium clade	Telephieae and Umbiliceae	*Hylotelephium*, *Kungia*, *Meterostachys*, *Orostachys*, *Phedimus*, *Pseudosedum*, *Rhodiola*, *Sinocrassula*, *Umbilicus*	160
	Sempervivum clade	Semperviveae	*Petrosedum*, *Sempervivum*, *Jovibarba*	60
	Aeonium clade	Aeonieae	*Aeonium*, *Aichryson*, *Monanthes*, *Hypagophytum*, *Sedum* p.p. (8 spp.)	75
	Leucosedum clade	Sedeae p.p.	*Pistorinia*, *Rosularia*, *Prometheum*, *Afrovivella*, *Sedella*, *Dudleya*, *Sedum* p.p. (ca. 120 spp.)	200
	Acre clade	Sedeae p.p.	*Cremnophila*, *Echeveria*, *Graptopetalum*, *Lenophyllum*, *Pachyphytum*, *Thompsonella*, *Villadia*, *Sedum* p.p. (ca. 345 spp.)	550

Species numbers were updated by reviewing names of new species validly published after 2007 from ipni.org. The monospecific genus *Perrierosedum* is not represented in this table since its taxonomic position remains unknown.

infrageneric taxa of *Sedum*, we added 70 newly sequenced accessions representing 64 species to our sample (see Appendix 1 for a full list of specimens and GenBank accession numbers). Twenty-seven of these species (incl. 21 *Sedum* spp.) were included in a molecular phylogenetic study for the first time. These 70 accessions were obtained from living collections, as silica-dried material or as herbarium material from E, JEPS, L, MJG, MSB, O, OSC, TARI, U, WAG and ZSS. The few samples taken from living plants grown at the Botanic Gardens Berlin-Dahlem, Mainz and Zürich (Sukkulenten-Sammlung) mostly had a well-documented collection record (see Appendix 1). This sampling resulted in ITS sequence data from 271 accessions representing 263 different taxa at specific or infraspecific rank, including 145 species of *Sedum* (without *Petrosedum*). Of the 186 infrageneric taxa of *Sedum* (not counting those comprising only species that have been transferred to other genera; see suppl. Table S1) we were able to identify in the taxonomic literature (Scopoli, 1777; Boissier, 1872; Schönland, 1891; Berger, 1930; Fröderström, 1930, 1931, 1932, 1935; Alexander, 1942; Clausen, 1942, 1943a,b, 1975, 1979; Clausen & Uhl, 1943, 1944; S.-H. Fu, 1965; Maire & Quézel, 1967; K.T. Fu, 1974; Uhl, 1977, 1978, 1980, 1992a; ’t Hart, 1978, 1991; Ohba, 1978; Calie, 1981; Denton, 1982; Gilbert, 1985; Eggli, 1992; ’t Hart & Alpınar, 1995, 1999; Mes, 1995a,b; Nesom & Turner, 1995; Fu & Ohba, 2001; ’t Hart & Bleij, 2005; Afferni, 2012, 2014; Santiago & al., 2015), 144 were represented in our ITS dataset. However, 19 of the non-monospecific infrageneric taxa were represented by only one species.

For our plastid dataset we compiled sequences of *matK*, *rps16* and the *trnL-trnF* spacer (in the following abbreviated as *trnL-F*), the most commonly sequenced markers in Sempervivoideae, from GenBank and expanded the sampling by sequencing these plastid regions for 44 additional taxa (see below). Considering the well-established phylogenetic relationship of Kalanchoideae as sister to Sempervivoideae (Van Ham, 1995; Van Ham & ’t Hart, 1998; Mort & al., 2001) we chose seven species from three genera of Kalanchoideae as outgroup in our ITS dataset and three species of the same genera in the plastid sequence dataset. For the combined dataset of ITS and plastid sequences as well as the dated phylogenetic analysis of ITS (see below), we expanded the outgroup by sampling additional species from Crassulaceae and those families most closely related to it (for details see “Outgroup” in Appendix 1).

Except for *Jovibarba* and *Sempervivum*, which we treated as distinct genera (Klein & Kadereit, 2015), taxonomic treatment followed Eggli (2005). Furthermore, because of substantial differences in their ITS sequences, we considered *Sedum hillebrandtii* Fenzl a species separate from *S. urvillei* DC. although it has been treated as a heterotypic synonym of *S. urvillei* by ’t Hart & Bleij (2005).

Assessment of morphological character states. — Information about morphological characters (including seed testa ornamentation) of the sampled *Sedum* taxa as well as all other genera of Sedeae was taken from Eggli (2005), Thiede & Eggli (2007) and the International Crassulaceae Network (www.crassulaceae.ch). For infrageneric taxa of *Sedum* the descriptions given in the respective protologues were used, and missing data were complemented as far as possible consulting ’t Hart & Bleij (2005). Finally, morphological descriptions in ’t Hart & Bleij (2005) were also used to search for possible apomorphies of such well-supported clades as retrieved in our phylogenies (see below), which did not correspond to any published infrageneric group of *Sedum*.

DNA extraction, amplification, and sequencing. — DNA was extracted from silica-dried or herbarium leaf material (accessions with a lab code in Appendix 1) with the exception of *Sedum potosinum* Rose of which alcohol material was used for DNA extraction. From two of the accessions (*Sedum obtusatum* A.Gray, *S. urvillei*) DNA was extracted using the NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany) based on the Cetyltrimethylammonium bromide (CTAB) lysis method according to the manufacturer’s protocol with a lysis time of 80 minutes. For all other accessions, the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) was used for DNA extraction following the manufacturer’s protocol with a lysis time of two hours.

Polymerase chain reactions (PCR) were performed in a total reaction volume of 25 µl. We amplified ITS using primers ITS 18S and ITS B (Hungerer & Kadereit, 1998; Muir & al., 2001). Settings for the PCR cycling were adopted from Klein & Kadereit (2015) with the following exceptions: 40 instead of 35 PCR cycles and an annealing temperature of 52°C instead of 57°C were implemented. For samples that did not yield any PCR product with this protocol, separate amplification of the two intergenic spacer regions ITS1 and ITS2 was performed using the primer pairs ITS A and ITS C, and ITS B and ITS D, respectively (Blattner, 1999). For the separate amplification of ITS1 and ITS2, the same cycling programme was used as for the amplification of the entire ITS region. The three plastid markers were amplified using the primer pairs trnK-F-bryo and trnK-2R for *matK* (Johnson & Soltis, 1994; Wicke & Quandt, 2009), rps16-F and rps16-R for *rps16* (Oxelman & al., 1997; Shaw & al., 2005), and Tab-C and Tab-F for *trnL-F* (Taberlet & al., 1991). The cycler protocol for the amplification of *matK* and *rps16* was the same as that used for ITS. PCR for *trnL-F*, however, required a higher annealing temperature of 56°C instead of 52°C.

PCR products were purified with exoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.) following the manufacturer’s protocol. PCR products were Sanger-sequenced on a 3730 DNA Analyzer at StarSEQ (Mainz, Germany).

Sequence alignment and phylogenetic analysis. — Sequence contigs were assembled using Sequencher (v.4.1.4; Gene Codes, Ann Arbor, Michigan, U.S.A.). Alignments (suppl. Appendices S1–S5) were carried out using MAFFT (v.7.402; Katoh & Standley, 2013) on the CIPRES Science Gateway (https://www.phylo.org; Miller & al., 2010) with standard settings for all four markers. The algorithm used by MAFFT was L-INS-i for the three plastid DNA markers and

FFT-NS-i for the ITS dataset, which contained a higher number of sequences. All alignments were reviewed for mistakes but corrected only at very few sites. Parsimony-informative indels were not coded prior to the phylogenetic analyses. Variability of the four analyzed molecular markers was assessed using PAUP* (v.4.0b10; Swofford, 2002). The best-fitting substitution model and partition scheme was determined for the concatenated alignment of all markers (*matK*, *rps16*, *trnL-F*, and ITS subdivided into ITS1, 5.8S and ITS2) using the corrected Akaike information criterion (AICc) in PartitionFinder2 (v.2.1.1; Lanfear & al., 2017). PhyML (v.3.0; Guindon & al., 2010) was also used for this analysis.

The alignments of the three plastid markers were concatenated to result in one large alignment of all plastid DNA sequences. In order to reduce missing data in this alignment as well as in the combined alignment of ITS and plastid sequences, we combined markers from different accessions of the same species (see Appendix 1 for species with more than one accession). To further reduce missing data in the alignment of plastid sequences, we combined three species pairs (i.e., *Sedum compactum* Rose and *S. obcordatum* R.T.Clausen, *Dudleya pulverulenta* (Nutt.) Britton & Rose and *D. viscida* (S.Watson) Moran, *Jovibarba globifera* subsp. *allionii* (Jord. & Fourr.) J.Parn. and *J. heuffelii* (Schott) Á.Löve & D.Löve) into one terminal each. In these three cases the two species combined were sister to each other with maximum bootstrap support in the maximum likelihood ITS analysis (see below).

Maximum likelihood (ML) analyses were carried out for ITS, the concatenated plastid sequence matrix and the combined ITS and plastid dataset using RAxML-HPC2 (v.8.2.10; Stamatakis, 2014) on XSEDE in the CIPRES Science Gateway (Miller & al., 2010). In the following, the ML analyses of the ITS, plastid and combined ITS and plastid sequence matrices will be referred to as ITS/ML, cpDNA/ML and combined/ML, respectively. The GTR-GAMMA model was used as substitution model, and bootstrapping was set to halt automatically. Bayesian analysis was performed for ITS and the combined nuclear and plastid dataset using MrBayes (v.3.2.7a; Ronquist & Huelsenbeck, 2003) on XSEDE in the CIPRES Science Gateway (Miller & al., 2010). BEAST (v.2.4.5; Bouckaert & al., 2014) instead of MrBayes was used for Bayesian analysis of the plastid sequence matrix and carried out on XSEDE in the CIPRES Science Gateway because BEAST performed better than MrBayes for the plastid sequence data. These analyses will be referred to as ITS/MrBayes, combined/MrBayes and cpDNA/BEAST, respectively. For both Bayesian analyses with MrBayes (ITS and combined datasets), two independent runs were performed with six chains each, and the temperature of the hot chain was set to 1. The metropolis-coupled Markov Chain Monte Carlo (MCMC) algorithm was executed for 100 million generations sampling every 10,000th generation with a burn-in of 25%. For the cpDNA/BEAST analysis, the MCMC was run for 30 million generations in four independent runs. We used the GTR+I+Γ substitution model, which was retrieved as best substitution model in PartitionFinder2 (Lanfear & al., 2017) for each of the partitions used (i.e., *matK*, *rps16* and *trnL-F*). Trees were sampled every 3000th generation, and the resulting log files of all four independent runs were checked for convergence and effective sampling sizes of at least 200 for each parameter, using Tracer (v.1.5; Rambaut & Drummond, 2007). The four independent runs were combined using LogCombiner (v.2.4.5; Bouckaert & al., 2014) after discarding a burn-in of 10%. A majority-rule consensus tree was calculated using TreeAnnotator (v.1.8.3; Rambaut & Drummond, 2016). The topology of each inferred phylogenetic tree was assessed using FigTree (v.1.3.1; Rambaut, 2009).

Comparison of the trees resulting from ML and Bayesian analyses of ITS and the plastid sequence data revealed 15 supported (bootstrap support ≥75% and/or posterior probability ≥0.95) conflicts between the ITS and plastid topologies within Sempervivoideae. All 21 taxa affected by these 15 conflicts are listed in Appendix 2 and were excluded from the combined ML analysis.

Molecular dating. — No fossils of Crassulaceae or with affinities to Crassulaceae are known. For this reason, we were only able to use secondary age estimates instead of fossil ages as calibration points for our analysis of clade ages. Stem and crown age estimates for Crassulaceae were kindly provided by Susana Magallón (Mexico City/Mexico; pers. comm.). As a result of her own work, an age of 105.94 Ma (93.43–120.59 Ma, 95% HPD) was inferred for the split between Crassulaceae and its sister group consisting of Haloragaceae, Penthoraceae, Tetracarpaeaceae and Aphanopetalaceae (Fishbein & al., 2001), i.e., the stem age of Crassulaceae, and 58.27 Ma (26.23–88.39 Ma, 95% HPD) as crown age of Crassulaceae. These two age estimates were implemented as secondary calibration points in our dating analysis with a normal distribution that included the whole 95% HPDs. A third calibration point, the age of Fuerteventura (21 Ma), the oldest of the Canary Islands, was used as maximum age of the most recent common ancestor of the clade comprising the Macaronesian endemics in the Aeonium clade (i.e., all sampled species of *Aeonium*, *Aichryson* and *Monanthes*), as had been done by Kim & al. (2008).

Divergence times were calculated using the partitioned ITS alignment in BEAST (Bouckaert & al., 2014) implemented in the CIPRES Science Gateway (Miller & al., 2010). We used a lognormal relaxed clock model and predefined all clades as monophyletic that had maximum support (bootstrap = 100 and posterior probability = 1) in both analyses of ITS (i.e., ITS/ML and ITS/MrBayes, respectively). Calculations were carried out using the birth-death process (Gernhard, 2008), and the ucld.mean parameter was specified to be gamma-distributed with 0.001 as lower and 1000 as upper limit. The GTR+I+Γ substitution model was used for both partitions of ITS (i.e., partition 1: ITS1 and ITS2 [suppl. Appendix S4]; partition 2: 5.8S [suppl. Appendix S5]). The analysis was run four times independently with an MCMC chain length of 100 million generations, sampling every 10,000th generation with a burn-in of 25%. The posterior analysis of the four independent runs was carried out in the same way as for the cpDNA/BEAST analysis.

■ RESULTS

Morphological character states. — Character states of eight traits often used in Crassulaceae systematics (i.e., life form, presence and quality of trichomes, phyllotaxis, inflorescence insertion, flower merism, sympetaly, number of stamens, seed testa ornamentation) are given in Fig. 1 for all species sampled in the ITS phylogeny (see below). Similarly, a matrix of character states relevant for the circumscription of all genera of Sedeae is given in Table 2. Diagnostic morphological character states of all infrageneric taxa of *Sedum* that we found in the taxonomic literature are summarized in columns J–M of suppl. Table S1, where the literature used is given in column I and listed in the spread sheet "literature cited". Finally, Table 3 summarizes the results of our search for possible synapomorphies of clades that were well supported in our phylogenies (see below).

Variability of molecular markers. — Variation in the ITS dataset was markedly higher than in the dataset of concatenated plastid markers. The ITS dataset (suppl. Appendix S2) contained 271 tips and 854 nucleotide positions of which 496 (58.1%) were parsimony informative. The plastid dataset of concatenated *matK*, *rps16* and *trnL-F* (suppl. Appendix S1) consisted of 130 tips and 3520 nucleotide positions of which 991 (28.2%) were parsimony informative. Of these 3520 positions 1239 were from *matK*, 1073 from *rps16* and 1208 from *trnL-F*. With 51.7% parsimony informative sites *matK* was almost as informative as ITS. The combined nuclear and plastid dataset (suppl. Appendix S3) with sequences of 163 tips, which also comprised species of Crassuloideae and Saxifragalean outgroup families had a total length of 4331 nucleotide positions of which 1703 (39.3%) were parsimony informative.

Phylogenetic relationships in subfam. Sempervivoideae. — Sempervivoideae received maximum support as a monophyletic group in all our analyses (Figs. 1–3, suppl. Fig. S1). In the following, we will describe our results with reference to the five major groups identified in the subfamily as described in previous studies (see Introduction).

(1) Telephium clade. – The three subgroups of the Telephium clade, i.e., *Umbilicus*, *Phedimus*/*Rhodiola* and *Hylotelephium* (non-monophyletic)/*Meterostachys*/*Orostachys* (non-monophyletic)/*Kungia*/*Sinocrassula*, formed a monophyletic group only in cpDNA/BEAST (Fig. 2A), where it was sister to the remainder of the subfamily. In all other analyses, the relationships among the three subgroups to the remaining lineages of the subfamily were unresolved.

(2) Sempervivum clade. – Both lineages of the Sempervivum clade, i.e., the Petrosedum clade, including *Sedum nanum* Boiss. of ser. *Nana* 't Hart & Alpınar, and *Sempervivum*/*Jovibarba*, were monophyletic in all analyses but never sister to each other. Because these two lineages were supported as successive sisters to the remainder of Sempervivoideae above the Telephium clade in cpDNA/ML (Fig. 2A), while the Petrosedum clade was supported as sister to the Aeonium clade in ITS/MrBayes (Fig. 1A), *Petrosedum* and *Sedum nanum* showed conflicting phylogenetic placements and consequently were removed from the combined analyses. The sister relationship of the Petrosedum clade with the Aeonium clade was also supported in the dating analysis of ITS (suppl. Fig. S1). *Sedum nanum* was consistently resolved as sister to *Petrosedum* (Figs. 1A, 2A, suppl. Fig. S1).

In ITS/MrBayes (Fig. 1A,B) *Sempervivum*/*Jovibarba* were part of a trichotomy with the Aeonium/Petrosedum clade and Sedeae (i.e., clades Acre and Leucosedum). This topology was essentially the same in the combined analyses (Fig. 3A, B) from which, however, the Petrosedum clade was excluded (see above).

(3) Aeonium clade. – The Aeonium clade, comprising *Aeonium* (incl. *Greenovia* Webb & Berthel.), *Aichryson*, *Monanthes* and *Hypagophytum* as well as *Sedum* species of ser. *Monanthoidea* Batt. ex Fröd., ser. *Caerulea* Fröd. and ser. *Pubescens* T.Mes, was monophyletic in all analyses. This clade was sister to Sedeae in both analyses of the plastid dataset, although not supported in cpDNA/BEAST (Fig. 2A), but sister to the Petrosedum clade in all analyses of ITS including the dating analysis (Fig. 1A, suppl. Fig. S1). In the combined analyses, from which the Petrosedum clade was excluded (Fig. 3A,B), the Aeonium clade was part of a trichotomy with *Sempervivum*/*Jovibarba* and Sedeae.

(4) Leucosedum clade and (5) Acre clade. – The clades Leucosedum and Acre together (i.e., Sedeae) were strongly supported as a monophyletic group in all analyses with the exception of ITS/ML (Fig. 1B) in which it received poor support. The Acre clade alone was monophyletic in all analyses. In ITS/ML, the Acre clade, several lineages/single species of the Leucosedum clade, *Sempervivum*/*Jovibarba* and the Aeonium and Petrosedum clades formed a large polytomy. This polytomy would become most apparent if branches supported by bootstrap values <75 (signified as "–") were collapsed in Fig. 1A,B. In ITS/MrBayes (Fig. 1B), a much smaller polytomy was formed by the Acre clade and three supported subgroups of the Leucosedum clade. Supported topological conflict between the analyses of ITS (Fig. 1) and cpDNA (Fig. 2) was found for nine *Sedum* species of clades Leucosedum and Acre (i.e., *S. album* L., *S. fragrans* 't Hart, *S. hakonense* Makino, *S. litoreum* Guss., *S. lydium* Boiss., *S. satumense* Hatus., *S. sedoides* (Decne.) Pau, *S. subtile* Miq. and *S. tenellum* M.Bieb.), which consequently were excluded from the combined nuclear and plastid analyses, together with the entire Petrosedum clade (see above) and six more conflicting genera (*Dudleya*, *Pistorinia*, *Rosularia*) or species of other genera (see Appendix 2).

As one of the goals of our study is to explore the phylogenetic positions of infrageneric groups of *Sedum* (listed in suppl. Table S1), these infrageneric groups will be assigned to the identified clades in the following paragraphs. It is important to note that several of the infrageneric taxa are named more than once, indicating that such taxa are non-monophyletic and that their members belong to more than one clade. Those infrageneric groups that we identified as monophyletic are listed in Table 3 (see Discussion).

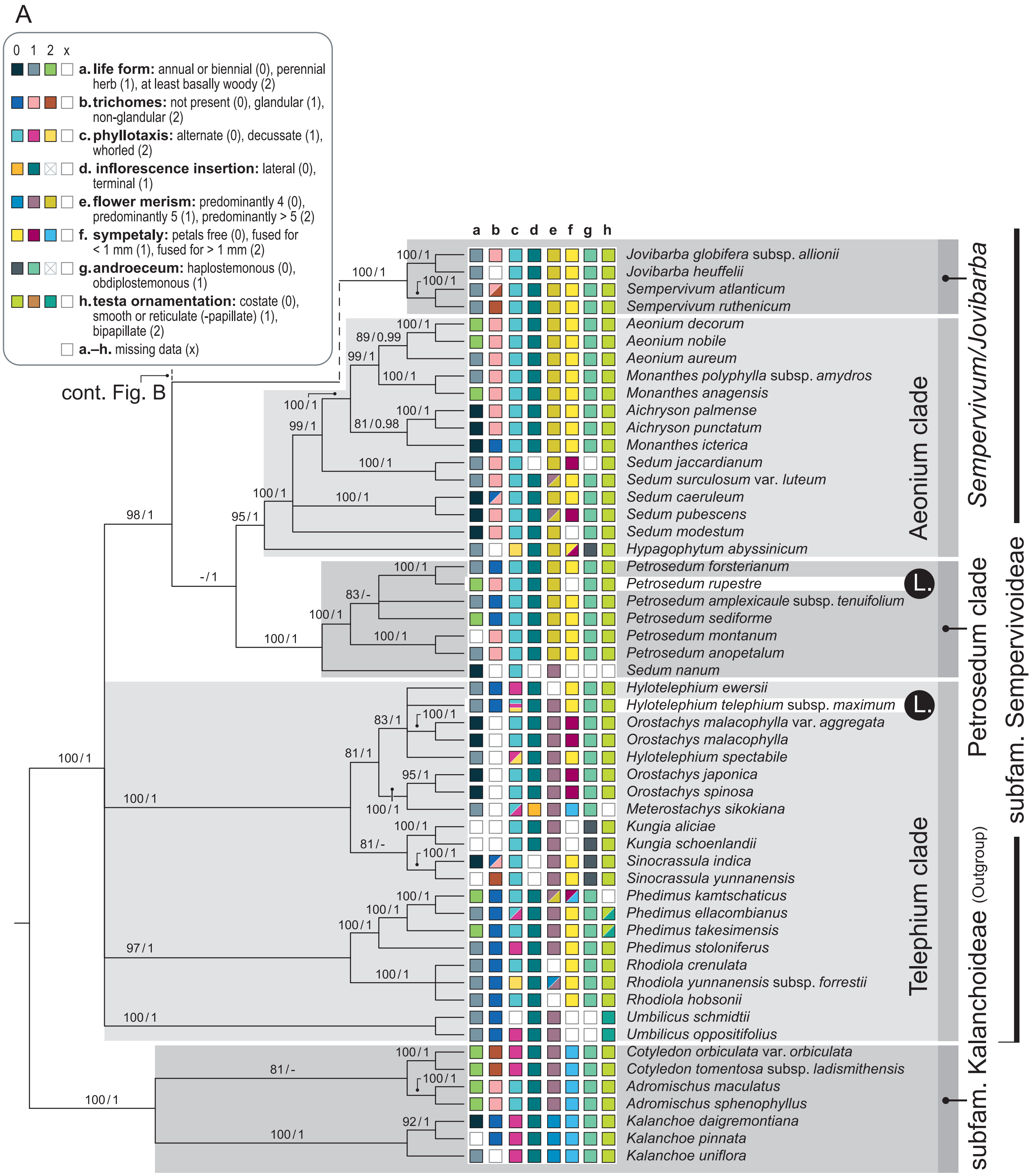

Fig. 1. Molecular phylogeny of Crassulaceae subfam. Sempervivoideae based on sequences of the internal transcribed spacer (ITS) of nuclear ribosomal DNA and inferred using maximum likelihood (ITS/ML) and Bayesian inference (ITS/MrBayes). Branch labels give bootstrap support values for ITS/ML (left) and posterior probabilities for ITS/MrBayes (right). Branches with bootstrap support <75 and posterior probability <0.95 were collapsed or marked "–" when support was only found in one of the two analyses. Species names first published by Linnaeus (1753) are highlighted and marked with "L.". Names of major clades as used in the main text are given. Character states for eight morphological traits are given for each tip of the phylogeny according to the colour code in the figure inset. The species of *Aichryson* sampled have been described as up to triennial by Nyffeler (2005).

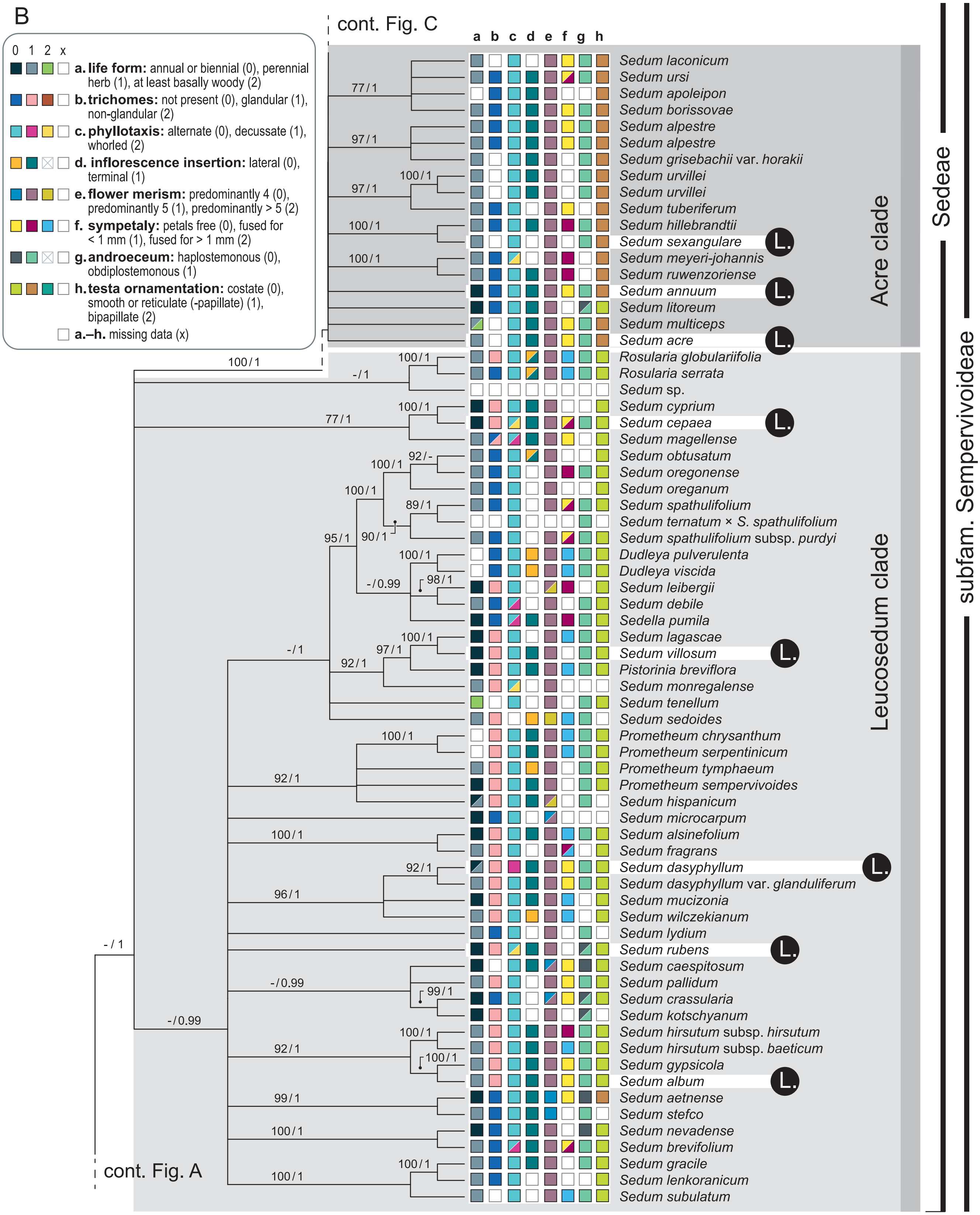

Fig. 1. Continued.

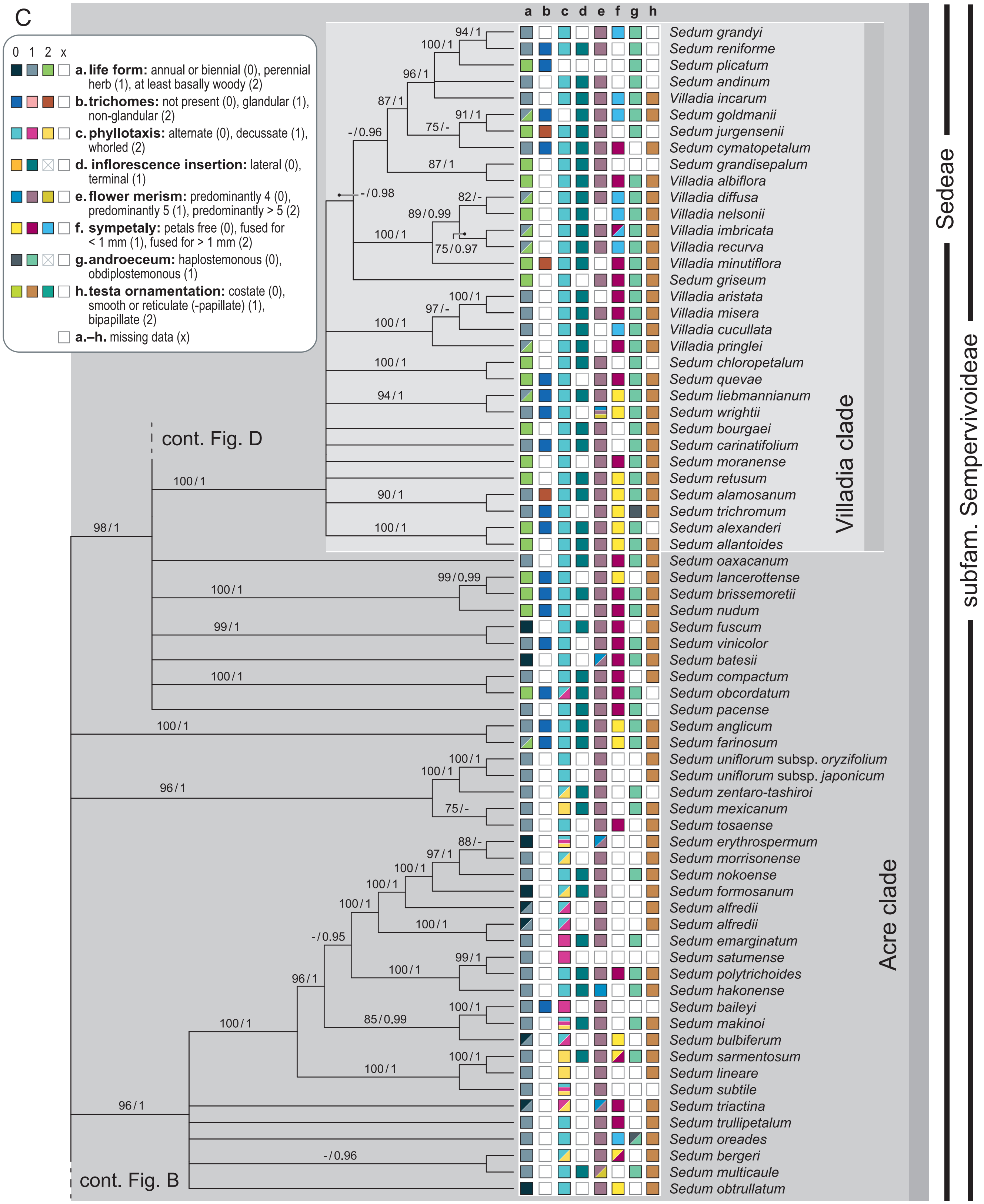

Fig. 1. Continued.

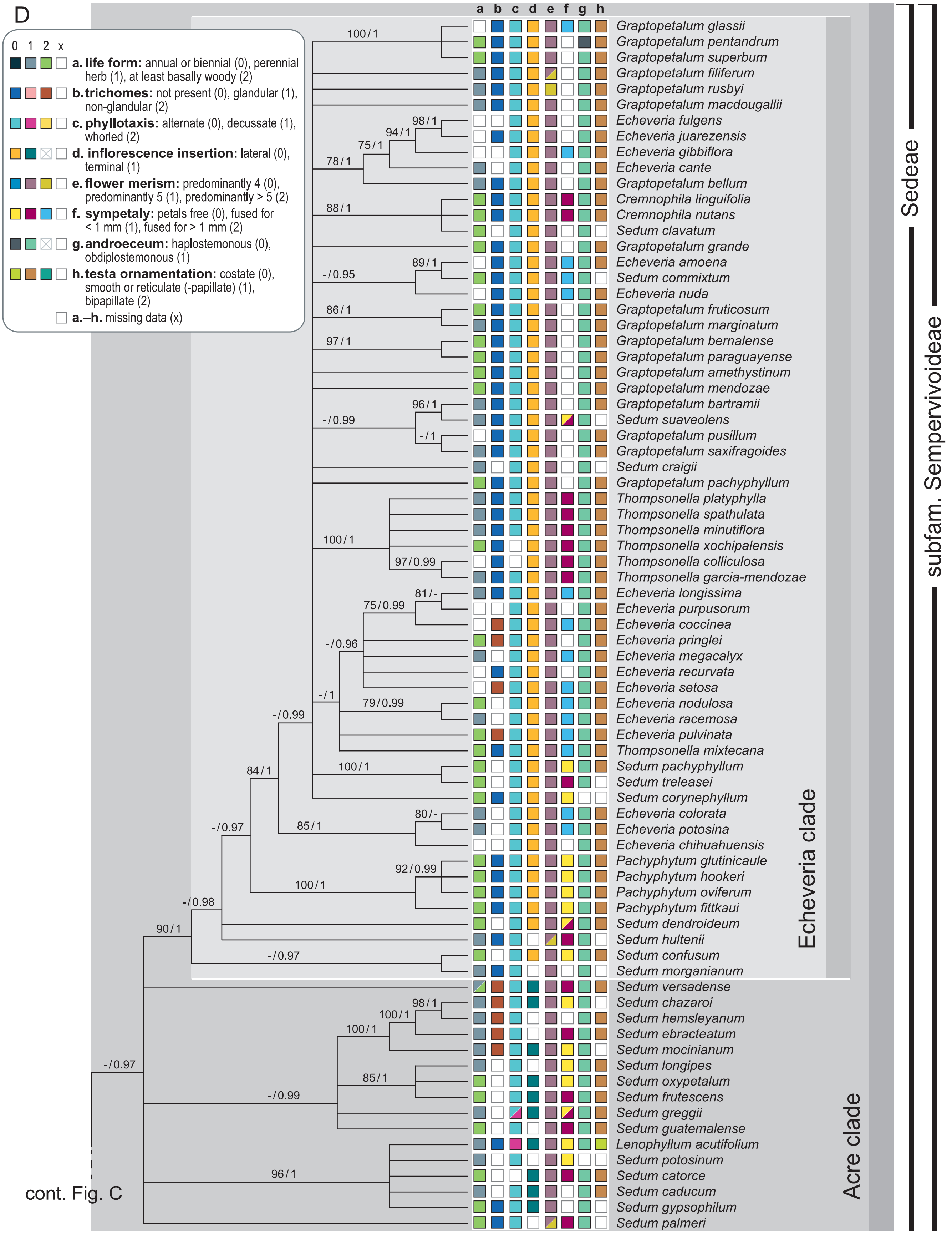

Fig. 1. Continued.

Table 2 (left part). Genera of the Leucosedum and Acre clades (= Sedeae) and their defining characters following Eggli (2005) and Thiede & Eggli (2007) unless other references are specified. *Afrovivella* was not sampled by us. *Cremnophila* has not been accepted as a genus separate from *Sedum* by Thiede & Eggli (2007).

Genus	Clade affiliation	Inferred phylogeny	Distribution	Life form	Phyllotaxis	Inflorescence insertion	Flower merism	Petal fusion
Afrovivella A.Berger	Leucosedum	Not sampled	Ethiopian highlands	Perennial herbs	Sessile rosettes	Lateral	5–7	Fused for 1/3 of corolla length
Cremnophila Rose	Acre	Unresolved	Mexico (Mexico, Morelos)	Perennial	Alternate, rosulate	Lateral	5	Slightly fused at base
Dudleya Britton & Rose	Leucosedum	Monophyletic	W U.S.A., NW Mexico	Perennial	Terminal rosettes	Lateral	(4–)5	Fused[a]
Echeveria DC.	Acre	Polyphyletic	S U.S.A. (Texas) to N Argentina	Perennial herbs to subshrubs	Alternate, rosulate, rarely scattered along stem	Lateral	5	Fused
Graptopetalum Rose	Acre	Polyphyletic	S U.S.A. (Arizona), Mexico	Perennial herbs to subshrubs	Terminal rosettes	Lateral	(4–)5, (7–10)	Free or slightly fused at base
Lenophyllum Rose	Acre	Monophyletic[b]	S U.S.A. (S Texas), NE Mexico	Perennial herbs	Decussate	Terminal	5	Free
Pachyphytum Link & al.	Acre	Monophyletic	E to C Mexico	Subshrubs	Alternate, rosulate	Lateral	5(–6)	Free[a]
Pistorinia DC.	Leucosedum	Only 1 sp. sampled	Iberian peninsula, N Africa	Annual herbs	Alternate	Terminal	5	Fused for ≥1/2 of corolla length
Prometheum (A.Berger) H.Ohba	Leucosedum	Monophyletic	N Greece to N Iran, incl. Caucasus	Annual to perennial herbs	Sessile rosettes, alternate on stems	Terminal or lateral	5	Fused for ≤1/2 of corolla length, never free
Rosularia (DC.) Stapf	Leucosedum	Monophyletic, but only 2 spp. sampled	SE Aegean islands, Cyprus, Near East, Karakorum, Himalayas, Altai	Perennial herbs	Sessile rosettes, alternate on stems	Terminal or lateral	5–9	Fused for 1/10 to 3/4 of corolla length
Sedella Britton & Rose	Leucosedum	Only 1 sp. sampled	W U.S.A.	Annual herbs	Decussate near base, alternate above	Terminal	5	Slightly fused at base
Sedum L.	Acre and Leucosedum	Polyphyletic	America, Eurasia, N, C and E Africa	Annual to perennial herbs to subshrubs	Alternate, rarely decussate or in whorls of 3 or 4, rarely rosulate	Terminal, rarely lateral	(3–)5 (–12)	Free or fused for up to 2/3 of corolla length
Thompsonella Britton & Rose	Acre	Paraphyletic	C and S Mexico	Perennial herbs to subshrubs	Sessile or terminal rosettes	Lateral	5	Shortly fused at base
Villadia Rose	Acre	Polyphyletic	S U.S.A., Mexico, Guatemala, Peru	Perennial herbs or subshrubs	Alternate	Terminal	(4–)5	± shortly fused at base

a – García & Chávez (2003); b – Carrillo-Reyes & al. (2009)

(Continues in the right part ↓)

(Continued from the left part ↓)

Table 2 (right part).

Genus	Petal colour	Number of stamens	Trichomes	Rosette branching sympodial	Leaf apex	Filament insertion	Seeds per follicle	Testa ornamentation
Afrovivella A.Berger	White, outside tinged reddish	2× petals	Glandular, leaf margins ciliate	No	Mucronate	n.s.	n.s.	Costate[c]
Cremnophila Rose	Greenish-white to yellow	2× petals	None	No	Rounded to broadly acute	n.s.	n.s.	n.s.
Dudleya Britton & Rose	White, yellow, orange or red	2× petals	None	Yes (when branched)	Obtuse-rounded to acutely pointed	At corolla base, antepetalous stamens slightly higher than antesepalous stamens	n.s.	Ribbed
Echeveria DC.	White to yellow, red to orange, rarely green(ish)	2× petals	None or non-glandular	No	Obtuse to acute, usually mucronate	Antepetalous stamens fused to corolla at top of nectar cavities, antesepalous stamens at top of corolla tube	n.s.	Smooth or reticulate
Graptopetalum Rose	Whitish or yellowish (to greenish), reddish to brown blotches, rarely uniformly coloured	2× petals, rarely 1× petals	None	No	Rounded to conspicuously mucronate	Antepetalous stamens fused to corolla, antesepalous stamens hardly to conspicuously fused to corolla and intercarpellar tissue	Many	Reticulate
Lenophyllum Rose	Yellow(ish)	2× petals	None	No	Rounded to acuminate	Antepetalous stamens fused to petals for ±1/2 their length	Many	Longitudinally striate
Pachyphytum Link & al.	White to pink, rarely orange to red(dish), inside in upper part often with red blotch	2× petals	None	No	Obtuse to acute, rarely mucronate	Antepetalous stamens fused to corolla, antesepalous stamens (almost) free	Many	Fairly smooth
Pistorinia DC.	Yellow, pink or purple, often finely spotted	2× petals	Glandular	No	Obtuse to subacute	Slightly below mouth of corolla tube	n.s.	Costate
Prometheum (A.Berger) H.Ohba	Yellow, cream, white, pink or red	2× petals	Glandular	No	Rounded to mucronate	n.s.	n.s.	Costate
Rosularia (DC.) Stapf	White, pale yellow, pink, pinkish-purple or pinkish-brown	2× petals	None or glandular	No	Never mucronate	n.s.	n.s.	Longitudinally striate

(Continues)

Table 2 (right part). Continued.

Genus	Petal colour	Number of stamens	Trichomes	Rosette branching sympodial	Leaf apex	Filament insertion	Seeds per follicle	Testa ornamentation
Sedella Britton & Rose	Bright to pale or greenish-yellow	1× petals, rarely 2× petals	None	No	Obtuse to rounded	Fused to petal bases	1	Longitudinally striate
Sedum L.	Yellow, white, pink, purple or reddish	2× petals, rarely 1× petals	None, glandular or non-glandular	No	Rounded to mucronate	Antepetalous stamens or all stamens (when petals are fused) fused to petal bases, rarely higher up	1 to many	Costate, bipapillate, or reticulate (-papillate)
Thompsonella Britton & Rose	Outside pale, inside dark purplish-red	2× petals	None	No	Rounded to mucronate	n.s.	Many	Reticulate with irregular longitudinal rows
Villadia Rose	Whitish to pink or reddish	2× petals	None or rarely non-glandular	No	Obtuse to acute	Basally fused to corolla for 0.6–2.8(–4) mm	n.s.	Centrally papillose (always?)

c – Eggli (1988); n.s. – not specified in the literature

In all ITS analyses (Fig. 1B–D, suppl. Fig. S1), the monophyletic Acre clade, beside several lineages/single species of *Sedum*, contained a predominantly North American clade with *Echeveria* (non-monophyletic), *Cremnophila* (included in *Sedum* by Thiede & Eggli, 2007), *Graptopetalum* (incl. *Tacitus* Moran; non-monophyletic), *Pachyphytum*, *Thompsonella* (non-monophyletic when including *T. mixtecana* J.Reyes & L.G.López), *Lenophyllum* and *Villadia* (non-monophyletic). In our combined analysis (Fig. 3C), *Cremnophila* and *Pachyphytum* were not sampled but the other genera listed were recovered in the American subclade of the Acre clade. In the analyses of ITS (Fig. 1C,D) and the combined ITS and plastid data (Fig. 3C), the Echeveria clade (*Graptopetalum glassii* Acev.-Rosas & Cházaro through *Sedum morganianum* E.Walther; *Echeveria fulgens* Lem. through *S. corynephyllum* Fröd., respectively) and the Villadia clade (*Sedum grandyi* Raym.-Hamet through *S. allantoides* Rose and *S. plicatum* Thiede & 't Hart through *S. alexanderi* Eggli, respectively) were recovered. These had been known before as well-supported subgroups of the American part of the Acre clade (Mort & al., 2001; Thiede & Eggli, 2007; Carrillo-Reyes & al., 2008, 2009; Nikulin & al., 2016). *Sedum* species of the Echeveria clade belong to the following infrageneric taxa: subg. *Pachysedum* (A.Berger) R.T.Clausen, sections *Craigia* R.T. Clausen, *Dendrosedum* A.Berger and *Pachysedum* A.Berger and the unranked taxon *Dendroideum* Fröd. (Americana Orthocarpia). *Sedum* representatives of the Villadia clade have been classified in the former genus *Altamiranoa* Rose, and in *Sedum* sections *Cockerellia* R.T.Clausen & C.H.Uhl ("subg. *Eusedum* (Boiss.) R.T.Clausen"), *Centripetalia* Alexander, *Fruticisedum* A.Berger, *Pachysedum* and *Leptosedum* A.Berger, series *Alamosana* Praeger, *Americana* A.Berger, *Compacta* A.Berger and *Moranensia* A.Berger ("sect. *Seda genuina* W.D.J.Koch") and the unranked taxa *Moranense* Fröd. (Americana Kyphocarpia), *Alamosanum* Fröd., *Andinum* Fröd., *Bourgaei* Fröd., *Conzattii* Fröd. and *Oxypetalum* Fröd. (Americana Orthocarpia). The remaining *Sedum* species of the American Acre clade, which did not fall into either of the clades Echeveria or Villadia, belong to the former genus *Altamiranoa*, *Sedum* sections *Dendrosedum*, *Fruticisedum* and *Sedastrum* (Rose) A.Berger, series *Acria* A.Berger, *Americana*, *Compacta*, *Oaxacana* A. Berger and *Pulchella* A.Berger ("sect. *Seda genuina*") and the unranked taxa *Moranense* and *Napiferum* Fröd. (Americana Kyphocarpia), *Alamosanum*, *Conzattii*, *Dendroideum*, *Longipes* Fröd., *Minimum* Fröd., *Oxypetalum* and *Sedastrum* Praeger (Americana Orthocarpia), the Parvum group and the *Sedum palmeri* group. Lastly, a subclade of three Macaronesian *Sedum* species (*S. lancerottense* R.P.Murray through *S. nudum* Aiton; Fig. 1C) belonging to ser. *Macaronesia* (Fröd.) Afferni was part of the basal polytomy of the American Acre clade.

Species of *Sedum* in the remaining (mainly Eurasian) part of the Acre clade formed a polytomy of eight well-supported subclades and four single species in the ITS analyses (Fig. 1B,C). Two of these subclades (*S. uniflorum* Hook. & Arn. through *S. tosaense* Makino and *S. erythrospermum* Hayata through *S. obtrullatum* K.T.Fu) are distributed in Asia and had previously been retrieved as clades by Ito & al. (2017). In their phylogenetic study of Taiwanese *Sedum*, Ito & al. (2017) sampled more *Sedum* species of these two clades than we did here. All other lineages in this part of the tree have a clearly western Eurasian/Mediterranean distribution, with the exception of *S. meyeri-johannis* Engl. and *S. ruwenzoriense* Baker f., which occur in parts of central and east Africa. *Sedum* species of the two Asian subclades belong to sections

Table 3. Infrageneric groups of *Sedum* (and groups of *Sedum* including other currently recognized genera) that are phylogenetically well-supported and clearly definable by morphology within their distributional range.

Name	Species comprised	Defining characters / Distribution	$n^{\#}$	Literature
Sect. *Centripetalia* Alexander	***S. alexanderi***, ***S. allantoides***	Leaves very thick (0.5–1.2 cm), petals free, narrow-based and with red blotches, flowers in terminal compound pleiochasia (Uhl, 1980), centripetal order of flowering / Mexico (Oaxaca)	29	Alexander (1942), Moran (1966), Uhl (1980)
Sect. *Gormania* (Britton) R.T.Clausen (incl. sect. *Oreganica* R.T.Clausen)	*S. albomarginatum*, *S. laxum*, *S. moranii*, *S. oblanceolatum*, ***S. obtusatum***, ***S. oreganum***, ***S. oregonense***	Leaves broadest towards the tips, leaf tips often retuse, petals basally fused and divergent upward / W U.S.A.	–	Britton & Rose (1903), Clausen (1942, 1975), Clausen & Uhl (1944), Denton (1982)
Sect. *Lanceolata* R.T.Clausen	*S. borschii*, ***S. debile***, *S. divergens*, *S. elrodii**, *S. lanceolatum*, ***S. leibergii***, *S. nuttallianum*, *S. radiatum*, *S. rupicola*, *S. stenopetalum*	Non-rhizomatous herbs, leaves narrow-elongate, petals free, yellow, flowers in tripartite cymes (Clausen, 1975) / C and W U.S.A., W Canada	8	Clausen (1975), Uhl (1977)
Sect. *Sedastrum* (Rose) A.Berger	***S. chazaroi***, ***S. ebracteatum***, *S. glabrum*, ***S. hemsleyanum***, *S. hintonii*, *S. jarocho*, ***S. mocinianum***, *S. piaxtlaense*, *S. roberti*	Basal rosettes present, leaves and inflorescences generally pubescent, inflorescences more or less paniculate (Britton & Rose, 1905), petals white, carpels basally concave / Mexico (except for NW and SE Mexico)	–	Britton & Rose (1905), Berger (1930), Clausen (1943b), Uhl (1992a), Pérez-Calix (1998), Carrillo-Reyes & Lomelí-Sención (2008), Jimeno-Sevilla & al. (2012), Santiago & al. (2015)
Ser. *Alba* A.Berger	***S. album***, ***S. gypsicola***	Plants sparsely glandular-pubescent, leaves alternate, obtuse or rounded, flowers pentamerous, pedicellate, sepals basally fused with the receptacle, petals white, follicles many-seeded, erect, seeds oblong or ovoid, with a costate testa and acute apex / Europe, N Africa (Morocco, Algeria), Near East	17	Berger (1930), ’t Hart (1991), ’t Hart & Alpınar (1995)
Ser. *Alpestria* A.Berger sensu Berger (1930)	***S. alpestre***, ***S. grisebachii***	Perennial herbs without glandular trichomes, leaves alternate, widest above the middle, with a short, never 3-lobed spur, usually not persistent when senescent, flowers confined to a terminal cyme (very rarely in axillary cymes), strictly pentamerous, petals yellow, nectar scales square or oblong-linear, follicles (pale) brown, never yellowish, seeds with a reticulate (-papillate) testa / mountains of C and S Europe and N Anatolia, Sardinia, Macedonia, Bulgaria and Greece	8	Berger (1930), Webb & al. (1993)
Ser. *Alsinefolia* A.Berger	***S. alsinefolium***, ***S. fragrans***	Plants densely glandular-pubescent, leaves basally rosulate, flowers pedicellate, in a lax inflorescence, sepals basally fused with the receptacle, petals basally fused, seeds with a costate testa and coronate apex / French-Italian Alps	–	Berger (1930), ’t Hart (1991)

(Continues)

Table 3. Continued.

Name	Species comprised	Defining characters / Distribution	$n^{\#}$	Literature
Ser. *Anglica* ’t Hart	***S. anglicum***, *S. arenarium*, ***S. farinosum******	Plants glabrous, sepals basally free, petals white, follicles stellate-patent, with inconspicuous lips; seeds with a reticulate testa and coronate apex / Atlantic parts of W Europe and N Africa, Madeira	12	’t Hart (1991)
Ser. *Cepaea* (W.D.J.Koch) Afferni	***S. cepaea***, *S. creticum*, ***S. cyprium***, *S. lampusae*, ***S. magellense***, *S. microstachyum*, *S. tristriatum*	Plants glandular-pubescent, leaves alternate, opposite or in whorls of 4, flat, often in basal rosettes (except *S. magellense*), flowers pentamerous, shortly pedicellate, sepals basally united, petals white or pink, follicles erect, testa costate / S and SE Europe (incl. Balkans and S Greek islands), Cyprus	–	Fröderström (1932), ’t Hart (1991), ’t Hart & Alpınar (1995), Afferni (2012)
Ser. *Dasyphylla* ’t Hart	***S. dasyphyllum***, ***S. mucizonia***, ***S. wilczekianum******	Plants densely glandular-pubescent, leaves alternate or decussate, obtuse or subacute, never in sessile rosettes, flowers pentamerous, pedicellate, petals white to pink, follicles many-seeded, erect, seeds with a costate testa and acute apex / C and S Europe, N Africa, Anatolia	–	’t Hart (1991), ’t Hart & Alpınar (1995)
Ser. *Macaronesia* (Fröd.) Afferni	***S. brissemoretii***, *S. fusiforme*, ***S. lancerottense***, ***S. nudum***	Petals yellow, sometimes tinged red / Madeira, Lanzarote	–	Fröderström (1932), Afferni (2014)
Parts of ser. *Alpestria* A.Berger sensu ’t Hart (1991)	***S. apoleipon***, ***S. borissovae***, ***S. laconicum***, ***S. ursi***	Plants without glandular trichomes, leaves alternate, never arranged in six rows, with a short and/or truncate, never 3-lobed spur, flowers strictly pentamerous, sepals up to 3 mm long, basally fused with the receptacle, petals yellow, seeds brown, never reddish, with a reticulate-papillate testa / Greece, S Ukraine, Turkey (mountains of W Anatolia), Israel and Lebanon (disjunct area)	–	’t Hart (1978, 1991), ’t Hart & Alpınar (1995)
	S. tuberiferum, ***S. urvillei***	Perennial herbs without glandular trichomes, leaves alternate, widest below the middle or (if widest at or above the middle) with a 3-lobed spur, dead leaves usually persistent, flowers strictly pentamerous, sepals basally fused with the receptacle, petals yellow, seeds with a reticulate-papillate testa / from Hungary throughout the Balkans, Turkey (C and S Anatolia) and Ukraine (Crimea); 800–2450 m	16	’t Hart (1978, 1991), Webb & al. (1993), ’t Hart & Alpınar (1995)
Part of ser. *Subrosea* ’t Hart incl. genus *Pistorinia* DC.	*Pistorinia brachyantha*, ***P. breviflora***, *P. hispanica*, ***Sedum lagascae***, *S. maireanum*, ***S. villosum***	Densely glandular-pubescent annual or biennial (rarely perennial) herbs, leaves terete to semiterete, sepals basally fused with the receptacle, stamens 10, seeds coronate, with a costate testa / N, C and W Europe (especially Iberian peninsula), Iceland, Greenland, E Canada, N Africa	15	’t Hart (1991), ’t Hart & Alpınar (1995)
Part of former *Altamiranoa* Rose	***S. goldmanii***, ***S. jurgensenii***	Plants perennial, flowers in cincinni (’t Hart & Bleij, 2005), petals basally fused for approximately 1–5 mm / Mexico	23	Britton & Rose (1903), Berger (1930), Fröderström (1935), Moran (1996)
Part of *Alamosanum* Fröd. [unranked]	***S. alamosanum***, ***S. trichromum***	Plants perennial, not woody, tufted, with short sterile shoots, leaves alternate, flowers pedicellate, petals free, carpels erect or nearly so / Mexico (Durango, Sonora)	–	Fröderström (1935)

(Continues)

Table 3. Continued.

Name	Species comprised	Defining characters / Distribution	$n^{\#}$	Literature
–	*S. churchillianum*, *S. epidendrum*, ***S. meyeri-johannis***, *S. mooneyi*, ***S. ruwenzoriense***	At least some leaves in whorls of three (leaves alternate throughout in *S. ruwenzoriense*), petals bright yellow / parts of C and E Africa	–	Gilbert (1985)
Echeveria clade	***S.* sect. *Pachysedum***, ***Cremnophila***, ***Echeveria***, ***Graptopetalum***, ***Pachyphytum***, ***Thompsonella***	Perennial shrubs to subshrubs without rhizomes, stems never short and thick, never unbranched nor sympodially branched, leaves alternate, rosulate (sessile or at stem tips), inflorescences lateral, nectar scales inconspicuous / America	–	Berger (1930), Clausen (1943a), Uhl (1978), García & Chávez (2003), Acevedo-Rosas & al. (2004a,b), Eggli (2005), Carrillo-Reyes & al. (2008, 2009)

Nomenclature of species follows ’t Hart & Bleij (2005). Species sampled by us are marked by bold font.
[#] Base chromosome numbers (n) are given only when shared by all species of a group. Information obtained from the literature cited and ’t Hart & Bleij (2005).
* Species tentatively placed in the respective group by ’t Hart & Bleij (2005).

Oreades (Fröd.) K.T.Fu and *Sedum* (sensu Fu & Ohba, 2001), groups 2 and 6 of sect. *Epeteium* Boiss., series *Bracteatae* (Fröd.) S.H.Fu (“sect. *Asiatica Genuina Kyphocarpia* Fröd.”), *Chauveardica* K.T.Fu, *Longifuniculata* K.T.Fu, *Oreades* K.T.Fu and *Trullipetala* K.T.Fu (sect. *Oreades* sensu K.T. Fu, 1974), *Chinensia* A.Berger, *Galioidea* A.Berger, *Hametiana* A.Berger, *Involucrata* Maxim. and *Orientalia* A.Berger (“sect. *Seda genuina*”) and the unranked taxa *Douglasii* Fröd. (Americana Kyphocarpia), *Bracteata* Fröd. and *Japonica* Maxim. (Asiatica Genuina Kyphocarpia) and *Oreades* Fröd. (Asiatica Genuina Orthocarpia). *Sedum anglicum* Huds. and *S. farinosum* Lowe have been classified as part of *Sedum* series *Alba* A.Berger (“sect. *Seda genuina*”) and *Anglica* ’t Hart or as part of the unranked taxon *Stoloniferum* Fröd. (Eurasiatica Kyphocarpia). The subclade *Sedum laconicum* Boiss. & Heldr. through *S. borissovae* Balk. solely contains species of *Sedum* ser. *Alpestria* A.Berger in the sense of ’t Hart (1991) and is, thus, one of the well-characterized monophyletic groups in Table 3. The remaining species in this basal polytomy of the Acre clade (i.e., *S. alpestre* Vill. through *S. acre* L.) have been classified in series *Acria* (“sect. *Seda genuina*”) and *Alpestria*, the Acre group in ’t Hart’s (1978) earlier circumscription, as well as the east African Meyerijohannis group of subg. *Sedum*. The well-supported sister relationship between *Sedum hillebrandtii* and *S. sexangulare* L. is reflected in Fenzl’s (1856) notion that the two species, together with *S. acre*, are closely related to each other. Berger (1930) classified both species in his ser. *Mitia* A.Berger (“sect. *Seda genuina*”), which, however, also comprises *S. laconicum*, and neither this latter species nor *S. acre* are supported as sister to this clade in our ITS phylogenies.

Similar to earlier studies of only plastid DNA (Van Ham, 1995; Van Ham & ’t Hart, 1998), the Leucosedum clade was resolved as monophyletic only in cpDNA/BEAST (Fig. 2A). In all other analyses, the position of *Sedum* ser. *Cepaea* (W.D.J.Koch) Afferni (of which *S. cepaea* L., *S. cyprium* A.K.Jacks. & Turrill and *S. magellense* Ten. were sampled here) relative to the remainder of the Leucosedum and Acre clades was unresolved (Figs. 1B, 3B, suppl. Fig. S1). In ITS/ML (Fig. 1B) the Leucosedum clade consisted of 20 lineages/single species, of which four lineages included *Dudleya*, *Sedella*, *Pistorinia*, *Prometheum* and *Rosularia*. The remaining 16 lineages/single species were exclusively composed of *Sedum* species that belong to the former genus *Sempervivella* Stapf, *Sedum* subg. *Telmissa* (Fenzl) H.Ohba, sections *Sempervivoides* Boiss. and *Telmissa* (Fenzl) Schönland, and groups 1, 5 and 6 of sect. *Epeteium*, series *Alba* and *Alsinefolia* A.Berger (“sect. *Seda genuina*”), *Aithales* (Webb & Berthel.) ’t Hart, *Cepaea*, *Confertiflora* ’t Hart, *Dasyphylla* ’t Hart, *Glauco-rubens* Fröd., *Gracile* ’t Hart & Alpınar, *Hirsuta* Fröd., *Macrosepala* (Regel & Schmalh.) Boriss., *Pedicellata* ’t Hart, *Rubra* Boriss., *Subrosea* ’t Hart, *Subulata* ’t Hart, *Telmissa* (Fenzl) ’t Hart & Alpınar and *Tenella* ’t Hart & Alpınar, subseries *Cepaea* and *Cretica* Afferni (ser. *Cepaea*), and the unranked taxa “*Epeteium eurasiaticum* Fröd.”, *Glauco-rubens* Fröd. and *Stoloniferum*

(Eurasiatica Kyphocarpia), *Album* Fröd., *Cepaea* W.D.J. Koch and *Hirsutum* Fröd. (Eurasiatica Orthocarpia), and the Crassularia group. *Sedum* species of the American subclade of the Leucosedum clade (i.e., *Sedum obtusatum* through *Sedella pumila* (Benth.) Britton & Rose in Fig. 1B) belong to sections *Lanceolata* R.T.Clausen and *Ternata* A.Berger (subg. *Sedum*), *Gormania*, *Oreganica* R.T.Clausen and *Rosulata* (A.Berger) R.T.Clausen & C.H.Uhl (subg. *Gormania* (Britton) R.T.Clausen), series *Rosulata* A.Berger and *Ternata* A.Berger (“sect. *Seda genuina*”) and the unranked

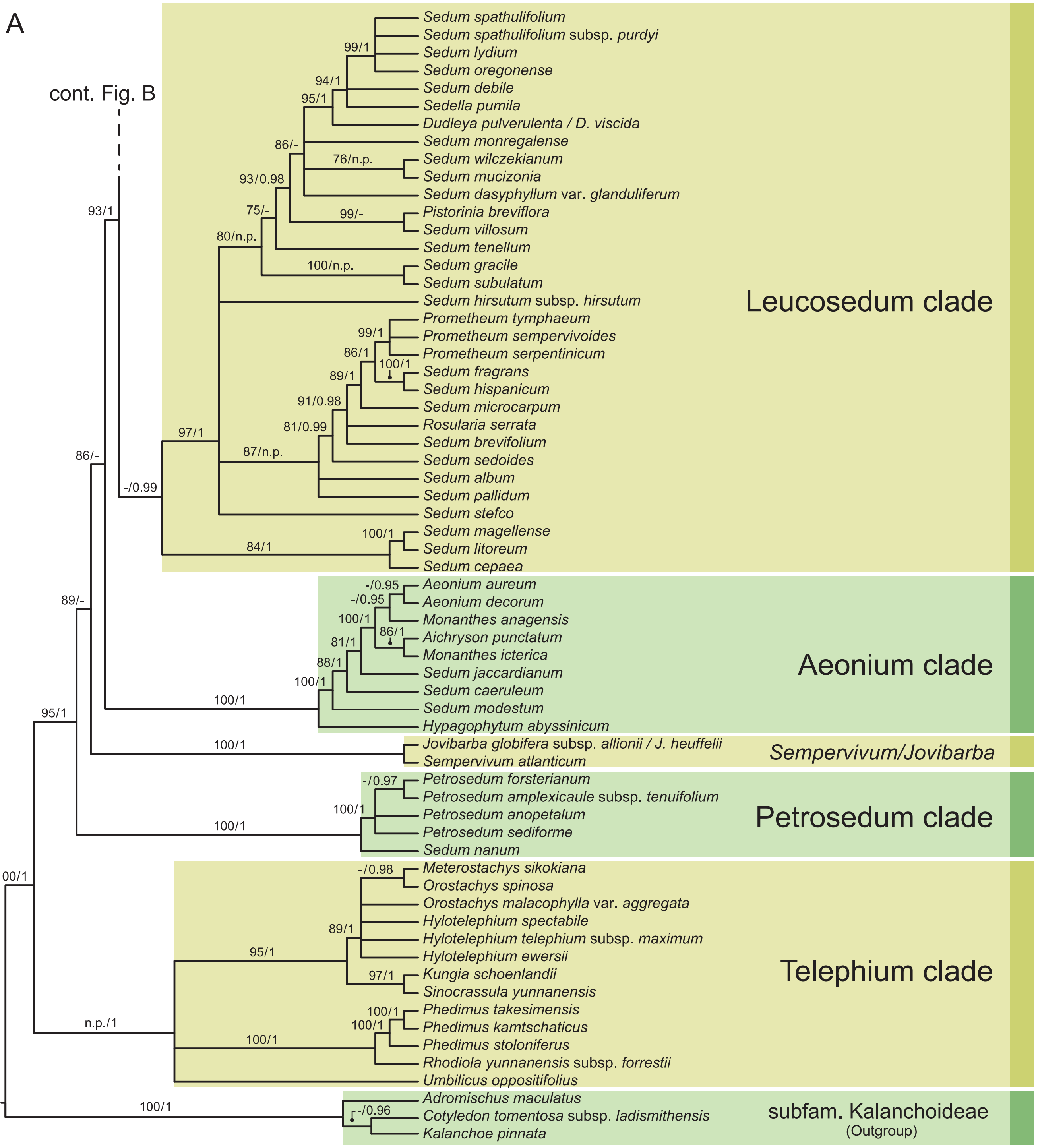

Fig. 2. Molecular phylogeny of Crassulaceae subfam. Sempervivoideae based on a concatenated sequence matrix of the plastid DNA markers *matK*, *rps16* and *trnL-trnF* and inferred using maximum likelihood (cpDNA/ML) and Bayesian inference (cpDNA/BEAST). Branch labels give bootstrap support values for cpDNA/ML (left) and posterior probabilities for cpDNA/BEAST (right; n.p.: clade not present; –: clade present but unsupported). Branches with bootstrap support <75 and posterior probability <0.95 were collapsed. Names of major clades as used in the main text are given.

taxa *Spathulifolium* Fröd. and *Ternatum* Fröd. (Americana Kyphocarpia) and *Alamosanum* (Americana Orthocarpia). *Sedum lagascae* Pau and *S. villosum* L., which were resolved as the closest relatives of *Pistorinia* (we only sampled *P. breviflora* Boiss.) whenever these species were sampled (Figs. 1B, 2A, suppl. Fig. S1), belong to ’t Hart’s (1991) ser. *Subrosea* (also containing the apparently more distantly related *S. nevadense* Coss.; Fig. 1B, suppl. Fig. S1), and these together were sister to *S. monregalense* Balb. of the monospecific ser. *Monregalensia* ’t Hart in our ITS phylogenies (Fig. 1B, suppl. Fig. S1). Lastly, *Prometheum* was resolved as sister to *Sedum hispanicum* L. of ser. *Glauco-rubens* in

Fig. 2. Continued.

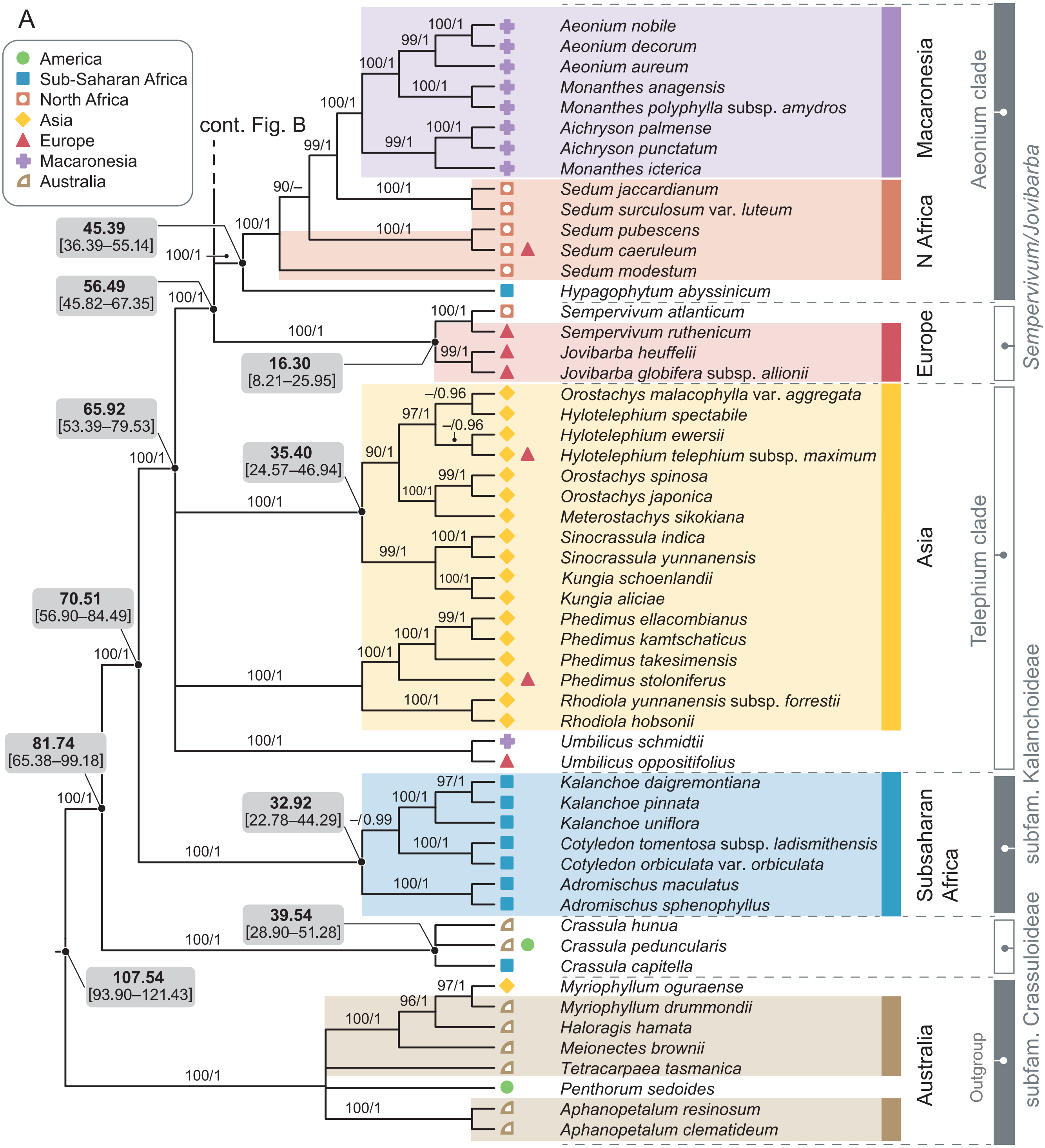

Fig. 3. Family-wide molecular phylogeny of Crassulaceae based on a concatenated sequence matrix of the combined nuclear and plastid DNA markers ITS, *matK*, *rps16* and *trnL-trnF* and inferred using maximum likelihood (combined/ML) and Bayesian inference (combined/MrBayes). Branch labels give bootstrap support values for combined/ML (left) and posterior probabilities for combined/MrBayes (right). Branches with bootstrap support <75 and posterior probability <0.95 were collapsed or marked "–" when support was only found in one of the two analyses. Node labels in gray boxes provide mean crown group age estimates and 95% confidence intervals in million years (see Table 4) as inferred in the dating analysis of ITS sequences (see suppl. Fig. S1 and Materials and Methods for more information). Geographical distribution is indicated by colour, and symbols as explained in the figure inset.

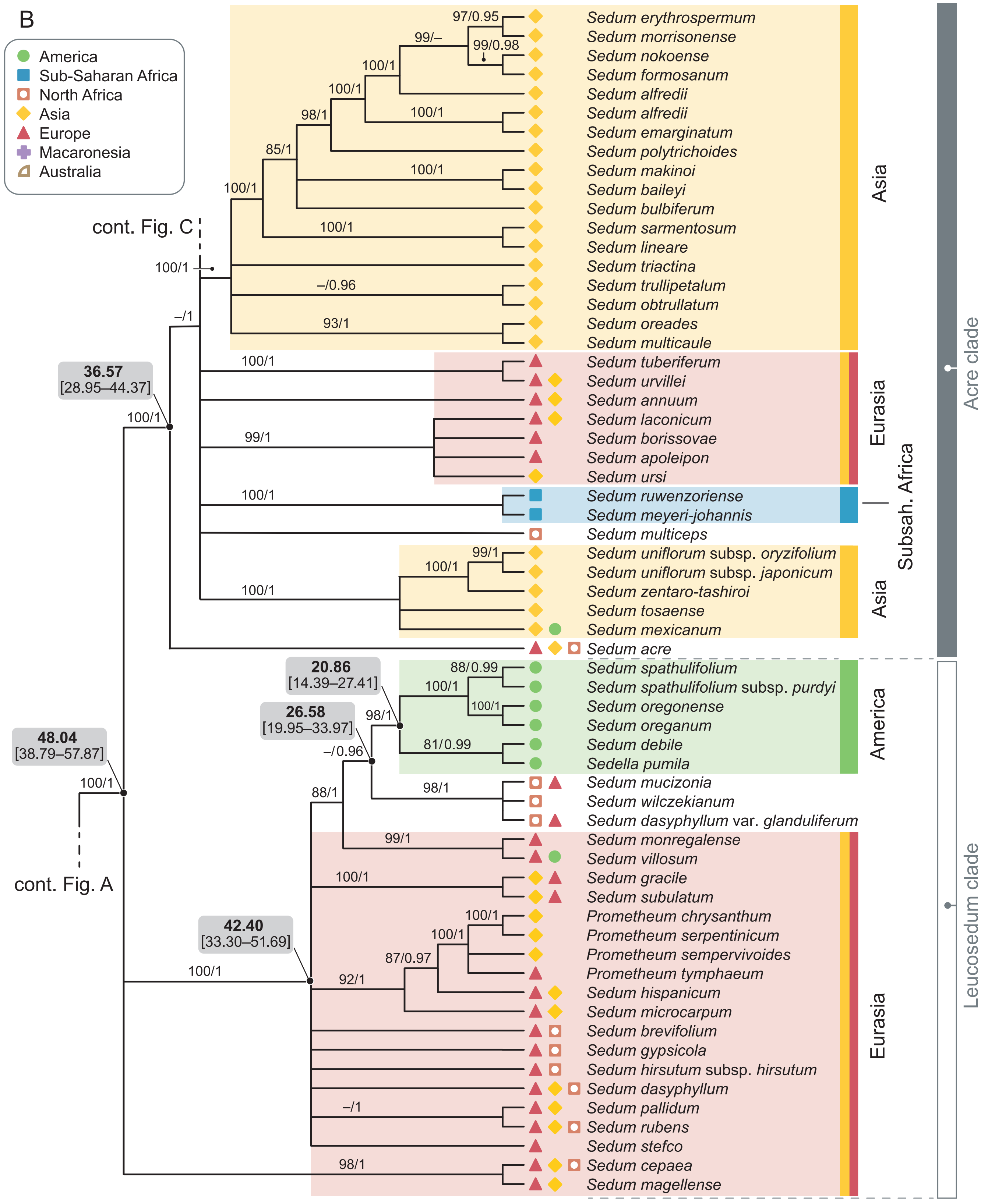

Fig. 3. Continued.

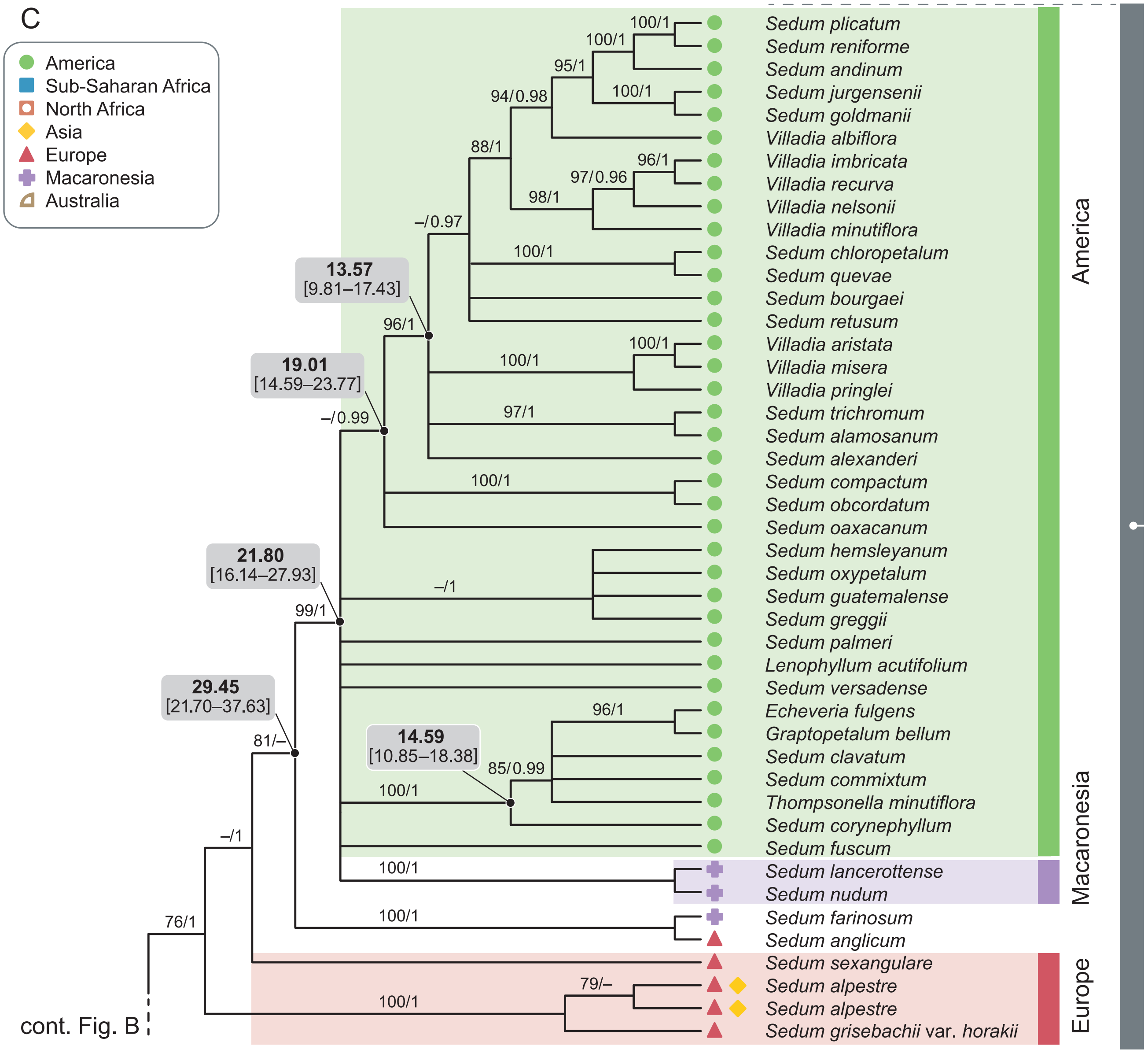

Fig. 3. Continued.

all analyses that included ITS sequence data (Figs. 1B, 3B, suppl. Fig. S1), but *S. hispanicum* is in turn most closely related to *S. fragrans* of ser. *Alsinefolia* in the analyses of plastid DNA (Fig. 2A).

In summary, four monophyletic major groups could be identified when the Telephium clade is considered as unsupported (supported only in cpDNA/BEAST, Fig. 2A). These are (1) the Petrosedum clade, (2) *Sempervivum*/*Jovibarba*, (3) the Aeonium clade and (4) clades Leucosedum plus Acre in which the latter is monophyletic and the former is monophyletic when plastid sequence data alone were used (Fig. 2A). While our plastid data resolved the Telephium clade and these four clades as successive sister clades (Telephium clade (Petrosedum clade (*Sempervivum*/*Jovibarba* (Aeonium clade, clades Leucosedum plus Acre)))), the Petrosedum clade was consistently recovered as sister to the Aeonium clade in our ITS analyses (Fig. 1A, suppl. Fig. S1).

Molecular dating. — The results of our dating analysis are shown in detail in suppl. Fig. S1, and stem and crown ages of the five major clades as well as other important clades are summarized in Table 4 and shown in the phylogeny based on combined nuclear and plastid sequence data (Fig. 3). After a burn-in of 25%, three of the four independent MCMC runs yielded an effective sampling size between 170 and 200 for one parameter (i.e., the mrcatime parameter of one of the clades that had been predefined as monophyletic). However, because the four runs converged to the same estimate for this parameter, we considered the analysis to be robust.

Table 4. Stem (**S**) and crown (**C**) age estimates of selected clades in the Crassulaceae chronogram (see suppl. Fig. S1) based on Bayesian inference using a lognormal relaxed clock.

Clade	Estimated age [95% confidence interval]
Crassulaceae **S**	107.54 [93.90–121.43]
Crassulaceae **C****	81.74 [65.38–99.18]
subfam. Crassuloideae **C****	39.54 [28.90–51.28]
subfam. Sempervivoideae **S***	70.51 [56.90–84.49]
subfam. Kalanchoideae **C****	32.92 [22.78–44.29]
subfam. Sempervivoideae **C****	65.92 [53.39–79.53]
Telephieae **C**** (Telephium clade)	35.40 [24.57–46.94]
Umbiliceae **S**$^{-}$ (Telephium clade)	63.43 [51.45–75.64]
Umbiliceae **C**$^{-}$ (Telephium clade)	56.54 [41.53–71.11]
Petrosedum + Aeonium clade **S****	56.49 [45.82–67.35]
Petrosedum **C****	31.93 [19.86–44.57]
Aeonium clade **S****	52.13 [42.06–62.60]
Aeonium clade **C****	45.39 [36.39–55.14]
Sempervivum/*Jovibarba* **S****	51.97 [42.26–62.66]
Sempervivum/*Jovibarba* **C****	16.30 [8.21–25.95]
Sedeae **C****	48.04 [38.79–57.87]
Leucosedum clade (excl. *Rosularia* and *Sedum* ser. *Cepaea*) **C****	42.40 [33.30–51.69]
American subclade of Leucosedum **S**$^{-}$	26.58 [19.95–33.97]
American subclade of Leucosedum **C****	20.86 [14.39–27.41]
Acre clade **C****	36.57 [28.95–44.37]
American subclade of Acre (incl. ser. *Macaronesia*) **S***	29.45 [21.70–37.63]
American subclade of Acre (incl. ser. *Macaronesia*) **C****	21.80 [16.14–27.93]
Echeveria clade **S**$^{-}$	17.01 [12.80–21.34]
Echeveria clade **C****	14.59 [10.85–18.38]
Villadia clade **S**$^{-}$	19.01 [14.59–23.77]
Villadia clade **C****	13.57 [9.81–17.43]

Age estimates are given in million years as mean and 95% confidence interval. Symbols following C or S indicate the inferred posterior probabilities (p.p.): $^{-}$ p.p. < 0.90; *0.90 ≤ p.p. < 0.95; ** p.p. ≥ 0.95

■ DISCUSSION

Backbone phylogeny and major clades in Sempervivoideae. — Our analysis of the largest taxon sample of Sempervivoideae analyzed so far using both ITS and, for a smaller sample, plastid DNA sequences led to the recognition of clades and relationships that had not been recognized before.

(1) The Telephium clade, never before resolved as supported monophyletic (Van Ham & ’t Hart, 1998; Mort & al., 2001; Mayuzumi & Ohba, 2004; Gontcharova & al., 2006), was supported as monophyletic in cpDNA/BEAST (Fig. 2A) in which it was sister to the remainder of Sempervivoideae. This clade had been suspected to be monophyletic based on the shared presence of a 6 bp insertion in *matK* by Mort & al. (2001). In all other analyses (Figs. 1A, 3A, suppl. Fig. S1), the three well-supported sublineages of this clade were part of a polytomy with the monophyletic remainder of Sempervivoideae.

(2) Based on our plastid dataset (Fig. 2A), the Petrosedum clade and *Sempervivum*/*Jovibarba* are successive sister clades to the remainder of the subfamily without the Telephium clade. In phylogenies of the ITS dataset, however, the Petrosedum clade was sister to the Aeonium clade (Fig. 1A, suppl. Fig. S1), and in our dated phylogeny of ITS (suppl. Fig. S1), *Sempervivum*/*Jovibarba* were supported as the closest relatives of Sedeae. *Petrosedum* and *Sempervivum*/*Jovibarba* before had been treated as the Sempervivum clade (Van Ham & ’t Hart, 1998), although they had never been resolved as supported sister to each other. Therefore, there is no evidence for Semperviveae in that circumscription as a monophyletic group.

(3) The Aeonium clade was resolved as sister to the Acre and Leucosedum clades by our plastid data (Fig. 2A), a relationship never supported before, whereas our dating analysis of ITS supported *Sempervivum*/*Jovibarba* as sister to the Acre and Leucosedum clades (suppl. Fig. S1). The Aeonium clade was in turn sister to the Petrosedum clade in all phylogenies of ITS (Fig. 1A, suppl. Fig. S1).

(4) Finally, clades Acre and Leucosedum, together constituting Sedeae, were strongly supported as one clade in all our phylogenies except ITS/ML (Fig. 1B) in which this clade was unsupported. The Acre clade alone received substantial support in all our phylogenetic analyses, too. However, in our ITS phylogeny (Fig. 1A,B) the monophyletic Acre clade was part of a large polytomy containing a number of lineages representing the Leucosedum clade (both ITS/ML and ITS/MrBayes), but also *Sempervivum*/*Jovibarba* and the Aeonium and Petrosedum clades (ITS/ML only). Such a topology of the Acre and Leucosedum clades had already been found before (Mort & al., 2001; Nikulin & al., 2016). In the backbone of our phylogeny, relationships clearly were least resolved in this part of the tree.

(1) Telephium clade. — The Telephium clade of each of our analyses consisted of three sublineages. (1) To our knowledge, recognition of *Umbilicus*, a genus of 13 species found mainly in the Mediterranean area, Macaronesia, southwest Asia, Arabia and north to east Africa (Thiede & Eggli, 2007), as a genus distinct from *Sedum* has never been questioned. (2) Although *Rhodiola* is a Linnaean genus and has been treated as distinct from *Sedum* in many modern Floras (e.g., Webb, 1993), other Floras included *Rhodiola* in *Sedum* (e.g., Jäger, 2005, but not Jäger, 2011). *Phedimus*, also often treated as part of *Sedum* (Webb, 1993), has been re-segregated from *Sedum* by, e.g., ’t Hart (1995) and Ohba & al. (2000). This clade of *Phedimus* and *Rhodiola* also contains what has been recognized as *Pseudosedum* (not sampled here), another *Sedum* segregate according to Thiede & Eggli (2007). Although *Pseudosedum* may be sister to *Rhodiola*, Mayuzumi & Ohba (2004) discussed the option of including the former in the latter. This would be supported by the findings of Gontcharova & al. (2006), who found *Pseudosedum* to be the closest relative to only part of *Rhodiola*. Gontcharova & al. (2006) also segregated *Aizopsis* Grulich from *Phedimus*. Following Thiede & Eggli (2007), the *Phedimus/Rhodiola* clade contains ca. 88 species (*Phedimus*: 18; *Pseudosedum*: 12; *Rhodiola*: ca. 58) mainly in Asia with few species in Europe (*Phedimus*, *Rhodiola*) and North America (*Rhodiola*). These first two sublineages of the Telephium clade together form Umbiliceae as understood by Thiede & Eggli (2007). (3) The third sublineage has been classified as Telephieae (Thiede & Eggli, 2007) and contains *Hylotelephium*, a segregate of *Sedum* (Ohba, 1977), *Meterostachys*, *Sinocrassula*, *Orostachys* and *Kungia*, a segregate of a non-monophyletic *Orostachys* with two species from southwestern China and probably sister to *Sinocrassula*; see Thiede & Eggli (2007) and our phylogenies of ITS/ML (Fig. 1A), cpDNA (Fig. 2A) and the combined dataset (Fig. 3A). Neither *Hylotelephium* nor *Orostachys* were recovered as monophyletic in our analyses (Figs. 1A, 2A, 3A, suppl. Fig. S1) and in the analyses by Mayuzumi & Ohba (2004) and Gontcharova & al. (2006), and *Meterostachys* is nested in this group. However, all phylogenies in which more than one species of *Orostachys* subsect. *Appendiculatae* (Boriss.) H. Ohba was sampled (Figs. 1A, 3A, suppl. Fig. S1) suggested monophyly of this subsection and its sister relationship to *Meterostachys*, as also found by Mayuzumi & Ohba (2004) and Gontcharova & al. (2006). According to Thiede & Eggli (2007), this sublineage (Telephieae) contains ca. 48 species (*Hylotelephium*: ca. 27; *Kungia*: 2; *Meterostachys*: 1; *Orostachys*: 11; *Sinocrassula*: 7) mainly in Asia with few species in Europe and North America (*Hylotelephium*).

(2) Petrosedum clade. — *Sedum nanum* of ser. *Nana* as well as all species sampled of *Sedum* ser. *Rupestria* A.Berger belong to this clade; ser. *Rupestria* had been recognized at generic rank as *Petrosedum* by Grulich (1984) and accepted as such by Thiede & Eggli (2007), who emphasized the embryological and phytochemical distinctness of this lineage. Thus, Mauritzon (1933) recognized a pattern of endosperm and proembryo formation unique to *Petrosedum rupestre* (L.) P.V.Heath and *P. anopetalum* (DC.) Grulich, the representatives of *Petrosedum* sampled by him. This pattern (Rupestre-Type) is characterized by a much-elongated suspensor of the proembryo, which is surrounded by only one layer of endosperm cells. In the field, *Petrosedum* can be distinguished on the basis of its polymerous instead of pentamerous flowers, which probably is the main reason for its traditional association with *Sempervivum*/*Jovibarba*. According to Grulich (1984), *Petrosedum* is further characterized by the presence of glandular trichomes or papillae (in most but not all species) on bracts, pedicels, sepals, filaments and carpels (see Fig. 1A for the occurrence of glandular trichomes in the sampled species), by distinctly rigid and acute leaf apices and by a completely apocarpous, slender and upright gynoecium, which is "slightly submerged in a 'receptaculum' formed by connate parts of sepals and enlarged pedicels". Representatives of *Sedum* ser. *Nana* do not share these features with *Petrosedum*. The pronounced distinctness of *Petrosedum* from *Sedum* has again been demonstrated more recently in a study of micromorphological characters of Mediterranean *Sedum* s.l. (Giuliani & al., 2018) sampling five *Petrosedum* species as well as representatives of the clades Telephium, Leucosedum and Acre. *Petrosedum* currently comprises 14 species native to Europe, the Levant and North Africa (Thiede & Eggli, 2007), but with the inclusion of *Sedum* ser. *Nana* (from the Near East), as suggested by our findings, species number would increase to 9 or 12, depending on whether *Sedum caroli-henrici* Kit Tan, *S. elburzense* Akhani & Assadi and *S. yildizianum* Sümbül indeed belong to this lineage as suggested by 't Hart & Alpınar (2000) and 't Hart & Bleij (2005). The inclusion of *Sedum* ser. *Nana* in *Petrosedum* or as sister to *Petrosedum* is further corroborated by Van Ham & 't Hart's (1998) earlier finding that *S. assyriacum* Boiss. also falls in the Petrosedum clade.

(3) Sempervivum/Jovibarba. — This clade comprises *Sempervivum* and *Jovibarba*, treated as one genus by Thiede & Eggli (2007) but as two separate genera in the most recent phylogenetic analysis of this group (Klein & Kadereit, 2015). Klein & Kadereit (2015) considered *Sempervivum* to contain 46, and *Jovibarba* to contain 2 species. Both genera are oreophytes found mainly in western Eurasian mountain ranges.

(4) Aeonium clade. — This clade comprises *Aeonium* (incl. *Greenovia*), *Aichryson*, *Monanthes*, *Hypagophytum* and approximately eight *Sedum* species of series *Monanthoidea*, *Caerulea* and *Pubescens*, which formed two or three successive sister clades to the first three genera in our phylogenies, although ser. *Monanthoidea* probably is not monophyletic (see suppl. Table S1 and Figs. 1A, 2A, 3A and suppl. Fig. S1). According to our findings, *S. jaccardianum* Maire & Wilczek and *S. surculosum* Coss. of ser. *Monanthoidea* might be the closest relatives of the Macaronesian genera of the Aeonium clade, but only full sampling of the *Sedum* species of interest will clarify relationships in this part of the phylogeny. Morphological and karyological similarities between some North African *Sedum* and the Macaronesian genera had already been pointed out by Uhl (1961b). *Hypagophytum abyssinicum* (Hochst. ex A.Rich.) A.Berger received strong support as the earliest branching species of the Aeonium clade in all our analyses (Figs. 1A, 2A, 3A, suppl. Fig. S1). This only species of the northwest Ethiopian *Hypagophytum* had previously been treated as part of the Crassula clade (Thiede & Eggli, 2007) and even included in *Crassula* L. by Stevens (2001–). The phylogenetic position of *Hypagophytum* that we found here had been suspected early by Fröderström (1930, 1932) and fits a biogeographical pattern also observed in other plant lineages (for other examples and discussion see Thiv & al., 2010). Whereas *Aichryson* and *Monanthes* are restricted to the Macaronesian Islands, and *Aeonium* is found mainly there with one species in Morocco and two species in east Africa (Jorgensen & Olesen, 2001), the *Sedum* series of this clade (listed above) are mostly northwest African in distribution with only *S. caeruleum* L. also found on several islands in the Mediterranean Sea. A phylogeny of the first three genera by Mort & al. (2002) had shown that *Monanthes* is not monophyletic when *M. icterica* (Webb ex Bolle) Christ is included, and that *Aeonium* is not monophyletic when *Greenovia* is recognized as a separate genus. The clade contains about 64 species (*Aeonium*: 39; *Aichryson*: 15; *Monanthes*: 10) plus approximately 8 species of *Sedum* (Mes, 1995a; 't Hart & Bleij, 2005; Thiede & Eggli, 2007), and the monospecific *Hypagophytum*.

(5) Clades Leucosedum and Acre. — Except for two early studies of cpDNA restriction site variation (Van Ham & 't Hart, 1998; 't Hart & al., 1999) based on rather sparse taxon samples (11 and 26 spp., respectively), the Leucosedum clade to our knowledge has never been recovered as monophyletic (Mort & al., 2001; Nikulin & al., 2016). In agreement with that, our phylogenetic tree of ITS sequences (Fig. 1B) shows clades Leucosedum and Acre, together constituting Sedeae of Thiede & Eggli (2007), as a polytomy of four clades. The largest of these four clades (*Graptopetalum glassii* through *Sedum acre* in Fig. 1B–D) constitutes the Acre clade and will be further discussed below.

One small clade in this polytomy consisted of a monophyletic *Rosularia* (represented here by *R. globulariifolia* (Fenzl) A.Berger and *R. serrata* (L.) A.Berger) and an unidentified *Sedum* species. The *Sedum* species of the remaining two clades, i.e., *S. cyprium* through *S. magellense* and *S. obtusatum* through *S. subulatum* (C.A.Mey.) Boiss., almost exclusively belong to *Sedum* subg. *Gormania* as defined by Thiede & Eggli (2007) on account of the presence of glandular trichomes (when plants are hairy), broadly sessile sepals and a costate seed testa (testa reticulate in *Sedum aetnense* Tineo) (see Fig. 1B). In a detailed micromorphological study, Giuliani & al. (2018) have furthermore shown that all species of subg. *Gormania* of their sample of Mediterranean *Sedum* s.l. have epidermal cells of a unique shape on the adaxial surface of the petals, found in none of the species of *Sedum* subg. *Sedum*, *Petrosedum* and the Telephium clade sampled by these authors. All genera included in the Leucosedum clade by Thiede & Eggli (2007), except for *Rosularia* and *Afrovivella*, of which the latter was not sampled by us, fell into the large *Sedum-obtusatum*-through-*S.-subulatum* clade, which received significant support in ITS/MrBayes (Fig. 1B), both analyses of the combined dataset (Fig. 3B) and the dated phylogeny (suppl. Fig. S1). In the analyses of plastid DNA (Fig. 2A), the same clade was supported as monophyletic with *Rosularia* nested within. In the large *Sedum-obtusatum*-through-*S.-subulatum* clade (Fig. 1B), a monophyletic *Dudleya* (for the phylogeny of *Dudleya*, see Yost & al., 2013) and *Sedella* (only one of three species sampled by us) together with several species of *Sedum* fell into a subclade that is exclusively North American in distribution (i.e., *Sedum obtusatum* through *Sedella pumila*). A monophyletic *Prometheum* was sister to *Sedum hispanicum*. *Afrovivella* has been claimed to be similar to *Prometheum* in seed characters (Eggli, 1988) and is therefore likely to belong to this clade, too. *Afrovivella* used to be included in *Rosularia* (Ohba, 1978; Eggli, 1988), as was the largest part of *Prometheum* until several *Rosularia* species were transferred to *Prometheum* (Ohba, 1978; 't Hart, 1995) to render both *Prometheum* and *Rosularia* monophyletic. Except for the North American *Dudleya*/*Sedella* subclade, all other lineages of the Leucosedum clade are distributed in the Old World (Fig. 3B). *Prometheum* is southwest Asian and southeast European (northern Greece) in distribution, at least part of *Rosularia* is distributed in Asia, and *Pistorinia* and most *Sedum* species of this clade are distributed in the Mediterranean area (Fig. 3B). Nine of the 15 supported conflicts between the ITS and plastid phylogenies were found in the Leucosedum clade, which makes it the lineage with the highest density of topological conflict. The well-supported clade of *Sedum cyprium* through *S. magellense* (Fig. 1B) corresponds to ser. *Cepaea* (three out of seven species sampled) and is thus one of the few morphologically well-characterized clades (see Table 3). Its sister relationship to the remainder of the Leucosedum clade was supported in the one analysis that also supported the Leucosedum clade as a monophyletic group, i.e., cpDNA/BEAST (Fig. 2A).

As indicated above, the largest of the four clades of Sedeae (*Graptopetalum glassii* through *Sedum acre*) is commonly referred to as the Acre clade (Thiede & Eggli, 2007) and in our ITS phylogeny (Fig. 1B–D) consisted of a large polytomy. Without exception, all *Sedum* species in the Acre clade as inferred by us belong to subg. *Sedum*. Species of this subgenus are characterized by the absence of glandular trichomes (plants glabrous or trichomes non-glandular), by basally free sepals that often are spurred and unequal in length, and by a reticulate, reticulate-papillate or papillate testa ornamentation (testa costate in *Sedum litoreum*; Thiede & Eggli, 2007) (Fig. 1B–D). Apart from a large clade (*Graptopetalum glassii* through *Sedum pacense* J.Meyrán in Fig. 1C,D) that except for the subclade *S. lancerottense* through *S. nudum* (= *Sedum* ser. *Macaronesia*) is American in distribution (Fig. 3C), the remaining clades and single species in the basal polytomy of the Acre clade contained only species of *Sedum*. The large clade of *Graptopetalum glassii* through *Sedum pacense* contained eight clades/species of *Sedum* plus partly other genera (Fig. 1C,D). One of these clades included a non-monophyletic *Villadia* and several lineages of *Sedum* (Fig. 1C). In this Villadia group (Carrillo-Reyes & al., 2009), non-monophyly of *Villadia* had also been found by Carrillo-Reyes & al. (2009). The largest of these eight clades, i.e., *Graptopetalum glassii* through *Sedum palmeri* S.Watson, was only supported in ITS/MrBayes and – among four other subclades/species – contained the always well-supported Echeveria group (Fig. 1D) of Carrillo-Reyes & al. (2009). The Echeveria group contained (besides several lineages/species of *Sedum*) *Echeveria*, *Cremnophila* (included in *Sedum* by Thiede & Eggli, 2007), *Graptopetalum* (incl. *Tacitus*; for a phylogenetic analysis see Acevedo-Rosas & al., 2004a,b), *Pachyphytum* and *Thompsonella*. Of the five genera (besides *Sedum*) recognized in the Echeveria group, *Pachyphytum* and *Thompsonella* appear to be monophyletic (see also Carrillo-Reyes & al., 2008, 2009), although the position of *T. mixtecana* is not always resolved, and monophyly of *Cremnophila* remains unresolved. *Echeveria* and *Graptopetalum* clearly are not monophyletic (see also Acevedo-Rosas & al., 2004a; Carrillo-Reyes & al., 2008, 2009). Another subclade in the polytomy containing the Echeveria clade comprised *Lenophyllum acutifolium* Rose, the only species of *Lenophyllum* sampled here, and four *Sedum* species (i.e., *S. potosinum* through *S. gypsophilum* B.L.Turner in Fig. 1D) that share a preference for limestone soils with *Lenophyllum* (Uhl, 1985; Carrillo-Reyes & al., 2009). The relationships of *Lenophyllum*, a genus of seven species, to other lineages of American Sedeae were unresolved in Mort & al. (2001) and Carrillo-Reyes & al. (2009), but Nikulin & al. (2016) had already inferred a close relationship of this genus to two of the *Sedum* species that we sampled of this lineage.

Biogeography of Sempervivoideae. — In view of limited phylogenetic resolution in many areas of our phylogenetic trees and non-representative geographical sampling of Sempervivoideae, we decided not to conduct a formal biogeographical reconstruction. Considering the geographical distribution of the first two lineages of the family, i.e., Crassuloideae and Kalanchoideae, mostly in sub-Saharan Africa (Fig. 3A), Van Ham & ’t Hart (1998) and Mort & al. (2001), the latter based on formal biogeographical analysis, hypothesized that the family originated there and then colonized the Northern Hemisphere. More specifically, Mort & al. (2001) postulated that, starting from southern Africa/Madagascar, the family spread through the Mediterranean region to reach eastern Europe and Asia. Our cpDNA/BEAST phylogeny of the Sempervivoideae, with the Telephium clade as first diverging lineage (Fig. 2A), might suggest a scenario in which, originating from sub-Saharan Africa, the subfamily colonized the Mediterranean region on the one hand and eastern Europe and Asia on the other hand simultaneously. However, in the absence of a formal biogeographical reconstruction, this is speculative.

The distribution of the remaining four major clades of the subfamily clearly supports the original hypothesis by Mort & al. (2001), i.e., expansion into the Mediterranean and neighbouring areas. Thus, *Petrosedum* and *Sempervivum*/*Jovibarba* are clearly European/Mediterranean in distribution (Fig. 3A, Petrosedum clade not sampled), and this area was reconstructed as ancestral for this clade by Mort & al. (2001). With the exception of the east African *Hypagophytum abyssinicum* and two *Aeonium* species (see above), the Aeonium clade is North African/Macaronesian in distribution (Fig. 3A), and this was reconstructed as ancestral area of this clade by Mort & al. (2001). Although origin of the Leucosedum clade was reconstructed as ambiguous by Mort & al. (2001), the Eurasian members of this group are predominantly European/Mediterranean in distribution, with some lineages extending into central Asia and the Himalayas (Fig. 3B). America was reached once in the evolution of the Leucosedum clade (Fig. 3B), and essentially the same pattern is found in the Acre clade, which also reached America once (Fig. 3C).

Mean and 95% confidence intervals of the stem group ages of the Telephieae, Umbiliceae, the Petrosedum/Aeonium clade, *Sempervivum*/*Jovibarba*, and Sedeae largely overlap (Fig. 3A, Table 4), with a slightly older stem age for Telephieae. The stem ages of these lineages range between 79.53 and 42.26 Ma, and there is no gap between the 95% confidence intervals of stem ages of any two lineages. These age estimates place the diversification of Northern Hemisphere Sempervivoideae (without the Telephium clade) at a time when the palaeogeography of southern Europe was radically different from today and the area essentially was an archipelago of smaller land masses in the Tethys and Paratethys Seas (Rögl, 1999; Scotese, 2001).

As regards the diversification of Sedeae, the age estimate for the crown group of 48.04 Ma (38.79–57.87 Ma) is older than any other diversification of species-rich lineages in the Mediterranean area known to us (see Vargas & al., 2018). Interestingly, the biogeography of Sempervivoideae (without the Telephium clade) strongly resembles that of Cistaceae, which also contain a Macaronesian clade and reached North America twice. The diversification of that lineage, however, started only in the middle Miocene (Guzmán & Vargas, 2009).

The very similar ages of all major clades might imply that lack of phylogenetic resolution in the subfamily is the result of a rapid radiation, and the palaeogeography of southern Europe at the time of arrival of the subfamily may have facilitated the more or less simultaneous origin of several distinct lineages in allopatry. However, hybridization as a cause of lacking phylogenetic resolution cannot be ruled out. ’t Hart (1991) showed that hybridization is possible within but not between those series of *Sedum* he recognized in western Eurasia, and hybridization within and among North American genera of Sempervivoideae is well documented (Uhl, 1963, 1976, 1992b, 1994; Carrillo-Reyes & al., 2008). In our phylogenetic analyses, 21 species – including *Dudleya*, *Petrosedum*, *Pistorinia* and *Rosularia* possibly in their entirety – were supported as incongruently placed in our nuclear and plastid trees. All these observations, together with the exceptionally high diversity of chromosome numbers in Crassulaceae (Uhl, 1992b), clearly show that hybridization and polyploidization must have played a large role in the evolution of the subfamily.

Patterns of morphological evolution in *Sedum* and Sempervivoideae. — The high degree of poly-/paraphyly of *Sedum* as well as non-monophyly of many other genera of Sempervivoideae as discussed above clearly illustrates that those morphological characters used to circumscribe *Sedum* and several other genera of the subfamily are misleading indicators of phylogenetic relationships. As discussed repeatedly (’t Hart & al., 1999; Mort & al., 2001), *Sedum* is defined by a rather unspecific and somewhat plesiomorphic flower morphology, and those characters used to define most other genera, such as fused vs. free petals, poly- vs. tetra- or pentamerous flower organ whorls and leaf arrangement, show pronounced homoplasy (see Fig. 1).

Considering the geographical distribution of lineages classified as *Sedum* and of lineages morphologically distinct from *Sedum* and recognized at generic rank, a certain pattern emerges: many of the lineages recognized as generically distinct from *Sedum* in the clades Aeonium, Leucosedum and Acre are found in areas that are geographically disjunct or remote from the Mediterranean and adjacent areas. Thus, the Aeonium clade contains three lineages recognized at generic rank on various Macaronesian islands. *Hypagophytum*, also part of the Aeonium clade, is distributed in east Africa (Fig. 3A). In the Acre clade, colonization of America (Fig. 3C) resulted in the origin of all genera in this part of the phylogenetic tree, i.e., *Lenophyllum*, *Cremnophila*, *Villadia*, *Echeveria*, *Graptopetalum* (incl. *Tacitus*), *Pachyphytum* and *Thompsonella* (Fig. 1C,D). However, this American clade also contains several lineages classified as *Sedum*. In the Acre clade, two lineages colonized Asia (*Sedum uniflorum* through *S. mexicanum Britton*, *S. erythrospermum* through *S. multicaule* Wall. ex Lindl.) and diversified there, and one lineage colonized east Africa (*S. ruwenzoriense* and *S. meyeri-johannis*) but remained species-poor (Fig. 3B). All these are treated as *Sedum*. Similar to the Acre clade, a large part of the North American lineage of the Leucosedum clade has been classified as generically distinct (*Dudleya*/*Sedella*). Two lineages of the Leucosedum clade colonized east Africa, namely a lineage of six species of *Sedum* subg. *Gormania* (Gilbert, 1985) of which we sampled *S. crassularia* Raym.-Hamet (Fig. 1B), and the monospecific *Afrovivella* (not sampled by us). Of these two lineages, only *Afrovivella* has been treated at generic rank. Of the other lineages of the Leucosedum clade described as separate genera, *Prometheum* is southwest Asian and southeast European in distribution (Fig. 3B), at least part of *Rosularia* is distributed in Asia, and *Pistorinia* is distributed in the Mediterranean area.

This pattern shows that accelerated phenotypic evolution, resulting in the origin of lineages recognized at genus rank, is often but not always linked to major shifts in geographical distribution. We cannot offer any explanation for this pattern.

Classification of *Sedum*. — Of the 15 species and 3 varieties of *Sedum* described by Linnaeus (1753) in *Species plantarum*, 10 species were sampled here. These 10 species (marked “L.” in Fig. 1) fell into four of the five major clades identified, i.e., the clades Telephium, Petrosedum, Leucosedum and Acre, and the remaining five Linnaean species are now classified in *Hylotelephium* and *Phedimus*. It thus is obvious that *Sedum* as understood by Linnaeus (1753) is highly polyphyletic, hence “Linnaeus’s folly”. As type of *Sedum*, *S. acre* was designated by ’t Hart in Jarvis (1992) to replace *S. telephium* L. as an earlier choice (Britton & Rose, 1905). Considering the segregation of *Sedum* sect. *Telephium* Gray as a separate genus, it was argued: “The adoption now of *S. telephium* as type would cause *Sedum* to be applied to the segregate genus (where recognized), and a new generic name would have to be adopted for about 500 species currently recognized in *Sedum* (McNeill & al., 1987: 384). *S*[*edum*]. *acre* is a later choice (Green, 1929) but has been fairly generally accepted (e.g., by Clausen, 1975) and falls within *S.* subg. *Sedum*. It would maintain usage irrespective of the generic concept adopted” (’t Hart in Jarvis, 1992: 569). As evident from the results of our and earlier phylogenies, subg. *Sedum*, corresponding to the Acre clade according to Thiede & Eggli (2007), is not mono- but poly- or paraphyletic by including the American genera of this clade. Also, a large number of lineages or individual species of *Sedum* are found in clades Leucosedum and Aeonium. Considering the position of *Sedum acre* in our ITS phylogeny (Fig. 1B) – as the only species of ser. *Acria* (see suppl. Table S1) it was part of the large basal polytomy of the Acre clade –, obviously much classificatory action is needed to arrive at a monophyletic *Sedum* provided classification is expected to recognize only monophyletic taxa (for pros and cons, see Schmidt-Lebuhn, 2012 and Stuessy & Hörandl, 2014).

As all former *Sedum* species of the Telephium clade have been combined in other genera (Ohba, 1977, 1978), *Petrosedum* is increasingly being accepted as a segregate genus (Grulich, 1984; Thiede & Eggli, 2007) and *Sempervivum*/*Jovibarba* have never been classified in *Sedum* after 1903 (see Eggli, 2005), a first option would be to include all genera of the Aeonium clade (which contains species of *Sedum*) and all those genera of Sedeae (clades Leucosedum and Acre) long recognized as segregates into a vastly expanded *Sedum*, although this would mean to abandon a number of well-established genera. In the Aeonium clade, this would affect *Aeonium* (39 spp.), *Aichryson* (15 spp.), *Monanthes* (10 spp.) and *Hypagophytum* (1 sp.). In Sedeae, this would affect (following the generic classification by Thiede & Eggli, 2007) *Afrovivella* (1 sp.), *Dudleya* (ca. 47 spp.), *Sedella* (3 spp.), *Pistorinia* (3 spp.), *Prometheum* (8 spp.) and *Rosularia* (20 spp.) of the Leucosedum clade, and *Villadia* (ca. 21 spp.), *Lenophyllum* (7 spp.), *Echeveria* (ca. 139 spp.), *Graptopetalum* (18 spp.), *Pachyphytum* (15 spp.) and *Thompsonella* (6 spp.) of the Acre clade. *Cremnophila* (2 spp.) was included in *Sedum* by Thiede & Eggli (2007), but no validly published name in *Sedum* exists for *Cremnophila linguifolia* (Lem.) Moran (Moran, 2005). In sum, species number in *Sedum* would increase from ca. 470 to ca. 820. As the Aeonium clade is one of the five major lineages identified by us and other authors before, and since it was recovered as sister to Sedeae by plastid data (Fig. 2A) but as sister to the Petrosedum clade by ITS data (Fig. 1A), we would argue for its exclusion from a newly circumscribed *Sedum* in order to achieve taxa (in this case tribes) of phylogenetic equivalency and more or less identical age, a criterion for classification advocated by, e.g., Avise & Johns (1999).

When not including the Aeonium clade in *Sedum*, the number of species in *Sedum* would increase from ca. 470 to ca. 755, and the approximately eight species of *Sedum* found in the Aeonium clade as well as the 2–5 species of *Sedum* ser. *Nana* found in the Petrosedum clade will have to be re-classified (see below). Exclusion of the Aeonium clade would limit the name *Sedum* to the well-supported Sedeae, i.e., clades

Leucosedum and Acre, and would require combination of all genera of these two clades other than *Sedum* in *Sedum*. As a third option, circumscription of *Sedum* as corresponding to the Acre clade, a well-supported and morphologically distinct clade in which the type *S. acre* is found, would require recognition (in addition to the *Sedum* species in the Aeonium clade) of probably a large number of lineages of the Leucosedum clade at generic rank. An alternative to this would be the recognition of the whole Leucosedum clade as another genus so as to avoid establishing numerous segregate genera with uncertain phylogenetic support in the Leucosedum clade. Finally, the probably most radical option would be to limit the name *Sedum* to *S. acre* alone. Whereas *S. acre* was mostly unresolved within the basal polytomy of the Acre clade (Figs. 1B, 2B), it was resolved as sister to the remainder of the whole Acre clade in combined/MrBayes (Fig. 3B). Restricting the name *Sedum* to *S. acre* would necessitate to classify the large number of *Sedum* lineages in the clades Aeonium, Leucosedum and Acre at generic rank.

Of the above four options (Aeonium clade plus Sedeae; Sedeae; Acre clade; *S. acre* alone), where inclusion of all members of Sempervivoideae in *Sedum* would represent a fifth option we dismissed above (see Linder & Verboom, 1996, for a very similar case in Poaceae: Danthonieae), we favour circumscription of *Sedum* as containing all members of Sedeae for mainly four reasons.

(1) Restriction of *Sedum* to *S. acre* would require recognition of a large number of new or resurrected genera. Consideration of the phylogeny obtained by us and other authors (Van Ham & ’t Hart, 1998; ’t Hart & al., 1999; Mort & al., 2001, 2002; Acevedo-Rosas & al., 2004a,b; Mayuzumi & Ohba, 2004; Gontcharova & al., 2006; Carrillo-Reyes & al., 2008, 2009; Yost & al., 2013; Klein & Kadereit, 2015; Nikulin & al., 2016; Ito & al., 2017) as well as careful study of morphological, karyological, phytochemical and geographical variation as described in the literature (Boissier, 1872; Schönland, 1891; Berger, 1930; Fröderström, 1930, 1931, 1932, 1935; Clausen, 1940, 1942, 1943a,b, 1959, 1975, 1978, 1979, 1981; Alexander, 1942; Clausen & Uhl, 1943, 1944; Uhl, 1961a,b, 1963, 1970, 1976, 1977, 1978, 1980, 1992a,b; S.-H. Fu, 1965; Uhl & Moran, 1973, 1999; K.T. Fu, 1974; Ohba, 1978; ’t Hart, 1978, 1991, 1995; Calie, 1981; Denton, 1982; Gilbert, 1985; Eggli, 1992, 2005; Nesom & Turner, 1995;’t Hart & Alpınar, 1995, 1999, 2000;’t Hart & al., 1999; K.T. Fu & Ohba, 2001; Afferni, 2012, 2014; Santiago & al., 2015) leads us to the conclusion that recognition of subclades as genera appears impossible in most cases, although some clearly definable sublineages do exist in the Leucosedum and Acre clades. All well-supported clades we found in our phylogenies that are characterized by a distinctive combination of morphological features within their area of distribution are listed in Table 3. We were not able to find any potential synapomorphies for any other well-supported clade. This lack of unifying characters may be due to incomplete knowledge of the species’ morphology rather than a hard absence of synapomorphies. While some *Sedum* segregates are widely accepted (*Hylotelephium* and *Phedimus*: Telephium clade; *Petrosedum*: Petrosedum clade; *Prometheum* and *Sedella*: Leucosedum clade; *Lenophyllum*: Acre clade; *Perrierosedum* (A.Berger) H.Ohba: incertae sedis), other efforts to segregate additional *Sedum* lineages at generic rank (e.g., Nuttall, 1818: *Diamorpha* Nutt.; Berger, 1930: *Mucizonia* A.Berger; Grulich, 1984: *Oreosedum* Grulich and *Asterosedum* Grulich; Löve & Löve, 1985a,b: six new genera, four of them monospecific; Král, 1987: *Helladia* M.Král) have not been accepted. Lastly, the fact that a considerable number of infrageneric taxa of *Sedum* are polyphyletic (see Results) demonstrates that previous taxonomic treatments have rarely been successful in identifying clades of *Sedum* based on morphological or karyological data.

(2) Restricting *Sedum* to the Acre clade and recognizing the Leucosedum clade as one new segregate genus seems very appealing since virtually all *Sedum* species of the Leucosedum clade belong to the morphologically distinct subg. *Gormania* and share at least the costate testa as one unifying character with all other genera of the Leucosedum clade (see Fig. 1B and Table 2). However, the Leucosedum clade was supported only in phylogenies inferred from plastid sequence data (Fig. 2A) (Van Ham & ’t Hart, 1998; ’t Hart & al., 1999). Furthermore, Thiede & Eggli (2007) clearly acknowledged the non-monophyly of subg. *Gormania*, and this notion is supported by the nested position of *Sedum aetnense* of subg. *Sedum* in the Leucosedum clade in our ITS phylogenies (Fig. 1B, suppl. Fig. S1). This – together with the fact that some *Sedum* species cannot be unambiguously assigned to one of the two subgenera (see the example of *S. litoreum* above under “(5) Clades Leucosedum and Acre”) – demonstrates the difficulties of characterizing the Acre clade as well as the Leucosedum clade as two distinct genera.

(3) Several (but not all) of the genera to be included in *Sedum* when containing the entire Sedeae are not monophyletic (*Villadia*, *Echeveria*, *Graptopetalum* and *Thompsonella* when including *T. mixtecana*; Carrillo-Reyes & al., 2008, 2009; see Table 2).

(4) Perhaps most importantly, definition of *Sedum* as proposed does not really alter its morphological circumscription and recognizability. Thus, when comparing the descriptions of all relevant genera as provided by Thiede & Eggli (2007) and as summarized here in Table 2, it becomes clear that essentially none of the characters used for the definition of other genera cannot also be found in *Sedum* as described by Thiede & Eggli (2007). The only exception to this is an autapomorphy of *Dudleya* (i.e., sympodial branching in *Dudleya* but nowhere else; see Table 2).

Description of *Sedum* in a broader circumscription. — If *Sedum* will be recognized as encompassing all members of Sedeae, our favoured solution of the “*Sedum* problem”, it should be circumscribed as follows. This circumscription is based on our literature search (see Materials and Methods):

Annual to perennial, monocarpic to polycarpic herbs or subshrubs, rarely monocarpic rosette plants; roots usually fibrous, less commonly tuberous or as taproots; stems usually with branched non-flowering shoots or sessile rosettes, rarely rhizomatous; plants glabrous or pubescent, indumentum (if present) glandular or non-glandular; leaves usually alternate, rarely decussate or in whorls of up to four, often arranged in terminal or sessile rosettes, mostly sessile, sometimes (semi-)petiolate, mostly (semi-)terete, sometimes flat, with an entire, rarely undulate, finely serrate or ciliate margin, apically rounded to mucronate; inflorescences formed in terminal or lateral position, many-flowered thyrses, simple cincinni or pleiochasia with single or double cincinni, or corymboid or compound thyrsoids with many cincinni, sometimes reduced to spikes, racemes or botryoids, rarely few-flowered thyrses or cymes with one or two cincinni; bracts usually present, often leaf-like; flowers (3–)5(–12)-merous, rarely haplostemonous, sessile to pedicellate; sepals broadly sessile and basally united or free and then often spurred, equal to strongly unequal, usually smaller than petals; petals free or connate at base for up to 3/4 of corolla length, spreading or erect, yellow, greenish, cream, white, pink, purple, orange or reddish, sometimes with reddish to brown blotches, never blue, often with distinct, mostly reddish keel and a dorsal subapical appendage; filaments free or connate with petals up to the mouth of the corolla tube when present; nectar scales variable, usually whitish, yellow or red, less commonly greenish or orange; carpels usually sessile with broad base and slightly connate at base, sometimes slightly sunken into a receptacle, less frequently completely free, rarely stipitate, usually erect during anthesis; stylodia usually slender and recurved during anthesis, or short and stigma ± sessile; follicles (sub-)erect or stellate-patent, without lips along ventral suture, or stellate-patent follicles sometimes with distinct lips, many- to few-seeded, rarely one-seeded and nut-like; seeds ovoid to ellipsoid, rarely fusiform or cylindrical; testa costate-bipapillate or reticulate-papillate to reticulate, sometimes smooth.

About 755 species, mostly in temperate and subtropical regions of North America, Europe, north Africa, Near East and Asia, a few species in Central and South America and central to east Africa.

Thiede & Eggli (2007) did not succeed in identifying phenotypic characters unique to their Sedeae. Equally, the above description of *Sedum* does not contain a single apomorphy by which the genus, very well supported in our molecular phylogenies, can be recognized, and due to all exceptions and rarely seen characters in the above genus description, it is not sufficient to unambiguously distinguish the newly circumscribed *Sedum* from every other genus of Sempervivoideae. This, however, is possible by considering the apomorphies of other genera of Sempervivoideae in comparison to their combination in some *Sedum* species. For example, *Sinocrassula* of tribe Telephieae is characterized by haplostemonous, strictly pentamerous flowers that are arranged in corymbose inflorescences on monocarpic rosettes. Although all four character states (haplostemonous and pentamerous flowers, corymbose inflorescences, monocarpic rosettes) can be found in *Sedum*, to our knowledge all haplostemonous species of *Sedum* are either not rosette plants or (*Graptopetalum glassii*, *G. pentandrum* Moran) have elongate instead of corymbose inflorescences. Similar arguments can be provided for all genera of Sempervivoideae outside *Sedum*.

When equating *Sedum* with Sedeae, and assuming that reclassification at generic rank of those former *Sedum* species found in the Telephium clade and *Petrosedum* is accepted (although not all genera in the Telephium clade are monophyletic; see Mayuzumi & Ohba, 2004, and Gontcharova & al., 2006), reclassification of *Sedum* is necessary in the Aeonium clade as well as for *Sedum* ser. *Nana*. As the Aeonium clade is subject of further study by us, we refrain from expanding on this issue.

When including all members of Sedeae in *Sedum* but recognizing several genera in three of the other four major groups, namely the clades Telephium, *Sempervivum*/*Jovibarba* and Aeonium, infratribal classification in Sempervivoideae is somewhat uneven. This, however, appears to be fully justified by the recognizability of monophyletic lineages and much better resolved phylogenetic relationships in these three groups.

Clearly, phylogenies can be translated into different classifications even when monophyly of taxa is accepted as primary criterion (for review see Humphreys & Linder, 2009), and this has recently resulted in a fierce debate about lycophyte and fern classification where, based on the same trees, between 51 families with 337 genera and 24 families with 212 genera were recognized (Christenhusz & Chase, 2018; Schuettpelz & al., 2018). Although our phylogeny is poorly resolved in many parts, and clearly more data and a more thorough taxon sampling are needed, we believe that our proposed solution of the "*Sedum* problem" is the best of all available options. However, it may only prove stable when the transfer of Sedeae genera into *Sedum* will be justified by profound knowledge of morphology and phylogenetic position of the species to be transferred. This is the major reason why we refrain from and advise against making new combinations at this stage.

■ AUTHOR CONTRIBUTIONS

JWK and JTK conceived the project. TFEM and JTK obtained plant material and performed the laboratory work. Data analysis was conducted by TFEM, JTK and GK. TFEM and JWK wrote an initial manuscript, and all authors contributed to the final version. This publication is part of TFEM's doctoral thesis. — TFEM, https://orcid.org/0000-0002-8656-8654; JTK, https://orcid.org/0000-0003-2736-8094; GK, https://orcid.org/0000-0003-0094-8769; JWK, https://orcid.org/0000-0002-9274-3359

■ ACKNOWLEDGEMENTS

We would like to thank the curators and personnel of E, JEPS, MSB, O, OSC and TARI, the Nederlands Centrum voor Biodiversiteit Naturalis (L, U, WAG), and the Sukkulenten-Sammlung Zürich (ZSS) for having provided material for DNA analysis. We are grateful to Suzanne Cubey and Lesley Scott (E), Andreas Fleischmann (MSB) and Urs Eggli (ZSS) for their assistance during visits. We thank Doris

Franke (Mainz/Germany) for her help with the design of figures, Susana Magallón (Mexico City/Mexico) for providing age estimates for Crassulaceae, Karla Yotoko (Viçosa, Minas Gerais/Brazil) for her assistance with running MrBayes, and Marie Claire Veranso-Libalah (Mainz/Germany) for help with formatting the text. Open access funding enabled and organized by Projekt DEAL.

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Appendix 1. Detailed information about specimens and GenBank accession numbers used in the molecular phylogenetic analyses.

Species (lab code, only provided for accessions with sequences generated by the authors); country; locality and additional information; voucher (+ herbarium code or Botanical Garden); ITS; *matK*; *rps16*; *trnL-F*. "–" indicates missing data or missing information and "*" newly generated sequences.

Outgroup: ***Aphanopetalum clematideum*** Domin; Australia; Western Australia; *Moody 421* (CONN); EF178792; EF179065; –; –. ***A. resinosum*** Endl.; Australia; New South Wales; *Moody 484* (CONN cult.); EF178791; EF179066; –; –. ***Glischrocaryon angustifolium*** (Nees) M.L.Moody & Les; Australia; Western Australia; *Moody 393* (CONN); EF178776; –; –; –. ***Gonocarpus montanus*** (Hook.f.) Orchard; Australia; New South Wales; *Moody 448* (CONN); EF178770; –; –; –. ***G. trichostachyus*** (Benth.) Orchard; Australia; Western Australia; *Moody 398* (CONN); EF178767; –; –; –. ***Haloragis hamata*** Orchard; Australia; Western Australia; *Cranfield 10585* (PERTH); EF178738; EF179012; –; –. ***Laurembergia repens*** (L.) P.J.Bergius; South Africa; Cape Town; *Williams 113 C* (HPBG); EF178735; –; –; –. ***Meionectes brownii*** Hook.f.; Australia; Victoria; *Moody 438* (CONN); EF178773; EF179047; –; –. ***Myriophyllum drummondii*** Benth.; Australia; Western Australia; *Moody 409* (CONN); EF178725; EF178999; –; –. ***M. oguraense*** Miki; Japan; Hyogo; *Kadono s.n.* (HYO); EF178705; EF178979; –; –. ***M. pedunculatum*** Hook.f.; Australia; Tasmania; *Les 643* (CONN); FJ870953; –; –; –. ***Penthorum sedoides*** L.; U.S.A.; Connecticut; *Moody 515* (CONN); EF178789; EF179063; –; –. ***Proserpinaca pectinata*** Lam.; U.S.A.; South Carolina; *Nelson 19432* (USCH); EF178788; –; –; –. ***Tetracarpaea tasmanica*** Hook.f.; Australia; New South Wales; *Wiecek 577*; EF178790; EF179064; –; –. ***Trihaloragis hexandra*** (F.Muell.) M.L.Moody & Les; Australia; Western Australia; *Bright 93* (PERTH); EF178759; –; –; –. **Crassulaceae:** ***Adromischus maculatus*** (Salm-Dyck) Lem.; –; –; (Huntington Botanical Garden 69133); AY692317; –; –; AY692287. ***A. maculatus*** (Salm-Dyck) Lem.; –; –; (Missouri Botanical Garden U6638); –; AF115575; –; –. ***A. nanus*** (N.E.Br.) Poelln.; –; –; *Bruyns 9493* (BOL); LN878913; –; –; –. ***A. sphenophyllus*** C.A.Sm.; –; –; (Huntington Botanical Garden 70042); AY692318; –; –; AY692288. ***Aeonium aureum*** (C.Sm. ex Hornem.) T.Mes; –; –; *Mort 1405* (WS); AY082110; AY082263; –; AY082227. ***A. decorum*** Webb ex Bolle; –; –; *Mort 1435* (WS); AY082130; AY082165; –; AY082239. ***A. nobile*** (Praeger) Praeger; –; –; *Mort 1459* (WS); AY082144; AF115582; –; AY082251. ***Aichryson palmense*** Webb ex Bolle; –; –; *Mort 1482* (WS); AY082104; AY082156; –; AY082222. ***A. punctatum*** (C.Sm. ex Link) Webb & Berthel.; –; –; *Mort 1495* (WS); AY082103; AF115587; –; AY082287. ***Cotyledon orbiculata*** L. s.str.; –; –; (Royal Botanic Gardens at Kew, DNA bank ID 25982); KC988280; –; –; –. ***C. orbiculata*** L. s.str.; –; –; *Mort 1341* (WS); –; AF115591; –; –. ***C. orbiculata*** L. s.str.; –; –; (Huntington Botanical Garden 77962); –; –; –; AY692299. ***C. tomentosa*** subsp. ***ladismithensis*** (Poelln.) Toelken; –; –; (Huntington Botanical Garden); AY692311; –; –; AY692302. ***C. tomentosa*** subsp. ***ladismithensis*** (Poelln.) Toelken; –; –; (Kirstenbosch Botanical Garden); –; AF115592; –; –. ***Crassula alstonii*** Marloth; South Africa; –; *Bruyns 13178* (BOL); MH503501; –; –; –. ***C. bergioides*** Harv.; South Africa; –; *Bruyns 13057* (BOL); MH503508; –; –; –. ***C. capitella*** Thunb.; –; –; (KBG 16848); AY596321; –; –; –. ***C. columnaris*** subsp. ***prolifera*** Friedrich; South Africa; –; *Bruyns 13182* (BOL); MH503520; –; –; –. ***C. dentata*** Thunb.; South Africa; –; *Jardine 2564* (BOL); MH503532; –; –; –. ***C. dodii*** Schönland & Baker f.; South Africa; –; *Bruyns 13195 a* (BOL); MH503535; –; –; –. ***C. elsiae*** Toelken; South Africa; –; *F. Walters s.n.* (BOL); MH503537; –; –; –. ***C. fascicularis*** Lam.; South Africa; –; *Bruyns 12934* (BOL); MH503542; –; –; –. ***C. glomerata*** P.J.Bergius; South Africa; –; *Bruyns 13004* (BOL); MH503545; –; –; –. ***C. hirtipes*** Harv.; South Africa; –; *Bruyns 13171* (BOL); MH503549; –; –; –. ***C. hunua*** A.P.Druce; New Zealand; South Auckland, Hunua, Wairoa Falls; (AK 288129); AY787406; –; –; –. ***C. hunua*** A.P.Druce; New Zealand; South Auckland, Hunua, Wairoa Falls; (AK 294737); –; –; –; EF436513. ***C. muricata*** Thunb.; South Africa; –; *Bruyns 13160* (BOL); MH503561; –; –; –. ***C. oblanceolata*** Schönland & Baker f.; South Africa; –; *Bruyns 13191* (BOL); MH503570; –; –; –. ***C. peduncularis*** F.Meigen; New Zealand; Tararua, Cape Turakirae Scientific Reserve; (AK 286751); AY787409; –; –; EF436508. ***C. pellucida*** L. s.str.; South Africa; –; *Bruyns 12943 a* (BOL); MH503580; –; –; –. ***C. sieberiana*** (Schult. & Schult.f.) Druce; New Zealand; South Auckland, Cornwallis; Puponga Point; (AK 285560); AY787412; –; –; –. ***C. tecta*** Thunb.; South Africa; –; *Bruyns 13159* (BOL); MH503606; –; –; –. ***C. umbellata*** Thunb.; South Africa; –; *Bruyns 13215* (BOL); MH503614; –; –; –. ***C. vaginata*** Eckl. & Zeyh. s. str.; South Africa; –; *Bruyns 13076* (BOL); MH503616; –; –; –. ***C. vestita*** Thunb.; South Africa; –; *Theron s.n.* (BOL); MH503619; –; –; –. ***Cremnophila linguifolia*** (Lem.) Moran; –; –; (Huntington Botanical Garden 91285); AY545679; –; –; –. ***C. nutans*** Rose; –; –; (Huntington Botanical Garden 88184); AY545680; –; –; –. ***Dudleya pulverulenta*** (Nutt.) Britton & Rose; Mexico; Baja California Norte; *A. Oceguera* (XAL); EF632171; –; EF632188; –.

Appendix 1. Continued.

D. pulverulenta (Nutt.) Britton & Rose; U.S.A.; California, Santa Barbara County, Coast; (UCSC SM24-A1122-2); –; –; –; JX960562. ***D. viscida*** (S.Watson) Moran; U.S.A.; California, San Diego County, Oceanside; (UCSC SM63); JX960530; –; –; –. ***D. viscida*** (S.Watson) Moran; –; –; (Rancho Santa Anna Botanical Garden 17710); –; AF115604; –; –. ***Echeveria amoena*** De Smet; Mexico; Veracruz; *P. Carrillo-Reyes & F. Nicolalde 4233* (IEB, XAL); EF632172; –; –; –. ***E. cante*** Glass & Mend.-Garc.; –; –; (Huntington Botanical Garden 26737); FJ753912; –; –; –. ***E. chihuahuensis*** Poelln.; –; –; (Huntington Botanical Garden 52395); FJ753913; –; –; –. ***E. coccinea*** (Cav.) DC.; –; –; *M. Kimanch 3356* (Huntington Botanical Garden); AY545682; –; –; –. ***E. colorata*** E.Walther; –; –; (Huntington Botanical Garden); AY545683; –; –; –. ***E. fulgens*** Lem.; –; –; *M. Kimanch 3331* (Huntington Botanical Garden); AY545684; –; –; AY540553. ***E. fulgens*** Lem.; –; –; *Mort 1596* (WS); –; AF115607; –; –. ***E. gibbiflora*** DC.; –; –; (Huntington Botanical Garden 42398); AY545685; –; –; –. ***E. juarezensis*** E.Walther; –; –; *P. Carrillo-Reyes & D. Cabrera-Toledo 5090* (XAL); FJ753914; –; –; –. ***E. longissima*** E.Walther; –; –; (Huntington Botanical Garden 22943); FJ753915; –; –; –. ***E. megacalyx*** E.Walther; Mexico; Oaxaca; *P. Carrillo-Reyes & F.Z. Vaz-de-Mello 4952* (XAL); FJ753916; –; –; –. ***E. nodulosa*** (Baker) Otto; Mexico; Oaxaca; *P. Carrillo-Reyes & M. Cházaro 1081* (XAL); EF632173; –; –; –. ***E. nuda*** Lindl.; –; –; *Kimnach 3358*; FJ753917; –; –; –. ***E. potosina*** E.Walther; –; –; (Huntington Botanical Garden 73689); AY545686; –; –; –. ***E. pringlei*** (S.Watson) Rose; –; –; *Hernández-Galavíz & Acevedo 101* (XAL); AY545687; –; –; –. ***E. pulvinata*** Rose; –; –; *A. Lau 14* (Huntington Botanical Garden 41554); AY545688; –; –; –. ***E. purpusorum*** A.Berger; –; –; *P. Carrillo-Reyes y D. Cabrera-Toledo 4472* (XAL); FJ753919; –; –; –. ***E. racemosa*** Schltdl. & Cham.; Mexico; Veracruz; *P. Carrillo-Reyes 4444* (XAL); FJ753920; –; –; –. ***E. recurvata*** L.Carruth.; –; –; (University of California at Berkeley 90-0768); FJ753921; –; –; –. ***E. setosa*** Rose & Purpus; –; –; (Huntington Botanical Garden); AY545689; –; –; –. ***Graptopetalum amethystinum*** (Rose) E.Walther; –; –; *Acevedo 1734* (XAL); AY545690; –; –; –. ***G. bartramii*** Rose; –; –; (Huntington Botanical Garden 92052); AY545691; –; –; –. ***G. bellum*** (Moran & J.Meyrán) D.R.Hunt; U.S.A.; California; –; JX960526; –; –; –. ***G. bellum*** (Moran & J.Meyrán) D.R.Hunt; –; –; *Mendoza* (XAL); –; –; –; AY540579. ***G. bellum*** (Moran & J.Meyrán) D.R.Hunt; –; –; (University of Washington Botanical Garden s.n.); –; AF115680; –; –. ***G. bernalense*** (Kimnach & Moran) V.V.Byalt; –; –; *Acevedo & Hernández-Galavíz 1722* (XAL); AY545692; –; –; –. ***G. filiferum*** (S.Watson) J.Whitehead; –; –; *Acevedo 1729* (XAL); AY545694; –; –; –. ***G. fruticosum*** Moran; –; –; *Acevedo & Hernández-Galavíz 1726* (XAL); AY545695; –; –; –. ***G. glassii*** Acev.-Rosas & Cházaro; –; –; *Acevedo 1724* (XAL); AY545696; –; –; –. ***G. grande*** Alexander; –; –; *Acevedo 1718* (XAL); AY545697; –; –; –. ***G. macdougallii*** Alexander; –; –; *Mendoza* (XAL); AY545698; –; –; AY540564. ***G. macdougallii*** Alexander; –; –; (University of Washington Botanical Garden s.n.); –; AF115611; –; –. ***G. marginatum*** Kimnach & Moran; –; –; *Acevedo 1733* (XAL); AY545699; –; –; –. ***G. mendozae*** Glass & Cházaro; –; –; *Acevedo 1732* (XAL); AY545700; –; –; –. ***G. pachyphyllum*** Rose; –; –; *Acevedo & Hernández-Galavíz 1711, 1715* (XAL); AY545701; –; –; –. ***G. paraguayense*** (N.E.Br.) E.Walther; –; –; *Johnson* (New York Botanical Garden 52299A); AY545702; –; –; –. ***G. pentandrum*** Moran; –; –; *Acevedo 1723* (XAL); AY545703; –; –; –. ***G. pusillum*** Rose; –; –; *Acevedo 1735* (XAL); AY545704; –; –; –. ***G. rusbyi*** (Greene) Rose; –; –; *Acevedo 1727, 1730* (XAL); AY545705; –; –; –. ***G. saxifragoides*** Kimnach; –; –; *Acevedo 1736* (XAL); AY545706; –; –; –. ***G. superbum*** (Kimnach) Acev.-Rosas; –; –; *Acevedo 1725* (XAL); AY545708; –; –; –. ***Hylotelephium ewersii*** (Ledeb.) H.Ohba; Russia; –; *M.Wakabayashi & al. 1993* (TI); AB088570; –; –; AB480641. ***H. ewersii*** (Ledeb.) H.Ohba; –; –; (Royal Botanic Gardens at Kew 197-80-01945); –; AF115615; –; –. ***H. spectabile*** (Boreau) H.Ohba; Japan; –; *Mayuzumi C00003* (TI); AB088575; –; –; AB480649. ***H. spectabile*** (Boreau) H.Ohba; –; –; –; –; AB038181; –; –. ***H. telephium*** subsp. ***maximum*** (L.) H.Ohba; Armenia; N Armenia; *H. Ohba 1999* (TI); AB088561; –; –; AB480654. ***Hypagophytum abyssinicum*** (Hochst. ex A.Rich.) A.Berger (JKC51); Ethiopia; Gondar, lower part of the Limalima escarpment on the road from Adi Arkay to Debark, Open, rocky slope with mosses, *Swertia* and *Hypagophytum*, 13.21°N/37.89°E; 2350 m; *I. Friis, G.S. Bidgood, W. Abebe, E. Getachew 12294* (WAG 0416928); MT336090*; MT181557*; MN381921*; MT155885*. ***Jovibarba globifera*** subsp. ***allionii*** (Jord. & Fourr.) J.Parn. (JKB52); Italy; 45.57°N/7.57°E; *J. Klein B 100825-05-164* (MJG 012909); KJ884135; –; KJ884341; MT155886*. ***J. heuffelii*** (Schott) Á.Löve & D.Löve (JKB149); Bulgaria; Kyustendil, Rila mts., Pastra, Rilska valley, 42.15°N/23.42°E; ca. 1700 m; *J. Klein G 110828-466* (MJG 012747); KJ884152; –; KJ884359; –. ***J. heuffelii*** (Schott) Á.Löve & D.Löve (JKB151); Bulgaria; 41.73°N/23.51°E; *J. Klein G 110830-482* (MJG 012920); –; MT181558*; –; MT155887*. ***Kalanchoe beauverdii*** Raym.-Hamet; –; –; –; AJ231305; –; –; –. ***K. beharensis*** Drake; –; –; –; AJ231317; –; –; –. ***K. daigremontiana*** Raym.-Hamet & H.Perrier; –; –; *Morgan 2151* (WS); U42527; AF274619; –; –. ***K. densiflora*** Rolfe; –; –; –; AJ231334; –; –; –. ***K. eriophylla*** Hils. & Bojer ex Tul.; –; –; –; AJ231345; –; –; –. ***K. linearifolia*** Drake; –; –; –; AJ231340; –; –; –. ***K. longifolia*** E.T.Geddes; –; –; –; AJ231335; –; –; –. ***K. marmorata*** Baker; –; –; –; AJ252911; –; –; –. ***K. millotii*** Raym.-Hamet & H.Perrier; –; –; –; AJ231337; –; –; –. ***K. pinnata*** (Lam.) Pers.; –; –; –; AJ231323; –; –; –. ***K. pinnata*** (Lam.) Pers.; –; –; *S.B. Davis 1290* (FLAS); –; GU135118; –; –. ***K. pinnata*** (Lam.) Pers.; Japan; Ogasawara; –; –; –; –; AB817448. ***K. pubescens*** Baker; –; –; –; AJ231347; –; –; –. ***K. rhombopilosa*** Mannoni & Boiteau; –; –; –; AJ231348; –; –; –. ***K. thyrsiflora*** Harv.; –; –; –; AJ231341; –; –; –. ***K. uniflora*** (Stapf) Raym.-Hamet; –; –; –; AJ231343; –; –; –. ***K. uniflora*** (Stapf) Raym.-Hamet; –; –; (University of Bonn Botanical Garden 05175); –; AF115623; –; –. ***Kungia aliciae*** (Raym.-Hamet) K.T.Fu; China; –; *S. Mayuzumi CH00061* (TI); AB480591; –; –; AB480632. ***K. schoenlandii*** (Raym.-Hamet) K.T.Fu; China; –; *S. Mayuzumi CH00063* (TI); AB480593; –; –; AB480634. ***Lenophyllum acutifolium*** Rose; –; –; (Huntington Botanical Garden (at New York Botanical Garden)); AY545709; –; –; –. ***L. acutifolium*** Rose; –; –; *Mort 1598* (WS); –; AF115625; –; –. ***Meterostachys sikokiana*** (Makino) Nakai; Japan; Nagasaki; *Mayuzumi & al. C00028* (TI); AB088579; –; –; AB480670. ***Monanthes anagensis*** Praeger; –; –; *Mort 1391* (WS); AY082114; AF115626; –; AY082231. ***M. icterica*** (Webb ex Bolle) Christ; –; –; *Santos*; AY082108; AF115629; –; AY082288. ***M. polyphylla*** subsp. ***amydros*** Nyffeler; –; –; *Mort 1433* (WS); AY082113; AF115627; –; AY082230. ***Orostachys japonica*** (Maxim.) A.Berger; Japan; –; *Mayuzumi & al. C00027* (TI); AB088576; –; –; AB480626. ***O. malacophylla*** (Pall.) Fisch.; Japan; Hokkaido; *Mayuzumi & al. C00011* (TI); AB088572; –; –; –. ***O. malacophylla*** var. ***aggregata*** (Makino) H.Ohba; Japan; Hokkaido; *Mayuzumi & al. C00012* (TI); AB088574; –; –; AB480616. ***O. spinosa*** (L.) C.A.Mey. ex A.Berger; Russia; Novosibirskaya Oblast; *Doronkin* (Sukkulenten-Sammlung Zürich 97 1570 /0); AB088577; –; –; AB480631. ***Pachyphytum fittkaui*** Moran; –; –; (Huntington Botanical Garden); FJ753925; –; –; –. ***P. glutinicaule*** Moran; –; –; *J. Meyrán 2692* (Huntington Botanical Garden); AY545710; –; –; –. ***P. hookeri*** (Salm-Dyck) A. Berger; –; –; (Huntington Botanical Garden); FJ753926; –; –; –. ***P. oviferum*** J.A.Purpus; –; –; (Huntington Botanical Garden 48603); FJ753928; –; –; –. ***Petrosedum amplexicaule*** subsp. ***tenuifolium*** (Sm.) Velayos (KB03); Spain; Montes de Toledo, Province of Cáceres, Guadalupe, rocky slopes in Villuercas peak; *F.J. Valtueña, C.G. Relinque* (MJG 024790); MT336100*; MT181567*; –; MT155896*. ***P. anopetalum*** (DC.) Grulich; Greece; Olympus; *Stephenson 5.28.043*; HE999672; –; –; –. ***P. anopetalum*** (DC.) Grulich; –; –; *Stephenson 5.28.032*; –; KX452242; –; –. ***P. forsterianum*** (Sm.) Grulich (JKB130); Spain; Montes de Toledo, Province of Cáceres, Guadalupe, rocky slopes in Villuercas peak, 39.48°N/5.40°W; *F.J. Valtueña, C.G. Relinque* (MJG 012903); KJ884125; MT181574*; KJ884334; MT155903*. ***P. montanum*** (Songeon & E.P.Perrier) Grulich; France; Alps; *Stephenson 5.27.15*; HE999667; –; –; –. ***P. rupestre*** (L.) P.V.Heath (TM52.1); Spain; Albacete, Fábrica de Riópar, Valle del Chorro, limestone cliffs near waterfall, shaded damp rocks by waterfall, 38°30′N/2°27′W, 1100 m; *P.F. Cannon, P.R. Crane, S.L. Jury & D.M. Moore* (E 00121627); MT336134*; –; –; –. ***P. sediforme*** (Jacq.) Grulich; Spain; Asturias las Agueras; *Gontcharov ESP_Astur_070724*; HE999683; –; –; –. ***P. sediforme*** (Jacq.) Grulich; Portugal; Beira Litoral, Vaguira Praia; *HRT-15429* (Utrecht University Botanic Gardens 15429); –; AF115640; –; X74286. ***Phedimus ellacombianus*** (Praeger) ’t Hart; South Korea; Daejeon; *A.K. Kim SB KRIBBSD2202*; JQ954564; –; –; JQ954571. ***P. kamtschaticus*** (Fisch. & C.A.Mey.) ’t Hart; South Korea; Seoul; *A.K. Kim PDBK 2010-0700*; JQ954563; –; –; JQ954570. ***P. kamtschaticus*** (Fisch. & C.A.Mey.) ’t Hart; –; –; (Utrecht University Botanic Gardens 33168); –; AF115653; –; –. ***P. kamtschaticus*** (Fisch. & C.A.Mey.) ’t Hart; China; Hebei, Xiaowutai mt.; *J.Q. Zhang 120613-03* (PEY); –; –; KJ570189; –. ***P. stoloniferus*** (S.G.Gmel.) ’t Hart (KB05); Georgia; Guria, Lesser Caucasus, mt. Bakhmaro, environs Fafara, 41.83°N/42.32°E, 2103 m; *S. Shetekauri* (MJG 024791); MT336091*; MT181559*; –; MT155888*. ***P. takesimensis*** (Nakai) ’t Hart; South Korea; Gangwondo; *A.K. Kim GAR 2010-AK02*; JQ954566; –; –; –. ***P. takesimensis*** (Nakai) ’t Hart; South Korea; Dokdo; –; –; NC_026065; NC_026065; NC_026065. ***Pistorinia breviflora*** Boiss. (JKC11); Morocco; –; *H. ’t Hart 67?-120 = HtH 32308* (ZSS); MT336092*; MT181560*; –; MT155889*. ***Prometheum chrysanthum*** (Boiss.) ’t Hart; Turkey; –; *Stephenson 4R022*; HE999634; KX452252; –; –. ***P. sempervivoides*** (M.Bieb.) H.Ohba (KB09); Georgia; Samtskhe-Javakheti, Caucasus, Aspindza distr., Vardzia, dry slope, 1400-1600 m; *S. Shetekauri* (MJG 024792); MT336093*; MT181561*; –; MT155890*. ***P. serpentinicum*** (Werderm.) ’t Hart (JKC06); Turkey; Mugla, Sandras Dagi, Cicekbaba Tepe, summit area, 2300 m; *K. Alpinar & ’t Hart AH873 = HtH 32081* (Sukkulenten-Sammlung Zürich 99 6713 /0); MT336094*; MT181562*; –; MT155891*. ***P. tymphaeum*** (Quézel & Contandr.) ’t Hart; –; –; *Doboš P.015*; LM993276;

Appendix 1. Continued.

KX452251; –; –. ***Rhodiola crenulata*** (Hook.f. & Thomson) H.Ohba; China; Xizang, Lazi Xian; *G.Y. Rao & al. 100813-01* (PEY); KF113691; –; –; –. ***R. hobsonii*** (Prain ex Raym.-Hamet) S.H.Fu; China; Xizang, Linzhi Xian; *J.Q. Zhang & al. 120717-05* (PEY); KF113701; KJ570460; KJ570224; KF113807. ***R. yunnanensis*** subsp. ***forrestii*** (Raym.-Hamet) H.Ohba; China; Yunnan, Shangri-La Xian; *J.Q. Zhang & al. 110730-02* (PEY); KF113696; KJ570452; KJ570216; KF113802. ***Rosularia globulariifolia*** (Fenzl) A.Berger (JKC52); Turkey; Antalia area; *P. Raes, Sedum Society seed distribution SSS1234* (Sukkulenten-Sammlung Zürich 12 0085 /0); MT336095*; MT181563*; –; MT155892*. ***R. serrata*** (L.) A.Berger (UM32); Turkey; Mersin, Taurus mts., Prov. Icel, Bolandiz, on limestone, 750m (*ex cult*. Botanical Garden Würzburg); (MJG 012926); KJ884124; MT181564*; KJ884332; MT155893*. ***Sedella pumila*** (Benth.) Britton & Rose (JKC16); U.S.A.; California, Shasta, near a vernal pool, ca. 250 yd south of the barn, ca. 1/4 mi south of the Coleman Canal, 2.8 mi (air) WSW of Darah Springs State Fish Hatchery, ca. 12 mi (air) ENE of Cottonwood; *L. Ahart 10145* (JEPS 104348); MT336096*; MT181565*; –; MT155894*. ***Sedum acre*** L.; Spain; Sierra Alhama; *Stephenson 5.01.011 diploid*; HE999636; –; –; –. ***S. acre*** L.; Estonia; –; *I. Hiiesalu 60*; –; –; –; HM590343. ***S. aetnense*** Tineo (JKC24); Italy; Nicolosi (mt. Etna area) in open area with fine lava dust, left of the road to the Refugio, ± 2 km outside the village; *B.K. Boom 46446* (L 1860263); MT336097*; –; –; –. ***S. alamosanum*** S.Watson; –; –; *P. Carrillo-Reyes & E. Ruiz-Sánchez 4767* (IBUG, IEB, XAL); FJ753929; –; FJ753847; –. ***S. album*** L. (JKC62); Albania; Korçë Province, along the road from Voskopojë to Gjorgjevicë, ca. 750 m west of where the road turns west to go to Gjorgjevicë, *Juniperus-Buxus sempervirens* scrub on serpentine outcrop in *Pinus* woodland, 40.58°N/20.59°E, 1360 m; *A. Moore & R.L. Welch 1540* (TARI 73097-1082); MT336098*; MT181566*; –; MT155895*. ***S. alexanderi*** Eggli; Mexico; Morelos; *P. Carrillo-Reyes & F.Z. Vaz-de-Mello 4257* (IEB, XAL); EF632174; –; EF632191; –. ***S. alfredii*** Hance; China; Guangdong, Guangzhou; (IBK 114924); FJ919951; FJ919941; –; –. ***S. alfredii*** Hance; China; East China; *G. Kokubugata 17191* (TNS); AB930260; LC258165; LC229432; LC229500. ***S. allantoides*** Rose; –; –; (Huntington Botanical Garden 90032); AY545712; –; –; –. ***S. alpestre*** Vill.; –; –; *Stephenson V001*; HE999639; –; –; –. ***S. alpestre*** Vill.; Turkey; Bursa, Uludag; (U); –; –; –; not deposited (Kim & al., 1996). ***S. alpestre*** Vill. (JKB199); Austria; Zechnerkarspitze/Schladminger Tauern near Weißpriach, exposed grassy scarp, 47.22°N/13.69°E, ca. 2420 m; *U. Hecker D2830* (MJG 012906); MT336103*; MT181570*; KJ884333; MT155899*. ***S. alsinefolium*** All. (TM37.1); Italy; Prov. Cuneo, E face of mt. Viso. Near the well at Crissolo Borgo, river Po; *O. Angerer* (MSB); MT336099*; –; –; –. ***S. andinum*** Ball; –; –; *P. Carrillo-Reyes y N.I. Ortega 5178* (IEB, USM); FJ753930; –; FJ753848; –. ***S. anglicum*** Huds. (O1); Norway; Telemark, Kragerø, v Rauane, N of „Storholme“, 0–10 m; *T. Berg* (O 89072); MT336101*; MT181568*; MN381922*; MT155897*. ***S. annuum*** L. (O2); –; –; (O 340653); –; MT181569*; MN381923*; MT155898*. ***S. annuum*** L. (TM51.1); Iceland; Medaldalur, valley on pingeyri peninsula, north-west Iceland; *R.W. Groves 07-08 1966* (E 00766661); MT336102*; –; –; –. ***S. apoleipon*** ’t Hart; Greece; –; *Stephenson 5.04.001*; HE999643; KX452276; –; –. ***S. baileyi*** Praeger; –; –; (LBG 0064555); FJ919935; FJ919945; –; –. ***S. batesii*** Hemsl.; –; –; *E. Ruiz-Sánchez 143* (XAL); FJ753931; –; –; –. ***S. bergeri*** Raym.-Hamet; China; West Mountain, Kunming, Yunnan; –; AY352897; –; –; –. ***S. borissovae*** Balk. (JKC64); –; Garden origin; (Sukkulenten-Sammlung Zürich 99 4604 /0); MT336104*; MT181571*; MN381924*; MT155900*. ***S. bourgaei*** Hemsl.; –; –; *E. Pérez y T. Platas 3224* (IEB); FJ753932; –; FJ753849; –. ***S. brevifolium*** DC. (m883); France; Languedoc-Roussilon, Pyrénées-Orientales, Porté-Puymorens, 42.55°N/1.84°E, 1700 m; *J. Klein I 120717-883* (MJG 025432); –; MT181572*; –; MT155901*. ***S. brevifolium*** DC.; Italy; Val Restonica; *Hornát S5*; LM993278; –; –; –. ***S. brissemoretii*** Raym.-Hamet; Portugal; Madeira; *Stephenson V003*; HE999645; –; –; –. ***S. bulbiferum*** Makino; Japan; –; *L. Niu 1999* (TI); AB088628; –; –; –. ***S. bulbiferum*** Makino; –; –; (Utrecht University Botanic Gardens 32795); –; AF115652; –; –. ***S. bulbiferum*** Makino; Japan; *T. Ito 416* (TNS); –; –; LC229434; –. ***S. bulbiferum*** Makino; South Korea; Seoul; –; –; –; –; JQ954574. ***S. caducum*** R.T.Clausen; –; –; (Huntington Botanical Garden 47970); FJ753933; –; –; –. ***S. caeruleum*** L. (JKC37); Malta; Mellieña Ridge just S of Mellieña. Shallow pockets of clay in very open garrigue vegetation on limestone rocks. Extremely common in Malta and Gozo in places that are otherwise ± devoid of vegetation, 100 m; *K.U. Kramer & L.Y.T. Westra 4198* (U 1219265); MT336105*; MT181573*; –; MT155902*. ***S. caespitosum*** (Cav.) DC. (TM61.1); Morocco; Near Amanouz (c. 35 km. ESE of Marrakech), rocky limestone slopes, ca. 500 m; *P. & J. Davis* (E 00764455); MT336106*; –; –; –. ***S. carinatifolium*** (R.T.Clausen) Pérez-Calix; –; –; *E. Pérez y E. Carranza 3155* (IEB); FJ753934; –; –; –. ***S. catorce*** G.L. Nesom; –; –; *E. Pérez* (IEB); FJ753935; –; –; –. ***S. cepaea*** L.; Greece; –; *Stephenson V022*; HE999646; KX452248; –; –. ***S. chazaroi*** P.Carrillo & J.A.Lomelí; –; –; *P. Carrillo-Reyes y J.A. Lomelí 5105* (GUADA, IBUG, IEB, XAL); FJ753936; –; –; –. ***S. chloropetalum*** R.T.Clausen; –; –; *P. Carrillo-Reyes & F.Z. Vaz-de-Mello 4958* (IEB, IBUG); FJ753937; –; FJ753850; –. ***S. clavatum*** R.T.Clausen; –; –; *J. Meyrán 1306* (Huntington Botanical Garden 73053); AY545713; –; –; AY540576. ***S. clavatum*** R.T.Clausen; –; –; (Huntington Botanical Garden 23982); –; AF115660; –; –. ***S. commixtum*** Moran & Hutchison; –; –; *P. Carrillo-Reyes & F.Z. Vaz-de-Mello 4954* (IEB, XAL); FJ753938; –; –; –. ***S. commixtum*** Moran & Hutchison; –; –; (University of Washington Botanical Garden 124); –; AF115656; –; –. ***S. compactum*** Rose; Mexico; Oaxaca; *P. Carrillo-Reyes & F.Z. Vaz-de-Mello 4929* (XAL); EF632175; –; EF632192; –. ***S. confusum*** Hemsl.; –; –; *Stephenson 0.06.001*; HE999648; –; –; –. ***S. corynephyllum*** Fröd.; –; –; *Acevedo 1713* (XAL); AY545715; –; –; AY540577. ***S. craigii*** R.T.Clausen; –; –; *Acevedo 1728* (XAL); AY545693; –; –; –. ***S. crassularia*** Raym.-Hamet (TM40.1); Kenya; W mt. Kenya, rocky outcrops N of Hausberg Valley, 250 m N of Nanyuki River, 3620 m; *H. Rehder* (MSB); MT336107*; –; –; –. ***S. cymatopetalum*** Fröd.; –; –; *Kimnach 2001.16*; FJ753939; –; –; –. ***S. cyprium*** A.K.Jacks. & Turrill (TM47.1); Cyprus; Tillyria, north slopes of mt. Zakharou. Roadside banks and clearings in *Pinus brutia* forest, 800–1000 m; *J.R. Edmondson & M.A.S. McClintock* (E 00361857); MT336108*; –; –; –. ***S. dasyphyllum*** L.; Italy; South Tyrol, Merano; *Gontcharov ITA_Toskana_070518*; HE999684; –; –; –. ***S. dasyphyllum*** L.; –; –; *Soltis & Soltis 2557*; –; AF115657; –; –. ***S. dasyphyllum*** L.; Italy; Liguria, Rocco; (U); –; –; –; not deposited (Kim & al., 1996). ***S. dasyphyllum*** var. ***glanduliferum*** (Guss.) Moris; France; Corsica; *Stephenson 5.38.006*; HE999650; KX452266; –; –. ***S. debile*** S.Watson; U.S.A.; –; *Stephenson V030*; HE999651; KX452265; –; –. ***S. dendroideum*** DC.; U.S.A.; California; –; JX960523; –; –; –. ***S. ebracteatum*** DC.; Mexico; –; *Stephenson 0.47.004*; HE999653; –; –; –. ***S. emarginatum*** Migo; China; Anhui Province; –; EU592006; –; –; –. ***S. emarginatum*** Migo; China; Zhejiang Province; –; –; –; –; KM111121. ***S. erythrospermum*** Hayata; Taiwan; Kaohsiung, Taoyuan Hsiang; *C. Tsutsumi 504* (TNS); AB906473; –; –; AB932631. ***S. farinosum*** Lowe; Portugal; Madeira, Pico Ruiva; *Stephenson V023*; HE999654; –; –; –. ***S. farinosum*** Lowe; –; –; (Utrecht University Botanic Gardens 29006); –; AF115658; –; –. ***S. formosanum*** N.E.Br.; Japan; Ryukyus, Izena Island; *G. Kokubugata 12224* (TNS); AB930266; LC258195; LC229462; LC229530. ***S. fragrans*** ’t Hart (JKC09); –; –; *R. Stephenson, Sedum Society seed distribution SSS1437* (Sukkulenten-Sammlung Zürich 14 0098 /0); MT336109*; MT181575*; –; MT155904*. ***S. frutescens*** Rose; Mexico; –; *Stephenson 0.29.0026*; HE999657; –; –; –. ***S. fuscum*** Hemsl.; –; –; *E. Pérez y G. Ocampo 4188* (IEB); FJ753941; –; FJ753851; –. ***S. goldmanii*** (Rose) Moran; Mexico; Veracruz; *C. Gallardo 3333* (XAL); EF632186; –; EF632204; –. ***S. gracile*** C.A.Mey. (JKC08); Georgia; Kartli, between Tbilisi and Didgori, at Didgori Monument, 1630 m; *A. Gröger & Lobin 302-9* (Sukkulenten-Sammlung Zürich 10 2021 /0); MT336110*; MT181576*; –; MT155905*. ***S. grandisepalum*** R.T.Clausen; –; –; *P. Carrillo-Reyes & F.Z. Vaz-de-Mello 4964* (XAL, IEB); FJ753942; –; –; –. ***S. grandyi*** Raym.-Hamet; –; –; (Huntington Botanical Garden 54390); FJ753943; –; –; –. ***S. greggii*** Hemsl. (SO2); Mexico; Hidalgo, San Vicente, near Barranca de los Marmoles, 2315 m; *R. Moran 7807* (ZSS A 25561); –; –; MN381925*; –. ***S. greggii*** Hemsl.; –; –; *A. Rodríguez* (IBUG); FJ753944; –; –; –. ***S. grisebachii*** var. ***horakii*** (Rohlena) ’t Hart; North Macedonia; Korab mts., Golem Korab, Kobilino pole; *Hornát S4*; LM993280; KX452282; –; –. ***S. griseum*** Praeger; Mexico; –; *Stephenson 0.30.002*; HE999660; –; –; –. ***S. guatemalense*** Hemsl.; –; –; *E. Ruiz-Sánchez 145* (XAL); FJ753945; –; FJ753852; –. ***S. gypsicola*** Boiss. & Reut. (JKC58); Spain; Jaén, Sierra de Segurra, Sierra la Cabrilla, summit area, 2050 m; *H. ’t Hart 41-245 = HtH 29467* (ZSS A 22032); –; MT181577*; –; MT155906*. ***S. gypsicola*** Boiss. & Reut. (TM86.1); Malta; Misraħ għar Daqquq (close to ġebel Ciantar), Fawwara, Siġġiewi, coastal garigue, specifically in karstic rock cavities and crevices at the very edge of the cliffs, often isolated from surrounding flora, 200–250 m; *S. Mifsud* (E 00653406); MT336111*; –; –; –. ***S. gypsophilum*** B.L.Turner; –; –; *P. Carrillo-Reyes y A. De Nova 4175* (XAL); FJ753946; –; –; –. ***S. hakonense*** Makino; Japan; –; *Mayuzumi C00005* (TI); AB088625; –; –; –. ***S. hakonense*** Makino; Japan; –; *T. Ito 623* (TNS); –; LC258168; LC229435; LC229503. ***S. hemsleyanum*** Rose; –; –; *P. Carrillo-Reyes & F.Z. Vaz-de-Mello 4858* (XAL); FJ753947; –; –; –. ***S. hemsleyanum*** Rose; –; –; *Brunner 2079a*; –; AF115661; –; –. ***S. hillebrandtii*** Fenzl (TM11.1); Austria; Vienna, district 19, 0,05–0,1 km NE of the crossroads Höhenstraße / Krapfenwaldgasse, 360–370 m; *Thomas Barta* (MSB); MT336112*; –; –; –. ***S. hirsutum*** All. s.str. (JKC57); Portugal; Madeira, Pico de Rancho, W of Camara, dry SE-facing rocks along the road to Gabo Girao, 400 m; *H. ’t Hart 39-38 = HtH 29010* (ZSS A 21960); MT336113*; –; MN381926*; –. ***S. hirsutum*** subsp. ***baeticum*** Rouy; United Kingdom; Gibraltar; *Stephenson V025*; HE999662; –; –; –. ***S. hispanicum*** L. (JKB126); Greece; Central Macedonia, Thessalonika, Chalkidiki, Chortiatis, Boudos, 40.60°N/23.10°E, 920 m; *J. Klein*

Appendix 1. Continued.

C 110622-01-325 (MJG 012914); KJ884126; MT181578*; KJ884335; MT155907*. ***S. hultenii*** Fröd.; Mexico; –; *Stephenson 0.13.001*; HE999664; –; –; –. ***S. jaccardianum*** Maire & Wilczek; –; –; (Utrecht University Botanic Gardens 32211); AY082100; AF115637; –; AY082220. ***S. jurgensenii*** (Hemsl.) Moran; –; –; *P. Carrillo-Reyes & F. Nicolalde 4488* (XAL, MEXU); FJ753949; –; FJ753853; –. ***S. kotschyanum*** Boiss. (TM38.2); Afghanistan; Dascht-i-Nawor, NE-border, salty plains, overgrazed, only on creek borders, 3100 m; *H. Freitag* (MSB 175990); MT336114*; –; –; –. ***S. laconicum*** Boiss. & Heldr. (TM87.1); Greece; Lakonias, Githiou, by the deserted village of Poliaravos. Rocky slopes and old terraced fields. Mostly schist, 36°48′N/22°25′E, 700–800 m; *Strid & Kit Tan* (E 00095641); MT336115*; –; –; –. ***S. laconicum*** Boiss. & Heldr.; –; –; (Utrecht University Botanic Gardens 31101); –; AF115642; –; –. ***S. lagascae*** Pau; Spain; Ávila: Gredos, western massif, Solana de Ávila, El Chorro, 1868 m; *B. García*; FJ897739; –; –; –. ***S. lancerottense*** R.P.Murray (TM28.1); Spain; Lanzarote, Haria; (Berlin-Dahlem Botanical Garden 350088610); MT336116*; –; –; –. ***S. lancerottense*** R.P.Murray; –; –; *Mort 1533* (WS); –; AF115641; –; –. ***S. leibergii*** Britton (TM84.1); U.S.A.; Oregon, Ochoco National Forest, 44°41.9′N/120°10′W; *R. Goff* (OSC 235062); MT336117*; –; –; –. ***S. lenkoranicum*** Grossh. (TM63.1); Iran; Elburz Mts., above Allamul, between Chalus and Karadj: NE facing slope among *Acer*, *Rosa*, *Lonicera* and *Crataegus* scrub, ca. 1980 m; *J.C. Archibald* (E 00356605); MT336118*; –; –; –. ***S. liebmannianum*** Hemsl.; –; –; *P. Carrillo-Reyes & F.Z. Vaz-de-Mello 4958* (XAL); FJ753952; –; –; –. ***S. lineare*** Thunb.; Japan; –; *Mayuzumi C00120* (TI); AB088623; –; –; AB089773. ***S. litoreum*** Guss. (TM68.1); Greece; Lakonias, Githiou, by the deserted village of Poliaravos. Rocky slopes and old terraced fields. Mostly schist, 36°48′N/22°25′E, 700–800 m; *Strid & Kit Tan* (E 00095642); MT336119*; –; –; –. ***S. litoreum*** Guss. (JKC10); Italy; Sicily, Prov. Palermo, on limestone rocks near Cap Mongerbino, 50 m; *H. ’t Hart 74002 = HtH 32919* (ZSS 32794); –; MT181579*; –; MT155908*. ***S. longipes*** Rose; –; –; *P. Carrillo-Reyes & A. Kennedy 4420* (XAL); FJ753953; –; –; –. ***S. lydium*** Boiss. (JKC01); –; Garden origin; *J. Klein L141207-1524* (Sukkulenten-Sammlung Zürich 99 1284 /a); –; MT181580*; –; MT155909*. ***S. lydium*** Boiss. (TM36.2); Turkey; Izmir, Nif dağĭ, south of Kemâlpaşa: above Üçoluk çeşmesi on the track to mt. Nifkarlĭğĭ tepesi, on a block of stone, 1280 m; *K.P. Buttler & E. Erben 17374* (MSB); MT336120*; –; –; –. ***S. magellense*** Ten. (JKC05); Yugoslavia?; Komovi [?] Mts., [...], 1900 m; *H. ’t Hart 8528 = HtH 8528* (ZSS A 22663); –; MT181581*; MN381927*; MT155910*. ***S. magellense*** Ten. (TM75.1); North Macedonia; Šar Planina, rocky slope below limestone crag above Popova Šapka, 8 km of Tetovo, in grassy fissures between rocks, 2100 m; *J.R. Edmondson* (E 00763253); MT336121*; –; –; –. ***S. makinoi*** Maxim.; Japan; –; *Mayuzumi C00086* (TI); AB088627; –; –; AB089779. ***S. makinoi*** Maxim.; Japan; –; *T. Ito 2325* (TNS); –; LC258169; LC229436; –. ***S. mexicanum*** Britton; Japan; –; *Mayuzumi C00001* (TI); AB088621; –; –; AB089783. ***S. meyeri-johannis*** Engl. (JKC46); Tanzania; Arusha, Mt. Meru, E slope, road to the crater, Jekukumia River, in forest of *Podocarpus* and *Juniperus procera*, 3.23°S/36.80°E, 2100 m; *B.E. Jonsell 2148* (WAG 0085547); MT336122*; MT181582*; MN381928*; MT155911*. ***S. microcarpum*** (Sm.) Schönland (UM18); Cyprus; 34.90°N/32.32°E; *J. Klein H 120224-721* (MJG); KJ884127; MT181583*; KJ884336; MT155912*. ***S. mocinianum*** Pérez-Calix; –; –; *Stephenson 0.52.001*; HE999666; –; –; –. ***S. modestum*** Ball; –; –; (Utrecht University Botanic Gardens 33112); AY082101; AF115639; –; AY082221. ***S. monregalense*** Balb. (TM57.1); Italy; Toscana, mt. Falterona, Pso la Calla, 4000 ft. Habitat: mixed (dec. & conif.) woodland, sandy loam + lf mould on stone, med. shade; *K.I. Ransome* (E 00763266); MT336123*; –; MN381929*; –. ***S. moranense*** Kunth; –; –; *P. Carrillo-Reyes & F. Nicolalde 4236* (XAL); FJ753954; –; –; –. ***S. morganianum*** E.Walther; –; –; (University of Washington Botanical Garden 205); FJ753955; –; –; –. ***S. morrisonense*** Hayata; Taiwan; –; *Kokubugata 10831* (TNS); AB906477; –; –; AB932630. ***S. morrisonense*** Hayata; –; –; (Utrecht University Botanic Gardens 36400); –; AF115651; –; –. ***S. morrisonense*** Hayata; Taiwan; Nantou, Ren‘ai; *T. Ito 1274* (TNS); –; –; LC229477; –. ***S. mucizonia*** (Ortega) Raym.-Hamet (JKC30); Spain; Jaén, Desfiladeros de Despenaperros, 45 km south of Valdepenas, rivulet dale on the north facing bank; *Stud. biol. Rheno-Trai. in itinere 1328* (U 1213696); MT336124*; MT181584*; –; MT155913*. ***S. multicaule*** Wall. ex Lindl.; Nepal; –; *F.Miyamoto & al. TI9596136* (TI); AB088631; –; –; AB089782. ***S. multiceps*** Coss. & Durieu (JKC55); Tunisia; –; *H. ’t Hart 25256 = HtH 25256* (Sukkulenten-Sammlung Zürich 99 4291 /0); MT336125*; MT181585*; MN381930*; MT155914*. ***S. nanum*** Boiss. (JKC60); Iran; Kohkilouyeh-Boirahmad, 27 km N of Sisakht, Kuhe Gol; *Jamzad & al. 69488* (TARI 69488); MT336126*; MT181586*; –; MT155915*. ***S. nevadense*** Coss. (TM70.1); Spain; Ad lacum Cerveriz (Saliencia, Somiedo, Asturias), locis muscosis, substrato calcareo; (E 00763275); MT336127*; –; –; –. ***S. nokoense*** Yamam.; Taiwan; Hualien, Hsiulin Hsiang; *G. Kokubugata 10426* (TNS); AB906478; –; –; –. ***S. nokoense*** Yamam.; Taiwan: Nantou, Ren’ai; *T. Ito 3196* (TNS); –; LC258219; LC229486; LC229554. ***S. nudum*** Aiton (JKC56); Portugal; Madeira, c. 1 km E of Pico do Facho, along an old road to Canichal, E of Machico, 250 m; *H. ’t Hart 39-15 = HtH 28995* (ZSS A 21512); MT336128*; MT181587*; MN381931*; MT155916*. ***S. oaxacanum*** Rose; Mexico; Oaxaca; *P. Carrillo-Reyes & D. Cabrera-Toledo 5092* (XAL); EF632176; –; EF632193; –. ***S. oaxacanum*** Rose; –; –; (Huntington Botanical Garden 72161); –; AF115664; –; –. ***S. obcordatum*** R.T.Clausen; –; –; *P. Carrillo-Reyes & F. Nicolalde 4440* (XAL); FJ753957; –; –; –. ***S. obcordatum*** R.T.Clausen; –; –; (Huntington Botanical Garden 79360); –; AF115665; –; –. ***S. obtrullatum*** K.T.Fu; China; Xizang, Bomi Xian; *G.Y. Rao 090726-02* (PEY); KF113732; –; –; KF113838. ***S. obtusatum*** A.Gray (TM19.1); –; Garden origin; *J. Klein L141207-1523* (Sukkulenten-Sammlung Zürich 99 1277 /a); MT336129*; –; –; –. ***S. oreades*** Raym.-Hamet; Nepal; –; *F.Miyamoto & al. TI9420140* (TI); AB088632; –; –; –. ***S. oreades*** Raym.-Hamet; China; Xizang, Baqing Xian; *G.Y. Rao 090803-03* (PEY); –; –; –; KF113839. ***S. oreganum*** Nutt.; U.S.A.; Oregon Yachats; *Stephenson 9.12.006*; HE999673; KX452264; –; –. ***S. oregonense*** (S.Watson) M.Peck (UM31); –; Garden origin; (Washington Park Arboretum, WPA00-26); KJ884128; MT181588*; KJ884337; MT155917*. ***S. oxypetalum*** Kunth; –; –; *E. Pérez 3509* (IEB); FJ753958; –; FJ753856; –. ***S. pacense*** J.Meyrán; –; –; *E. Pérez 2750* (IEB); FJ753959; –; –; –. ***S. pachyphyllum*** Rose; –; –; *P. Carrillo-Reyes & F.Z. Vaz-de-Mello 4912* (IEB, XAL); FJ753960; –; –; –. ***S. pallidum*** M.Bieb. (KB10); Georgia; Samegrelo-Zemo Svaneti, Mestia valley, Central Greater Caucasus, Svaneti, gravelly layer on a scree slope N of Mestia, 43.10°N/42.74°E, ca. 1600 m; *H.-J. Zündorf 26534* (MJG 012904); MT336130*; MT181589*; –; MT155918*. ***S. palmeri*** S.Watson; –; –; *Acevedo & Hernández-Galavíz 1721* (XAL); AY545717; –; –; AY540578. ***S. plicatum*** Thiede & ’t Hart; –; –; *P. Carrillo-Reyes y M. Chocce 5173* (IEB, USM); FJ753961; –; FJ753857; –. ***S. polytrichoides*** Hemsl.; Japan; –; *Mayuzumi & al. C00029* (TI); AB088626; –; –; AB089772. ***S. polytrichoides*** Hemsl.; Japan; –; *T. Ito 396* (TNS); –; LC258172; LC229439; –. ***S. potosinum*** Rose (TM90.1); –; Garden origin (Botanical Garden Szeged); (MJG 024657); MT336131*; –; –; –. ***S. pubescens*** Vahl (TM82.1); Algeria; Oued Sebaou between Tizi Ouzou and Makouda, steep schist slopes of river valley, with *Sedum caeruleum*, 50–100 m; *Davis* (E 00764482); MT336132*; –; –; –. ***S. pubescens*** Vahl; Tunisia; Jebel Ichkeul; *HRT-31627*; –; –; –; X80551. ***S. quevae*** Raym.-Hamet; –; –; *P. Carrillo-Reyes y D. Cabrera-Toledo 4496* (XAL); FJ753962; –; FJ753858; –. ***S. reniforme*** (H.Jacobsen) Thiede & ’t Hart; –; –; *P. Carrillo-Reyes y M. Chocce 5174* (IEB, USM); FJ753963; –; FJ753859; –. ***S. retusum*** Hemsl.; –; –; *S. Zamudio & E. Pérez 9908* (IEB); FJ753964; –; FJ753860; –. ***S. rubens*** L. (TM58.1); Morocco; 22 km NE of Tafraout on road to Ait Baha, rocky slopes facing E, 1550 m; *Davis & King* (E 00764489); MT336133*; –; –; –. ***S. rubens*** L.; Turkey; Van, N of Muradiye, valley of the river Bendimahi, 1800 m; (ISTE 60911); –; –; –; not deposited (’t Hart & Alpınar, 1999). ***S. ruwenzoriense*** Baker f. (JKC45); Rwanda; Sabyinyo, Ruhengeri prefecture, on SE-facing rocks, among *Philippia johnstonii*, 1.4°S/29.6°E, 3300 m; *P.R.J. Bamps 3235* (WAG 0085546); MT336135*; MT181590*; MN381932*; MT155919*. ***S. sarmentosum*** Bunge; Japan; –; *Mayuzumi C00008* (TI); AB088624; –; –; –. ***S. sarmentosum*** Bunge; –; –; –; –; NC_023085; NC_023085; NC_023085. ***S. satumense*** Hatus.; Japan; Kyoto; *Stephenson V017*; HE999676; –; –; –. ***S. satumense*** Hatus.; Japan; –; *T. Ito 2295* (TNS); –; LC258174; LC229441; LC229509. ***S. sedoides*** (Decne.) Pau; –; –; *Stephenson V036*; HE999677; –; –; –. ***S. sedoides*** (Decne.) Pau; –; –; (University of Bonn Botanical Garden 15409); –; AF115677; –; –. ***S. sexangulare*** L.; Slovenia; Vrsic; *Stephenson 5.09.005*; HE999679; –; –; –. ***S. sexangulare*** L.; Poland; –; –; –; KJ746206; –; KJ746397. ***Sedum* sp.**; U.S.A.; –; –; HE999691; –; –; –. ***S. spathulifolium*** Hook. (JKC17); U.S.A.; California, Butte, on the east bank of Big Chico Creek, on the west side of the dirt road which is Ten Mile House Road, Upper Bidwell Park, about 3/4 mile (air) west of Highway 32, about 10 miles northeast of Chico; *L. Ahart 15772* (JEPS 115506); MT336136*; –; –; MT155920*. ***S. spathulifolium*** subsp. ***purdyi*** (Jeps.) R.T.Clausen; –; –; *Stephenson 9.15.001*; HE999687; KX452263; –; –. ***S. stefco*** Stef. (JKC07); Bulgaria; Bansko - Trigrad, about a 3-hour drive; *Stephenson* (Sukkulenten-Sammlung Zürich 10 0181 /0); MT336137*; MT181591*; MN381933*; MT155921*. ***S. suaveolens*** Kimnach; –; –; *Kimnach & Sánchez-Mejorada 1784* (Huntington Botanical Garden); AY545707; –; –; –. ***S. subtile*** Miq.; Japan; –; *A. Shimizu & al. 1999* (TI); AB088622; –; –; AB089775. ***S. subtile*** Miq.; –; –; –; –; KX452273; –; –. ***S. subulatum*** (C.A.Mey.) Boiss. (JKC61); Azerbaijan; between Vaighan and Veinagh, 1000 m; *Khatamsaz & Farzaneh 73097* (TARI 73097); –; MT181592*; –; MT155922*. ***S. subulatum*** (C.A.Mey.) Boiss.; Turkey; Ararat; *Stephenson V027*; HE999689; –; –; –. ***S. surculosum*** var. ***luteum*** (Emb.) Maire; Morocco; High Atlas; *Hornát S2*; LM993288; KX452239; –; –. ***S. tenellum*** M.Bieb. (KB11); Georgia; Samtskhe-Javakheti, Bakuriani, Caucasus,

Appendix 1. Continued.

Tskhratskaro Pass, Mt. Tskhratskharo, alpine meadow, 41.69°N/43.53°E, 2687 m; *S. Shetekauri* (MJG 024793); MT336138*; MT181593*; –; MT155923*. ***S. ternatum*** Michx.; –; –; (Missouri Botanical Garden 951462); –; AF115670; –; –. ***S. ternatum*** Michx. **x** ***S. spathulifolium*** Hook.; –; –; *Stephenson 9.18.004*; HE999633; –; –; –. ***S. tosaense*** Makino; Japan; –; *A. Iwamoto 2000* (TI); AB088620; –; –; AB089787. ***S. treleasei*** Rose; –; –; *D. Cabrera-Toledo y F. Nicolalde 4* (XAL); FJ753966; –; –; –. ***S. triactina*** A.Berger; Nepal; –; *F. Miyamoto & al. TI9596091* (TI); AB088629; –; –; AB089780. ***S. trichromum*** R.T. Clausen; –; –; *P. Carrillo-Reyes & E. Ruiz-Sánchez 4733* (XAL); FJ753967; –; FJ753861; –. ***S. trullipetalum*** Hook.f. & Thomson; Nepal; –; *F. Miyamoto & al. TI9420132* (TI); AB088630; –; –; AB089781. ***S. tuberiferum*** Stoj. & Stef.; Bulgaria; Lower Mesta Valley; *Stephenson 5.00.004*; HE999692; KX452278; –; –. ***S. uniflorum*** subsp. ***japonicum*** (Sieb. ex Miq.) H.Ohba; Japan; –; *Mayuzumi & al. C00030* (TI); AB088617; –; –; AB089784. ***S. uniflorum*** subsp. ***japonicum*** (Sieb. ex Miq.) H.Ohba; –; –; –; –; KX452274; –; –. ***S. uniflorum*** subsp. ***oryzifolium*** (Makino) H.Ohba; Japan; –; *Mayuzumi C00016* (TI); AB088618; –; –; –. ***S. uniflorum*** subsp. ***oryzifolium*** (Makino) H.Ohba; South Korea; Dokdo; –; –; NC_027837; NC_027837; NC_027837. ***S. ursi*** ’t Hart; Turkey; Baba Dagi summit; *Stephenson 5.11.003*; HE999693; KX452279; –; –. ***S. urvillei*** DC. (JKC63); Bulgaria; Warna, Aksakovo, Black Sea Coast, Slanchevo, 43.227426°N/27.705904°E, 114 m; *J. Klein J130820-1344* (TARI 73097-1344); MT336139*; MT181594*; MN381934*; MT155924*. ***S. urvillei*** DC. (TM13.1); Bulgaria; Northern Coast of the Black Sea, Probiti Kamini, between columns of stone, 43°13′42″N/27°42′22″E, 124 m; *Gr. Bot. Exk. Nr. 10* (MJG 024786); MT336140*; –; –; –. ***S. versadense*** C.H.Thomps.; –; –; *P. Carrillo-Reyes & D. Cabrera-Toledo 4498* (IEB, XAL); FJ753968; –; FJ753862; –. ***S. villosum*** L. (O3); Norway; Oppland, Lom, Gokkerdalen at the foot of mt. Lauvhø, ca. 1150 m; *R.Y. Berg* (O 257620); MT336141*; MT181595*; –; MT155925*. ***S. vinicolor*** S.Watson; –; –; *P. Carrillo-Reyes & E. Ruiz-Sánchez 4779* (IEB, XAL); FJ753969; –; –; –. ***S. wilczekianum*** Font Quer (JKC04); Morocco; Distr. Tarquist, N of Beni-Boufrah, near Torres-de-Alcala, 5 m; *S.L. Jury & Springate 11326* (Sukkulenten-Sammlung Zürich 99 4481 /0); MT336142*; MT181596*; –; MT155926*. ***S. wrightii*** A.Gray; –; –; *P. Carrillo-Reyes & A. de Nova 4582* (XAL); FJ753970; –; –; –. ***S. zentaro-tashiroi*** Makino; Japan; –; *Ohba 1998* (TI); AB088619; –; –; AB089785. ***Sempervivum atlanticum*** (Ball) Ball (JKB216); Morocco; 31.21°N/7.85°W; *D.R. Letz, S. Španiel, P. Mered‘a 43C* (MJG 024784); KJ884188; MT181597*; KJ884385; MT155927*. ***S. ruthenicum*** W.D.J.Koch ex Schnittsp. & C.B.Lehm. (JKB168); Ukraine; 49.35°N/26.09°E; *D.R. Letz, I. Hodálová, P. Mered’a DRL048* (MJG); KJ884292; –; KJ884483; –. ***Sinocrassula indica*** (Decne.) A.Berger; China; –; *S. Mayuzumi C0040C* (TI); AB480611; –; –; –. ***S. indica*** (Decne.) A.Berger; –; –; *Cody*; –; AF115679; –; –. ***S. indica*** (Decne.) A.Berger; China; –; *S. Akiyama 2000* (TI); –; –; –; AB480665. ***S. yunnanensis*** (Franch.) A.Berger; –; –; *L.Y. Chen*; KC988288; KC988295; –; –. ***S. yunnanensis*** (Franch.) A.Berger; China; –; *S. Mayuzumi C00115* (TI); –; –; –; AB480669. ***Thompsonella colliculosa*** Moran; Mexico; Michoacán; *P. Carrillo-Reyes & E. Pérez-Calix 2714* (IBUG, IEB, GUADA); EF632177; –; –; –. ***T. garcia-mendozae*** P.Carrillo & Pérez-Calix; Mexico; Michoacán; *P. Carrillo-Reyes & E. Pérez-Calix 3186* (IEB); EF632178; –; –; –. ***T. minutiflora*** (Rose) Britton & Rose; Mexico; Puebla; *P. Carrillo-Reyes & D. Cabrera-Toledo 4262* (XAL); EF632179; –; EF632200; –. ***T. minutiflora*** (Rose) Britton & Rose; –; –; (Huntington Botanical Garden 72129); –; AF115681; –; –. ***T. minutiflora*** (Rose) Britton & Rose; –; –; (Huntington Botanical Garden 88162); –; –; –; AY540580. ***T. mixtecana*** J.Reyes & L.G.López; Mexico; Guerrero; *P. Carrillo-Reyes & E. Pérez-Calix 2747* (IBUG, IEB, GUADA); EF632180; –; –; –. ***T. platyphylla*** Rose; Mexico; Guerrero; *P. Carrillo-Reyes & E. Pérez-Calix 2727* (IBUG, IEB, GUADA); EF632182; –; –; –. ***T. spathulata*** Kimnach; Mexico; Oaxaca; *P. Carrillo-Reyes & D. Cabrera-Toledo 4914* (XAL); EF632183; –; –; –. ***T. xochipalensis*** Gual, S.Peralta & Pérez-Calix; Mexico; Guerrero; *P. Carrillo-Reyes & E. Pérez-Calix 2734* (IBUG, IEB); EF632184; –; –; –. ***Tylecodon racemosus*** (Harv.) Toelken; Namibia; –; *Bruyns 9476b* (BOL); MH503627; –; –; –. ***T. singularis*** (R.A.Dyer) Toelken; Namibia; –; *Bruyns 8373* (BOL, MO, NBG, WIND); MH503628; –; –; –. ***T. torulosus*** Toelken; –; –; *Bruyns 9124* (BOL); LN878945; –; –; –. ***T. ventricosus*** (Burm.f.) Toelken; South Africa; –; *Bruyns 12927* (BOL); MH503629; –; –; –. ***Umbilicus oppositifolius*** (Ledeb.) Ledeb. (KB08); –; Garden origin (Botanischer Garten der Johannes Gutenberg-Universität Mainz); (MJG); KJ884131; MT181598*; –; MT155928*. ***U. schmidtii*** Bolle; Cape Verde; São Nicolau, Monte Gordo, Rib. dos Calhaus, 16.63°N/24.36°W; *Duarte & al. 4125c* (LISC); KP279434; KP279376; –; KP279334. ***Villadia albiflora*** (Hemsl.) Rose; –; –; *P. Carrillo-Reyes & D. Cabrera-Toledo 4495* (XAL); FJ753971; –; FJ753863; –. ***V. aristata*** Moran; Mexico; Nuevo León; *J. Cortés-Aguilar* (IBUG); EF632185; –; EF632202; –. ***V. cucullata*** Rose; –; –; *M. Kristen y J. Etter 2056* (XAL); FJ753972; –; –; –. ***V. diffusa*** Rose; –; –; *F. Nicolalde 1461* (XAL); FJ753973; –; FJ753864; –. ***V. imbricata*** Rose; Mexico; Oaxaca; *P. Carrillo-Reyes & F.Z. Vaz-de-Mello 4926* (XAL); EF632187; –; EF632203; –. ***V. incarum*** (Ball) Baehni & J.F.Macbr.; –; –; *P. Carrillo-Reyes & N.I. Ortega 5179* (IEB, USM); FJ753974; –; FJ753865; –. ***V. minutiflora*** Rose; –; –; *P. Carrillo-Reyes & D. Cabrera-Toledo 5095* (IEB); FJ753975; –; FJ753866; –. ***V. misera*** (Lindl.) R.T. Clausen; –; –; *P. Carrillo-Reyes & F. Nicolalde 4357* (XAL); FJ753976; –; FJ753867; –. ***V. nelsonii*** Rose; –; –; *P. Carrillo-Reyes y J.A. Lomelí-Sención 3709* (GUADA, XAL); FJ753977; –; FJ753868; –. ***V. pringlei*** Rose; –; –; *P. Carrillo-Reyes & E. Ruiz-Sánchez 4800* (XAL); FJ753978; –; FJ753869; –. ***V. recurva*** Moran, Kimnach & C.H.Uhl; –; –; *P. Carrillo-Reyes & F.Z. Vaz-de-Mello 4972* (XAL); FJ753979; –; FJ753870; –.

Appendix 2. Taxa with supportedly conflicting positions in the phylogenies based on nuclear and plastid sequence data.

Dudleya pulverulenta (Nutt.) Britton & Rose
D. viscida (S.Watson) Moran
Graptopetalum macdougallii Alexander
Petrosedum amplexicaule subsp. *tenuifolium* (Sm.) Velayos
P. anopetalum (DC.) Grulich
P. forsterianum (Sm.) Grulich
P. sediforme (Jacq.) Grulich
Pistorinia breviflora Boiss.
Rosularia serrata (L.) A.Berger
Sedum album L.
S. fragrans ’t Hart
S. hakonense Makino

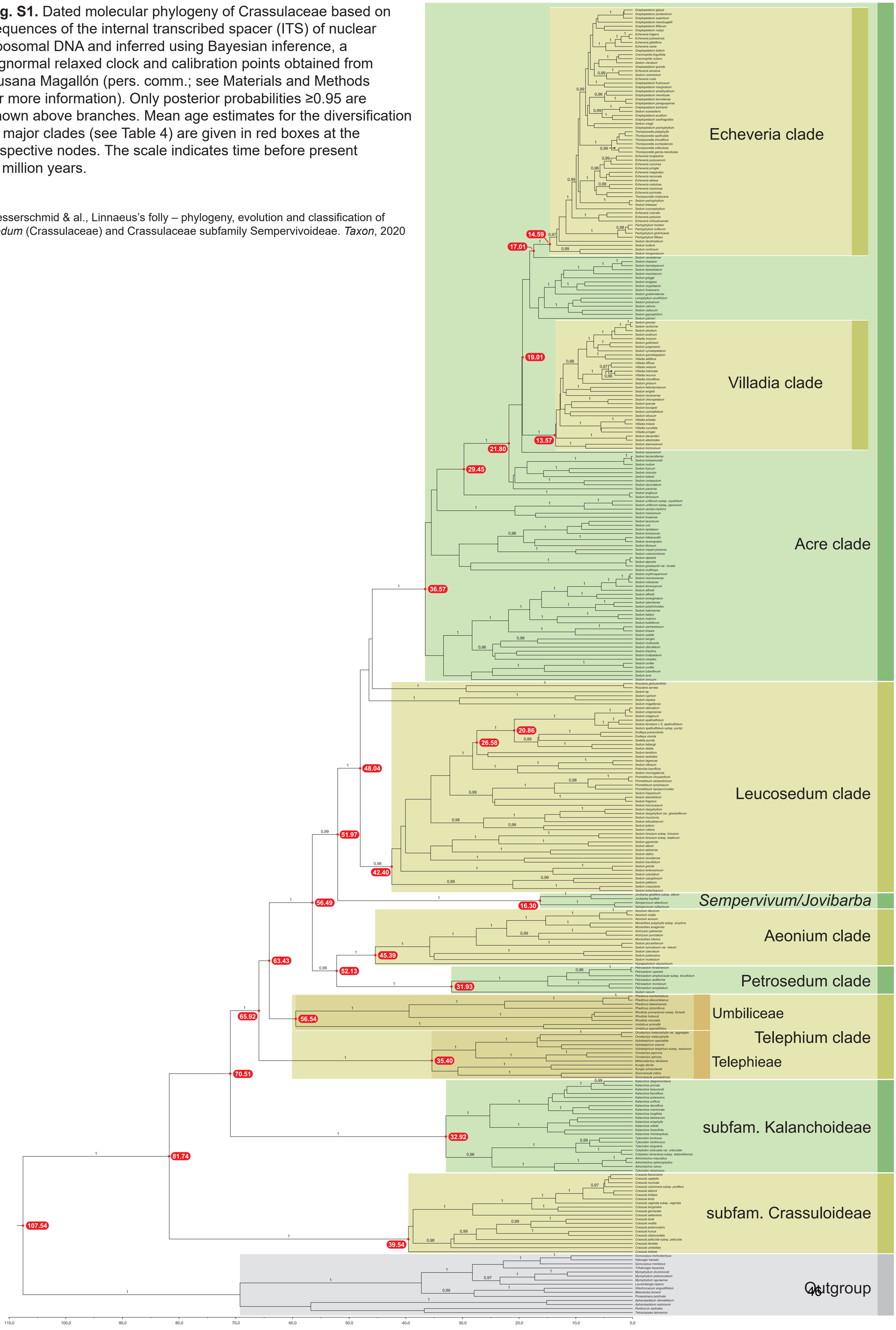
(Continues)

Appendix 2. Continued.

S. litoreum Guss.
S. lydium Boiss.
S. nanum Boiss.
S. satumense Hatus.
S. sedoides (Decne.) Pau
S. subtile Miq.
S. tenellum M.Bieb.
Villadia diffusa Rose
V. incarum (Ball) Baehni & J.F.Macbr.

Fig. S1. Dated molecular phylogeny of Crassulaceae based on sequences of the internal transcribed spacer (ITS) of nuclear ribosomal DNA and inferred using Bayesian inference, a lognormal relaxed clock and calibration points obtained from Susana Magallón (pers. comm.; see Materials and Methods for more information). Only posterior probabilities ≥0.95 are shown above branches. Mean age estimates for the diversification of major clades (see Table 4) are given in red boxes at the respective nodes. The scale indicates time before present in million years.

Messerschmid & al., Linnaeus's folly – phylogeny, evolution and classification of *Sedum* (Crassulaceae) and Crassulaceae subfamily Sempervivoideae. *Taxon*, 2020



2. Processes of evolutionary diversification in *Aeonium* (Crassulaceae), the most species-rich genus of Macaronesian plant radiations

Abstract

Oceanic archipelagos are regarded as natural laboratories for the study of evolution and hold a wealth of examples of endemic plant radiations. The Canary Islands form an archipelago central to the Macaronesian floristic region and have a rich history of botanical research. The most species-rich and ecologically diverse plant radiation (largely) endemic to the Canary Islands is the *Aeonium* alliance (i.e., tribe Aeonieae of Crassulaceae subf. Sempervivoideae). We studied the biogeographical and ecological setting of diversification processes in *Aeonium* on the basis of a well-sampled and dated phylogeny inferred using a modified ddRADseq approach. Under the assumption of an adaptive radiation, as often proposed for *Aeonium*, we hypothesized that (1) within-island sympatric diversification events are more frequent than between-island allopatric diversification, that (2) sympatric diversification is more frequently associated with ecological divergence of sister lineages than allopatric diversification, and that (3) ecological differentiation and reproductive barriers between taxa are better established on those islands that have a longer history of colonization by *Aeonium* and have received fewer *Aeonium* taxa by secondary colonization. The results of our ancestral area reconstruction showed that *Aeonium* is one of only few Canary Island radiations that successfully back-colonized the African continent twice. With a Biogeographical Stochastic Mapping (BSM) analysis, we found sympatric and allopatric diversification events in almost equal numbers rather than a dominance of sympatric diversification. This illustrates the importance of both geographical isolation and ecological speciation in island plant radiations. In lineages that diversified within single islands, morphological and ecological divergence was more pronounced than in lineages with frequent allopatric diversification, but only the difference in morphological divergence was significant. Sister lineages derived from sympatric diversification events show a higher diversity of growth forms than allopatric sister lineages. These findings imply that diversification in *Aeonium* resulted both from adaptive and geographical processes, as would be expected in an island radiation. Finally, those islands that were found to have the longest continuous history of (primary) colonization by *Aeonium* (e.g., Tenerife and La Gomera) also had the lowest percentages of actually co-occurring and hybridizing taxon pairs (relative to the number of possible combinations of any two taxa) when compared to islands that received *Aeonium* taxa more recently (e.g., La Palma and El Hierro), indicating a higher degree of ecological differentiation and reproductive isolation between taxa after longer periods of shared island colonization.

Keywords

adaptive radiation; Aeonieae; ancestral area reconstruction; biogeographical stochastic mapping; biogeography; diversification; evolution on islands; molecular dating

1. Introduction

Oceanic islands and archipelagos have long been considered natural laboratories where evolutionary and biogeographical processes that are complex on a continental scale are simplified and easier to study (Whittaker and Fernández-Palacios, 2007). Patterns of biodiversity on islands have fostered ideas and theories about island biogeography (Carlquist, 1965, 1974; MacArthur and Wilson, 1967) and the theory of evolution itself (Darwin, 1859). Another advantage of islands for the study of evolution is their greater number in comparison to continents, providing "necessary replications in natural 'experiments' by which evolutionary hypotheses can be tested" (MacArthur and Wilson, 1967). Due to their geographical isolation and limited size, they are characterized by a high degree of endemism but relatively low overall species numbers in comparison to continents (Whittaker and Fernández-Palacios, 2007). The floras and faunas of oceanic archipelagos are rich in examples of monophyletic lineages that diversified into distinct species often endemic to one or few islands, lineages that are commonly referred to as island radiations (e.g., Silvertown et al., 2005). Examples of such island radiations are the Caribbean *Anolis* lizards (Losos et al., 1998), Hawaiian honeycreepers (Lerner et al., 2011), lobeliads (Givnish et al., 2009) and silverswords (Baldwin, 2003), and the *Aeonium* alliance (Liu, 1989; Mort et al., 2002), *Echium* L. (Böhle et al., 1996) and *Argyranthemum* Webb (Francisco-Ortega et al., 1996; White et al., 2020) of the Macaronesian floristic region. Most of these examples are thought to have diversified by the process of adaptive radiation, i.e., "the rise of a diversity of ecological roles and attendant adaptations in different species within a lineage" in the definition by Givnish (1997). However, other examples of island radiations such as the Aegean *Nigella arvensis* complex (Comes et al., 2008) have been interpreted as non-adaptive radiations, because no appreciable diversity of ecological roles among the taxa belonging to the radiation could be identified.

Aeonium Webb & Berthel. (including *Greenovia* Webb. & Berthel., Crassulaceae; Fig. 1) is an iconic group of succulent plants that enjoys high popularity in horticulture and botanical research (Praeger, 1932; Lems, 1960; Liu, 1989; Lösch, 1990; Mes and 't Hart, 1996; Jorgensen and Frydenberg, 1999; Jorgensen and Olesen, 2001; Mort et al., 2002, 2007; Kim et al., 2008; Esfeld et al., 2009; Thiv et al., 2010a; Harter et al., 2015; Brilhante et al., 2021). With its 40 currently accepted species distributed predominantly in the Canary Islands (Table 1), *Aeonium* belongs to the most species-rich radiation of the Macaronesian flora (Jorgensen and Olesen, 2001), the *Aeonium* alliance, which also includes *Aichryson* Webb & Berthel. (15 spp.) and *Monanthes* Haw. (11 spp.; Praeger, 1932; Liu, 1989; Mes, 1995; Mort et al., 2002; Rebmann, 2013; Bañares Baudet, 2015a,b). This group shows outstanding diversity in growth form

(Lems, 1960; Liu, 1989; Mes and 't Hart, 1996), ecological niche and photosynthetic pathway (Tenhunen et al., 1982; Lösch, 1990; Pilon-Smits et al., 1992; Mort et al., 2007), and has therefore been among the most important systems for the study of adaptive radiations of island plants (Givnish, 2010; Thiv et al., 2010a) and the evolution of insular woodiness (Mes et al., 1996; Van Huysduynen et al., 2021). As in a number of other lineages of Crassulaceae, e.g., *Echeveria* DC. (Uhl, 1992) or *Kalanchoe* (Smith et al., in press), many species of *Aeonium* are interfertile and produce a high number of naturally occurring hybrids (Bañares Baudet, 2015a; Arango Toro, 2017, 2019).

Within the *Aeonium* alliance, *Aichryson* and *Monanthes* have been considered strictly endemic to the Macaronesian Islands (Bañares Baudet, 2015a). However, a population of *Aichryson tortuosum* Webb & Berthel. has been documented in Morocco (Sant et al., 2017), and it is not yet clear whether this is an introduced population or the result of natural dispersal. With the exception of the only two monospecific sections of *Aeonium*, i.e., sects. *Patinaria* (Lowe) A.Berger and *Pittonium* A.Berger which are endemic to Madeira (Table 1), all other sections include species that are endemic to one or several of the Canary Islands. However, three species of sect. *Aeonium* are endemic to the African continent (*A. korneliuslemsii* H.Y.Liu, *A. leucoblepharum* Webb ex A.Rich. and *A. stuessyi* H.Y.Liu) and one to the Cape Verde Islands (*A. gorgoneum* J.A.Schmidt; Table 1). Twenty-seven of the 37 insular species of *Aeonium* are single-island endemics (SIEs) while the remaining 10 species are multi-island endemics (MIEs). Four of these 10 MIE species contain infraspecific taxa that occupy different islands.

While most species of *Aichryson* as well as *Monanthes icterica* (Webb ex Bolle) Christ are annual or short-lived perennial herbs, the remaining species of *Monanthes* and *Aeonium* are perennial herbs or subshrubs, with the exception of *A. glandulosum* (Aiton) Webb & Berthel. and *A. tabuliforme* Webb & Berthel. which are mostly biennial (Liu, 1989). This dichotomy in habit is reflected in the molecular phylogenies of the *Aeonium* alliance with *Aeonium* and perennial *Monanthes* forming a monophyletic group sister to *Aichryson* (Mes, 1995; Mort et al., 2002; Kim et al., 2008; Messerschmid et al., 2020). While the relationships of *Monanthes icterica* were ambiguous in the study by Mort et al. (2002), with phylogenies based on plastid DNA markers resolving this species as sister to *Aichryson*, and the ITS (Internal Transcribed Spacer regions of nuclear ribosomal DNA) phylogeny resolving it as sister to the *Aeonium* plus *Monanthes* s.str. clade, it was always supported as sister to *Aichryson* in a more recent molecular phylogenetic analysis (Messerschmid et al., 2020). The closest relatives of the *Aeonium* alliance are eight northwest African species of *Sedum* L. series *Caerulea* Fröd., *Monanthoidea* Batt. ex Fröd. and *Pubescens* T.Mes which form a grade basal to the *Aeonium* alliance (see Chapter 3; Messerschmid et al., in preparation). These eight species share polymerous flowers (Fröderström, 1932) and, partly, some karyological features (Uhl, 1961) with species of the *Aeonium* alliance. The molecular phylogenetic analysis of subf. Sempervivoideae by Messerschmid et al. (2020) recently confirmed the notion of Fröderström (1930) and Uhl (1961) that the Ethiopian herbaceous geophyte *Hypagophytum abyssinicum* (Hochst. ex A.Rich.) A.Berger, the only species of the genus, is closely related to the *Aeonium* alliance and the eight northwest African species of *Sedum*. All these (*Aeonium* alliance, *Sedum* p.p., *Hypagophytum* A.Berger) make up tribe Aeonieae of subf. Sempervivoideae.

Webb and Berthelot (1836–1840) segregated *Aeonium*, *Aichryson* and *Greenovia* from *Sempervivum* L. as Haworth (1821) before had done for *Monanthes*. *Greenovia* differs from all (other) *Aeonium* species by its highly polymerous (20- to 35-merous) flowers, but shares its lack of hypogynous nectar glands with species of *Aeonium* sects. *Petrothamnium* (Webb ex Christ) H.Y.Liu and *Chrysocome* Webb ex Christ (see Table 1 for sectional affiliation of all *Aeonium* species). Mes (1995), who transferred all species of *Greenovia* into *Aeonium* because his phylogenies based on nuclear and plastid DNA sequences resolved *Greenovia diplocycla* Webb ex Bolle as nested within *Aeonium*, gave *Greenovia* sectional rank within *Aeonium*. The nested position of *Greenovia* in *Aeonium* was later corroborated by Mort et al. (2002) who sampled three of the four species of *Greenovia* and > 80% of the species of *Aeonium* s.str. Apart from sect. *Greenovia*, *Aeonium* has been divided into eight more sections (Table 1) of which sect. *Leuconium* A.Berger is the most species-rich and is characterized by white to reddish flowers and ciliate leaf margins. This sectional classification of *Aeonium* was mainly developed by Berger (1930), Praeger (1932), Liu (1989), Mes (1995) and Bañares Baudet (2015a). Early molecular phylogenetic studies (Mes, 1995; Mes and 't Hart, 1996) provided some evidence for the monophyly of sect. *Leuconium* and sect. *Aeonium*, but this could not be substantiated in the better-sampled study by Mort et al. (2002). Mort et al. (2002), however, resolved their sample of sect. *Canariensia* (Christ) Praeger as monophyletic. Furthermore, the monophyly of sect. *Goochiae* (Christ) Praeger, after exclusion of *A. lindleyi* subsp. *viscatum* (Bolle) Bañares, was supported in two of the more recent phylogenetic studies (Mort et al., 2002; Thiv et al., 2010a), implying that *A. lindleyi* Webb & Berthel. is not monophyletic. Apart from these findings, monophyly of sections and relationships among them remained unclear.

Knowledge of the age of *Aeonium* is key to understanding its evolution but has long been discussed controversially. While Praeger (1932) rightly suspected Macaronesian Aeonieae to be a relatively young group, Lems (1960) later hypothesized that the East African *A. leucoblepharum* should be regarded as an old link between the Macaronesian flora and "the

East and South African center of the Crassulaceae", referring to the early-diverging subfamilies Crassuloideae and Kalanchoideae of Crassulaceae. However, molecular phylogenetic studies (Mes, 1995; Mes and 't Hart, 1996; Mes et al., 1996; Jorgensen and Frydenberg, 1999; Mort et al., 2002) consistently placed *A. leucoblepharum* within the crown group of *Aeonium*, implying that this species originated from back-colonization of the African continent instead of being derived from South African ancestors. Given the poor resolution of phylogenetic relationships within *Aeonium* using ITS, Jorgensen and Frydenberg (1999) concluded that the lineage must have diversified recently and rapidly. This notion was later confirmed by phylogenetic studies dating the diversification of the Macaronesian Aeonieae (also including *Aichryson* and *Monanthes*) to approximately 6.73 (Kondraskov et al., 2015) and 15.2 million years (myr; Kim et al., 2008). In order to answer the fundamental question whether the radiation of *Aeonium* is an adaptive radiation (Jorgensen and Olesen, 2001; Mort et al., 2007; Thiv et al., 2010a), a fully resolved phylogeny and chronogram and a formal biogeographical analysis are needed.

So far molecular phylogenetic studies of *Aeonium* have been unsuccessful in resolving the phylogeny of the genus (Van Huysduynen et al., 2021), mainly because data from restriction fragment length polymorphism (RFLP) analyses (Mes and 't Hart, 1996) or sequences of only few molecular markers (Mort et al., 2002) have not been sufficiently informative. Thanks to advances in next-generation sequencing methods, the past years have seen improvements in our phylogenetic and evolutionary knowledge of important Macaronesian plant radiations, e.g., *Tolpis* Adans. (Asteraceae; Mort et al., 2015), *Micromeria* Benth. (Lamiaceae; Curto et al., 2018), *Argyranthemum* (Asteraceae; White et al., 2020) and *Helianthemum* Mill. (Cistaceae; Albaladejo et al., 2021). All these studies used high-throughput sequencing methods for reduced-representation libraries of genomic DNA, such as genotyping-by-sequencing (GBS; Elshire et al., 2011) or restriction-site associated DNA sequencing (RADseq; Baird et al., 2008; Andrews et al., 2016). However, due to the usually short sequence reads obtained with these methods, most of these studies (except Curto et al., 2018) based their phylogenetic analyses on datasets of concatenated DNA loci which precludes consideration of independently evolving sequence markers potentially containing differing and incongruent phylogenetic signal (Kubatko and Degnan, 2007; Pirie, 2015).

We here present a largely resolved and dated phylogeny of *Aeonium* sampling all currently accepted species and using a modified ddRADseq library preparation protocol with which long loci can be generated (Hühn et al., 2021) and which allows us to deploy coalescent-based summary methods. Based on the results of our phylogenetic analyses, we will discuss the sectional classification of *Aeonium* and identify the taxonomic changes necessary to render all sections monophyletic, and will identify species that are not monophyletic. The main aims of our biogeographical analysis are to identify migration between islands and out of the Canary Islands, and to quantify the relative contributions of allopatric (between-island) and sympatric (ecological and within-island) speciation to the diversification in *Aeonium*. Under the assumption of an adaptive radiation, we hypothesize that (1) sympatric diversification events should have been more frequent than allopatric diversification events, that (2) clades that diversified within single islands should have evolved higher morphological and ecological diversity than clades that diversified by repeated dispersal between islands, and that (3) habitat partitioning and reproductive barriers between different *Aeonium* taxa should be better established on (mostly older) islands which were colonized long ago and were later subject to only few instances of secondary colonization than on (mostly younger) islands which were colonized by *Aeonium* more recently and frequently.

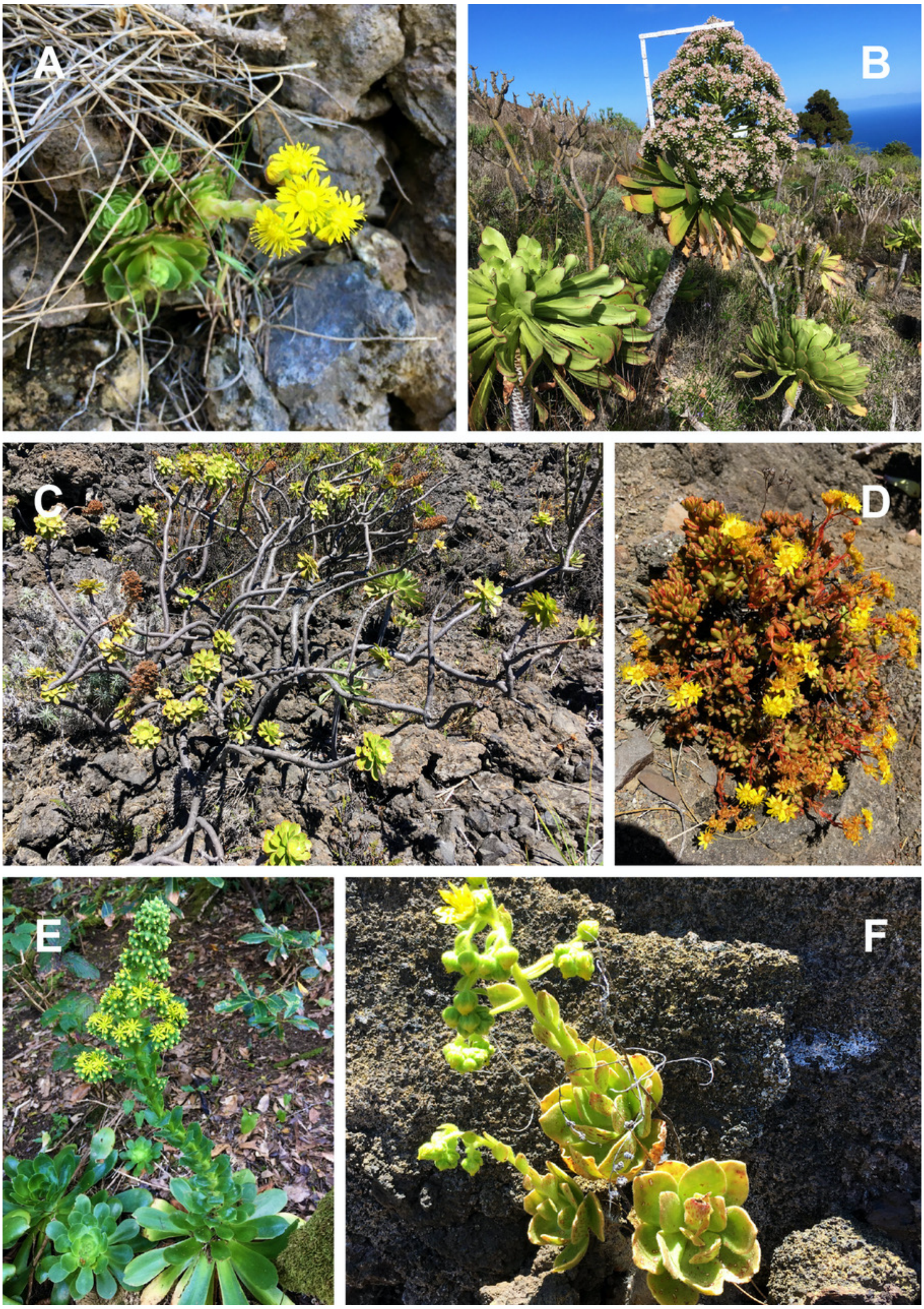


Fig. 1. Aspects of some species of *Aeonium*. (A) Sect. *Greenovia*: *A. aizoon*, a small (up to 15 cm high) rosette plant endemic to Tenerife, with ± 20-merous nectary-lacking flowers. (B) Sect. *Leuconium*: *A. urbicum* subsp. *meridionale*, a tall (1.5–2 m high) monocarpic plant with unbranched woody stem and hundreds of white flowers. Both subspecies of *A. urbicum* are endemic to Tenerife. (C) Sect. *Aeonium*: *A. arboreum* subsp. *holochrysum*, a distantly branched, spacious and tall (up to 1.5 m high) shrub with yellow flowers appearing in winter, distributed across El Hierro, La Palma and Tenerife. (D) Sect. *Petrothamnium*: *A. sedifolium*, a small (up to 25 cm high), densely branched shrublet with little thick and clavate leaves, native to La Palma, La Gomera and Tenerife. (E) Sect. *Canariensia*: *A. cuneatum*, a sturdy, herbaceous rosette plant with distinctly glaucous leaves endemic to the laurel forest of northern Tenerife (Teno and Anaga). (F) Sect. *Chrysocome*: *A. smithii*, a shrublet with densely hairy branches and leaves featuring conspicuous reddish tannic stripes.

Table 1. Distribution, sectional affiliation and ploidy level of all currently accepted *Aeonium* species following Bañares Baudet (2015a) for Canary Island endemics and Mes (1995) for taxa distributed outside the Canary Islands. C: Gran Canaria, EA: East Africa (including the southwestern part of the Arabian Peninsula), F: Fuerteventura, G: La Gomera, H: El Hierro, L: Lanzarote, Mc: Morocco, Md: Madeira, P: La Palma, T: Tenerife, V: Cape Verde

Section	Species	Subspecies/Variety	H	P	G	T	C	F	L	Mc	EA	Md	V	Ploidy
Aeonium	*A. arboreum* Webb & Berthel.	subsp. *arboreum*					X							$2n^{a}$, $4n^{b,c}$
		subsp. *holochrysum* (H.Y.Liu) Bañares	X	X	X	X								$2n^{a,b,d,e}$, $4n^{c}$
	A. balsamiferum Webb & Berthel.							X	X					$4n^{a,b,c}$
	A. gorgoneum J.A.Schmidt												X	$2n^{a}$, $4n^{e}$
	A. korneliuslemsii H.Y.Liu									X				$4n^{a}$
	A. leucoblepharum Webb ex A.Rich.										X			$2n^{a}$, $4n^{c}$
	A. simsii (Sweet) Stearn						X							$2n^{b}$, $3n^{a,b}$, $4n^{b,c,e}$
	A. stuessyi H.Y.Liu										X			unknown
	A. undulatum Webb & Berthel.						X							$4n^{a,b,c}$
Canariensia	*A. canariense* (L.) Webb & Berthel.	subsp. *canariense*				X								$2n^{a,b,d,e}$
		subsp. *christii* (Burchard) Bañares	X	X										$2n^{a,b,d,e}$
		subsp. *latifolium* (Burchard) Bañares			X									$2n^{a,b,e}$
		subsp. *virgineum* (Webb) Bañares					X							$2n^{a,b,e}$
	A. cuneatum Webb & Berthel.					X								$2n^{a,b,e}$
	A. tabuliforme Webb & Berthel.					X								$2n^{a,b,d,e}$
Chrysocome	*A. smithii* Webb & Berthel.					X								$2n^{a,b,d,e}$
	A. spathulatum (Hornem.) Praeger		X	X	X	X	X							$2n^{a,b,d,e}$

Goochiae	*A. goochiae* Webb & Berthel.			X										$2n^{a,b,d}$
	A. lindleyi Webb & Berthel.	subsp. *lindleyi*				X								$2n^{a,b,d,e}$
		subsp. *viscatum* (Bolle) Bañares			X									$2n^{a,b,e}$
Greenovia	*A. aizoon* (Bolle) T.Mes					X								$2n^{e}$
	A. aureum (C.Sm. ex Hornem.) T.Mes					X	X							$2n^{b,e}$
	A. diplocyclum (Webb ex Bolle) T.Mes		X	X	X									$2n^{b,e}$, $4n^{b,c}$
	A. dodrantale (Willd.) T.Mes					X								$2n^{b,e}$
Leuconium	*A. appendiculatum* Bañares				X									$2n^{e}$
	A. castello-paivae Bolle				X									$2n^{a,b,e}$
	A. ciliatum Webb & Berthel.					X								$2n^{a,b,d,e}$
	A. davidbramwellii H.Y.Liu			X										$2n^{a,e}$
	A. decorum Webb ex Bolle	var. *alucense* Bañares & M.Marrero			X									unknown
		var. *decorum*			X	X								$2n^{a,b,e}$
	A. escobarii Rebmann & Malkm.-Huss.			X										unknown
	A. gomerense (Praeger) Praeger				X									$2n^{a,b,e}$
	A. haworthii Webb & Berthel.					X								$4n^{a,b,c,e}$
	A. hierrense (Murray) J.Pitard & L.Proust		X											$2n^{a}$
	A. lancerottense (Praeger) Praeger								X					$2n^{a,b}$
	A. mascaense Bramwell					X								unknown
	A. nobile (Praeger) Praeger			X										$2n^{a,b,e}$
	A. percarneum (Murray) J.Pitard & L.Proust						X							$2n^{a,b,e}$
	A. pseudurbicum Bañares					X								$2n^{e}$

	A. urbicum (C.Sm. ex Hornem.) Webb & Berthel.	subsp. *meridionale* (Bañares) Bañares				X								$2n^{e}$
		subsp. *urbicum*				X								$2n^{a,b,d}$, $4n^{c}$
	A. valverdense (Praeger) Praeger		X											$2n^{a}$, $4n^{c}$
	A. volkeri E.Hern. & Bañares					X								$4n^{e}$
Patinaria	*A. glandulosum* (Aiton) Webb & Berthel.											X		$2n^{a,e}$
Petrothamnium	*A. saundersii* Bolle				X									$2n^{a,e}$
	A. sedifolium (Webb ex Bolle) J.Pitard & L.Proust			X	X	X								$2n^{a,b,d,e}$
Pittonium	*A. glutinosum* (Aiton) Webb & Berthel.											X		$2n^{a,b,e}$

[a] Liu (1989)

[b] Uhl (1961)

[c] Mes (1995)

[d] Suda et al. (2005)

[e] Brilhante et al. (2021)

2. Materials and methods

2.1. Taxon sampling

All 40 species and all infraspecific taxa of *Aeonium* except for *A. decorum* var. *alucense* Bañares & M.Marrero were sampled for DNA analysis. Of these, five species and one subspecies had never been included in a molecular phylogeny before. *Monanthes brachycaulos* (Webb & Berthel.) Lowe and *M. muralis* (Webb ex Bolle) Hook.f. were chosen as outgroup because *Monanthes* (excl. *M. icterica*) is sister to *Aeonium*. Overall, 49 accessions of *Aeonium* and two accessions of *Monanthes* were sampled (see Appendix for a full list of accessions). With the exception of *A. leucoblepharum* and *A. sedifolium* (Webb ex Bolle) J.Pitard & L.Proust, for which two different accessions were sampled, each taxon was represented by one sample. The majority of accessions were collected and rapidly silica-dried during field work by the author with Patrícia dos Santos and Miguel A. Brilhante (both University of Lisbon) on La Palma, La Gomera, Tenerife and Gran Canaria between March and May 2019. The remaining accessions were taken from mostly well-documented collections of living plants or from field work by Eberhard Fischer (Ethiopia), Michael Benedito (Madeira), Nadine Bobon (La Palma and Gran Canaria) and Ángel Bañares Baudet (El Hierro, La Gomera and Tenerife). Putative hybrids, identified morphologically, were excluded from our sample in order to avoid topological conflict resulting from hybridization.

2.2. Compilation of morphological, ecological and species-occurrence data

Morphological character states (including information about growth form and flowering time) to be mapped onto the *Aeonium* phylogeny were obtained from Liu (1989), Nyffeler (2003), Rebmann (2013) and Bañares Baudet (2015a). We followed Bañares Baudet's (2015a) definition of growth forms and consequently distinguished nanophanerophytes from chamaephytes, with nanophanerophytes having vegetative buds > 25 cm above ground. Climate zones of the Canary Islands were characterized in terms of the predominant thermotype and ombrotype (Rivas-Martínez et al., 2017) of each area. Thermo- and ombrotype are ordinal categories of the thermicity index and ombrothermic index, respectively (see Tables 1 and 2 in Rivas-Martínez et al., 1997). The thermicity index is defined as 10 times the sum of the mean annual temperature, the mean temperature of the coldest month minima and the mean temperature of the coldest month maxima. The ombrothermic index is defined as annual precipitation divided by 10 times the sum of mean monthly temperatures, counting (for the numerator as well as the denominator) only those months with mean temperature above 0°C

(Rivas-Martínez et al., 1997). The thermo- and ombrotypes prevailing in the distribution range of each species were taken from Bañares Baudet (2015a) for the Canary Island species, from Capelo et al. (2005) for the two species endemic to Madeira, and from Rivas-Martínez et al. (2011) for *A. korneliuslemsii* from Morocco. Information about the altitudinal range of species was compiled from Bañares Baudet (2015a) when available, and complemented with data by Voggenreiter (1974) for *A. ciliatum* Webb & Berthel., by Bramwell (1982) for *A. mascaense* Bramwell, by Liu (1989) for *A. castello-paivae* Bolle, species of sect. *Chrysocome*, as well as for all species from outside the Canary Islands, by Bañares Baudet (1999) for *A. urbicum* subsp. *meridionale* (Bañares) Bañares, and by Nyffeler (2003) for *A. diplocyclum* (Webb ex Bolle) T.Mes. Using the distribution maps provided by Liu (1989) and Bañares Baudet (2015a), we compiled a co-occurrence matrix for all species of *Aeonium* and noted the occurrence of natural hybrids between each pair of co-occurring species following Praeger (1929), Bañares Baudet (2015a,c), Mottram (2015) and Arango Toro (2017, 2019). Degree of sympatry (Chesser and Zink, 1994) for each pair of lineages that were the product of a well-supported diversification event in our phylogenetic analyses (see below) was calculated using the equation by Barraclough and Vogler (2000):

$$degree\ of\ sympatry\ = \frac{area\ of\ overlap}{range\ size\ of\ lineage\ with\ smaller\ range}$$

This was only possible for diversification events within the Canary Islands because detailed distribution maps were only available for the Canary Island species (Bañares Baudet, 2015a). This served to quantify the proportion of sympatric and allopatric diversification.

2.3. DNA extraction, library preparation and sequencing

The ddRADseq (double digest RADseq) approach we chose for sequencing reduced-representation libraries of genomic DNA necessitated the usage of high molecular weight DNA. DNA was extracted either from silica-dried material, when this yielded DNA of sufficiently high quality and integrity, or from flash-frozen leaves of living specimens. For the dried material, the DNeasy Plant Mini kit (Qiagen, Hilden, Germany) was used following the manufacturer's protocol but with an extended lysis time between one and two hours using 50% more lysis buffer (and later accordingly more binding buffer) than recommended by the protocol. Flash-frozen whole leaves or (when necessary, see Appendix) leaf epidermal shavings were ground manually in liquid nitrogen with small amounts of sea sand (Carl Roth

GmbH, Karlsruhe, Germany). DNA was isolated from the homogenized material using the NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol for CTAB-based extraction. Here, the volumes of the lysis and binding buffers were doubled, and lysis time was extended to one hour to obtain optimal results. For both extraction protocols, an extended time of one hour was allowed for the elution of DNA from the column membrane to increase the yield. DNA quality was checked on 0.8% agarose gels and DNA concentrations were quantified using a Qubit 3.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, U.S.A.).

The ddRADseq libraries were prepared according to a previously described laboratory protocol for sequencing genomic DNA from *Aichryson* using the two rare-cutter restriction endonucleases (REs) BamHI (G'GATCC) and KpnI (GGTAC'C) (Hühn et al., 2021) with the following modifications: In order to include samples with a DNA concentration < 40 ng/µL, we did several digestion reactions with 5 µL of DNA isolate per reaction, so that a total amount of 200 ng genomic DNA per sample could be used. For adapter ligation following digestion, 8 µL of each adapter solution were used per 200 ng of sample DNA in a total reaction volume of 50 µL. The optimal amount of adapters had been determined before by adapter titration. In this preliminary procedure, different amounts of adapters were ligated to the enzymatically digested DNA in separate assays, and the amount of adapters at which saturation of adapters relative to DNA fragments was first observed (checked on Bioanalyzer electropherograms) was chosen for the final library preparations. After multiplexing, purification and size selection of the pools, 10 low-cycle PCRs per pool were carried out separately and were subsequently re-pooled. After final purification, the libraries were sequenced on an Illumina MiSeq (San Diego, CA, U.S.A.; Reagent Kit v3 600-cycle) with 300bp paired-end reads, either at StarSEQ GmbH (Mainz, Germany) or at Macrogen Inc. (Seoul, Republic of Korea).

2.4. Data treatment and sequence assembly

Sequence data quality was assessed using FastQC v0.11.4 (Andrews, 2010). The sequencing quality of the reverse (R2) reads was consistently lower than that of the forward (R1) reads (see Electr. Suppl. 1). The library was demultiplexed using *ipyrad* v0.9.52 (Eaton and Overcast, 2016), once for each restriction site of the two REs used. Adapter sequences were trimmed/removed using cutadapt v2.3 (Martin, 2011). Because diploid and tetraploid individuals differ in the number of alleles present in their genomes, the demultiplexed sequence data from diploid and potentially tetraploid samples were analysed separately throughout the first assembly steps, i.e., (1) clustering of the reads within samples based on sequence similarity into putative loci (in-sample clustering, ISC) and (2) consensus calling of alleles. Information about chromosome numbers, genome size and inferred ploidy levels of the species sampled was taken from the literature (Uhl, 1961; Liu, 1989; Mes, 1995; Suda et al., 2005; Brilhante et al., 2021; see Table 1). Samples from species without information on ploidy level were treated as tetraploid because this minimized the potential error of merging different alleles into one. In order to find the most suitable clustering thresholds (CTs) for data assembly, we used an empirical approach based on the evaluation of several metrics of the *ipyrad* pipeline. First, with the *ipyrad* assembly steps 1–5 ISC was carried out, and the resulting number of clusters (clusters_total), read depth (avg_depth_total), number of flagged paralogs (filtered_by_maxH) and heterozygosity were visualized in the form of box- and scatterplots as a function of the tested CT range (0.81–0.99) using SPSS Statistics v23 (IBM Corp., 2015). To detect signals of over- and undermerging of reads into clusters using these plots, the most appropriate CT value for ISC was chosen as described by Hühn et al. (2021), independently for diploid and tetraploid samples. The resulting assemblies of clusters within samples were then merged and in turn tested for the CT value most suitable for between-sample clustering (BSC, steps 6 and 7 of the *ipyrad* pipeline; values from 0.81 to 0.99 tested). In order to identify the CT above which loci are subject to undermerging (Paris et al., 2017), the metrics corresponding to the resulting BSC runs were assessed with respect to the number of new polymorphic loci that were gained when the CT was increased by 0.01. Performing a BLASTN (ncbi-blast-2.2.28+; Camacho et al., 2009) search of all loci against four Crassulaceae reference plastomes (see Hühn et al., 2021, for GenBank accession numbers), loci of the plastid genome were removed from the final assembly. Additionally, all loci containing zero parsimony informative sites (PIS) were removed from the assembly prior to phylogenetic analyses.

2.5. Phylogenetic analyses

We analysed the final assembly in four different ways using Maximum Likelihood (ML) implemented in RAxML-NG v0.9.0 (Kozlov et al., 2019). (1) The full assembly of all loci was concatenated into a supermatrix using FASconCAT v1.11 (Kück and Meusemann, 2010), from which the phylogeny was inferred (all/RAxML). (2) Individual gene trees of all loci of the same dataset were inferred using RAxML-NG and were subsequently used for species tree inference in ASTRAL-III v5.7.4 (Zhang et al., 2018; all/ASTRAL). Based on the uneven distribution of locus lengths and sample coverage throughout the locus length range (Electr. Suppl. 2), we filtered loci from the assembly so that only loci between 320 and 500 nucleotides (nt) long were retained, in the following referred to as the 320–500 dataset. Thus, the numerous loci with lengths below 320 nt and the much fewer loci with lengths above 500 nt (Electr. Suppl. 2) were removed. These loci probably were the product of incorrect assembly resulting from the consistently lower quality of reverse reads compared to forward reads (see Electr. Suppl. 1), leading to biased locus and sample coverage. (3) The resulting reduced dataset was in turn again concatenated into a supermatrix using FASconCAT for phylogenetic inference in RAxML (320–500/RAxML), and (4) individual gene trees were inferred for the same, reduced dataset and used for species tree inference in ASTRAL-III (320–500/ASTRAL).

For each RAxML run, 1000 bootstrap replicates and the GTR+Γ substitution model were pre-defined. All ASTRAL runs were performed with default settings, and multilocus bootstrapping (Seo, 2018) was used in order to obtain branch support values for the resulting species trees.

2.6. Molecular Dating

Because there are no known fossils with affinity to the Crassulaceae, only secondary age estimates can be used for calibrating Crassulaceae phylogenies (Messerschmid et al., 2020). To obtain an accurate age estimate for calibrating the dating analysis of our *Aeonium* phylogeny, a Crassulaceae-wide, partitioned alignment of ITS sequences (Messerschmid et al., 2020) was used and expanded by 11 accessions of *Aichryson*, *Monanthes* and *Aeonium* (see Appendix for all accessions used in this study) in order to better represent the Macaronesian *Aeonium* alliance. The dating analysis for this ITS dataset was carried out using BEAST v2.4.8 (Bouckaert et al., 2014) implemented in the CIPRES Science Gateway (Miller et al., 2010). The most recently published estimates (Ramírez-Barahona et al., 2020) of the stem (116.25 million years ago (Ma); 95% HPD = 92.58–148.07 Ma) and crown ages (72.57 Ma; 95% HPD = 49.96–96.16 Ma) of Crassulaceae were used as calibration points. Settings for the BEAST analyses were the same as described by Messerschmid et al. (2020). Four independent runs were performed and the resulting log files were checked for convergence and sufficiently high sampling size of each parameter using Tracer v1.5 (Rambaut and Drummond, 2007). Although the effective sampling size of the treeLikelihood parameter was only 152 in one of the runs, we used this estimate because it converged to a value similar to those obtained in the other runs. The runs were combined using LogCombiner v2.4.5 (Bouckaert et al., 2014) pre-defining a burn-in of 10%, and a maximum clade credibility (MCC) tree was constructed using TreeAnnotator v.1.8.3 (Rambaut and Drummond, 2016) and inspected using FigTree v1.3.1 (Rambaut, 2009).

We used BEAST v2.6.4 (Bouckaert et al., 2019) on two Lenovo workstations (ThinkStation P620 Tower) for the dating analysis of our *Aeonium* phylogeny inferred from ddRADseq data.

We pre-defined all those clades as monophyletic that had high bootstrap support (BS > 95) in all four phylogenetic analyses of the ddRADseq data for *Aeonium* (see (1–4) in paragraph 2.5 above). The age estimate for the split between *Aeonium* and *Monanthes* as obtained from the Crassulaceae-wide dating analysis (see above; i.e., 11.62 Ma; 95% HPD = 6.56–17.10 Ma) was taken as tertiary calibration point for the divergence time estimation in the *Aeonium* phylogeny. Initial dating analyses using all loci of the 320–500 dataset resulted in unreasonably recent divergence times (e.g., 3.90–3.92 Ma for the split between *Aeonium* and *Monanthes*, an estimate lying far off the range of the prior). This most probably was due to the relatively high average proportion of missing data (referred to as missingness in the following) per locus (76.8 ± 18.7%) in this dataset (see 3.1 below). A similar association between high missingness and the inference of younger clade ages had recently been discovered for *Carex* sect. *Schoenoxiphium* (Nees) Baill. (Villaverde et al., 2021). Following these findings, the final analyses were performed with a dataset that contained sequence data for at least twenty taxa at every locus (called dataset min20tax in the following), corresponding to 42% missingness across the dataset. Also, a log normal instead of a normal prior for the divergence time between *Aeonium* and *Monanthes* was used, ticking 'mean in real space' in BEAUti v2.6.3 (Bouckaert et al., 2019), because of the potentially greater error associated with normal distribution for secondary calibration points (Schenk, 2016). The topologies inferred from the 320–500/RAxML as well as the 320–500/ASTRAL analyses were simultaneously used as starting trees. As for the family-wide dating analysis, four independent runs were carried out with each of the two starting trees, and the four respective runs were checked for convergence and combined as described above. To control whether the choice of the min20tax dataset introduced any significant bias to divergence time estimates and topology inferred in BEAST, we repeated the analyses as for the min20tax dataset for datasets with at least 15 and at least 40 taxa at every locus (min15tax and min40tax, respectively), and for three more datasets that contained loci randomly chosen from the pool of loci in the 320–500 dataset adding up to the same number as in the three datasets with deliberately chosen loci (i.e., 58 loci as in the min40tax dataset, 357 loci as in min20tax, and 550 loci as in min15tax).

2.7. Biogeographical reconstruction of ancestral distribution area

Prior to the formal biogeographical analysis, the age of each supported node in the MCC tree of the dated *Aeonium* phylogeny inferred using the topology of the 320–500/RAxML analysis as starting tree (see Fig. 2 and paragraph 2.6 above) was plotted against the degree of sympatry between the corresponding sister lineages. Because the dating analysis based on the 320–

500/ASTRAL tree as starting tree (Suppl. Fig. S1) yielded exactly the same topology as the analysis based on the 320–500/RAxML tree (Fig. 2), and divergence time estimates were virtually identical in these two analyses (see 3.2 below), no additional plot of sympatry versus node age was done for the former dating analysis.

The dated MCC tree obtained using the 320–500/RAxML topology as starting tree was used for an ancestral area reconstruction (AAR) analysis using the R package BioGeoBEARS (v1.1.2; Matzke, 2014, 2018a). Other R packages used for this analysis were rexpokit (v0.26.6.7; Matzke et al., 2020) and cladoRcpp (v0.15.1; Matzke, 2018b). The seven main islands of the Canarian Archipelago as well as Madeira, the Cape Verde Islands as a whole, Morocco and East Africa (including the southwestern part of the Arabian Peninsula) were treated as separate geographical regions in the AAR analysis, resulting in eleven distinct areas. The analysis was time stratified using four distinct time strata to account for the young age of Madeira (5 myr; Geldmacher et al., 2000), La Palma and El Hierro (1.7 and 1.1 myr, respectively; Van den Bogaard, 2013) relative to the root age estimate for the *Aeonium* phylogeny. Extant and ancestral taxa were allowed to occupy a maximum number of five geographical areas because *A. spathulatum* (Hornem.) Praeger, the taxon with the highest number of occupied areas, occurs on five of the Canary Islands. This AAR analysis was not repeated with the dated tree obtained using the 320–500/ASTRAL topology as starting tree because of its high similarity to the other dated MCC tree, as pointed out above. Subsequent to the completion of the AAR analysis, biogeographical stochastic mapping (BSM; Matzke, 2016; Dupin et al., 2016) as implemented in BioGeoBEARS was carried out on the model that received the highest log-likelihood (LnL) and corrected Akaike information criterion model weight (AICc_wt) scores (i.e., BAYAREALIKE+J, see 3.3 below) in order to simulate and quantify stochastically mapped cladogenetic and anagenetic events in the evolution of *Aeonium*. Fifty individual stochastic maps were simulated, matching the default settings.

2.8. Calculation of morphological and ecological divergence between sister lineages

In order to address our hypothesis that sister clades that diverged in sympatry on single islands should be morphologically and ecologically more distant than sister clades that diversified by dispersal between islands (i.e., founder-event speciation), we calculated Raw Euclidean Distances (REDs; Lloyd, 2016) as a measure of morphological and ecological divergence between well-supported sister lineages. More precisely, we identified those nodes that were present in all phylogenetic analyses (including the dating analysis) and received high support (BS = 100 and posterior probability ≥ 0.99) in at least one of these analyses. For these nodes, we separately calculated the morphological and ecological REDs between the respective sister lineages using the matrix of morphological characters (i.e., flower merism and characters k–t in Fig. 3) and characteristics of the habitat (i.e., altitude, thermotype and ombrotype; illustrated in Fig. 3), respectively. The internal topology of the always well-supported Teneriffan subclade of sect. *Leuconium* (i.e., *A. ciliatum* through *A. haworthii* Webb & Berthel. in Figs. 2 and 3, nodes 46–49 in Fig. 4) was inconsistently recovered across our phylogenetic analyses, but diversification in this subclade was reconstructed as exclusively sympatric (see Electr. Suppl. 3). Therefore, we summarized the morphological and ecological divergence arising from diversification in this subclade by calculating mean values of all morphological and ecological REDs, respectively, for the different topologies as recovered by our concatML (Fig. 2), ASTRAL (Fig. 3) and dating analyses (Fig. 4). Thus, to account for phylogenetic uncertainty in this Teneriffan subclade of sect. *Leuconium*, we replaced the REDs calculated for individual nodes by these two mean values. We then compared the magnitude of morphological and ecological REDs between those nodes that, according to the results of our BSM analysis (see 2.7 above), were associated with sympatric diversification on the one hand and those nodes that were associated with allopatric founder-event diversification on the other hand.

3. Results

3.1. Sequencing, parameter optimization in *ipyrad* and locus properties of the final assembly

The Illumina sequencing reactions yielded a total of 13,921,303 paired-end reads which is equivalent to an average number of 272,967 (± 146,466) paired-end reads per sample. Of these, 13,914,286 (i.e., 99.95%) reads passed quality filtering. *Aeonium undulatum* Webb & Berthel., *A. balsamiferum* Webb & Berthel. and *A. gorgoneum* had by far the highest number (680,738, 623,623 and 621,070, respectively) of reads while *A. lindleyi* subsp. *viscatum* and *A. nobile* (Praeger) Praeger had the lowest number of reads, 32,710 and 88,491, respectively.

The box- and scatterplots of the relevant assembly metrics as obtained from *ipyrad* after ISC against the tested clustering thresholds (Suppl. Fig. S2) revealed similar patterns for the samples treated as diploid and tetraploid. For both groups, an onset of exponential increase of the number of clusters (clusters_total in Suppl. Fig. S2) became apparent at CT values between 0.92 and 0.94 indicating incipient undermerging in this CT range. In the same range, the decrease in number of rejected clusters due to high heterozygosity (filtered_by_maxH in Suppl. Fig. S2) was steepest, and the decreasing slope of average read depth (avg_depth_total in Suppl. Fig. S2) gained steepness. A cubic polynomial regression for the heterozygosity metrics set (heterozygosity in Suppl. Fig. S2) revealed maximum heterozygosity at CT 0.92 for the tetraploid and at CT 0.93 for the diploid samples. Thus, for the tetraploids CT 0.92 was identified as the most suitable clustering threshold, and the same CT value was also chosen for the diploids because the other metrics indicated that undermerging had an impact on the ISC already at CT 0.93. After merging all samples of the ISC runs performed with a CT value of 0.92, the results of the BSC test (Electr. Suppl. 4) showed the characteristic 'hockey-stick signal' (Paris et al., 2017) when the number of new polymorphic loci was plotted against CT, with an initial increase again at CT 0.92. This signal, acting as indicator of incipient undermerging, coincided with a minimum of missingness in the assembled dataset (percentage missing sites = 73.17% in Electr. Suppl. 4) at the same CT value of 0.92. Although the cumulative number of single nucleotide polymorphisms (SNPs) and PIS both reached a maximum at a CT value of 0.93, this was not considered the most suitable CT value for the same reason as in the ISC of diploid samples. Therefore, 0.92 was chosen as CT value also for BSC in order to minimise the impact of undermerging.

Because *A. lindleyi* subsp. *viscatum* only had 24 loci in the assembly, it was removed from the dataset for the final assembly. After removing the loci of the plastome as well as those loci without PIS, the resulting assembly comprised 50 samples and 4,280 loci with a total length of 1,529,544 nt and an average length of 357.4 nt per locus (total length range 49–617 nt). The number of SNPs and PIS amounted to 89,093 (20.8 ± 13.3 SNPs per locus) and 49,209 (11.5 ± 9.4 PIS per locus), respectively. With an average of 11.2 samples per locus, the average missingness per locus was relatively high (77.6 ± 17.5%). When only the locus length range of 320–500 nt was retained (320–500 dataset; see 2.5 above), the number of loci was reduced to 2,218 (51.8% of the total number of loci) and the total length of the assembly was 884,786 nt (398.9 ± 49.8 nt per locus). This reduced assembly had a higher number of SNPs and PIS per locus (22.8 ± 13.1 and 13.1 ± 10.4, respectively) and a slightly lower average missingness per locus (76.8 ± 18.7%) than the dataset comprising all loci.

3.2. Phylogenetic relationships and age estimates for the diversification of *Aeonium*

All four phylogenies, i.e., all/RAxML, 320–500/RAxML (Fig. 2), all/ASTRAL and 320–500/ASTRAL (Fig. 3), strongly supported *Aeonium* sect. *Goochiae* (*A. lindleyi* subsp. *viscatum* was excluded from these analyses) as monophyletic. In the following this is referred to as the Goochiae clade (*A. goochiae* Webb & Berthel. and *A. lindleyi* in Fig. 3). This clade was also well supported when *A. lindleyi* subsp. *viscatum* was included (Suppl. Fig. S3) in spite of its very low locus coverage (see 3.1 above). In this latter analysis the two subspecies of *A. lindleyi* were supported as sister to each other (BS = 97). However, the position of the Goochiae clade relative to other lineages differed between analyses. While it was resolved as earliest-branching lineage of *Aeonium* in all/RAxML, it formed a polytomy with two larger clades in 320–500/RAxML. The smaller of these two clades (i.e., *A. aureum* (C.Sm. ex Hornem.) T.Mes through *A. saundersii* Bolle in Fig. 2) was not recovered by either of the ASTRAL analyses (see Fig. 3). This smaller clade comprises sects. *Chrysocome*, *Greenovia* and *Petrothamnium*. These sections have flowers which lack hypogynous nectary scales (see morphological characters in Fig. 3). They formed a supported clade in our ML analyses of concatenated sequence data (i.e., the concatML analyses; Fig. 2) but not in our species-tree analyses (Fig. 3). Within this nectary-lacking group, sect. *Greenovia* was well supported as monophyletic, and relationships among the four species of this section were consistently well resolved in all analyses. Section *Petrothamnium* was only monophyletic in both concatML phylogenies, and sect. *Chrysocome* was only supported as monophyletic in 320–500/RAxML (Fig. 2). In summary, sects. *Goochiae* and *Greenovia* were concordantly supported as monophyletic sections, while the inferred relationships of the remaining taxa in the basal part of the phylogeny differed between analyses (Figs. 2, 3). While a nectary-lacking clade was supported in both concatML analyses, the ASTRAL analyses recovered the nectary-lacking species in a basal grade including the Goochiae clade.

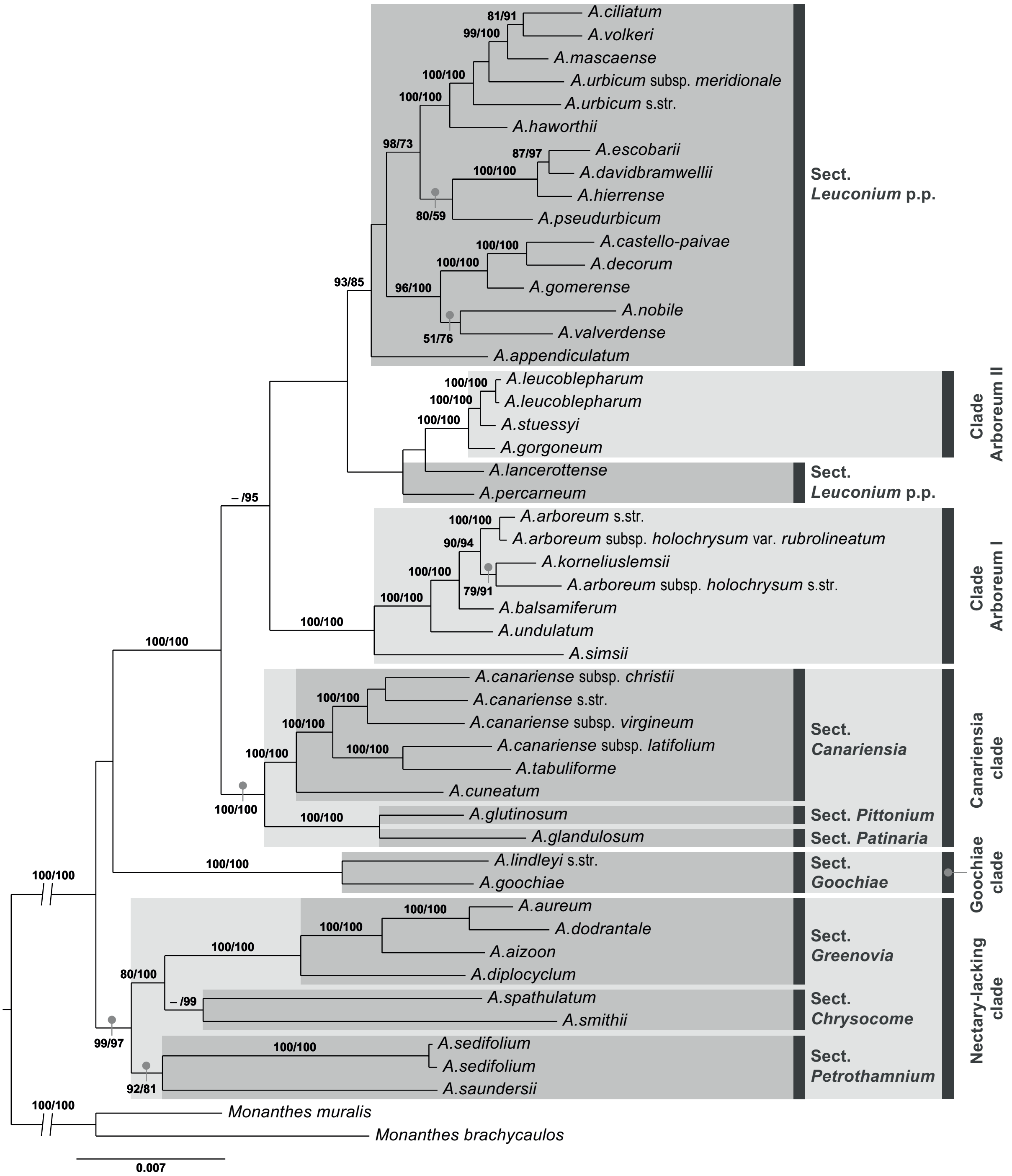


Fig. 2. Maximum Likelihood (ML) phylogeny of *Aeonium* inferred from concatenated supermatrices (concatML). Numbers above branches indicate bootstrap values (only shown if at least one of them is ≥ 75) obtained from the complete dataset of all 4,280 loci and from the dataset only comprising the 2,218 loci in the 320–500 nt length range (left and right values, respectively). Dashes (–) signify clades that were supported in 320–500/RAxML but not recovered in all/RAxML. The branches arising directly from the root were shortened (branch length = 0.019) for the purpose of better visibility. Clade names and affiliations to sections are indicated to the right of the species names. Topology and branch lengths correspond to the results obtained with the 320–500 dataset.

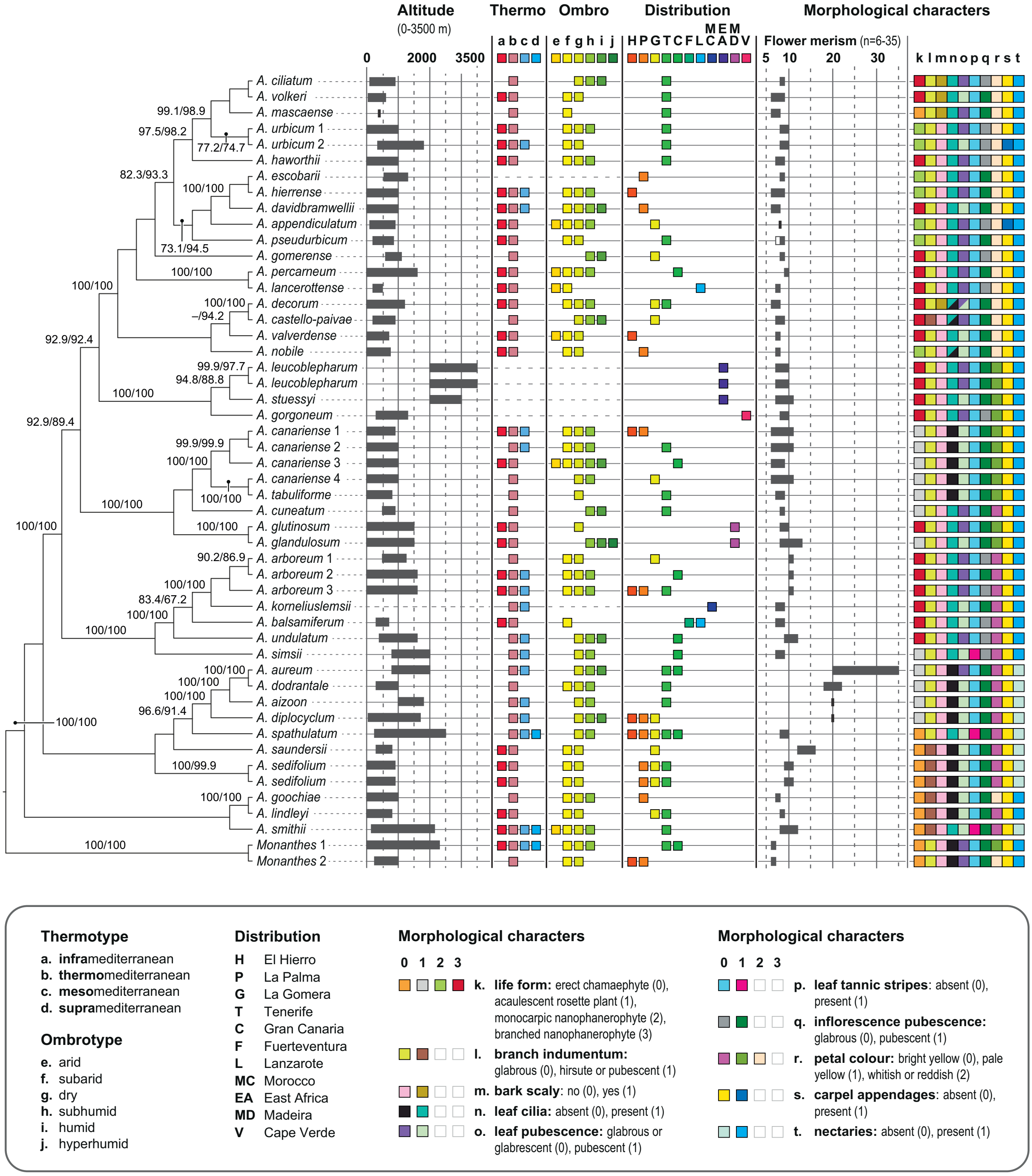


Fig. 3. Species tree of *Aeonium* inferred using ASTRAL-III. Numbers above branches indicate bootstrap values (only shown if at least one of them is ≥ 75) obtained from the species-tree analysis of all 4,280 individual RAxML gene trees and from those 2,218 gene trees inferred from loci in the 320–500 nt length range (left and right values, respectively). The dash (–) signifies a clade that was supported in 320–500/ASTRAL but not recovered in all/ASTRAL. The topology corresponds to the results obtained with the 320–500 dataset. Information about the species' geographical distribution, the altitude range covered across the distribution area and the thermo- and ombrotype of their habitats is given to the right of the taxon names. Missing data with respect to altitude, thermo- and ombrotype is indicated with dashed horizontal lines. Further to the right, morphological character states are coded and specified by the inset figure legend below. *A. urbicum* 1: *Aeonium urbicum* s.str.; *A. urbicum* 2: *A. urbicum* subsp. *meridionale*; *A. canariense* 1: *A. canariense* subsp. *christii*; *A. canariense* 2: *A. canariense* s.str.; *A. canariense* 3: *A. canariense* subsp. *virgineum*; *A. canariense* 4: *A. canariense* subsp. *latifolium*; *A. arboreum* 1: *A. arboreum* subsp. *holochrysum* var. *rubrolineatum*; *A. arboreum* 2: *A. arboreum* s.str.; *A. arboreum* 3: *A. arboreum* subsp. *holochrysum* s.str.; *Monanthes* 1: *M. brachycaulos*; *Monanthes* 2: *M. muralis*

The larger clade, *A. ciliatum* through *A. glandulosum* in Fig. 2 and *A. ciliatum* through *A. simsii* (Sweet) Stearn in Fig. 3, was always well supported and contains species of the remaining sections, i.e., sects. *Aeonium*, *Canariensia*, *Leuconium*, *Patinaria* and *Pittonium*. In this clade, the different analyses resolved different clades as earliest-branching lineage. The phylogenies inferred from species-tree analyses in ASTRAL-III (Fig. 3) supported a clade comprising all Canarian taxa of sect. *Aeonium* plus *A. korneliuslemsii* from Morocco as earliest-diverging lineage (*A. arboreum* 1 through *A. simsii* in Fig. 3, i.e., clade Arboreum I in Fig. 2). In the concatML phylogenies, the position of earliest-diverging lineage above the nectary-lacking clade and the Goochiae clade was taken by an always well-supported clade comprising all taxa of sects. *Canariensia*, *Patinaria* and *Pittonium* (*A. canariense* subsp. *christii* (Burchard) Bañares through *A. glandulosum* in Fig. 2). Within this clade, henceforth referred to as the Canariensia clade, the monospecific sects. *Patinaria* and *Pittonium* (i.e., *A. glandulosum* and *A. glutinosum* (Aiton) Webb & Berthel., respectively) were most closely related to each other and sister to the monophyletic sect. *Canariensia* in all analyses. In the all/RAxML phylogeny, both clade Arboreum I and the Canariensia clade were well supported as clades, but formed a polytomy with a small clade comprising the extra-Canarian taxa of sect. *Aeonium* (*A. leucoblepharum* through *A. gorgoneum* in Fig. 2) without *A. korneliuslemsii* from Morocco, i.e., clade Arboreum II, two single species (*A. lancerottense* (Praeger) Praeger and *A. percarneum* (Murray) J.Pitard & L.Proust; both sect. *Leuconium*) and one large clade (*A. ciliatum* through *A. appendiculatum* Bañares in Fig. 2) containing taxa only of sect. *Leuconium*. The extra-Canarian species of sect. *Aeonium* also formed a supported clade in all other analyses, and in both ASTRAL phylogenies all species of sect. *Leuconium* were well supported as a clade, i.e., the Leuconium clade. To summarize relationships in the larger clade above the nectary-lacking clade/grade and the Goochiae clade, the Canariensia clade comprised a monophyletic sect. *Canariansia* that was supported as sister to the Madeiran species *A. glandulosum* and *A. glutinosum*. Two distinct clades with representatives of sect. *Aeonium* were recovered, one containing the Canarian species and *A. korneliuslemsii* (clade Arboreum I), the other containing the East African and Cape Verde species (clade Arboreum II). Section *Leuconium* was supported as monophyletic only in the ASTRAL analyses, while it formed an unsupported paraphyletic group in relation to clade Arboreum II in the concatML analyses.

The chronogram of the *Aeonium* phylogeny obtained from the BEAST analysis of the min20tax dataset using the topology of the 320–500/RAxML analysis as starting tree is shown in Fig. 4. The corresponding age estimates for nodes that were well supported in the dating analysis, including those nodes that were well supported in all phylogenetic analyses, are summarized in Table 2, along with the degree of sympatry between the corresponding sister lineages (see 3.3 below). The BEAST analysis of the same min20tax dataset but with the topology of the 320–500/ASTRAL analysis as starting tree (Suppl. Fig. S1) resulted in a topology identical and divergence time estimates nearly identical to those obtained when using the 320–500/RAxML topology as starting tree. Therefore, only the results of the BEAST analysis with the 320–500/RAxML topology as starting tree are described and discussed. The divergence between *Aeonium* and *Monanthes* was dated to the Upper Miocene, 7.76 Ma (95% HPD = 3.42–12.70 Ma), and thus towards the younger range of the prior for the root (11.62 Ma; 95% HPD = 6.56–17.10 Ma). The onset of diversification of *Aeonium* was dated to the Pliocene (4.62 Ma; 95% HPD = 1.74–8.11 Ma), a time when El Hierro and La Palma had not yet formed (Van den Bogaard, 2013). Mean age estimates for the diversification within sects. *Chrysocome* and *Petrothamnium* were all older than 3 myr (Fig. 4), and the diversification of all other sections started later. The divergence between these sections and sect. *Greenovia* as well as between clade Arboreum I, the Canariensia clade and the larger clade comprising sect. *Leuconium* and clade Arboreum II (i.e., *A. mascaense* through *A. nobile* in Fig. 4) took place between 3 and 4 Ma. The split of sects. *Patinaria* and *Pittonium* from sect. *Canariensia* and thus the colonization of Madeira was dated to 2.48 Ma (95% HPD = 0.93–4.42 Ma). The colonization of the African continent – 1.10 Ma (95% HPD = 0.37–2.01 Ma) by the East African lineage giving rise to *A. leucoblepharum* and *A. stuessyi*, and 0.27 Ma (95% HPD = 0.08–0.50 Ma) by the Moroccan *A. korneliuslemsii* – and of the Cape Verde Islands (0.10 Ma; 95% HPD = 0.01–0.22 Ma) took place much later. The onset of diversification in sect. *Leuconium* was dated to 2.40 Ma (95% HPD = 0.91–4.28 Ma). This diversification as well as the incipient diversification of sects. *Greenovia* (2.11 Ma; 95% HPD = 0.71–3.82 Ma) and *Canariensia* (1.97 Ma; 95% HPD = 0.71–3.54 Ma) and clade Arboreum I (2.01 Ma; 95% HPD = 0.66–3.68 Ma) took place after the onset of the Pleistocene glaciation cycles (Gibbard et al., 2010). Section *Goochiae* (1.65 Ma; 95% HPD = 0.49–3.18 Ma) and the Madeiran species *A. glandulosum* and *A. glutinosum* (1.26 Ma; 95% HPD = 0.40–2.40 Ma) diverged later.

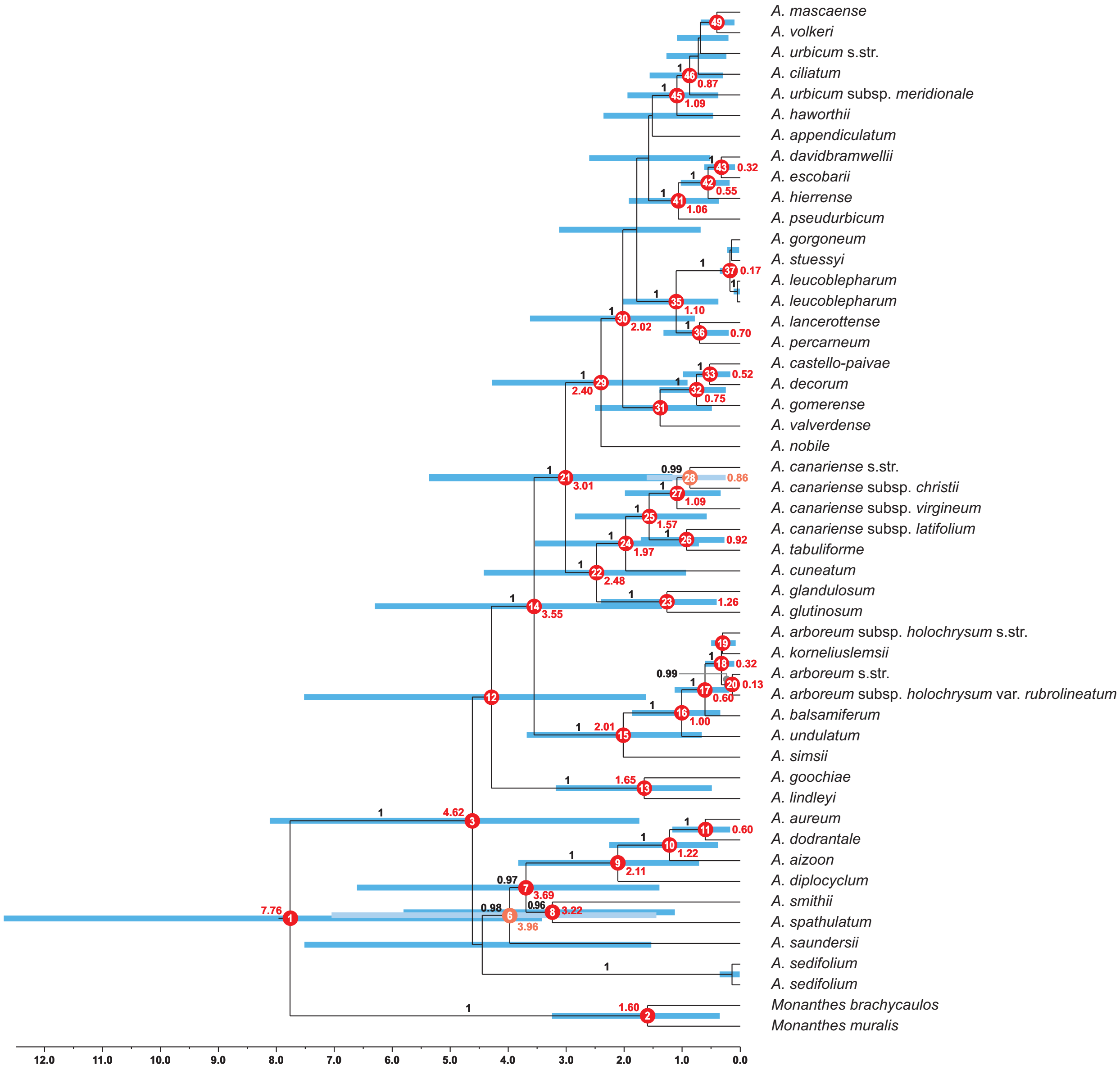


Fig. 4. Chronogram of *Aeonium* inferred using BEAST, based on the sequence dataset of those 357 loci in the 320–500 nt length range that featured sequence information for at least twenty different samples (referred to as min20tax dataset in the text). The topology inferred from the 320–500/RAxML analysis (see Fig. 2) was used as starting tree for this BEAST analysis. Only posterior probabilities ≥ 0.85 are shown above branches. Mean age estimates are given for each supported node (posterior probability ≥ 0.95) in red font, and the blue error bars indicate the respective 95% highest posterior density (HPD) intervals. For nodes 6 and 28, age estimates and error bars received paler colours for a better contrast to neighbouring nodes. The scale shows time in million years before present. See also Table 2 for a summary of the stem and crown age estimates of well-supported clades.

Table 2. Age estimates in million years for all supported nodes (posterior probability ≥ 0.95 unless otherwise specified) of the chronogram of *Aeonium* (Fig. 4). For clade names, see Fig. 2. For relationship between node age and degree of sympatry see Fig. 6.

Node number[a]	Clade name	Node age [95% confidence interval]	Degree of sympatry between corresponding sister lineages
1	*Aeonium* + *Monanthes*	7.76 [3.42–12.70]	not assessed
3	*Aeonium*	4.62 [1.74–8.11]	unsupported[b]
6	*A. saundersii* + sects. *Chrysocome* and *Greenovia*	3.96 [1.44–7.05]	1
7	Sects. *Chrysocome* + *Greenovia*	3.69 [1.39–6.61]	0.56
8	Sect. *Chrysocome*	3.22 [1.12–5.80]	0.67
9	Sect. *Greenovia*	2.11 [0.71–3.82]	0
10	*A. aizoon*, *A. aureum* + *A. dodrantale*	1.22 [0.38–2.25]	0.71
11	*A. aureum* + *A. dodrantale*	0.60 [0.17–1.17]	0.13
12[c]	Goochiae clade, Canariensia clade, sects. *Aeonium* and *Leuconium*	4.23 [1.63–7.52]	1
13	Goochiae clade	1.65 [0.49–3.18]	0
14	Canariensia clade + sects. *Aeonium* and *Leuconium*	3.55 [1.34–6.30]	0.65
15	Clade Arboreum I	2.01 [0.66–3.68]	0.56
16	*A. undulatum*, *A. balsamiferum*, *A. korneliuslemsii* + *A. arboreum* s.l.	1.00 [0.34–1.86]	0.90
17	*A. balsamiferum*, *A. korneliuslemsii* + *A. arboreum* s.l.	0.60 [0.19–1.13]	0
18	*A. korneliuslemsii* + *A. arboreum* s.l.	0.32 [0.10–0.60]	0 (unsupported[b])
19	*A. korneliuslemsii* + *A. arboreum* subsp. *holochrysum* s.str.	0.27 [0.08–0.50]	0 (unsupported[b])
20	*A. arboreum* s.str. + *A. arboreum* subsp. *holochrysum* var. *rubrolineatum*	0.13 [0.02–0.28]	0
21	Canariensia clade, clade Arboreum II + sect. *Leuconium*	3.01 [1.16–5.37]	0.77
22	Canariensia clade	2.48 [0.93–4.42]	0
23	Sects. *Patinaria* + *Pittonium*	1.26 [0.40–2.40]	not assessed
24	*A. cuneatum*, *A. canariense* s.l. + *A. tabuliforme*	1.97 [0.71–3.54]	1

25	*A. canariense* s.l. + *A. tabuliforme*	1.57 [0.57–2.85]	0.61
26	*A. canariense* subsp. *latifolium* + *A. tabuliforme*	0.92 [0.27–1.71]	0
27	*A. canariense* without *A. canariense* subsp. *latifolium*	1.09 [0.33–1.99]	0
28	*A. canariense* subsp. *christii* + *A. canariense* s.str.	0.86 [0.25–1.61]	0
29	Clade Arboreum II + sect. *Leuconium*	2.40 [0.91–4.28]	0.93
30[c]	Clade Arboreum II + sect. *Leuconium* without *A. nobile*	2.02 [0.78–3.62]	0.42
31[c]	*A. valverdense*, *A. gomerense*, *A. castello-paivae* + *A. decorum*	1.37 [0.48–2.50]	0
32	*A. gomerense*, *A. castello-paivae* + *A. decorum*	0.75 [0.24–1.39]	1
33	*A. castello-paivae* + *A. decorum*	0.52 [0.17–0.99]	0.33
35	*A. lancerottense*, *A. percarneum*, clade Arboreum II	1.10 [0.37–2.01]	0
36	*A. lancerottense* + *A. percarneum*	0.70 [0.20–1.32]	0
37	Clade Arboreum II	0.17 [0.04–0.35]	not assessed (unsupported[b])
41	*A. pseudurbicum*, *A. hierrense*, *A. davidbramwellii* + *A. escobarii*	1.06 [0.37–1.92]	0
42	*A. hierrense*, *A. davidbramwellii* + *A. escobarii*	0.55 [0.18–1.02]	0
43	*A. davidbramwellii* + *A. escobarii*	0.32 [0.09–0.62]	not assessed
45	*A. haworthii*, *A. urbicum* s.l., *A. ciliatum*, *A. volkeri* + *A. mascaense*	1.09 [0.37–1.94]	1
46[c]	*A. urbicum* s.l., *A. ciliatum*, *A. volkeri* + *A. mascaense*	0.87 [0.29–1.56]	0
49[c]	*A. volkeri* + *A. mascaense*	0.35 [0.10–0.68]	0

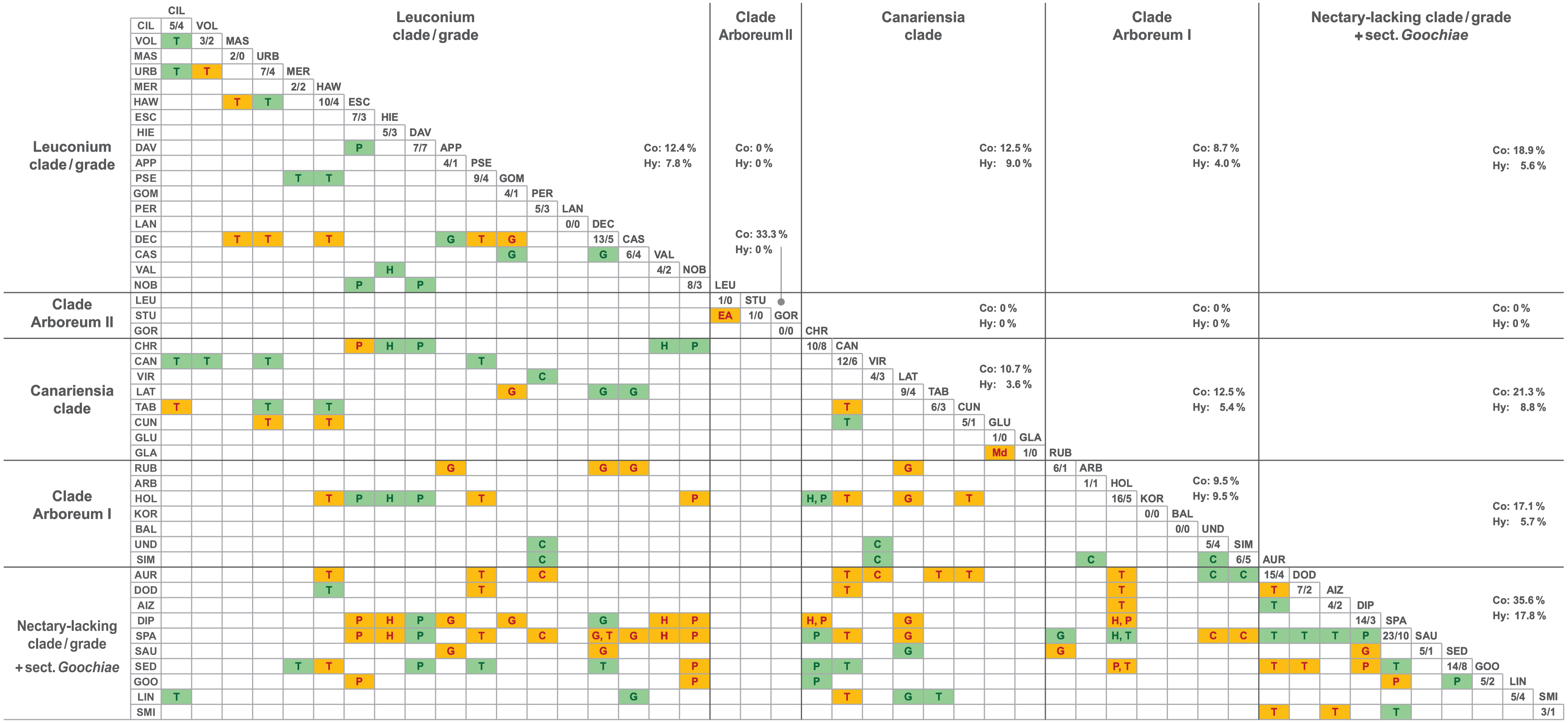
[a] Node numbers as specified in Fig. 4.

[b] Posterior probability of at least one of the two sister lineages < 0.85. In such cases the degree of sympatry is only given when lineages are allopatric (degree of sympatry = 0).

[c] Posterior probability for this node or for at least one of the two subordinate nodes < 0.95, but ≥ 0.85

3.3. Biogeography and co-occurrence of *Aeonium* species

The co-occurrence matrix for all species of *Aeonium* is shown in Fig. 5, where additional information on naturally occurring hybrids between any two of all species can be found. The nectary-lacking group contains, among others, those species that are most widespread across the Canary Islands, with *A. spathulatum* as the only taxon (on the level of species and below) occurring on five islands and with *A. sedifolium* and *A. diplocyclum* as two of three taxa that occur on three islands (Fig. 3). Against this background it is not surprising that species of the basal grade (i.e., all nectary-lacking species and species of sect. *Goochiae*) were shown to co-occur and hybridize with the highest percentage of other species of the same grade (35.6 and 17.8%, respectively) as well as other species of other clades/grades (on average 14.3 and 9.6% respectively; Fig. 5). Disregarding the exclusively extra-Canarian clade Arboreum II, the basal grade again co-occurs and hybridizes with the highest proportion of species of other clades or grades (on average 19.1 and 12.8%, respectively), followed by the Canariensia clade (15.4 and 7.7%, respectively), the Leuconium clade/grade (13.4 and 6.2%, respectively) and clade Arboreum I (12.8 and 5.0%, respectively). Concerning co-occurrence and hybridization of species within individual clades/grades, the basal grade is followed by the Leuconium clade/grade (12.4 and 7.8%, respectively), the Canariensia clade (10.7 and 3.6%, respectively) and clade Arboreum I (9.5% co-occurrence of taxa; Fig. 5). However, those two taxa of clade Arboreum I that co-occur also hybridize with each other (Fig. 5).



		CIL	VOL	MAS	URB	MER	HAW	ESC	HIE	DAV	APP	PSE	GOM	PER	LAN	DEC	CAS	VAL	NOB	LEU	STU	GOR	CHR	CAN	VIR	LAT	TAB	CUN	GLU	GLA	RUB	ARB	HOL	KOR	BAL	UND	SIM	AUR	DOD	AIZ	DIP	SPA	SAU	SED	GOO	LIN	SMI
Leuconium clade/grade	CIL	5/4																																													
	VOL	T	3/2																																												
	MAS			2/0																																											
	URB	T	T		7/4																																										
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	HAW			T	T		10/4																																								
	ESC							7/3																																							
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	DEC			T	T		T				G	T	G			13/5																															
	CAS												G			G	6/4																														
	VAL								H									4/2																													
	NOB							P		P									8/3																												
Clade Arboreum II	LEU																			1/0																											
	STU																			EA	1/0																										
	GOR																					0/0																									
Canariensia clade	CHR							P	H	P								H	P				10/8																								
	CAN	T	T		T							T												12/6																							
	VIR													C											4/3																						
	LAT												G			G	G									9/4																					
	TAB	T			T		T																	T			6/3																				
	CUN				T		T																	T				5/1																			
	GLU																												1/0																		
	GLA																												Md	1/0																	
Clade Arboreum I	RUB										G					G	G									G					6/1																
	ARB																															1/1															
	HOL						T	P	H	P		T							P				H, P	T		G		T					16/5														
	KOR																																	0/0													
	BAL																																		0/0												
	UND													C											C											5/4											
	SIM													C											C							C				C	6/5										
Nectary-lacking clade/grade + sect. Goochiae	AUR						T					T		C										T	C		T	T					T			C	C	15/4									
	DOD						T					T												T									T					T	7/2								
	AIZ																																T					T		4/2							
	DIP							P	H	P	G		G			G		H	P				H, P			G							H, P								14/3						
	SPA							P	H	P		T		C		G, T	G	H	P				P	T		G					G		H, T			C	C	T	T	T	P	23/10					
	SAU										G					G										G					G										G		5/1				
	SED					T	T			P		T				T			P				P	T									P, T					T	T		P	T		14/8			
	GOO							P											P				P																			P		P	5/2		
	LIN	T															G							T		G	T																			5/4	
	SMI																																					T		T		T					3/1

Fig. 5. Co-occurrence matrix of all species and subspecies of *Aeonium*. Taxa are sorted in clades (see Fig. 2 for clade names) and their names are abbreviated by the first three letters of their species/subspecies/variety epithet. Boxes filled ochre indicate a co-occurrence of the two taxa defining the coordinates of the box, boxes filled green indicate the knowledge of a natural hybrid between the two respective taxa. The letters in coloured boxes specify where the co-occurrence of two species or their hybrids are located, for the geographical code of these letters see Table 1 or Fig. 3. Numbers in the diagonal boxes below the abbreviated taxon names summarize the number of co-occurrences and number of natural hybrids with other *Aeonium* taxa (left and right of the slash, respectively) for each taxon. Percentages marked "Co" and "Hy" indicate the proportions of co-occurrences and natural hybrids, respectively, for each pairing of two clades, where the number of possible pairings between all taxa of the two respective clades is considered 100%.

We were able to assess the degree of sympatry (see Table 2) for 27 of the 29 diversification events represented by supported (posterior probability ≥ 0.95) sister lineages of the dated phylogeny (Fig. 4). In the two remaining supported sister lineages, distribution maps of the species in question were not available from Bañares Baudet (2015a). Fifteen of these 27 diversification events (55.6%) were associated with at least some degree of sympatry among the extant respective sister lineages, and four (14.8%) were associated with full sympatry (degree of sympatry = 1). Figure 6 shows the scatterplot of the degree of sympatry between supported sister lineages of the dated phylogeny versus the age of the respective nodes (as summarized in Table 2). There is a significant positive correlation between the degree of sympatry and node age (r = 0.571; P < 0.001) indicating that sister lineages deriving from younger diversification events are more often separated geographically than sister lineages that diverged earlier. None of the pairs of sister lineages with fully overlapping distribution ranges (degree of sympatry = 1) diverged later than 0.75 Ma, and the latest diversification event of this kind is the divergence of *A. gomerense* (Praeger) Praeger from the lineage comprising *A. castello-paivae* and *A. decorum* Webb ex Bolle (Table 2, Fig. 6). On the other hand, none of the diversification events giving rise to lineages that are fully separated geographically (degree of sympatry = 0) took place earlier than 2.48 Ma when Madeira was colonized by the ancestor of *A. glandulosum* and *A. glutinosum* (Table 2, Fig. 6).

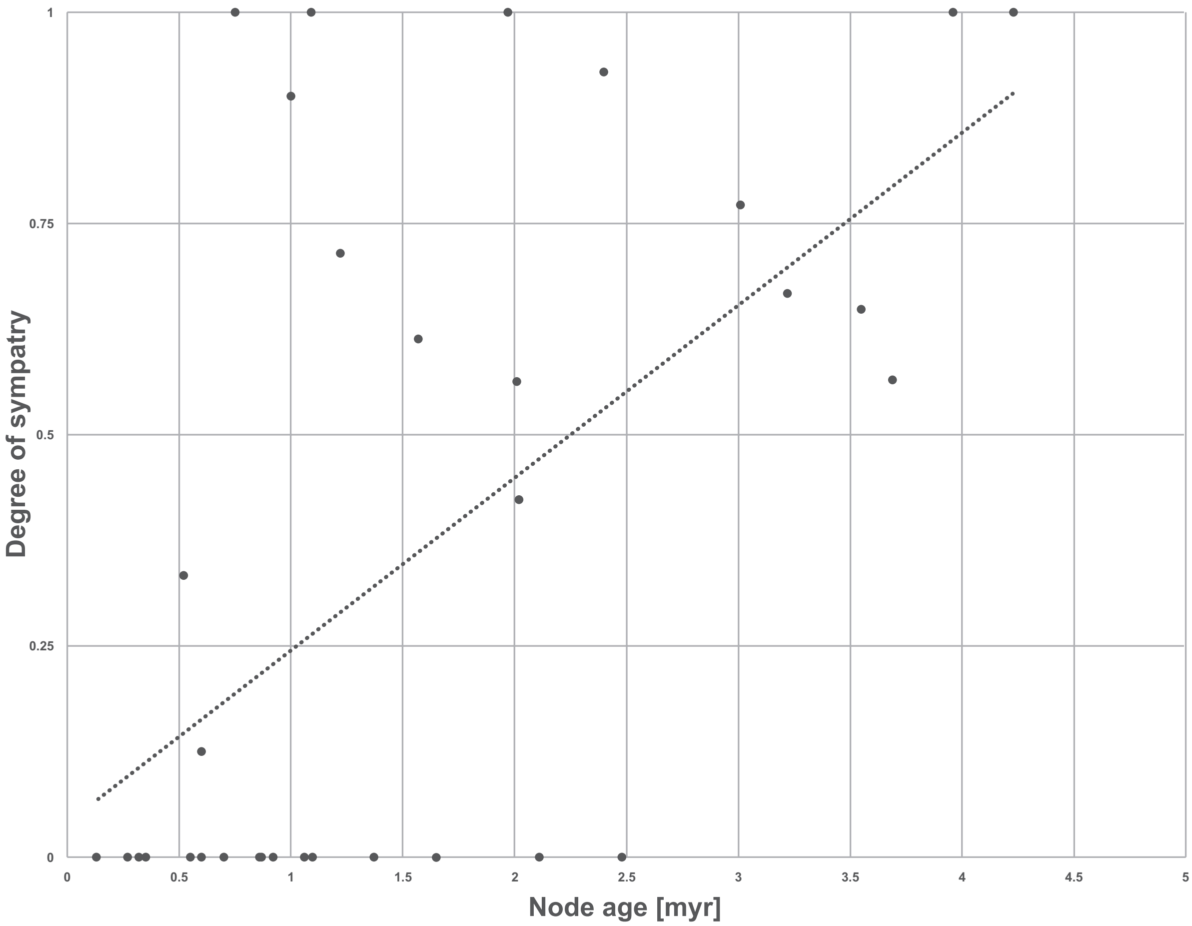

Fig. 6. Degrees of sympatry between extant *Aeonium* lineages that diversified from each supported node in the dated *Aeonium* phylogeny (see Fig. 4) plotted against the age of the respective nodes. Coefficient of determination (R^2) of the linear regression = 0.326

The AAR analysis in BioGeoBEARS identified BAYAREALIKE+J as the best model (LnL = -173.4; AICc_wt = 1.0; Table 3) fitting the input data (i.e., dated phylogeny and distribution matrix of all *Aeonium* taxa). This was followed by the DEC+J model receiving the second highest scores (LnL = -180.9; AICc_wt = $5.0 \cdot 10^{-4}$). We will not discuss the results of the latter model because of concerns regarding the method's bias towards underestimating anagenetic, time-dependent range evolution (Ree and Sanmartín, 2018). The BSM analysis revealed founder events (32.9%, interpreted as speciation following inter-island dispersal), anagenetic dispersal events (33.5%, interpreted as inter-island dispersal without speciation) and sympatric diversification events (33.6%, interpreted as speciation on the same island/area) as biogeographical processes that were more or less equally frequent in the evolution of *Aeonium* (Table 4). Hence, among the cladogenetic events only, sympatric diversification was more or less as frequent (50.5%) as allopatric diversification (49.5%). It is important to note that in BAYAREALIKE models it is not possible to infer estimates of intra-range (subset) speciation and vicariance (Matzke, 2018a), which are interpreted as sympatric speciation in one of several areas/islands occupied by a progenitor species and allopatric speciation in distinct areas/islands occupied by a progenitor species, respectively.

Table 3. Comparison of parameters obtained from different models in the ancestral area reconstruction analysis in BioGeoBEARS. The results for BAYAREALIKE+J, i.e., the model with the best fit based on log-likelihood (LnL) and the corrected Akaike information criterion model weight (AICc_wt), are highlighted in bold.

Model	LnL	*d*	*e*	*j*	AICc	AICc_wt
DEC	-197.4	0.051	0.180	0	399.0	$1.2 \cdot 10^{-10}$
DEC+J	-180.9	0.026	0.042	0.049	368.4	$5.0 \cdot 10^{-4}$
DIVALIKE	-195.8	0.054	0.140	0	395.9	$5.6 \cdot 10^{-10}$
DIVALIKE+J	-184.0	0.030	0.043	0.041	374.6	$2.4 \cdot 10^{-5}$
BAYAREALIKE	-207.8	0.055	0.520	0	419.9	$3.4 \cdot 10^{-15}$
BAYAREALIKE+J	**-173.4**	**0.021**	**0.044**	**0.050**	**353.3**	**1.0**

d: dispersal rate
e: extinction rate
j: rate of founder-event speciation (jump dispersal)

Table 4. Absolute and percentage mean incidence (± standard deviation) of biogeographical events recorded in 50 individual biogeographical stochastic maps (BSMs) simulated for the evolution of *Aeonium* under the BAYAREALIKE+J model. Cladogenetic dispersal corresponds to founder-event speciation (parameter *j* in BioGeoBEARS). Cladogenesis in sympatry and anagenetic dispersal correspond to parameters *y* and *d* in BioGeoBEARS, respectively. Note that this model does not allow for intra-range (subset) speciation and for vicariance.

	dispersal	sympatry	sum
cladogenesis	24.24 (± 1.61); 32.9%	24.76 (± 1.61); 33.6%	49.00 (± 0.00); 66.5%
anagenesis	24.64 (± 3.02); 33.5%	–	24.64 (± 3.02); 33.5%
sum	48.88 (± 3.24); 66.4%	24.76 (± 1.61); 33.6%	73.64 (± 3.02); 100%

The BSM analysis furthermore identified Tenerife as by far the most important starting point for dispersal events, as, on average, 41.73% of all recorded cladogenetic and anagenetic dispersal events (i.e., parameters *j* and *d* in BioGeoBEARS, respectively) originated from Tenerife (Table 5). Most of the remaining dispersal events had their origin on La Gomera (14.73%), Gran Canaria (12.32%), La Palma (10.02%) and El Hierro (7.82%), while less than one dispersal event per simulation originated from Fuerteventura, Madeira, the Cape Verde Islands or Morocco (Table 5). When only dispersal between the western Canary Islands, which host a greater number of *Aeonium* taxa, is considered, these numbers amount to 48.62%

(Tenerife), 18.27% (La Gomera), 12.61% (Gran Canaria), 11.90% (La Palma) and 8.60% (El Hierro). As receivers of dispersal events, islands were much more similar to each other in frequency. La Palma acted as sink in 18.94%, La Gomera in 16.41%, Gran Canaria in 16.12%, El Hierro in 14.73% and Tenerife in 10.72% of all dispersal events (Table 5). When considering dispersal only between the western Canary Islands, the corresponding figures are 24.93% (La Palma), 22.10% (La Gomera), 20.04% (Gran Canaria), 19.92% (El Hierro) and 13.02% (Tenerife). Fuerteventura, Madeira, the Cape Verde Islands and the continental African areas, on average, were colonized only once or twice per simulation (Table 5).

Most of the simulated sympatric cladogenetic events (i.e., parameter *y* in BioGeoBEARS) occurred on Tenerife. On average, 14.82 (± 2.64), i.e., 59.9% of the 24.76 recorded sympatric speciation events took place on Tenerife (Electr. Suppl. 5). In all 50 simulations of the BSM analysis, the five speciation events that gave rise to the Teneriffan subclade within sect. *Leuconium* (i.e., *A. haworthii* through *A. ciliatum* in Fig. 7) were inferred to have taken place on Tenerife (Electr. Suppl. 3). In addition to speciation events in this subclade, nodes 10 (i.e., the split between *A. aizoon* (Bolle) T.Mes and *A. aureum* + *A. dodrantale* (Willd.) T.Mes), 11 (i.e., the split between *A. aureum* and *A. dodrantale*), 24 (i.e., the split between *A. cuneatum* Webb & Berthel. and *A. canariense* s.l.), 25 (i.e., the initial diversification of *A. canariense* s.l.) and 40 (see Fig. 4 for node numbers) were among those speciation events most frequently associated with sympatric speciation on Tenerife (Electr. Suppl. 3). On La Gomera, on average 2.88 sympatric speciation events per simulation occurred (Electr. Suppl. 5), most frequently inferred for nodes 32 and 33, i.e., the diversification events that gave rise to *A. gomerense*, *A. decorum* and *A. castello-paivae* (Electr. Suppl. 3). However, *A. gomerense* was not recovered as closest relative of *A. decorum* and *A. castello-paivae* in the ASTRAL analyses (Fig. 3). La Palma, Gran Canaria and Madeira hosted approximately one sympatric speciation event each, i.e., the diversification of *A. davidbramwellii* H.Y.Liu and *A. escobarii* Rebmann & Malkm.-Huss. (node 43, inferred in 50/50 BSMs), that between *A. simsii* and the remainder of clade Arboreum I (node 15, inferred in 41/50 BSMs) and that between *A. glandulosum* and *A. glutinosum* (node 23, inferred in 50/50 BSMs), respectively (Electr. Suppl. 3). Another single sympatric diversification event in East Africa was inferred in 48/50 BSMs for the split between *A. leucoblepharum* and the last common ancestor of *A. stuessyi* and *A. gorgoneum* (node 37; Electr. Suppl. 3). Other speciation events in sympatry were inferred sporadically and inconsistently (Electr. Suppl. 5).

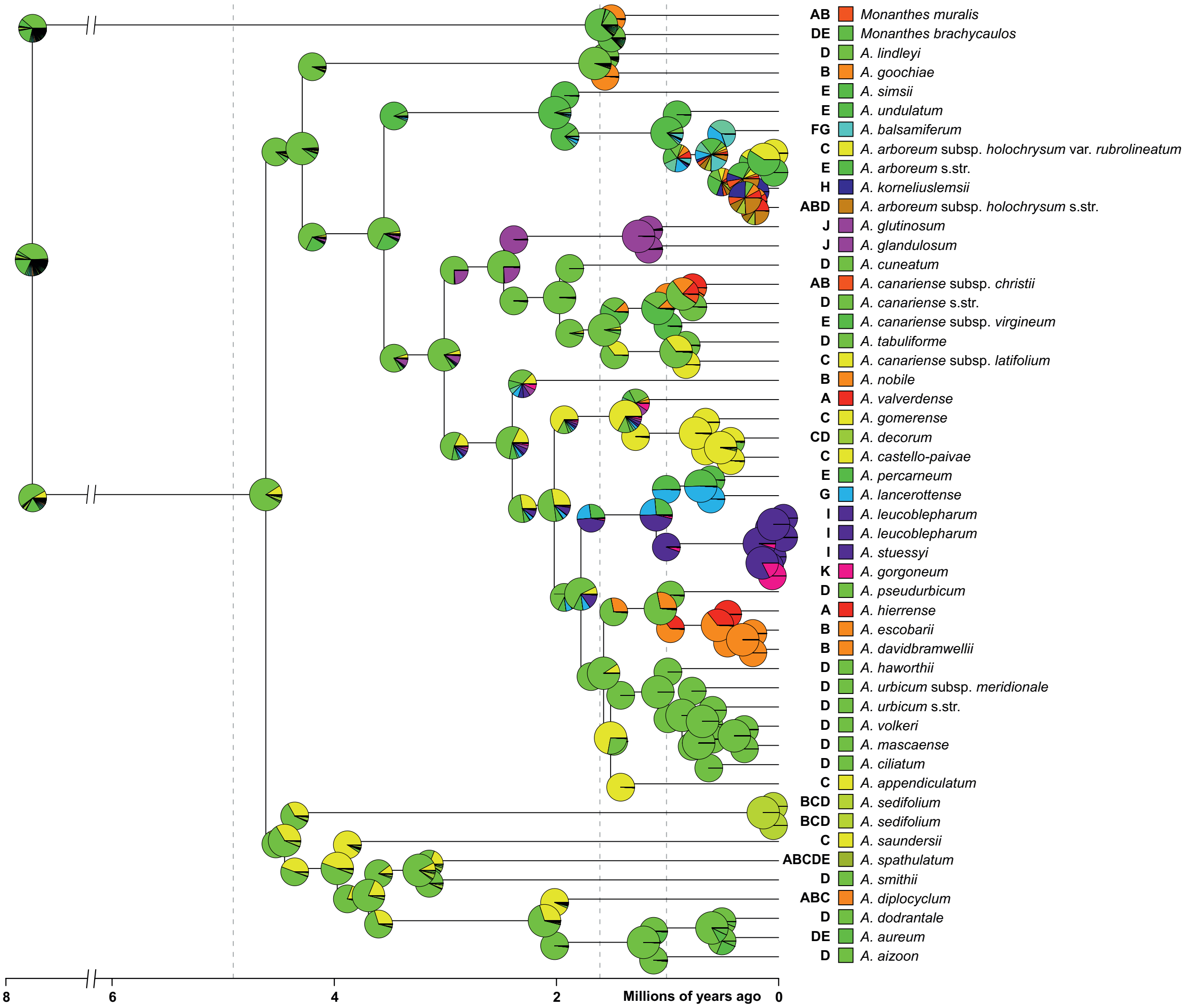

Fig. 7. Ancestral area reconstruction of the dated *Aeonium* phylogeny (see Fig. 4) obtained using the BAYAREALIKE+J model in BioGeoBEARS. Pie charts indicate the relative probability of each area or combination of areas for each node and each branch, and rectangles at the tips indicate the recent distribution area or combination of areas for each sampled taxon of the phylogeny. The branches arising directly from the root were shortened (as indicated by two parallel streaks on the time scale) for the purpose of better visibility. Dashed vertical lines represent the age of Madeira (circa 5 myr), La Palma (circa 1.7 myr) and El Hierro (circa 1.1 myr). A: El Hierro, B: La Palma, C: La Gomera, D: Tenerife, E: Gran Canaria, F: Fuerteventura, G: Lanzarote, H: Morocco, I: East Africa, J: Madeira, K: Cape Verde.

Table 5. Number of cladogenetic + anagenetic dispersal counts (mean ± standard deviation) between islands/areas averaged across 50 individual biogeographical stochastic maps (BSMs) simulated for the evolution of *Aeonium* under the BAYAREALIKE+J model. Direction of dispersal events is from the row state to the column state. At the end of each row the average number (absolute and relative) of dispersal events originating from the corresponding island/area is given. In turn, at the bottom of each column, the respective number of dispersal events terminating on the corresponding island/area is given. C: Gran Canaria, EA: East Africa, F: Fuerteventura, G: La Gomera, H: El Hierro, L: Lanzarote, Mc: Morocco, Md: Madeira, P: La Palma, T: Tenerife, V: Cape Verde

	H	P	G	T	C	F	L	Mc	EA	Md	V	Sum
H	–	1.74 (± 0.90)	0.36 (± 0.56)	0.28 (± 0.50)	0.54 (± 0.79)	0.18 (± 0.44)	0.20 (± 0.40)	0.46 (± 0.54)	0.02 (± 0.14)	0.02 (± 0.14)	0.02 (± 0.14)	3.82 7.82%
P	2.60 (± 0.97)	–	0.44 (± 0.73)	0.40 (± 0.61)	0.60 (± 0.64)	0.12 (± 0.33)	0.20 (± 0.45)	0.42 (± 0.50)	0.06 (± 0.24)	0.06 (± 0.24)	0.00 (± 0.00)	4.90 10.02%
G	1.22 (± 0.74)	1.52 (± 0.93)	–	2.36 (± 1.45)	1.10 (± 0.86)	0.18 (± 0.44)	0.18 (± 0.39)	0.14 (± 0.40)	0.20 (± 0.45)	0.12 (± 0.33)	0.18 (± 0.39)	7.20 14.73%
T	2.38 (± 1.32)	3.86 (± 1.39)	5.70 (± 1.64)	–	4.56 (± 1.05)	0.48 (± 0.58)	0.74 (± 0.88)	0.46 (± 0.73)	0.80 (± 0.73)	1.10 (± 0.68)	0.32 (± 0.62)	20.40 41.73%
C	0.56 (± 0.58)	1.34 (± 1.00)	1.00 (± 0.88)	1.38 (± 1.38)	–	0.16 (± 0.42)	0.74 (± 0.66)	0.04 (± 0.20)	0.54 (± 0.65)	0.20 (± 0.45)	0.06 (± 0.24)	6.02 12.32%
F	0.06 (± 0.24)	0.08 (± 0.27)	0.04 (± 0.20)	0.06 (± 0.31)	0.14 (± 0.40)	–	0.48 (± 0.54)	0.00 (± 0.00)	0.04 (± 0.20)	0.02 (± 0.14)	0.02 (± 0.14)	0.94 1.92%
L	0.08 (± 0.27)	0.28 (± 0.45)	0.12 (± 0.33)	0.20 (± 0.40)	0.50 (± 0.51)	0.54 (± 0.58)	–	0.04 (± 0.20)	0.24 (± 0.43)	0.02 (± 0.14)	0.00 (± 0.00)	2.02 4.13%
Mc	0.00 (± 0.00)	0.10 (± 0.36)	0.08 (± 0.34)	0.06 (± 0.24)	0.08 (± 0.27)	0.00 (± 0.00)	0.02 (± 0.14)	–	0.00 (± 0.00)	0.04 (± 0.20)	0.00 (± 0.00)	0.38 0.78%
EA	0.14 (± 0.35)	0.10 (± 0.30)	0.10 (± 0.36)	0.10 (± 0.30)	0.26 (± 0.53)	0.02 (± 0.14)	0.22 (± 0.42)	0.00 (± 0.00)	–	0.02 (± 0.14)	1.00 (± 0.00)	1.96 4.01%
Md	0.10 (± 0.30)	0.10 (± 0.30)	0.14 (± 0.40)	0.28 (± 0.64)	0.04 (± 0.20)	0.00 (± 0.00)	0.02 (± 0.14)	0.00 (± 0.00)	0.00 (± 0.00)	–	0.02 (± 0.14)	0.70 1.43%

V	0.06 (± 0.24)	0.14 (± 0.35)	0.04 (± 0.20)	0.12 (± 0.44)	0.06 (± 0.24)	0.02 (± 0.14)	0.02 (± 0.14)	0.04 (± 0.28)	0.04 (± 0.20)	0.00 (± 0.00)	–	0.54 1.10%
Sum	7.20 14.73%	9.26 18.94%	8.02 16.41%	5.24 10.72%	7.88 16.12%	1.70 3.48%	2.82 5.77%	1.60 3.27%	1.94 3.97%	1.60 3.27%	1.62 3.31%	48.88 100.00%

Tenerife was inferred as ancestral area at the crown node of *Aeonium* (Fig. 7; node 3 in Fig. 4). Although the geographical areas occupied by the ancestors of the nectary-lacking clade/grade were not reconstructed unambiguously, only La Gomera and Tenerife received noteworthy probabilities as ancestral areas of this clade/grade. Therefore, with the exception of the crown node of sect. *Chrysocome* (node 8 in Fig. 4) for which Tenerife was reconstructed as ancestral area, all internal nodes of the nectary-lacking clade/grade older than 2 myr had either La Gomera or Tenerife as ancestral area (Fig. 7). Tenerife was reconstructed as ancestral area for the most recent common ancestor (MRCA) of *A. aizoon*, *A. aureum* and *A. dodrantale*. Considering the distribution of the extant species of the nectary-lacking clade/grade, half of which are MIEs, this pattern suggests that range expansions must have been frequent and most probably took place subsequent to speciation events. This is especially true for the colonization of El Hierro and La Palma, islands that are younger than most of the diversification events in this group. The MRCA of clades Goochiae and Canariensia plus sects. *Aeonium* and *Leuconium* most probably lived in Tenerife (Fig. 7), but the corresponding node (i.e., node 12) received no support in the dating analysis (Fig. 4) and was not at all recovered in the ASTRAL analyses (Fig. 3). For the following nodes of the phylogenetic backbone, separating clade Arboreum I (node 14), the Canariensia clade (node 21), *A. nobile* etc., the AAR was inconclusive (Fig. 7). Gran Canaria was inferred as ancestral area for the two basal-most nodes in clade Arboreum I (nodes 15 and 16). For the following nodes, i.e., for the ancestors of the East Canarian *A. balsamiferum*, MIE *A. arboreum* s.l. and *A. korneliuslemsii* from Morocco, the ancestral distribution area could not be inferred conclusively. Therefore, the starting point for the colonization of Morocco remains elusive.

The ancestral area of the crown node of the Canariensia clade (i.e., node 22 in Fig. 4) was either Tenerife (74.5%) or Madeira (23.3%), and the direct descendants of this diversification event, i.e., the MRCAs of sects. *Patinaria* plus *Pittonium* (node 23) and of sect. *Canariensia* (node 24) were unambiguously distributed on Madeira and Tenerife, respectively (Fig. 7). This means that Madeira most probably was reached from Tenerife. *Aeonium canariense* s.l., incl. *A. tabuliforme*, originated on Tenerife before diversifying by repeated founder-event speciation to different islands. In the Leuconium clade/grade, the ancestors of the Gomeran endemics *A. gomerense*, *A. decorum* and *A. castello-paivae* (nodes 32 and 33) were distributed on La Gomera, the ancestor of *A. davidbramwelii* and *A. escobarii* (node 43) on Gran Canaria and the ancestral lineage of the Teneriffan subclade (nodes 45–49) on Tenerife. The source area of the East African taxa was not resolved because the ancestral areas for nodes 34 and 35 (only the latter was well supported in the dating analysis but neither were recovered by the ASTRAL analyses) were ambiguous (Fig. 7). The Cape Verde Islands most probably were reached from Africa since *A. gorgoneum* was nested in the predominantly East African clade Arboreum II.

3.4. Morphological and ecological divergence between sister lineages derived from sympatric and allopatric speciation events

We identified 17 nodes (listed in Table 6) that were consistently recovered in all phylogenetic analyses and received high support in at least one of these analyses. Four additional nodes in the Teneriffan subclade of sect. *Leuconium* (nodes 46–49 in Fig. 4) were inconsistently recovered across the phylogenetic analyses but were always associated with sympatric diversification on Tenerife (Table 6 and Electr. Suppl. 3). As at least 41 of the 50 BSMs (82%; corresponding to node 15 in Table 6) were consistent with either sympatric or allopatric diversification, we consider our classification of these altogether 21 diversification events as sympatric or allopatric as robust. Twelve of these nodes corresponded to sympatric diversification events and nine nodes to allopatric diversification events. Most of the sympatric diversification events took place on Tenerife, and one diversification event each occurred on La Gomera, Gran Canaria and Madeira (Table 6). The allopatric diversification events mostly involved migration between Tenerife and another island (La Gomera, La Palma, Gran Canaria or Madeira). The morphological REDs ranged between 0 and 1.86 among the sympatric diversification events (1.9 for the inconsistently recovered node 48) and between 0 and 1.20 among the allopatric diversification events. The ecological REDs ranged between 0.4 and 1.13 among sympatric and between 0.35 and 1.06 among allopatric diversification events. The difference in morphological REDs between sympatric (1.29 ± 0.54) and allopatric diversification events (0.74 ± 0.39) was significant (two-sided *t*-test, $p < 0.05$), but the difference in ecological REDs (0.86 ± 0.23 and 0.66 ± 0.25, respectively) was not ($p = 0.10$; Fig. 8).

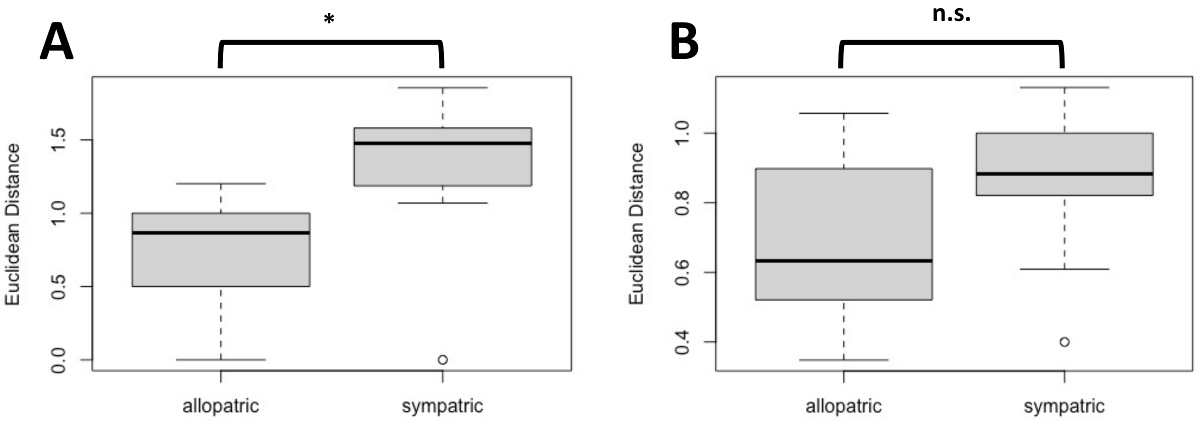

Fig. 8. Boxplots of Raw Euclidean Distances (REDs) in (A) morphological characters and (B) ecological habitat characteristics between well-supported sister lineages (see paragraph 2.8 for details concerning selection of sister lineages). REDs are separated by the biogeographical correlate (allopatric or sympatric) of the diversification event giving rise to the respective sister lineages. Raw data contributing to these boxplots are listed in Table 6.

Table 6. Morphological and ecological Raw Euclidean Distances (REDs) between sister lineages corresponding to those nodes that were recovered in all phylogenetic analyses and received high support in at least one of these analyses. For each node, the biogeographical correlate of diversification (i.e., allopatric or sympatric diversification) as reconstructed in the BSM analysis is given, along with the islands on which diversification took place and the percentage of BSMs supporting this scenario. Numbers in brackets following morphological REDs refer to the numbers of morphological characters (of 10 characters observed, i.e., characters k–t in Fig. 3) in which the two sister lineages differ from each other. In addition to the morphological and ecological REDs, the distances in terms of growth form and the three habitat characteristics examined (i.e., altitude, thermo- and ombrotype) are given individually, with 0 corresponding to full identity and 1 corresponding to full disparity.

Node number[a]	Sister lineage 1	Sister lineage 2	Biogeographic correlate of diversification	Morphological RED	Ecological RED		
				Growth form	Altitude	Thermotype	Ombrotype
9	*A. diplocyclum*	remainder of sect. *Greenovia*	94 % allopatric (92 % Tenerife - La Gomera)	1.07 (1/10)	0.38		
				0.00	0.28	0.00	0.25
10	*A. aizoon*	*A. aureum* + *A. dodrantale*	100 % sympatric (100 % Tenerife)	1.07 (1/10)	0.88		
				0.00	0.53	0.50	0.50
11	*A. aureum*	*A. dodrantale*	90 % sympatric (90 % Tenerife)	1.30 (1/10)	1.13		
				0.00	0.88	0.50	0.50
13	*A. goochiae*	*A. lindleyi*	100 % allopatric (96 % Tenerife - La Palma)	1.20 (1/10)	0.67		
				0.00	0.20	0.50	0.33
15	*A. simsii*	remainder of clade Arboreum I	82 % sympatric (82 % Gran Canaria)	1.66 (4/10)	0.85		
				1.00	0.60	0.33	0.50
16	*A. undulatum*	*A. balsamiferum*, *A. korneliuslemsii* + *A. arboreum* s.l.	100 % allopatric (ambiguous)	0.87 (2/10)	0.65		
				0.00	0.25	0.33	0.50
17	*A. balsamiferum*	*A. korneliuslemsii* + *A. arboreum* s.l.	100 % allopatric (ambiguous)	0.81 (2/10)	1.06		
				0.00	0.75	0.33	0.67

20	*A. arboreum* subsp. *holochrysum* var. *rubrolineatum*	*A. arboreum* s.str.	100 % allopatric (100 % Gran Canaria - La Gomera)	1.00 (1/10)	0.92		
				0.00	0.53	0.67	0.33
22	*A. glandulosum* + *A. glutinosum*	sect. *Canariensia*	100 % allopatric (98 % Tenerife - Madeira)	1.00 (3/10)	0.90		
				0.50	0.33	0.67	0.50
23	*A. glandulosum*	*A. glutinosum*	100 % sympatric (100 % Madeira)	1.50 (2/10)	1.00		
				1.00	0.00	0.00	1.00
24	*A. cuneatum*	remainder of sect. *Canariensia*	98 % sympatric (98 % Tenerife)	1.86 (3/10)	1.08		
				0.00	0.60	0.67	0.60
25	*A. tabuliforme* + *A. canariense* subsp. *latifolium*	remainder of *A. canariense*	84 % sympatric (84 % Tenerife)	0.00 (0/10)	0.40		
				0.00	0.00	0.67	0.60
26	*A. tabuliforme*	*A. canariense* subsp. *latifolium*	100 % allopatric (100 % Tenerife - La Gomera)	0.50 (0/10)	0.54		
				0.00	0.20	0.00	0.50
27	*A. canariense* subsp. *virgineum*	*A. canariense* s.str. + *A. canariense* subsp. *christii*	98 % allopatric (92 % Tenerife - Gran Canaria)	0.33 (0/10)	0.52		
				0.00	0.00	0.33	0.40
28	*A. canariense* s.str.	*A. canariense* subsp. *christii*	100 % allopatric (ambiguous)	0.00 (0/10)	0.35		
				0.00	0.10	0.33	0.00
33	*A. castello-paivae*	*A. decorum*	94 % sympatric (94 % La Gomera)	1.58 (3/10)	0.82		
				0.00	0.42	0.50	0.50
45	*A. haworthii*	remainder of the Teneriffan Leuconium subclade	100 % sympatric (100 % Tenerife)	1.19 (5/10)	0.61		
				0.50	0.44	0.33	0.25

46[b]	*A. urbicum* subsp. *meridionale*	*A. urbicum* s.str., *A. ciliatum*, *A. mascaense* + *A. volkeri*	100 % sympatric (100 % Tenerife)	1.47 (5/10)	0.76		
				0.50	0.64	0.33	0.50
47[b]	*A. ciliatum*	*A. urbicum* s.str., *A. mascaense* + *A. volkeri*	100 % sympatric (100 % Tenerife)	1.17 (4/10)	0.73		
				0.50	0.20	0.50	0.50
48[b]	*A. urbicum* s.str.	*A. mascaense* + *A. volkeri*	100 % sympatric (100 % Tenerife)	1.90 (4/10)	0.56		
				1.00	0.45	0.00	0.33
49[b]	*A. mascaense*	*A. volkeri*	100 % sympatric (100 % Tenerife)	1.44 (2/10)	1.22		
				1.00	1.00	0.50	0.50
Mean of Teneriffan *Leuconium* subclade[c]	-	-	100 % sympatric (100 % Tenerife)	1.48 (3.25/10)	0.91		
				0.63	0.62	0.39	0.51

[a] Node numbers as specified in Fig. 4

[b] These nodes in the Teneriffan subclade of sect. *Leuconium* were retrieved in the dating analysis only. The REDs corresponding to these nodes were replaced by the mean values given in the bottom row of this table.

[c] These values are REDs averaged across the nodes of the Teneriffan subclade of sect. *Leuconium* above node 45 (i.e., the inconsistently resolved nodes 46–49) as recovered in all our phylogenetic analyses. See paragraph 2.8 (above) for more detailed information.

4. Discussion

4.1. Implications for the sectional classification of *Aeonium*

The results of our phylogenetic analyses suggest that relatively few changes are necessary to the sectional classification of *Aeonium*. Most importantly, species of sect. *Greenovia* always formed a well-supported clade nested within *Aeonium* (Figs. 2, 3), and *Greenovia* should thus retain its sectional rank. Although this had already been suggested in earlier phylogenetic studies (Mes, 1995; Jorgensen and Frydenberg, 1999; Mort et al., 2002), but never with a complete taxon sample, inclusion of *Greenovia* in *Aeonium* is not accepted by all authors (e.g., Arango Toro, 2021). Sections *Goochiae* and *Canariensia* were always recovered as clades (Figs. 2, 3), but the latter without support in 320–500/ASTRAL (Fig. 3). Within sect. *Goochiae*, the two subspecies of *A. lindleyi* were sister to each other (Suppl. Fig. S3), in contrast to previous findings (Jorgensen and Frydenberg, 1999; Mort et al., 2002) where *A. lindleyi* subsp. *viscatum* (treated as *A. viscatum* Webb ex Bolle by these authors) was resolved as sister to a weakly supported clade comprising *A. lindleyi* s.str. and *A. goochiae* or as sister to sect. *Greenovia*, respectively. Our results, however, justify the inclusion of *A. lindleyi* subsp. *viscatum* in *A. lindleyi*. For the remaining two sections with nectary-lacking species (i.e., sects. *Chrysocome* and *Petrothamnium*), our phylogenies were less conclusive. Section *Petrothamnium* was supported as a clade in both concatML analyses, and sect. *Chrysocome* was only supported as monophyletic in 320–500/RAxML (Fig. 2). In this latter phylogeny, sect. *Petrothamnium* was sister to a clade consisting of sects. *Chrysocome* and *Greenovia*. The only other topology that is in supported conflict to such relationships is the phylogeny recovered in 320–500/ASTRAL where *A. spathulatum* is supported as sister to sect. *Greenovia* (Fig. 3). Nevertheless, we conclude that no changes to the classification of these sections are necessary because there is no unequivocal evidence against their monophyly.

The two monospecific sections endemic to Madeira (sect. *Patinaria* with *A. glandulosum* and sect. *Pittonium* with *A. glutinosum*) were always well supported as sister to each other, together forming the sister clade to sect. *Canariensia* (Figs. 2, 3). Although their morphological distinctness – with rosette plants in sect. *Patinaria* and shrubs up to 1.5 m tall in sect. *Pittonium* – impedes their classification as one section, this would be desirable as it would indicate the common ancestry of these two Madeiran endemics and increase the phylogenetic information content of the classification. A section comprising *A. glandulosum* and *A. glutinosum* can be defined as follows: Biennial to perennial rosette plants or shrubs up to 1.5 m tall; stems and branches completely glabrous; bark of the stem, when present, smooth and without prominent ridges along the edges of leaf scars; leaves without tannic stripes, with unicellular cilia at the margins, distinctly sticky, at least when young, in shrubs; leaf cilia of rosette plants bead-shaped; inflorescence pubescent; petals pale yellow; carpels without ventral appendages, hypogynous nectar glands present. Madeira, Porto Santo and Deserta Grande.

Within sect. *Canariensia*, the consistently recovered paraphyly of *A. canariense* s.l., with *A. canariense* subsp. *latifolium* (Burchard) Bañares from La Gomera most closely related to *A. tabuliforme* from northern Tenerife, necessitates either segregation of *A. canariense* subsp. *latifolium* from *A. canariense* and its elevation to the rank of a species, or inclusion of *A. tabuliforme* in *A. canariense* s.l. Before the taxonomic treatment by Liu (1989), *A. canariense* s.l. had been treated as four distinct SIE species. Segregation of *A. canariense* subsp. *latifolium* (= *A. subplanum* Praeger) from *A. canariense* would justify re-instatement of these four species, and species names already exist. The strong similarity in habit of *A. canariense* subsp. *latifolium* and *A. tabuliforme* had previously been observed by Lösch (1990) and is based on the conspicuously flattened rosettes of the former that resemble the perfectly plank-shaped rosettes of the latter. Also, Mes and 't Hart (1996), who sampled all four taxa of *A. canariense* s.l. in an RFLP analysis of plastid DNA, had already inferred *A. canariense* subsp. *latifolium* (as *A. subplanum*) and *A. tabuliforme* as closest relatives.

Species of sect. *Aeonium* were consistently recovered in two distinct and well-supported clades that were never sister to each other, i.e., clades Arboreum I and Arboreum II in Fig. 2. These two clades are geographically very distinct because the members of clade Arboreum I are distributed across the Canary Islands and Morocco, while species of clade Arboreum II occur on the Cape Verde Islands (*A. gorgoneum*) and in East Africa. Also, species of these two clades can easily be distinguished from each other by the shape of their leaf rosettes, seasonality of leaf shedding and by the colour of their petals. With the exception of *A. simsii*, a decumbent rosette plant (see character k in Fig. 3), species of clade Arboreum I shed their older leaves in summer and only keep the youngest leaves in relatively small and flat rosettes (Liu, 1989; Lösch, 1990). Most of them, although not *A. balsamiferum*, furthermore have distinctly flattened rosette centres with the youngest leaves tightly appressed to the older ones, especially during summer. All representatives of clade Arboreum II are shrubs without seasonal leaf shedding and always have rosettes in which the youngest leaves arise from the centre of the rosette at an acute angle (Lösch, 1990). Lastly, the petals of taxa in clade Arboreum I are a very bright yellow, while petals of species in clade Arboreum II are much paler (see character r in Fig. 3). Based on these morphological characters distinguishing clades Arboreum I and II, *A. leucoblepharum*, *A. stuessyi* and *A. gorgoneum* could be segregated from sect. *Aeonium* to render this section monophyletic. However, Liu (1989) considered these three species to be closely related to *A. glutinosum* and grouped them into sect. *Pittonium*, now a monospecific section containing *A. glutinosum*. Therefore, a new sectional name should be given to the species of clade Arboreum II, while *A. glutinosum* could be classified together with *A. glandulosum* in another section (see above).

Lastly, all sampled species of sect. *Leuconium* formed a well-supported clade in the species-tree analyses in ASTRAL (Fig. 3), but not in the concatML analyses (Fig. 2). In the latter case, *A. percarneum* and *A. lancerottense* were part of a polytomy with clades Arboreum I and Arboreum II and the remainder of sect. *Leuconium*. This incongruent phylogenetic placement of *A. percarneum* and *A. lancerottense*, those two species of sect. *Leuconium* found on Gran Canaria and Lanzarote, respectively, had also been found by Jorgensen and Frydenberg (1999) and Jorgensen and Olesen (2001) who interpreted this incongruency as an indicator of a hybridogenous origin of these two species. While these authors compared the topologies obtained from their analysis of nuclear ribosomal DNA sequences with a previously published plastid RFLP analysis (Mes and 't Hart, 1996), we only included loci of the nuclear genome. However, mainly because the phylogenetic position of *A. percarneum* and *A. lancerottense* was unsupported in the concatML analyses (Fig. 2), a more thorough examination of alternative topologies supported by different loci of our dataset or a phylogenetic network analysis are necessary in order to find further evidence for a potential hybrid origin of these species. With the results currently at hand, no taxonomic changes in sect. *Leuconium* seem to be necessary.

4.2. Biogeography and age of diversification in the evolution of *Aeonium*

While Kim et al. (2008) had estimated the split between *Aeonium* and *Monanthes* to approximately 11.08 Ma, which is very close to the result of our Crassulaceae-wide dating analysis of ITS sequence data (11.62 Ma), the dating analysis based on ddRAD sequences of *Aeonium* and *Monanthes* resulted in an age of 7.76 (± 4.64) Ma for this split. This estimate is intermediate between the estimate inferred by Kim et al. (2008) and the estimate for the stem age of the clade comprising *Aeonium* + *Monanthes* inferred by Kondraskov et al. (2015) who used newly sequenced and previously published sequence data of the plastid marker *matK* as basis for their dating analysis sampling all three genera of Aeonieae from the Canary Islands. Because *Monanthes* has its centre of diversity on Tenerife, with eight of eleven species occurring there and three species endemic to Tenerife, it seems likely that the ancestral range of the common ancestor of *Aeonium* and *Monanthes* was also, at least partly, Tenerife, although this was not inferred conclusively, and only two species of *Monanthes* were sampled here.

Although three of the eight species of the nectary-lacking clade (Fig. 2) occur (among others) on El Hierro and/or La Palma, these islands did not play a part in the origin of these species because they diversified earlier than the emergence of these two westernmost of the Canary Islands. The same applies to the diversification of *Aeonium* into distinct sections. Most of the currently accepted sections of *Aeonium* originated before the emergence of El Hierro and La Palma, and the exceptions to this, i.e., sects. *Patinaria* and *Pittonium* endemic to Madeira as well as the East African clade of sect. *Aeonium* (i.e., clade Arboreum II in Fig. 2), are completely independent of the existence of El Hierro and La Palma (Fig. 7). Instead, speciation by founder events between Tenerife and either La Gomera to the west, Gran Canaria to the east or Madeira to the north, as well as sympatric cladogenesis within Tenerife were inferred to play the major part in the emergence of the sections of *Aeonium* (Electr. Suppl. 3) between approximately 4.6 and 2.5 Ma. When cladogenetic events within Tenerife are interpreted as sympatric speciation in this time period, it needs to be considered that this island most probably originated from three palaeo-islands (i.e., Adeje, Anaga and Teno) that were separated from each other by sea straits between circa 8 and 3.5 Ma (Caujapé-Castells et al., 2017). Therefore, diversification that was reconstructed to have taken place in this timespan on Tenerife could be the result of founder-event speciation between palaeo-islands instead of sympatric speciation. Dispersal to the eastern Canary Islands Fuerteventura and Lanzarote earlier than 2 Ma was inferred as rather unlikely because the probability of any ancestral taxon older than 2 myr distributed on either or both of these islands was consistently lower than 5%. The MRCA of *A. percarneum*, *A. lancerottense* and the East African clade Arboreum II which existed between 1.1 and 1.7 Ma (Fig. 4; for discussion of alternative phylogenetic placement of *A. percarneum* and *A. lancerottense* see 4.1 above), was the oldest ancestral taxon with a probability greater 8% (namely 24%) of colonizing the eastern Canary Islands. This pattern of a late colonization of Fuerteventura and/or Lanzarote has also been found in many other endemic lineages of the Canarian flora, such as the closely related *Aichryson* (Hühn et al., 2021), but also in *Argyranthemum* (White et al., 2020) and *Helianthemum* (Albaladejo et al., 2021). This pattern differs from a stepping-stone model of colonization from older to younger islands (Juan et al., 2000), i.e., from east to west in case of the Canary Islands. Colonization of the westernmost islands after initial colonization of Fuerteventura and Lanzarote has been proposed for *Olea europaea* L. (Hess et al., 2000) and for one of the two lineages of *Lotus* sect. *Pedrosia* (Lowe) Valdés that are native to the Canaries (Jaén-Molina et al., 2021).

Back-colonization of the African continent took place twice independently in the evolution of *Aeonium*, i.e., migration of *A. korneliuslemsii* to Morocco not earlier than 0.27 Ma (95% HPD

= 0.08–0.50 Ma) and migration of the MRCA of clade Arboreum II to East Africa and the southwestern part of the Arabian Peninsula (i.e., the Eritreo-Arabian region) between 0.17 and 1.10 Ma (95% HPD = 0.04–2.01 Ma). Both age estimates are younger than the estimates by Kim et al. (2008; 1.28 ± 0.21 Ma and 2.95 ± 1.32 Ma), but the topology of their *Aeonium* phylogeny differed strongly from ours. The source area for colonization of the African continent could not be reconstructed unequivocally. Back-colonization of the African continent after diversification on the Canary Islands has also been suggested for, among others, *Matthiola* W.T.Aiton (Brassicaceae, migration to Morocco; Jaén-Molina et al., 2009), *Lotus* L. (Fabaceae, migration to Northwest Africa; Jaén-Molina et al., 2021), *Euphorbia regis-jubae* Webb & Berthel. (Euphorbiaceae, migration to Morocco; Sun et al., 2016) and *Tolpis* (migration to Europe or North Africa; Grünstäudl et al., 2017). Only in case of *E. regis-jubae* migration to North Africa could be reconstructed to have taken place from either Fuerteventura or Lanzarote, while the area of origin was not explicitly reconstructed in the other examples. However, the closest relatives of the Moroccan populations of *Matthiola longipetala* subsp. *viridis* (Conti) Maire occur on Lanzarote and Gran Canaria (Jaén-Molina et al, 2009), suggesting an origin from eastern Canary Islands for this back-colonization. Possibly, preference for habitats of sparse vegetation cover and little competition in (semi-)arid environments may have facilitated back-colonization of Africa in all examples above as well as in *Aeonium*. Thus, *A. korneliuslemsii* and *A. leucoblepharum* are common on rocks, banks and cliffs (Liu, 1989). Only *A. stuessyi* seems to have adapted to an epiphytic and occasionally epilithic lifestyle (Liu, 1989). All phylogenetic analyses supported a sister relationship of the Cape Verde species *A. gorgoneum* to either *A. stuessyi* alone or to *A. stuessyi* plus the second East African species, *A. leucoblepharum*. Probably because the dated phylogeny that was used for the AAR analysis supported the former relationship, and because *A. gorgoneum* was thus nested within the East African clade, migration from (East) Africa to the Cape Verde Islands was reconstructed. However, if the latter relationship of *A. gorgoneum* as sister to both East African species had been the foundation of the AAR analysis, the direction of migration (either from the Cape Verde Islands to the Eritreo-Arabian region or the other way around) might not have been reconstructed as clearly. In any case, a long-distance dispersal event between these two very far-apart areas (distance approximately 7.500 km) would be one possible explanation for the disjunct distribution and was put forward by Thiv et al. (2010b) who argued that the numerous small seeds produced by *Aeonium* species may have facilitated long-distance dispersal. Furthermore, disjunct distributions of closely related plant taxa on the Cape Verde or Canary Islands on the one hand and in the Eritreo-Arabian region on the other are not uncommon and have also been found in, e.g., *Camptoloma* Benth. (Scrophulariaceae; Culshaw et al., 2021), *Campylanthus* Roth (Plantaginaceae; Thiv et al., 2010b) and *Cicer* L. (Fabaceae; Javadi et al., 2007). More generally, disjunct distribution ranges around the margins of the African continent (including the Macaronesian archipelagos to the West) are known as the Rand Flora pattern (Christ, 1910; Pokorny et al., 2015) which has been explained by vicariance of lineages initially widespread across northern Africa that subsequently went extinct in the central part of the distribution range due to aridification and emergence of the Sahara, approximately 6–7 Ma (Senut et al., 2009). Although this date for the onset of aridification in the Sahara is much older than the age of disjunction we inferred for clade Arboreum II (see above), the Sahara area went through later periods of increased humidity, witnessed by fossil pollen of tropical and subtropical trees (Watrin et al., 2009), and could therefore possibly have been inhabited by the MRCA of clade Arboreum II. The latest phase of such a 'green Sahara' has been dated to only 6.000 years ago (i.e., much later than our age estimate for the split between *A. gorgoneum* and the Eritreo-Arabian *Aeonium* species) and is known as the mid-Holocene humid period (Schuster et al., 2006).

Our sympatry versus node age plot (Fig. 6) obtained from the dated phylogeny (Fig. 4) somewhat resembles the results of a simulation of cladogenesis and species' range movements (Barraclough and Vogler, 2000) when the model runs under a balanced proportion of sympatric and allopatric diversification events, occasional large-scale shifts of species' ranges and a relatively high general frequency of range shifts (Fig. 3B in Barraclough and Vogler, 2000). The results of our BSM analysis indicated that the relative proportion of cladogenetic events associated with dispersal on the one hand and sympatry on the other (interpreted as allopatric and sympatric speciation, respectively) was virtually the same (Table 4) and therefore are well in line with this model of range movement and cladogenesis. Conclusions about the frequency of range shifts in the evolution of *Aeonium* can be drawn less easily in comparison with Barraclough and Vogler (2000) because these authors (1) based their simulations on relative instead of absolute time units, (2) did not take into account extinction and (3) modelled species' range shifts in a continental rather than insular framework. In a recently published BSM analysis of *Argyranthemum*, a genus endemic to the Canary Islands, Madeira and Selvagem Pequena, White et al. (2020) inferred, on average, 21.28 dispersal events (i.e., the sum of range expansion and vicariance counts in their Table 3) across their phylogeny of 40 *Argyranthemum* taxa with a root age of 2.5 myr. Their mean of 8.5 dispersal events per myr is slightly higher than our 48.88 dispersal events across a timespan of 7.76 myr, i.e., 6.3 dispersal events per myr. However, the biogeographical model with the best fit obtained by White et al. (2020) was

DIVALIKE and therefore did not take into account founder-event speciation, unlike the BAYAREALIKE+J model used in our study.

4.3. Secondary contact, co-occurrence and hybridization among *Aeonium* species

The observation that plant radiations in the Canary Islands, especially species-rich radiations, are mostly monophyletic, a pattern that is best explained by a single colonization event, has led to the theory that niche pre-emption by the earliest successful colonizers prevented the establishment of later colonizers closely related to a given radiation (Silvertown, 2004). This idea is consistent with the general dynamic model of island biogeography (Whittaker et al., 2008) and has been developed and supported by later studies (Carine et al., 2004; Silvertown et al., 2005). Previous studies have clearly shown that the Macaronesian clade of tribe Aeonieae, as well as *Aeonium*, are monophyletic lineages (Mes, 1995; Mort et al., 2002), implying that the Macaronesian Aeonieae, the most species-rich radiation native to the Canary Islands, too, are the product of a single colonization event of these islands and subsequent diversification. However, considering the biogeographical history within *Aeonium*, a more differentiated picture emerges with respect to the role of niche pre-emption in the evolution of *Aeonium*.

The results of our AAR analysis show that some of the islands were successfully colonized several times independently and hence after the arrival of other *Aeonium* species on the same islands. For example, the MRCA of clade Arboreum I (node 15 in Fig. 4; age estimate: 2.01 myr) represents the oldest lineage with a probability > 25% (i.e., 89.6%) of having colonized Gran Canaria, and Gran Canaria was later colonized by (1) *A. canariense* subsp. *virgineum* (Webb) Bañares (node 27), (2) *A. percarneum* (node 36), (3) *A. arboreum* s.str. (node 20), and (4) *A. aureum* (node 11). Timing of the arrival of the exceptionally widespread *A. spathulatum* on Gran Canaria is unknown, but very unlikely to have taken place prior to the colonization of Gran Canaria by the MRCA of clade Arboreum I (see Electr. Suppl. 3). In this example, the two species that diversified *in situ* early after the first colonization of Gran Canaria (i.e., *A. simsii* and *A. undulatum*) co-occur and hybridize frequently when growing in the same area (Bañares Baudet, 2015a). They considerably differ from each other in growth form (decumbent rosette plants in *A. simsii*, ramified erect shrubs in *A. undulatum*) and leaf morphological characters (leaves puberulent and with remarkably long cilia and tannic stripes in *A. simsii*, but glabrous, inconspicuously ciliate and without tannic stripes in *A. undulatum*, see Fig. 3). *Aeonium arboreum* s.str. grows in sympatry only with *A. simsii*, but all other species that arrived on Gran Canaria after its first colonization by the MRCA of clade Arboreum I live in sympatry with both *A. simsii* as well as *A. undulatum*. Only *A. spathulatum*, otherwise the most widely hybridizing *Aeonium* species, does not hybridize with *A. simsii* or *A. undulatum*. Given that *A. spathulatum* only hybridizes with taxa that have at least some diploid individuals (see Fig. 5 and Table 1), it seems likely that *A. spathulatum* might only be able to hybridize with other diploid plants. Despite this observation, different ploidy levels do not always appear to constitute an unbreachable reproductive barrier because, e.g., the diploid *A. canariense* subsp. *virgineum* hybridizes with *A. simsii* as well as *A. undulatum*, when co-occurring with them (Fig. 5). However, plants that appear to be hybrids in nature do not always have to be the offspring of the hypothesized parental species nor do they have to be hybrids at all (see the discussion whether *A. mascaense* is a hybrid species in Liu (1989) and Jorgensen and Frydenberg (1999)), and this should be further investigated for proposed hybrids in *Aeonium* by means of targeted crossing experiments. Another possible reproductive barrier between co-occurring species could be non-overlapping flowering times (McNeilly and Antonovics, 1968) and this has been repeatedly discussed for *Aeonium*, too (Jorgensen and Olesen, 2001; Esfeld et al., 2009). However, we only found two species pairs (i.e., *A. aureum* and *A. tabuliforme* on Tenerife and *A. escobarii* and *A. goochiae* on La Palma; data from Bañares Baudet, 2015a) with non-overlapping flowering periods among those co-occurring species pairs that do not produce natural hybrids (see boxes that are filled ochre in Fig. 5). Consequently, the remaining 72 co-occurring species pairs overlap in flowering time, which makes differentiation of flowering time seem unlikely to constitute a major barrier to gene flow in *Aeonium*.

Similar to the colonization of Gran Canaria, a number of secondary colonization events were also reconstructed for La Gomera. *Aeonium* has probably been present on this island for the past 3.96 myr (95% HPD = 1.44–7.05 myr), because the ancestral lineage of *A. saundersii* (node 6 in Fig. 4; Table 2) was simulated as an inhabitant of La Gomera in 46 out of 50 BSMs (Electr. Suppl. 3). After the arrival of *A. saundersii*, a comparatively rare species restricted to warm rock escarpments, La Gomera was reached independently by numerous lineages: (1) *A. sedifolium*, (2) the widespread *A. diplocyclum* and *A. spathulatum* (either they reached La Gomera independently or the MRCA of sects. *Chrysocome* and *Greenovia* was distributed on La Gomera), (3) *A. lindleyi* subsp. *viscatum*, (4) *A. arboreum* subsp. *holochrysum* var. *rubrolineatum* (Svent.) H.Y.Liu, (5) *A. canariense* subsp. *latifolium*, (6) *A. appendiculatum*, and (7) the clade of *A. gomerense*, *A. decorum* and *A. castello-paivae* (Fig. 7). Of these, lineages (1) and (2) may not have colonized La Gomera later than *A. saundersii* after all, because in 12 out of 50 BSMs La Gomera was reconstructed as ancestral area of the MRCA of the nectary-lacking clade (node 6; Electr. Suppl. 3) which comprises, among others, *A.*

saundersii, *A. sedifolium*, *A. diplocyclum* and *A. spathulatum*. Among the Gomeran species of the nectary-lacking clade, only *A. diplocyclum* and *A. saundersii* co-occur, and no natural hybrids between them are known (Fig. 5). Also, *A. sedifolium* does not co-occur with any other *Aeonium* species on La Gomera and only occupies a very small area there, possibly due to its recent migration to La Gomera. However, the time of arrival of *A. sedifolium* on La Gomera was not simulated concordantly across BSMs. Altogether, 49% of all possible pairings of species occurring on La Gomera co-occur and 18% of all possible pairings hybridize in nature. Both of these proportions are lower than on Gran Canaria where 67% of all possible species pairings co-occur and 43% of all possible pairings produce natural hybrids (see preceding paragraph). On the other hand, the highest proportions of co-occurrence and hybrid formation are found on La Palma where 83% of all possible taxon pairings co-occur and 44% hybridize, and on El Hierro where 80% co-occur and 40% hybridize.

In contrast to the four islands just discussed that received numerous *Aeonium* taxa after their first colonization by *Aeonium*, Tenerife, which acted most frequently as source area, was colonized secondarily only in two instances: (1) along the ancestral lineage of *A. arboreum* subsp. *holochrysum* s.str. (nodes 16–19), a subspecies that also colonized El Hierro and La Palma, and (2) by *A. decorum* which is widespread on La Gomera but locally restricted to the surroundings of Barranco de Masca in Tenerife (Electr. Suppl. 3). Other dispersal events from other islands to Tenerife were inconsistently simulated in the BSM analysis (Electr. Suppl. 3). With only these two well-supported secondary colonization events and an unknown number of less probable secondary colonization events, Tenerife only received 13% of all dispersal events among the western Canary Islands (see 3.3 above). In addition, Tenerife also has the lowest proportions of co-occurrence and hybrid formation among these islands with only 36 and 15%, respectively, of all possible species pairings. Together with our finding that Tenerife and La Gomera have the oldest record of continuous colonization by *Aeonium*, followed by Gran Canaria, La Palma and El Hierro, a clear pattern emerges: different taxa of *Aeonium* are more likely to co-occur and hybridize with one another on islands that were colonized by *Aeonium* more recently. The only outlier in this pattern is El Hierro which, as youngest of the Canary Islands, should have the highest proportions of co-occurrence and hybrid formation. However, El Hierro is surpassed by La Palma, possibly because relatively few taxa of *Aeonium* have (yet) arrived on El Hierro. Tenerife, on the other hand, where *Aeonium* most probably originated and where the highest number of sympatric diversification events was reconstructed (see 3.3 above), has the lowest proportion of co-occurrence and hybrid formation among *Aeonium* taxa indicating a high level of ecological differentiation and reproductive isolation between these taxa.

4.4. Morphological and ecological divergence of sister lineages

Reproductive isolation between diverging populations that may later become species can be attained in two different ways, namely ecologically via divergent/disruptive selection in different environmental conditions or geographically via the emergence of geographical barriers to dispersal (Gillespie et al., 2020). In case of an island radiation as dealt with here these two mechanisms of diversification can be equated with sympatric and allopatric diversification, respectively. Hence, sympatric diversification is expected to be linked to ecological divergence. Although allopatric diversification is initially independent of ecology, ecological divergence between lineages originating from allopatric diversification may occur, especially through interaction with other closely related species (Gillespie et al., 2020) that, for example, may have reached the same island earlier. Under the assumption of adaptive radiation as underlying mechanism in the evolution of *Aeonium*, (1) a high proportion of sympatric relative to allopatric diversification events and (2) stronger ecological divergence resulting from sympatric than from allopatric diversification can be expected. However, our BSM analysis revealed that sympatric and allopatric diversification occurred an essentially equal number of times (Table 4). This suggests a much more balanced role of ecological and geographical drivers of diversification in *Aeonium* than, e.g., in *Argyranthemum* where 71.2% of diversification events were recently shown to have occurred within single islands (White et al., 2020). However, *Aeonium* is a considerably older genus than *Argyranthemum* (stem age 7.76 versus 2.5 myr), and thus has had more opportunity for the repeated colonization of individual islands. Our comparison of ecological REDs between sister lineages derived from sympatric and allopatric diversification events (henceforth referred to as sympatric and allopatric sister lineages, respectively) indicates slightly higher ecological divergence in sympatric vs. allopatric diversification, but this difference was not significant (Fig. 8). This failure to detect a significant difference in ecological REDs may be due to the shortage and coarseness of the habitat-related data investigated in this study, namely altitude and categorical data for thermo- and ombrotype prevailing in the distribution ranges of species. However, this deficiency may soon be amended by an in-depth species distribution modelling analysis (dos Santos et al., in preparation). In contrast, morphological REDs between sympatric sister lineages were significantly higher than those between allopatric sister lineages (Fig. 8). Additionally, sympatric sister lineages were more frequently differentiated from each other in terms of their growth form (mean RED ± standard deviation: 0.35 ± 0.35) when compared to allopatric sister lineages (0.06 ± 0.17; see Table 6). Growth form, in turn, has been discussed as an important indicator of the occupied ecological niche in adaptive radiations of plants (e.g., Lowrey, 1995), and especially of *Aeonium* (Lösch, 1990; Mes and 't Hart, 1996; Jorgensen and Olesen, 2001; Mort et al., 2007).

In summary, we found clear signs of stronger divergence in terms of morphology and ecology among sympatric rather than allopatric sister lineages, and this may be the result of ecologically divergent selection in sympatric diversification. To demonstrate this further, the patterns of diversification and the resulting morphological and ecological divergence for well-supported clades of our phylogenies (as listed in Table 6) can be examined in more detail.

Species of sect. *Greenovia* are relatively uniform in the morphological characters investigated here (see Fig. 3 for characters discussed here). They are acaulescent rosette plants, but flower merism varies considerably among them, with up to 35-merous flowers in *A. aureum* but ± 20-merous flowers in the remaining species. *Aeonium diplocyclum*, the earliest-branching species of this section (node 9), is the only species absent from Tenerife, and the corresponding allopatric diversification is associated with only little ecological divergence (Table 6). *Aeonium diplocyclum* occupies a wide altitudinal range and largely overlaps with the other *Greenovia* species with respect to the climatic variables (Fig. 3). By contrast, the two sympatric diversification events in this clade (nodes 10 and 11) are associated with more extensive ecological divergence, because *A. dodrantale* occurs in slightly drier habitats at lower elevation, and *A. aizoon* occurs in cooler sites on mountain ridges or southeast-facing rock escarpments (Bañares Baudet, 2015a). In terms of morphology, *A. aureum* is distinguished from the other species by its high flower merism, as mentioned above, and completely glabrous leaves.

The allopatric diversification between *A. lindleyi* on Tenerife plus La Gomera and *A. goochiae* on La Palma is concomitant with the highest morphological divergence of allopatric sister lineages recorded by us (morphological RED = 1.2; Table 6). This is due to the striking difference in petal colour (reddish-white in *A. goochiae* and bright yellow in *A. lindleyi*) and slight differences in the range of flower merism (Fig. 3). Also, leaf succulence, a trait not investigated here, strongly differs between these two species, with distinctly flat and hardly succulent leaves in *A. goochiae* (Lösch, 1990). The ecological divergence between *A. goochiae* and *A. lindleyi* consits mainly in the preference of slightly cooler and more humid sites by *A. goochiae* which is also the reason why its altitudinal range extends into higher ranges typical of the lower transitional zone of the laurel forest.

With the exception of the basal split between *A. simsii* and the remainder of clade Arboreum I (node 15) that took place on Gran Canaria, all other supported diversification events in this clade (nodes 16, 17 and 20) were allopatric. This is linked to a strong morphological divergence between *A. simsii* (an acaulescent rosette plant with distinct foliar tannic stripes) and all other taxa of clade Arboreum I (branched nanophanerophytes without foliar tannic stripes). Ecological differentiation between *A. simsii* and the remaining taxa of clade Arboreum I is mainly expressed in the slightly higher altitudinal range of *A. simsii*, but *A. simsii* co-occurs with *A. undulatum* and *A. arboreum* s.str., other Gran Canarian species of clade Arboreum I (Fig. 5). All remaining diversification events in this clade were allopatric, because all taxa above *A. undulatum* occur on different islands (Fig. 3). Therefore, this clade is an example of a largely geographical radiation. It is noticeable that the taxa of this clade (without *A. simsii*) are comparatively uniform in morphology, some of them only differing from others in the pubescence of leaves and/or inflorescences (see characters o and q in Fig. 3). However, the ecological divergence between the East Canarian *A. balsamiferum* and *A. arboreum* s.l. (incl. *A. korneliuslemsii*) is the highest recorded in this clade, because *A. balsamiferum* is restricted to hotter and more arid habitats within a relatively narrow altitudinal range.

The initial diversification event of the Canariensia clade that was prompted by the colonization of Madeira (node 22) was associated with the highest morphological and ecological divergence (1.0 and 0.9, respectively) among allopatric diversification events in this clade. This was brought about mainly by the shrubby growth form of *A. glutinosum* and slightly higher flower merism in *A. glandulosum*, the latter species sharing the acaulescent habit with the sister lineage sect. *Canariensia* (Fig. 3). Therefore, the sympatric diversification between *A. glutinosum* and *A. glandulosum* (node 23) on Madeira was associated with a shift in growth form, but also in leaf pubescence and leaf viscidity (the latter character not assessed here but nevertheless remarkable). Also, *A. glandulosum* is a strictly biennial species (Lösch, 1990) restricted to (very) humid habitats along the northern coast of Madeira, somewhat similar to *A. tabuliforme* (Liu, 1989) which is endemic to Tenerife. Two more diversification events in this clade were sympatric, having taken place on Tenerife, namely the split between *A. cuneatum* and the remainder of sect. *Canariensia* (node 24) and the split between *A. tabuliforme* + *A. canariense* subsp. *latifolium* and the other subspecies of *A. canariense* (node 25). The former diversification event was associated with the highest morphological divergence recorded here (i.e., 1.86; see Table 6), because *A. cuneatum* is very different from *A. canariense* s.l. (incl. *A. tabuliforme*) by having ciliate, glabrous and distinctly glaucous leaves and a slightly different petal colour. It is much more narrowly distributed than *A. canariense* s.l. and restricted to humid and either terrestrial or epiphytic sites in the laurel forest. By contrast, node 25 is associated with the lowest morphological and ecological divergence (0.0 and 0.4, respectively) among all sympatric diversification events. The sister lineages belonging to this node do not differ in any of the morphological characters considered by us. The two Teneriffan taxa of this clade, namely *A. tabuliforme* and *A. canariense* s.str. co-occur, but the former occupies a narrower distribution area and ecological space (Fig. 3; Bañares Baudet, 2015a). Similar to the *A. balsamiferum* / *A. arboreum* s.l. subclade of clade Arboreum I (see above), *A. canariense* s.l. is strikingly uniform in morphology and altitudinal range, and its (sub)species only differ slightly in preferred thermo- and ombrotype (Fig. 3) resulting in low morphological and ecological REDs for the corresponding allopatric sister lineages (Table 6).

The sympatric diversification of *A. decorum* and *A. castello-paivae* on La Gomera (node 33) resulted in a strong morphological divergence of the two species, not only affecting the morphological characters investigated here (branches puberulent below the leaf rosettes in *A. castello-paivae*, branches with prominent ridges along the edges of leaf scars in *A. decorum*), but also in flower morphology, with *A. castello-paivae* having distinctly recurved petals in large flowers (Bañares Baudet, 2015a) that produce a high amount of nectar (Stefan Abrahamczyk, unpublished results) and *A. decorum* having smaller flowers and lower nectar volumes. Although they co-occur (Fig. 5), *A. decorum* prefers warmer and more arid habitats in comparison to *A. castello-paivae* (Fig. 3).

Lastly, morphological and ecological divergence resulting from the purely sympatric diversification events in the Teneriffan subclade of sect. *Leuconium* (i.e., *A. ciliatum* through *A. haworthii* in Figs. 2, 3 and 7, and *A. mascaense* through *A. haworthii* in Fig. 4) were more difficult to analyse because relationships in this subclade were not consistently recovered and largely unsupported in our phylogenetic analyses. However, the consistently sympatric diversification within this subclade allowed for averaging the morphological and ecological REDs calculated for the respective individual nodes across phylogenies. The resulting mean values of morphological (i.e., 1.48 ± 0.26) and ecological RED (i.e., 0.92 ± 0.27) were above average when compared to other sympatric diversification events (Table 6; see also paragraph 3.4 above). The reason for the high morphological divergence in this subclade is the heterogeneity in growth form (with branched shrubs or dwarf shrubs in *A. ciliatum*, *A. volkeri* E.Hern. & Bañares and *A. mascaense*, and unbranched monocarpic nanophanerophytes in *A. urbicum* s.l.), bark ornamentation on stems and branches, and pubescence of leaves and inflorescences (Fig. 3). Also, the ecological space occupied differs slightly between the taxa of this subclade as *A. ciliatum* grows in more humid regions, and the distribution of *A. urbicum* subsp. *meridionale* extends into cooler ranges of higher altitude. However, the remaining taxa are ecologically more or less similar but in part separated geographically, such that *A. volkeri* is endemic to the Anaga massif while *A. haworthii* and *A. mascaense* (the latter extinct in the wild; Brilhante et al., 2021) are limited to the Teno massif. Only *A. urbicum* s.str. occupies a larger distribution area bridging the Teno and Anaga massifs (Bañares Baudet, 2015a).

In summary of the above, the high morphological and ecological diversity of taxa that diversified sympatrically, such as the Teneriffan and Gomeran subclades of sect. *Leuconium*, is strikingly different from that seen in clades that diversified in different geographical areas such as *A. canariense* s.l. incl. *A. tabuliforme* and the *A. balsamiferum* / *A. arboreum* s.l. subclade of clade Arboreum I. One could argue that these clades are not equal in age and accordingly not comparable. Indeed, the clade of *A. canariense* s.l. incl. *A. tabuliforme* is distinctly older (1.57 myr; 95% HPD = 0.57–2.85 myr) than the *A. balsamiferum* / *A. arboreum* s.l. subclade (0.6 myr; 95% HPD = 0.19–1.13 myr) and the two subclades of sect. *Leuconium* (95% HPD of both subclades taken together: 0.24–1.94 myr; Table 2). However, the clade of *A. canariense* s.l. is also a morphologically and ecologically more or less uniform clade, although its older age would have allowed for more time to produce a similar degree of diversity as in the sympatrically diversified subclades of sect. *Leuconium*. This again indicates that sympatric diversification within single islands is more strongly associated with morphological and ecological diversification and therefore has a greater likeness to an adaptive radiation than has geographical diversification by repeated migration to different islands.

We conclude that the diversification of *Aeonium* has both adaptive and non-adaptive components. Evidence for adaptive processes in the evolution of *Aeonium* is to be found in the high diversity of ecological roles among the species of *Aeonium*. Most notably, the different growth forms (Mes and 't Hart, 1996; Mort et al., 2007) as well as the variable expression patterns of predominantly facultative Crassulacean Acid Metabolism (CAM) photosynthesis (Lösch, 1990; Pilon-Smits et al., 1992) found in *Aeonium* species likely constitute the attendant adaptations necessary to fulfil these ecological roles. Furthermore, we presented evidence for numerous diversification events that happened within single islands (Fig. 7), and the high potential for co-existence of different *Aeonium* taxa indicates niche differentiation to such a degree that these taxa do not outcompete each other. On the other hand, the relationship of node age and degree of sympatry between the lineages defined by each node (Fig. 6) matches a biogeographical model that assumes a balanced proportion of sympatric and allopatric diversification (Barraclough and Vogler, 2000), as was also evidenced by the results of our BSM analysis. Also, there are several examples of allopatric diversification events corresponding to largely geographical radiations in the *Aeonium* phylogeny, e.g., in clade Arboreum I and the Canariensia clade (see above), in which only little morphological and ecological diversity has developed. Therefore, allopatric diversification as an initially non-adaptive process has played a more or less equally important role as sympatric diversification, and such a varied pattern is to be expected in an island radiation with a certain potential for dispersal by a high number of small seeds.

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Supplementary material:

Appendix. Detailed information about specimens used in this study.

Species; lab code (only for accessions with sequences generated by the author) or GenBank accession number for ITS (only for accessions that were not generated by the author); material used for extraction; country; locality and additional information; date of collection; voucher (+ herbarium code or Botanical Garden). "–" indicates missing data or missing information. "*" after the lab code indicates that the sample was only used for the Crassulaceae-wide dating analysis of ITS sequences.

Outgroup: ***Aichryson bituminosum*** Bañares; A02_J03*; whole dried leaves; Spain; Gran Canaria, Roque del Saucillo, 1640 m; 06.03.2018; *J. Los A02_J03*. ***A. divaricatum*** (Aiton) Praeger; –*; whole dried leaves; Portugal; Madeira, entre Pico do Gato y Macizo de Pico das Torres, cabecera de Riveira Fajã da Nogueira, cara este, laderas umbrosas en matorral denso con brezos y retamas, 1590–1625 m; 24.07.2006; *Á. Marrero* (LPA-029204). ***A. tortuosum*** **subsp.** ***bethencourtianum*** (Bolle) Bañares; A37_J50*; whole dried leaves; Spain; Fuerteventura, Montana de la Muda; –; *J. Los A37_J50*. ***Monanthes brachycaulos*** (Webb & Berthel.) Lowe; NB73.1; whole dried leaves; Spain; Gran Canaria, Artanara, 851 m; 06.03.2018; *N. Bobon M02_N2*. ***M. minima*** (Bolle) Christ; M04_N5*; whole dried leaves; Spain; Tenerife, Igueste San Andres, 36 m; 13.03.2018; *N. Bobon M04_N5*. ***M. muralis*** (Webb ex Bolle) Hook.f.; NB111; whole dried leaves; Spain; La Palma, Monte de Luna, 730 m; 09.04.2018; *N. Bobon M37_N45* (MJG-030605). ***M. wildpretii*** Bañares & S.Scholz; M32_N40*; whole dried leaves; Spain; Tenerife, Chinamada, 711 m; 06.04.2018; *N. Bobon M32_N40*. ***Aeonium*** **Webb & Berthel.:** ***Aeonium aizoon*** (Bolle) T.Mes; TM201.1; flash-frozen leaves; Spain; Tenerife, cliff at the walking path between Barranco de Chacorche and Barranco de Chese, Pinar tinerfeño, E-facing slope, growing together with *Hypericum* *reflexum* and *Monanthes brachycaulos*; 09.05.2019; *P. dos Santos, T. Messerschmid & M. Brilhante 2019-21* (MJG-028144). ***A. appendiculatum*** Bañares; TM246.2; flash-frozen leaves; Spain; La Gomera, near Alajeró, ca. 850 m; 09.2020; *Á. Bañares* 116 (TFC-53764). ***A. arboreum* ssp. *holochrysum*** (H.Y.Liu) Bañares s.str.; TM152.1; whole dried leaves; Spain; Tenerife, above road TF-38 and Restaurante Boca Tauce, ca. 1.5 km N of the village Chirche, Lava flows on a WSW-facing slope, growing together with *Pinus canariensis*, *Kleinia neriifolia*, euphorbs, *Aeonium spathulatum* and *A. urbicum* var. *meridionale*; 06.05.2019; *P. dos Santos, T. Messerschmid & M. Brilhante 2019-12* (MJG-028169). ***A. arboreum* subsp. *holochrysum* var. *rubrolineatum*** (Svent.) H.Y.Liu; TM211.1; flash-frozen leaves; Spain; La Gomera, near Roque Agando, 1000 m; 08.2013; *Á. Bañares 104* (TFC-53757). ***A. arboreum*** Webb & Berthel. s.str.; TM153.1; whole dried leaves; Spain; Gran Canaria, at the road GC-704 from San Fernando to Lomo del Peñón; 01.05.2019; *P. dos Santos & M. Brilhante 37*. ***A. aureum*** (C.Sm. ex Hornem.) T.Mes; UM2.1; dried flower buds; Spain; Tenerife, Barranco above the road TF-21 from Las Cañadas del Teide to Aguamansa, growing together with *Pterocephalus lasiospermus*, *Silene vulgaris*, *Carlina salicifolia*, *Spartocytisus supranubius*, *Pinus canariensis* and *Aeonium canariense* s.str.; 09.05.2019; *P. dos Santos, T. Messerschmid & M. Brilhante 2019-18* (MJG-028137). ***A. balsamiferum*** Webb & Berthel.; TM178.1; flash-frozen leaves; –; Garden origin; *S. Scholz* (MJG-030599). ***A. canariense*** (L.) Webb & Berthel. s.str.; TM169.1; whole dried leaves; Spain; Tenerife, walking path from Las Bodegas into the laurel forest; 05.05.2019; *P. dos Santos, T. Messerschmid & M. Brilhante 2019-06* (MJG-028140). ***A. canariense* subsp. *christii*** (Burchard) Bañares; TM189.1; flash-frozen leaves; Spain; La Palma, Barranco de las Angústias; 12.04.2019; *P. dos Santos & M. Brilhante 27*. ***A. canariense* subsp. *latifolium*** (Burchard) Bañares; TM133.1; dried flower buds; Spain; La Gomera, walking path between Roque de Agando and La Laja, ca. 900 m W of La Laja; 13.05.2019; *P. dos Santos, T. Messerschmid & M. Brilhante 2019-22* (MJG-028166). ***A. canariense* subsp. *virgineum*** (Webb) Bañares; TM165.1; flash-frozen leaves; Spain; Gran Canaria, Barranco de los Tilos, ca. 1.2 km NE of Lomo del Peñón; 30.04.2019; *P. dos Santos & M. Brilhante s.n.* (MJG-028177). ***A. castello-paivae*** Bolle; TM137.1; dried flower buds; Spain; La Gomera, above the road GM-1 from Las Rosas to Agulo, ca. 700 m NW of Agulo; 15.05.2019; *P. dos Santos, T. Messerschmid & M. Brilhante 2019-30* (MJG-028146). ***A. ciliatum*** Webb & Berthel.; TM135.2; whole dried leaves; Spain; Tenerife, walking path from Las Bodegas into the laurel forest; 05.05.2019; *P. dos Santos, T. Messerschmid & M. Brilhante 2019-08* (MJG-028175, MJG-030006). ***A. cuneatum*** Webb & Berthel.; TM134.1; dried flower buds; Spain; Tenerife, El Bailadero, above the road TF-123 from El Bailadero to Las Bodegas, laurel forest, growing together with *Erica arborea*, *Canarina canariensis*, *Phyllis nobla* and figworts; 05.05.2019; *P. dos Santos, T. Messerschmid & M. Brilhante 2019-10* (MJG-028154). ***A. cuneatum*** Webb & Berthel.; FJ11*; whole dried leaves; Spain; Tenerife, Sierra de Anaga; –; –. ***A. davidbramwellii*** H.Y.Liu; TM202.1; flash-frozen leaves; Spain; La Palma, Barranco de las Angústias; 15.04.2019; *P. dos Santos & M. Brilhante 30* (MJG-028176). ***A. davidbramwellii*** H.Y.Liu; AY082129*; –; Spain; La Palma; –; *Mort 1458* (WS). ***A. decorum*** Webb ex Bolle s.str.; UM3.1; dried flower buds; Spain; La Gomera, ca. 400 m WNW of La Laja; 13.05.2019; *P. dos Santos, T. Messerschmid & M. Brilhante 2019-24* (MJG-028181). ***A. diplocyclum*** (Webb ex Bolle) T.Mes; TM195.1; flash-frozen leaves; Spain; La Gomera, walking path between Roque de Agando and La Laja, ca. 900 m W of La Laja; 13.05.2019; *P. dos Santos, T. Messerschmid & M. Brilhante 2019-23* (MJG-028155). ***A. dodrantale*** (Willd.) T.Mes; TM196.1; flash-frozen leaves; Spain; Tenerife, slope close to the road TF-436, ca. 600 m WNW of Santiago del Teide; 08.05.2019; *P. dos Santos, T. Messerschmid & M. Brilhante 2019-17* (MJG-028182). ***A. escobarii*** Rebmann & Malkm.-Huss.; TM209.1; flash-frozen leaves; Spain; La Palma, near Las Mimbreras, ca. 650 m; 05.2017; *Á. Bañares 115* (TFC-53758). ***A.***

glandulosum Webb & Berthel.; TM224.2; flash-frozen leaves; Portugal; Madeira, escarpment near Continente supermarket, Ribeira Brava; spring 2020; *M. Benedito s.n.* (MJG-030876). ***A. glutinosum*** (Aiton) Webb & Berthel.; FJ21; whole dried leaves; Portugal; Madeira; –; *R. Lösch s.n.* (MJG-030600). ***A. gomerense*** (Praeger) Praeger; TM155.1; whole dried leaves; Spain; La Gomera, at the road CV-14 from Las Poyatas to Benchijigua; 20.05.2019; *P. dos Santos & M. Brilhante s.n.* ***A. goochiae*** Webb & Berthel.; TM184.1; flash-frozen leaves; Spain; La Palma, along the new road between the access road to Los Franceses and Barlovento, above the road, 420 m; 21.04.1993; *U. Eggli & R. Nyffeler 2340* (ZSS-013038). ***A. gorgoneum*** J.A.Schmidt; TM185.1; flash-frozen leaves; Cape Verde; Santo Antão; –; *R. Lösch s.n.* (MJG-030602). ***A. haworthii*** Webb & Berthel.; FJ05; whole dried leaves; Spain; Tenerife, Tierra de Trigo, 550 m; spring 2005; *K. Esfeld s.n.* (MJG-030601). ***A. hierrense*** (Murray) J.Pitard & L.Proust; TM210.1; flash-frozen leaf epidermal shavings; Spain; El Hierro, near Tiñor, ca. 900 m; 12.2019; *Á. Bañares 67* (TFC-53760). ***A. korneliuslemsii*** H.Y.Liu; FJ01; whole dried leaves; Morocco; Anti-Atlas, Kerdous massif, between Tanout, Igui Ifred and Agni, "Djbel Imzi", on sandstone and siltstone in crevices, moderate amount of humus, probably nutrient rich (and probably containing lime), ca. 600 m; 02.04.2005; *Erpenbach & Levejohann s.n.* (MJG-030604). ***A. lancerottense*** (Praeger) Praeger; TM197.1; flash-frozen leaves; Spain; Lanzarote, Famara massif, southwesternmost foothill to the bungalow colony, basalt rocks, ca. 200 m; 08.04.1990; *E. Royl Ro 384* (MJG-030603). ***A. leucoblepharum*** Webb ex A.Rich.; TM157.2; whole dried leaves; Somalia; Puntland, Bari Region, Boosaaso Distr., hills above Galgala; 1969 or 1971; Lavranos 7304 or 9009 (MJG-030608). ***A. leucoblepharum*** Webb ex A.Rich.; TM238.1; flash-frozen leaves; Yemen; Sumara pass, gorge leading up to Himyasitik fort, 2400 m; 27.09.1977; *Lavranos & Newton 15996* (ZSS-4877). ***A. lindleyi*** Webb & Berthel. s.str.; TM190.1; flash-frozen leaves; Spain; Tenerife, Anaga; 04.04.2019; *P. dos Santos & M. Brilhante 15* (MJG-028148). ***A. lindleyi* subsp. *viscatum*** (Bolle) Bañares; AY082154*; –; Spain; La Gomera; –; *Mort 1432* (WS). ***A. lindleyi* subsp. *viscatum*** (Bolle) Bañares; TM198.1; whole dried leaves; Spain; San Sebastián de La Gomera, Valle San Sebastián, above Barranco Seco, near route from San Sebastián to Tunél de la Cumbre (GM-1); 02.12.1985; *W. Rauh 66936*. ***A. mascaense*** Bramwell; TM207.1; flash-frozen leaves; –; Garden origin; –; *Á. Bañares 79* (TFC-53761). ***A. nobile*** (Praeger) Praeger; TM191.1; flash-frozen leaves; Spain; La Palma, Mirador de El Time, above the road LP-1 to Argual; 13.04.2019; *P. dos Santos & M. Brilhante 28*. ***A. percarneum*** (Murray) J.Pitard & L.Proust; TM192.1; flash-frozen leaves; Spain; Gran Canaria, at the road GC-606 from Carrizal de Tejeda to El Toscón; 26.04.2019; *P. dos Santos & M. Brilhante 34* (MJG-028171). ***A. percarneum*** (Murray) J.Pitard & L.Proust; AY082146*; –; Spain; Gran Canaria; –; *Mort 1400* (WS). ***A. pseudurbicum*** Bañares; TM160.1; dried flower buds; Spain; Tenerife, plateau above road TF-436, close to the parking lot of the village Masca; 08.05.2019; *P. dos Santos & T. Messerschmid 2019-15* (MJG-028172). ***A. saundersii*** Bolle; TM188.1; flash-frozen leaves; Spain; La Gomera; –; (MJG-030606). ***A. sedifolium*** (Webb ex Bolle) J.Pitard & L.Proust; TM187.1; flash-frozen leaves; Spain; Tenerife, Guía de Isora, S of Morro de los Cerrillos, Parque Nacional del Teide, 2000 m; 03.04.1977; *W. Rauh KaI/23* (MJG-030607). ***A. sedifolium*** (Webb ex Bolle) J.Pitard & L.Proust; TM242.1; flash-frozen leaves; Spain; Tenerife, plateau above road TF-436, close to the parking lot of the village Masca; 08.05.2019; *P. dos Santos & T. Messerschmid 2019-16* (MJG-028162). ***A. simsii*** (Sweet) Stearn; TM193.1; flash-frozen leaves; Spain; Gran Canaria, Barranco del Palmar, just above the road GC-216; 26.04.2019; *P. dos Santos & M. Brilhante s.n.* (MJG-028183). ***A. smithii*** Webb & Berthel.; TM199.1; flash-frozen leaves; Spain; Tenerife, cliff next to the walking path between Barranco de Chacorche and Barranco de Chese; 09.05.2019; *P. dos Santos, T. Messerschmid & M. Brilhante 2019-20* (MJG-028174). ***A. spathulatum*** (Hornem.) Praeger; UM1.1; dried flower buds; Spain; Tenerife, above the road TF-21 from Las

Cañadas del Teide to Aguamansa, ca. 3.5 km NE from Las Cañadas del Teide; 09.05.2019; *P. dos Santos, T. Messerschmid & M. Brilhante 2019-19* (MJG-028143). ***A. stuessyi*** H.Y.Liu; TM203.1; flash-frozen leaves; Ethiopia; Lalibela, 2800-3000 m; 02.03.2019; *E. Fischer*. ***A. tabuliforme*** Webb & Berthel.; TM212.1; flash-frozen leaf epidermal shavings; Spain; Tenerife, above Los Carrizales, ca. 650 m; 01.2020; *Á. Bañares 128* (TFC-53762). ***A. undulatum*** Webb & Berthel.; TM95.1; whole dried leaves; Spain; Gran Canaria, Tejeda, 900 m; 24.04.1985; *W. Rauh 66886* (MJG-030610). ***A. urbicum*** (C.Sm. ex Hornem.) Webb & Berthel. s.str.; TM131.1; dried flower buds; Spain; Tenerife, ca. 1 km S of the village Teno Alto; 03.05.2019; *P. dos Santos, T. Messerschmid & M. Brilhante 2019-04* (MJG-028157, MJG-028170). ***A. urbicum* subsp. *meridionale*** Bañares; TM200.1; flash-frozen leaves; Spain; Tenerife, above road TF-38 and Restaurante Boca Tauce, ca. 1.5 km N of the village Chirche; 06.05.2019; *P. dos Santos, T. Messerschmid & M. Brilhante 2019-13* (MJG-028173). ***A. valverdense*** (Praeger) Praeger; TM213.1; flash-frozen leaf epidermal shavings; Spain; El Hierro, below Valverde, ca. 450 m; 12.2017; *Á. Bañares 32* (TFC-53763). ***A. volkeri*** E.Hern. & Bañares; TM194.1; flash-frozen leaves; Spain; Tenerife, Anaga, ca. 1.2 km N of Valle Crispín; 29.03.2019; *P. dos Santos & M. Brilhante 22* (MJG-028139).

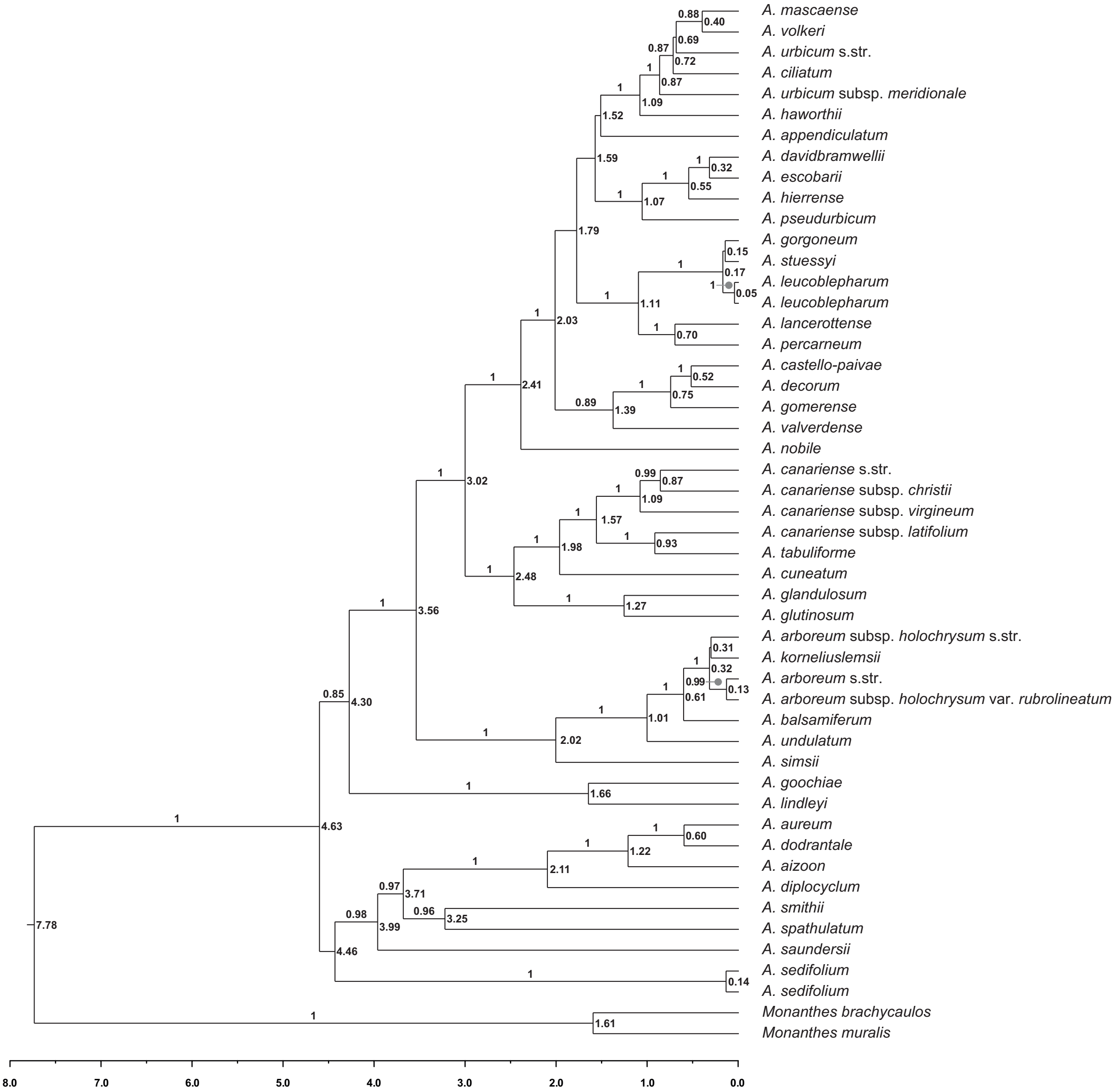
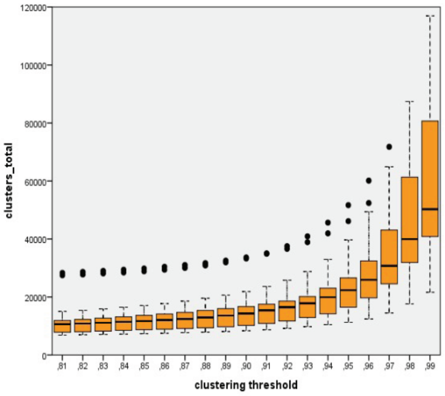
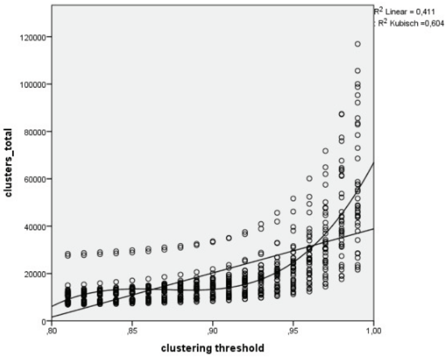
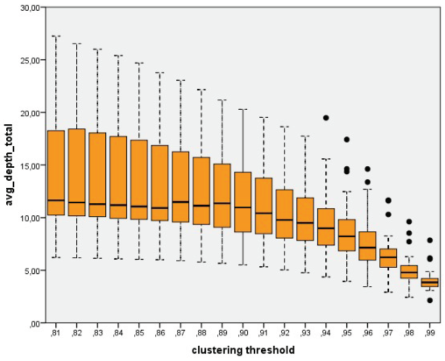


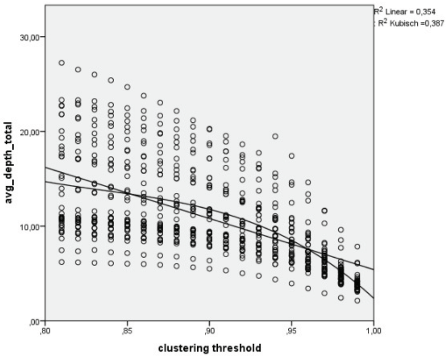
Fig. S1. Chronogram of *Aeonium* inferred using BEAST, based on the sequence dataset of those 357 loci in the 320–500 nt length range that featured sequence information for at least twenty different samples (referred to as min20tax dataset in the text). The topology inferred from the 320–500/ASTRAL analysis (see Fig. 3) was used as starting tree for this BEAST analysis. Only posterior probabilities ≥ 0.85 are shown above branches. Mean age estimates are given at each node. The scale shows time in million years before present.

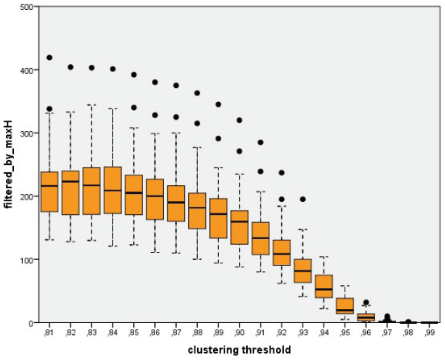
ISC diploids

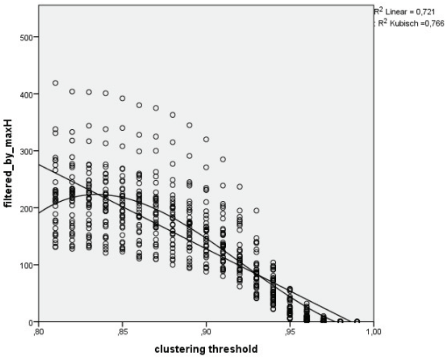


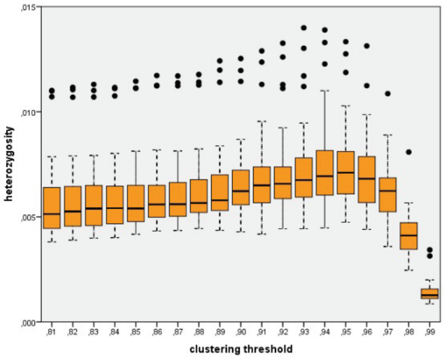


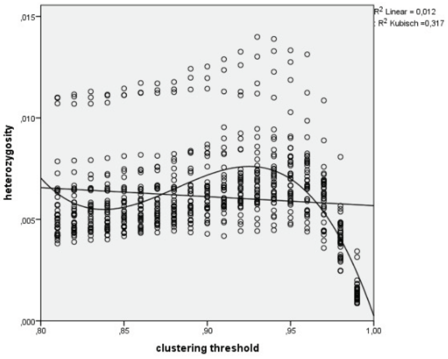












ISC tetraploids

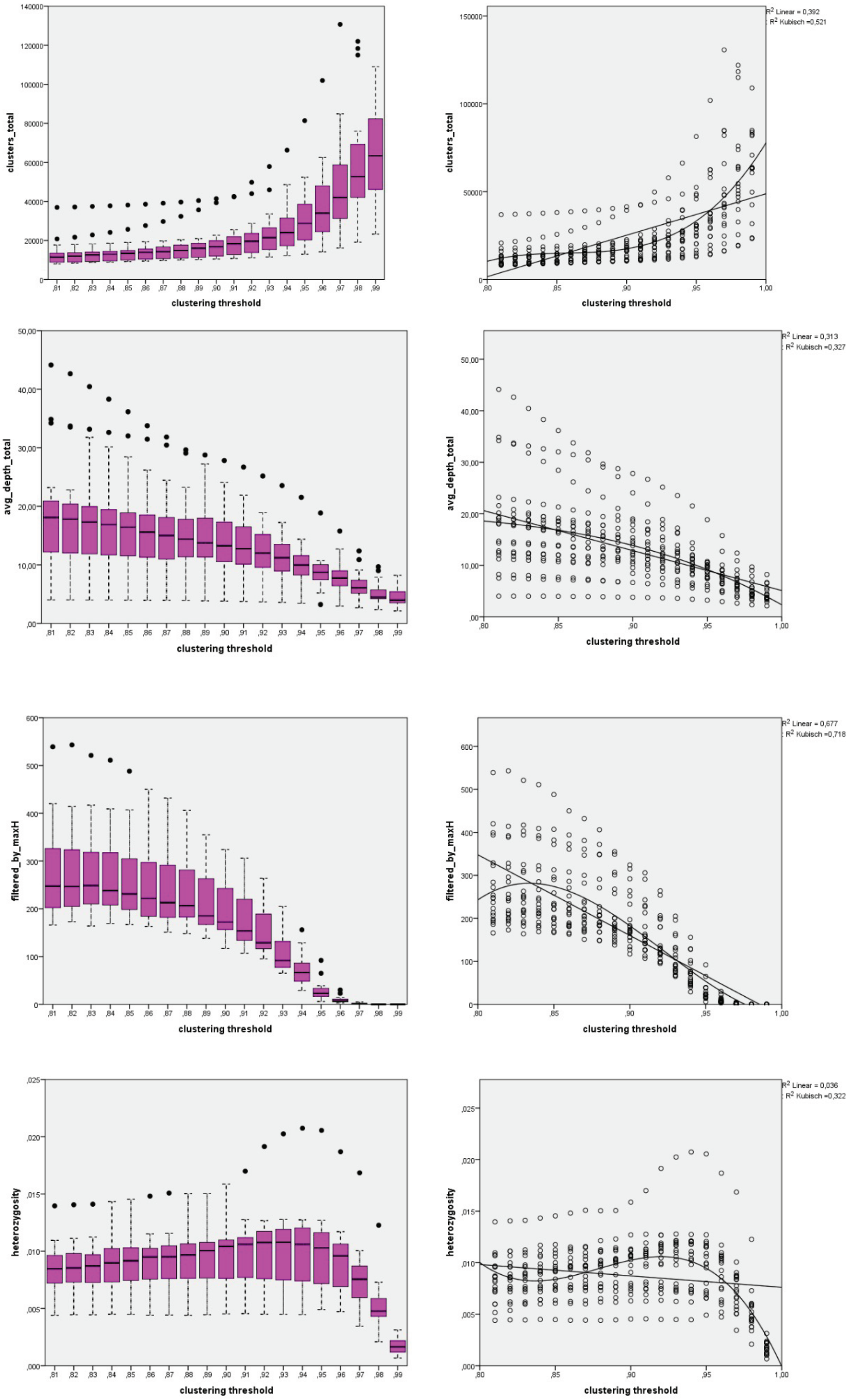


Fig. S2. Box- and scatterplots of assembly metrics obtained from *ipyrad* over the course of in-sample clustering for different clustering thresholds (CT 0.81–0.99 were tested in intervals of 0.01). See paragraph 2.4 for more information on the method and paragraph 3.1 for an interpretation of the plots.

Fig. S3. Maximum Likelihood (ML) phylogeny of *Aeonium*, including *A. lindleyi* subsp. *viscatum*, inferred from a concatenated supermatrix of all assembled loci. Numbers above branches indicate bootstrap values (only shown if ≥ 75). The branches arising directly from the root were shortened (branch length = 0.017) for the purpose of better visibility.

Electronic supplement (see attached CD):

Electr. Suppl. 1. Multiple FastQC (MultiQC) reports summarizing sequence counts, sequence quality and other statistics for all samples included in our ddRADseq laboratory analysis.

Electr. Suppl. 2. Properties of all 4,280 loci of our final assembly that was used for the all/RAxML and all/ASTRAL analyses. Properties correspond (from left to right) to ID number, locus length (nt), numbers of covered samples, single nucleotide polymorphisms (SNPs), parsimony informative sites (PIS) and variable sites (VAR), locus variability and percentage of missing samples for each locus. The diagrams at the top show the number of samples relative to the length of each locus (scatter diagram) and the locus length distribution across the whole assembly.

Electr. Suppl. 3. Fifty simulations of range shifts and migration over the dated *Aeonium* phylogeny (see Fig. 4) obtained from our Biogeographical Stochastic Mapping analysis. A: El Hierro, B: La Palma, C: La Gomera, D: Tenerife, E: Gran Canaria, F: Fuerteventura, G: Lanzarote, H: Morocco, I: East Africa, J: Madeira, K: Cape Verde.

Electr. Suppl. 4. Assembly metrics and corresponding diagrams for between-sample clustering using *ipyrad* with different clustering thresholds (CT 0.81–0.99 were tested in intervals of 0.01). See paragraph 2.4 for more information on the method and paragraph 3.1 for an interpretation of the plots.

Electr. Suppl. 5. Counts of sympatric diversification events over the 50 simulations of our Biogeographical Stochastic Mapping analysis (see Electr. Suppl. 3). Counts are ordered in columns by the geographical areas in which the sympatric diversification events occurred. The penultimate and last row of the table indicate mean number and standard deviation, respectively, of sympatric diversification events averaged over the 50 simulations. B: La Palma, C: La Gomera, D: Tenerife, E: Gran Canaria, F: Fuerteventura, G: Lanzarote, H:Morocco, I: East Africa, J: Madeira, K: Cape Verde.

3. Phylogeny of the *Sedum* species of tribe Aeonieae (Crassulaceae), closest relatives to the Macaronesian *Aeonium* alliance, and a tentative proposal for a new generic classification of tribe Aeonieae

Abstract

Molecular phylogenies of Crassulaceae subf. Sempervivoideae have identified five main clades of which three clades, i.e., Petrosedum clade, Aeonium clade and tribe Sedeae, contain different species of *Sedum*, thereby rendering *Sedum*, the largest genus of Crassulaceae, non-monophyletic. If *Sedum* were to be expanded to encompass all genera of the well-supported tribe Sedeae, a possible solution of this '*Sedum* problem' proposed in the most recent phylogenetic study of subf. Sempervivoideae (Chapter 1), little change to the morphological descriptions of *Sedum* found in the literature would be needed, but it would necessitate a transfer of the *Sedum* species of the Petrosedum and Aeonium clades into segregate genera. We here address this problem for the Aeonium clade for which previous studies have shown that the Ethiopian *Hypagophytum abyssinicum* and species of *Sedum* sers. *Caerulea*, *Monanthoidea* and *Pubescens* form a basal grade of successive sister lineages to the well-supported Macaronesian *Aeonium* alliance, the most species-rich plant radiation of the Macaronesian floristic region. So far, only five of the altogether eight species of the above-mentioned three series of *Sedum* had been sampled in previous phylogenetic studies. We present the first phylogenies of the Aeonium clade with a full sample of the species of *Sedum* sers. *Caerulea*, *Monanthoidea* and *Pubescens* which are distributed in Northwest Africa, including the Macaronesian Enclave in Morocco, and on some Western Mediterranean islands. Molecular phylogenies were inferred from sequence data of two nuclear (ITS and ETS) and four plastid markers (*matK*, *rps16*, *trnL-trnF* and *psbA-trnH*) in order to investigate (1) whether inclusion of the three relevant *Sedum* species that had not been sampled before (i.e., *S. gattefossei*, *S. maurum* and *S. versicolor*) into their respective infrageneric taxa is justified by their relationships to the other *Sedum* species and (2) which *Sedum* species or clade of *Sedum* species is closest relative to the Macaronesian *Aeonium* alliance. Our results suggest that *S. gattefossei* and *S. versicolor* are most closely related to the Macaronesian *Aeonium* alliance, but they fail to resolve whether these are sister to the Macaronesian *Aeonium* alliance or instead nested within this clade. A close relationship between *S. gattefossei* and *S. versicolor* would be supported by concordant morphological characters (similarity in habit and an absence of a leaf indumentum), but *S. gattefossei* shares a common chromosome number with *Aichryson* of the Macaronesian *Aeonium* alliance, while chromosome numbers are unknown for *S. versicolor*. We further identified three well-supported clades that exclusively comprise *Sedum* species, i.e., (1) *S. pubescens* plus *S. maurum*, (2) *S. caeruleum* and (3) *S.* ser. *Monanthoidea*, that could be segregated from *Sedum* and are distinguished from each other by a combination of morphological character states.

Keywords

Aeonieae, biogeography, classification, Macaronesian floristic region, molecular phylogenetics

1. Introduction

Phylogenetic studies of Crassulaceae subf. Sempervivoideae have led to the recognition of five major clades within the subfamily, the Telephium clade, Petrosedum clade, *Sempervivum*/*Jovibarba*, Aeonium clade and tribe Sedeae, of which the last can be further subdivided in two well-supported clades, i.e., the Leucosedum and Acre clades (Van Ham and 't Hart, 1998; Mort et al., 2001; Messerschmid et al., 2020). Except for the Telephium clade and *Sempervivum*/*Jovibarba*, all these clades contain, among representatives of other genera, species currently classified in *Sedum* L., the most species-rich genus of Crassulaceae (Messerschmid et al., 2020). The polyphyly of *Sedum* resulting from these diverse phylogenetic relationships of its species has been termed the '*Sedum* problem' (Mort et al., 2010) and has been causing difficulties in the circumscription and classification of a monophyletic *Sedum* (Thiede and Eggli, 2007; Nikulin et al., 2016; Messerschmid et al., 2020). Recently, Messerschmid et al. (2020) proposed to expand *Sedum* to include all genera currently included in tribe Sedeae. Regardless of whether this proposal will be followed or not, the phylogenetic position of the type species of *Sedum*, *S. acre* L., in the Acre clade will make it necessary that *Sedum* needs to comprise at least part of the taxa of the Acre clade. Therefore, unless *Sedum* should be expanded even beyond tribe Sedeae, the *Sedum* species of the Aeonium and Petrosedum clades need to be segregated from *Sedum* if a generic classification of subf. Sempervivoideae on the basis of monophyletic lineages (or holophyletic lineages in the definition by Stuessy and Hörandl, 2014) is desired.

The Aeonium clade is probably best known for the Macaronesian *Aeonium* alliance, a lineage of three genera (i.e., *Aeonium* Webb & Berthel., *Aichryson* Webb & Berthel. and *Monanthes* Haw.) that is the most species-rich plant radiation of the Macaronesian floristic region (Jorgensen and Olesen, 2001). Some species of *Aeonium*, such as the Moroccan *A. korneliuslemsii* H.Y.Liu and the East African *A. leucoblepharum* Webb ex A.Rich., have long been known to be native to continental Africa (Richard, 1847; Berger, 1930; Praeger, 1932). The possible occurrence of *Aichryson* and *Monanthes* in Africa has been a matter of debate because of the controversial classification of two Moroccan species currently classified in *Sedum*, i.e., *S. gattefossei* Batt. & Jahand. and *S. surculosum* Coss. While *S. gattefossei* was originally described as a new species of *Sedum* (Battandier and Jahandiez, 1921), along with the explicit remark of its resemblance to *Aichryson* in overall habit but dissimilarity from *Aichryson* in the shape of its hypogynous nectar glands, Bramwell (1968) later transferred *S. gattefossei* into *Aichryson* based on the discovery of the same chromosome number ($n = 15$) in this species as in the majority of *Aichryson* species (Uhl, 1961). This chromosome number sets

S. gattefossei apart from the other annual Moroccan *Sedum* species with known chromosome numbers. *Sedum surculosum* was discovered and described as a new species independently by Cosson (1873) and Ball (1873). While the former assigned the currently accepted name, the latter named this species *Monanthes atlantica* Ball (Mes and 't Hart, 1994). These two species thus illustrate the morphological and/or karyological similarity of at least some NW African species of *Sedum* to members of the Macaronesian *Aeonium* alliance. Such similarities have been discussed (Berger, 1930; Fröderström, 1932; Uhl, 1961; Maire and Quézel, 1967) for all species of *Sedum* sers. *Caerulea* Fröd. (*S. caeruleum* L. and *S. maurum* Humbert & Maire), *Monanthoidea* Batt. ex Fröd. (*S. jaccardianum* Maire & Wilczek, *S. modestum* Ball and *S. surculosum*) and *Pubescens* T.Mes (*S. gattefossei*, *S. pubescens* Vahl and *S. versicolor* Coss. ex Raym.-Hamet), following the classification by Mes (1995a). Molecular phylogenetic studies (Mes, 1995a,b; Van Ham and 't Hart, 1998; Mort et al., 2001, 2002; Nikulin et al., 2016) have indeed confirmed that some species of these three series are closely related to the Macaronesian *Aeonium* alliance. However, only five of the overall eight *Sedum* species have been sampled in phylogenetic analyses so far. Consequently, we do not yet know whether the serial classification of these *Sedum* species is reflected in their phylogenetic relationships, nor do we know which lineage is most closely related to the Macaronesian *Aeonium* alliance. In addition to the Macaronesian *Aeonium* alliance and the above three series of *Sedum*, Messerschmid et al. (2020) recently identified *Hypagophytum abyssinicum* (Hochst. ex A.Rich.) A.Berger, the only species of *Hypagophytum* A.Berger, as a further member of tribe Aeonieae. This NW Ethiopian species was shown to be the earliest-branching lineage within the Aeonium clade (Messerschmid et al., 2020).

Like the other members of tribe Aeonieae, *Sedum* sers. *Caerulea*, *Monanthoidea* and *Pubescens* are characterized by polymerous flowers (> 5 parts per whorl), a feature that has usually been associated with *Sempervivum* L., *Jovibarba* Opiz and *Petrosedum* Grulich (= *Sedum* ser. *Rupestria* A.Berger), but they differ in several morphological characters from these three genera. They are distributed in NW Africa with their centre of diversity in Morocco and, only with *S. caeruleum*, on several western Mediterranean islands (Fig. 1). Some of the species of these three series also occur in the so-called "Macaronesian Enclave" (Peltier, 1973) which is defined by the presence of plant taxa that otherwise are endemic to the Canary Islands, such as *Euphorbia regis-jubae* Webb & Berthel. (Euphorbiaceae), *Helianthemum canariense* (Jacq.) Pers. (Cistaceae) and *Drusa glandulosa* (Poir.) H.Wolff ex Engl. (Apiaceae; Peltier, 1982). While Peltier (1982) restricted the Macaronesian Enclave to the immediate vicinity of the Atlantic coast, Sunding (1979) expanded this region to further inland based on its extended definition as a region where "[a] great number of plant taxa [...] show greater affinity to taxa in the Macaronesian islands than to taxa on the mainland outside the enclave". The Macaronesian Enclave, also encompassing the point of shortest distance to the Canary Islands, has since then often been discussed as a potential source area for the colonization of the Macaronesian Islands (Caujapé-Castells et al., 1999; Hess et al., 2000; Jaén-Molina et al., 2009; Caujapé-Castells, 2011). However, the Mediterranean region has been suggested as an alternative source area for the colonization of the Canaries by numerous plant lineages (e.g., Carine et al., 2004 and references therein; Carine et al., 2010; Affenzeller et al., 2018).

The main aims of this study are (1) to resolve the phylogenetic position of those species of the above three series of *Sedum* which have not been sampled before, i.e., *S. gattefossei*, *S. maurum* and *S. versicolor*, in order to test the hypothesis that their relationships justify membership in tribe Aeonieae, and (2) to identify the closest relative(s) of the Macaronesian *Aeonium* alliance. To these ends, we sampled all eight *Sedum* species of tribe Aeonieae in a molecular phylogenetic analysis of two markers of the nuclear genome, namely ITS and ETS (internal and external transcribed spacers of nuclear ribosomal DNA, respectively), and four markers of the plastid genome, i.e., *matK*, *rps16* and the intergenic spacers *trnL-trnF* (in the following abbreviated as *trnL-F*) and *psbA-trnH*.

2. Materials and methods

2.1. Species sample

All species of *Sedum* ser. *Caerulea* (*S. caeruleum*, *S. maurum*), ser. *Monanthoidea* (*S. jaccardianum*, *S. modestum*, *S. surculosum*) and ser. *Pubescens* (*S. gattefossei*, *S. pubescens*, *S. versicolor*) were sampled in order to fully cover those infrageneric taxa of *Sedum* that were found or discussed to be close relatives of the Macaronesian *Aeonium* alliance (see Introduction). *Sedum maurum*, *S. gattefossei* and *S. versicolor* have never been sampled before in a molecular phylogenetic study. To infer whether these species are most closely related to the other representatives of the *Sedum* series they belong to rather than to other clades of the Sempervivoideae phylogeny, we also sampled two species each of *Sempervivum*/*Jovibarba* and the Telephium, Petrosedum, Leucosedum and Acre clades. Furthermore, we sampled *Hypagophytum abyssinicum* of tribe Aeonieae and four species of the Macaronesian *Aeonium* alliance, namely two species of *Aeonium* and one species each of *Aichryson* and *Monanthes*. All accessions sampled in this study, including voucher information and GenBank accession numbers, are listed in Appendix 1, and Fig. 1 shows the geographical localities of the sampled accessions.

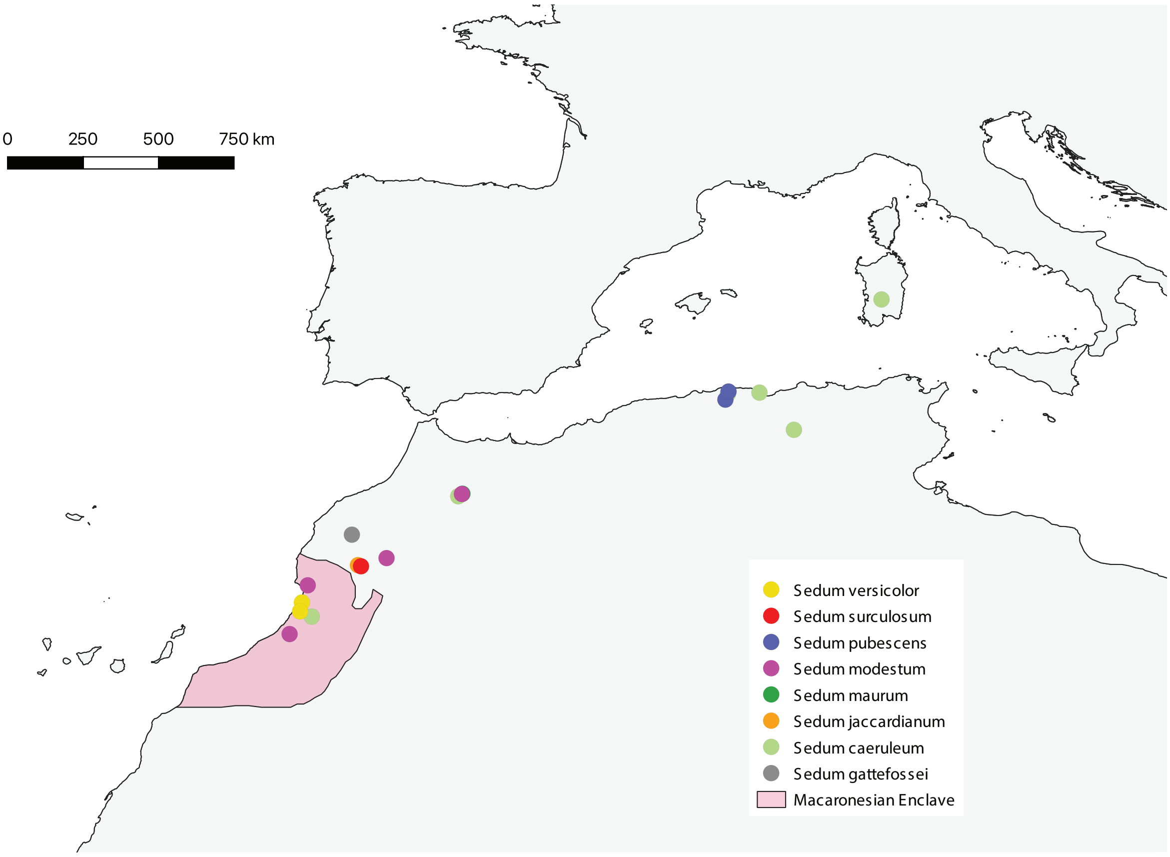


Fig. 1. Georeferenced localities for the *Sedum* species of tribe Aeonieae in NW Africa and the Mediterranean region sampled in this study. The Macaronesian Enclave in Morocco is outlined (shape approximately redrawn from Sunding, 1979).

2.2. DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from dried leaf material or dried flower buds using the NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany) based on the Cetyltrimethylammonium bromide (CTAB) lysis method according to the manufacturer's protocol, but with an extended lysis time of 60 minutes and frequent inversions of the lysate every 2–3 minutes during incubation at 65°C. After lysis, we introduced one additional centrifugation at 13,000 g for 5 minutes in order to discard most of the solid particles prior to loading the clear lysate onto the NucleoSpin filter. Finally, elution was carried out twice for 60 minutes instead of 5 minutes. The modifications to the protocol were intended to increase the DNA yield of the material which was up to 94 years old (see *S. maurum* in Appendix 1).

Because DNA was highly degraded in most of the samples, polymerase chain reactions (PCRs) were designed to amplify short fragments rather than the entire molecular markers using primer pairs ITS A and ITS C and ITS B and ITS D or, where this proved to be more successful, ITS

A and ITS 2, ITS B and ITS 3, ITS 5 and ITS 2 or ITS 4 and ITS 3 for ITS (ITS A–D: Blattner, 1999; ITS 2–5: White et al., 1990), Semp ETS 5 and Semp IGS 4R (Klein and Kadereit, 2015) and Semp ETS 1 (Klein and Kadereit, 2015) and 18S-IGS (Baldwin and Markos, 1998) for ETS, and matK 710F (Plunkett at al., 1996) and Als11R and matK 980F (both published in Harbaugh et al., 2010) and trnK-2R (Johnson and Soltis, 1994) for *matK*. Because the *matK* fragments could not be amplified for some of the samples, we designed the internal primers matK-AeonF (5'-CCC ATC CAT CTC GAA ATA TTG G-3') and matK-AeonR (5'-CCS GAA ATG GGT TTC TTT AG-3') that were used in combination with trnK-F-bryo (Wicke and Quandt, 2009) and trnK-2R, respectively. For the amplification of short stretches of *rps16*, we designed the internal primers rps16-internF (5'-GTA TGT TGC TGC CAT TTT TGA AAG G-3') and rps16-internR (5'-CTT GTT CCG GAA TCC TTT ATC-3') that were used in combination with rps16-R (Shaw et al., 2005) and rps16-F (Oxelman et al., 1997), respectively. We used primer pairs Tab-C and Tab-D and Tab-E and Tab-F (Taberlet et al., 1991) for the amplification of *trnL-F*, and primer pair psbAF and trnHR (Sang et al., 1997) for *psbA-trnH*. Settings for the PCR cycling for amplification of ITS1 (primer pairs ITS A+C, A+2 and 5+2), *rps16* and *trnL-F* as well as the ETS region amplified with primer pair Semp ETS 1 and 18S-IGS were the same as described in Messerschmid et al. (2020) for ITS, but with the following modifications: 35 instead of 40 PCR cycles and an elongation temperature of 68 instead of 72°C were implemented because we used another Taq polymerase (New England Biolabs, Ipswich, Massachusetts, U.S.A) than these authors. For the amplification of ITS2 (primer pairs ITS B+D, B+3 and 4+3) and the other region of ETS (primer pair Semp ETS 5 and Semp IGS 4R) as well as for *matK*, the annealing temperature was lowered to 50°C, while it was raised to 56°C for the amplification of *psbA-trnH*. In addition, annealing and elongation times were extended to 60 and 150 seconds, respectively, in the PCR cycles for *matK*. PCR products were purified using the ExoSap-IT PCR Clean-up Kit (Affymetrix, Santa Clara, California, U.S.A.) following the manufacturer's protocol. When PCR products yielded double bands on an agarose gel, the band of the appropriate bp length was cut out and purified using the NucleoSpin Gel and PCR Clean-up Kit (Macherey-Nagel). The purified PCR products were Sanger-sequenced by StarSEQ (Mainz, Germany) using the same primers as used for the PCR amplifications.

2.3. Sequence editing and alignment

Sequence reads were edited based on the chromatograms obtained from the sequencing reactions using Sequencher (v4.8; Gene Codes, Ann Arbor, Michigan, U.S.A.). Sequence contigs were aligned using MAFFT (v.7.471; Katoh and Standley, 2013) on the CIPRES

Science Gateway (https://www.phylo.org; Miller et al., 2010) with standard settings. Alignments of individual markers were subsequently revised and manually edited where necessary. In order to achieve sequence datasets with as little missing data as possible, we complemented the newly generated sequences (see asterisks in Appendix 1) with appropriate sequence data downloaded from NCBI GenBank (www.ncbi.nlm.nih.gov/genbank/). We furthermore combined the available sequence information of two different accessions of *Jovibarba heuffelii* (Schott) Á.Löve & D.Löve (accessions JKB151 and JKB156) as well as of two different species of *Sempervivum* (i.e., *S. arachnoideum* L. and *S. atlanticum* (Ball) Ball) and of the Leucosedum clade (i.e., *Sedum borissovae* Balk. and *S. laconicum* Boiss. & Heldr.) into single terminals to complete our alignments as far as possible.

2.4. Phylogenetic analyses

Alignments of individual molecular markers as well as concatenated datasets of the two nuclear markers, the four plastid markers, and all six markers (combined dataset) were used for Maximum-Likelihood (ML) and Bayesian phylogenetic analyses. *Phedimus stoloniferus* (S.G.Gmel.) ’t Hart and *Umbilicus oppositifolius* (Ledeb.) Ledeb. were always defined as outgroup because the Telephium clade is known to be the earliest-branching lineage within subf. Sempervivoideae (Mort et al., 2001; Messerschmid et al., 2020). The most appropriate substitution model was inferred for the alignments of each molecular marker using PartitionFinder (v2.2.1; Lanfear et al., 2017), with ITS subdivided into three partitions, i.e., ITS1, 5.8S rDNA and ITS2. ML phylogenetic trees were inferred using RAxML-NG (v1.0.0; Kozlov et al., 2019) on the online RAxML BlackBox (https://raxml-ng.vital-it.ch) or using RAxML (v8.2.12; Stamatakis, 2014) on the CIPRES Science Gateway. Bootstrapping was set to be halted automatically. Bayesian inference (BI) of phylogenetic trees was carried out using MrBayes (v3.2.7a; Ronquist and Huelsenbeck, 2003) on the CIPRES Science Gateway with two independent Markov Chain Monte Carlo (MCMC) runs of 100 million generations and 6 chains for each sequence dataset. Reference tree and log files were stored at every 10,000th generation after a burn-in of the first 10 million generations (= 10%). Phylogenetic trees were visualized using FigTree (v1.3.1; Rambaut, 2009).

As there were no supported conflicts between any two phylogenies inferred from single molecular markers, we concatenated markers into a nuclear, a plastid and a combined nuclear and plastid supermatrix. Phylogenies were inferred separately from these three concatenated datasets because the taxon sample differed between them due to the lack of nuclear DNA sequences for *S. versicolor*. Nevertheless, this species was included in the analyses of the combined nuclear and plastid sequence dataset.

3. Results

3.1. PCR amplification and sequencing

For the newly sampled *Sedum* species, we were able to sequence ITS, *rps16* (both markers sequenced for *S. gattefossei* and *S. maurum*) and *psbA* (*S. gattefossei* and *S. versicolor*). However, we did not succeed in sequencing any of the nuclear markers for *S. versicolor*. Therefore, *S. versicolor* could only be sampled for the phylogenetic analysis of the plastid dataset and the combined plastid and nuclear sequence dataset. Because the only material of these three species available to us was collected in the 1920s and 1930s, we frequently amplified and sequenced fungal DNA or obtained low-quality sequence reads. Therefore, these sequences in general needed to be trimmed to a greater extent than sequences of the other accessions.

3.2. Phylogenetic relationships of the *Sedum* species of tribe Aeonieae

Phylogenies that were inferred from single molecular markers received little overall support and did not show supported conflicts among each other (see paragraph 2.4 above; Appendix 2). In the phylogenies of the three concatenated sequence datasets (i.e., nuclear, plastid and combined nuclear and plastid supermatrices), tribe Aeonieae was consistently recovered as a well-supported monophyletic group, i.e., the Aeonium clade, and the three *Sedum* species that we here sampled for the first time were always recovered within this Aeonium clade. The remaining four major clades of subf. Sempervivoideae were recovered as supported clades in the combined (Fig. 2) and the plastid phylogeny (Fig. 3), but the Acre clade and samples of the Leucosedum clade were part of a polytomy with the Petrosedum clade, Aeonium clade and *Sempervivum*/*Jovibarba* in the nuclear phylogeny (Fig. 4). In the plastid and the combined phylogenies, the Telephium clade, Petrosedum clade and *Sempervivum*/*Jovibarba* were successive sister to tribe Aeonieae + tribe Sedeae. However, the backbone was not resolved in the nuclear phylogeny (Fig. 4).

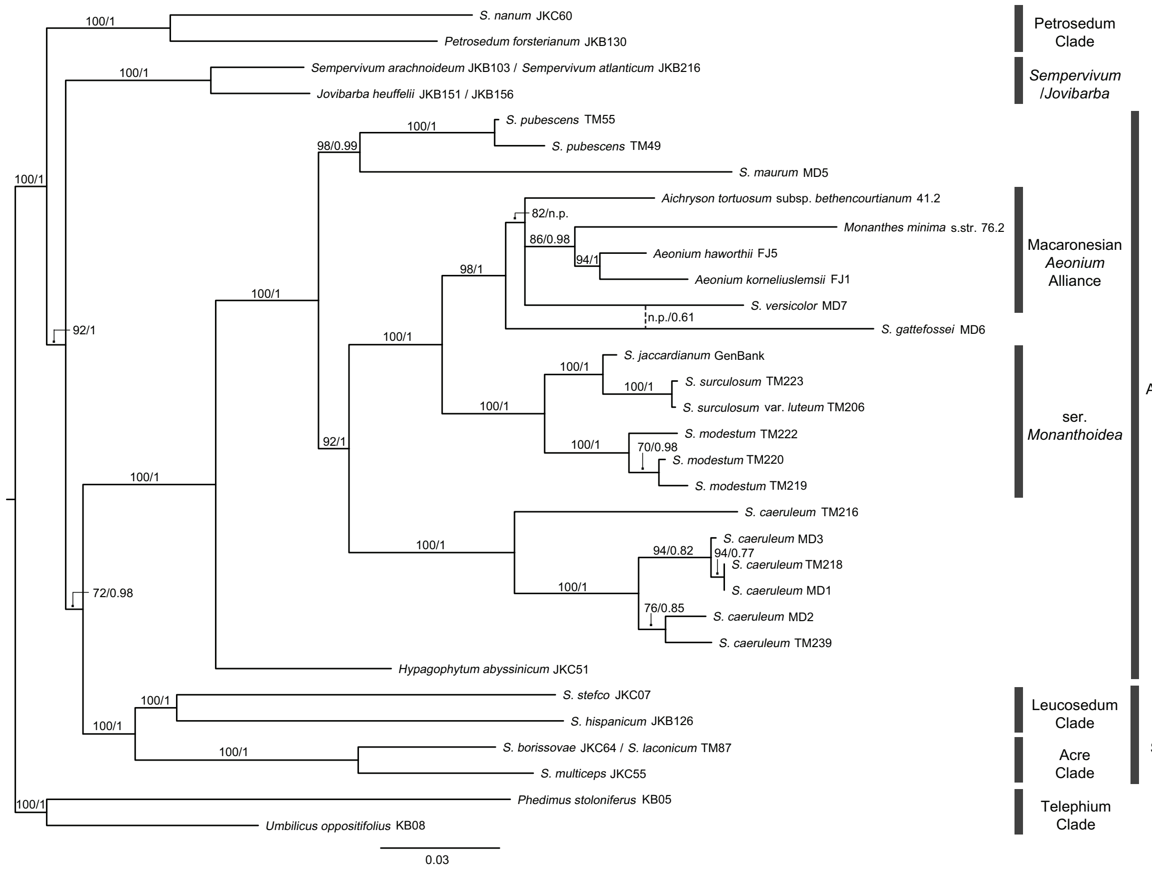


Fig. 2. Molecular phylogeny of Crassulaceae subf. Sempervivoideae with a complete sample of the *Sedum* species of tribe Aeonieae as inferred from the combined sequence dataset of concatenated nuclear (ITS, ETS) and plastid markers (*matK*, *rps16*, *trnL-F*, *psbA-trnH*). Numbers above branches indicate bootstrap support values of the ML analysis (left) and posterior probabilities of the BI analysis (right). Branches with bootstrap support <75 and posterior probability (p.p.) <0.95 were collapsed. "n.p." (not present) signifies that the corresponding clade was not recovered in the Bayesian phylogeny (solid branch) where *S. versicolor* was unsupported (p.p. = 0.61) sister to *S. gattefossei*, instead (dashed line).

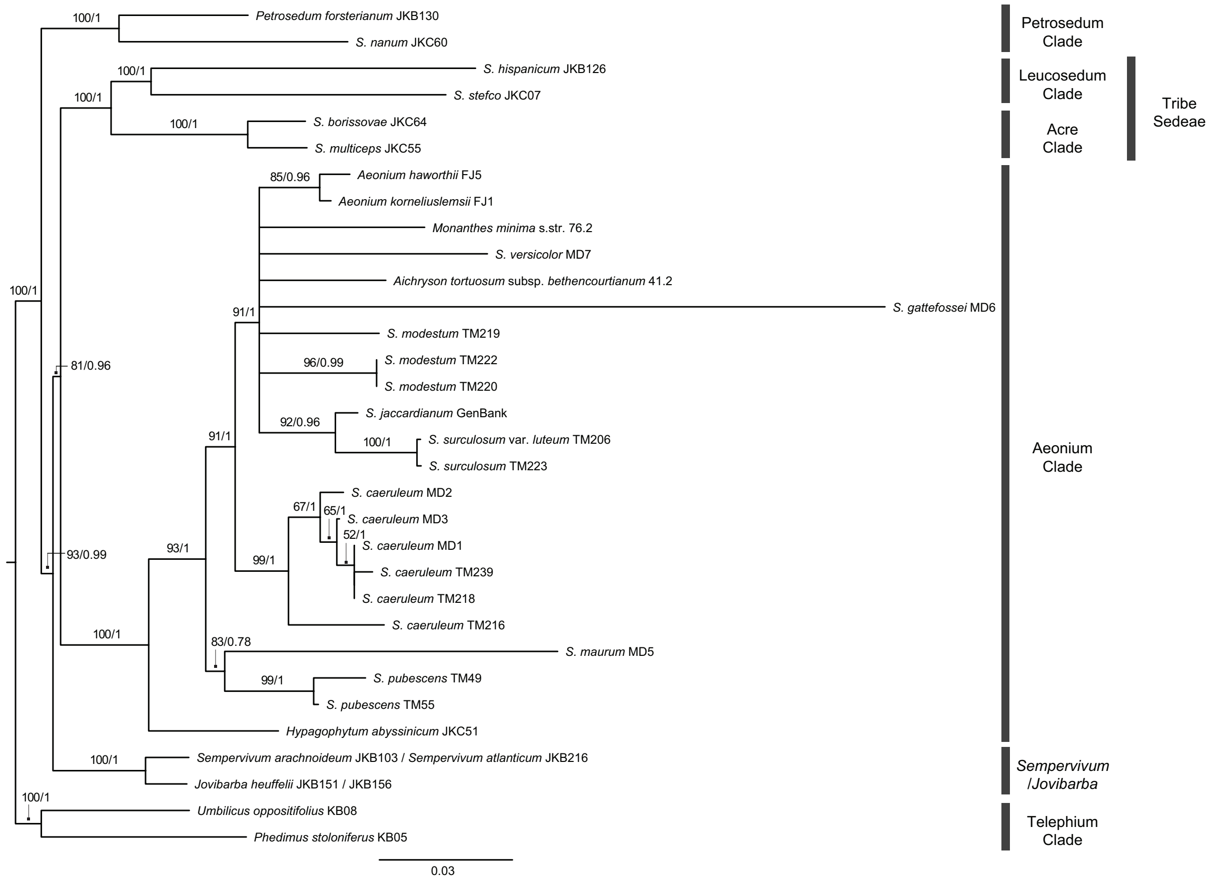


Fig. 3. Molecular phylogeny of Crassulaceae subf. Sempervivoideae with a complete sample of the *Sedum* species of tribe Aeonieae as inferred from the concatenated sequence dataset of plastid markers (*matK*, *rps16*, *trnL-F*, *psbA-trnH*). Numbers above branches indicate bootstrap support values of the ML analysis (left) and posterior probabilities of the BI analysis (right). Branches with bootstrap support <75 and posterior probability (p.p.) <0.95 were collapsed.

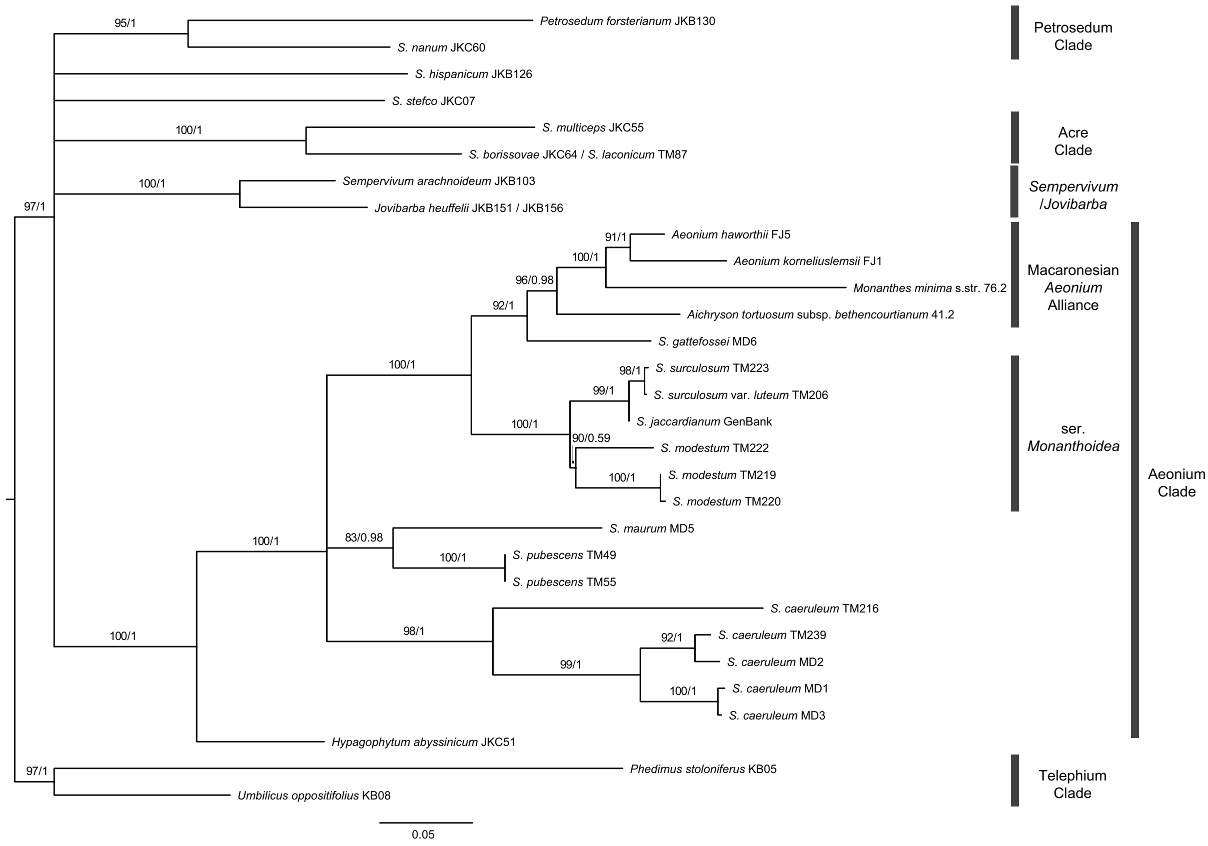


Fig. 4. Molecular phylogeny of Crassulaceae subf. Sempervivoideae with a complete sample of the *Sedum* species of tribe Aeonieae as inferred from the concatenated sequence dataset of nuclear markers (ITS, ETS). Numbers above branches indicate bootstrap support values of the ML analysis (left) and posterior probabilities of the BI analysis (right). Branches with bootstrap support <75 and posterior probability (p.p.) <0.95 were collapsed.

Hypagophytum abyssinicum was supported as earliest-branching lineage of the Aeonium clade in all phylogenetic analyses (Figs. 2–4). *Sedum pubescens* and *S. maurum* were always supported as closest relatives to each other, and all sampled accessions of *S. caeruleum* always formed a supported clade. Among the accessions of *S. caeruleum*, accession TM216 from the Middle Atlas was always supported as sister to the remaining accessions, while the only accession sampled from a Mediterranean island (i.e., TM239; see Appendix 1 for detailed information about all accessions) was consistently nested among NW African accessions of *S. caeruleum*. The two clades *S. pubescens* plus *S. maurum* and *S. caeruleum* were supported as successive sister to the remainder of the Aeonium clade above *Hypagophytum* in the plastid (Fig. 3) and the combined phylogeny (Fig. 2), while analyses of the nuclear markers did not resolve relationships among these clades (Fig. 4). All species of *Sedum* ser. *Monanthoidea* were recovered in a well-supported clade of the nuclear (Fig. 4) and combined phylogenies (Fig. 2) in which *S. jaccardianum* was supported as sister to a monophyletic *S. surculosum* in all phylogenetic analyses. Finally, in the nuclear phylogeny (Fig. 4) in which *S. versicolor* could not be included due to lacking sequence data, *S. gattefossei* was supported as sister to the

Macaronesian *Aeonium* alliance, i.e., a clade comprising all accessions of *Aichryson*, *Aeonium* and *Monanthes*, where *Aichryson* and *Monanthes* were successive sister to a monophyletic *Aeonium*. In the plastid and the combined phylogenies, the Macaronesian *Aeonium* alliance was not supported as a clade (Fig. 3). Instead, the three Macaronesian genera were part of a larger polytomy with *S. versicolor*, *S. gattefossei*, *S. modestum* (one single accession and a small clade of accessions TM220 and TM222) and a clade comprising *S. jaccardianum* and *S. surculosum*. In the phylogenies of the combined nuclear and plastid dataset, genera of the Macaronesian *Aeonium* alliance were part of a polytomy with *S. versicolor* (ML) or *S. versicolor* plus *S. gattefossei* (BI). In the ML analysis of the combined dataset, the extended clade of *S. versicolor* and the Macaronesian *Aeonium* alliance was in turn supported as sister to *S. gattefossei*. However, the BI phylogeny of the combined dataset (also Fig. 2) resolved *S. versicolor* as closest relative of *S. gattefossei*, but this relationship was not supported (posterior probability = 0.61). Because the Macaronesian *Aeonium* alliance was not supported as a clade in the combined phylogeny but was instead recovered in a polytomy with *S. versicolor* (and *S. gattefossei*), the Macaronesian *Aeonium* alliance could either be a monophyletic lineage or instead contain *S. versicolor* (and *S. gattefossei*).

4. Discussion

The phylogenetic backbone and hence the relationships among the five major clades of subf. Sempervivoideae was only resolved in our combined (Fig. 2) and plastid phylogenies (Fig. 3) and was identical to the relationships inferred in a recent phylogenetic study of a much larger taxon sample and a dataset of plastid markers (Fig. 2 in Messerschmid et al., 2020). Our results show that *Sedum* sers. *Caerulea*, *Monanthoidea* and *Pubescens* have rightly been assumed to belong to tribe Aeonieae or to be close relatives of the Macaronesian *Aeonium* alliance (Battandier and Jahandiez, 1921; Fröderström, 1932; Uhl, 1961; Mes and 't Hart, 1994; Mes, 1995b). *Sedum* ser. *Monanthoidea* was the only monophyletic infrageneric taxon of *Sedum* in our analyses, while *S. maurum* of ser. *Caerulea* was closest relative to *S. pubescens* of ser. *Pubescens* instead of to *S. caeruleum*. In a phylogenetic study of ITS2 and *trnL-F*, Mes (1995b) had already recovered a monophyletic ser. *Monanthoidea*, but with his incomplete sample of *Sedum* species, ser. *Monanthoidea* was supported as sister lineage to the Macaronesian *Aeonium* alliance, a finding referred to in later publications (e.g., 't Hart and Bleij, 2005). With a complete sample of the *Sedum* species of tribe Aeonieae, our phylogenies indicate that *S. gattefossei* and *S. versicolor* of ser. *Pubescens* are more closely related to the Macaronesian

Aeonium alliance than to *S. pubescens*, which renders ser. *Pubescens* non-monophyletic. Because of the conflicting but unsupported position of *S. versicolor* in the ML and BI analyses of the combined nuclear and plastid dataset, it remains unclear whether *S. versicolor* is more closely related to *S. gattefossei* or whether *S. versicolor* alone is most closely related to the Macaronesian *Aeonium* alliance. A third possibility, in view of the polytomy in this part of the phylogeny, would be a nested position of *S. versicolor*, possibly together with *S. gattefossei*, within the Macaronesian *Aeonium* alliance (see paragraph 3.2 above). Such a topology could be explained by a Canary Island origin of the ancestral lineage(s) of *S. gattefossei* and *S. versicolor* that later back-colonized the African continent. In summary, our phylogenetic topology implies that there are four possible candidates for sister lineage of the Canarian genera: (1) If *S. gattefossei* and *S. versicolor* were nested within the Macaronesian *Aeonium* alliance, *Sedum* ser. *Monanthoidea* would represent its sister lineage. However, if *Aichryson*, *Aeonium* and *Monanthes* together form a monophyletic lineage, which is the prevalent concept of this group (e.g., Mes, 1995b; Fairfield et al, 2004; Kim et al., 2008; Mort et al., 2010; Nikulin et al., 2016; Messerschmid et al., 2020), (2) *S. gattefossei*, (3) *S. versicolor* or (4) these two species together could be sister to the Macaronesian *Aeonium* alliance. This problem can only be solved with more thorough sampling of these two *Sedum* species, denser sampling of the Macaronesian *Aeonium* alliance (especially more *Aichryson* species), and particularly the use of more and more variable molecular markers. Considering the apparent rarity of *S. gattefossei* and *S. versicolor* this will not be easy to achieve.

If the classificatory proposal by Messerschmid et al. (2020) were to be followed and all members of tribe Sedeae were transferred into *Sedum*, the *Sedum* species of tribe Aeonieae would need to be transferred into other genera. The three species of the monophyletic *Sedum* ser. *Monanthoidea* differ from representatives of *Sedum* sers. *Caerulea* and *Pubescens* by having more or less distinctly petiolate leaves (Maire and Quézel, 1967). In addition, two of them, namely *S. jaccardianum* and *S. surculosum*, are perennials, whereas *S. modestum* and the *Sedum* species of the remaining two series are annuals. When segregating ser. *Monanthoidea* from *Sedum* the resurrection of the genus *Monanthella* A.Berger might be a first option. *Monanthella* had been introduced by Berger (1930) as a monotypic genus to segregate the peculiar *S. jaccardianum* from *Sedum*. However, *Monanthella* is an invalidly published name (Mes and 't Hart, 1994; 't Hart and Bleij, 2005), so that a new name will have to be found. In contrast to the monophyletic *Sedum* ser. *Monanthoidea*, the phylogenetic relationships presented here suggest that sers. *Caerulea* and *Pubescens* are not monophyletic and would therefore need to be re-classified in order to arrive at monophyletic segregate genera of *Sedum*.

The five species of *Sedum* sers. *Caerulea* and *Pubescens* were recovered in two distinct clades (1 and 2) and one lineage (3) comprising additional taxa: (1) a clade comprising *S. pubescens* and *S. maurum*, (2) *S. caeruleum* and (3) *S. versicolor* and *S. gattefossei* together with the Canarian genera. Although the last of these lineages contains the three genera of the Macaronesian *Aeonium* alliance, a closer relationship of *S. versicolor* and *S. gattefossei* would be supported by their close resemblance in overall habit (Maire, 1928) and by sharing completely glabrous and smooth leaves (Maire and Quézel, 1967). In combination with petal colour, leaf pubescence is an excellent morphological character to distinguish between the three lineages in which species of sers. *Caerulea* and *Pubescens* were recovered: (1) *Sedum maurum* and *S. pubescens* differ from the remaining three species by having glandular-pubescent leaves, (2) *S. caeruleum*, having glabrous leaves, is unique in the blue to white colour of its petals, a character that had originally caused its affiliation to the white-flowered *S. maurum* in ser. *Caerulea*, and (3) *S. gattefossei* and *S. versicolor* share their glabrous leaves with *S. caeruleum* but differ by having yellow petals that sometimes turn purple in *S. versicolor* (Maire and Quézel, 1967; 't Hart and Bleij, 2005). If the three lineages will be classified at generic rank, as we prefer, new names will have to be found for all of them. However, support for a sister relationship of *S. gattefossei* and *S. versicolor* is yet missing, and these species might be nested in the Macaronesian *Aeonium* alliance (see above). The chromosome number of *S. versicolor* is unknown, but *S. gattefossei* has the same chromosome number ($n = 15$) as the majority of *Aichryson* species (Uhl, 1961) while *Monanthes icterica* (Webb ex Bolle) Christ, which has been supported as sister to *Aichryson* in several phylogenetic studies (Mes et al., 1997; Nikulin et al., 2016; Messerschmid et al., 2020), has a chromosome number of $n = 10$ (Mort et al., 2002). The common chromosome number in *S. gattefossei* and *Aichryson* could indicate a common origin of the two lineages and was the reason for inclusion of *S. gattefossei* in *Aichryson* (Bramwell, 1968). On the other hand, the shape of hypogynous nectar glands lacking digitate or horn-like appendages and the sessile and completely glabrous leaves of *S. gattefossei* and *S. versicolor* would suggest a close relationship of these two *Sedum* species to each other. In these character states they clearly differ from *Aichryson*.

Our study is one of few investigations that identified the likely closest relatives of a Macaronesian plant radiation by fully sampling the relevant mainland taxa. In an extensive literature search, Carine et al. (2004) compiled information from molecular phylogenies about the closest relatives of Macaronesian endemic plants and concluded that most of the closest continental relatives are distributed in the western Mediterranean region (including NW Africa). Of the 32 plant lineages endemic to the Macaronesian Islands listed by Carine et al.

(2004), 21 could be resolved as sister to a supported continental lineage, but completeness of sampling of continental species that could potentially be close relatives of the island radiation was not considered. Although we cannot guarantee that we sampled all potential closest relatives of the Macaronesian *Aeonium* alliance because this would ultimately require sampling of all species of Crassulaceae subf. Sempervivoideae, we did sample all *Sedum* species of those infrageneric taxa of *Sedum* that had been shown to be closely related to the Macaronesian *Aeonium* alliance in earlier phylogenetic studies (Mes, 1995a,b; Van Ham and 't Hart, 1998; Mort et al., 2001, 2002), including species that have been hypothesized to be close relatives of the Macaronesian *Aeonium* alliance on the grounds of morphological (Battandier and Jahandiez, 1921; Berger, 1930; Fröderström, 1932) and karyological similarities (Uhl, 1961). We conclude that the probably closest relatives of the Macaronesian *Aeonium* alliance (either *S. versicolor* alone or *S. versicolor* + *S. gattefossei*) are annual herbs that occur, at least partly, in the Macaronesian Enclave in Morocco (Fig. 1). Apart from the implication that the perennial woody habit evolved (at least twice) in the Macaronesian *Aeonium* alliance on the Canary Islands (Mes et al., 1996; Mort et al., 2002), our findings also add another case in which the closest relative of a Canary Island radiation is distributed in the Macaronesian Enclave. However, this need not mean that their ancestors occurred in the Macaronesian Enclave at the time when the Canary Islands were colonized. In comparison to other plant radiations of the Macaronesian Islands, the MRCA of the *Aeonium* alliance reached the Canary Islands relatively early, namely around 15.2 million years ago (Kim et al., 2008). This time falls into the Betic crisis (Kim et al., 2008), a time of frequent temperature fluctuations that caused sea level changes. This may mean that some of the seamounts north of Lanzarote, some of them in close proximity to the southwest tip of the Iberian Peninsula, could have emerged above sea level at the time of colonization of the Macaronesian Islands (Caujapé-Castells, 2011). Also, range shifts of the ancestral *Sedum* species of tribe Aeonieae seem likely in the face of climatic fluctuations. Therefore, the question whether the MRCA of the *Aeonium* alliance migrated to the Canary Islands from Morocco, occupied a much larger area throughout northern Africa before the aridification and formation of the Sahara Desert (Senut et al., 2009), or originated from the Iberian Peninsula, remains elusive. As regards the second of these possibilities, a wide distribution range even beyond northern Africa of the ancestral lineage of tribe Aeonieae is suggested by the Rand Flora pattern (Pokorny et al., 2015) of the extant *Hypagophytum abyssinicum* in Ethiopia and the *Sedum* species of tribe Aeonieae mainly in NW Africa. Such a distribution pattern is found in several different plant lineages, e.g., *Camptoloma* Benth. (Scrophulariaceae; Culshaw et al., 2021) or *Globularia* L., *Poskea* Vatke and *Campylanthus*

Roth (Plantaginaceae; Affenzeller et al., 2018), and can be explained by the initial occupation of a large distribution range throughout northern or central Africa that was later broken apart by climatic changes and aridification of the Sahara.

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Supplementary material:

Appendix 1. Detailed information about specimens and GenBank accession numbers used in the molecular phylogenetic analyses.

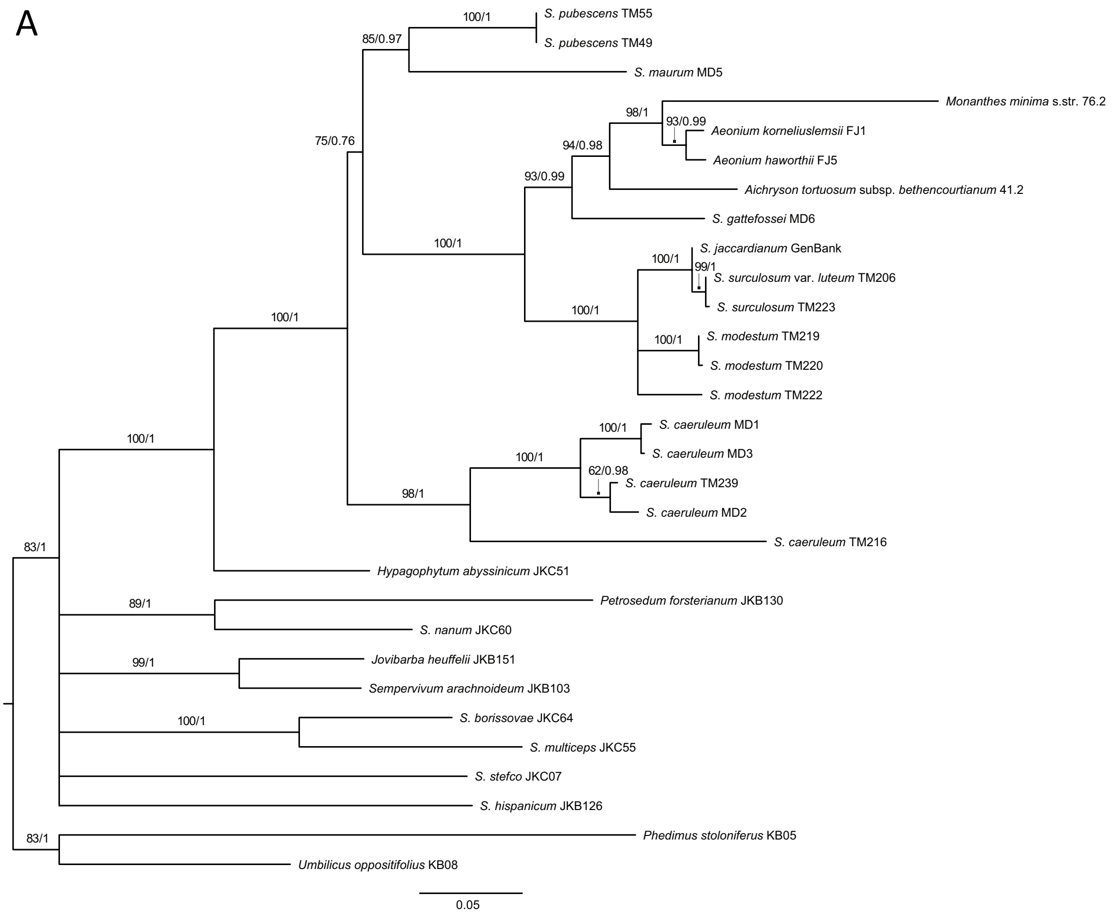
Species (lab code, only provided for accessions with sequences generated by the author); country; locality and additional information; year of collection; voucher (+ herbarium code or Botanical Garden); ITS; ETS; *matK*; *rps16*; *trnL-F*; *psbA-trnH*. "–" indicates missing data or missing information and "*" newly generated sequences.

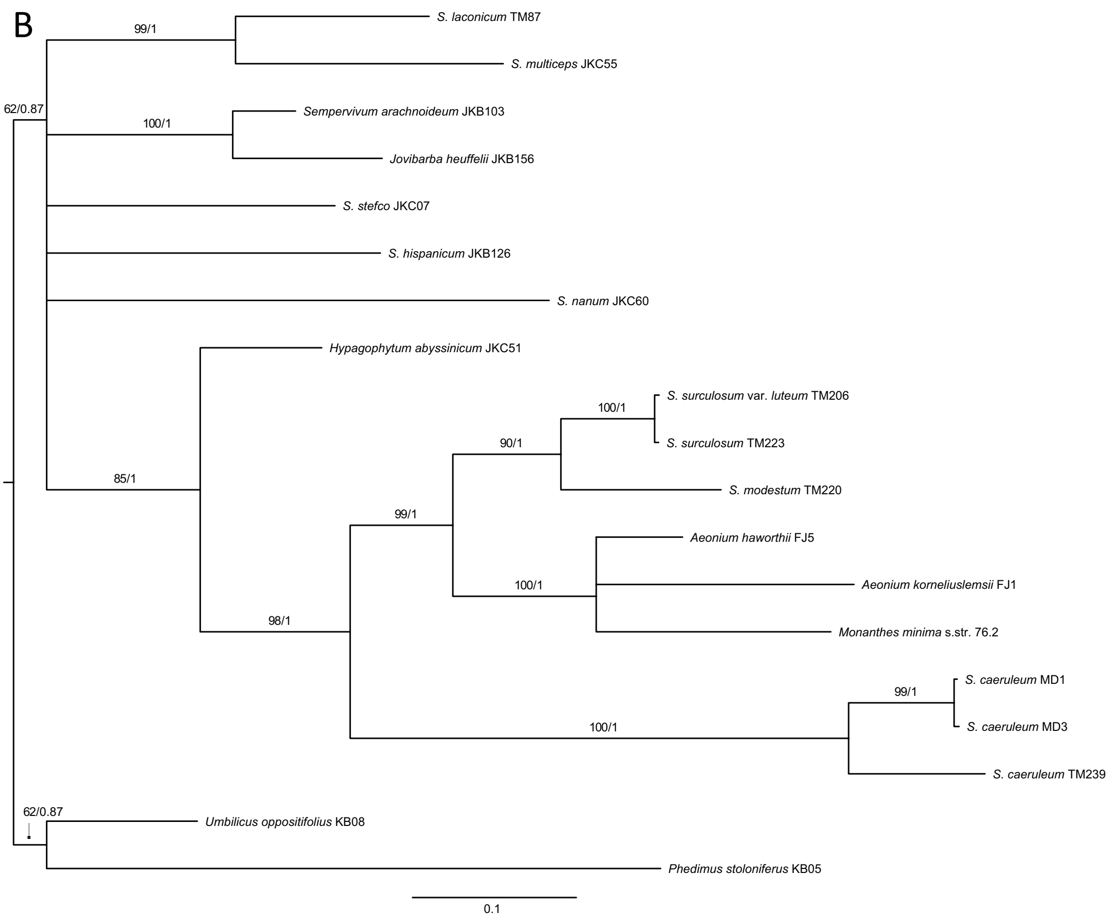
Outgroup: ***Jovibarba heuffelii*** (Schott) Á.Löve & D.Löve (JKB151); Bulgaria; 41.73°N/23.51°E; 2011; *J. Klein G 110830-482* (MJG 012920); KJ884153; –; MT181558; –; MT155887; OM337480*. ***J. heuffelii*** (Schott) Á.Löve & D.Löve (JKB156); North Macedonia; 41.02°N/21.20°E; 2011; *J. Klein G 110903-511*; –; KJ883937; –; KJ884362; –; –. ***Petrosedum forsterianum*** (Sm.) Grulich (JKB130); Spain; Montes de Toledo, Province of Cáceres, Guadalupe, rocky slopes in Villuercas peak, 39.48°N/5.40°W; –; *F.J. Valtueña, C.G. Relinque* (MJG 012903); KJ884125; –; MT181574; KJ884334; MT155903; OM337479*. ***Phedimus stoloniferus*** (S.G.Gmel.) 't Hart (KB05); Georgia; Guria, Lesser Caucasus, mt. Bakhmaro, environs Fafara, 2103 m, 41.83°N/42.32°E; 2011; *S. Shetekauri* (MJG 024791); MT336091; OM337437*; MT181559; OM337435*; MT155888; OM337486*. ***Sedum borissovae*** Balk. (JKC64); –; Garden origin; –; (Sukkulenten-Sammlung Zürich 99 4604 /0); MT336104; –; MT181571; MN381924; MT155900; OM337485*. ***S. hispanicum*** L. (JKB126); Greece; Central Macedonia, Thessalonika, Chalkidiki, Chortiatis, Boudos, 920 m, 40.60°N/23.10°E; 2011; *J. Klein C 110622-01-325* (MJG 012914); KJ884126; OM337447*; MT181578; KJ884335; MT155907; OM337477*. ***S. laconicum*** Boiss. & Heldr. (TM87.1); Greece; Lakonias, Githiou, by the deserted village of Poliaravos. Rocky slopes and old terraced fields. Mostly schist, 700–800 m, 36.80°N/22.42°E; 1991; *Strid, Kit Tan* (E 00095641); –; OM337443*; –; –; –; –. ***S. multiceps*** Coss. & Durieu (JKC55); Tunisia; –; –; *H. 't Hart 25256 = HtH 25256* (Sukkulenten-Sammlung Zürich 99 4291 /0); MT336125; OM337449*; MT181585; MN381930; MT155914; OM337483*. ***S. nanum*** Boiss. (JKC60); Iran; Kohkilouyeh-Boirahmad, 27 km N of Sisakht, Kuhe Gol; 1991; *Jamzad & al. 69488* (TARI 69488); MT336126; OM337439*; MT181586; OM337434*; MT155915; OM337484*. ***S. stefco*** Stef. (JKC07); Bulgaria; Bansko - Trigrad, about a 3-hour drive; 2007; *Stephenson* (Sukkulenten-Sammlung Zürich 10 0181 /0); MT336137; OM337448*; MT181591; MN381933; MT155921; OM337481*. ***Sempervivum arachnoideum*** L. (JKB103); Italy; Trentino; –; *G. Cristofoloni*; KJ884163; KJ883944; –; KJ884370; OM337471*; OM337476*. ***S. atlanticum*** (Ball) Ball (JKB216); Morocco; 31.21°N/7.85°W; –; *D.R. Letz, S. Španiel, P. Mered‘a 43C* (MJG 024784); –; –; MT181597; –; –; –. ***Umbilicus oppositifolius*** (Ledeb.) Ledeb. (KB08); –; Garden origin (Botanischer Garten der Johannes Gutenberg-Universität Mainz); –; (MJG); KJ884131; OM337438*; MT181598; OM337436*; MT155928; OM337487*. **Aeonieae:** ***Aeonium haworthii*** Webb & Berthel. (FJ5); Spain, Tenerife, Tierra de Trigo, 550 m; 2005; *K. Esfeld* (MJG 030601); OM200090*; OM337442*; –; OM337418*; OM337456*; OM337473*. ***A. korneliuslemsii*** H.Y.Liu (FJ1); Morocco; Anti-Atlas, Kerdous mountains, between Tanout, Igui Ifred and Agni, "Djbel Imzi", 29.74°N/9.25°W, ca. 600m; 2005; *Erpenbach, Levejohann*; OM200089*; OM337441*; –; OM337417*; OM337455*; OM337472*. ***Aichryson tortuosum* subsp. *bethencourtianum*** (Bolle) Bañares (JL41.2); Spain; Fuerteventura, Montaña de la Muda, 28.57°N/13.96°W, 686 m; 2018; *Á.*

Bañares; OM200092*; –; OM337454*; OM337420*; OM337458*; OM337475*. ***Hypagophytum abyssinicum*** (Hochst. ex A.Rich.) A.Berger (JKC51); Ethiopia; Gondar, lower part of the Limalima escarpment on the road from Adi Arkay to Debark, open, rocky slope with mosses, *Swertia* and *Hypagophytum*, 2350 m, 13.21°N/37.89°E; 2005; *I. Friis, G.S. Bidgood, W. Abebe, E. Getachew 12294* (WAG 0416928); MT336090; OM337446*; MT181557; MN381921; MT155885; OM337482*. ***Monanthes minima*** Christ s.str. (NB76.2); Spain; Tenerife, Igueste San Andres, 36 m; 2018; *N. Bobon M04_N5*; OM200091*; OM337440*; –; OM337419*; OM337457*; OM337474*. ***Sedum caeruleum*** L. (MD1); Algeria; Wilaya Tizi Ouzou, Djurdjura massif, 4 km NE below the pass of Tizi N'Kouilal, calcareous escarpments by the road, 1300 m, 36.48°N/4.27°E; 1984; *D. Podlech 39132* (MSB 132633); OM200093*; OM337452*; –; OM337421*; OM337459*; OM337488*. ***S. caeruleum*** L. (MD2); Algeria; Wilaya Batna, Massif de l'Aurès, 2 km above Tarhil on the road from Biskra to Arris, calcareous escarpments, 830 m, 35.12°N/6.23°E; 1984; *D. Podlech 38 640* (MSB 132632); OM200094*; –; –; OM337422*; OM337460*; OM337489*. ***S. caeruleum*** L. (MD3); Algeria; Wilaya Bejaia, northern Djurdjura, 8 km W of El Kseur on the road to Tizi Ouzou, siliceous rocks, 420 m, 36.68°N/4.80°E; 1984; *D. Podlech 39318* (MSB 132636); OM200095*; OM337453*; –; OM337423*; OM337461*; OM337490*. ***S. caeruleum*** L. (TM216); Morocco; province Meknes, Middle Atlas, Ifrane, exit on the road towards Boulemane, calcareous rocks and (xeric) grassland, 1680 m, 33.52°N/5.12°W; 1987; *W. Lippert 22901* (M 0307505); OM200096*; –; –; OM337424*; OM337462*; OM337493*. ***S. caeruleum*** L. (TM218); Morocco; Agadir, Col du Kerdouss, in rupestribus secus viam, solo calcareo persicco, 930 m; 1985; *C. Blanché, J. Fernández Casas, J. Molero, J.M. Montserrat, A. Romo 9234* (M 0307501); –; –; –; –; –; OM337494*. ***S. caeruleum*** L. (TM239); Italy; Sardinia, Giara di Gesturi; 2010; *J. Manner, W. Schumacher* (University of Bonn Botanical Garden IT-0-BONN-33206); OM200097*; OM337451*; –; OM337425*; OM337463*; OM337499*. ***S. gattefossei*** Batt. & Jahand. (MD6); Morocco; Rehamna, Sidi Ali, on dry rocks, 410 m; 1933; *J. Gattefossé* (B 10 1052784); OM200099*; –; –; OM337427*; –; OM337478*. ***S. jaccardianum*** Maire & Wilczek; –; –; –; (Utrecht University Botanic Gardens 32211); AY082100; –; AF115637; –; AY082220; AY082179. ***S. maurum*** Humbert & Maire (MD5); Morocco; Northern Middle Atlas; 1926; *R. Maire* (B 10 1052783); OM200098*; –; –; OM337426*; –; –. ***S. modestum*** Ball (TM219); Morocco; province Beni-Mellal, Middle Atlas, Cascades d'Ouzoud, 960-1060 m, 32.03°N/6.72°W; 1986; *D. Podlech 41495* (MSB 132568); OM200104*; –; –; OM337432*; OM337468*; OM337495*. ***S. modestum*** Ball (TM220); Morocco; province Marrakesh, High Atlas, 7 km N of Asni on the road to Marrakesh, on siliceous rocks, 1050 m, 31.28°N/7.97°W; 1989; *D. Podlech 45903* (MSB 132567); OM200105*; OM337445*; –; –; OM337469*; OM337496*. ***S. modestum*** Ball (TM222); Morocco; province Agadir, western High Atlas, environs Cascades d'Imouzzer, 4 km SW of Imouzzer-Ida-Outanen, 850 m, 30.68°N/9.48°W; 1997; *D. Podlech 53773* (MSB 138003); OM200106*; –; –; OM337433*; OM337470*; OM337497*. ***S. pubescens*** Vahl (TM49); Algeria; K1, Djurdjura, 5 km S of Boghni, olive groves, round base of olive trunks, 330 m; 1975; *Davis* (E 00764484); OM200100*; –; –; OM337428*; OM337464*; OM337500*. ***S. pubescens*** Vahl (TM55); Algeria; K1, just below Makouda (N of Tizi Ouzou), steep conglomerate banks, 300 m; 1975; *Davis* (E 00764483); OM200101*; –; –; OM337429*; OM337465*; OM337501*. ***S. surculosum*** Coss. (TM223); Morocco; High Atlas, province Marrakesh, environs Oukaimeden, on siliceous substrate, 2700–3200 m, 31.18°N/7.85°W; 1991; *Sammet, Ilitz* (M); OM200103*; OM337450*; –; OM337431*; OM337467*; OM337498*. ***S. surculosum* var. *luteum*** (Emb.) Maire (TM206); –; –; 1992; *L. Gallo* (Collection Ray Stephenson 8.08.002); OM200102*; OM337444*; –; OM337430*; OM337466*; OM337492*. ***S. versicolor***

Coss. ex Raym.-Hamet (MD7); Morocco; Souss-Massa, Draâ, Tiznit, rocailles calcaires, 29.77°N/9.81°W; 1931; *É. Jahandiez 78* (BM 013399459); –; –; –; –; –; OM337491*.

Appendix 2. Phylogenetic trees inferred from single molecular markers: (A) ITS, (B) ETS, (C) *matK*, (D) *rps16*, (E) *trnL-F*, (F) *psbA-trnH*. Numbers above branches indicate bootstrap support (BS) values from the Maximum-Likelihood analyses (left) and posterior probability (p.p.) values from the Bayesian Inference analyses. Branches that received little support (BS < 75 and p.p. < 0.95) were collapsed, except for the branches bearing the whole in- and outgroup.

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Jovibarba heuffelii JKB156
S. stefco JKC07
S. hispanicum JKB126
S. nanum JKC60
Hypagophytum abyssinicum JKC51
100/1
S. surculosum var. luteum TM206
90/1
S. surculosum TM223
85/1
S. modestum TM220
99/1
Aeonium haworthii FJ5
100/1
Aeonium korneliuslemsii FJ1
98/1
Monanthes minima s.str. 76.2
99/1
S. caeruleum MD1
100/1
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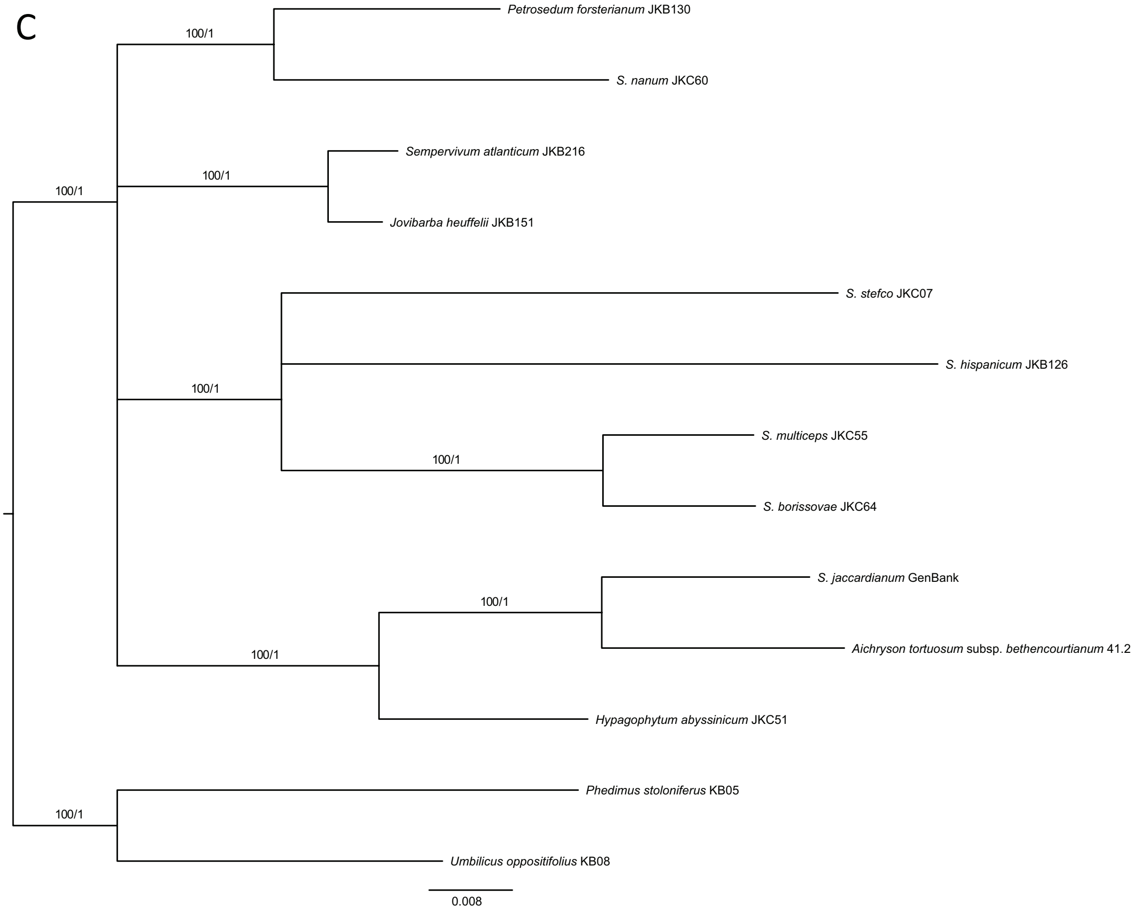
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S. nanum JKC60
Sempervivum atlanticum JKB216
100/1
100/1
Jovibarba heuffelii JKB151
S. stefco JKC07
S. hispanicum JKB126
100/1
S. multiceps JKC55
100/1
S. borissovae JKC64
S. jaccardianum GenBank
100/1
Aichryson tortuosum subsp. bethencourtianum 41.2
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Hypagophytum abyssinicum JKC51
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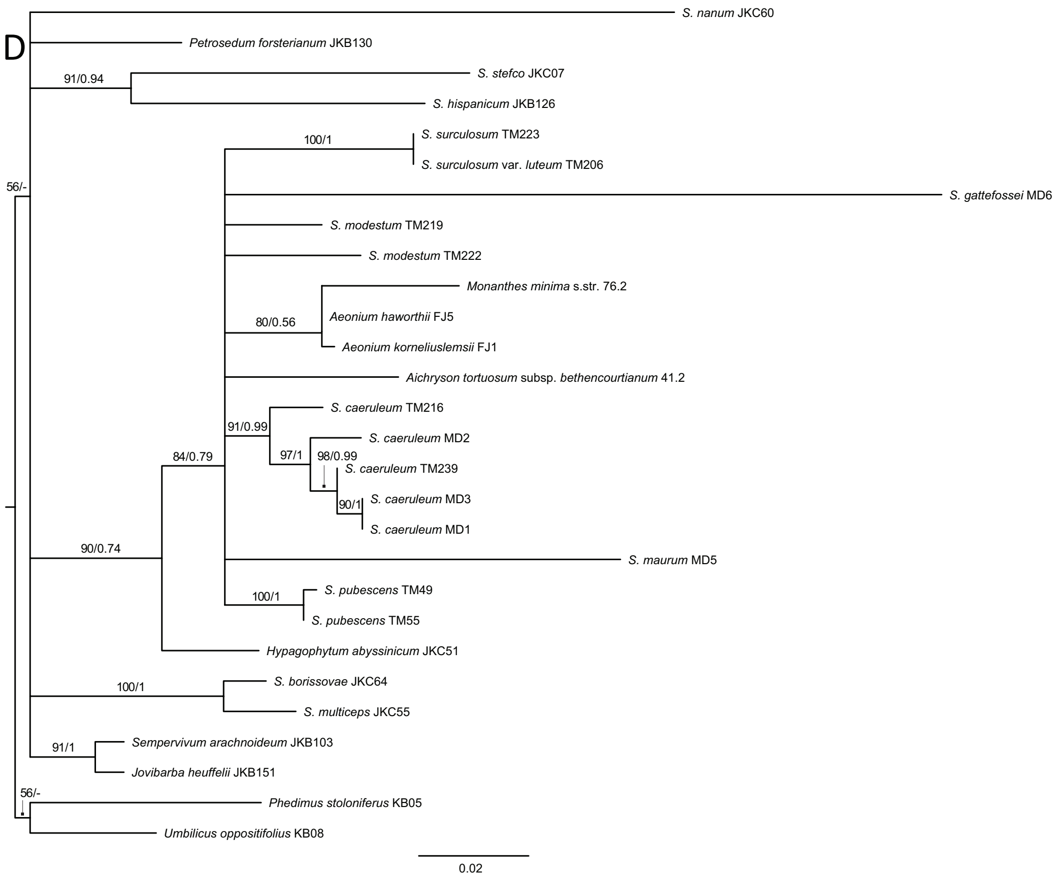
100/1
Umbilicus oppositifolius KB08
0.008


D
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91/0.94
S. stefco JKC07
S. hispanicum JKB126
100/1
S. surculosum TM223
S. surculosum var. luteum TM206
56/-
S. gattefossei MD6
S. modestum TM219
S. modestum TM222
Monanthes minima s.str. 76.2
80/0.56
Aeonium haworthii FJ5
Aeonium korneliuslemsii FJ1
Aichryson tortuosum subsp. bethencourtianum 41.2
S. caeruleum TM216
91/0.99
S. caeruleum MD2
84/0.79
97/1
98/0.99
S. caeruleum TM239
90/1
S. caeruleum MD3
S. caeruleum MD1
90/0.74
S. maurum MD5
S. pubescens TM49
100/1
S. pubescens TM55
Hypagophytum abyssinicum JKC51
S. borissovae JKC64
100/1
S. multiceps JKC55
91/1
Sempervivum arachnoideum JKB103
Jovibarba heuffelii JKB151
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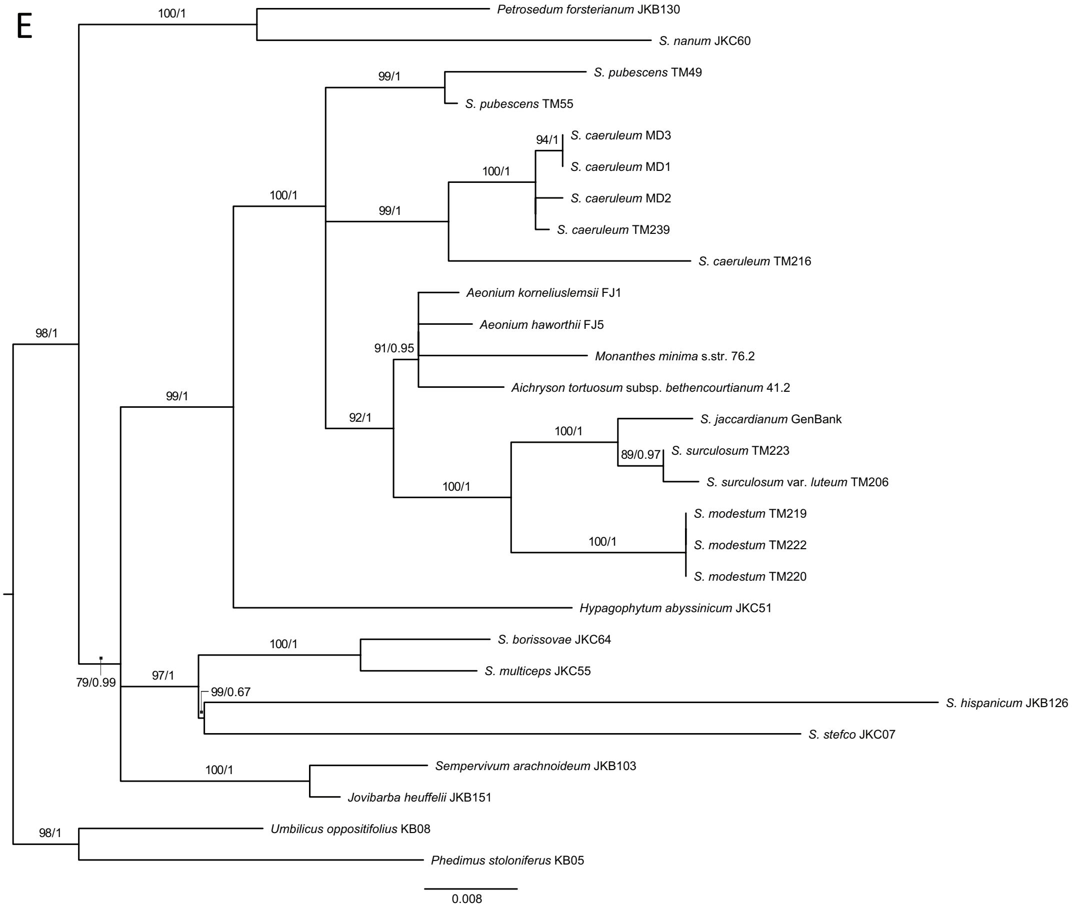
Phedimus stoloniferus KB05
Umbilicus oppositifolius KB08
0.02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100/1
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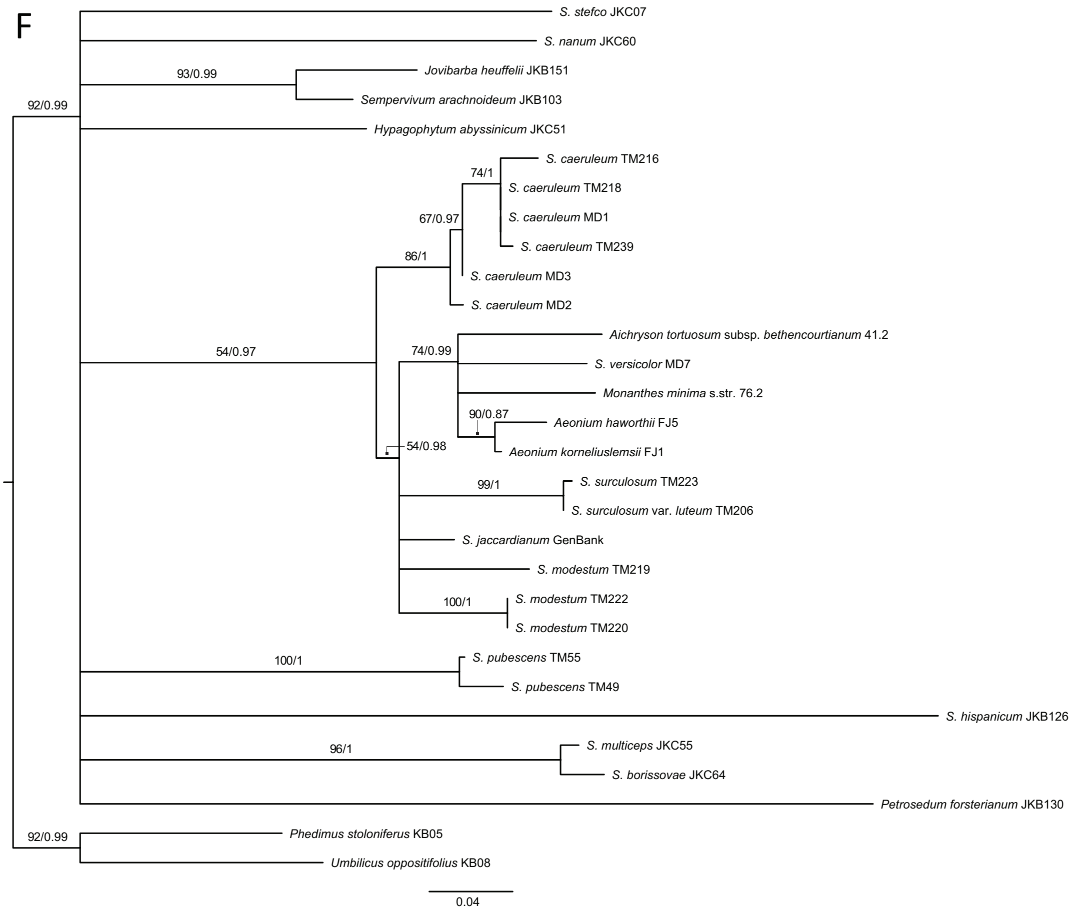
S. nanum JKC60
99/1
S. pubescens TM49
S. pubescens TM55
94/1
S. caeruleum MD3
100/1
S. caeruleum MD1
100/1
S. caeruleum MD2
99/1
S. caeruleum TM239
S. caeruleum TM216
Aeonium korneliuslemsii FJ1
Aeonium haworthii FJ5
98/1
91/0.95
Monanthes minima s.str. 76.2
Aichryson tortuosum subsp. bethencourtianum 41.2
99/1
92/1
S. jaccardianum GenBank
100/1
89/0.97
S. surculosum TM223
S. surculosum var. luteum TM206
100/1
S. modestum TM219
100/1
S. modestum TM222
S. modestum TM220
Hypagophytum abyssinicum JKC51
100/1
S. borissovae JKC64
S. multiceps JKC55
79/0.99
97/1
99/0.67
S. hispanicum JKB126
S. stefco JKC07
100/1
Sempervivum arachnoideum JKB103
Jovibarba heuffelii JKB151
98/1
Umbilicus oppositifolius KB08
Phedimus stoloniferus KB05
0.008


F
S. stefco JKC07
S. nanum JKC60
93/0.99
Jovibarba heuffelii JKB151
Sempervivum arachnoideum JKB103
92/0.99
Hypagophytum abyssinicum JKC51
S. caeruleum TM216
74/1
S. caeruleum TM218
67/0.97
S. caeruleum MD1
S. caeruleum TM239
86/1
S. caeruleum MD3
S. caeruleum MD2
Aichryson tortuosum subsp. bethencourtianum 41.2
54/0.97
74/0.99
S. versicolor MD7
Monanthes minima s.str. 76.2
90/0.87
Aeonium haworthii FJ5
54/0.98
Aeonium korneliuslemsii FJ1
99/1
S. surculosum TM223
S. surculosum var. luteum TM206
S. jaccardianum GenBank
S. modestum TM219
100/1
S. modestum TM222
S. modestum TM220
100/1
S. pubescens TM55
S. pubescens TM49
S. hispanicum JKB126
96/1
S. multiceps JKC55
S. borissovae JKC64
Petrosedum forsterianum JKB130
92/0.99
Phedimus stoloniferus KB05
Umbilicus oppositifolius KB08
0.04

4. Summary

The Crassulaceae are a family of leaf succulent plants comprising about 1410 species, with centres of diversity in South Africa and Mexico, but with similarly high diversity and floristic importance in Macaronesia. The Crassulaceae can be divided into three subfamilies, each characterized as monophyletic and morphologically distinct lineages. While the South African diversity centre exclusively hosts representatives of the subfamilies Crassuloideae and Kalanchoideae, the most species-rich subfamily Sempervivoideae has its distribution focus in the Northern Hemisphere. Previous phylogenetic studies in the Crassulaceae have identified a sister relationship of Sempervivoideae and Kalanchoideae, as well as five supported major clades within Sempervivoideae. Among several other genera, *Sedum* is represented in three of these five clades, making *Sedum* a non-monophyletic genus.

Two chapters of this dissertation deal with the molecular phylogenetics of *Sedum* and related genera and aim at making monophyletic and morphologically definable lineages the basis of a proposed generic classification of the Sempervivoideae. In addition to these questions of systematics and classification, the phylogenetic analyses presented here have also served to answer biogeographical and evolutionary biological questions, such as the mechanisms and consequences of diversification.

The most important conclusion from the first study, a molecular phylogenetic investigation of the entire subfamily Sempervivoideae, is firstly the proposal to transfer all 14 currently accepted genera of the phylogenetically well-supported tribe Sedeae into *Sedum*. With this option, common morphological descriptions of *Sedum* found in the literature can essentially remain unaltered. Secondly, this study was able to show that migrations from the ancestral range (probably the area of the former Tethys and Paratethys) had repeatedly led to increased morphological diversification in the evolution of the Sempervivoideae, which in taxonomic history has led to the description of several genera besides *Sedum*, especially for the American and Asian taxa.

The object of the second and third study was tribe Aeonieae, which contains the most species-rich plant radiation of the Macaronesian Islands, the *Aeonium* alliance comprising *Aeonium*, *Aichryson* and *Monanthes*. Using a next-generation sequencing approach, I was able to contribute to a well-resolved phylogeny and biogeography of all 40 *Aeonium* species. It could be shown that sympatric diversification events had led to a more pronounced morphological and partly ecological divergence of the sister groups than allopatric diversification events had. Finally, the third study dealt with the mainly Northwest African *Sedum* species of tribe

Aeonieae, which for the first time included all relevant *Sedum* species of the Aeonieae in a molecular phylogenetic analysis. This allowed me to identify the possibly closest relatives of the above-mentioned Macaronesian genera and to discuss a possible concept for a genus classification in the Aeonieae.

5. Zusammenfassung

Die Dickblattgewächse (Crassulaceae) sind eine Familie blattsukkulenter Pflanzen und umfassen circa 1410 Arten mit Schwerpunkten der Diversität in Südafrika und Mexiko aber mit einer ebenfalls hohen Diversität und floristischen Bedeutung in Makaronesien. Die Crassulaceae lassen sich in drei Unterfamilien gliedern, die jeweils als monophyletische und morphologisch distinkte Abstammungslinien charakterisiert sind. Während das südafrikanische Diversitätszentrum ausschließlich Vertreter der Unterfamilien Crassuloideae und Kalanchoideae beherbergt, hat die artenreichste Unterfamilie Sempervivoideae ihren Verbreitungsschwerpunkt in der Nordhemisphäre. Vorausgegangene phylogenetische Untersuchungen in den Crassulaceae haben ein Schwesterverhältnis von Sempervivoideae und Kalanchoideae sowie fünf unterstützte Hauptverwandtschaftskreise (Kladen) innerhalb der Sempervivoideae identifiziert. Neben mehreren anderen Gattungen ist *Sedum* in drei dieser fünf Kladen vertreten, womit *Sedum* eine nicht-monophyletische Gattung darstellt.

Zwei Kapitel der vorliegenden Dissertation befassen sich mit der molekularen Phylogenetik von *Sedum* und verwandten Gattungen und verfolgen das Ziel, monophyletische und morphologisch definierbare Abstammungslinien zur Grundlage einer vorgeschlagenen Gattungsklassifikation der Sempervivoideae zu machen. Neben diesen Fragen zur Systematik und Klassifikation haben die hier vorgestellten phylogenetischen Analysen aber auch dazu gedient, biogeographische und evolutionsbiologische Fragen, etwa nach den Mechanismen und Folgen von Diversifizierung, zu beantworten.

Die wichtigste Schlussfolgerung aus der ersten Studie, einer molekular-phylogenetischen Untersuchung der gesamten Unterfamilie Sempervivoideae, ist zum einen der Vorschlag, alle 14 derzeit akzeptierten Gattungen der phylogenetisch gut unterstützten Tribus Sedeae in *Sedum* zu überführen. Mit dieser Option können die gängigen morphologischen Beschreibungen von *Sedum* im Wesentlichen bestehen bleiben. Zum anderen konnte diese Studie zeigen, dass Migrationen aus dem anzestralen Areal (wahrscheinlich dem Gebiet der ehemaligen Tethys und Paratethys) in der Evolution der Sempervivoideae wiederholt zu verstärkter morphologischer Diversifizierung geführt hatten, was in der taxonomischen Geschichte zur Beschreibung mehrerer Gattungen neben *Sedum* vor allem für die amerikanischen und asiatischen Taxa geführt hat.

Das Objekt der zweiten und dritten Studie war die Tribus Aeonieae, die mit den Gattungen *Aeonium*, *Aichryson* und *Monanthes* die artenreichste Pflanzenradiation der Makaronesischen Inseln beinhaltet. Mit Hilfe eines Next-Generation-Sequencing-Ansatzes war es mir möglich, zu einer gut aufgelösten Phylogenie und Biogeographie aller 40 *Aeonium*-Arten beizutragen. Dabei konnte gezeigt werden, dass sympatrische Diversifizierungsereignisse zu einer stärker ausgeprägten morphologischen und zum Teil ökologischen Divergenz der Schwestergruppen geführt hatten als allopatrische Diversifizierungsereignisse. Mit den hauptsächlich nordwestafrikanischen *Sedum*-Arten der Tribus Aeonieae befasste sich schließlich die dritte Studie, welche zum ersten Mal alle relevanten *Sedum*-Arten der Aeonieae in einer molekular-phylogenetischen Analyse einschloss. Damit konnte ich die möglicherweise engsten Verwandten der oben genannten makaronesischen Gattungen identifizieren und ein mögliches Konzept für eine Gattungsklassifikation in den Aeonieae diskutieren.

6. List of tables

7. List of figures

9. Curriculum vitae

Curriculum vitae

Thibaud Felix Edmond Messerschmid

Gender: Male

Date and place of birth: 26. November 1991, Starnberg

Nationalities: German and Swiss

Marital status: Married

Address: Fendter Str. 12, 82380 Peißenberg

E-Mail: t.messerschmid@lmu.de

Education

2017– **PhD in Biology, Johannes Gutenberg-Universität (JGU) Mainz**
Dissertation: „Phylogenetics and Evolution of Crassulaceae subf. Sempervivoideae“ (Supervisor: Prof. Joachim W. Kadereit, PhD)

2014–2016: **MSc. in Biology, Julius-Maximilians-Universität (JMU) Würzburg**
Main subjects: Ecophysiology of Plants and Pharmaceutical Biology
Master thesis: „Quantification of cuticular and stomatal contributions to leaf transpiration during water limitation in plant species of contrasting ecology“ (Supervisor Prof. Dr. Markus Riederer)
Final mark: 1.2

2011–2014: **BSc. in Biology, Ludwig-Maximilians-Universität (LMU) München**
Main subjects: Systematic Botany and Mycology, Molecular Plant Sciences, Ecology, Evolutionary Biology, and Anthropology
Bachelor thesis: „Molekulare Identifizierung von chinesischen Heilpflanzen – DNA-Barcoding an Arten der Gattung *Glycyrrhiza* L. (Fabaceae)“ (Supervisor: Prof. Dr. Günther Heubl)
Final mark: 1.3

2002–2011: **Grammar school education, Gymnasium Weilheim i. OB**
A-level subjects: Biology, Ancient Greek, Latin, and Religious Education
Final mark: 1.0

1998–2002: **Primary Education**

Positions

2021– **Research fellow, LMU München**
Research and teaching at the chair of Systematics, Biodiversity and Evolution of Plants

2017–2021: **Research assistant, JGU Mainz**
Research and teaching at the Institute of Organismic and Molecular Evolution as well as the Institute of Molecular Physiology

January–March 2017: **Research assistant, JMU Würzburg**
Continuative research building on the results of my Master thesis

October–December 2013: **Student assistant, LMU München**
Working on a digitization project for herbarium type specimens funded by the Mellon Foundation (Botanische Staatssammlung München)