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Five molecular markers reveal extensive morphological homoplasy and reticulate evolution in the *Malva* alliance (Malvaceae)

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ABSTRACT

The *Malva* alliance is a well-defined group with extensive morphological homoplasy. As a result, the relationships among the taxa as well as the evolution of morphological traits have remained elusive and the traditional classifications are highly artificial. Using five molecular markers (nuclear ITS, plastid *mat*K plus *trn*K, *ndh*F, *trn*L-*trn*F, *psb*A-*trn*H), we arrived at a phylogenetic hypothesis of this group, the genera *Alcea*, *Althaea* and *Malvalthaea* being studied here for the first time with molecular data. *Althaea* and, in particular, *Lavatera* and *Malva* are highly polyphyletic as currently circumscribed, because their diagnostic characters, the number and degree of fusion of the epicalyx bracts, evolve in a highly homoplasious manner. In contrast, fruit morphology largely agrees with the molecularly delimited groups. Hybrid origins confirmed for the genus *Malvalthaea* and for *Lavatera mauritanica* and hybridization in the group of ruderal small-flowered mallows underline the importance of reticulate evolution in shaping the history of this group and complicating the interpretation of morphological evolution.

1. Introduction

Traditional classifications, from Linnean times to deep into the second half of the 20th century, have largely relied on the translation of a suite of morphological characters into taxonomic ranks, without using any explicit method to treat taxonomic information. Notwithstanding conceptual and methodological progress in the second half of the 20th century allowing a more objective evaluation of the phylogenetic signal contained in the morphological characters, this signal may be distorted by a high level of homoplasy (Nyffeler et al., 2005; Pfeil et al., 2002; Ranker et al., 2004; Scotland et al., 2003). Biologically meaningful causes of homoplasy include convergent/parallel evolution and reticulation (with or without polyploidization). These processes are not mutually exclusive and are amply known from angiosperms (Arnold, 1997; Grant, 1981; Otto and Whitton, 2000; Stebbins, 1950). Assessment of homoplasy, whether a morphological character is to be interpreted as symplesiomorphy or as a synapomorphy, requires an independently derived hypothesis on the phylogenetic relationships of the group of interest. Although not immune to homoplasy, molecular data provide the most important alternative.

An excellent example for taxonomic problems caused by homoplasy of morphological characters is provided by *Malva* and related genera (Malvaceae, tribe Malveae). This group includes mainly perennial herbs of Mediterranean to Southwestern Asian distribution, with main centers of diversity in the Western Mediterranean Basin (*Malva*, *Lavatera*, *Althaea*) and the Middle East (*Alcea*). Based on morphology, the genera *Malva*, *Lavatera*, *Althaea* and *Alcea* have been grouped into the so-called *Malva* alliance (Bates, 1968). Molecular data suggest that *Malope* (Tate et al., 2005) and *Kitaibela* (former *Malope* alliance: Bates, 1968; Bates and Blanchard, 1970) are closely related to the *Malva* alliance, while a third genus, *Malvalthaea*, has been largely neglected. While the morphology-based circumscriptions of the small genera *Malope* (2–3 Mediterranean species: Cullen, 1966; Nogueira et al., 1993; Webb, 1968), *Kitaibela* (1 species in Southeast Europe: Webb, 1968) and *Malvalthaea* (1–3 lignified perennial species from the Caucasus and Northern Iran: Iljin, 1949; Riedl, 1976) are uncontroversial, those of *Malva*, *Lavatera*, *Althaea* and *Alcea* are not. The reason is that different authors emphasized different (often single) characters as the differential ones, but, as in other malvaceous groups such as the Hibisceae (Pfeil et al., 2002), these are burdened with extensive homoplasy.

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Linnaeus (1753), using features from the epicalyx (number of segments and degree of fusion), redefined the circumscription of *Alcea*, *Althaea*, *Malva* and *Lavatera* already established by Tournefort (1700, 1706). Although criticized as highly artificial (Alefeld, 1862; Krebs, 1994a,b; Medikus, 1787; Ray, 1995) and replaced by alternative systems relying on fruit characters (Krebs, 1994a,b; Medikus, 1787; Ray, 1995), the Linnean classification scheme was followed by many others (e.g., Baker, 1890; de Candolle, 1805b, 1824; Fernandes, 1968a,b) and is still the most frequently used in modern floras (e.g., Flora Europaea, Flora USSR, Flora Iberica). According to this system, the c. 12 perennial and annual species of *Malva* (native to Eurasia with the center in the western Mediterranean, introduced elsewhere: Dalby, 1968; Morton, 1937), are characterized by three (sometimes two) free epicalyx bracts, while the c. 20 species of *Lavatera* (Mediterranean herbs and shrubs with highest diversity in the western Mediterranean, a few shrubby species in California and Mexico, Ethiopia and Western Australia; Fernandes, 1968b) have also three, but fused epicalyx bracts. Species with higher numbers of fused segments (6–12) were included in *Althaea* (5–6 species in Eurasia and particularly the Mediterranean: Tutin, 1968) and *Alcea* (c. 60 mainly eastern Mediterranean to southwest Asian species: Pakravan, 2001; Riedl, 1976; Zohary, 1963b).

When using fruit morphology and anatomy as diagnostic character, a system irreconcilable with the widely used Linnean one is obtained. The majority of species can be assigned to two main groups. The first one has fruits with fused mericarps that open when ripe releasing the seed, while the walls remain attached to a more or less developed carpophore thus forming tiny hyaline flaps. This fruit type is intermediate between a schizocarp and a capsule, and is called lavateroid, since it is found in the type species of *Lavatera*, *L. trimestris* (Ray, 1995). In contrast, the second group possesses true schizocarps, with thick-walled sharp-edged mericarps that do not release the seed, but detach from the carpophore either separately or as a whole (e.g., in *M. nicaeensis*), without leaving any remnants. This fruit type is called malvoid, since it is found in the type species of *Malva*, *M. sylvestris* (Ray, 1995). Malvoid and lavateroid fruits are, however, found in both *Malva* and *Lavatera* regardless of their current generic assignment. *Malva moschata* and allies (sect. *Bismalva*) produce typical lavateroid fruits, while *Lavatera cretica* and related taxa (sections *Anthema* and *Axolopha*) are clearly malvoid. In fact, without observing the epicalyx it would be difficult to separate species as close morphologically as *L. cretica* and *M. sylvestris* (as noted by Medikus, 1787, and Fernandes, 1968a,b). Nevertheless, the malvoid/lavateroid boundaries are blurred by the existence of taxa with intermediate morphology. Some *Malva* species (namely *M. aegyptia*, *M. cretica* and *M. trifida*) possess fruits not assignable to any of the above types, with mericarps of rounded abaxial surface similar to these of *Althaea* (Alefeld, 1862). In *Alcea,* a unique pseudobilocular mericarp is found (Zohary, 1963b). *Malope* and *Kitaibela* possess mericarps arranged in globose heads, but these differ in their development (van Heel, 1995).

Further complications, and possibly part explanation for the observed homoplasy, stem from the occurrence of reticulate evolution. Based on the intermediacy of morphological characters, the genus *Malvalthaea* has been hypothesized to be of hybrid origin between *Althaea hirsuta* and *Malva aegyptia* (Iljin, 1949). Many of the species, for which karyological information is available, are polyploids (up to 16-ploid; Luque and Devesa, 1986; Escobar, unpublished data), and, although no explicit hypotheses in this direction have been proposed, some of the polyploids might be of allopolyploid origin with putative intermediate or mixed morphology.

The use of a single character for group delimitation has often led to the recognition of artificial taxa (Grant, 2003), and the *Malva* alliance appears to be no exception. As a result, the natural affinities among taxa were misinterpreted, and character evolution remained obscure. Molecular phylogenetic investigations based on nuclear ITS have already been applied in the tribe Malveae (Fuertes Aguilar et al., 2002; Ray, 1995; Tate et al., 2005), but with restricted taxon sampling within the *Malva* alliance due to different study foci. *Alcea*, *Althaea* and *Malvalthaea* as well as some potentially phylogenetically distinct *Malva* species with unusual fruits (e.g., *Malva trifida* or *M. cretica*), and poorly known or only recently described species (e.g., *Lavatera abyssinica*, *L. plazzae*, *L. maroccana*), have never been studied molecularly. As a result, the phylogenetic position and relationships of these taxa remain unknown.

The aim of this study is to establish a solid hypothesis on the phylogenetic relationships of the genera of the *Malva* alliance, identify possible cases of hybrid speciation, and assess the evolution of morphological characters with emphasis on those important for the groups' systematic treatment. Specifically, we address the following questions: (1) are the genera as currently circumscribed monophyletic, in particular *Malva*, *Lavatera* and *Althaea*? What are the phylogenetic relationships of the suggested generic segregates *Navaea* (for the Canarian *L. phoenicea*: Webb and Berthelot, 1836), *Saviniona* (Californian, Mexican and Canary *Lavatera* species with malvoid fruits: Greene, 1912; Webb and Berthelot, 1836) or *Dinacrusa* (for the annual *Althaea* species plus *M. cretica*, *M. aegyptia*, *M. trifida* and *Malvalthaea*: Krebs, 1994b)? What are the phylogenetic relationships between *Althaea* and *Alcea*, which are sometimes merged within a single genus (Baker, 1890; de Candolle, 1824)? What are the phylogenetic relationships of *Lavatera trimestris*, the morphologically very divergent single diploid species of the group sometimes treated as monotypic genus (de Candolle, 1805a,c; Luque and Devesa, 1986)? (2) Is the genus *Malvalthaea* of hybrid origin as hypothesized before? Are any of the highly polyploid taxa of allopolyploid origin, and if so, which taxa were involved? (3) How did key morphological characters in the group evolve, including woody habit, epicalyx structure and fruit types? Which of these characters are synapomorphies and can be used for circumscription of natural units? To this end, we obtained sequences for up to five molecular markers (nrDNA ITS, plastid non-coding *psb*A-*trn*H, *trn*L-*trn*F, and plastid coding *ndh*F, *mat*K) from a wide array of species, often with multiple accessions, of the *Malva* alliance and analyzed those using maximum parsimony and Bayesian inference. Additionally, important morphological characters were scored for all taxa and analyzed using this new phylogenetic framework, which is by far the most comprehensive one for this group so far.

2. Materials and methods

2.1. Taxon sampling

Forty-seven species covering all genera of the *Malva* alliance plus *Anisodontea malvastroides* and *Malvella sherardiana* as outgroup species based on previous molecular work (Tate et al., 2005) have been studied (Table 1). Samples came from field collection in the Mediterranean area, living material grown by the authors in the experimental greenhouse at the Real Jardín Botánico, Madrid (partly raised from unambiguously identifiable material from the index seminum), or herbarium specimens (Table 1). Vouchers of all specimens used were deposited at the herbarium MA (Real Jardín Botánico, Madrid). Multiple individuals per species were analyzed when material was available, resulting in a total of 425 sequences (Tables 1 and 3), all of which are deposited in GenBank under Accession Nos. EF419430–EF419769 and EU346763–EU346849).

Table 1
Material studied.

Species	Voucher	ITS	*psb*A-*trn*H	*trn*L-*trn*F	*mat*K	*ndh*F	Origin
Alcea aucheri Alef.	ang615	EF419543	—	—	—	—	Austria, Wien: Botanischer Garten
Alcea pallida Bess	PE140	EF419545	EF419661	EF419767	—	—	Iran, Quasr-el-Shirin
Alcea rosea L.	PE422	EF419544	EF419662	EF419766	EU346805	EU346847	Austria, Wien: Botanischer Garten
Althaea armeniaca Ten	PE427	EF419542	EF419660	EF419735	EU346763	EU346807	Ukraine, Danube Delta (Index Seminum)
Althaea cannabina L.	PE345	EF419540	EF419657	EF419732	—	—	Spain, Madrid: Chinchón
Althaea cannabina L.	PE471	EF419541	EF419660	EF419734	—	—	Greece, Edessa
Althaea cannabina L.	PE594	EF419539	EF419657	EF419733	EU346764	EU346810	Austria, Wien: Botanischer Garten
Althaea hirsuta L.	PE270	EF419510	EF419659	EF419717	EU346794	EU346808	Italy, Sardegna: Perdasdefogu
Althaea hirsuta L.	PE356	EF419509	EF419658	EF419716	—	—	Turkey, Aydin
Althaea hirsuta L.	PE454	EF419508	EF419621	EF419718	—	—	Italy, Abruzzo: L'Aquila
Althaea hirsuta L.	PE455	EF419507	EF419622	EF419719	—	—	Spain, Barcelona: Cabacés
Althaea hirsuta L.	PE456	—	EF419623	EF419720	—	—	Spain, Álava: Orviso
Althaea hirsuta L.	PE458	—	EF419624	EF419721	—	—	Romania, Dobrogea: Babadag
Althaea longiflora Boiss. & Reut.	PE362	EF419500	—	—	—	—	Spain, Ciudad Real: Alhambra
Althaea longiflora Boiss. & Reut.	PE461	EF419502	EF419638	EF419725	—	—	Spain, Badajoz: Gévora
Althaea longiflora Boiss. & Reut.	PE462	EF419503	EF419639	EF419726	—	—	Spain, Badajoz: Magacela
Althaea longiflora Boiss. & Reut.	PE596	EF419501	EF419640	EF419724	EU346795	EU346809	Morocco, Marrakech
Althaea ludwigii L.	PE459	EF419504	EF419641	—	—	—	Morocco, Taza
Althaea ludwigii L.	PE460	EF419505	EF419642	EF419722	—	—	Morocco, Ouarzazate
Althaea ludwigii L.	PE616	EF419506	EF419643	EF419723	EU346796	EU346812	Iran, Tehran
Althaea officinalis L.	PE330	EF419537	EF419656	EF419727	—	—	Spain, Madrid: Aranjuez
Althaea officinalis L.	PE511	—	EF419653	EF419729	EU346765	EU346811	Spain, Zamora: Río Duero
Althaea officinalis L.	PE512	EF419538	EF419654	EF419730	—	—	France, Haute Corse: L'Aliso
Althaea officinalis L.	PE513	—	EF419655	EF419731	—	—	Bulgaria, Varna: Nos Cernija
Althaea officinalis L.	PE604	EF419536	EF419652	EF419728	—	—	Spain, Ciudad Real: Alhambra
Anisodontea malvastroides (Baker f.) D.M. Bates	PE067	EF419547	—	—	EU346803	EU346848	South Africa, Cape Town
Kitaibela vitifolia Willd.	PE111	—	—	—	EU346804	EU346849	Index Seminum
Lavatera abyssinica Hutch. & E.A. Bruce	PE383	EF419461	EF419579	EF419709	—	—	Spain, Madrid: Jardín Botánico
Lavatera acerifolia Cav.	PE134	—	EF419577	EF419687	EU346778	EU346820	Spain, Tenerife: Los Gigantes
Lavatera acerifolia Cav.	PE135	EF419459	—	—	—	—	Spain, Fuerteventura: Antigua
Lavatera agrigentina Tineo	PE308	EF419430	EF419553	EF419670	EU346769	EU346814	Italy, Sicilia: Agrigento
Lavatera arborea L.	PE153	EF419466	EF419585	EF419704	—	—	Spain, Albacete: Tobarra
Lavatera arborea L.	PE239	EF419469	EF419586	EF419706	—	—	Italy, Sardegna: Capo Caccia
Lavatera arborea L.	PE252	EF419467	EF419587	EF419707	EU346779	EU346821	Italy, Sardegna: Alghero
Lavatera arborea L.	PE378	EF419468	—	EF419705	—	—	Spain, Lugo: Ribadeo
Lavatera assurgentiflora Kellogg	PE570	EF419460	EF419578	EF419708	EU346780	EU346819	Spain, Madrid: Jardín Botánico
Lavatera assurgentiflora Kellogg	PE325	EF419548	—	—	—	—	USA, California (Index Seminum)
Lavatera bryoniifolia Mill.	PE141	EF419439	EF419550	EF419666	EU346768	EU346815	Greece, Crete: Rethimnion
Lavatera bryoniifolia Mill.	PE552	EF419440	—	—	—	—	Greece, Agios Ioannis
Lavatera cretica L.	PE031	EF419470	EF419589	EF419688	—	—	Spain, Badajoz: Don Benito
Lavatera cretica L.	PE076	—	EF419588	—	—	—	Portugal, Alto Alentejo: Évora
Lavatera cretica L.	PE235	EF419471	EF419590	EF419690	EU346783	EU346813	Italy, Sardegna: Alghero
Lavatera cretica L.	PE599	EF419472	EF419591	EF419689	—	—	Morocco, Sidi Yahya
Lavatera flava Desf.	PE000	EF419434	EF419552	—	—	—	Morocco, Taourirt
Lavatera flava Desf.	PE414	EF419433	EF419551	EF419669	EU346772	EU346818	Morocco, Al-Hoceima
Lavatera maritima Gouan	PE200	EF419457	EF419573	EF419712	EU346781	EU346822	Spain, Zaragoza: Calatayud
Lavatera maritima Gouan	PE329	EF419456	EF419572	EF419711	—	—	Italy, Sardegna: Cala Gonone
Lavatera maritima Gouan	PE404	EF419458	EF419574	EF419713	—	—	Spain, Murcia: Los Belones
Lavatera maritima Gouan	PE405	—	EF419575	—	—	—	Spain, Almería: Mojácar
Lavatera maritima Gouan	PE598	EF419455	EF419576	EF419710	—	—	Morocco, Gorges du Zegzel
Lavatera maroccana Maire	PE346	EF419453	EF419563	EF419681	EU346777	EU346823	Spain, Sevilla: Las Cabezas de San Juan
Lavatera maroccana Maire	PE515	EF419454	EF419564	EF419682	—	—	Morocco, Taza
Lavatera mauritanica Durieu	PE137	EF419463	EF419581	EF419691	—	—	Spain, Almería: Alborán
Lavatera mauritanica Durieu	PE318	EF419464	EF419583	—	—	—	Portugal, Algarve, Ponta de Sagres
Lavatera mauritanica Durieu	PE319	—	EF419582	EF419692	—	—	Portugal, Algarve, Cabo de São Vicente
Lavatera mauritanica Durieu	PE630	EF419465	EF419584	EF419693	EU346782	EU346824	Morocco, Mediouna
Lavatera oblongifolia Boiss.	PE144	EF419441	EF419560	EF419665	EU346767	EU346825	Spain, Almería
Lavatera olbia L.	PE004	EF419442	EF419561	EF419668	—	—	Spain, Baleares: Mahón
Lavatera olbia L.	PE451	EF419443	EF419562	EF419667	EU346766	EU346826	Italy, Sardegna: San Giovanni di Sinis
Lavatera phoenicea Vent.	PE002	EF419526	EF419644	EF419763	—	—	Spain, Tenerife, Anaga
Lavatera phoenicea Vent.	PE628b	EF419527	EF419645	EF419764	—	—	Spain, Tenerife, Anaga
Lavatera phoenicea Vent.	PE629	EF419528	EF419646	EF419765	EU346802	EU346828	Spain, Tenerife: Teno
Lavatera plazzae Atzei	PE285	EF419444	EF419549	EF419664	EU346773	EU346829	Italy, Sardegna: Porto Torres
Lavatera plebeia Sims	PE634	EF419462	EF419580	—	EU346784	EU346827	Australia, South Australia, Adelaide
Lavatera punctata All.	PE348	EF419446	EF419566	EF419677	—	—	Turkey, Aydin
Lavatera punctata All.	PE450	EF419445	EF419565	EF419678	EU346776	EU346830	Turkey, Aydin
Lavatera punctata All.	PE555	—	—	EF419679	—	—	Greece, Amfilokhia
Lavatera thuringiaca L.	PE353	EF419452	EF419567	EF419680	EU346775	EU346831	Russia, Burgistan: Pyatigorsk
Lavatera thuringiaca L.	PE559	EF419451	—	—	—	—	Austria, Wien: Botanischer Garten
Lavatera triloba ssp. *pallescens* (Moris) Nyman	PE354	EF419431	EF419555	EF419672	—	—	Spain, Baleares: Sa Foradada
Lavatera triloba ssp. *pallescens* (Moris) Nyman	PE564	EF419432	EF419554	EF419671	EU346770	EU346817	Spain, Baleares: Isla Colom
Lavatera triloba L. ssp. *triloba*	PE117	EF419435	EF419556	EF419673	—	—	Spain, Ciudad Real: Almedina
Lavatera triloba L. ssp. *triloba*	PE169	EF419438	EF419557	EF419676	—	—	Spain, Badajoz: Usagre
Lavatera triloba L. ssp. *triloba*	PE357	EF419436	EF419558	EF419674	EU346771	EU346816	Spain, Murcia: Alhama
Lavatera triloba L. ssp. *triloba*	PE359	EF419437	EF419559	EF419675	—	—	Spain, Almería: Vélez Blanco

Table 1 (*continued*)

Species	Voucher	ITS	*psb*A-*trn*H	*trn*L-*trn*F	*mat*K	*ndh*F	Origin
Lavatera trimestris L.	PE181	EF419448	EF419569	EF419684	—	—	Spain, Cáceres: Logrosán
Lavatera trimestris L.	PE233	EF419449	EF419570	EF419685	—	—	Italy, Sardegna: Alghero
Lavatera trimestris L.	PE308	EF419450	EF419568	EF419686	—	—	Spain, Cádiz: Alcalá de los Gazules
Lavatera trimestris L.	PE595	EF419447	EF419571	EF419683	EU346774	EU346832	Morocco, Rif: Chefchaouen
Malope malacoides L.	PE279	—	EF419651	—	—	—	Italy, Sardegna: Laconi
Malope malacoides L.	PE415	EF419535	EF419650	EF419760	—	—	Morocco, Rif: Tetouan
Malope malacoides L.	PE600	EF419534	—	EF419761	—	—	Morocco, Rif: Fnidek
Malope malacoides L.	PE605	—	—	EF419762	EU346800	EU346833	Spain, Cádiz: Algodonales
Malope trifida Cav.	PE070	EF419532	EF419648	EF419758	—	—	Spain, Huelva: El Portil
Malope trifida Cav.	PE394	EF419529	EF419647	—	—	—	Morocco, Sidi Kacem
Malope trifida Cav.	PE499	EF419533	EF419649	EF419759	—	—	Morocco, Rif: Khenichet
Malope trifida Cav.	PE550	EF419530	—	—	EU346801	EU346834	Morocco, Fès
Malope trifida Cav.	PE601	EF419531	—	EF419757	—	—	Morocco, Rif: Khenichet
Malva aegyptia L.	PE351	EF419520	EF419632	EF419740	EU346798	EU346835	Spain, Zaragoza: Bujaraloz
Malva aegyptia L.	PE465	EF419519	EF419630	EF419741	—	—	Spain, Alicante: Santa Pola
Malva aegyptia L.	PE466	EF419516	—	—	—	—	Greece, Crete: Rethimnion
Malva aegyptia L.	PE467	EF419518	—	—	—	—	Greece, Karpathos
Malva aegyptia L.	PE468	EF419517	EF419631	EF419742	—	—	Spain, Toledo: Yepes
Malva alcea L.	PE338	EF419493	EF419609	EF419747	EU346790	EU346840	Spain, Ávila: Mijares
Malva alcea L.	PE440	EF419492	EF419610	EF419745	—	—	France, Marnay-Sur-Seine
Malva alcea L.	PE539	EF419491	EF419611	EF419746	—	—	Spain, Toledo: Navamorcuende
Malva cretica ssp. *althaeoides* (Cav.) Dalby	PE350	—	EF419629	—	—	—	Spain, Cádiz: Algodonales
Malva cretica ssp. *althaeoides* (Cav.) Dalby	PE389	EF419513	EF419626	—	—	—	Spain, Jaén: Aldeaquemada
Malva cretica ssp. *althaeoides* (Cav.) Dalby	PE390	EF419514	—	—	—	—	Spain, Alicante: Castell de Castells
Malva cretica ssp. *althaeoides* (Cav.) Dalby	PE391	EF419515	EF419627	—	—	—	Spain, Alicante: Vall de Gallinera
Malva cretica ssp. *althaeoides* (Cav.) Dalby	PE463	EF419512	EF419628	EF419744	EU346797	EU346837	Spain, Málaga: Carratraca
Malva cretica Cav. ssp. *cretica*	PE361	—	—	EF419743	—	—	Italy, Sicilia: Palermo
Malva hispanica L.	PE149	EF419488	EF419606	EF419715	—	—	Spain, Badajoz: Cabeza del Buey
Malva hispanica L.	PE602	EF419489	EF419607	EF419714	—	—	Spain, Badajoz: Guadajira
Malva hispanica L.	PE631	EF419490	EF419608	—	EU346793	EU346838	Morocco, Ounara
Malva moschata L.	PE322	EF419495	EF419616	EF419750	—	—	Spain, La Rioja: Rasillo de Cameros
Malva moschata L.	PE493	—	EF419617	EF419748	—	—	Spain, Lérida: Bausent
Malva moschata L.	PE494	—	EF419618	EF419749	—	—	Spain, Lérida: Alins
Malva moschata L.	PE496	EF419496	EF419615	EF419751	—	—	Spain, Guipúzcoa: Legazpia
Malva moschata L.	PE499	—	EF419619	EF419752	—	—	Spain, León: La Uña
Malva moschata L.	PE593	EF419494	—	EF419753	EU346792	EU346841	France, Pyrenées Orientales: Lortet
Malva neglecta Wallr.	PE349	EF419478	EF419597	EF419702	EU346788	EU346842	Spain, Valladolid: Encinas de Esgueva
Malva neglecta Wallr.	PE632	EF419479	EF419598	—	—	—	Italy, Sardegna: Lago Cuga
Malva nicaeensis All.	PE032	EF419473	EF419592	EF419699	—	—	Spain, Badajoz: Talarrubias
Malva nicaeensis All.	PE105	—	EF419594	—	—	—	Italy, Sardegna: Uri
Malva nicaeensis All.	PE097	EF419474	EF419593	EF419700	—	—	Spain, Toledo: Yepes
Malva nicaeensis All.	PE105	EF419475	—	—	—	—	Spain, Ciudad Real: Alhambra
Malva nicaeensis All.	PE228	EF419477	EF419596	EF419701	EU346785	EU346843	Italy, Sardegna: Lago Cuga
Malva nicaeensis All.	PE633	EF419476	EF419595	—	—	—	Morocco, Berkane
Malva parviflora L.	PE005	EF419483	EF419604	EF419694	—	—	Spain, Badajoz: Guadajira
Malva parviflora L.	PE059	EF419484	EF419601	EF419696	—	—	Portugal, Alto Alentejo: Vendas Novas
Malva parviflora L.	PE249	EF419485	EF419603	EF419695	EU346786	EU346844	Italy, Sardegna: San Giovanni di Sinis
Malva parviflora L.	PE296	EF419486	EF419602	EF419697	—	—	Spain Sevilla: Morón de la Frontera
Malva sylvestris L.	PE001	EF419480	EF419599	EF419698	EU346787	EU346845	Spain, Madrid: Retiro
Malva sylvestris L.	PE515	EF419481	—	—	—	—	Morocco, Oujda
Malva sylvestris L.	PE635	EF419482	EF419600	—	—	—	Portugal, Alto Alentejo: Vendas Novas
Malva tournefortiana L.	PE189	EF419497	EF419612	EF419755	EU346791	EU346839	Spain, Badajoz: Talarrubias
Malva tournefortiana L.	PE479	EF419499	EF419614	EF419754	—	—	Morocco, Oukaimeden
Malva tournefortiana L.	PE489	EF419498	EF419613	EF419756	—	—	Portugal, Tras-os-Montes: Mogadouro
Malva trifida Cav.	PE352	EF419524	EF419634	EF419739	—	—	Spain, Navarra: Fraile Alto
Malva trifida Cav.	PE254	EF419525	EF419636	EF419738	—	—	Spain, Lérida: Balaguer
Malva trifida Cav.	PE392	EF419522	EF419633	—	—	—	Spain, Madrid: San Martín de la Vega
Malva trifida Cav.	PE393	EF419523	EF419635	—	EU346799	EU346836	Spain, Huesca: Fraga
Malva trifida Cav.	PE452	—	—	EF419736	—	—	Spain, Soria: Monteagudo de las Vicarías
Malva trifida Cav.	PE453	EF419521	—	EF419737	—	—	Spain, Madrid: Aranjuez
Malva verticillata L.	PE442	EF419487	EF419605	EF419703	EU346789	EU346846	Germany, Leipzig (Index Seminum)
Malvalthaea transcaucasica Iljin	PE628	EF419511	EF419637	—	—	—	Azerbaijan, Saliani: Chaladzh
Malvella sherardiana (L.) Jaub. & Spach	PE325	EF419546	EF419663	EF419768	EU346806	—	Spain, Córdoba: Montoro

2.2. DNA isolation, PCR and sequencing

Total genomic DNA was isolated using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Total DNA was checked on 1.5% agarose gels to test the amount and quality of the extractions. PCR products for nuclear ITS and the four plastid regions *psb*A-*trn*H, *trn*L-*trn*F, *mat*K-*trn*K, *ndh*F were obtained using puReTaq Ready-To-Go PCR Beads (GE Healthcare, Munich, Germany), with 1:10 diluted stock DNA. 1 μl DMSO per reaction was added, and for old or difficult herbarium material 4% BSA was used. PCR programs were run in GeneAmp PCR System 9700 (PE Applied Biosystems, Foster City, CA) and MJ Research PTC 200 (MJ Research, Waltham, MASS) thermocyclers. Primers and PCR conditions are given in Table 2. PCR products were checked on 1.5% agarose gel (Pronadisa, Madrid, Spain), stained with ethidium bromide, and then purified with UltraClean PCR Clean-up Kit (MoBio, Carlsbad, CA). Cleaned PCR products were se-

quenced at the DNA Sequencing Service of CIB, CSIC (Madrid, Spain; http://www.secugen.es). Due to the extensive amount of cloning necessary to sufficiently assess intra- and inter-specific sequence variation we refrained from cloning, but sequenced instead multiple accessions of many species.

2.3. Data analysis

Sequences were aligned with the programs ClustalX (Thompson et al., 1997) and, due to the lack of ambiguous regions, easily edited manually in BioEdit version 7.0.5.2 (Hall, 1999). We additionally checked for the preserved regions in ITS1 (Liu and Schardl, 1994) and in ITS2 (Hershkovitz and Zimmer, 1996) to identify possible pseudogenes. Inversions in the hairpin structure in *psb*A-*trn*H (positions 28–101) were reverse complemented (Löhne and Borsch, 2005).

Given that the plastid genome behaves as a single linked region and that the single regions exhibited low levels of variation (see Section 3), the four plastid markers (*psb*A-*trn*H, *trn*L-*trn*F, *mat*K-*trn*K, *ndh*F) were concatenated *a priori*. Congruence with the nuclear ITS partition was tested using the Incongruence Length Difference tests (ILD, Farris et al., 1994) implemented as partition homogeneity test in PAUP* 4.0b10 (Swofford, 2000) excluding constant characters. Including all taxa, significant incongruence was found (see Section 3), so the test was repeated after sequential removal of taxa likely responsible for these incongruences, until the result was no longer significant. For all ILD tests, 100 replicates were used, each with 1000 random stepwise addition replicates, holding and saving 10 trees per replicate, with tree bisection-reconnection (TBR) branch swapping and a significance level of 0.01 (Cunningham, 1997).

Maximum parsimony phylogenetic analyses were conducted using PAUP* 4.0b10 (Swofford, 2000) on three data sets (ITS, joint plastid matrix, joint total matrix). Gaps were coded following the simple method of Simmons and Ochoterena (2003), as implemented in IndelCoder provided by the software SeqState 1.25 (Müller, 2005). For each data set, heuristic searches were conducted with 1000 random stepwise addition replicates, holding and saving 10 trees per replicate, with TBR branch swapping, and all characters treated as equally weighted and unordered. Bootstrap support analyses (Felsenstein, 1985) were performed running 100 bootstrap replicates, each with 1000 replicates of random sequence addition, equal weighting and TBR branch swapping, holding a maximum of 10 trees per replicate.

Bayesian analyses (Huelsenbeck et al., 2001) were conducted with MrBayes 3.1 (Ronquist and Huelsenbeck, 2003). The best fit models were determined using hierarchical likelihood ratio tests and the AIC as implemented in Modeltest version 3.06 (Posada and Crandall, 1998, 2001). This was the General Time-Reversible (GTR) + G + I model as best fitting the ITS and plastid datasets. For each analysis (conducted at the University of Oslo Bioportal, http://www.bioportal.uio.no/), four simultaneous runs with one cold and three heated chains each (using the default heating parameters) with random starting tree were run for 5,000,000 generations, with tree sampling every 500 generations and using default priors. This was more than enough to allow standard deviation of split frequencies to stabilize at levels lower than 0.01. The first 2000 trees (20%) of each run were discarded as burnin and a 50% majority rule consensus tree was constructed.

Thirteen morphological, discrete qualitative characters used as diagnostic characters by different authors, were coded for all taxa as unordered and equally weighted (Appendix A) and analysed using maximum parsimony in PAUP with the same settings as above. The evolution of four diagnostic characters (number and degree of fusion of epicalyx bracts, life form and fruit morphology) was analyzed on the posterior set of trees from the ITS Bayesian analysis, thus taking phylogenetic uncertainty into account. The analysis was performed using unordered maximum parsimony as implemented in Mesquite ver. 2.5 (available from http://mesquiteproject.org). The results are summarized on the majority rule consensus tree of the posterior set of trees.

3. Results

3.1. Sequence characteristics

For ITS, a region spanning 778 bp (including the adjacent regions of the 18S and 26S genes) was sequenced (Table 3). In all sequences, the preserved domain in ITS1 (Liu and Schardl, 1994) and the six conserved regions in ITS2 (Hershkovitz and Zimmer, 1996) were present, with the exception of a single accession of *L. mauritanica* with numerous point mutations, which was therefore omitted from all analyses. The ITS sequences included 277 variable characters, from which 225 were parsimony-informative. The ITS data set includes a total of 73 additive polymorphic sites (APS, as defined by Fuertes Aguilar et al., 1999) amounting for 9.2% of total positions, appearing in 27 out of 40 studied taxa. Most of them were autapomorphies, but 16 appeared to be shared. The frequency of polymorphic sites was not related to especially high chromosome numbers, as the majority of affected species were hexaploids. This was especially evident for the ruderal mallows. For example, *Malva parviflora* and *M. sylvestris* shared three polymorphic positions, while *Lavatera arborea* and the 12-ploid *L. mauritanica* shared four. In *Malvalthaea transcaucasica*, three positions were shared with both *Althaea hirsuta* and *Malva aegyptia*.

Table 2
Primers and PCR conditions. fw, forward; rev, reverse.

Marker	Primer	Sequence	PCR conditions
ITS	P1A (fw.) P4 (rev.)	Fuertes Aguilar et al. (1999).	95 °C 1 min, followed by 35 cycles 95 °C 1 min, 52 °C 1 min and 72 °C 1 min, 72 °C 10 min
psbA-trnH	PSB (fw.) TRN (rev.)	5′-CGA AGC TCC ATC TAC AAA TGG-3′ 5′-ACT GCC TTG ATC CAC TTG GC-3′	95 °C 1 min, followed by 35 cycles 95 °C 1 min, 53 °C 30 s and 72 °C 1 min, 72 °C 10 min
trnL-trnF	e (fw.) f (rev.)	Taberlet et al. (1991).	95 °C 1 min, followed by 35 cycles 95 °C 1 min, 52 °C 30 s and 72 °C 1 min, 72 °C 10 min
matK plus trnK	trnK570F (fw.) matK390F (fw.) matK530R (rev.) matK1300R (rev.) matK1200F (fw.)	5′-TCC AAA ATC AAA AGA GCG ATT GG-3′ 5′-CGA TCT ATT CAT TCA ATA TTT C-3′ 5′-GTT CCA ATT CCA ATA CTC GTG AAG-3′ 5′-CGA AGT ATA TAC TTC ATT CGA TAC A-3′ 5′-GAY TCT GAT ATT ATC AAC CGA TTT G-3′	95 °C 1 min, followed by 35 cycles 95 °C 1 min, 55 °C 1.5 min and 72 °C 1 min, 72 °C 10 min
ndhF	ndhF (fw.) ndhR (rev.)	Pfeil et al. (2002) Pfeil et al. (2002)	Pfeil et al. (2002).

Table 3
Analysed data matrices and parsimony analysis statistics. MNPT, joint cpDNA analysis; IMNPT, joint analysis; Trees, number of trees; CI, consistency index; HI, homoplasy index; RI, retention index.

	ITS	*psb*A-*trn*H	*trn*L-F	*mat*K	*ndh*F	MNPT	IMNPT
Taxa	92	115	112	44	43	43	39
Characters	778	659	464	1986	1845	2932	3772
Constant	501	572	414	1769	1777	2552	3060
Variable	277	87	50	217	68	380	712
Parsimony-uninformative	52	41	15	127	39	175	284
Parsimony-informative	225	46	35	90	29	205	428
Indels	67	99	29	43	5	130	199
Trees	9380	400	9860	7520	9580	10	2
Tree length	649	109	51	267	93	662	1194
CI	0.5613	0.7794	0.9487	0.7029	0.5472	0.5621	0.5462
HI	0.4387	0.2206	0.0513	0.2971	0.4528	0.4379	0.4538
RI	0.9172	0.9667	0.9934	0.8686	0.8209	0.8076	0.8111

We sequenced four different chloroplast markers spanning 4954 characters (Table 3). The most variable markers were the spacers *trn*L-*trn*F (464 bp) and *psb*A-*trn*H (470 bp), with 50 and 87 variable and 35 and 46 parsimony-informative characters, respectively. Less variation was encountered in the (mainly) coding regions *mat*K-*trn*K (1986 bp) and *ndh*F (1845 bp) with 217 and 68 variable characters, respectively, only 90 and 29 being parsimony-informative. The *trn*L-*trn*F sequences displayed the greatest amount of sequence diversity, and length differences due to a repeated sequence motif of 100 bp in the 3′-end of the spacer region occurring among the malvoid taxa. Only a few other indels were detected. The most significant were a 7 bp insertion at position 215 exclusive to *Alcea*, and a 9 bp insertion at position 95 characteristic of the perennial *Althaea*. A reduced AT microsatellite spanned positions 152–176, interrupted by a GTG conserved triplet. For *psb*A-*trn*H, sequence variation was complex due to the presence of multiple indels, some of them autapomorphic. The annual *Althaea*, *M. cretica* and *M. aegyptia* possessed large deletions in the region spanning positions 462–559. An AT microsatellite of reduced size appeared between positions 354 and 387. The inversion (relative to the outgroup taxa) in the *psb*A-*trn*H spacer is present in all taxa. The alignment and the phylogenetic trees are available in TreeBase (www.treebase.org) under study number SN3815.

3.2. Phylogenetic relationships

3.2.1. ITS

The maximum parsimony strict consensus tree and the Bayesian 50% majority rule tree (Fig. 1) are topologically nearly identical (tree statistics in Table 3), exceptions being restricted to minor differences in the arrangement of some terminals from the ruderal malvoid taxa (such as *Malva neglecta* or *Lavatera cretica*). Therefore, we only present the results from the Bayesian analysis. The species of *Malva* and *Lavatera* plus the annual species of *Althaea* plus *Malvalthaea* fall into a well-supported monophyletic clade (bootstrap support BS 99, Bayesian posterior probability PP 1.00), called hereinafter the core *Malva* alliance, which itself is sister to *Lavatera phoenicea* (BS 99, PP 1.00). Subsequent sister groups are the genus *Malope* and a well-supported clade (BS 100, 1.00) including *Alcea*, *Kitaibela* and the perennial *Althaea* species, hereinafter called the *Alcea* clade.

Within the core *Malva* alliance, the majority of species are found in the sister groups (BS < 50, PP 0.95) of the malvoid clade (BS 99, PP 1.00) with malvoid fruits and the lavateroid clade (BS < 50, PP 0.88) with lavateroid fruits. Within the malvoid clade, a clade including *Lavatera acerifolia* and *L. maritima* (BS 74, PP 0.70) and one including *L. arborea* and *L. mauritanica* (BS 98, PP 1.00) were consecutive sister taxa (BS 90–98, PP 0.99–1.00) to the hereinafter named core malvoid clade (BS 90, PP 0.99). The core malvoid clade includes three clades with unclear relationships to each other, the extra-Mediterranean shrubby *Lavatera* species (*L. assurgentiflora*, *L. plebeia* and *L. abyssinica*, from the Californian Channel Islands, Australia and Ethiopia, respectively; BS 97, PP 1.00) and two comprising annual malvoids (*M. sylvestris, M. parviflora*, *M. verticillata*, *M. neglecta*: BS 54, PP 0.58; *L. cretica*, *M. nicaeensis*: BS 96, PP 1.00). In several cases, regarding *Malva neglecta* or *Lavatera cretica*, sequences from different accessions failed to form monophyletic groups and appeared intermixed in short-branched terminal clades. Within the lavateroid clade, only *Malva* sect. *Bismalva* (BS 100, PP 1.00) and the perennial *Lavatera* species (BS 81, PP 1.00) received significant support. Within the latter, the taxa of *L.* sect. *Olbia* appear in two weakly supported clades (*L. olbia* and *L. oblongifolia*; *L. plazzae* and *L. bryoniifolia*) as consecutive sisters to the members of the *L. triloba* aggregate (*L. agrigentina*, *L. flava*, *L. triloba*; BS 81, PP 1.00). Five monospecific lavateroid clades (each BS > 95, PP 1.00) were collapsed at the base of the core *Malva* alliance in the bootstrap analysis: *L. maroccana*, *L. punctata*, *L. thuringiaca*, *L. trimestris* and *Malva hispanica*. Consecutive sister to the clade composed of the malvoid and lavateroid clades are two small clades of the annual *Althaea* species plus *Malva cretica* (annual *Althaea* clade; BS 62, PP 0.98) and of *Malva aegyptia*, *M. trifida* plus *Malvalthaea transcaucasica* (*Malva aegyptia* clade; BS 97, PP 1.00), respectively.

3.2.2. Plastid markers

Single marker analyses produced poorly resolved trees, whose topological differences were not statistically significant (data not shown). Maximum parsimony and Bayesian analyses of the cpDNA matrix resulted in essentially identical trees (Fig. 2, tree statistics given in Table 3). As for the nuclear data, the *Malope* clade (BS 100, PP 1.00) and the *Alcea* clade (BS 88, PP 1.00) are subsequent sister taxa to the core *Malva* alliance (BS 97, PP 1.00). Resolution within the core *Malva* alliance was lower than for the nuclear data set with several lineages with unclear relationships to each other. These include a truncated malvoid clade (without the clade of *L. maritima* and *L. acerifolia*; BS 98, PP 1.00) with several moderately supported subclades of annual ruderal species; several lineages belonging to the lavateroid clade, which, however, fail to cluster together, such as *Malva* sect. *Bismalva* (BS 100, PP 1.00), a clade of *L. agrigentina* and *L. flava* (BS 96, PP 1.00) and one of, among others, *L. bryoniifolia* and *L. triloba* (BS 94, PP 1.00); the *Malva aegyptia* clade (BS 100, PP 1.00), but without *Malvalthaea*, which instead groups with *Malva cretica* and *Althaea hirsuta*; the failure of inference of the annual *Althaea* clade. Phylogenetic positions strongly differing from the ones inferred from nuclear ITS data were also observed for *Lavatera phoenicea* (nested within the core *Malva* alliance, BS < 50, PP 0.96), *L. mauritanica* (nested within the ruderal malvoids; BS 82, PP 1.00), *L. trimestris* and *L. plazzae*, consecutive

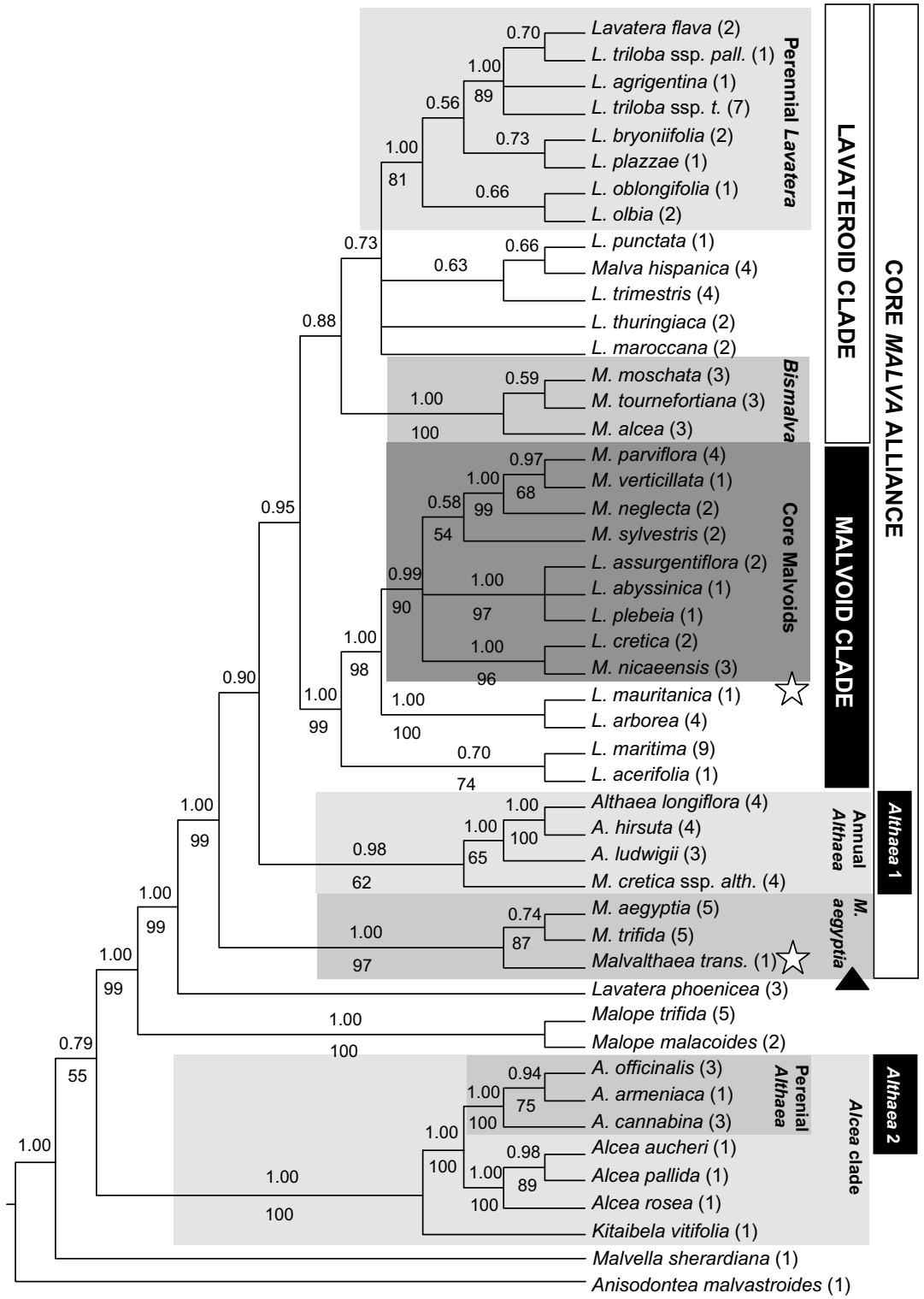

Fig. 1. ITS Bayesian 50% majority rule tree. Stars indicate hybrid speciation events. The triangle marks the isolated position of *L. phoenicea*. Values above branches indicate posterior probabilities, those below bootstap support values (only when higher than 50%). The number of accessions appears in brackets after the species name.

sister taxa to the truncated malvoid clade (BS < 50, PP 0.96 and 1.00, respectively).

3.2.3. Joint analysis

The ILD test detected statistically significant incongruence ($P = 0.01$) between plastid and nuclear data. To test for the extent of this incongruence, several species were sequentially removed from the analysis, starting with the putative hybrids *L. mauritanica* and *Malvalthaea transcaucasica* as evident sources of incongruence. As the ILD test results remained significant ($P = 0.01$), three more species that showed labile phylogenetic positions (*L. phoenicea*, *L. trimestris* and *L. plazzae*) were additionally removed, resulting in the lack of significant incongruence ($P = 0.21$).

The joint analysis of all markers of this reduced data set yielded well resolved trees with substantially elevated bootstrap support values (Fig. 3, tree statistics in Table 3). The

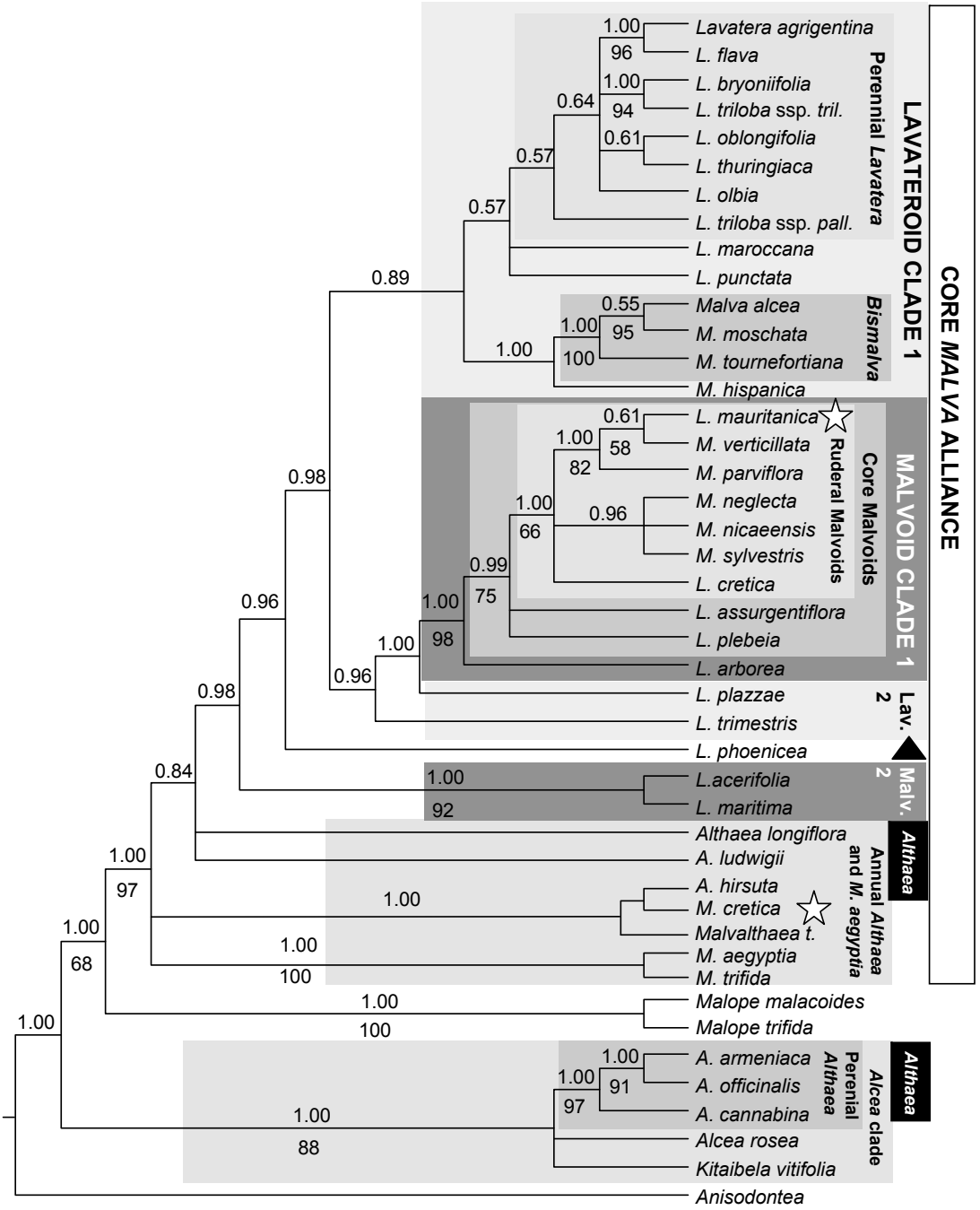

Fig. 2. Total plastid Bayesian 50% majority rule tree. Stars indicate hybrid speciation events. The triangle marks the isolated position of *L. phoenicea*. Values above branches indicate posterior probabilities, those below bootstap support values (only when higher than 50%).

resulting topology was largely similar to the ITS one (Fig. 1) and compatible with the joint plastid tree (Fig. 2). The *Malope* and the *Alcea* clade (both BS 100, PP 1.00) are consecutive sister to the core *Malva* alliance clade (BS 100, PP 1.00). Within the core *Malva* alliance, the relationships of the annual *Althaea* clade (BS < 50, PP 1.00) and the *M. aegyptia* clade (BS 100, PP 1.00) to the clade of the lavateroid (BS<50, PP 1.00) and the malvoid clade (BS 99, PP 1.00) are poorly resolved. *Malva* sect. *Bismalva* (BS 100, PP 1.00) plus the divergent *Malva hispanica* were sister to the remaining lavateroid taxa, including a clade of perennial *Lavatera* species (BS 70, PP 0.87). Within the malvoid clade, the clade of *L. maritima* and *L. acerifolia* (BS 94, PP 1.00) is sister to the remaining taxa, and, as in ITS, *L. arborea* is sister to the core malvoid clade (BS 98, PP 1.00). Within the core malvoids, the ruderal mallows form a highly supported clade (BS 85, PP 1.00).

3.2.4. Morphological character analysis

A matrix of fourteen diagnostic characters used in the taxonomy of the *Malva* alliance was analysed (Appendix A). Trees were poorly or very poorly resolved with very low bootstrap support scores (data not shown). Nevertheless, several classical morphological groups were recognisable. The shrubby malvoid *Lavatera* species clustered together along with *L. phoenicea* and the other malvoid, mostly ruderal species. The perennial *Althaea* species clustered together (BS 53), while the annual *Althaea* species failed

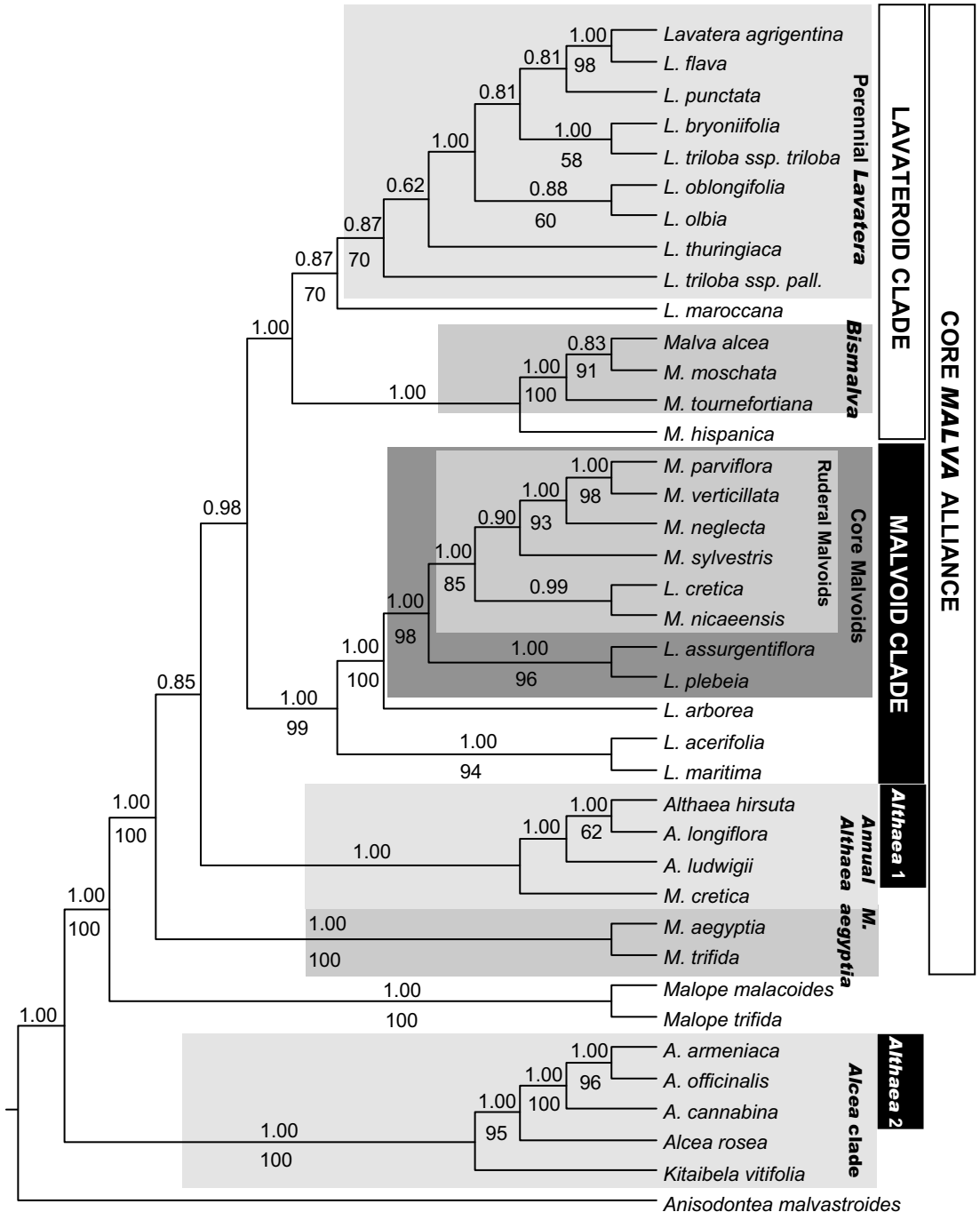

Fig. 3. Joint Bayesian 50% majority rule consensus tree without conflict species (see text). Values above branches indicate posterior probabilities, those below bootstap support values (only when higher than 50%).

to group in the same clade as the perennial congeners, but instead grouped with *M. cretica*. Other recognizable groups were the *Malope* clade (BS 71), the shrubby *Lavatera* of section *Olbia* (BS 56), and the *L. triloba* aggregate (BS 51).

The evolution of four morphological characters (life form, number of epicalyx bracts, degree of fusion of epicalyx bracts and fruit type) was investigated on the posterior set of trees derived from the Bayesian analysis of the ITS data, thus taking phylogentic uncertainty into account. The results are shown on the majority rule consensus tree in Fig. 4. ITS data were chosen because they result in the best resolved topologies among those including all taxa studied; using the respective plastid topologies results in minor changes only, not affecting our overall conclusions (data not shown). The ancestral number of epicalyx bracts in the *Malva* alliance was inferred to be three, with reductions to two bracts occurring twice independently in *M. hispanica* and the *M. aegyptia* clade (Fig. 4A). Multifid epicalyces (with six or more segments) were inferred to have appeared independently three times, in *L. plazzae*, the annual *Althaea* clade and the *Alcea* clade. The epicalyx bracts are inferred to ancestrally have been free, followed by an early shift to fused bracts in early diverging groups (the *Alcea* clade) and multiple independent reversals to free bracts in *Malope*, the *Malva aegyptia* clade, the malvoid *Lavatera* species, *M.* sect. *Bismalva* and *M. hispanica* (Fig. 4B). The ancestor of the *Malva* alliance was a perennial,

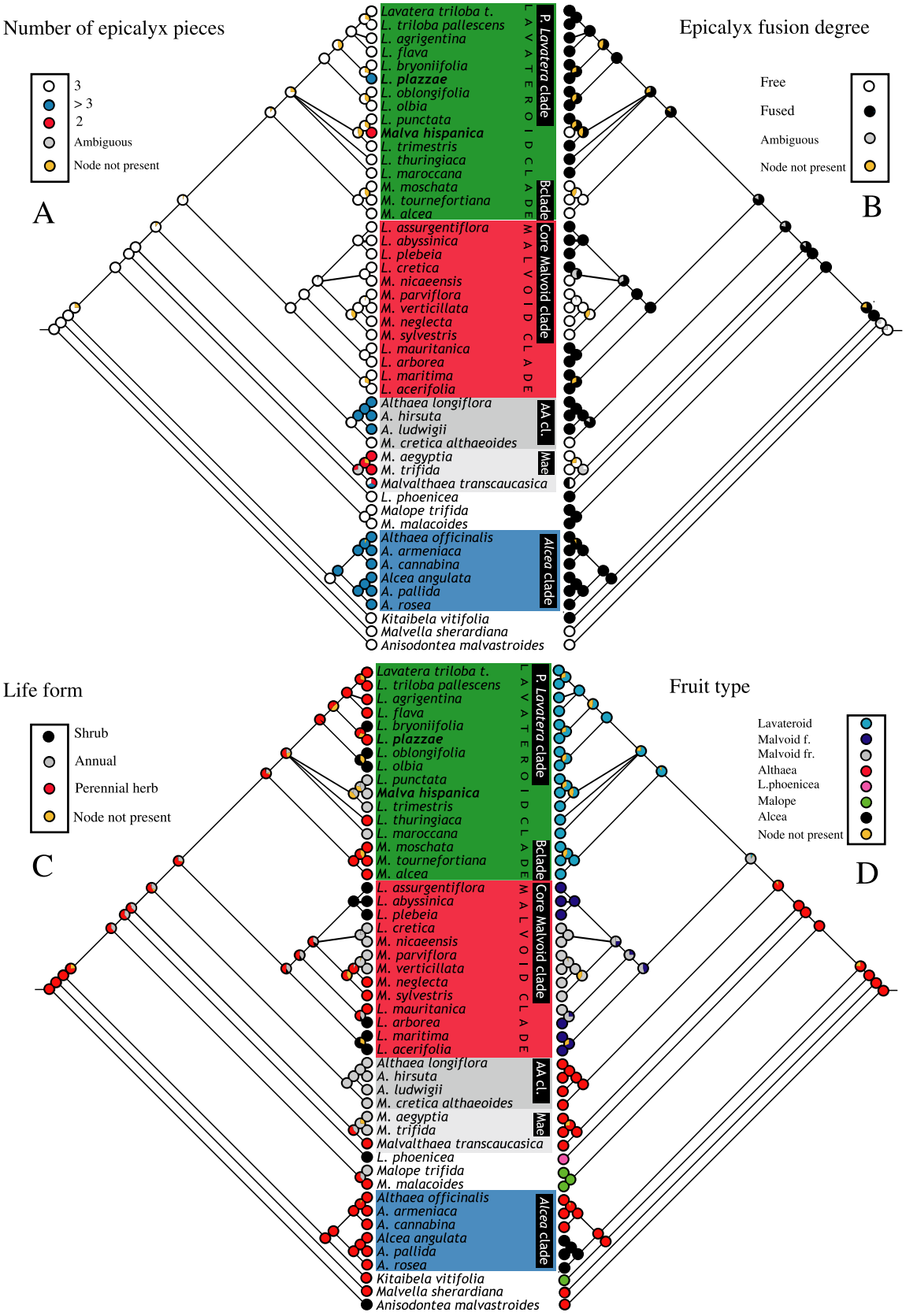

Fig. 4. Evolution of morphological characters in the *Malva* alliance. A, number of epicalyx pieces. B, epicalyx fusion degree. C, life form. D, fruit type. *P. Lavatera* clade, Perennial *Lavatera* clade; B clade, *Bismalva* clade; AA cl., Annual *Althaea* clade; Mae, *Malva aegyptia* clade. Malvoid f., Malvoid fused; Malvoid fr., Malvoid free. Morphologically divergent lavateroids with > 3 (*L. plazzae*) or 2 (*M. hispanica*) epicalyx pieces are given in bold. For details see text.

although it was not possible to determine whether shrubby or herbaceous, with six or seven independent changes to annual life form (Fig. 4C). Fruit types are clade-characteristic, with all the representatives of the lavateroid and malvoid clades possessing lavateroid

or malvoid fruits, respectively (Fig. 4D). Within the malvoids, the fused mericarp type found among the ruderal mallows was inferred to be derived from a more primitive, non-fused mericarp malvoid fruit. The *Alcea* (pseudobilocular) type was restricted to the homonymous clade, while the *Malope* type (globose fruits of unordered or verticillately arranged carpels) appeared twice, independently in *Malope* and *Kitaibela vitifolia* (*Alcea* clade).

4. Discussion

4.1. Extensive morphological homoplasy in diagnostic characters obscures recognition of natural lineages

With the exceptions of the small genera *Malope*, *Kitaibela*, and *Malavalthaea* (see Section 4.2) and the still understudied *Alcea* with many species in the Middle East (Zohary, 1963a), molecular data strongly suggest that the current classification does not reflect monophyletic lineages. This is particularly pronounced in *Malva* and *Lavatera*, where the relationships inferred from molecular data strongly contrast with the traditional classification based upon the number and degree of fusion of the epicalyx bracts. Both characters are inferred to have undergone multiple independent changes and accordingly high levels of homoplasy (Fig. 4A and B), rendering *Lavatera* and *Malva* in their current circumscription highly unnatural (in the sense of non-monophyletic) groups. In contrast, all species possessing thick-walled, indehiscent schizocarps (malvoid fruits) cluster together in a well-supported clade (malvoid clade; Figs. 1–3, 4D), regardless of their inclusion in traditional *Lavatera* or *Malva*. These species additionally share a chromosome base number of $x = 7$ with small chromosomes and consequently low DNA amounts ($1C_x$-values around 0.240 pg: Escobar García et al., 2004) and are high polyploids ranging from the widespread hexaploid up to the 16-ploid level in *L. cretica* (Luque and Devesa, 1986, and Escobar unpublished data). Within the malvoid clade two morphological transitions can be observed. One concerns fruit morphology, from true schizocarps with mericarps released individually when ripe in the basal malvoid clades (*L. maritima*, *L. arborea*) to mericarp fusion in the ruderal mallows of the core malvoids, which possess, in addition to the characteristic tiny fasciculate flowers subtended by leaf-like bracts, thicker pericarps and mature fruits dispersing as a single diaspore. The second change is from shrubs in *L. maritima*, *L. acerifolia*, *L. arborea* and the morphologically often very similar extra-Mediterranean species of the core malvoids (e.g., *L. plebeia*, L. *assurgentiflora*) to annual herbs (e.g., *M. parviflora, M. nicaeensis*). This transition occurred more than once and is in line with the remarkable plasticity observed in some species. For instance, *L. arborea* is a shrubby perennial under benign conditions, while it behaves as an annual in harsh environments, and *L. cretica* and *M. sylvestris* show a tendency to perennate when thriving in mild habitats.

Fruits with fused mericarps that release the seeds when ripe (lavateroid fruit) characterize the lavateroid clade. With the data currently available it is, however, not possible to determine, whether this fruit type is a symplesiomorphy of a paraphyletic lavateroid grade or a synapomorphy of a monophyletic lavateroid clade. Several well-supported lineages within the lavateroid clade with unclear relationships to each other merit further mentioning. *Malva* sect. *Bismalva* (*M. moschata, M. tournefortiana* and *M. alcea*) comprises perennial herbs with a dimorphic indumentum of stellate and simple hairs and solitary flowers subtended by leaf-like bracts. The clade of perennial *Lavatera* includes species of the traditional sections *Olbia* and *Glandulosae*. Section *Olbia* (*L. olbia*, *L. oblongifolia*, *L. bryoniifolia*) includes mostly evergreen shrubs with solitary flowers in terminal bracteate racemes, a characteristic carpophore longer than the mericarps and a monomorphic indumentum of stellate hairs. Those of sect. *Glandulosae* (*L. triloba*, *L. flava*, *L. agrigentina*) display axillary fasciculate flowers, leaf-like bracts, and a dimorphic indumentum of glandular and stellate hairs. Additionally, the Sardinian endemic *L. plazzae* (Atzei, 1995), a tall perennial herb with paniculate terminal ebracteate inflorescences and a unique epicalyx of 3–6 lobes, also belongs here. The species of the perennial *Lavatera* clade have fruits with 13–20 mericarps, while the remaining *Lavatera* and *Malva* species have up to 10(−15). Species of the perennial *Lavatera* clade also share the same chromosome number ($2n = 6x = 44$), and have larger chromosomes with higher DNA amounts ($1C_x$-values around 1.10 pg, Escobar, unpublished data) than the malvoid species.

The exact phylogenetic position of a few other species within the lavateroid clade remains obscure. This includes *L. trimestris*, which possesses a unique umbrella-like carpophore covering the mericarps and has therefore sometimes been treated as the monotypic genus *Stegia* (an invalid name due to the fact that *L. trimestris* is the type of genus *Lavatera*) or section *Stegia* within *Lavatera* (Alefeld, 1862; de Candolle, 1805c). Morphologically similar species are *L. maroccana*, with an umbrella-like carpophore not covering the mericarps, and *L. punctata* with an extended, but not umbrella-like carpophore, all three species sharing extended, campanulate, fused epicalyces of mucronate bracts, sometimes with tiny lobes between the three main ones. Nevertheless, none of the molecular analyses support close affinities among those taxa, and these morphological similarities might therefore be homoplasious as well.

The phylogenetic position of the Canary Islands endemic *Lavatera phoenicea* is unclear, being resolved either as sister to the core *Malva* alliance (ITS, this study, and low copy nuclear genes, Escobar, unpublished data) or as sister of the malvoid clade (plastid data), sharing with it the indehiscent thick-walled mericarps. This species is morphologically very divergent, possessing an unusually high number of mericarps (30–40) that bear two horn-like protuberances, a deciduous epicalyx, articulate flower stalks and a unique nectary-structure. Therefore, Webb and Berthelot (1836) segregated this species as monotypic genus *Navaea*. The second Canary Islands species, *L. acerifolia* of the malvoid clade, is not closely related to *L. phoenicea*, but to the western Mediterranean *L. maritima*, and thus represents a second independent island colonization (Fuertes Aguilar et al., 2002). The extra-Mediterranean species *L. assurgentiflora* and its relatives from the Californian Channel Islands (*L. insularis*, *L. lindsayi*, *L. occidentalis*, *L. venosa*) are morphologically astonishingly similar to *L. acerifolia*, all of them with maple-like leaves covered with tiny stellate hairs and slightly zygomorphic flowers with a flexuous staminal column. Therefore, they were included into the genus *Saviniona* (*L. acerifolia*: Webb and Berthelot, 1836) by Greene (1912), but they are not each other's closest relatives (Figs. 1–3), rendering *Saviniona* paraphyletic. This remarkable morphological similarity (Greene, 1912) might be a symplesiomorphy of the malvoid clade or the result of convergent evolution as adaptation to, for instance, dry island environments and similar pollinators. Interestingly, the North American species are closely related to the other extra-Mediterranean *Lavatera* taxa from Ethiopia and Australia. The core *Malva* alliance is a clear Western Mediterranean-centered group, but the radiation to tropical Africa, North America and Australia pose a complex biogeographic pattern which is hardly comparable to any other plant group and requires further study.

The lack of congruence of molecular data and current classification also affects the comparatively small genus *Althaea*. Its species, which against previous assertions (Baker, 1890; de Candolle, 1824; Willdenow, 1800) are clearly distinct from those of *Alcea* (Medikus, 1787; Pakravan, 2001; Riedl, 1976; Townsend, 1980; Zohary, 1963b,c), fall into two morphologically distinct groups traditionally recognized as sections, which turn out not to be each other's closest relatives. Instead, the perennial species of sect. *Althaea* are sister to *Alcea*, while the annual species of sect. *Hirsutae* are

more closely related to *Malva cretica*. *Althaea* in the Linnean circumscription shares with *Alcea* the number of epicalyx bracts (five or more), but differs by possessing a terete staminal column and mericarps lacking endoglossa, an inner fruit wall extension unique to *Alcea*. A morphological link between the annual *Althaea* species and the annual mallows *M. cretica* and *M. aegyptia* has long been recognized based on fruit characters and life form, and Alefeld (1862) proposed a new classification alternative to the Linnaean one, including the annual *Althaea*, *M. aegyptia* and *M. cretica* within a single group. Later, Krebs (1994b) grouped these taxa plus the genus *Malvalthaea* (unknown to Alefeld) within the genus *Dinacrusa*, using characteristics of the epicalyx for infrageneric classification. This classification scheme is, however, also not supported by our data, which link *M. cretica* with the annual *Althaea*, and *Malvalthaea* either with *Malva aegyptia* (ITS data) or *Althaea hirsuta* (cpDNA). Due to the lack of morphological synapomorphies, it is currently not possible to characterize these two clades.

4.2. Phylogenetic incongruence, hybrid speciation and introgression among the malvoids

Phylogenetic relationships in the Malveae appear to be significantly shaped by reticulate events. Even using the very conservative approach employed here by considering only cases with strongly supported, but contradictory inferences from the nuclear and the plastid data, several unambiguous cases of reticulate evolution are found. Given that several other cases of contradicting, yet not mutually well-supported relationships are found (e.g., *L. plazzae*) and that cases, where the ITS copies converge towards the maternal parent (Brochmann et al., 1996; Álvarez and Wendel, 2003), will remain undetected, we consider the following examples as mere tips of the iceberg.

The first case is *Malvalthaea transcaucasica* from primary steppes in southwestern Asia. Due to an ambiguous epicalyx shape intermediate between *M. aegyptia* and *Althaea hirsuta* (3–7 linear-lanceolate epicalyx pieces which are never all fused) it has been named *Malvalthaea* (Iljin, 1924, 1949). Actually, it groups with those taxa in the nuclear and plastid data set, respectively (Figs. 2 and 3), confirming its hybrid (maybe allopolyploid, but no karyological data available) origin. The fact that *Malvalthaea* is a lignified perennial, while both *M. aegyptia* and *Althaea hirsuta* are annuals with no hybrids between them known, suggests an ancient origin of this taxon.

An allopolyploid origin is strongly suggested for *Lavatera mauritanica*, a dodecaploid species ($2n = 12x = 84$) grouping with hexaploid *L. arborea* ($2n = 6x = 40,42,44$) in the nuclear data, but with ruderal *Malva* species, most of them also being hexaploids ($2n = 6x = 42$) in the mostly maternally inherited (Harris and Ingram, 1991) plastid data (Figs. 1 and 2). Morphologically, both *L. mauritanica* and *L. arborea* share unique petals with dark bases.

Among the closely related small-flowered ruderal malvoids, different accessions of *M. neglecta* do not form a cohesive group, but group either with *M. nicaeensis* and *Lavatera cretica* (data not shown) or with *M. verticillata*. This is probably due to hybridization, a well known phenomenon among the hexaploid small-flowered mallows (*M. neglecta*, *M. nicaeensis*, *M. parviflora* and *M. sylvestris*), which has caused a high number of names describing the variability and morphological diversity of hybrids (see Sennen, 1910, 1932).

4.3. Possible generic circumscriptions within the Malva alliance

The current delimitation of *Malva*, *Lavatera* and *Althaea* is clearly artificial and untenable. Both *Malva* and *Lavatera* include taxa that could be separated taking into consideration their fruit morphology in malvoids and lavateroids, depending on whether they are more related to *Malva sylvestris* (typus of *Malva*) or to *Lavatera trimestris* (typus of *Lavatera*). With the data currently available, it is not possible to determine whether lavateroids are monophyletic or paraphyletic.

Two alternative approaches are possible: (1) splitting the *Malva* alliance into a number of small and clearly monophyletic entities, or (2) merging both lavateroids and malvoids into a single genus, which for nomenclatural reasons would have to be named *Malva*. The first approach would lead to the recognition of up to 12 independent genera, only the circumscriptions of *Malope*, *Kitaibela* and *Alcea* remaining unaltered. *Lavatera* would retain only its typus, *L. trimestris*, and maybe the morphologically close but phylogenetically unclear *L. maroccana* and *L. punctata*. The perennial species of *Lavatera* could remain as segregates in genus *Olbia* Medik., with the exception of *L. thuringiaca*, which does not seem to be closely related to any of these taxa. The same would apply for *Malva hispanica*, a lavateroid of isolated position. The lavateroid *Malva* could be easily transferred to the genus *Bismalva* Medik., and the malvoid *Lavatera* (*L. acerifolia*, *L. maritima* and *Lavatera* section *Anthema*) could be moved to *Malva* L., as already suggested by Webb and Berthelot (1836). Compared to *Lavatera,* the circumscription of *Malva* would remain fairly stable, major changes concerning the addition of malvoid *Lavatera* species and the exclusion of *M. aegyptia* and *M. trifida*, which are neither malvoid nor lavateroid. Regarding *Althaea*, it is clear that the annual species need to be removed from this genus, but our data do not suggest any obvious taxonomic solution. *Lavatera phoenicea*, whose phylogenetic position differs strongly among different molecular and morphological markers, could be recognized again as monotypic genus *Navaea* Webb & Berthel. Until the position of such *incertae sedis* taxa is better understood, for example by applying low copy molecular markers, splitting the *Malva* alliance appears not recommendable.

The other approach, applying the name *Malva* to all malvoid and lavateroid taxa, does not seem to be very useful, as it would result in a morphologically very diverse and thus hardly diagnosable genus. Therefore, we are in favor of keeping the current taxonomy of the group transferring only very clear cases, such as the malvoid *Lavatera* to genus *Malva*.

4.4. Conclusions

Several of the traditionally used morphological characters in Malveae, in particular features of the epicalyx and life form, are hampered by extensive homoplasy, rendering them of limited suitability for generic diagnoses, which, at least in part, is connected to a significant level of reticulate evolution (with or without chromsome number changes). On a more positive note, fruit morphology largely agrees with the limits of phylogenetic lineages, although their assessment as symplesiomorphies or as synapomorphies is not always clear (e.g., the lavateroid fruit). Accordingly, the current circumscriptions of *Althaea*, *Lavatera* and *Malva* based on epicalyx features cannot be retained, since these render them polyphyletic. Further studies employing other molecular markers, in particular low copy genes, will be necessary to address the not yet unambiguously resolved relationships of, for instance, the lavateroid clade or *L. phoenicea*, and eventually propose a modern classification in this notoriously difficult group.

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Appendix A

Data matrix of morphological characters. Unknown character states are indicated with ?, polymorphic ones with v. Leaf sequence. 0 homophyllous (entire leaves), 1 homophyllous (lobate leaves), 2 heterophyllous. LifeF. Life form. 0 Annual, 1 perennial herb, 2 shrub. NEpic. Number of epicalyx pieces. 0 three, 1 two, 2 more than three. FEpic. Fusion degree of the epicalyx pieces. 0 free, 1 fused. IEpic. Insertion point of the epicalyx bracts. 0 calyx base, 1 flower stalk, 2 calyx pieces. Fruit. Fruit type. 0 malvoid fused, 1 malvoid, 2 lavateroid, 3 undifferentiated, 4 *Malope*, 5 *L. phoenicea*, 6 *Alcea*. NMeric. Number of mericarps. 0 up to 15, 1 up to 25, 2 up to 20, 3 up to 40. Carpoph. Carpophore. 0 not surpassing the fruit, 1 slightly surpassing the fruit, 2 clearly surpassing the fruit. Ploid. Ploidy level. 0 hexaploid, 1 octoploid, 2 16-ploid, 3 tetraploid. StamCol. Staminal column. 0 of pentagonal section, 1 of circular section. FlowerAr. Flower arrangement. 0 solitary or geminated, 1 fascicles. Infl. Inflorescence. 0 terminal, 1 not terminal. Ind. Indumentum. 0 simple hairs, 1 stellate hairs, 2 mixed simple and stellate (or pluriradiate) hairs, 3 glandular hairs.

TAXON	LeafS.	LifeF.	NEpic.	FEpic.	IEpic.	Fruit	NMeric.	Carpoph.	Ploid.	StamCol.	FlowerAr.	Infl.	Ind.
Alcea aucheri	0	1	1	1	0	6	3	1	0	0	0	0	2
Alcea rosea	0	1	1	1	0	6	3	1	0	0	0	0	2
Althaea armeniaca	1	1	1	1	0	3	2	0	1	1	0	1	1
Althaea cannabina	1	1	1	1	0	3	2	0	1	1	1	1	1
Althaea hirsuta	2	0	1	1	0	3	1	0	0	1	0	1	2
Althaea longiflora	2	0	1	1	0	3	1	0	3	1	0	1	2
Althaea ludwigii	2	0	1	1	0	3	1	0	0	1	0	1	2
Althaea officinalis	0	1	1	1	0	3	2	0	0	1	1	1	1
Lavatera abyssinica	1	2	0	1	0	1	0	0	0	1	1	1	1
Lavatera acerifolia	1	2	0	1	0	1	0	0	0	1	0	1	1
Lavatera agrigentina	0	1	0	1	0	2	1	2	0	1	1	1	3
Lavatera arborea	0	2	0	1	0	1	0	0	0	1	1	1	1
Lavatera assurgentiflora	1	2	0	1	0	1	0	0	0	1	0	1	1
Lavatera bryoniifolia	1	2	0	1	0	2	1	1	0	1	0	0	1
Lavatera cretica	0	0	0	1	0	0	0	0	2	1	1	1	1
Lavatera flava	0	1	0	1	0	2	1	2	0	1	1	1	3
Lavatera maritima	0	2	0	1	0	1	0	0	0	1	0	1	1
Lavatera maroccana	0	0	0	1	0	2	1	2	0	1	0	1	2
Lavatera mauritanica	0	1	0	1	0	0	0	0	0	1	1	1	1
Lavatera oblongifolia	0	2	0	1	0	2	1	1	0	1	0	0	1
Lavatera olbia	1	2	0	1	0	2	1	1	0	1	0	0	1
Lavatera phoenicea	1	2	0	1	0	5	3	2	0	1	0	1	1
Lavatera plazzae	0	1	0	1	0	2	1	2	?	1	1	0	2
Lavatera plebeia	1	2	0	1	0	1	0	0	0	1	1	1	1
Lavatera punctata	2	0	0	1	0	2	1	1	0	1	0	1	2
Lavatera thuringiaca	2	1	0	1	0	2	1	0	0	1	1	1	1
Lavatera triloba	0	1	0	1	0	2	1	2	0	1	1	1	3
Lavatera triloba ssp.pallescens	0	1	0	1	0	2	1	2	0	1	1	1	3
Lavatera trimestris	0	0	0	1	0	2	0	1	0	1	0	1	2
Malope malacoides	0	1	0	1	0	4	1	0	0	1	0	0	0

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