Molecular Phylogenetics and Evolution 63 (2012) 97–112

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Molecular phylogenetics, historical biogeography, and chromosome number evolution of *Portulaca* (Portulacaceae)

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ARTICLE INFO

Article history:
Received 6 October 2011
Accepted 16 December 2011
Available online 27 December 2011

Keywords:
Chromosome number evolution
Biogeography
Divergence times
Phylogeny
Portulaca
Portulacaceae

ABSTRACT

Portulaca is the only genus in Portulacaceae and has ca. 100 species distributed worldwide, mainly in the tropics and subtropics. Molecular data place the genus as one of the closest relatives of Cactaceae, but phylogenetic relationships within *Portulaca* are barely known. This study samples 59 species of *Portulaca*, 10 infraspecific taxa, and three cultivars, including multiple samples of widespread species. The sampled taxa represent all subgenera in the classifications of von Poellnitz (1934), Legrand (1958), and Geesink (1969) and come from around the world. Nuclear ITS and chloroplast *ndhF*, *trnT-psbD* intergenic spacer, and *ndhA* intron DNA sequences were analyzed using maximum likelihood and Bayesian methods to produce a hypothesis of relationships within *Portulaca*. Divergence times were estimated using Hawaiian endemics for calibration, and biogeographical patterns were examined using a Bayes–DIVA approach. In addition, the evolution of chromosome numbers in the genus was investigated using probabilistic models. The analyses strongly support the monophyly of *Portulaca*, with an age of the most recent common ancestor (MRCA) of 23 Myr. Within *Portulaca* are two major lineages: the OL clade (comprising opposite-leaved species) distributed in Africa, Asia, and Australia, and the AL clade (comprising alternate to subopposite-leaved species), which is more widespread and originated in the New World. *Sedopsis*, a genus sometimes recognized as distinct from *Portulaca* based on a long corolla tube, is nested within the OL clade and does not merit taxonomic recognition. Samples of *Portulaca grandiflora*, *Portulaca halimoides*, and *Portulaca oleracea* were found to be non-monophyletic. It is hypothesized that the ancestral distribution area of *Portulaca* included southern hemisphere continents and Asia. The OL clade remained restricted to the Old World (except *Portulaca quadrifida*, a pantropical weed), while the AL clade, with a South American origin, was able to disperse multiple times to other continents. The base chromosome number for *Portulaca* is inferred to be $x = 9$, although the analysis was primarily based on the available data for the AL clade. A number of chromosome number change events (polyploidization, demi-polyploidization, gain, and loss) were shown to have occurred in the genus, especially within the Oleracea clade.

1. Introduction

Portulaca L. is the only genus that remains in Portulacaceae Juss. following the proposal of splitting the family into four (Anacampserotaceae Eggli and Nyffeler, Montiaceae Raf., Portulacaceae, and Talinaceae Doweld; Nyffeler and Eggli, 2010) in response to molecular evidence that the family as traditionally circumscribed is not monophyletic (e.g., Hershkovitz and Zimmer, 1997; Applequist and Wallace, 2001; Nyffeler, 2007). The relationship of *Portulaca* to Anacampserotaceae, Cactaceae Juss., and Talinaceae is uncertain (Hershkovitz and Zimmer, 1997; Applequist and Wallace, 2001; Applequist et al., 2006; Nyffeler, 2007; Nyffeler and Eggli, 2010; Ocampo and Columbus, 2010), but together they form a well-supported clade (ACPT clade; Nyffeler, 2007) within suborder Cactineae (Caryophyllales; sensu Thorne, 2007). Although research on the relationships inside Cactineae has been an active focus of attention, a limited number of species of *Portulaca* have been employed in these studies; therefore, phylogenetic relationships within the genus have remained largely unknown.

Portulaca is distributed worldwide, mainly in the tropics and subtropics, with centers of diversity in South America and Africa. The number of species in the genus is uncertain, with estimates ranging from a few morphologically variable species (Geesink, 1969) to over 100 (von Poellnitz, 1934; Legrand, 1958, 1962). Species of *Portulaca* include annuals and herbaceous perennials, usually with hairs (conspicuous or inconspicuous; Fig. 1A and B) in the axils of the leaves, but in some species the hairs extend around the stem (*Portulaca quadrifida* L.) or are replaced by lanceolate scales (e.g., *Portulaca wightiana* Wall. ex Wight & Am.; Fig. 1C). Leaves are alternate (Fig. 1A), opposite (Fig. 1D) or subopposite

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doi:10.1016/j.ympev.2011.12.017

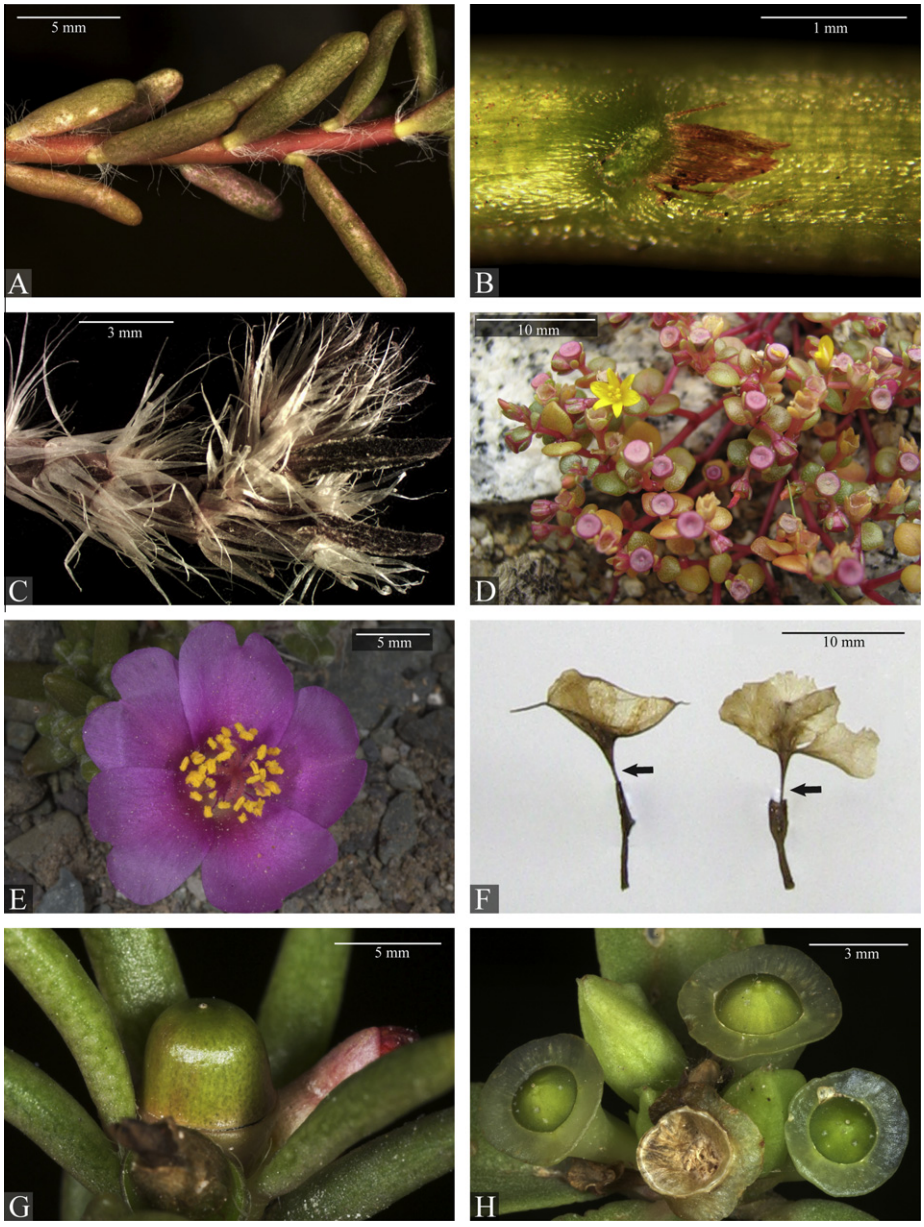

Fig. 1. Morphological features of *Portulaca*. (A) *P. californica*, showing conspicuous leaf axilar hairs and alternate leaf arrangement. (B) Inconspicuous axilar hairs are shown after a leaf is removed from the stem of a specimen of *P. oleracea* subsp. *granulatostellulata*. (C) Lanceolate scales found in the stems of *P. wightiana* (*Burgoyne 3616*, PRE). (D) *P. bicolor*, showing opposite leaves and cymose inflorescences. (E) Flower of *P. confertifolia*. (F) Flowers of *P. armitii*, showing an evident floral tube (black arrows; *McDonald KRM4815*, BRI). (G) Capsules of *P. tuberosa*. (H) *P. umbraticola* subsp. *umbraticola*, showing a wing at the dehiscence line of the capsule.

(alternate at the base of the stem, with short internodes towards the apex, giving the impression of opposite leaves in the upper part of the plant), with a short petiole (Fig. 1A). The flowers, sessile or rarely pedicellate, are arranged in cymose inflorescences (Fig. 1D), heads or are solitary, surrounded by a whorl of leaves. Geesink (1969) considered solitary flowers or those arranged in heads in species of subgen. *Portulaca* to be the result of a reduction from the cymose inflorescences found in the subgen. *Portulacella*. Sepals and petals are typically persistent in fruit. Petals usually range from four to five in number (Fig. 1E) (in some cases more), and are shortly connate at the base; only a few Old World species form an evident corolla tube (Fig. 1F), treated by some botanists as members of the genus *Sedopsis* (Engl.) Exell & Mendoça (e.g., Exell and Mendoça, 1937). [von Poellnitz (1934) and Legrand (1962) considered sepals and petals to be modified bracts, while Geesink (1969) supported a traditional point of view for the perianth parts. Recent studies have shown that sepals and petals in Portulacaceae and other Caryophyllales are of bracteal origin (Ronse De Craene, 2008; Brockington et al., 2009) and are not homologous to the floral parts of other dicots. Nevertheless, to avoid confusion, the traditional terminology is used here.] The ovary is semi-inferior, multilocular at the base, becoming unilocular towards the apex, with free-central placentation. The single style has 2–12 lobes (Fig. 1E). The fruit is a many-seeded, circumscissile capsule with a lid that is flat to hemispheric (Fig. 1G and H), campanular or conical. The seeds are reniform and compressed, and sculpturing is known to be morphologically diverse (e.g., Danin et al., 1978; Matthews and Levins, 1986). Most species grow in disturbed or pioneer habitats, especially rocky and shallow soils, usually in full sun.

The circumscription of the genus has been relatively stable although Exell and Mendoça (1937) erected *Sedopsis* to include species with an evident corolla tube and four stamens. Plants for-

mally considered as members of *Sedopsis* include four African species [*Sedopsis carrisoana* Exell & Mendoça, *Sedopsis hereroensis* (Schinz) Exell & Mendoça, *Sedopsis saxifragoides* (Welw.) Exell & Mendoça, and *Sedopsis sedoides* (Welw.) Exell & Mendoça] and two Australian endemics (*Sedopsis armitii* (F. Muell.) Poelln. and *Sedopsis filsonii* (J.H. Willis) J.H. Willis). However, the genus has not been widely accepted and most botanists recognize its species as members of *Portulaca*.

Although a number of species had already been described by the beginning of the 20th century, and a few infrageneric taxa had been proposed (Engelmann, 1850; Mueller, 1859, 1877; Kuntze, 1898; Engler, 1915), it was not until 1934 that von Poellnitz produced the first monographic study of *Portulaca*. He recognized 104 species and based his classification on the presence (*Portulaca planooperculata* Kuntze = *Portulaca umbraticola*) or absence of a flat and discoid lid of the capsule (subgen. *Discoportulaca* Kuntze and subgen. *Euportulaca* Speg., respectively). Later, Legrand (1958) recognized 126 species worldwide, although his studies were biased towards the New World. He proposed a classification based mainly on inflorescence and floral characters and composed of six subgenera: four in the Old World [*Dichocalyx* (F. Muell.) D. Legrand, *Enantiophylla* D. Legrand, *Portulacella*, *Siphonopetalum* (F. Muell.) D. Legrand], one in the New World (*Portulacelloides* D. Legrand), and one worldwide (*Portulaca*). His sectional and subsectional categories are complex and most of the time difficult to use in practice. Geesink (1969) produced the most recent classification of *Portulaca*, which contrasts with Legrand's (1958) in its simplicity. He focused on Asian and Australian species, however, and did not thoroughly study the African and New World taxa. Geesink considered *Portulaca* to have no more than 15 species worldwide and recognized two subgenera: *Portulaca* with flowers arranged in heads or solitary, and the endemic Australian *Portulacella* with cymose inflorescences. Subgenus *Portulaca* was further divided into sect. *Neossia* D. Legrand, with opposite leaves, and sect. *Portulaca*, with alternate leaves; the latter includes the well-known *Portulaca oleracea* (subsect. *Portulaca*) and *Portulaca pilosa* L. (subsect. *Stellulatotuberculatae* Poelln.). Although Geesink's classification does not take into account all species in the genus, his work has been followed by most botanists (e.g., Danin et al., 1978; Kim and Carr, 1990; Gilbert, 1994; Gilbert and Phillips, 2000; Eggli, 2002; Nyffeler, 2007).

Within Cactineae, *Portulaca* is the only taxon whose members have C_4 photosynthesis, although C_3–C_4 intermediacy has been detected in *P. cryptopetala*, a South American endemic (Voznesenskaya et al., 2010). Furthermore, species of the genus have both NADP-ME and NAD-ME C_4 biochemical variants, and display different types of Kranz leaf anatomy that seem to be correlated with certain clades within *Portulaca* (Voznesenskaya et al., 2010; Ocampo et al., unpublished data).

It has been suggested that *Portulaca* has a base chromosome number of $x = 4$ or 9 (Matthews et al., 1994), while others argue that it is $x = 10$ (Turner, 1994). The most common haploid number (n) is 9, but the range varies from $n = 4$ in *Portulaca suffrutescens* Engelm. (Matthews et al., 1994) to $n = 27$ in certain polyploid subspecies of *P. oleracea* L. (Danin et al., 1978). Polysomaty (different ploidy levels in different organs of the same plant) has been detected in *Portulaca grandiflora* Hook. (Bouharmont, 1965; Mishiba and Mii, 2000). Only two putative hybrids have been reported to occur naturally in the wild (Danin et al., 1978; Kim and Carr, 1990), while artificial hybrids (Bouharmont, 1965; Kim and Carr, 1990; Ichimura and Suto, 1998) have been produced.

The place of origin of *Portulaca* is uncertain, although Geesink (1969) suggested that the ancestral lineage is located in Australia because members of subgen. *Portulacella* (F. Muell.) D. Legrand are found there and have a cymose inflorescence, which he considered ancestral. In support of this, he pointed out that the greatest diversity of species is in the southern hemisphere. Sage (2005) also supported the idea of a southern hemisphere origin of *Portulaca*, but he recognized that the lack of phylogenetic knowledge has been an impediment for hypothesizing a more specific place of origin.

Portulaca is of no major economic importance, except that *P. grandiflora* (with single or multiple whorls of petals) and *P. umbraticola* Kunth are cultivated as ornamentals, and *P. oleracea* is used as vegetable (Mitich, 1997; El Jack, 2004) and is considered one of the most widely distributed and pernicious weeds in the world (Coquillant, 1951; Singh and Singh, 1967; Caton et al., 2004). Because *P. oleracea* is widespread and easily available, much research has focused on its nutritional value (Oliveira and De Carvalho, 1975; Obied et al., 2003), omega-3 fatty acids content (Omara-Alwala et al., 1991; Liu et al., 2000; Simopoulos et al., 2005), and pharmacological effects (Habtemariam et al., 1993; Chan et al., 2000; Radhakrishnan et al., 2001; Chen et al., 2003; Rashed et al., 2003; Karimi et al., 2004; Lim and Quah, 2006).

In this study we provide a hypothesis of the evolutionary relationships among species of *Portulaca* by analyzing DNA sequences from four loci: ITS (nuclear), the *ndhF* gen, the *trnT-psbD* intergenic spacer and the *ndhA* intron (chloroplast). The resulting phylogenetic hypothesis was then used to infer divergence times and historical biogeography of the genus, as well as the evolution of chromosome numbers.

2. Materials and methods

2.1. Taxon sampling

The study includes 80 samples of *Portulaca* representing 59 species, 10 subspecies, and three cultivars (Table 1). The sampled taxa represent all subgenera that have been proposed (von Poellnitz, 1934; Legrand, 1958; Geesink, 1969), and multiple samples are included for some taxa: *Portulaca amilis* Speg. (2), *Portulaca halimoides* L. (2), *P. oleracea* subsp. *granulatostellulata* (Poelln.) Danin & H.G. Baker (2), *P. oleracea.* subsp. *nitida* Danin & H.G. Baker (3), *P. oleracea* subsp. *papilatostellulata* Danin & H.G. Baker (3), *P. pilosa* (4), and *P. quadrifida* (2). The taxonomy of *P. oleracea* has been controversial. While it has been considered by some as a complex composed of multiple subspecies (Danin et al., 1978) or a group of microspecies (Ricceri and Arrigoni, 2000; Danin and Reyes-Betancort, 2006; Danin et al., 2008), others recognize it as a polymorphic taxon (e.g., Legrand, 1962; Matthews et al., 1993; Matthews, 2003). For the purposes of this study, samples that belong to the *P. oleracea* complex were considered at the subspecific level following Danin et al. (1978), except *Portulaca canariensis* Danin & Reyes-Bet. and *Portulaca trituberculata* Danin, Domina & Raimondo, which do not have a subspecific combination.

Relationships within suborder Cactineae remain unclear (e.g., Hershkovitz and Zimmer, 1997; Applequist and Wallace, 2001; Nyffeler, 2007; Nyffeler and Eggli, 2010; Ocampo and Columbus, 2010), and the sister group of Portulacaceae is not known with certainty. Anacampserotaceae, Cactaceae, and Talinaceae are the closest relatives to Portulacaceae; therefore *Talinopsis frutescens* A. Gray, *Pereskia aculeata* Mill., and *Talinum paniculatum* (Jacq.) Gaertn., representing each of these families, respectively, were selected to serve as outgroups.

2.2. DNA extraction, PCR, and sequencing

Sources of DNA included fresh leaf material or leaves dried in silica gel, and, in a few instances, herbarium specimens and DNA aliquots obtained from colleagues. Total genomic DNA was extracted from 10 mg of dried material or 20 mg of fresh tissue using

Table 1
List of taxa and voucher information for samples of *Portulaca* and outgroups used in this study.

Taxon	*Collection number* (herbarium)	Provenance	GenBank accession number			
			ITS	*ndhF*	*trnT-psbD*	*ndhA* intron
P. amilis Speg.	Ocampo et al. 1556 (RSA, SI)	Argentina	JF508527	JF508674	JF508757	HQ241593
	Matthews & Soule s.n. (RSA)	USA	JF508528	JF508675	JF508758	JF508609
P. armitii F. Muell.	Ocampo et al. 1750 (BRI, RSA)	Australia	JF508529	JF508676	JF508759	JF508610
P. australis Endl.	Ocampo et al. 1747 (BRI, RSA)	Australia	JF508531	JF508678	JF508761	JF508612
P. bicolor F. Muell.	Ocampo et al. 1753 (BRI, RSA)	Australia	JF508532	JF508679	JF508762	HQ241594
P. cf. bicolor	Ocampo et al. 1726 (BRI, RSA)	Australia	JF508530	JF508677	JF508760	JF508611
P. biloba Urb.	Carter 18168 (RSA)	USA	JF508533	JF508680	JF508763	JF508613
P. californica D. Legrand	Ocampo & Columbus 1529 (RSA)	Mexico	JF508534	JF508681	JF508764	HQ241595
P. canariensis Danin & Reyes-Bet.	Reyes-Betancort s.n (RSA, from seed)	Canary Islands	JF508535	JF508682	JF508765	JF508614
P. confertifolia Hauman	Ocampo et al. 1619 (RSA, SI)	Argentina	JF508536	JF508683	JF508766	JF508615
P. constricta M.G. Gilbert	Kilian et al. 5916 (B)	Yemen	JF508537	JF508684	JF508767	JF508616
P. cryptopetala Speg.	Ocampo et al. 1540 (RSA, SI)	Argentina	JF508538	JF508685	JF508768	HQ241596
P. decipiens Poelln.	Ocampo et al. 1758 (BRI, RSA)	Australia	JF508539	JF508686	JF508769	JF508617
P. digyna F. Muell.	Ocampo et al. 1749 (BRI, RSA)	Australia	JF508540	JF508687	JF508770	JF508618
P. echinosperma Hauman	Ocampo et al. 1638 (RSA, SI)	Argentina	JF508541	JF508688	JF508771	HQ241597
P. elatior Mart. ex Rohrb.	Ocampo 1708cv (RSA)	Caribbean (cultivated)	JF508542	JF508689	JF508772	HQ241598
P. eruca Hauman	Ocampo et al. 1645 (RSA, SI)	Argentina	JF508543	JF508690	JF508773	JF508619
P. filifolia F. Muell.	Ocampo et al. 1733 (BRI, RSA)	Australia	JF508544	JF508691	JF508774	JF508620
P. fluvialis D. Legrand	Ocampo et al. 1581 (RSA, SI)	Argentina	JF508545	JF508692	JF508775	JF508621
P. foliosa Ker Gawl.	Ocampo 1772cv (RSA)	Tropical Africa (cultivated)	JF508546	JF508693	JF508776	JF508622
P. fulgens Griseb.	Ocampo et al. 1636 (RSA, SI)	Argentina	JF508547	JF508694	JF508777	JF508623
P. giliesii Hook.	Ocampo et al. 1545 (RSA, SI)	Argentina	JF508548	JF508695	JF508778	JF508624
P. grandiflora Hook.	Ocampo et al. 1662 (RSA, SI)	Argentina	JF508549	JF508696	JF508779	JF508625
P. grandiflora Hook. cv.	Ocampo 1403cv (RSA)	Cultivated	JF508550	JF508697	JF508780	JF508626
P. guanajuatensis G. Ocampo	Ocampo 1482 (RSA)	Mexico	JF508551	JF508698	JF508781	HQ241599
P. halimoides L.	Ocampo 1474 (RSA)	Mexico	JF508552	JF508699	JF508782	JF508627
	O'Brien s.n. (RSA)	USA	JF508553	JF508700	JF508783	JF508628
P. hereroensis Schinz	Roodt 223 (PRE)	Botswana	JF508554	JF508701	JF508784	JF508629
P. howellii (D. Legrand) Eliasson	Jaramillo 3332 (CDS)	Galápagos Islands	JF508555	JF508702	JF508785	HQ241601
P. intraterranea J.M. Black	Ocampo et al. 1748 (BRI, RSA)	Australia	JF508556	JF508703	JF508786	JF508630
P. johnstonii Henrickson	Columbus 5076 (RSA)	Mexico	JF508557	JF508704	JF508787	JF508631
P. lutea Sol. ex G. Forster	Morden 1575 (HAW)	Hawaii	JF508558	JF508705	JF508788	JF508632
P. massaica S.M. Phillips	Cruse-Sanders s.n. (RSA)	Tanzania	JF508559	JF508706	JF508789	HQ241600
P. matthewsii G. Ocampo	Ocampo 1425 (RSA)	Mexico	JF508560	JF508707	JF508790	JF508633
P. mexicana P. Wilson	Ocampo & Morales 1461 (RSA)	Mexico	JF508561	JF508708	JF508791	JF508634
P. molokiniensis Hobdy	Perlman 12643 (RSA)	Hawaii	JF508562	JF508709	JF508792	HQ241602
P. mucronulata D. Legrand	Ocampo et al. 1598 (RSA, SI)	Argentina	JF508563	JF508710	JF508793	JF508635
P. oblonga Peter	Mboya 877 (MO)	Tanzania	JF508564	JF508711	JF508794	JF508636
P. obtusa Poelln.	Ocampo et al. 1591 (RSA, SI)	Argentina	JF508565	JF508712	JF508795	JF508637
P. oleracea subsp. oleracea	Guertin 365 (ARIZ)	USA	JF508578	JF508725	JF508808	JF508650
subsp. granulatostellulata (Poelln.) Danin & H.G. Baker	Chhetri 1065 (RSA)	Nepal	JF508569	JF508716	JF508799	JF508641
	Cerros 2493 (RSA)	Dominican Republic	JF508568	JF508715	JF508798	JF508640
subsp. impolita Danin & H.G. Baker	André 8501 (RSA)	USA	JF508570	JF508717	JF508800	JF508642
subsp. nicaraguensis Danin & H.G. Baker	Reyes-Betancort 40511 (ORT)	Canary Islands	JF508571	JF508718	JF508801	JF508643
subsp. nitida Danin & H.G. Baker	Danin & Domina s.n. (PAL)	Italy	JF508573	JF508720	JF508803	JF508645
	Ocampo et al. 1553 (RSA, SI)	Argentina	JF508572	JF508719	JF508802	JF508644
	Thomas 3834 (BRI)	Australia	JF508567	JF508714	JF508797	JF508639
subsp. papillatostellulata Danin & H.G. Baker	Ocampo 1486 (RSA)	Mexico	JF508575	JF508722	JF508805	JF508647
	Ocampo & Columbus 1512 (RSA)	Mexico	JF508566	JF508713	JF508796	JF508638
	Ocampo 1783cv (RSA, from seed)	Afghanistan	JF508576	JF508723	JF508806	JF508648
subsp. sativa (Haw.) Celak.	Danin & Hadjikyriakou CY2004/22a (RSA, from seed)	Cyprus	JF508577	JF508724	JF508807	JF508649
P. oligosperma F. Muell.	Ocampo et al. 1751 (BRI, RSA)	Australia	JF508579	JF508726	JF508809	JF508651
P. papulifera D. Legrand	Ocampo et al. 1569 (RSA, SI)	Argentina	JF508580	JF508727	JF508810	JF508652

P. perennis R.E. Fr.	Ocampo et al. 1606 (RSA, SI)	Argentina	JF508581	JF508728	JF508811	JF508653
P. pilosa L.	Ocampo 1782cv (RSA)	Cuba (cultivated)	JF508584	JF508731	JF508814	JF508656
	Ocampo et al. 1718 (BRI, RSA)	Australia	JF508582	JF508729	JF508812	JF508654
	Nortrup s.n. (UNCC)	USA	JF508585	JF508732	JF508815	HQ241603
	Cerros 2487 (RSA)	Mexico	JF508586	JF508733	JF508816	JF508657
P. pusilla Kunth	Gröger 927 (MO)	Venezuela	JF508587	JF508734	JF508817	JF706266
P. quadrifida L.	Cruse-Sanders s.n. (RSA)	Tanzania	JF508588	JF508735	JF508818	HQ241604
	Cerros 2498 (RSA)	Dominican Republic	JF508589	JF508736	JF508819	JF508658
P. retusa Engelm.	Baker 16325 (ARIZ)	USA	JF508590	JF508737	JF508820	JF508659
P. rotundifolia R.E. Fr.	Ocampo et al. 1611 (RSA, SI)	Argentina	JF508591	JF508738	JF508821	JF508660
P. rubricaulis Kunth	Simá et al. 2433 (MO)	Mexico	JF508592	JF508739	JF508822	JF508661
P. rzedowskiana G. Ocampo	Ocampo 1124 (IEB)	Mexico	JF508593	JF508740	JF508823	JF508662
P. sclerocarpa A. Gray	Morden 1828 (HAW)	Hawaii	JF508594	JF508741	JF508824	HQ241607
P. smallii P. Wilson	Herkenham s.n. (UNCC)	USA	JF508595	JF508742	JF508825	JF508663
P. suffrutescens Engelm.	Ocampo & Columbus 1505 (RSA)	Mexico	JF508597	JF508744	JF508827	JF508665
P. tingoensis J.F. Macbr.	Ocampo et al. 1615 (RSA, SI)	Argentina	JF508598	JF508745	JF508828	JF508666
P. trituberculata Danin, Domina & Raimondo	Danin & Domina SC55 (PAL; RSA from seed)	Italy	JF508574	JF508721	JF508804	JF508646
P. tuberosa Roxb.	Ocampo et al. 1737 (BRI, RSA)	Australia	JF508599	JF508746	JF508829	JF508667
P. umbraticola Kunth subsp. umbraticola	Ocampo et al. 1586 (RSA, SI)	Argentina	JF508603	JF508750	JF508833	JF508670
subsp. coronata (Small) J.F. Matthews & Ketron	Faircloth s.n. (UNCC)	USA	JF508601	JF508748	JF508831	JF508669
subsp. lanceolata J.F. Matthews & Ketron	Ocampo & Columbus 1527 (RSA)	Mexico	JF508602	JF508749	JF508832	HQ241605
P. umbraticola cv. 'wildfire mixed'	Ocampo 1485cv (RSA)	Cultivated	JF508600	JF508747	JF508830	JF508668
P. villosa Cham.	Perlman 13305 (PTBG)	Hawaii	JF508604	JF508751	JF508834	HQ241606
P. wightiana Wall. ex Wight & Arn.	Burgoyne 3613 (PRE)	Namibia	JF508605	JF508752	JF508835	JF508671
P. yecorensis Henrickson & T. Van Devender	Columbus 5006 (RSA)	Mexico	JF508606	JF508753	JF508836	JF508672
Portulaca sp.	Burgoyne 7943 (PRE)	South Africa	JF508583	JF508730	JF508813	JF508655
Portulaca sp. nov.	Ocampo et al. 1754 (BRI, RSA)	Australia	JF508596	JF508743	JF508826	JF508664
Outgroups						
Pereskia aculeata Mill. (Cactaceae)	NA (ZSS)	Americas (cultivated)	JF508526	JF508673	JF508756	HQ241587
Talinopsis frutescens A. Gray (Montiaceae)	Ocampo 1480 (RSA)	Mexico	JF508607	JF508754	JF508837	HQ241613
Talinum paniculatum (Jacq.) Gaertn. (Talinaceae)	Ocampo & Morales 1458 (RSA)	Mexico	JF508608	JF508755	JF508838	HQ241618

the modified CTAB method of Doyle and Doyle (1987) or DNeasy kits (Qiagen, Valencia, California, USA). DNA extracted via the CTAB method was quantified and diluted to a concentration of ca. 10 ng/μl, whereas the concentration of DNA obtained with DNeasy kits was not determined because similar concentrations are usually acheived using this method.

Nuclear ribosomal (ITS, comprising ITS1, the 5.8S gene, and ITS2) and chloroplast (protein-coding *ndhF*, *trnT-psbD* intergenic spacer, and *ndhA* intron) DNA loci were selected for this study. Because there are no previous phylogenetic studies focused on *Portulaca*, two of the molecular markers were selected from the literature on suborder Cactineae. In particular, ITS was used by Hershkovitz and Zimmer (1997), and Applequist and Wallace (2001) employed *ndhF* at the subordinal level. These loci provided informative characters for resolving relationships among the few species of *Portulaca* sampled in these studies. Screening of the fast-evolving chloroplast regions in Shaw et al. (2007) showed that the *trnT-psbD* intergenic spacer and the *ndhA* intron yielded the most informative sites, hence these markers were selected for this study.

Primers used for amplification and sequencing were as follows: ITS—18SF and 26SR (Prince, 2010) and, for herbarium material, internal primers 5.8SF and 5.8SR (Prince and Kress, 2006); *ndhF*—2110R (Prince and Kress, 2006) and 1B, 5C, and 8B (Applequist and Wallace, 2001); *ndhA* intron and *trnT-psbD* intergenic spacer—primers in Shaw et al. (2007), but the latter proved difficult to sequence, hence two sequencing primers were designed (IF3 – GTG ATG GAG AAT GTA TGC GGG and IR1 – TCA ACC ATT TCC GAG CAC CGC). Amplifications were performed in 25 μl reactions with 0.62 unit of *Taq* DNA polymerase (Promega, Madison, Wisconsin, USA), 2.5 μl of ammonium sulfate buffer, 0.5 pM each of forward and reverse primers, 0.25 mM $MgCl_2$, 0.25 mM dNTPs, 0.25 μl of BSA 100X for a final concentration of 1%, and 1 μl of DNA template in a Robocycler 96 or RoboCycler Gradient 96 thermal cycler (Stratagene, La Jolla, California, USA). PCR cycles were as follows: (1) initial denaturation at 94 °C for 4 min, (2) 35 cycles of denaturation at 94 °C for 1 min, primer annealing at 50–54 °C for 1 min, and primer extension at 72 °C for 1 min for ITS, 2.5 min for *ndhF*, and 1.5 min for *trnT-psbD* and *ndhA*, and (3) final elongation for 7 min at 72 °C. PCR products were purified by the PEG precipitation protocol (Johnson and Soltis, 1995) or by adding 3 μl of a solution containing 0.2 μl each of Antarctic phosphatase and exonuclease I (New England Biolabs, Ipswich, Massachusetts, USA) and incubating for 30 min at 37 °C, followed by 20 min at 80 °C. Cycle sequencing was carried out in both directions with ABI Prism Big Dye Terminator solution (Applied Biosystems, Foster City, California, USA) using half the reaction size recommended by the manufacturer. Products were cleaned using Sephadex G-50 columns (GE Healthcare, Anaheim, California, USA), and read on an ABI Prism 3130xl automated sequencer (Applied Biosystems, Foster City, California, USA). Resulting fragments were contiged and edited using Sequencher 4.2.2 (Genes Codes Corp., Ann Arbor, Michigan, USA). ITS sequences were clean and only seven samples contained one polymorphic site, thus no cloning was performed. All but 18 *ndhA* intron sequences in this study were newly generated and deposited in GenBank (Table 1).

2.3. Sequence alignment and phylogenetic analyses

Sequences were aligned using MUSCLE version 3.7 (Edgar, 2004), followed by manual alignment with Se–Al version 2.0a11 (Rambaut, 2002). Individual markers were analyzed using Bayesian inference under Markov Chain Monte Carlo (MCMC; Yang and Rannala, 1997) in MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003) and maximum likelihood (ML; Felsenstein, 1973) in RAxML version 7.2.6 (Stamatakis, 2006). Bayesian and ML analyses were conducted using the best-fit model of evolution provided by MrModeltest version 2.3 (Nylander, 2004) under the Akaike Information Criterion (AIC; Akaike, 1974). The model selected for ITS and *ndhF* was a general time reversible model (GTR; Tavaré, 1986) plus parameters for proportion of invariant sites (I; Reeves, 1992) and a gamma-distributed rate variation (G; Yang, 1993). For *trnT-psbD* and *ndhA* the model selected was GTR + G. Bayesian analyses were run with two replicates for 10,000,000 generations. Trees were saved every 100th generation, and the burn-in value for obtaining a 50% majority-rule consensus tree was set to ignore the first 25% of trees, to only include trees after stationarity was reached. Clade support was determined by Bayesian posterior probabilities (p.p.; Rannala and Yang, 1996; Li et al., 2000) and nonparametric bootstrap (BS; Felsenstein, 1985) from 1000 replicates performed simultaneously with the ML search under the option "–f a".

Because the incongruence length difference test (Farris et al., 1995) has been shown to be problematic (Barker and Lutzoni, 2002), we assessed combinability of the different loci by comparing the topologies and nodal support from the ML and Bayesian analyses. Results of the individual analyses revealed no supported topological conflicts among individual markers (support in this study $\geqslant$ 75% BS and $\geqslant$ 0.95 p.p.), thus a combined data matrix comprising all four loci was assembled, partitioned by locus, and analyzed as explained above. Because RAxML can only employ one model of evolution for a partitioned data set, the ML analysis of the combined data matrix was performed using the GTRGAMMA model (GTR + G).

In addition, we conducted the Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) in RAxML to determine whether the data supports the currently recognized species boundaries of *P. halimoides*, *P. oleracea*, and *P. grandiflora*, which were found to be non-monophyletic. We compared our phylogenetic hypothesis (obtained by the analysis of the combined data matrix) with alternative topologies that enforced monophyly of the aforementioned species, using the topological constraint function also available in RAxML.

2.4. Divergence dates

Estimation of divergence times was accomplished using the program BEAST version 1.5.2 (Drummond and Rambaut, 2007). The combined data matrix was analyzed, assigning the best-fit model of evolution from MrModeltest to each locus. Because of the lack of unambiguous fossil evidence for *Portulaca* and the entire suborder (see Hershkovitz and Zimmer, 2000), calibration of the phylogeny was based on the age of selected Hawaiian islands that have narrowly endemic species (*Portulaca molokiniensis* R.W. Hobdy and *Portulaca sclerocarpa* A. Gray). The divergence between *Portulaca howellii* (D. Legrand) Eliasson and *P. molokiniensis* was set to an age of 1.03 ± 0.18 My, which is the age of Kahoolawe (Naughton et al., 1980), the oldest island where the latter species is found, and the divergence between *P. villosa* Cham. and *P. sclerocarpa* was calibrated at 0.43 ± 0.02 My, the oldest reported age for the island of Hawaii (Kohala volcano; McDougall and Swanson, 1972) to which *P. sclerocarpa* is endemic (for more details see Ocampo and Columbus, 2010). To estimate divergence times, eight independent analyses were run with a relaxed clock (uncorrelated lognormal; Drummond et al., 2006) and a Yule birth rate prior (Drummond and Rambaut, 2007) for 10,000,000 generations, saving every 1000th tree. Trace files were loaded into Tracer version 1.4.1 (Rambaut and Drummond, 2007) to look for an Effective Sample Size > 200 for all parameters sampled from the MCMC. Tree information from the independent analyses was combined and summa-

rized on the Bayesian 50% majority-rule consensus tree produced from the same combined data set, discarding the first 8000 trees as burn-in. FigTree version 1.2.3 (Rambaut, 2008) was used for visualizing the results on divergence dates.

2.5. Historical biogeography

Analysis of potential ancestral distribution areas for clades and taxa of *Portulaca* was conducted in the program S-DIVA version 1.5c (Yu et al., 2010). The software implements a Bayesian approach to dispersal-vicariance analysis (DIVA; Ronquist, 1996), following the method of Nylander et al. (2008) which estimates optimized areas over a set of trees, thereby accounting for uncertainty in the phylogenetic estimate. The Bayes–DIVA analysis was performed using 1000 random trees after the burn-in period from the MrBayes run. Because polytomies in the target tree are not accepted by S-DIVA, the topology of the Bayesian *allcompat* tree of the combined data matrix was used for the analysis. Distribution areas were coded on a large, primarily continental scale, although Central America and the Caribbean islands were treated apart from North America. Because some taxa are widespread, occurring in multiple regions, assigning distribution areas to the places where the samples were collected could potentially bias the reconstruction towards areas that were more heavily sampled for this study. Therefore, in order to use the known distribution ranges of wide-ranging (i.e., in multiple regions) taxa in this analysis, the random trees from the MrBayes run and the Bayesian *allcompat* tree were pruned to include only one sample per taxon and coded as present in multiple areas. Exceptions were *P. halimoides*, *P. pilosa*, and some subspecies of *P. oleracea* with multiple samples that were found to be non-monophyletic (subspp. *granulatostellulata*, *nitida*, and *papillatostellulata*, although support for the relationships among them is lacking), in which case their distributions were coded according to the area where they were collected for this study. Commercially cultivated samples were excluded from the biogeographical analysis. S-DIVA reconstructions were estimated without restrictions on the number of inferred areas at each node.

2.6. Evolution of chromosome numbers

The evolution of chromosome numbers in *Portulaca* was studied using the program chromEvol version 1.2 (Mayrose et al., 2010), which estimates polyploidization and chromosome loss events at ancestral nodes by implementing a series of likelihood models. Besides considering polyploidization and the addition and deletion of individual chromosomes, chromEvol also takes into account demi-polyploidization. The latter is defined as an increase of 1.5× in the chromosome number due to the union of a reduced and an unreduced gamete (Mayrose et al., 2010), giving rise, for instance, to triploid and hexaploid plants. The Bayesian *allcompat* tree was pruned in order to include only those taxa with chromosome numbers reported in the literature, except for *P. grandiflora* and *P. pilosa*, which were found not to be monophyletic and were also excluded. Samples of the three subspecies of *P. oleracea* that were not monophyletic were included in the analysis because there was no strong support for those relationships. The program was run with the default parameters except, because branch lengths are relatively long at the base of the phylogeny, the parameter "_branchMul" was set to 0.01 to re-scale the tree. In instances were a taxon has reports of dissimilar chromosome numbers, the most recent count was used.

MrBayes analyses were run in Bioportal at the University of Oslo (Norway; www.bioportal.uio.no), while RAxML and BEAST analyses were performed using the computer cluster of the Center for Comparative Genomics at the California Academy of Sciences (San Francisco, California, USA).

3. Results

3.1. Phylogenetic analyses

Bayesian trees from separate and combined analyses of sequences from the four loci are shown in Figs. S1-S4 (supplementary figures in the online version) and Fig. 2 (topologies of the ML trees are similar and thus are not shown). In all, *Portulaca* is monophyletic with strong support (⩾90% BS, ⩾0.95 p.p.). With the exception of the ITS analysis, two early divergent clades were recovered: one of opposite-leaved species (OL clade) and another whose species have alternate to subopposite leaves (AL clade). In the ITS trees, the AL clade is resolved, whereas the species with opposite leaves do not form a clade (Fig. S1). Almost every analysis recovered the following monophyletic groups with moderate (75–89% BS) to strong support (shown in Fig. 2): African–Asian and Australian clades within the OL clade; *P. cryptopetala* and Oleracea, Pilosa, and Umbraticola clades within the AL clade, although the relationships among them were not always supported. Analyses of the combined data resulted in a significant increase in clade support compared to analyses of individual markers (Fig. 2). The analyses indicated that *P. grandiflora* (including one wild-collected plant and one cultivated specimen), *P. halimoides*, and *P. oleracea* are not monophyletic, which was confirmed by the SH test (Table 2).

3.2. Divergence dates

Divergence time estimates are shown in a chronogram in Fig. 2. Mean values for the age of the most recent common ancestor (MRCA) and the maximum and minimum values for the 95% highest posterior density interval for selected nodes are presented in Table 3. The age of the MRCA for *Portulaca* is 23 (6.9–43) My. The age of the MRCA for the OL and AL clades are 18.6 (5.7–34.9) and 17.55 (5.2–32.2) My, respectively.

3.3. Historical biogeography reconstructions

Reconstruction of ancestral distribution patterns is shown in Fig. 3. Fifty dispersal events were needed to obtain an optimal reconstruction. The place of origin of *Portulaca* was not recovered with certainty but three of the four possibilities are in the southern hemisphere (Africa, Asia, Australia, and South America). A similar pattern, but restricted to the Old World, was observed for the OL clade, with equal probability values for the inclusion/exclusion of Asia. In contrast, South America was estimated as the ancestral area for the AL clade, *P. cryptopetala* and the Oleracea, and Pilosa clades with high probability values. The ancestral area for the Umbraticola clade was recovered as widely distributed in North and South America. Multiple long-distance events are frequent inside the AL clade, which has representatives in all distribution areas as delimited in the study. Species of the OL clade are confined to the Old World and Australia, although *P. quadrifida* is found in the Caribbean, apparently derived from a dispersal event from Africa or Asia.

3.4. Evolution of chromosome numbers

The model favored by chromEvol was "Linear_rate_demi" (log-likelihood = −128.3, AIC = 266.5) that takes into account equal rates of polyploidy and demi-polyploidy, and the gains and losses are linearly dependent on the current chromosome number (Mayrose et al., 2010). The inferred ancestral chromosome numbers are shown in Fig. 4. The estimated base number for *Portulaca* was $x = 9$ (0.99 probability). The estimated chromosome number

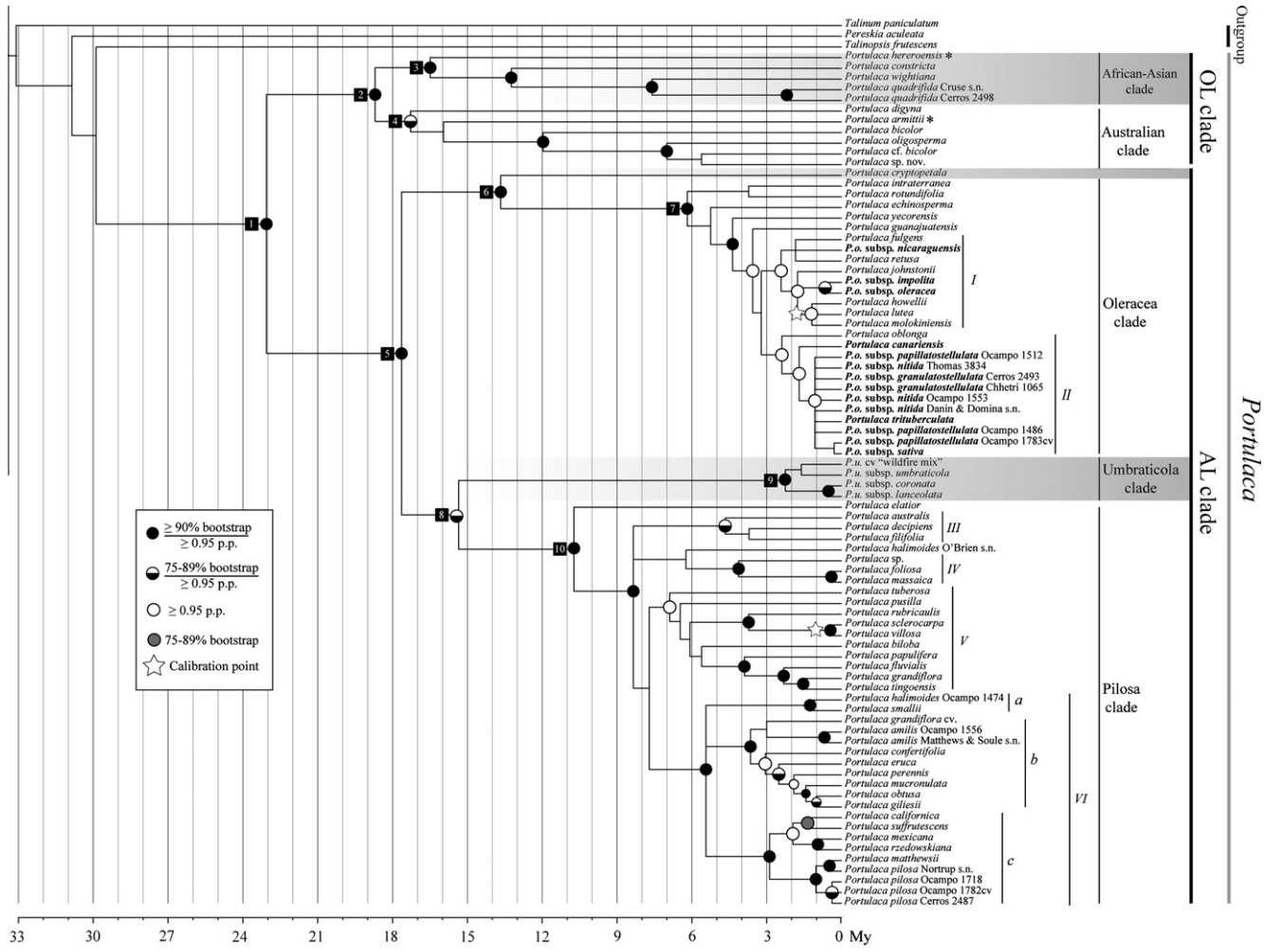

Fig. 2. Chronogram of *Portulaca*. Dates (in millions of years) obtained from a BEAST (Drummond and Rambaut, 2007) analysis of a combined matrix of ITS, *ndhF*, *trnT-psbD* spacer, and *ndhA* intron sequences. Results are displayed on the Bayesian 50% majority-rule tree inferred from the combined data set. Stars indicate calibration points. Nodes with BS values ⩾75% and posterior probabilities ⩾0.95 are indicated. Ages of selected nodes (black boxes) are provided in Table 3. *P.o.* = *Portulaca oleracea*; *P.u.* = *Portulaca umbraticola*. Names in bold indicate the segregate species or subspecies of *P. oleracea*. ∗ = species that have been treated by some botanists as members of *Sedopsis*.

Table 2
Results of testing alternative phylogenetic hypotheses using the Shimodaira–Hasegawa (SH) test in RAxML (Stamatakis, 2006).

Hypothesis	Likelihood	Difference in likelihood	Significant with SH test?	Outcome
Best ML tree	−23465.324837	NA	NA	NA
P. grandiflora monophyly	−23716.459642	−251.134805	Yes	Reject
P. halimoides monophyly	−23659.979311	−194.654475	Yes	Reject
P. oleracea monophyly	−23725.862678	−260.537842	Yes	Reject

Table 3
Estimated ages for the most recent common ancestor (MRCA) of *Portulaca* and selected clades, expressed in millions of years. Nodes labeled in Fig. 2.

Node	MRCA of	95 % Highest posterior density		
		Mean	Lower	Upper
1	*Portulaca*	23	6.9	43
2	OL clade	18.6	5.7	34.9
3	African–Asian clade	16.4	4.9	30.8
4	Australian clade	17.2	5.2	32.2
5	AL clade	17.5	5.2	32
6	*P. cryptopetala* + Oleracea clade	13.6	3.8	25.3
7	Oleracea clade	6.2	2	11.6
8	Umbraticola + Pilosa clade	15.2	4.5	28.2
9	Umbraticola clade	2.2	0.3	4.8
10	Pilosa clade	10.7	3	19.8

change events are shown in Table 4. Tetraploids and hexaploids were only found in the Oleracea and Umbraticola clades.

4. Discussion

4.1. Phylogenetic relationships

The present study significantly expands the sampling of *Portulaca* used in previous studies (Hershkovitz and Zimmer, 1997; Applequist and Wallace, 2001; Nyffeler, 2007; Nyffeler and Eggli, 2010; Ocampo and Columbus, 2010) and again shows that the genus is monophyletic. In addition, results from analyses of all loci except ITS (Fig. S1) are congruent with the findings of Ocampo and Columbus (2010) in that there are two major early divergent clades within *Portulaca* (AL and OL clades; Figs. S1–S4 and Fig. 2). The

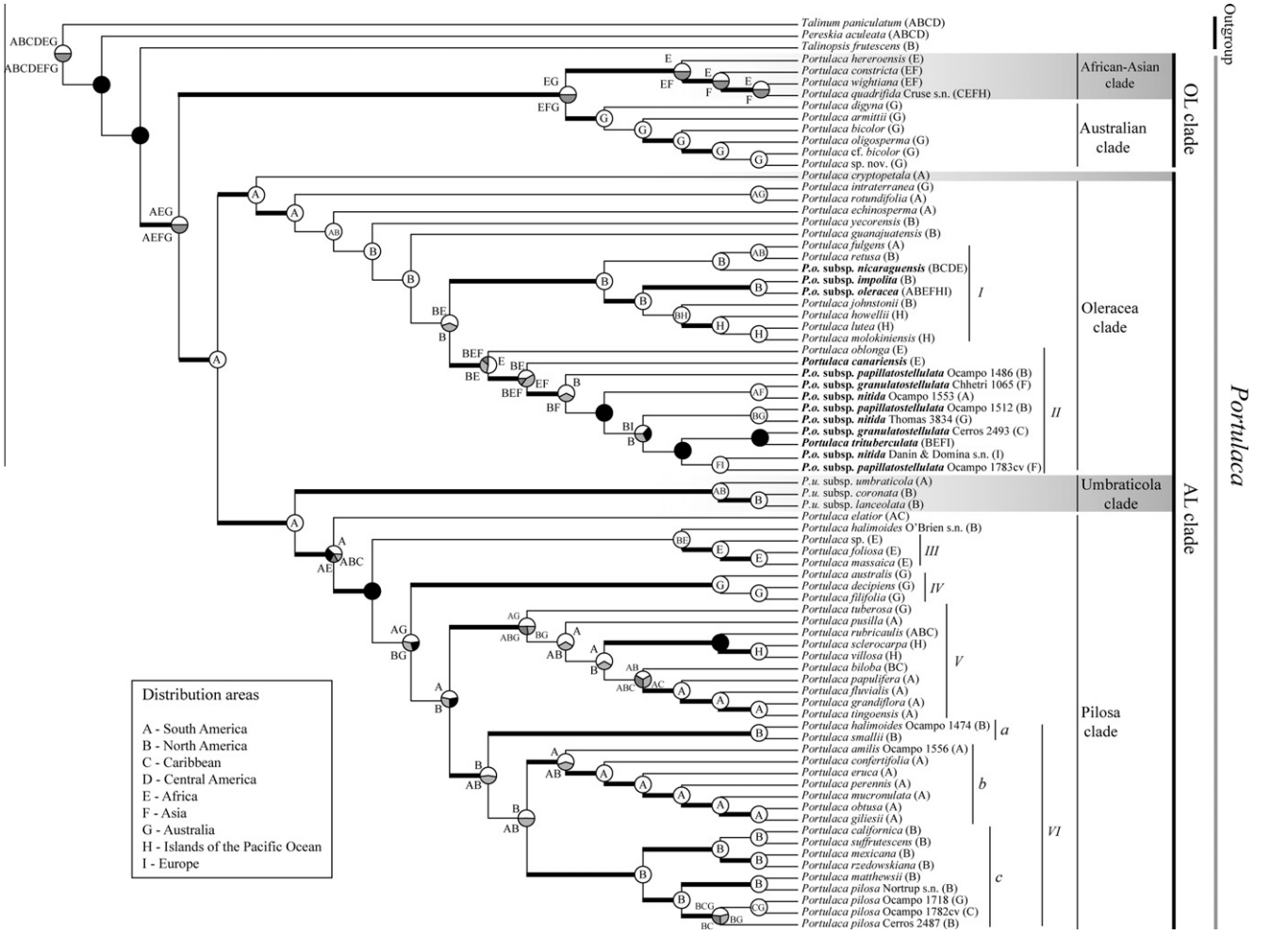

Fig. 3. Biogeographical analysis of *Portulaca*. Pruned Bayesian *allcompat* tree from analysis of a combined matrix of ITS, *ndhF*, *trnT-psbD* spacer, and *ndhA* intron sequences. Biogeographical reconstructions are displayed in the form of a pie chart at each node, representing the probability for each alternative ancestral area derived from a dispersal-vicariance analysis (DIVA; Ronquist, 1997) as implemented in the program S-DIVA (Yu et al., 2010) and optimized over 1000 trees randomly sampled from the MrBayes run (Ronquist and Huelsenbeck, 2003). The analysis included one sample per taxon, except when a taxon did not resolve as monophyletic (*Portulaca halimoides*, *P. pilosa*, and *P. oleracea.* subsp. *granulatostellulata*, subsp. *nitida*, and subsp. *papillatostellulata*). Commercially available cultivars were excluded from the analysis. Black portions of the pie charts represent multiple reconstructed ancestral ranges with similar probability values. Letters after each taxon name represent its distribution. Thicker branches represent ⩾0.95 posterior probabilities. *P.o.* = *Portulaca oleracea*; *P.u.* = *Portulaca umbraticola*. Names in bold indicate the segregate species or subspecies of *P. oleracea*.

relationships among the species were not always supported in the analyses of individual loci, but the combined data matrix provided a substantially more resolved and better supported phylogeny. Therefore, the discussion will focus on phylogenetic relationships resulting from analyses of the combined data matrix.

The subgeneric circumscriptions proposed by von Poellnitz (1934) and Legrand (1958) are not reflected in the phylogeny, whereas Geesink's (1969) classification is supported to some extent. Geesink divided *Portulaca* into two subgenera, *Portulaca* and *Portulacella*, mainly characterized by flowers solitary or arranged in heads in the former versus cymose inflorescences in the latter. Subgenus *Portulacella* is monophyletic in our study and is equivalent to the African–Asian clade, but the position of this clade renders subgen. *Portulaca* (=all other clades) paraphyletic (Fig. 2).

A new classification of the genus based on the phylogenetic evidence will be presented elsewhere. Here, we discuss the phylogeny in detail with respect to the existing taxonomy, morphology, geography, and chromosome number variation.

4.1.1. Opposite-leaved clade

The OL clade, strongly supported as monophyletic, has species with opposite leaves that are distributed in Africa, Asia, and Australia. The exception is *P. quadrifida*, which is a pantropical weed, including the Caribbean. The OL clade is formed of two well-supported sister lineages, the African–Asian clade and Australian clade.

4.1.1.1. African–Asian clade. Although the taxon sample is limited, the African–Asian clade corresponds to Geesink's (1969) sect. *Neossia*, although he positioned it in subgen. *Portulaca* because of the presence of solitary flowers or a few flowers arranged in heads. Geesink considered the contracted inflorescence to be homologous to that found in subgen. *Portulaca*; however, according to the phylogenetic relationships, flowers arranged in heads may have evolved independently in *Portulaca*. The African–Asian clade is also characterized by the presence of hairs and/or scales around the stem nodes. The scales seem to be formed of connate hairs, although further study is needed to verify this. This group is especially diverse in Africa, with ca. 14 species, but is also found in Asia (*P. dhofarica* M.G. Gilbert, *P. kuriensis* M.G. Gilbert, and *P. wightiana*; Gilbert and Phillips, 2000), Australia (*P. clavigera* Geesink), and one species is a pantropical weed (*P. quadrifida*). The inclusion of *P. clavigera*, the only native Australian member of the group and known just from the type specimen, in future

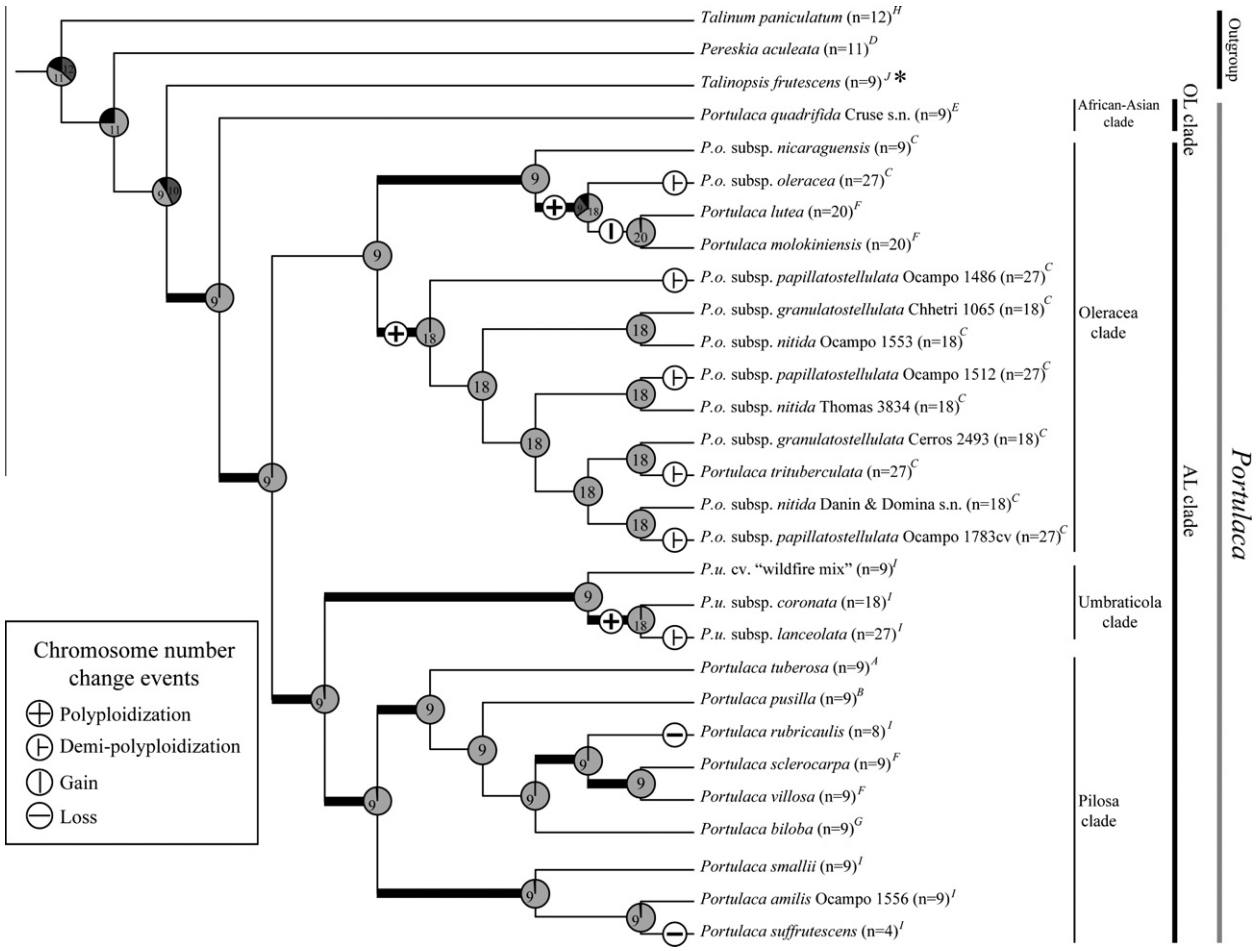

Fig. 4. Chromosome number evolution in *Portulaca* inferred from chromEvol (Mayrose et al., 2010) over a pruned Bayesian *allcompat* tree from a combined matrix of ITS, *ndhF*, *trnT-psbD* spacer, and *ndhA* intron sequences. Only taxa with reported chromosome numbers are included. Excluded were *P. grandifolia* and *P. pilosa* because they are not monophyletic in this study. The subspecies of *P. oleracea* that are not monophyletic (subspp. *granulatostellulata*, *nitida*, and *papillatostellulata*) were included because the relationships among them were not supported. Inferred ancestral haploid numbers (*n*) are displayed in a pie chart at each node, representing the probability of a particular haploid number. Black portions of the pie charts represent three or more haploid numbers with similar probability values. A summary of chromosome number change events is provided in Table 4. References for chromosome numbers: [A] Raghavan and Srinivasan, 1941; [B] Steiner, 1944; [C] Danin et al., 1978; [D] Leuenberger, 1986; [E] Nyananyo, 1987; [F] Kim and Carr, 1990; [G] Matthews et al., 1992; [H] Xu et al., 1992; [I] Matthews et al., 1994; [J] Rowley, 1994. *The chromosome number for *Talinopsis frutescens* (Anacampserotaceae) is not known, but the base number for the family is $x = 9$ (Rowley, 1994), which is used here for the analysis. Thicker branches represent $\geqslant 0.95$ posterior probabilities. *P.o.* = *Portulaca oleracea*; *P.u.* = *Portulaca umbraticola*.

Table 4
Chromosome number changes in *Portulaca*, as estimated by chromEvol (Mayrose et al., 2010).

Event	Number	Clade (number of events)
Polyploidization	3	Oleracea (2), Umbraticola (1)
Demi-polyploidization	6	Oleracea (5), Umbraticola (1)
Gain	1	Oleracea (1)
Loss	2	Pilosa (2)

studies is of particular interest to determine if it belongs to this clade.

The genus *Sedopsis* was created by Exell and Mendoça (1937) to accommodate the African *S. saxifragoides* and *S. sedoides* which have flowers with an evident corolla tube and four stamens. Neither species could be sampled for this study. However, in spite of the shared floral traits, these species differ in stem pubescence and inflorescence type: hairs around the stem nodes and flowers in heads in *S. saxifragoides*, and reduced leaf axil hairs and flowers in cymose inflorescences in *S. sedoides*. Because these characters are consistent with the African–Asian clade and Australian clade (discussed below), respectively, we predict that the two species will resolve apart in these clades (i.e., that *Sedopsis* is not monophyletic). *Portulaca armitii* F. Muell. (Australian clade) and *P. hereroensis* Schinz (African–Asian clade) each have an evident corolla tube and were included in this study. Results indicate that they belong to different lineages, providing direct evidence that the corolla tube evolved at least twice. Expanded sampling within the OL clade will enhance our understanding of the evolution of the floral tube in *Portulaca*.

4.1.1.2. Australian clade. Results of this study agree, with moderate (ML) to strong (Bayesian inference) statistical support, with Geesink's (1969) recognition (as subgen. *Portulacella*) of a group of about six Australian species that have highly reduced hairs in the leaf axils and flowers arranged in cymes. Some authors have described plants in this group as glabrous (e.g., Legrand, 1958, 1962; Geesink, 1969), but inconspicuous axillary hairs are present (von Poellnitz, 1934; see also Ogburn and Edwards, 2009). This study does not include *P. rhodesiana* R.A. Dyer & E.A. Bruce and *P. sedoides* Welw. (*S. sedoides*), African taxa with cymose inflorescences and flowers with evident corolla tubes that may belong to this clade. Further studies that incorporate these species are desirable in order to determine their place in the phylogeny, which will

contribute to the understanding of corolla evolution in this group. Voznesenskaya et al. (2010) reported a NADP-malic enzyme (NADP-ME) C_4 biochemistry with Portulacelloid-type anatomy in the Australian *P.* cf. *armitii*. This anatomical configuration has been found in other members of the OL cladeand may represent a synapomorphy for the group (Ocampo et al., unpublished data).

4.1.2. Alternate to subopposite-leaved clade

The AL clade is strongly supported as monophyletic and sister to the OL clade (Fig. 2). Members of this monophyletic group have alternate to subopposite leaves and flowers arranged in heads. This clade, which is consistent with Geesink's sect. *Portulaca* (1969), has the largest number of species of the family [more than 100 according to Legrand (1958)] and is distributed worldwide. This lineage has as members *P. cryptopetala* and three principal strongly-supported groups: the Oleracea, Umbraticola and Pilosa clades. The relationships among them are moderately to strongly supported, and the latter two were found to be sister to *P cryptopetala* + Oleracea clade (Fig. 2). These major phylogenetic groups within the AL clade are discussed below.

4.1.2.1. Portulaca cryptopetala. This South American species is strongly supported as sister to the Oleracea clade, though it has morphological, anatomical, and photosynthetic features that differentiate it from the species of its sister group. *Portulaca cryptopetala* has conspicuous axillary hairs and is the only member of *Portulaca* that is a C_3–C_4 intermediate (Voznesenskaya et al., 2010) with C_3 leaf anatomy (Voznesenskaya et al., 2010; Ocampo and Columbus, 2010), which are unique in a genus of otherwise Kranz (C_4) species. The species has been collected in Argentina, Bolivia, Brazil, Paraguay, and Uruguay and is usually associated with riparian environments. Legrand (1962) considered it a very variable species; he recognized five varieties mainly based on petal coloration and seed micromorphological features [the specimen used in this study may correspond to *P. cryptopetala* var. *poellnitziana* (D. Legrand) D. Legrand.]. Further work is warranted to study the species throughout its distribution range to confirm monophyly and to verify its known photosynthetic characteristics.

4.1.2.2. Oleracea clade. This group, which comprises ca. 20 species distributed around the world, is strongly supported as monophyletic and is consistent with Geesink's (1969) subsect. *Portulaca*. They have subopposite leaves (except *P. echinosperma*, with alternate leaves) with short axillary hairs that are often inconspicuous. Members of this group usually have an apical constriction of the capsule that envelopes a few seeds, a feature seen elsewhere only in *P. constricta* M.G. Gilbert (African–Asian clade); in addition, their sepals often are keeled. Voznesenskaya et al. (2010) reported a NAD-ME activity and an Atriplicoid-type leaf anatomy for *P. molokiniensis* and *P. oleracea*.

Early-divergent members of this group include taxa endemic to Australia (*P. intraterranea*), North America (*P. guanajuatensis* and *P. yecorensis*), and South America (*P. echinosperma* and *P. rotundifolia*). A basal grade is formed by these five species, except *P. intraterranea* and *P. rotundifolia,* which were recovered as sisters, although with low support. *Portulaca yecorensis* (an annual with distinctive red stems and leaves) and *P. guanajuatensis* (a perennial with a napiform root), narrow endemics from northern and central Mexico, respectively, were sequentially sister to the rest of the members of the clade.

Within the Oleracea clade is the *P. oleracea* complex of 15 taxa (nine sampled in this study) that are morphologically quite similar to one another and are treated as segregate species or subspecies of *P. oleracea* based on chromosome number, seed size, and micromorphological differences (Danin et al., 1978; Ricceri and Arrigoni, 2000; Danin and Reyes-Betancort, 2006; Danin et al., 2008). The phylogenetic reconstruction does not support the monophyly of *P. oleracea*, samples of which resolve in clades *I* and *II* (Fig. 2), discussed below. Although the relationships between and within these clades have lower support compared to many other areas of the *Portulaca* phylogeny, the SH test also rejected the monophyly of the *P. oleracea* as currently circumscribed.

Clade *I* comprises mainly taxa with restricted distributions, usually found in undisturbed habitats, though two are widely distributed. Endemic New World taxa include the perennial, high-altitude *Portulaca fulgens* Griseb. from Argentina, and annual species endemic to arid regions of the southern USA and northern Mexico: *Portulaca johnstonii* Henrickson (with long, flat, and acute projections on the margins of the seeds), *P. oleracea* subsp. *impolita* Danin & H.G. Baker, and *Portulaca retusa* Engelm. (the latter sometimes considered a synonym of *P. oleracea*, although it is distinguished by strongly tuberculate seeds with a metallic coloration). In addition, there is a clade of species restricted to Pacific Ocean islands: *P. howellii* (Galápagos), *P. molokiniensis* (Hawaii), and *P. lutea* Sol. ex G. Forster (commonly found throughout the Pacific Ocean insular system). Widely distributed taxa with weedy behavior include *P. oleracea* subsp. *nicaraguensis* Danin & H.G. Baker (characterized by a bluish wax covering the seed), which ranges from Florida and the Caribbean to the Yucatán Peninsula and Central America, and recently was reported from the Canary Islands (Danin and Reyes-Betancort, 2006; a sample from there was included in this study), and subsp. *oleracea*, which has been collected throughout the world and is supported as sister to the North American subsp. *impolita*. Danin et al. (2008) determined that the type of *P. oleracea* (LINN 625.1) corresponds to subsp. *stellata* Danin & H.G. Baker (sensu Danin et al., 1978); therefore, they concluded that subsp. *oleracea* should be applied to what has been called subsp. *stellata* or *P. stellata* (Danin & H.G. Baker) Ricceri & Arrigoni, and specimens formerly attributed to subsp. *oleracea* should be called *P. trituberculata* (recovered in Clade *II*, see below), which is followed here.

Clade *II*, on the other hand, includes taxa that occupy naturally disturbed and/or human-modified habitats, with the exception of *P. canariensis*, which seems to prefer undisturbed places (Danin and Reyes-Betancort, 2006). There is support for *P. canariensis* and *P. oblonga* Peter, both African taxa, as sequentially basal to the rest of the clade. The remaining samples belong to *P. trituberculata* and a number of subspecies of *P. oleracea*, but relationships among them are unresolved in this study. The subspecies and segregate species of *P. oleracea* are circumscribed according to seed micromorphology and size (the latter has been proposed to be positively correlated with ploidy level) (Danin et al., 1978; Danin and Reyes-Betancort, 2006; Danin et al., 2008), but other authors have suggested that seed characters are unreliable for taxonomy in the group (Legrand, 1962; Matthews and Levins, 1986; Matthews et al., 1993, 1994). The results of this study could not confirm the monophyly of the subspecies in Clade *II*, thus the taxonomic utility of seed features in this group could not be established.

There are no known diagnostic morphological features to differentiate members of Clade *I* from Clade *II*, but it is clear that the group that Danin et al. (1978) and subsequent studies considered as the *P. oleracea* complex is not monophyletic. In order to study the evolution of this group, other species have to be considered (especially taxa from Clade *I* that were not included in previous studies), and multiple samples of the same subspecies (including those not sampled here) must be taken into account. In addition, future research must use additional data that can clarify the relationships among these taxa, so questions of monophyly and rank can be addressed. For the time being, description of new taxa and nomenclatural transfers from the subspecific to specific rank (e.g., Ricceri and Arrigoni, 2000; Danin and Reyes-Betancort, 2006; Danin et al., 2008) are strongly discouraged until new insights into the evolution and morphology of these taxa are obtained.

4.1.2.3. Umbraticola clade. Geesink (1969) ambiguously mentioned the annual *P. umbraticola* as member of subsect. *Stellulatotuberculatae*. The presence of a wing at the dehiscence line of the capsule is a potential synapomorphy for this clade that has *P. umbraticola* as its only member. The plants are usually erect, often with limited branching, and have highly reduced hairs in the axils of the alternate leaves. The species is distributed from the southern USA to Argentina and tends to be weedy. Three subspecies are recognized, differing mainly by flower color. Subspecies *coronata* (Small) J.F. Matthews & Ketron and *lanceolata* J.F. Matthews & Ketron are endemic to North America, while subsp. *umbraticola* is found in South America. The widely used cultivar *P. umbraticola* 'wildfire mixed' (Matthews et al., 1992) produces flowers of many colors, and is sister to subsp. *umbraticola* (though without support), possibly indicating that the cultivar was selected from individuals of this subspecies, as suggested by Legrand (1962). Sister to this clade are the North American subspecies, which are strongly supported as sisters. An Atriplicoid-type leaf anatomy and NADP-ME C_4 activity has been reported for this lineage (*P. umbraticola* 'wildfire mixed'; Voznesenskaya et al., 2010).

4.1.2.4. Pilosa clade. This strongly supported lineage includes *P. pilosa*, a species that was placed by Geesink (1969) in subsect. *Stellulatotuberculatae* along with *P. macrorrhiza* Geesink (not sampled here). He recognized an extraordinary amount of morphological variability in *P. pilosa*, referring to it as the *P. pilosa* complex and reduced many species to synonymy and subspecific rank (Geesink, 1969). There are no potential morphological synapomorphies for the Pilosa clade, although all species have alternate leaves with conspicuous hairs in the axils, features also found in *P. cryptopetala*. The flowers are generally arranged in heads, although there are some cases where the capitulum is reduced to a very few flowers or the flowers are solitary (*P. pusilla* Kunth). Members of this clade (*P. amilis*, *P. grandiflora*, and *P. pilosa*) have been reported to have a Pilosoid-type leaf anatomy with a NADP-ME C_4 activity (Voznesenskaya et al., 2010).

The analyses show *P. elatior* Mart. ex Rohrb., a species distributed in northern South America and the Caribbean, as the earliest diverging member of the Pilosa clade. Within the clade there are four major clades (labeled *III–VI* in Fig. 2) with moderate to strong nodal support, although the relationships among them are uncertain. Though there are no clear morphological synapomorphies for these clades, some have a well-defined geographical distribution.

Clade *III* is a group of endemic Australian species that were treated by Geesink (1969) as conspecific with *P. pilosa*, although *P. decipiens* Poelln. was recognized as a subspecies. However, these species represent a lineage distinct from *P. pilosa* (Clade *VI*, Fig. 2).

Clade *IV* includes exclusively African species, and the phylogenetic analyses include the yellow-flowered *P. halimoides* collected in the North American Mojave Desert (*O'Brien s.n.*, from California, USA), as its early divergent lineage; however, this position is uncertain due to the lack of support. In this study, the two samples identified as *P. halimoides* do not form a clade. The species is characterized by its small flowers and pedicellate small capsules. The white-flowered sample of the species collected in central Mexico (from rock oucrops within a disturbed oak forest) was recovered as member of Clade *VI* (see below). The *O'Brien s.n.* sample may correspond to *P. parvula* A. Gray, treated as a synonym of *P. halimoides* by Legrand (1962) and Matthews (2003). Future studies should include multiple samples of what is considered as *P. halimoides* from its known distribution (southern USA and the Caribbean to Brazil), in order to evaluate the taxonomic status of both species.

Clade *V* is supported only by the Bayesian analysis, where *P. tuberosa* Roxb. [another Australian species considered by Geesink (1969) to be a synonym of *P. pilosa*] and *P. pusilla* (an Amazonian species from Colombia and Venezuela, treated by Legrand (1958, 1962) in subgen. *Portulacelloides*, and tentatively placed by Geesink (1969) as a member of subgen. *Portulaca*) have an uncertain placement due to a lack of support. The remaining species are distributed in the New World with exception of two Hawaiian endemics (*P. sclerocarpa* and *P. villosa*) that form a clade sister to *P. rubricaulis*, which ranges from Florida through the Caribbean to southeastern Mexico and north coast of South America. Coelho et al. (2010) synonymized *P. rubricaulis* under *P. halimoides*, although our findings indicate they are independent lineages. In addition, the characteristically sessile, relatively large capsule and the usually depressed capsule lid of *P. rubricaulis* Kunth (Ocampo, 2003) contrasts with the hemispheric lid of the small, pedicellate capsule of *P. halimoides*, which, as mentioned above, is not monophyletic as currently circumscribed. Interestingly, the two samples of *P. grandiflora* included in this study fall in two different places inside the Pilosa clade (Fig. 2). The wild-collected sample is in clade *V*, nested within a group of South American species. The cultivar sample is in Clade *VI* (discussed below).

Clade *VI* is divided into three main clades (labeled *a–c* in Fig. 2) and all are strongly supported, although relationships among them are unresolved. Clade *a* is formed of *P. halimoides* (*Ocampo 1474*) and *Portulaca smallii* P. Wilson, rock outcrop species from Mexico and the southeastern USA, respectively. *P. smallii* has been considered by some authors to be derived from *P. pilosa* (Cotter and Platt, 1959; Matthews and Levins, 1985), although the molecular phylogenies indicate they belong to different lineages.

Clade *b* is composed of South American species, although *P. amilis* is recently introduced to North America (Judd and Wunderlin, 1981; Bennett, 1982; Matthews and Levins, 1985). *Portulaca grandiflora* is not monophyletic: a sample of a cultivated plant resolved in clade *b*, while a sample from the wild is in Clade *V* (Fig. 2). The cultivated sample used in this study comes from a plant in central Mexico (Querétaro) having magenta flowers and one whorl of petals. The species is considered to have a number of cultivars with flowers of different colors and some are characterized by having flowers with a double whorl of petals (Legrand, 1962). Future phylogenetic studies should include more samples of wild-collected *P. grandiflora* and cultivars to determine origins and clarify taxonomy. This is especially important because the species is often used in studies (e.g., Bouharmont, 1965; Ku et al., 1981; Mishiba and Mii, 2000; Guralnick et al., 2002; Muhaidat et al., 2007; Ogburn and Edwards, 2009), yet few clearly indicate the origin of the sample (e.g., Nyananyo, 1986). It is likely these were cultivars because they are easy to obtain, and not wild-collected plants from South America.

Clade *c* includes North American species and *P. pilosa*, which is distributed worldwide. The group is strongly supported as monophyletic, and *P. matthewsii* and *P. pilosa* are found as sister to the clade formed by the remaining species. The latter clade comprises perennial species with tuberous or fasciculate roots (except *P. californica* D. Legrand) and yellow flowers (exception *P. rzedoskiana* G. Ocampo, with magenta flowers). It has been suggested that *P. mexicana* P. Wilson and *P. pilosa* are conspecific (Rzedowski, 2001), but our results suggest that although samples of those taxa are found in the same clade, they correspond to different lineages.

All samples determined as *P. pilosa* in this study form a clade with *P. matthewsii* G. Ocampo from central Mexico. All have fibrous roots, magenta flowers, and the lid of the capsule is hemispheric except in *P. matthewsii*, which has a campanulate lid; further study is needed to investigate the taxonomic status of *P. matthewsii*. *Portulaca pilosa* has been considered a morphologically variable species, and a number of infraspecific taxa have been erected to account for this diversity (e.g., Legrand, 1962; Geesink, 1969); however, some of these taxa are found not to be closely related to *P. pilosa* [e.g., *P. decipiens* (Clade *III*) and *P. tuberosa* (Clade *V*) that

were considered by Geesink (1969) as a subspecies and as a synonym of the type subspecies, respectively]. Future studies should include multiple samples from its known worldwide distribution, although special caution should be exercised because it is not unusual to find *Portulaca* specimens misidentified as *P. pilosa*.

4.2. Portulaca: A relatively young lineage with successful intercontinental dispersal

Divergence times in the genus were calculated using estimated ages for specific Hawaiian islands, as done in other studies (e.g., Chacón et al., 2006; Ocampo and Columbus, 2010; VanderWerf et al., 2010), because the ages of particular islands or groups of islands can be correlated with some narrow endemic species of *Portulaca*. This approach was employed here due to the absence of an unambiguous fossil record for Cactineae (see Hershkovitz and Zimmer, 2000). Van Campo (1976) reported the existence of *P. oleracea* pollen in the Miocene of Europe; however, pollen morphology for the family is homogeneous, making it difficult to identify samples to species level, and can be confused with that of Nyctaginaceae and Polygonaceae (Erdtman, 1952). Photographs of putative *P. oleracea* pollen in Van Campo (1976) were examined for this study (1976, Plate 24, Figs. 18 and 19), but the generic determination of the sample could not be confirmed. In the absence of an unambiguous fossil record for the genus and suborder, calibrating the phylogeny using the geological record of some Hawaiian islands seems appropriate, pending new evidence that may improve our knowledge of the temporal scale for relationships within *Portulaca*. However, the estimated lineage ages resulting from this study should be interpreted with care due to the wide range of the 95% highest posterior distributions (Table 3), and because dating nodes with these Hawaiian "hotspots" includes the possibility that the selected taxa existed before the rise of the volcanic islands (see Heads, 2005). In addition, it has been suggested that this calibration method may underestimate ages in the tens of millions of years (Heads, 2009).

Divergence time analysis puts the age of the MRCA of *Portulaca* at 23 (6.9–43) Myr. This date contrasts with the estimate of ca. 9 Myr obtained by Ocampo and Columbus (2010) using the same calibration strategy, and the results of Christin et al. (2011) who calibrated their phylogeny with fossils of taxa outside of Cactineae. This difference in ages is consistent with other studies that have shown a larger taxon sample may produce older estimates (e.g., Linder et al., 2005; Pirie et al., 2005). The age of the stem node of *Portulaca* (ca. 30 Myr; Fig. 2) agrees with Christin et al. (ca. 28 Myr; 2011), who showed that C_4 eudicots are not younger than their monocot counterparts.

Biogeographical analysis should also be interpreted with care due to topological ambiguity. Some ancestral area reconstructions presented in this study may change if alternative topologies are considered, especially in those subclades where the terminal taxa were coded for different regions (e.g., relationships within the Oleracea clade). In our study, results are inconclusive about the place of origin of *Portulaca* (Fig. 3) but do suggest that the ancestral area at ca. 23 Myr (Miocene) was in the southern hemisphere, less likely Asia. Sage (2005) suggested a southern hemisphere origin of C_4 photosynthesis for *Portulaca*, due to the high diversity of species in that part of the world. The ages of the MRCA of the OL and AL clades are 18.6 (5.7–34.9) Myr and 17.5 (5.2–32) Myr (Fig. 2), respectively, and the geographic analysis indicates that these lineages were isolated from each other at that time (Fig. 3).

The place of origin of the OL clade is inconclusive as well, but Africa, Asia, and Australia—to which the sampled OL species except one are restricted—are all suggested; only *P. quadrifida*, a pantropical weed, strays beyond these continents, but its nested position within the clade points to a more recent introduction in the Caribbean. Sampled species in the African–Asian and Australia clades occur only in their respective regions, which would suggest no interchange, but other species in these regions remain to be sampled, in particular the African *P. rhodesiana* and *P. sedoides* (putative members of the Australian clade) and the Australian *P. clavigera* (predicted to belong to the African clade).

The MRCA of the AL clade is shown to have originated in South America (Fig. 3). The continents were almost in their present positions at the time of the estimated age of origin of the clade, thus long-distance dispersal is invoked to explain the current distributions of the descendent lineages, which is consistent with the high number of dispersal events (50) estimated by the Bayes–Diva approach to obtain the optimal reconstruction. The main dispersal events from South America to other regions, particularly to North America, took place within the Oleracea and Pilosa clades during the Miocene and Pliocene (ca. 11–4 Myr; Figs. 2 and 3). Diversification and dispersal of *P. umbraticola* to North America was not achieved until the Pliocene–Pleistocene transition (2.2 Myr), perhaps facilitated by the Central American land bridge established ca. 3 Myr (Coates and Obando, 1996). Major dispersal events from North America to other regions also occurred, including dispersals back to South America (*P. fulgens*). Species of the Oleracea and Pilosa clades colonized Africa, Australia, and Hawaii multiple times and diversified into local lineages (Fig. 3). Our results agree with Wagner et al. (1999) that the Hawaiian endemics are the result of two independent colonization events. As well, the inferred intercontinental dispersals are consistent with Clayton et al. (2009), who showed that long-distance dispersal among continents was common after the late Oligocene.

The geographical origin of *P. oleracea* has been a controversial topic. Africa is one hypothesis (Ridley, 1930), which contrasts with the suggestion that *P. oleracea* is European or Asian (e.g., Bryant, 1783; DeCandolle, 1886; Williamson, 1905; Gray et al., 1908; Frankton, 1955; Taylor, 1990; Hernández Bermejo and León, 1994; Whitson et al., 2001) or from the New World (Rusby, 1921). Some have said the species was introduced to the New World (DeCandolle, 1886; Taylor, 1990; Hernández Bermejo and León, 1994), although there are reports of it in North America prior to European colonization (Gray and Trumbull, 1883; Chapman et al., 1973; Byrne and McAndrews, 1975). The molecular phylogenies show *P. oleracea* is not monophyletic as currently circumscribed (Fig. 2). Within clade *I*, subsp. *oleracea* is show to have a North American origin, with a MRCA age of ca. 0.7 Myr, and it is now distributed as a weed worldwide. On the other hand, there are a number of subspecific and segregate species of *P. oleracea* that form clade *II*, with a MRCA age of ca. 1 Myr. Clade *II* is inferred with highest probability to have originated in Africa, although North America and Asia are also suggested. Adding more representatives and samples of the *P. oleracea* clade complex will likely improve our knowledge of geographic origins, although ancestral distribution areas can be difficult to estimate when taxa having wide distribution ranges are involved, as shown in other groups within Cactineae (Applequist and Wallace, 2001; Ocampo and Columbus, 2010).

The reconstructions show that the ancestral species of the AL clade began to disperse from South America to other continents ca. 11 Myr, although extant species have no obvious mechanism to disperse great distances. Species usually have a dehiscent capsule (indehiscent in *P. sclerocarpa*, a Hawaiian endemic), and the seeds are small (typically < 1.3 mm long). Ridley (1930) suggested that the seeds may be dispersed long distances by birds. Water dispersal has also been hypothesized, including intercontinental (by sea) and intracontinental dispersal, as seeds have been demonstrated to remain viable after floating for a number of weeks in distilled and sea water (Ridley, 1930; Danin et al., 1978). Tropical storms, acting as a wind dispersal mechanism, have also been

proposed as a means to distribute plants and/or seeds from the Caribbean to the southeastern USA (Matthews et al., 1991). In addition, humans are a very effective dispersal agent (Ridley, 1930) and have widely propagated species (intentionally or inadvertently), especially in urban areas, agricultural fields, along roads and at ports. Remarkably, there are major weedy species from all major clades in this study: *Portulaca bicolor* (Australian clade; Australia) and *P. quadrifida* (African–Asian clade; tropical regions) in the OL clade, and, in the AL clade, members of the *P. oleracea* complex (Oleracea clade; cosmopolitan), *P. pilosa* (Pilosa clade; tropical and subtropical regions), and *P. umbraticola* (Umbraticola clade; the Americas), which underscores the dispersal and adaptive capabilities of some species of *Portulaca* to a range of disturbed habitats.

4.3. Evolution of chromosome numbers in Portulaca

Uncertainty about the base chromosome number for the genus, which has been proposed as $x = 4$, 9 or 10 (Matthews et al., 1994; Turner, 1994), seems to emanate from a lack of a robust hypothesis of phylogenetic relationships. The reconstruction presented in Fig. 4 shows with high probability (0.99) that $x = 9$ is the ancestral base number for *Portulaca*. It has been suggested that Anacampserotaceae may be closely related to *Portulaca* because both have species with $n = 9$ (Nyffeler, 2007).

Chromosome number data are limited for *Portulaca*, particularly for the OL clade for which only counts from *P. quadrifida* have been reported. More data are available for species in the AL clade, which allows for some assessment of chromosome number change. The best supported model for chromosome number evolution in *Portulaca* indicates that gain, loss, polyploidization, and demi-polyploidization events were all factors, particularly within the Oleracea clade (Table 4; Fig. 4). The Pilosa clade is shown to have only chromosome losses, which are also restricted to that clade. *Portulaca suffrutescens*, distributed in the southern USA and northern Mexico, has the lowest known haploid number in the genus, $n = 4$, which led Matthews et al. (1994) to consider it as a potential base number for the genus; however, $n = 4$ is recovered here as derived, resulting from the loss of five chromosomes.

The results support Carr (1998) in that polyplody evolved prior to the dispersal of ancestral lineages to Hawaii, as shown for *P. lutea* and *P. molokiniensis* (the former widely distributed on the islands of the Pacific Ocean; Figs. 3 and 4). In addition, it is inferred that the ancestral tetraploid lineage ($n = 18$) gained two chromosomes after it colonized the Pacific, which may have contributed to the successful establishment of the two daughter species in new niches (see Fawcett and Van de Peer, 2010).

Within the Oleracea and Umbraticola clades, hexaploids ($n = 27$) are recovered as being the result of demi-polyploidization events in tetraploid ancestors near the tips of the tree, which suggests that hexaploids are relatively new lineages (ca. 1 Mya; Figs. 2 and 4). It is interesting that the tetraploid and hexaploid taxa are weedy (except *P. umbraticola* subsp. *coronata* from the southeastern USA; Matthews et al., 1992). This is consistent with the fact that many weeds are polyploids (Barrett, 1988), which may enable them to increase their fitness in disturbed habitats and expand their distribution range (Radosevich et al., 1997; Soltis and Soltis, 2000).

Polyploidy may occur in *Portulaca* mainly via autopolyploidy because self-fertilization (cleistogamy) seems to be predominant (Geesink, 1969; Matthews et al., 1994; Phillips, 2002). Reports of natural hybridization are rather rare [e.g., Kim and Carr (1990) reported a putative hybrid between *P. oleracea* and *P. lutea* in Hawaii, with $n = 47$], which may indicate that allopolyploidy is uncommon. It is possible that polyploidy and chromosome gains have been important factors in species diversification and increasing the colonization capabilities in the family. Obtaining chromosome counts from more species in the OL clade should bring additional interesting insights.

Our analysis of DNA sequence data provides a hypothesis of relationships within *Portulaca*, where major clades and the relationships among them receive support. Most of these clades have anatomical and morphological features that will be used elsewhere to emend the classification of the genus. Current research efforts include expanding the taxon sample, especially by adding African and Asian species and more samples from widespread taxa (e.g., *P. halimoides*, *P. pilosa*, members of the *P. oleracea* complex) and commercially cultivated varieties. This increased taxon sampling will allow us to clarify the taxonomic status of some species that were shown to be non-monophyletic in this study. In particular, collection of new material will be especially valuable in a genus with a limited set of morphological variable characters and with generally poorly preserved herbarium specimens. Additionally, further taxon sampling will be of interest to examine in more detail the evolution of *Portulaca*, including distribution patterns and interesting adaptations (e.g., photosynthetic variants).

Acknowledgments

We are grateful to Lucinda McDade for her helpful comments and to Wendy Applequist for reviewing earlier versions of this manuscript; James André, Rosa Cerros-Tlatilpa, Hari Chhetri, Jennifer Cruse-Sanders, Avinoam Danin, Giannantonio Domina, Patricia Jaramillo, Norbert Kilian, James Matthews, Clifford Morden, Bart O'Brien, Alfredo Reyes-Betancort, and Megan Thomas for providing samples for this study; Urs Eggli at Sukkulenten-Sammlung Zürich, Dylan Hannon and John Trager at the Huntington Botanical Gardens, Michelei Kikuchi at the National Tropical Botanical Garden, Rob Nicholson at Smith College, David Orr at the Waimea Arboretum Foundation, and Ernesto Sandoval at the University of California, Davis, for access to their living collections; Priscila Burgoyne at the Pretoria National Herbarium, Héctor Osorio at the Museo Nacional de Historia Natural (Uruguay), Bryan Simon at the Queensland Herbarium, Fernando Zuloaga at the Instituto de Botánica Darwinion, the Missouri Botanical Garden, the National Plant Germplasm System (USA), and the University of Arizona Herbarium for access to herbarium collections; James André, Stephen Dreher, Amanda Ingram, Tasha LaDoux, Oscar Morales, Sarah Siedschlag, Valerie Sosa, and Sula Vanderplank for their companionship and assistance during fieldwork. We thank Itay Mayrose for his suggestions on the use of the program chromEvol. This study was supported by Rancho Santa Ana Botanic Garden, the Cactus and Succulent Society of America, Claremont Graduate University, the Claremont University Club, and The Community Foundation serving the Riverside and San Bernardino Counties. Financial support to G.O. was provided by Rancho Santa Ana Botanic Garden, The Fletcher Jones Foundation, Comisión Nacional de Ciencia y Tecnología (Mexico), Fundación Prywer (Mexico), and the Instituto de Ecología, A.C. (Mexico).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.12.017.

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