Molecular phylogeny of *Euphorbia* subg. *Esula* sect. *Aphyllis* (Euphorbiaceae) inferred from nrDNA and cpDNA markers with biogeographic insights

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Abstract *Euphorbia* subg. *Esula* (Euphorbiaceae) has recently been shown, using molecular analyses, to contain a clade with a disjunct distribution in Macaronesia, South Africa and the Eritreo-Arabian region, and being primarily made up of members of sect. *Tithymalus* subsect. *Pachycladae* and sect. *Tirucalli*. To delimitate this disjoint group, we carried out phylogenetic analyses of the internal transcribed spacer (nrITS) using a broad sampling, with emphasis on subg. *Esula*. Subsequently, we carried out phylogenetic analyses focused on this clade using nuclear (ITS, ETS) and chloroplast (*trnL-trnF*, *psbA-trnH*, *ycf3-trnS*, *trnG*, *atpB-rbcL*, *trnK-matK*, *trnT-trnL*) markers, with the aim of resolving the phylogenetic relationships within the group and reconstructing its biogeographic history. Our results showed that sect. *Tithymalus* subsect. *Pachycladae* and sect. *Tirucalli* are polyphyletic. Section *Aphyllis* is recircumscribed to comprise the *Pachycladae* core clade and part of sect. *Tirucalli*. Low resolution within sect. *Aphyllis* and incongruences between nuclear and chloroplast phylogenies may be due to hybridization. Section *Aphyllis* should have originated in the Mediterranean area; its disjunct distribution is probably due to vicariance, resulting from fragmentation of a wider distribution area in North Africa caused by the aridification of the climate during the late Miocene-Pliocene.

Keywords Eritreo-Arabian region; *Euphorbia*; Macaronesian region; *Pachycladae*; Rand Flora; South Africa; *Tirucalli*

■ INTRODUCTION

Euphorbia L. (Euphorbiaceae, Euphorbioideae) is considered the second largest genus in the angiosperms, including ca. 2000 species (Oudejans, 1990). It comprises a large diversity of life forms, including annual and perennial herbs, trees, spiny succulents and non-spiny pencil-like succulents, mainly distributed in temperate and tropical habitats. *Euphorbia* can be easily recognised by the presence of latex in the stems and the organisation of the reduced flowers in a structure named the cyathium, which acts as a pseudanthium. Recent molecular studies have shown that the traditional infrageneric classification does not agree with the natural circumscription of monophyletic lineages and have detected four clades within *Euphorbia* s.l. (A–D; Steinmann & Porter, 2002), which correspond to four newly circumscribed subgenera: subg. *Rhizanthium* (Boiss.) Wheeler, subg. *Chamaesyce* Raf., subg. *Euphorbia* and subg. *Esula* Pers. (Bruyns & al., 2006).

Subgenus *Esula* comprises approximately 400 herbaceous and shrubby species primarily distributed in temperate regions of the Northern Hemisphere. Most of the species have cyathia with nectariferous involucral glands, as well as a caruncle on their seeds. Subgenus *Esula* (formerly referred to as clade B; Steinmann & Porter, 2002; Bruyns & al., 2006) is the sister clade to the rest of *Euphorbia* (Horn & al., 2009; Zimmermann & al., 2010).

This new delineation of subg. *Esula* largely corresponds to section *Tithymalus* Boiss. (Steinmann & Porter, 2002). Boissier (1862) created within sect. *Tithymalus* the unranked category *Pachycladae* Boiss. and divided it into two groups (Table 1). The first group, *Canarienses et Mediterraneae*, comprises woody spurges; eight species endemic to the Macaronesian region; one species mainly distributed in the Canary Islands but also found in West Africa and Yemen (*E. balsamifera* Aiton) and one Mediterranean species (*E. dendroides* L.). The second group, *Polynesicae et Sundaieae*, comprises Australasian species from the Fiji Islands, New Zealand, Norfolk Island and Malesia.

The group *Canarienses et Mediterraneae* has undergone notable changes throughout history with regard to its rank and circumscription (Table 1). Pax & Hoffmann (1931) considered the species in this group as subsect. *Pachycladae* (Boiss.) Pax within sect. *Tithymalus*. In several regional floristic treatments, the group is described within several taxonomic ranks (Vindt, 1953; Radcliffe-Smith & Tutin, 1968; Radcliffe-Smith, 1982; Benedí & al., 1997).

Molecular phylogenies have determined that sect. *Tithymalus* subsect. *Pachycladae* is polyphyletic, as the species traditionally included in this group belong to several independent clades (Molero & al., 2002; Steinmann & Porter, 2002; Bruyns & al., 2006): (1) the *Pachycladae* core clade, which includes some species from sect. *Tithymalus* subsect. *Pachycladae* −*Euphorbia atropurpurea* Brouss. ex Willd., *E. bravoana* Svent., *E. piscatoria* Aiton, *E. regis-jubae* J. Gay and *E. tuckeyana* Steud. ex Webb; (2) *Euphorbia balsamifera*, related with other species from subg. *Lyciopsis* (Boiss.) Wheeler sect. *Somalica*

S. Carter; (3) *Euphorbia stygiana* H.C. Watson and *E. mellifera* Aiton, two lauroid trees from the Canary Islands, Madeira and Azores that are not closely related to the *Pachycladae* core clade; and finally (4) *Euphorbia dendroides*, also excluded from the *Pachycladae* core clade. These four clades of species previously calssified in sect. *Tithymalus* subsect. *Pachycladae* are scattered throughout subg. *Esula* and subg. *Rhizanthium*.

Section *Tirucalli* Boiss., which was later defined at the subgeneric level by Carter (1985), was first described to include a group of "pencil-like" species, defined as subsucculent shrubs with cylindrical photosynthetic stems and reduced deciduous leaves. The East/South African and Arabian elements of sect. *Tirucalli* comprise two morphologically well-differentiated groups. First, the *Euphorbia mauritanica* L. complex, described by Leach (1975) and later confirmed by Carter (1992), includes species with foliar bracts, absence of glandular stipules and pleiochasia with 3–8 rays. The members of this complex appeared related to the aforementioned *Pachycladae* core clade in molecular phylogenies (Steinmann & Porter, 2002; Bruyns & al., 2006). Second, *E. tirucalli* L. and related species would constitute the "true" *Tirucalli*. This group, characterised by the presence of scarious bracts, glandular stipules and congested synflorescences, is placed in subg. *Euphorbia* (Steinmann & Porter, 2002; Bruyns & al., 2006). Boissier (1862) also included two Macaronesian species within sect. *Tirucalli*: *Euphorbia aphylla* Brouss. ex Willd., which was originally classified in the monotypic section *Aphyllis* Webb & Berthel. (Webb & Berthelot, 1842); and *E. lamarckii* Sweet. However, both species also belong to the *Pachycladae* core clade (Molero & al., 2002).

Despite these findings, many species traditionally classified in sect. *Tithymalus* subsect. *Pachycladae* and sect. *Tirucalli* have never been included in any comprehensive molecular phylogenetic analysis and their closest relatives remain unknown.

The disjunct geographic distribution of sect. *Tithymalus* subsect. *Pachycladae* and allies in Macaronesia and eastern/southern Africa, southern Arabia and Socotra represents an

Table 1. Historical sectional classifications of the most relevant species included in this study.

Webb & Berthelot (1842)	Boissier (1862)	Pax & Hoffmann (1931)
Sect. *Aphyllis* Webb & Berthel.	**Sect. *Tirucalli* Boiss.**	**Sect. *Tithymalus* Boiss.**
E. aphylla	*E. aphylla*	**Subsect. *Pachycladae* (Boiss.) Pax**
Sect. *Balsamis* Webb & Berthel.	*E. dregeana*	*E. atropurpurea*
E. balsamifera	*E. larica*	*E. balsamifera*
Sect. *Tithymalus* Tourn.[a]	*E. lateriflora*	*E. berthelotii*
†† ***Esula* Haw.[a]**	*E. mauritanica*	*E. bourgeana*
**** Frutescentes***	*E. obtusifolia* (= *E. lamarckii*)	*E. dendroides*
E. atropurpurea	*E. schimperi*	*E. fidjiana*
E. obtusifolia (= *E. lamarckii*)	*E. tirucalli*	*E. glauca*
E. piscatoria	**Sect. *Tithymalus* Boiss.**	*E. mellifera*
E. regis-jubae	**[§] *Pachycladae* Boiss.**	*E. norfolkiana*
**** Arborescentes***	**** Canariensis et Mediterraneae***	*E. piscatoria*
E. mellifera	*E. atropurpurea*	*E. plumerioides*
	E. balsamifera	*E. regis-jubae*
	E. berthelotii	*E. stygiana*
	E. bourgeana	*E. tuckeyana*
	E. dendroides	**Subsect. *Galarrhoei* (Boiss.) Pax**
	E. mellifera	*E. usambarica*
	E. piscatoria	**Sect. *Euphorbium* Boiss.**
	E. regis-jubae	**Subsect. *Tirucalli* (Boiss.) Pax**
	E. stygiana	*E. dregeana*
	E. tuckeyana	*E. gummifera*
	**** Polynesicae et Sundaicae – Species anomale***	*E. lateriflora*
	E. glauca	*E. mauritanica*
	E. fidjiana	*E. tirucalli*
	E. norfolkiana	
	E. plumerioides	

[a] This name was not validly used in this work.

example of a phytogeographic pattern found in other plant groups (reviewed by Andrus & al., 2004), collectively known as the Rand Flora (Christ, 1892; Le Houérou, 1995). This disjunct distributional pattern has been explained by two alternative hypotheses. Based on ecological and floristic studies (Quézel, 1978; Sunding, 1979; Bramwell, 1985; Médail & Quézel, 1999) as well as recent phylogenetic studies (Park & al., 2001; Moore & al., 2002; Andrus & al., 2004; Thiv & al., 2010), vicariance has been proposed as the most reliable explanation. Aridification of the Mediterranean basin during the Upper Miocene and Pliocene (Axelrod, 1975) would have resulted in two refugial distribution centres, one in the eastern and one in the western part of northern Africa, for the flora that previously occupied a continuous strip in this area. In contrast, Thulin (1994), Francisco-Ortega & al. (1999), Carine (2005), Galley & Linder (2006) and Sanmartín & al. (2008) postulated that the disjunct distribution could be due to more recent, post-aridification long-distance dispersal events between the Horn of Africa and the Macaronesian region, followed by fast diversification events in each of these regions. The southern African Flora has also been considered part of the Rand Flora, following the "African track" proposed by Linder & al. (1992). This route connects southern Africa with East Africa and, via the Sahara, with Macaronesia and the Mediterranean region. Two directions of this migration route have been proposed for different plant groups: northwards from southern Africa to the Horn of Africa and then the Macaronesian and Mediterranean areas (Bellstedt & al., 2008; Galbany-Casals & al., 2009) or southwards from the Mediterranean area and the Horn of Africa to southern Africa (Levyns, 1964; Axelrod & Raven, 1978; McGuire & Kron, 2005).

Throughout this paper, the Macaronesian biogeographic region is meant to include five archipelagos in the Atlantic Ocean (Azores, Madeira, Cape Verde, Selvagens Islands, and Canary Islands), one continental enclave in Morocco (Sunding, 1979; Barbero & al., 1992; García-Verdugo & al., 2010) and Cape Espichel in Portugal (Pedro, 1942; Carine & al., 2004). The origin of the Macaronesian archipelagos is volcanic and their age ranges from 20.6 Ma for Lanzarote Island to 1.77 Ma for El Hierro Island (Geldmacher & al., 2001; Carracedo & al., 1998; Holm & al., 2006). Several groups of plants seem to have diversified in the Macaronesian archipelagos following a single colonization event (Francisco-Ortega & al., 1997, 1999, 2001; Helfgott & al., 2000; Allan & al., 2004) while others have reached these archipelagos multiple times (Cuenoud & al., 2000; Hess & al., 2000; Park & al., 2001; Percy & Cronk, 2002; Kilian & al., 2010).

The aims of our study were twofold: first, to provide a general phylogenetic framework for sect. *Tithymalus* subsect. *Pachycladae* and its relatives based on a broad sampling of *Euphorbia* species and using sequences of the nrITS region, widely employed for inferring *Euphorbia* phylogenies (Molero & al., 2002; Steinmann & Porter, 2002; Bruyns & al., 2006; Zimmermann & al., 2010); second, to provide a robust hypothesis of relationships in the disjunct clade of subg. *Esula* using a subset of taxa, with focused sampling in sect. *Tithymalus* subsect. *Pachycladae* and sect. *Tirucalli*, and explore its biogeographic history. To achieve our second aim, we expanded upon our ITS dataset by adding sequences from the nrDNA ETS region and seven non-coding chloroplast markers. In addition, we investigated the sectional classification within subg. *Esula*, with particular focus on the western Mediterranean species.

■ MATERIALS AND METHODS

Plant material. — A general sampling of the genus *Euphorbia* included the four subgenera previously identified in *Euphorbia* (Steinmann & Porter, 2002; Bruyns & al., 2006) and a representation of African, American and Asian species. In addition, a wide sampling of Mediterranean species of subg. *Esula* was done. A total of 154 taxa were included, of which 95 were newly sequenced and 59 downloaded from GenBank. As to the main group of study, all members of *Euphorbia* sect. *Thithymalus* subsect. *Pachycladae* were included (11 species) as well as 21 species from sect. *Tirucalli*, including members of the *E. mauritanica* complex and some morphologically similar ones of unknown phylogenetic position. Accession numbers and sources of material are given in the Appendix. Nomenclature follows Govaerts (2010).

DNA extraction, amplification and sequencing. — Total genomic DNA, from fresh or silica gel-dried leaf tissues, taken from field collections or herbarium specimens (in the herbaria BCN, K, W), was extracted using the DNeasy Plant Mini extraction kit (Qiagen, Hilden, Germany) modified by adding 5 µl of proteinase K at 20 mg/ml (Pereira, pers. comm.) in order to avoid the interference of secondary compounds that occur in *Euphorbia*.

Plant material from the Kew herbarium (K) was extracted following the CTAB method from Saghai-Maroof & al. (1984) as modified by Doyle & Dickson (1987) and Palmer & al. (1989). The DNA from old herbarium specimens was highly degraded and the extractions were concentrated with the commercial kit DNA Clean Concentrator-5 (Zymo Research Group, Orange, California, U.S.A.), following the manufacturer's instructions.

Two nrDNA regions—the internal transcribed spacer (ITS1, 5.8S, ITS2) and the external transcribed spacer (ETS)—and seven cpDNA regions (the *trnL-trnF* non-coding region, including the *trnL* intron and the *trnL-trnF* intergenic spacer; the *psbA-trnH* intergenic spacer; the non-coding *ycf3-trnS* region; the non-coding *trnG* region; the region between the *atpB* and *rbcL* genes and the first codons of the *rbcL* gene; the *trnK-matK* spacer; and the *trnT-trnL* non-coding region) were amplified using the universal primers indicated in Table 2.

Polymerase chain reactions (PCR) were performed in 25 µl volumes composed of 10% 10× AmpliTaq buffer, 10% 25 mM $MgCl_2$, 10% 2 mM dNTPs mix, 4% primers at 5 µM, 1 U AmpliTaq DNA polymerase (Applied Biosystems, Foster City, California, U.S.A.) and 2 µl of template DNA of an unknown concentration. This was filled up to 25 µl with distilled sterilised water. We added 0.5 µl DMSO (dimethyl sulfoxide; Sigma-Aldrich, Schnelldorf, Germany) in nrDNA regions and 2.5 µl BSA (bovine serum albumin; New England Biolabs,

Ipswich, Massachusetts, U.S.A.) at 400 ng/µl in cpDNA regions to enhance the PCR reaction.

The PCR conditions varied for each DNA region and consisted of a preheat for 1 min 35 s to 5 min at 94°C–96°C, followed by 30 to 35 cycles of the following steps: 30 s to 1 min at 94°C, 30 s to 1 min 30 s at 52°C–58°C and 30 s to 3 min at 72°C. A final extension phase of 10 to 15 min at 72°C was conducted. Annealing temperatures for each primer pair are given in Table 2.

PCR products were purified with either QIAquick Purification Kit (Qiagen, Valencia, California, U.S.A.) or DNA Clean & Concentrator-5.

Direct sequencing of the amplified DNA segments was performed with a Big Dye Terminator v.3.1 kit (Applied Biosystems) following the protocol recommended by the manufacturer. Nucleotide sequencing was carried out at the Serveis Científico-Tècnics of the University of Barcelona on an ABI PRISM 3700 DNA analyser (Applied Biosystems).

Alignments and phylogenetic analyses. — Sequences were edited visually with BioEdit v.7.0.9.0 (Hall, 1999) and aligned by hand or with the T-Coffee software (Notredame & al., 2000) and refined by hand. In all datasets, several areas of ambiguous alignment and/or autapomorphic indels were excluded. Alignments are available upon request from the corresponding author.

Dataset 1 included ITS sequences of 152 *Euphorbia* taxa and two related genera coded as outgroups (*Dichostemma glaucescens* Pierre and *Triadica sebifera* (L.) Small), selected following Steinmann & Porter (2002). Dataset 2 included sequences of nine markers (the two nrDNA regions and the seven cpDNA regions mentioned above) of 26 species, 22 of them recovered as clade 1 (Fig. 1) in the analysis of the first dataset, and four additional species coded as outgroups based on the analysis of dataset 1: *E. biumbellata* Poir., *E. dendroides*, *E. megalatlantica* Ball and *E. terracina* L. DNA of *Euphorbia lateriflora* Schumach. and *E. calamiformis* P.R.O. Bally & S. Carter was highly degraded and it could not be amplified for the *trnK-matK* marker. We coded the sequences of these species as missing data for this region in the combined matrix of dataset 2.

We carried out a partition homogeneity test (incongruence length difference, ILD; Farris & al., 1995a, b) to test the

Table 2. Primers description and amplification conditions used in this study.

Region	Primer	Direction	Sequences 5′ → 3′	Reference	T (°C)
ITS	ITS1[a]	F	TCCGTAGGTGAACCTGCGG	White & al., 1990	57
	ITS2[a,b]	R	GCTGCGTTCTTCATCGATGC		
	ITS3[a]	F	GCATCGATGAAGAACGCAGC		
	ITS4[a,b]	R	TCCTCCGCTTATTGATATGC		
	1460F[a]	F	TGTACACACCGCCCGT	Nickrent & al., 1994	
	307R[a,b]	R	TTGGGCTGCATTCCCA		
ETS	18SIGS[a]	R	GAGACAAGCATATGACTACTGGCAGGATCAACCAG	Baldwin & Markos, 1998	54
	ETSR38[a,b]	F	GGYGGTGCATGAGTGGTGATWY	Yang & al., pers. comm.	
trnL intron and *trnL-trnF* spacer	trnL-c[a]	F	CGAAATCGGTAGACGCTACG	Taberlet & al., 1991	58
	trnL-d[a,b]	R	GGGGATAGAGGGACTTGAAC		
	trnL-e[a]	F	GGTTCAAGTCCCTCTATCCC		
	trnL-f[a,b]	R	ATTTGAACTGGTGCAACGAG		
psbA-trnH	psbAF[a]	F	GTTATGCATGAACGTAATGCTC	Sang & al., 1997	53
	trnHR[a,b]	R	CGCGCATGGTGGATTCACAAATC		
trnT-trnL	A2[a,b]	F	CAAATGCGATGCTCTAACCT	Cronn & al., 2002	52
	trnB[a,b]	R	TCTACCGATTTCGCCATATC		
ycf3-trnS	SP43122F[a]	F	ATTGGCYACAAYTGAAAAGG	Hershkovitz, 2006	54
	SP44097R[a,b]	R	ATTCGAACCCTCGGTAAACA		
trnG	5′ trnG-2G[a,b]	F	GCGGGTATAGTTTAGTGGTAAAA	Shaw & al., 2005	54
	3′ trnGUUC[a,b]	R	GTAGCGGGAATCGAACCCGCATC		
trnK-matK	trnK-3914F[a,b]	F	TGGGTTGCTAACTCAATGG	Johnson & Soltis, 1995	52
	matK-1168R[a,b]	R	ATTGAATGAATTGATCGTA		
atpB-rbcL	2[a,b]	F	GAAGTAGTAGGATTGATTCTC	Savolainen & al., 1994	52
	5[a,b]	R	TACAGTTGTCCATGTACCAG		

F, Forward; R, Reverse; T (°C), annealing temperature.
[a] Primer used for amplifications.
[b] Primer used for sequencing.

heterogeneity of phylogenetic signals between the nuclear and chloroplast markers on dataset 2. ILD significance values were calculated in TNT v.1.1 (Goloboff & al., 2008) with the INCTST script (kindly provided by the authors of the program) using 1000 replicates.

Two matrices were used for the analyses of dataset 2: the first matrix was composed of the two nrDNA markers combined, and the second matrix was composed of the seven cpDNA markers combined.

For the three total matrices generated from both datasets, maximum parsimony (MP) and Bayesian inference (BI) analyses were conducted. Phylogenetic trees were constructed with PAUP* v.4.0b10 (Swofford, 2002) employing MP with heuristic searches consisting of 1000 replicates of random taxon addition with MULPARS in effect and tree bisection reconnection (TBR) branch swapping, and saving all the most parsimonious trees, except for dataset 1 where we used 1000 iterations with the constraint of saving no more than 1000 trees with a length ≥ 3085 due to memory restrictions. Parsimony-uninformative positions were excluded. In the case of dataset 2, MP analyses used indels as additional characters. Indel codification was done with IndelCoder v.1.0 (Müller, 2006) using the Modified Complex Indel Coding (MCIC) algorithm. After computing the strict consensus tree, bootstrap analyses (BS; Felsenstein, 1985) were performed using 1000 replicates of heuristic search with the default options except for dataset 1, where we used 1000 iterations with random taxa addition and no swapping. Nodes with BS ≥ 75 were considered as significantly supported.

Bayesian inference estimation was calculated with MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). In order to select the best-fit model of substitution for each region, the Akaike information criterion (Akaike, 1973) was used as implemented in the MrModeltest v.2.3 program (Nylander, 2004). Best-fit models of substitution chosen by each region are detailed in Table 3. For the Bayesian analyses of dataset 2, we set up a partitioned analysis to apply the parameters of the most appropriate model to each region. We ran two independent analyses with 12 million generations sampling every 1000 generations, in the case of dataset 1, and two independent analyses with 3 million generations sampling every 1000 generations for dataset 2 until they reached stationary frequencies (final split frequency between the two runs, $P < 0.01$). The first 25% of the total trees generated (3000 for dataset 1 and 750 trees for dataset 2) were eliminated before summarising the posterior distribution and computing a 50% majority-rule consensus tree. Nodes with posterior probability (PP) ≥0.95 were considered statistically supported.

■ RESULTS

The main characteristics and statistics of both datasets are summarised in Table 3. Parsimony and Bayesian analyses were congruent in topology and generally showed the same supported clades. For this reason, only the 50% majority-rule consensus tree from Bayesian analyses of dataset 1 (Fig. 1) and one of the most parsimonious phylograms from the MP analyses of dataset 2 (Fig. 2) are shown.

Phylogenetic analyses of dataset 1 (Fig. 1). — The four main clades correspond to the four lineages (subg. *Rhizanthium*, subg. *Chamaesyce*, subg. *Euphorbia*, subg. *Esula*) previously identified by other authors (Steinmann & Porter, 2002; Bruyns & al., 2006; Zimmermann & al., 2010). Subgenus *Esula* is recovered as a clade but without statistical support. Eight main supported clades are recovered within subg. *Esula* (clades 1–8). Clade 1 (BS = 76%, PP = 1) includes the *Pachycladae* core clade and 11 species from sect. *Tirucalli*. *Euphorbia dendroides* and other West Mediterranean species constitute the sister clade to clade 1 (PP = 0.98). Most of the remaining clades (2 to 8) include a mix of species from different taxonomic sections and will be further commented on in the discussion.

Subgenus *Euphorbia* is not statistically supported in our analyses. *Euphorbia tirucalli* and its closest relatives are included in subg. *Euphorbia*, constituting the "true" *Tirucalli* clade (clade 9; BS = 98%, PP = 1). *Euphorbia bariensis* S. Carter, *E. dhofarensis* S. Carter and *E. uzmuk* S. Carter & J.R.I. Wood appear as part of the "true" *Tirucalli* clade. The succulent species endemic to Macaronesia (*E. canariensis* L., *E. handiensis* Burchard) and from West Morocco (*E. resinifera* O. Berg) are included in subg. *Euphorbia*, constituting a robust clade with *E. drupifera* Thonn. and *E. meenae* S. Carter, from Tropical Africa and India, respectively (clade 10; BS = 100%, PP = 1).

Subg. *Chamaesyce* (BS = 76%, PP = 1) is sister to subg. *Euphorbia*. Subg. *Rhizanthium* (PP = 0.97) includes some species traditionally classified in sect. *Tirucalli* (*E. larica* Boiss., *E. masirahensis* Ghaz.) that appear closely related to *E. balsamifera* (clade 11; PP = 0.98).

Phylogenetic analyses of dataset 2 (Fig. 2). — The results of the ILD test ($P = 0.001$) and several hard incongruities detected in the topologies, obtained with the independent analyses, did not support the possibility of combining the nrDNA markers with the cpDNA markers.

nrDNA analyses (Fig. 2A). — A main clade constituted by the eastern/southern African, Arabian and Madagascan species traditionally classified in sect. *Tirucalli* is strongly supported (BS = 93%, PP = 1). Within this clade, *E. stolonifera* Marloth ex A.C. White, R.A. Dyer & B. Sloane, endemic to the Cape Floristic Region, is shown in a clade also including *E. berotica* N.E. Br., *E. calamiformis*, *E. gossypina* Pax, *E. nubica* N.E. Br., *E. papilionum* S. Carter and *E. schimperi* C. Presl, species from East and central Africa and Arabia (BS = 86%, PP = 1). Within this clade, *E. nubica* and *E. schimperi* appear closely related to each other (BS = 95%, PP = 1) and are included in a clade supported only by Bayesian analyses (PP = 1) together with *E. calamiformis* and *E. gossypina*.

Regarding the Macaronesian species, two main supported clades are detected: the first one includes *E. anachoreta* Svent., *E. berthelotii* Bolle ex Boiss., *E. pedroi* Molero & Rovira and *E. piscatoria* (PP = 0.95) and the second one is composed of *E. lamarckii* and *E. regis-jubae* (BS = 82%, PP = 1). The position of the other species is unresolved.

cpDNA analyses (Fig. 2B). — The analyses of cpDNA recover *E. tuckeyana* as sister to the rest of the species (BS =

Table 3. Main characteristics of each DNA region used in the phylogenetic analyses.

	Dataset 1			Dataset 2							
	ITS	ITS	ETS	nrDNA combined	*trnL-trnF*	*trnT-trnL*	*ycf3-trnS*	*psbA-trnH*	*trnG*	*atpB-rbcL*	*trnK-matK*	cpDNA combined
Number of taxa	154	26	26	26	26	26	26	26	26	26	24	26
Alignment length	634	636	342	978	1152	435	483	593	638	959	980	5240
Length range	531–581	629–635	337–342	968–974	874–1091	387–412	340–480	449–524	620–630	896–934	963–976	4653–4934
Variable characters (%)	344 (54.26)	152 (23.90)	78 (22.80)	230 (23.52)	181 (15.71)	33 (7.58)	47 (9.73)	58 (9.78)	36 (5.64)	86 (8.97)	65 (6.63)	506 (9.65)
Parsimony-informative characters (%)	97 (15.30)	68 (10.69)	39 (11.40)	107 (10.94)	118 (10.24)	25 (5.74)	39 (8.07)	46 (7.75)	18 (2.82)	29 (3.02)	37 (3.77)	279 (5.32)
Indel length	1–39	1	1–2	1–2	1–39	1–13	1–39	1–20	1–19	1–13	1–22	1–39
Informative indels	–	6	11	17	37	2	9	5	6	5	3	67
Tree length	3085	108	56	167	150	39	70	72	22	40	58	272
No. of most parsimonious trees	104	25	27	122	647020	23509	802	2398	254	1	94	54
Consistency index	0.26	0.74	0.82	0.75	0.81	0.64	0.57	0.69	0.82	0.78	0.71	0.75
Retention index	0.74	0.83	0.87	0.83	0.85	0.79	0.74	0.83	0.90	0.87	0.83	0.82
Homoplasy index	0.74	0.26	0.18	0.25	0.19	0.36	0.43	0.31	0.18	0.23	0.29	0.25
Maximum sequence divergence within ingroup (%)	35.03	6.24	3.03	4.73	9.40	3.19	4.59	4.79	2.30	4.77	1.42	3.31
Model of evolution (Akaike criterion)	SYM+I+G	GTR+G	GTR+G		GTR+G	F81+I	HKY+G	GTR+G	GTR+I	F81+G	GTR+I	

100%, PP = 1), which are grouped in two main clades. One of them is constituted by the species traditionally classified in sect. *Tirucalli* (BS = 85%, PP = 1), and the other is constituted by the Macaronesian species (BS = 93%). Two main clades are resolved in the African and Arabian group: one is constituted by *E. calamiformis*, *E. lateriflora*, *E. nubica* and *E. usambarica* Pax (BS = 97%, PP = 1) and a second one is constituted by the rest of species (PP = 1). Within the first clade, *E. usambarica* is sister to the rest of the species. *Euphorbia calamiformis* and *E. lateriflora* constitute a strongly supported clade within this group (BS = 94%). Within the second clade, two main clades are also resolved: one constituted by *E. berotica*, *E. gossypina*, *E. papilionum* and *E. schimperi* (PP = 1) and the other one is composed of species from South Africa and Madagascar (*E. mauritanica*, *E. orthoclada* Baker and *E. stolonifera*; PP = 0.96). *Euphorbia gossypina* and *E. berotica* constitute a strongly supported clade (BS = 100%, PP = 1) within the first clade and *E. mauritanica* and *E. stolonifera* constitute a clade only supported by Bayesian analyses (PP = 1) within the second clade.

Among the Macaronesian species, two main clades are recovered. The first clade (PP = 0.97) shows *E. piscatoria* as sister species to a clade including *E. anachoreta*, *E. aphylla*, *E. bourgeana* J. Gay ex Boiss., *E. bravoana*, *E. pedroi* and *E. regis-jubae* (BS = 75%, PP = 1). Within this clade, two additional clades are recovered. One consists of *E. anachoreta*, *E. pedroi* and *E. regis-jubae* (PP = 1) and the other clade includes *E. bourgeana* and *E. bravoana* (BS = 91%, PP = 1). The second main clade within the Macaronesian species is constituted of *E. atropurpurea* and *E. lamarckii* (BS = 96%, PP = 1).

Some notable differences are found between nrDNA and cpDNA analyses. Among the Macaronesian species, the position of *E. berthelotii*, *E. lamarckii* and *E. regis-jubae* is incongruent between the nrDNA and the cpDNA analyses, and among the African-Arabian species, the position of *E. calamiformis*, *E. nubica* and *E. schimperi* is also inconsistent.

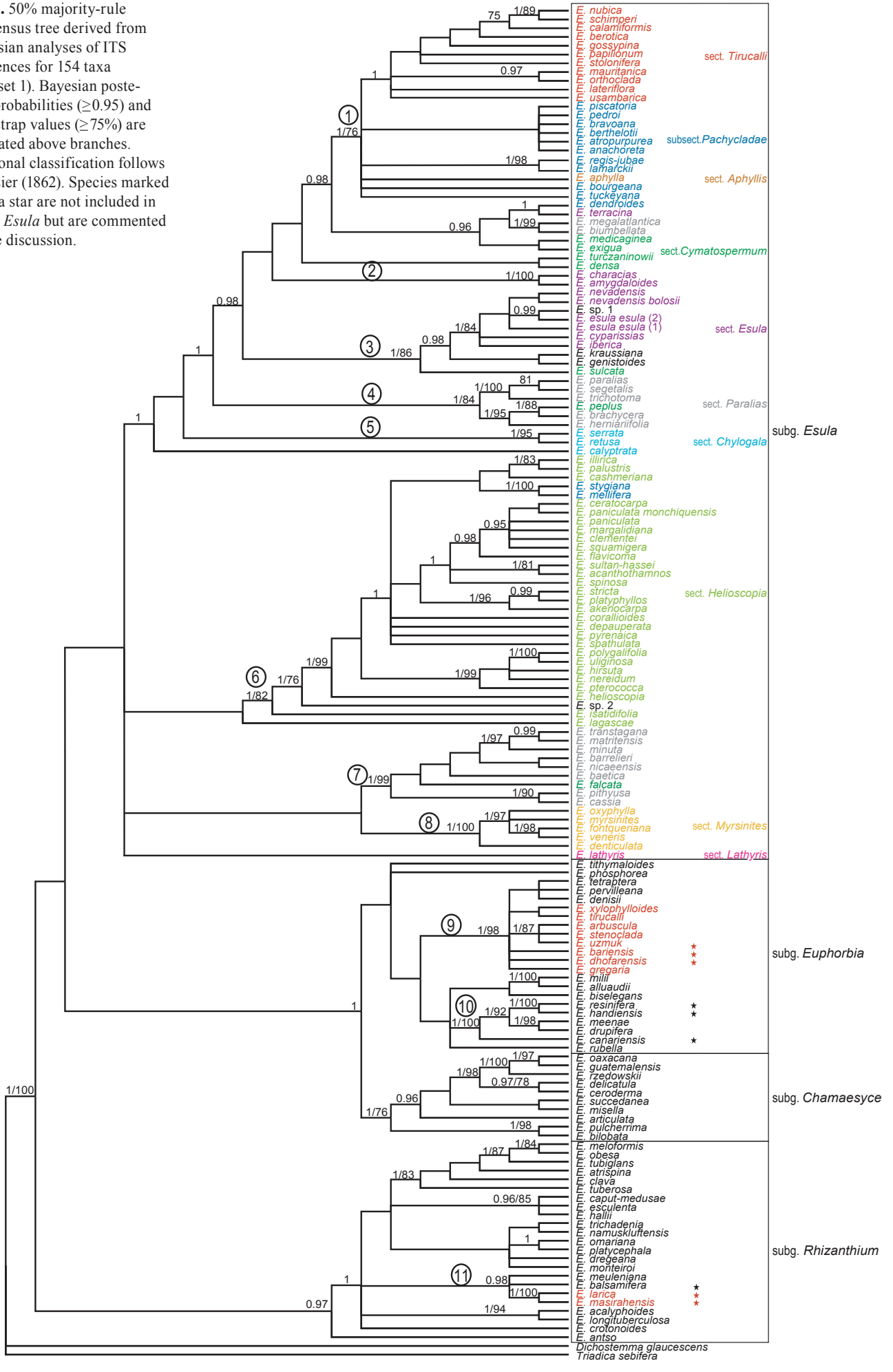

Fig. 1. 50% majority-rule consensus tree derived from Bayesian analyses of ITS sequences for 154 taxa (dataset 1). Bayesian posterior probabilities (≥0.95) and bootstrap values (≥75%) are indicated above branches. Sectional classification follows Boissier (1862). Species marked with a star are not included in subg. *Esula* but are commented in the discussion.

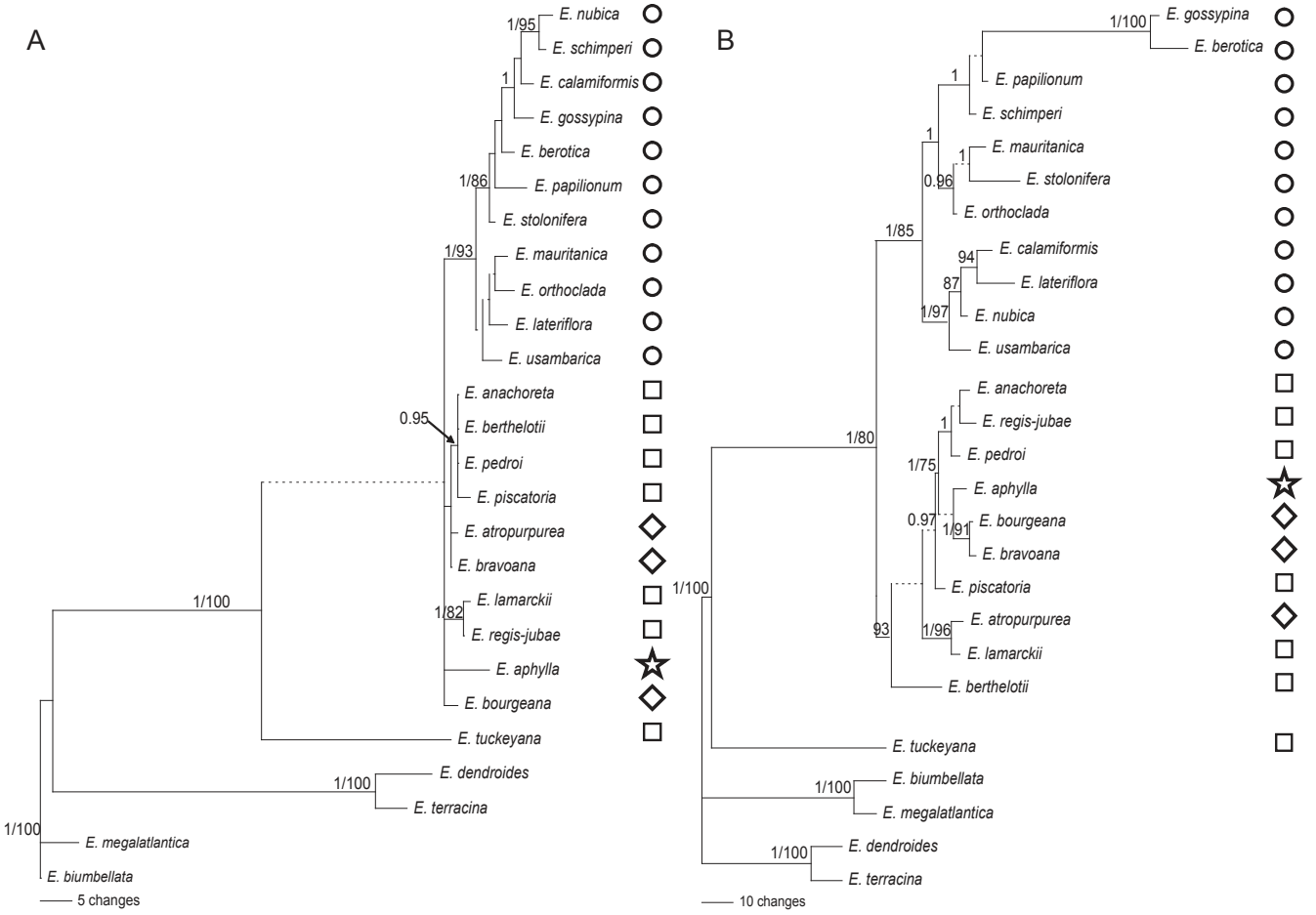

Fig. 2. Phylogram of one of the most parsimonious trees from the maximum parsimony analyses of combined nuclear **(A)** and chloroplast sequences **(B)** for dataset 2. Discontinuous branches do not appear in the topology of the strict consensus tree. Bayesian posterior probabilities (≥0.95) and bootstrap values (≥75%) are indicated above branches. The traditional groups previously considered within sect. *Aphyllis* as currently circumscribed, are indicated as: circle, sect. *Tirucalli*; square, subsect. *Pachycladae*, *Euphorbia lamarckii* complex; rhombus, subsect. *Pachycladae*, *Euphorbia atropurpurea* complex; star, sect. *Aphyllis* sensu Webb & Berthelot (1842).

■ DISCUSSION

Phylogenetic relationships in *Euphorbia* and taxonomic implications. — Our analyses based on ITS sequences provide no statistical support for subg. *Esula* (Fig. 1). Monophyly of subg. *Esula* has been demonstrated in the past based on analyses of chloroplast regions receiving moderate bootstrap support (BS = 86% in Steinmann & Porter, 2002) or based on analyses of nuclear regions with strong support of Bayesian PP (PP = 1 in Bruyns & al., 2006; PP = 1 in Zimmermann & al., 2010). The lack of statistical support for subg. *Esula* in our analysis could be explained by the high substitution rate known for ITS and by the sampling differences, since 13, 38 and 39 taxa from subg. *Esula* were sampled in the last three works mentioned, respectively, compared to the 97 taxa included in our analyses.

Although the existence of the *Pachycladae* core clade (Fig. 1, clade 1) within subg. *Esula* was already known from previous phylogenies (Steinmann & Porter, 2002; Bruyns & al., 2006), we demonstrate for the first time that several additional species from sect. *Tirucalli*, previously classified in the *E. mauritanica* complex based on morphologic studies (*E. berotica*, *E. gossypina*, *E. lateriflora* [Leach, 1975], and *E. papilionum* [Carter, 1992]) belong to this clade. In the present study, the affinities with the *E. mauritanica* complex are newly confirmed by molecular analyses and the phylogenetic relationships for *E. calamiformis*, *E. nubica* and *E. orthoclada* are established for the first time.

The first available name for members of clade 1 (Fig. 1) at the sectional level is sect. *Aphyllis* (Table 1). Based on our results, we propose sect. *Aphyllis* to include *E. aphylla* and the members of the traditional sect. *Tithymalus* subsect. *Pachycladae* and sect. *Tirucalli* included in clade 1. Species of this newly circumscribed sect. *Aphyllis* share some morphological characters (Fig. 3A–L): they are dendroid shrubs with green succulent young stems (except for *E. usambarica*, which has non-succulent stems); leaves can be absent (in *E. aphylla*) or persistent until fructification, but in most cases they are soon deciduous and leave prominent and callose scars in the stem;

stipules are absent; synflorescences are pseudo-umbellate with equal or sub-equal radii length and leafy sub-cyathial bracts; 4–5 rounded, truncate, emarginate or two-horned cyathium glands are always present; capsules are smooth (rarely granulate) and wider than high; and seeds are carunculate.

Euphorbia dendroides, traditionally classified in sect. *Tithymalus* subsect. *Pachycladae* (Table 1), is placed in the sister clade to sect. *Aphyllis*. This taxon differs from the species of sect. *Aphyllis* in having non-photosynthetic subsucculent young stems covered by a brown rhytidome and laterally compressed seeds.

The incongruities between nuclear and chloroplast markers that precluded a combined analysis (see results) could be due to incomplete lineage sorting or hybridisation events, though the last explanation is more probable in this case as hybridisation processes have been reported between several Macaronesian species of this group (Molero & Rovira, 2005). No evidence of hybridisation has been reported for the African species of sect. *Aphyllis*, but it could be feasible since incongruities are also found on this part of the tree.

Our phylogenies based on nrDNA and cpDNA sequences (Fig. 2) show some correspondence with morphological

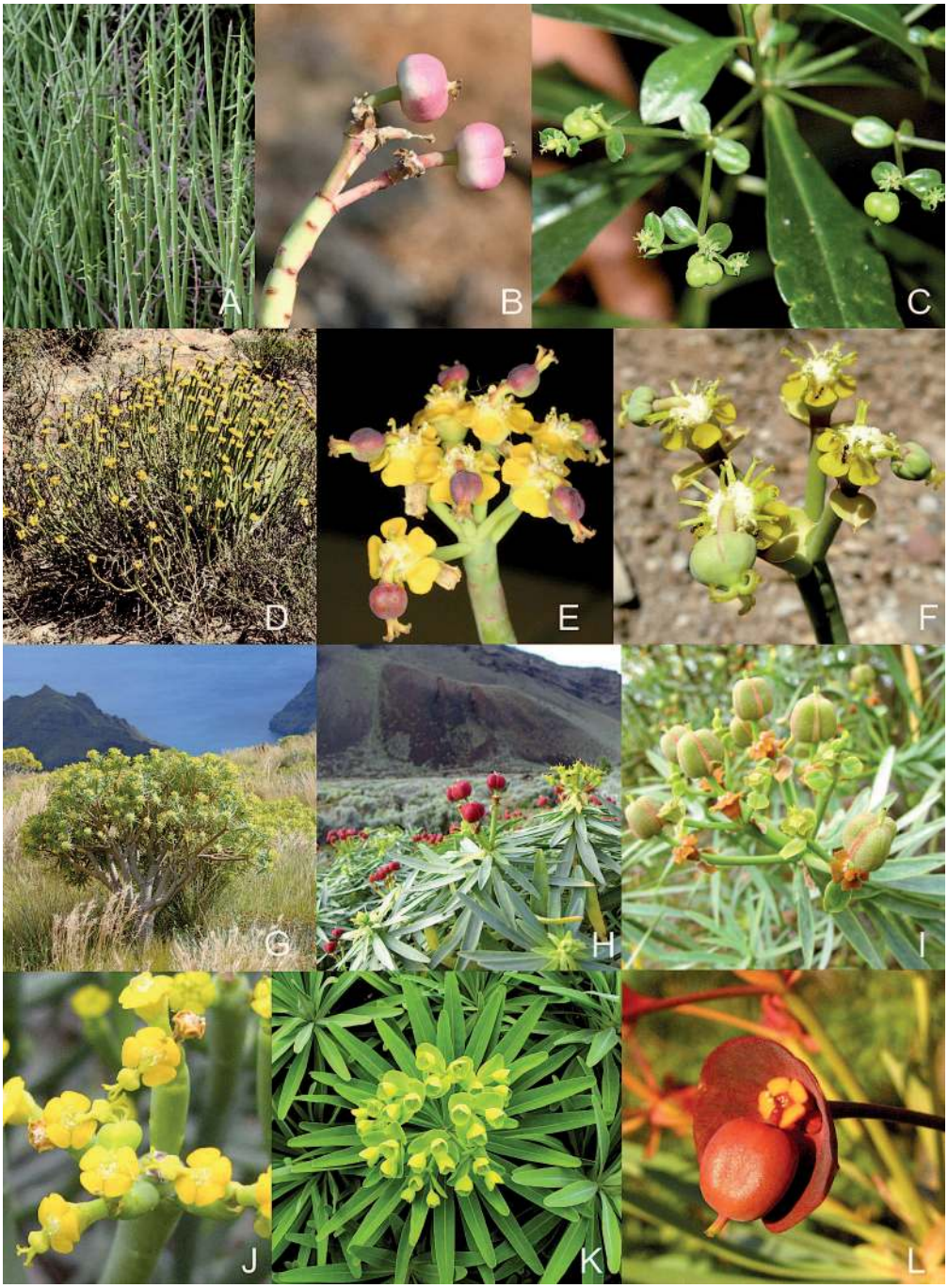

Fig. 3. Morphology and habit of some members of *Euphorbia* sect. *Aphyllis*, as currently circumscribed. **A–B,** habit and capsules of *E. gossypina*, from Kenya; **C,** synflorescence of *E. usambarica* from Kenya; **D–E,** habit and synflorescence of *E. stolonifera*, from the Cape Province, South Africa; **F,** synflorescence of *E. schimperi* from Yemen; **G,** habit of *E. berthelotii* from La Gomera, Canary Islands, Spain; **H,** habit of *E. lamarckii* from El Hierro, Canary Islands, Spain; **I,** synflorescence of *E. piscatoria*, from Madeira, Portugal; **J,** synflorescence of *E. aphylla* from Tenerife, Canary Islands, Spain; **K,** synflorescene of *E. bourgeana* from Tenerife, Canary Islands, Spain; **L,** capsule of *E. atropurpurea* from Tenerife, Canary Islands, Spain. Photographs by J. Morawetz (A–C), A. Moller (D–E), A. Susanna (F), J. Molero (G) and L. Barres (H–L).

characters. Two main groups can be distinguished within sect. *Aphyllis*. First, the African-Arabian group, constituting a strongly supported clade in both trees and characterized by xerophilous plants with patent or erecto-patent lateral ramification of the stems, small and deciduous loosely distributed leaves, small deciduous bracts and pseudoverticillate synflorescences congested on the apex of the stems with undivided radii (Fig. 3A–F). Secondly, the Macaronesian group, only supported in the cpDNA analyses, characterized by xerophilous and mesophylous plants with a pseudoumbellate ramification of the stems, wide deciduous leaves (when present) arranged spirally on young branches and leaving conspicuous leaf scars, persistent bracts, and lax pleiochasial synflorescences divided 1–2 times (Fig. 3G–L). *Euphorbia tuckeyana*, which is suggested to be sister to the rest of the species of sect. *Aphyllis* only by the cpDNA analyses (BS = 100%, PP = 1; Fig. 2B), resembles the species of the African-Arabian clade in its type of ramification but it has pleiochasial synflorescences like the other Macaronesian species. *Euphorbia aphylla* (Fig. 3J) also shares some characters with the African species, like the presence of small deciduous bracts and CAM photosynthesis (Mies & al., 1996). However, the chromosome number ($2n = 20$) and karyotype characteristics tie this species to the rest of Macaronesian species (Molero & al., 2002). *Euphorbia usambarica* has wide lanceolate leaves and two horned nectariferous glands (Fig. 3C), like most of the Macaronesian species. Wide leaves are also present in *E. lateriflora* and *E. orthoclada*, while the rest of the African-Arabian species have narrow leaves. However, variations of this character are probably more influenced by ecological factors than by phylogenetic signal. The long branch lengths recovered for *E. gossypina* and *E. berotica* in the cpDNA analyses (Fig. 2B) indicate that those two species have accumulated a high number of mutations, and would probably have diverged more anciently than the other lineages.

Macaronesian species have been classified in two complexes according to morphological characteristics (Molero & al., 2002): the *E. lamarckii* complex and the *E. atropurpurea* complex (Figs. 2 and 4). Species belonging to the *E. lamarckii* complex are *E. anachoreta*, *E. berthelotii*, *E. lamarckii*, *E. pedroi*, *E. piscatoria*, *E. regis-jubae* and *E. tuckeyana*. They all have deciduous leaves, simple pleochasial synflorescences with small and free sub-cyathial bracts (Fig. 3H–I) and smooth to rugulose seeds. In contrast, the *E. atropurpurea* complex includes *E. atropurpurea*, *E. bourgeana* and *E. bravoana*, all possessing persistent leaves, double pleochasial synflorescences with large and fused sub-cyathial bracts, at least at the base (Fig. 3K–L), and strongly ornamented seeds. The two complexes are not supported as monophyletic groups. However, in the nrDNA tree there is no incongruence of this morphological classification and the phylogenetic relationships obtained, since the two statistically supported clades are exclusively composed of members of the *E. lamarckii* complex (Fig. 2A). On the contrary, in the cpDNA-based phylogeny (Fig. 2B) we found strong support for *E. atropurpurea* and *E. lamarckii*, each belonging to a different complex, to be sister species (BS = 96%, PP = 1). A mix of species from both complexes is recovered in another well-supported clade (BS = 75%, PP = 1).

With regard to other groups in subg. *Esula*, our results show that most of the traditional sections do not correspond to monophyletic groups (Fig. 1). *Euphorbia amygdaloides* L. and *E. characias* L. are members of clade 2 (BS = 100%, PP = 1), but they are not placed in the main clade of sect. *Esula* Dumort. (clade 3; BS = 86%, PP = 1), where they are traditionally classified. Clade 3 also includes *E. kraussiana* Bernh. ex Krauss and *E. genistoides* P.J. Bergius, to our knowledge not previously classified in any section and *E. sulcata* Lens ex Loisel., from sect. *Cymatospermum* (Prokh.) Prokh. *Euphorbia falcata* L. and *E. peplus* L., also from sect. *Cymatospermum*, appear instead in two different clades, clade 4 (BS = 84%, PP = 1) and clade 7 (BS = 99%, PP = 1), respectively; both clade 4 and clade 7 are composed of members of sect. *Paralias* Dumort. Clade 5 encompasses two species from sect. *Chylogala* (Fourr.) Prokh. (BS = 95%, PP = 1), but the phylogenetic position of *E. calyptrata* Coss. & Kralik is unresolved. All members of sect. *Helioscopia* Dumort., except *E. lagascae* Spreng., constitute clade 6 (BS = 82%, PP = 1), which also includes the Macaronesian lauroid trees *E. mellifera* and *E. stygiana*, previously included in sect. *Tithymalus* subsect. *Pachycladae*. Distinctive characters define this Macaronesian clade within sect. *Helioscopia*, such as the tree habit condition, lauroid leaves, paniculate cymose synflorescence, tuberculate capsule, smooth seeds and an exclusive chromosome and base chromosome number ($2n = 44$, $x = 11$; Molero & al., 2002). Monophyletic groups in clade 6 cannot be delimited based on the presence of smooth or tuberculate ovaries (or capsules), as Steinmann & Porter (2002) suggested. *Euphorbia isatidifolia* Lam. is the sister species to the rest of the species of clade 6. This species possesses some exclusive characteristics in the group, like the presence of tubers, yellow latex and non-horned nectariferous glands. Its related species probably lie among species from sect. *Holophyllum* (Prok.) Prok., from central Asia. The position of *E. lagascae* remains unresolved, though a relationship with sect. *Helioscopia* in the tree topology is detected. With the available data, sect. *Myrsinites* (Boiss.) Lojac. (clade 8, BS = 100%, PP = 1) is the only monophyletic section and is populated by orophyte narrow endemics from the Mediterranean basin, characterised by the presence of nectariferous glands with dilatated horns. The phylogenetic position of *E. lathyris* L., the only member of sect. *Lathyris* Dumort., is not resolved in our results, though it is recovered as sister species to the rest of subg. *Esula* in another study including many genes from all three genomic compartments (Horn & al., 2009).

Molecular evidence demonstrates that sect. *Tirucalli* is polyphyletic. Carter (1992) already suggested the division of this section into two different groups on the basis on morphological characteristics, but our study reveals that this group has at least three different origins (Fig. 1). The first group (clade 9; BS = 98%, PP = 1) was named the "*Tirucalli* alliance" by Bruyns & al. (2006) and is placed in subg. *Euphorbia*. *Euphorbia tirucalli* and related species populate this clade; these species are related to other species from Madagascar and southwestern Africa. *Euphorbia bariensis* and *E. dhofarensis*, from Somalia and Oman, are shown to be members of this alliance for the first time. The second group, as described

above, is comprised of the *E. mauritanica* complex and related species (clade 1; BS = 76%, PP = 1), now members of sect. *Aphyllis*. Finally, *E. larica* and *E. masirahensis*, from Iran and the Arabian Peninsula, are placed within subg. *Rhizanthium* and constitute clade 11 (PP = 0.98) along with *E. balsamifera*, a widely distributed species in West Africa, Yemen and the Canary Islands, and *E. meuleniana* O. Schwartz from Yemen.

Biogeographical implications. — The origin of Macaronesian endemic plant lineages has been studied by many authors. The oceanic origin of the islands implies long-distance dispersal as the more reliable hypothesis to explain the source of colonisation. Sister groups of several endemic Macaronesian taxa proposed by molecular and morphological approaches populate a range of areas like Eurosiberia (Vargas & al., 1999), East Africa (reviewed by Andrus & al., 2004) or South Africa (Sunding, 1979), but the origin of the Macaronesian flora is mostly the West Mediterranean basin (see examples in Kim & al., 2008).

Our phylogenetic reconstruction suggests that the Macaronesian species of sect. *Aphyllis* have their closest relatives on the Horn of Africa, southern Africa and the southern Arabian Peninsula, and thus are part of the Rand Flora (Fig. 4). Recent molecular dating of genus *Euphorbia* estimates the split between the African-Arabian and Macaronesian species to be 5 Ma old (Horn & al., in prep.). This agrees with a vicariance scenario as the aridification process, which occurred in the late Miocene-Pliocene (Axelrod, 1975) in northern Africa, acted as an important ecological barrier. The arid belt would have fragmented the continuous subtropical flora in this area and left two relict distribution centres in refugia areas, explaining the present disjunct distribution of sect. *Aphyllis*. This process should always be associated to a long-distance dispersal event to the oceanic islands. Since most of the Afro-Arabian species of sect. *Aphyllis* are distributed in the Horn of Africa (Fig. 4), a migration from this area to southern Africa could have taken place following the African track (Linder & al., 1992). A

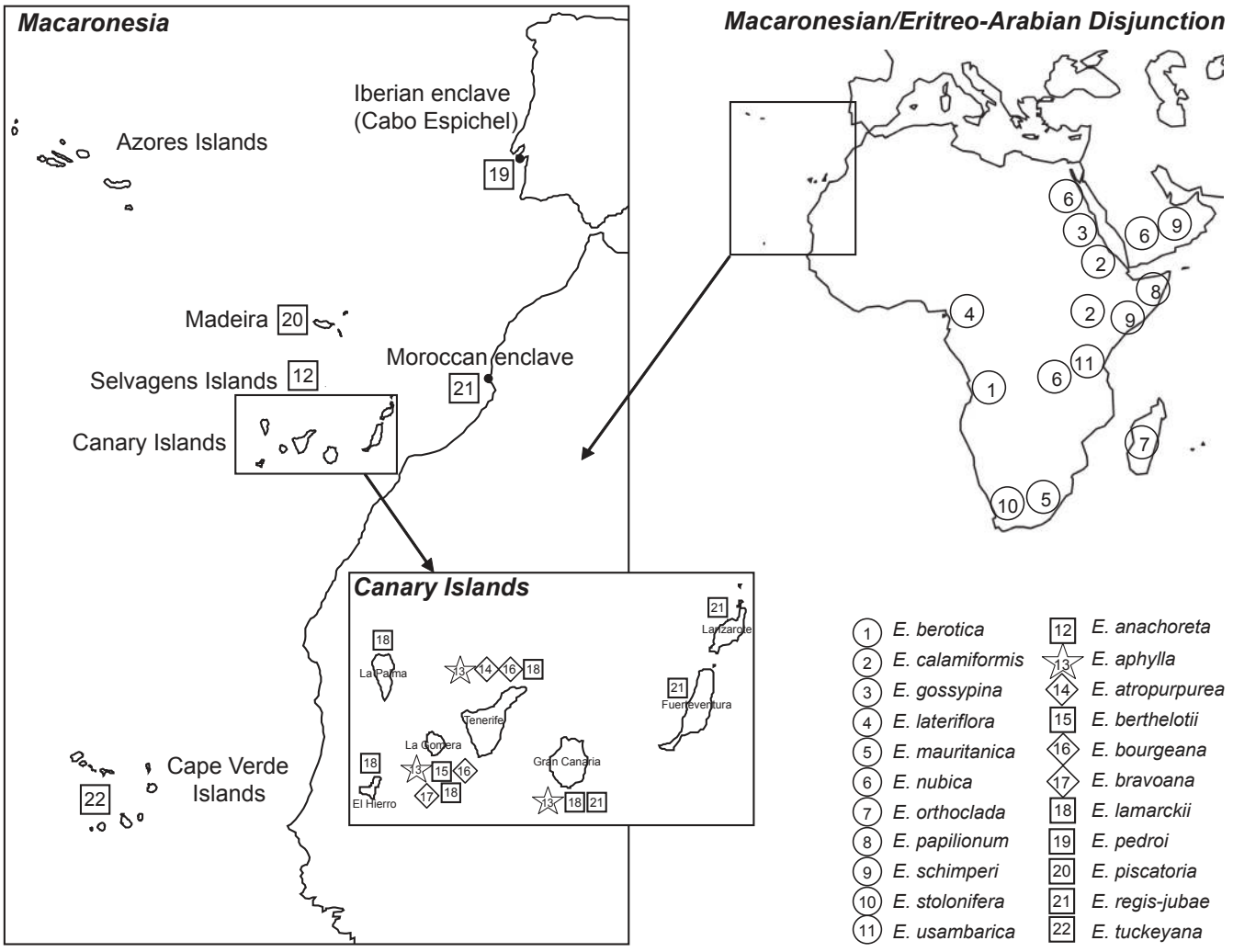

Fig. 4. Geographical distribution of the species belonging to sect. *Aphyllis* as currently circumscribed. The traditional groups previously considered within sect. *Aphyllis* are indicated as: circle, sect. *Tirucalli*; square, subsect. *Pachycladae*, *Euphorbia lamarckii* complex; rhombus, subsect. *Pachycladae*, *Euphorbia atropurpurea* complex; star, sect. *Aphyllis* sensu Webb & Berthelot (1842).

long-distance dispersal event from southern Africa to Madagascar would have originated the endemic *E. orthoclada*. In this case, vicariance seems improbable since the isolation of Madagascar from other Godwana landmasses is estimated to be 120 Ma (Rabinowitz & al., 1983).

From our results (Fig. 2) it is unclear whether members of sect. *Aphyllis* dispersed to Macaronesia from continental masses once or more than once. Although the isolated position of *E. tuckeyana* in both nuclear and chloroplastic analyses could suggest at least an independent colonisation of Cape Verde Archipelago, the rest of the species could have diversified after a unique dispersion event from the continent, since they are shown as monophyletic in cpDNA based analyses (Fig. 2B). Moreover, hybridisation, which seems to be recurrent in this group (Molero & Rovira, 2005), is common in plant groups presenting a single colonisation event (Carine & al., 2004). The phylogenetic position of the endemic *E. pedroi* from Cape Espichel (Figs. 2 and 4) could be explained through two hypotheses: (1) a back-colonisation from Macaronesia, probably from the closest archipelago, Madeira; or (2) the permanence of this species as a relict from the subtropical flora existing in the Mediterranean basin during the Miocene. The same two hypotheses could explain the presence of *E. regis-jubae* in the west coast of Morocco (Figs. 2 and 4). However, the short branches observed in the phylogram in both species (Fig. 2), and the short distance between the two distribution areas in the case of *E. regis-jubae* (Fig. 4), could make back-colonisation the most probable hypothesis for both cases.

An independent colonisation of Macaronesia by other members of subg. *Esula* is evident from our results (Fig. 1, clade 6). A Mediterranean herbaceous ancestor from sect. *Helioscopia* would have given origin to the lauroid trees *E. stygiana*, endemic to the Azores Islands, and *E. mellifera*, distributed in the Canary Islands and Madeira. The present distribution of these species could be explained either by independent dispersal events from the Mediterranean area to the different archipelagos, as has been reported for other plant groups (Carine & al., 2004), or a step-stone colonisation via Madeira. The acquisition of a woody habit in the ancestor of *E. mellifera* and *E. stygiana* provides another example of derived insular woodiness, a phenomenon also detected in subg. *Chamaesyce* in Hawaii (Koutnik, 1999). The acquisition of woodiness in islands was first reported by Carlquist (1974) and has been widely documented in different plant groups from several volcanic archipelagos (e.g., Jorgensen & Olesen, 2001; Helfgott & al., 2000; García-Maroto & al., 2009, among others).

The presence of the succulents *E. canariensis* and *E. handiensis* from subg. *Euphorbia*, both endemic to the Canary Islands, in clade 10 (Fig. 1) and their grouping with species from Morocco, tropical Africa and India suggests at least two additional independent colonisations of the Macaronesian region by *Euphorbia*.

In summary, the Macaronesian endemic *Euphorbia* have originated from at least five independent colonisation events: three in subg. *Esula* (two independent events in sect. *Aphyllis* and one additional event in sect. *Helioscopia*—*E. mellifera* and *E. stygiana*) and two in subg. *Euphorbia* (*E. canariensis* and *E. handiensis*, independently).

Finally, two modes of speciation have been postulated to occur in endemic lineages of oceanic islands (Whittaker & Fernández-Palacios, 2007; Carine & Schaefer, 2010): adaptive ecological radiation and allopatric speciation. Within sect. *Aphyllis*, the pattern found is very complex and both types seem to have occurred. The fact that members of the *E. lamarckii* complex mainly occupy xerophytic habitats in low- and mid-altitude areas while members of the *E. atropurpurea* complex mainly occur in mesophytic and sub-hygrophytic areas associated with evergreen laurisilva suggests a role for radiation into different ecological habitats. However, allopatric speciation among different archipelagos also seems to have occurred within members of the *E. lamarckii* complex, each endemic to one archipelago or Macaronesian enclave (Fig. 4) but with the same ecological preferences. Furthermore, dispersal among islands must have occurred repeatedly within the group because most species are present in more than one island. Also, the low divergence among sequences of different species suggests recent radiation of the group, hybridisation events, or both. Population genetic studies using more variable molecular markers (i.e., fingerprinting markers) on a complete sampling of the different species across their whole distributions are needed for a better understanding of the origin and evolution of this group in Macaronesia.

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Appendix. Taxa, voucher information and GenBank accession numbers employed in molecular analyses. GenBank accession numbers marked with superindex were already published ([1]Molero & al., 2002; [2]Steinmann & Porter, 2002; [3]Bruyns & al., 2006). GenBank accession numbers for new sequences are in the following order: ITS, ETS, *trnL-trnF*, *psbA-trnH*, *ycf3-trnS*, *trnG*, *atpB-rbcL*, *trnK-matK*, *trnT-trnL*. When only one accession number is indicated, it corresponds to ITS.

Dichostemma glaucescens Pierre, Gabon, AF537584[2]. ***Euphorbia acalyphoides*** Hochst. ex Boiss., Kenya, AF537576[2]. ***E. acanthothamnos*** Heldr. ex Sart., Greece, Crete, Melindaou Peak, *Riina 1562* (MICH), HQ900573. ***E. akenocarpa*** Guss., Spain, Cádiz, Vejer de la Frontera, *Barres & al. s.n.* (BCN 53041), HQ900574. ***E. alluaudii*** Drake, Madagascar, AF537468[2]. ***E. amygdaloides*** L., Spain, Barcelona, Sant Iscle de Vallalta, *Molero 19/2007* (BCN 48887), HQ900575. ***E. anachoreta*** Svent., Portugal, Selvagens Islands, Pequeño Pitón (cultivated) (BC), HQ900576, HQ900393, HQ900495, HQ900419, HQ900547, HQ900445, HQ900367, HQ900471, HQ900521. ***E. antso*** Denis, Madagascar (cultivated), AF537579[2]. ***E. aphylla*** Brouss. ex Willd., Spain, Canary Islands, Tenerife, Montaña del Taco, *Molero 2/2007* (BCN), HQ900577, HQ900394, HQ900496, HQ900420, HQ900548, HQ900446, HQ900368, HQ900472, HQ900522. ***E. arbuscula*** Balf. f., Socotra (cultivated), AF537496[2]. ***E. atrispina*** N.E. Br., Republic of South Africa (cultivated), AF537568[2]. ***E. articulata*** Burm., Virgin Islands, AF537446[2]. ***E. atropurpurea*** Brouss. ex Willd., Spain, Canary Islands, Tenerife, Masca, Los Carrizales, *Molero 5/2007* (BCN), HQ900579, HQ900395, HQ900497, HQ900421, HQ900549, HQ900447, HQ900369, HQ900473, HQ900523. ***E. baetica*** Boiss., Spain, Cádiz, Parque Natural de Breña y Marismas de Barbate, *Molero 18/2007* (BCN), HQ900580. ***E. balsamifera*** Aiton subsp. ***balsamifera***, Spain, Canary Islands (cultivated) (BC), HQ900581. ***E. bariensis*** S. Carter, Somalia, *Osman & al. 10500* (K), HQ900582. ***E. barrelieri*** Savi, Italy, Toscana, Grosseto, Castiglione della Pescaia, *Vilatersana 1235 & al.* (BC), HQ900583. ***E. berotica*** N.E. Br., Angola, Huila, between Pocolo and Quihita, *Gouveia & Barbosa 10720* (LISC 2247), HQ900584, HQ900396, HQ900498, HQ900422, HQ900550, HQ900448, HQ900370, HQ900474, HQ900524. ***E. berthelotii*** Bolle ex Boiss., Spain, Canary Islands, La Gomera, San Sebastián de la Gomera, near Santiago Beach, *Molero 25/2007* (BCN), HQ900585, HQ900397, HQ900499, HQ900423, HQ900551, HQ900449, HQ900371, HQ900475, HQ900525. ***E. bilobata*** Engelm., U.S.A., AF537435[2]. ***E. biselegans*** Bruyns, Tanzania (cultivated), AF537470[2]. ***E. biumbellata*** Poir., Spain, Barcelona, Sant Pol de Mar, *Molero 26/2007* (BCN), HQ900586, HQ900398, HQ900500, HQ900424, HQ900552, HQ900450, HQ900372, HQ900476, HQ900526. ***E. bourgeana*** J. Gay ex Boiss., Spain, Canary Islands, La Gomera, Parque Nacional de Garajonay, Los Noruegos, *Molero 11/2007* (BCN), HQ900587, HQ900399, HQ900501, HQ900425, HQ900553, HQ900451, HQ900373, HQ900477, HQ900527. ***E. brachycera*** Engelm., U.S.A., AF537533[2]. ***E. bravoana*** Svent., Spain, Canary Islands, La Gomera, Punta de la Sardina, *Fernández-López 08/2007* (BCN), HQ900588, HQ900400, HQ900502, HQ900426, HQ900554, HQ900452, HQ900374, HQ900478, HQ900528. ***E. calamiformis*** P.R.O. Bally & S. Carter, Kenya, *Carter & Stannard 558* (K), HQ900589, HQ900401, HQ900503, HQ900427, HQ900555, HQ900453, HQ900375, –, HQ900529. ***E. calyptrata*** Coss. & Kralik, Morocco, Tarfaya, near Tan-Tan, *Batriu & al. s.n.* (BCN 49739), HQ900590. ***E. canariensis*** L., Spain, Canary Islands (cultivated) (BC), HQ900591. ***E. caput-medusae*** L., Republic of South Africa (cultivated), AF537574[2]. ***E. cashmeriana*** Royle, Afghanistan, Nuristan, Nishei, *Edelberg 781* (W 1964–14520), HQ900592. ***E. cassia*** Boiss., Cyprus, road B8 from Lemesos to Trimiklini, *Galbany 2035 & al.* (BC), HQ900593. ***E. ceratocarpa*** Ten., Italy, Sicily, Messina, Santo Stefano di Camastra, *Vilatersana 1141 & al.* (BC), HQ900594. ***E. ceroderma*** I.M. Johnst., Mexico, AF537389[2]. ***E. characias*** L., Spain, Barcelona, Parc de Montjuïc, *Barres 21* (BC), HQ900595. ***E. clava*** Jacq., Republic of Sotuh Africa (cultivated), AF537569[2]. ***E. clementei*** Boiss. subsp. ***clementei***, Morocco, Midelt, Jebel Ayachi, *Molero s.n.* (BCN 49327), HQ900596. ***E. coralloides*** L., Italy, Sicily, Agrigento, south shore of Lake Arancio, *Vilatersana 1161 & al.* (BC), HQ900597. ***E. crotonoides*** Boiss., Tanzania, AF537578[2]. ***E. cyparissias*** L., Spain, Girona, Olot, *Barres 37* (BC), HQ900598. ***E. delicatula*** Boiss., Mexico, AF537393[2]. ***E. dendroides*** L., Italy, Sardinia, Capo Caccia, *Barres 26 & Mameli* (BC), HQ900599, HQ900402, HQ900504, HQ900428, HQ900556, HQ900454, HQ900376, HQ900479, HQ900530. ***E. denisii*** Oudejans, Madagascar (cultivated), AF537497[2]. ***E. densa*** Schrenk, Pakistan, Quetta, Yaro near Bostan, 40 km NNE Quetta versus Pishin, *Rechinger 28952* (W 1984–11717), HQ900600. ***E. denticulata*** Lam., Iran, Luristan, Baghbanan, SE Khorramabad, *Rechinger 47845* (W 1981–05279), HQ900601. ***E. depauperata*** Hochst. ex A. Rich., Malawi, AF537556[2]. ***E. dhofarensis*** S. Carter, Oman, Dhofar, 30 km past Mugsail on Sarfait Rd, 2 km past police checkpoint, *Morawetz 324* (MICH), HQ900602. ***E. dregeana*** E. Mey. ex Boiss., South Africa, *Becker 897* (MICH), HQ900603. ***E. drupifera*** Thonn., Africa (cultivated), AF537480[2]. ***E. esculenta*** Marloth, Republic of South Africa (cultivated), AF537575[2]. ***E. esula*** L. subsp. ***esula*** (1), Spain, Granada, Sierra Nevada Natural Park, Campos de Otero, *Vilatersana 1831 & al.* (BC), HQ900605. ***E. esula*** L. subsp. ***esula*** (2), Spain, Tarragona, Falset, *Molero & Vallverdú s.n.* (BCN), HQ900604. ***E. exigua*** L., Spain, Girona, L'Escala, Mas Vilanera, *Barres 44 & al.* (BC), HQ900606. ***E. falcata*** L., Spain, Girona, El Far de l'Empordà, *Barres 42 & al.* (BC), HQ900607. ***E. flavicoma*** DC. subsp. ***flavicoma***, Spain, Alacant, Torremanzanas, Puerto del Restonar, *Molero s.n.* (BCN 53024), HQ900608. ***E. fontqueriana*** Greuter, Spain, Balearic Islands, *Sáez s.n.* (BCB), HQ900609. ***E. genistoides*** P.J. Bergius, South Africa, AM040770[3]. ***E. gossypina*** Pax, Tanzania, *Richards 23502* (K), HQ900610, HQ900403, HQ900505, HQ900429, HQ900557, HQ900455, HQ900377, HQ900480, HQ900531. ***E. gregaria*** Marloth, Republic of South Africa (cultivated), AF537527[2]. ***E. guatemalensis*** Standl. & Steyerm., Mexico, AF537408[2]. ***E. hallii*** R.A. Dyer, Republic of South Africa (cultivated), AF537573[2]. ***E. handiensis*** Burchard, Spain, Canary Islands, Fuerteventura (cultivated) (BC), HQ900611. ***E. helioscopia*** L. subsp. ***helioscopia***, Spain, Barcelona, Montserrat Mountain, near Santa Cecília, *Barres 6 & al.* (BC), HQ900612. ***E. herniariifolia*** Willd., Greece, Crete, Pachnes Peak, 20 km from Aradena, *Riina 1571* (MICH), HQ900613. ***E. hirsuta*** L., Spain, Cádiz, Tarifa, *Molero 21/2007 & Rovira* (BCN), HQ900614. ***E. iberica*** Boiss., Armenia, Tavush province, N area of Berd, *Vitek 05–0912* (W 2008–06796), HQ900615. ***E. illirica*** Lam., Spain, Girona, Olot, near Ravell, *Barres 39* (BC), HQ900616. ***E. isatidifolia*** Lam., Spain, Lleida, La Sentiu de Sió, Serra de les Guineus, *Barres 30 & al.* (BC), HQ900617. ***E. kraussiana*** Bernh. ex Krauss, South Africa, AF537548[2]. ***E. lagascae*** Spreng., Spain, Murcia, Puerto Lumbreras, *Molero 18/2008 & al.* (BCN 53042), HQ900618. ***E. lamarckii*** Sweet, Spain, Canary Islands, Tenerife, Barranco del Infierno, *Molero 6/2007* (BCN), HQ900619, HQ900404, HQ900506, HQ900430, HQ900558, HQ900456, HQ900378, HQ900481, HQ900532. ***E. larica*** Boiss., Oman, Al-Dakhiliyah, Jebel Akhdar, *Morawetz 350* (MICH), HQ900620. ***E. lateriflora*** Schumach., Ghana, *Hall 46017* (K), HQ900621, HQ900405, HQ900507, HQ900431, HQ900559, HQ900457, HQ900379, –, HQ900533. ***E. lathyris*** L., Spain, Girona, Olot, *Barres 36* (BC), HQ900622. ***E. longituberculosa*** Hochst.

Appendix. Continued.

ex Boiss., East tropical Africa (cultivated), AF537577[2]. ***E. margalidiana*** Kuhbier & Lewej., Spain, AF334252[1]; AF334267[1]. ***E. masirahensis*** Ghaz., Oman, Sharqiya, Masirah Island, NE part of island, *Morawetz 348* (MICH), HQ900623. ***E. matritensis*** Boiss., Spain, Toledo, Santa Cruz del Retamar, *Molero 11/2008 & al.* (BCN 53037), HQ900624. ***E. mauritanica*** L., South Africa, Western Cape, N12 from De Rust to Oudtshoorn, *Becker & al. 857* (MICH277), HQ900625, HQ900406, HQ900508, HQ900432, HQ900560, HQ900458, HQ900380, HQ900482, HQ900534. ***E. medicaginea*** Boiss., Spain, Cádiz, Algeciras, *Molero 22/2007 & Rovira* (BCN), HQ900626. ***E. meenae*** S. Carter, India, AF537483[2]. ***E. megalatlantica*** Ball, Morocco, Midelt, Jebel Ayachi, *Molero 07/2007* (BCN), HQ900627, HQ900407, HQ900509, HQ900433, HQ900561, HQ900459, HQ900381, HQ900483, HQ900535. ***E. mellifera*** Aiton, Spain, Canary Islands, La Gomera, Parque nacional de Garajonay, *Molero 12/2007* (BCN), HQ900628. ***E. meloformis*** Ait., Republic of South Africa (cultivated), AF537565[2]. ***E. meuleniana*** O. Schwartz, Yemen (cultivated), AF537572[2]. ***E. milii*** Des Moul., Madagascar (cultivated), AF537461[2]. ***E. minuta*** Loscos & J. Pardo, Spain, Lleida, Llardecans, *Barres 33 & al.* (BC), HQ900629. ***E. misella*** S. Watson, Mexico, AF537384[2]. ***E. monteiroi*** Hook., Botswana, AF537563[2]. ***E. myrsinites*** L., Italy, Basilicata, Potenza, Pollino National Park, *Vilatersana 1132 & al.* (BC), HQ900630. ***E. namuskluftensis*** L.C. Leach, Namibia (cultivated), AF537562[2]. ***E. nereidum*** Jahand. & Maire, Morocco, Beni-Mellal, *Montserrat s.n.* (BC), HQ900631. ***E. nevadensis*** Boiss. & Reut., Spain, Granada, Sierra Nevada, *Molero s.n.* (BCN 34062), JF279604. ***E. nevadensis*** subsp. ***bolosii*** Molero & Rovira, Spain, Barcelona, Montserrat Mountain, *Hilpold s.n. & Pramsohler* (BC), HQ900632. ***E. nicaeensis*** All., Spain, Barcelona, near Monistrol de Montserrat, *Barres 3 & al.* (BC), HQ900633. ***E. nubica*** N.E. Br., Ethiopia, *Friis & al. 8288* (K), HQ900634, HQ900408, HQ900510, HQ900434, HQ900562, HQ900460, HQ900382, HQ900484, HQ900536. ***E. oaxacana*** B.L. Rob. & Greenm., Mexico, AF537373[2]. ***E. obesa*** Hook.f., Republic of South Africa (cultivated), AF537566[2]. ***E. omariana*** M.G. Gilbert, Ethiopia, AF537560[2]. ***E. orthoclada*** Baker, Madagascar, *Riina 1739* (MICH), HQ900635, HQ900409, HQ900511, HQ900435, HQ900563, HQ900461, HQ900383, HQ900485, HQ900537. ***E. oxyphylla*** Boiss., Spain, Toledo, El Real de San Martín, *Molero 12/2008 & al.* (BCN 53036), HQ900636. ***E. palustris*** L., Spain, Girona, L'Escala, Cinclaus, *Barres 34 & al.* (BC), HQ900637. ***E. paniculata*** Desf. subsp. ***paniculata***, Spain, Huelva, between Los Marines and Fuenteheridos, *Molero 16/2008 & al.* (BCN 53039), HQ900639. ***E. paniculata*** subsp. ***monchiquensis*** (Franco & P. Silva) Vicens, Molero & C. Blanché, Portugal, Algarve, Nave Redonda, *Molero 14/2008 & al.* (BCN), HQ900638. ***E. papilionum*** S. Carter, Somalia, *Thulin & al. 9415* (K), HQ900640, HQ900410, HQ900512, HQ900436, HQ900564, HQ900462, HQ900384, HQ900486, HQ900538. ***E. paralias*** L., Spain, Tarragona, Torredembarra Beach, *Molero 6/2008* (BCN), HQ900641. ***E. pedroi*** Molero & Rovira, Portugal, Sesimbra, *Riina 1585* (MICH), HQ900642, HQ900411, HQ900513, HQ900437, HQ900565, HQ900463, HQ900385, HQ900487, HQ900539. ***E. peplus*** L., Spain, Barcelona, Parc de Montjuïc, *Barres 16* (BC), HQ900643. ***E. pervilleana*** Baill., Madagascar (cultivated), AF537518[2]. ***E. phosphorea*** Mart., Brazil (cultivated), AF537512[2]. ***E. piscatoria*** Aiton, Portugal, Madeira (cultivated) (BC), HQ900644, HQ900412, HQ900514, HQ900438, HQ900566, HQ900464, HQ900386, HQ900488, HQ900540. ***E. pithyusa*** L. subsp. ***pithyusa***, Spain, Balearic Islands, Minorca, Fornells, *Benedí & Montes s.n.* (BC 869609), HQ900645. ***E. platycephala*** Pax, Tanzania, AF537561[2]. ***E. platyphyllos*** L., Spain, Tarragona, Cornudella de Montsant, La Venta d'en Pubill, *Molero s.n.* (BCN), HQ900646. ***E. polygalifolia*** Boiss. & Reut., Spain, Cantabria, Puerto del Escudo, *Molero & Rovira s.n.* (BCN 53621), HQ900647. ***E. pterococca*** Brot., Spain, Cádiz, Tarifa, *Molero 20/2007 & Rovira* (BCN), HQ900648. ***E. pulcherrima*** Willd. ex Klotzsch, Mexico, AF537432[2]. ***E. pyrenaica*** Jord., Spain, Cantabria, Fuente Dé, Peña Vieja, *Molero & Rovira s.n.* (BCN 53619), HQ900649. ***E. regis-jubae*** J. Gay, Morocco, Sous-Massa-Drâa, Tiznit, Sidi Daoud, *Batriu & al. s.n.* (BCN), HQ900650, HQ900413, HQ900515, HQ900439, HQ900567, HQ900465, HQ900387, HQ900489, HQ900541. ***E. resinifera*** O. Berg, Morocco, Beni-Mellal, road to Azilal, *Molero s.n.* (BC), HQ900651. ***E. retusa*** Forssk., Morocco, Guelmin-Es-Smsa, Guelmin, between Targwa m-Mayt and Mansoura, Ahmed, *Batriu & al. s.n.* (BCN), HQ900652. ***E. rubella*** Pax, East Tropical Africa (cultivated), AF537487[2]. ***E. rzedowskii*** McVaugh, Mexico, AF537399[2]. ***E. schimperi*** C. Presl, Ethiopia, *Riina 1675* (MICH), HQ900653, HQ900414, HQ900516, HQ900440, HQ900568, HQ900466, HQ900388, HQ900490, HQ900542. ***E. segetalis*** L., Spain, Barcelona, Montserrat Mountain, *Barres 5 & al.* (BC), HQ900654. ***E. serrata*** L., Spain, Barcelona, Montserrat Mountain, *Barres 9 & al.* (BC), HQ900655. ***E.* sp. 1,** Russia, AF537545[2]. ***E.* sp. 2,** Armenia, SE of Yerevan Province, *Vitek 05-1171* (W 2008-06492), HQ900578. ***E. spathulata*** Lam., U.S.A., AF537552[2]. ***E. spinosa*** L., Italy, Basilicata, Potenza, road from Maratea to Trecchina, *Vilatersana 1121 & al.* (BC), HQ900656. ***E. squamigera*** Loisel., Spain, Valencia, La Safor, *Molero & Rovira s.n.* (BCN 53020), HQ900657. ***E. stenoclada*** Baill., Madagascar, AM040791[3]. ***E. stolonifera*** Marloth ex A.C. White, R.A. Dyer & B. Sloane, voucher 1: South Africa, AM040792[3], voucher 2: South Africa, Karoo National Park, *Becker 1109* (MICH), –, HQ900415, HQ900517, HQ900441, HQ900569, HQ900467, HQ900389, HQ900491, HQ900543. ***E. stricta*** L., Austria, AF537559[2]. ***E. stygiana*** H.C. Watson, Portugal, Azores Islands, AF334247[1]; AF334262[1]. ***E. succedanea*** L.C. Wheeler, Mexico, AF537403[2]. ***E. sulcata*** Lens ex Loisel., Spain, Lleida, La Sentiu de Sió, Serra Gran, *Barres 32 & al.* (BC), HQ900659. ***E. sultan-hassei*** Å. Strid et al., Greece, Crete, Gorges d'Aradena, *Riina 1568* (MICH), HQ900660. ***E. terracina*** L., Spain, Sevilla, *Molero 23/2007 & Rovira* (BCN), HQ900661, HQ900416, HQ900518, HQ900442, HQ900570, HQ900468, HQ900390, HQ900492, HQ900544. ***E. tetraptera*** Baker, Madagascar, AF537526[2]. ***E. tirucalli*** L., Africa and Madagascar (cultivated), AF537479[2]. ***E. tithymaloides*** L., Guatemala, AF537494[2]. ***E. transtagana*** Boiss., Portugal, Lisbon, Fernão Ferro, *Molero 13/2008 & al.* (BCN 53035), HQ900662. ***E. trichadenia*** Pax, Zimbabwe and Angola (cultivated), AF537564[2]. ***E. trichotoma*** Kunth, Belize, AF537534[2]. ***E. tuberosa*** L., Republic of South Africa (cultivated), AF537570[2]. ***E. tubiglans*** Marloth ex R.A. Dyer, Republic of South Africa (cultivated), AF537567[2]. ***E. tuckeyana*** Steud. ex Webb, Portugal, Cape Verde, Santiago Island (cultivated) (BC), HQ900663, HQ900417, HQ900519, HQ900443, HQ900571, HQ900469, HQ900391, HQ900493, HQ900545. ***E. turczaninowii*** Kar. & Kir., China, AF537543[2]. ***E. uliginosa*** Welw. ex Boiss., Spain, Galicia, A Coruña, Zas, *Molero & Rovira s.n.* (BCN 53605), HQ900664. ***E. usambarica*** Pax subsp. ***usambarica***, Tanzania, *Gereau & Lovett 3021* (K), HQ900665, HQ900418, HQ900520, HQ900444, HQ900572, HQ900470, HQ900392, HQ900494, HQ900546. ***E. uzmuk*** S. Carter & J.R.I. Wood, Yemen, Nashima, *Aldasoro 14569 & Susanna* (BCN), HQ900666. ***E. veneris*** M.S. Khan, Cyprus, near Prodromos road to Platres, *Galbany 2039 & al.* (BC), HQ900667. ***E. xylophylloides*** Brongn. ex Lem., Madagascar (cultivated), AF537467[2]. ***Triadica sebifera*** (L.) Small, China (cultivated), AF537586[2].