Universidade de Aveiro Departamento de Biologia

2018

Ana Carla Ramos da Silva Gonçalves

Revisão taxonómica do género *Calendula* L. (Asteraceae – Calenduleae) na Península Ibérica e Marrocos

Taxonomic revision of the genus *Calendula* L. (Asteraceae – Calenduleae) in the Iberian Peninsula and Morocco

2018

Universidade de Aveiro Departamento de Biologia
2018

Ana Carla Ramos da Silva Gonçalves

Revisão taxonómica do género *Calendula* L. (Asteraceae – Calenduleae) na Península Ibérica e Marrocos

Taxonomic revision of the genus *Calendula* L. (Asteraceae – Calenduleae) in the Iberian Peninsula and Morocco

Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia, realizada sob a orientação científica do Doutor Paulo Cardoso da Silveira, Professor auxiliar do Departamento de Biologia da Universidade de Aveiro e da e da Doutora Maria da Conceição Lopes dos Santos, Professora Catedrática da Faculdade de Ciências da Universidade do Porto.

Apoio financeiro da FCT e do FSE no âmbito do III Quadro Comunitário de Apoio através de uma bolsa – Programa Ciência Global (SFRH/BD/51464/2011).

Apoio financeiro do Laboratório Associado CESAM - Centro de Estudos do Ambiente e do Mar (AMB/50017) financiado por fundos nacionais através da FCT/MCTES e cofinanciado pelo FEDER (POCI-01-0145-FEDER-007638), no âmbito do Acordo de Parceria PT2020, e Compete 2020

o júri / the juri

Presidente / President:

Doutor Amadeu Mortágua Velho da Maia Soares
Professor Catedrático do Departamento de Biologia da Universidade de Aveiro.

Vogais / Members:

Doutor António Xavier de Barros e Cunha Pereira Coutinho
Professor Auxiliar do Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade de Coimbra.

Doutor Rubim Manuel Almeida da Silva
Professor Auxiliar do Departamento de Biologia da Faculdade de Ciências da Universidade do Porto.

Doutora Maria Helena Abreu Silva
Professora Auxiliar do Departamento de Biologia da Universidade de Aveiro

Doutor Paulo Cardoso da Silveira
Professor Auxiliar do Departamento de Biologia da Universidade de Aveiro.
(orientador)

agradecimentos

Aos orientadores Dr. Paulo Silveira e Dr.ª Conceição Santos, meu profundo agradecimento pelo apoio, incentivo, críticas, sugestões e disponibilidade para orientar e ajudar.

Aos co-autores das publicações efetuadas Helena Oliveira, João Loureiro, Sílvia Castro, Jorge Paiva, Estrela Figueiredo, Sofia Nora, Teresa Ribeiro, Maria Leonor Morais, Ahmed Ouhammou e, Rachid Amirouche, bem como alguns revisores anónimos, pelo contributo e enriquecimento do trabalho.

À Violeta Barrios pela ajuda na revisão dos estatutos de conservação IUCN.

Aos projetos *Flora Iberica* e *Flore Pratique du Maroc*, nomeadamente aos respetivos responsáveis, Carlos Aedo e Mohamed Fennane, Mohamed Ibn Tattou, e Jalal El Oualidi.

Aos Centro de Ecologia Funcional e Instituto Superior de Agronomia, agradeço a prontidão e o auxílio prestado para realizar trabalhos laboratoriais.

A todos os herbários que disponibilizaram material para estudar e respetivos curadores (ABH, AL (ENSA), ARAN, B, BC (IBB), BCN, BM, BONN, BR, C, COI, E, ELVE, FI, G, GAT, GDA (GDAC), HAL, JACA, JAEN, JE, K, LD, LEB, LISI, M, MA, MACB, MAF, MARK, MGC, MPU, O, P, PO, RAB, RNG, SALA, SANT, SEV, TFMC, US, VAL and W).

À FCT – Programa ciência Global e ao CESAM pelo apoio financeiro.

À minha família, em especial aos meus pais, Ana e Carlos Gonçalves.

A todos que direta ou indiretamente contribuíram para a realização deste trabalho.

Muito obrigada.

palavras-chave

Compositae, conteudo em ADN, Morfologia, Nomenclatura, Número cromossomatico,

resumo

O género *Calendula* L. (Asteraceae - Calenduleae) inclui, dependendo do autor, 10 a 25 espécies, distribuídas essencialmente na bacia do Mediterrâneo. A taxonomia deste género é considerada extremamente difícil, devido à grande variabilidade morfológica, discutivel relevância de alguns dos caracteres utilizados para distinguir suas espécies (por exemplo, a forma de vida: anual ou perene, o hábito: erecto ou difuso, a forma das folhas, o indumento, o tamanho e a cor dos capítulos e a morfologia dos aquénios), mas também devido à hibridização e poliploidização. Apesar dos inúmeros estudos que foram publicados, não foi alcançado um acordo sobre a classificação e os caracteres utilizados para discriminar as suas espécies. Um estudo taxonómico do género *Calendula* foi realizado para a Península Ibérica e Marrocos, com o objectivo de (1) verificar a variabilidade morfológica, (2) confirmar o número de cromossomas, (3) aumentar as estimativas de conteúdo em ADN, (4) reavaliar a delimitação e a circunscrição dos taxa, e (5) reavaliar e redefinir as descrições e caracteres úteis para os distinguir. Para alcançar uma robustês taxonómica satisfatória, foram realizados extensos trabalhos de campo, análise morfométrica detalhada, abordagens corológicas, cariológicas e quanto ao conteúdo em ADN. Para a Península Ibérica, quatro espécies foram reconhecidas, incluindo nove subespécies (entre essas duas novas subespécies foram descritas). Para Marrocos, incluindo alguns taxa da Argelia e Tunisia, foram reconhecidas 13 espécies (duas novas e uma mudança nomenclatural), incluindo 15 subespécies (entre essas oito novas subespécies foram descritas). Para corroborar os resultados obtidos e avaliar as relações evolutivas e filogenéticas entre os taxa, estudos que utilizem diferentes métodos moleculares, tais como ITS, microsatélites ou outros marcadores moleculares, devem ser utilizados.

keywords Chromosome number, Compositae, nuclear DNA content, Morphology, Nomenclature

abstract

The genus *Calendula* L. (Asteraceae - Calenduleae) includes, depending on the author, 10 to 25 species, distributed mainly in the Mediterranean basin. The taxonomy of this genus is considered to be extremely difficult, due to a great morphological variability, doubtfull relevance of some of the characters used to distinguish its species (e.g. the life form: annual or perennial; the habit: erect or diffuse, shape of the leaves, indumentum, relative size of the capitula and colour of disc or ray florets, achene morphology), but also due to the hybridization and polyploidization. Despite the numerous studies that have been published, no agreement on the classification and characters used to discriminate between taxa has been reached. A taxonomic study of the genus *Calendula* was conducted for the Iberian Peninsula and Morocco, aiming at (1) access the morphological variability between and within taxa, (2) confirm the chromosome numbers, (3) increase the nuclear DNA content estimations, (4) re-evaluate taxa delimitations and circumscription, and (5) reassess, and redefine, the descriptions and characters useful to distinguish *taxa*. In order to achieve a satisfying taxonomic core, extensive fieldwork, detailed morphometric analysis, chorological, karyological and genome size studies were conducted. For the Iberian Peninsula, four species were recognized, including nine subspecies (between these two new subspecies were described). For Morocco, including some taxa from Algeria and Tunisia 13 species were recognized (two new species and a nomenclatural change), including 15 subspecies (among these eight new subspecies were described). To corroborate the results obtained and to evaluate the evolutionary relationships among taxa, phylogenetic studies using molecular methods, such as ITS, microsatellites or other molecular markers, should be used.

‘*Genus difficillimum et in quo characteres nondum satis explorati probatique…*’

Boissier (1849)

‘*Species ob achenia valde variabilia difficile enucleando et cultura iterum probandse.*’

Boissier (1875)

‘*Ce genre est très difficile, et peu d’auteurs son d’accord sur la valeur des espèces qui y ont eté établies*’

Rouy (Rouy 1903)

‘*Groupe de petites espèces variables et difficiles à limiter.*’

Battandier & Trabut (1890)

‘Il genere Calendula è straordinariamente
interessante sotto diversi punti di vista, e principalmente per l’eterocarpia’
Lanza (1919, p. 4)

Index

list of figures

list of tables

List of abbreviations and terms used

The terminology and abbreviations commonly employed in this thesis are defined below:

acc. – The name of a taxon that is considered to be the correct name or the most acceptable name, based on recent botanical references.

auct. (*auctorum*) – Abbreviation for the author.

auct. non – The use of '*auct. non*' denotes a common misapplication or misinterpretation of a species name, i.e., a taxon that was identified erroneously as the named species, but not in the sense of the original author.

basionym – The original or first validly described name on which a new combination or name at new rank is based. A basionym has priority over other subsequently published names given to the same species by different authors. If the species is transferred to a different genus, the specific epithet is retained.

basionym author – The author of a basionym. If the species is transferred to a different status, the specific epithet will be retained and the name of the basionym author will be placed in parentheses before the author of the new combination, hence the alternate name of parenthetical author for the basionym author of a new combination.

comb. (*combination*) – A name of a taxon below the rank of genus, consisting of the name of a genus combined with one or two epithets.

comb. illeg. (*combinatio illegitimum*) – An illegitimate combination. Any combination that is not legitimate according to the rules of the ICBN.

comb. inval. (*combinatio invalidum*) – An invalid combination. Any combination that is invalid according to the rules of the ICBN.

epithet – used for the words in a combination other than the generic name and any rank-denoting term; hyphenated words are equivalent to a single word.

et al. (*et aliorum*) – The abbreviation for 'and others'. When more than 2 authors appear in a citation, the abbreviation '*et al.*' is placed after the first author. This same method is used to refer to bibliographic citations in the text which are authored by several people.

ex – The word '*ex*' is used to denote the work of the publishing author when a name is described and attributed to a previous author whose work was not validly published. Both authors should be listed in the original name.

f.– The abbreviation for '*filius*' (son of) or '*filium*' (the daughter of).

forma (forme) – An infraspecific (lower) taxonomic rank below the rank of variety, denoting minor differences in morphology, such as variations in the colour of petals or fruit. The rank of forma can be abbreviated as '**fa.**' to avoid confusion with the abbreviation '***f***.' for *filius*.

holotype – Specimen or illustration used by the author, or designated by the author as the nomenclatural type.

illeg. name (*illigitemum*) – A validly published name that is not in accordance with specified rules of the ICBN.

in – The word '*in*' is used to denote a taxon described by one author occurring in the work of a different author.

isolectotype – A duplicate specimen of the lectotype.

isoneotype – A duplicate specimen of the neotype.

isotype – A duplicate specimen of the holotype.

ICBN – International Code of Botanical Nomenclature.

IPNI – International Plant Name Index.

IUCN – International Union for Conservation of Nature.

lectotype – A specimen or illustration designated from the original material as the nomenclatural type if no holotype was indicated at the time of publication, or if the holotype is missing, or if a type is found to belong to more than one taxon.

neotype – A specimen or illustration selected to serve as nomenclatural type if no original material is extant or as long as it is missing (Art. 9.7 of the ICBN).

new comb. (combinatio nova) – A new name of rank lower than genus based on a legitimate, previously published name, which is its basionym and which provides the final epithet of the new combination.

nom. nud. (nomen nudum) – A designation of a new taxon published without a description or diagnosis or reference to a description or diagnosis

nom. illeg. (nomen illegitimum) – An illegitimate name, i.e., that is validly published, but which does not follow one or more rules of the ICBN. Later homonyms, later isonyms, superfluous names, and autonyms are various types of illegitimate names.

nom. inval. (nomen invalidum) – An invalid name, i.e., a name that was not validly published according to the rules of the ICBN, or a name that was not accepted by the author in the original publication, for example, if the name was suggested as a synonym of an accepted name.

p.p. (*pro parte*) – The abbreviation for '*in part*'. This abbreviation is placed after the name of a synonym to indicate that some, but not all, individuals identified as this species are synonymous with the accepted species.

s.l. (*sensu lato*) – In the broad sense.

sensu – Means 'in the sense of,' referring to an erroneous identification made according to another author's concept or sense of the species rather than the original author's intention.

sp. – The abbreviation for a single species. The abbreviation 'sp.' is used in combination with a genus name when referring to a plant whose specific identity has not yet been determined.

spp. – The abbreviation for two or more species. The abbreviation 'spp.' is used in combination with a genus name when referring to a group of plants of a single genus whose specific identity has not been determined.

subsp. – The abbreviation for the taxonomic rank of subspecies applied to infraspecific taxa (below species level) that show large-scale of geographic differences.

synonym – Two or more different scientific names that refer to the same taxon. They may be names based on the same type (nomenclatural synonyms) or names based on different types that are judged to be the same taxa (taxonomic synonyms). Synonyms are rejected in favour of the accepted name.

taxon (pl. taxa) – A general term referring to a group of like organisms of any taxonomic rank, including genus, species, or subspecies.

type specimen – A plant specimen (herbarium specimen or illustration) designated by an author to represent the species that he or she has described.

valid name – A name that was validly published according to the rules of the ICBN. A validly published name must include a description of the new taxon or reference to a previous description of the taxon. A validly published name must also be recognized by the author when it is proposed, that is, it cannot be proposed as a synonym of another name. Validly published names may be legitimate or illegitimate.

var. (varietas) – The abbreviation for the taxonomic rank of variety. An infraspecific taxonomic rank below subspecies but above forma.

Chapter 1 - Introduction

1. Contextualization

The genus *Calendula* L. includes 10 to 25 species, depending on the taxonomic treatment (Heyn *et al.* 1974; Ohle 1974, 1975a, b; Meikle 1976a). It is the only genus of the tribe Calenduleae (Asteraceae) confined to the Mediterranean region (Norlindh 1946; Heyn *et al.* 1974; Norlindh 1977a; Nordenstam 2007), and is characterised by its heterocarpy, wide range of cytological variability, hybridization events, and occurrence of intermediate forms (Heyn and Joel 1983; Ruiz de Clavijo 2005).

The genus occupies diversified habitats, from the coast to high mountains, usually colonising unpredictable environments (Ruiz de Clavijo 2005). The most important centre of diversity is in the SW-Mediterranean region, where *taxa* with low chromosome numbers occur (Norlindh 1946; Nora *et al.* 2013).

The taxonomy and evolutionary history of *Calendula* are particularly complex (Lanza 1919; Norlindh 1977a; Heyn and Joel 1983). Thus, uncertainties persist regarding the number of *taxa*, their morphological characterization, circumscription and phylogenetic relationships.

Despite several studies that have been published on *Calendula* (Lanza 1919; Nègre 1958, 1961; Heyn *et al.* 1974; Ohle 1974, 1975b; Meikle 1976a), the genus has never been the object of a taxonomic revision including all taxa, and no agreement on the classification and characters used to discriminate between species has been reached.

This taxonomic revision was started after a challenge endorsed to Dr. Paulo Silveira, supervisor of this thesis, to be responsible for the studies of the genus *Calendula* in the Iberian Peninsula for the *Flora iberica* project (Castroviejo, 1986-ongoing), coordinated by the Royal Botanical Garden of Madrid (CSIC, Spain).

To study the relationships between *Calendula*, some studies were performed: (1) pollen morphology (Antunes *et al.* 2007); (2) karyology and nuclear DNA content (Nora *et al.* 2013); (3) *Calendula* treatment for Flore Pratique du Maroc (Gonçalves *et al.* 2014), and (4) *Calendula* treatment for Flora Iberica (Silveira and Gonçalves, in press).

Here, revised taxonomic treatments of *Calendula* for the Iberian Peninsula and Morocco are provided, based on extensive fieldwork and detailed morphometric, chorological, karyological and genome size studies.

2. The Mediterranean area

The Mediterranean region harbours around 25 000 species of vascular plants, 8.3% of the world's species (Myers *et al.* 2000; Lavagnini *et al.* 2006). The Mediterranean includes not so much the diversity of species, but a remarkable number of endemics (13 000 species or 4.3% of the global endemic plants), predominantly herbs, and sub-shrubs (Myers *et al.* 2000; Lavagnini *et al.* 2006), many of which restricted to islands, peninsulas or isolated in mountain ranges. This diversity has been greatly influenced by geomorphological, and climate changes (Thompson 2005).

Despite encompassing an exceptional biodiversity hotspot, much of the Mediterranean flora is unprotected from habitat destruction, overexploitation and biological invasions. In *Calendula,* several examples of endangered *taxa* can be pointed out, like *C. suffruticosa* subsp. *maritima* in Italy, *C. suffruticosa* subsp. *carbonelli* in Spain, or *C. suffruticosa* subsp. *monardii* in Algeria, restricted populations, threatened by urban growth, among other menaces.

3. The family Asteraceae

Diversity and distribution

The Asteraceae (*Compositae*), with over 1 600 genera, and *ca.* 24 000 species (22 000 to 30 000 species, depending on the authors), is the largest flowering plant family, representing more than 9% of the angiosperms. It is a cosmopolitan family, adapted to temperate or subtropical climates (Mediterranean region, South Africa, south of Australia, Mexico, south-eastern USA, and arid areas of South America), under-represented in the tropical wet forests, and completely absent in Antarctica (Cronquist 1981; Bremer 1994; Funk *et al.* 2005, 2009b; Heywood *et al.* 2007; Kadereit and Jeffrey 2007).

This family includes diverse plants, regarding the habit, life cycles, habitat, pollination, and seed dispersion (Cronquist 1981; Jeffrey 2007). Many species are ruderal, especially abundant in disturbed areas. A significant number, especially in mountains ranges, are endemic.

Origin and diversification

There has been wide controversial discussion concerning the origin of the Asteraceae family. For example, the estimated dates of its origin varies from the Miocene (20 Ma) to Cretaceous (69.5 Ma), or earlier (Funk *et al.* 2005). A few dates could be estimated from fossils, or by comparing phylogenetic trees of the Asteraceae with closely related families. Some important records include: Miocene pollen reports of *Nassauvieae* (25.5 Ma), *Barnadesioideae* (23 Mya), and *Calyceraceae* (21–22 Mya) for southern South America (Barreda *et al.* 2008; Barreda *et al.* 2010a; Palazzesi *et al.* 2010; Panero and Crozier 2016); pollen fossil of *Artemisia* has been recorded from the Eocene to Oligocene (34 Mya) from north-western China (Hobbs and Baldwin 2013); three fossil records of *Mutisiapollis* sp. (*Mutisieae)* was found in the Oligocene from Australia and southern South America (Barreda *et al.* 2010a); pollen records of *Mutisiapollis viteauensis* (*Mutisieae*) dated to approximately in the Palaeocene to Eocene, was found in south-eastern African deposits (Zavada and de Villiers 2000; Barreda *et al.* 2010a); other pollen records from the Paleocene to Eocene are assigned to subfamilies *Mutisioideae* (47.5 Mya), and *Carduoideae* (38 – 42 Mya) from Australia, and southern South America (Barreda *et al.* 2010a, b).

More recent studies of evolutionary rates of Asteraceae, using cpDNA phylogeny, placed the origin approximately in the late Cretaceous (69.5 Mya) to the Eocene (57 Mya) (Panero and Crozier 2016) (Figure 1).

Additionally, Katinas *et al.* (2013) studied how Asteraceae expanded its area of origin and become so widespread. Different scenarios were proposed, considering three vectors of dispersion: (1) birds; (2) wind and (3) floating islands. This study suggested that early-branching lineages of Asteraceae probably dispersed from South America to Africa along with an island chain formed by the Rio Grande Rise and the Walvis Ridge, transported by birds, possibly combined with rafting and/or sweepstakes.

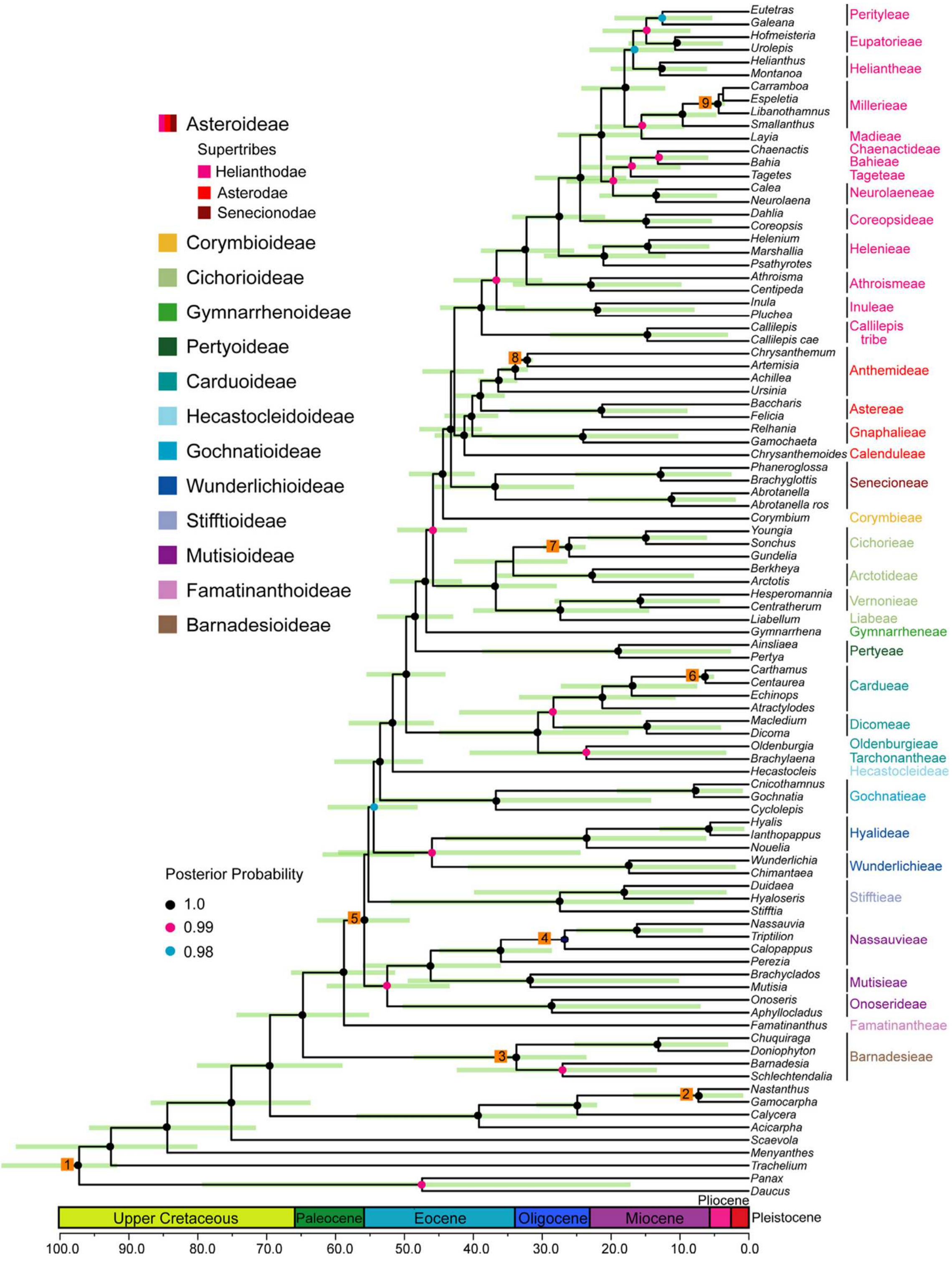


Figure 1 – Evolutionary divergence times for Asteraceae and closely related families (Panero and Crozier 2016)

Classification and phylogeny

The taxonomic history of Asteraceae began with Cassini (1816a, b, 1817, 1819). He made an exhaustive morphological study about '*le style et le stigmate des Synanthérés*' (Figure 2), grouping genera into tribes. His classification includes 19 tribes, comprising nine genera. The following works on Asteraceae (Lessing 1832; De Candolle 1838; Bentham 1873; Hoffmann 1894) were merely improvements on Cassini's tribal classification (Bonifacino *et al.* 2009).

Afterward, Carlquist (1976), studying morphological characters, divided the family into two large subfamilies (*Asteroideae* and *Cichorioideae*). Wagenitz (1976) recognised the same subfamilies but suggested a different classification from Carlquist. He placed the *Eupatorieae* within the *Asteroideae* instead of in the *Cichorioideae*.

Since then, several classification systems have been proposed, based on morphological (Bremer 1987; Kadereit and Jeffrey 2007), chemical (Calabria *et al.* 2007), and molecular phylogenetic analyses (Bremer *et al.* 1992; Bremer 1994; Panero and Funk 2002; Funk *et al.* 2005, 2009a; Jeffrey 2007).

The current classification recognises 12 monophyletic subfamilies, and 43 tribes (Funk *et al.* 2009b). The subfamily *Asteroideae* contains more than 65% of the species of the family, including the tribe Calenduleae. This tribe is one of the smallest tribes of the Asteraceae, including *ca.* 120 species, placed in 12 genera. It is distributed into two main biodiversity centres, in the South African and Mediterranean regions, respectively.

Although the family is well defined, the molecular phylogeny of many genera are poorly known (Heywood 2009). The results of phylogenetic studies have clarified substantially the relationships of the Asteraceae (Panero and Funk 2002), but more studies are needed to understand intraspecific levels (e.g.: 'rogue' genera).

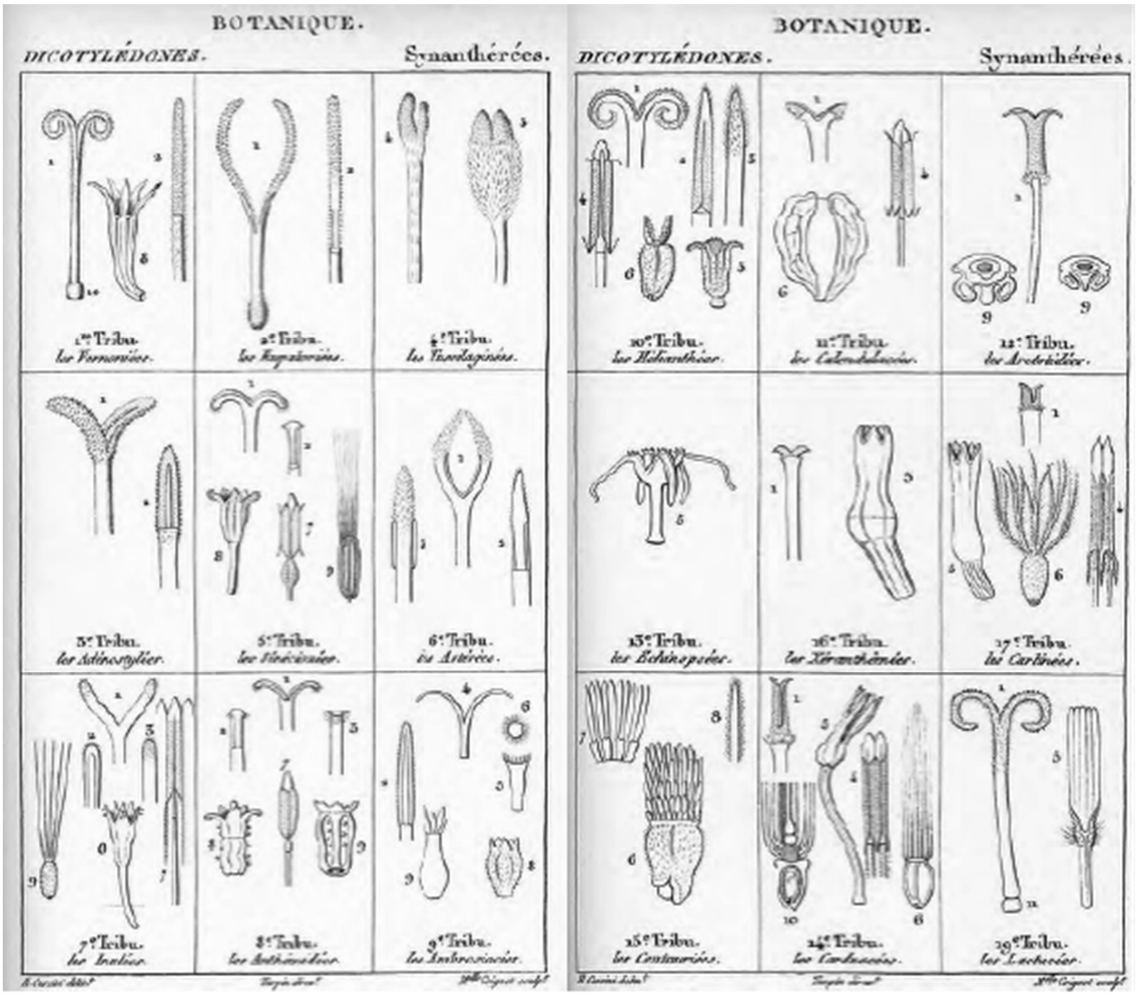


Figure 2 – Morphological traits of Asteraceae as seen by Cassini, taken from Bonifacino *et al.* (2009)

Morphology

The family is characterised by (1) the inflorescence (a head or *capitulum*) with florets (ray or disc) arranged on a receptacle, surrounded by bracts (involucre of phyllaries); by (2) anthers fused in a ring with the pollen pushed or brushed out by the style; and by (3) the presence of achenes (cypsela) usually with a pappus (Bremer 1994; Kadereit and Jeffrey 2007; Funk *et al.* 2009b). A morphological description of this family was extensively revised by Anderberg *et al.* (2007), and Devesa and Quintanar (2015).

Economic importance

The Asteraceae includes numerous genera used as ornamentals (e.g.: *Chrysanthemum*, *Dahlia, Gerbera*, etc.). Other well-known genera include cultivated species for food or oil, such as *Cynara* (includes artichokes), *Lactuca* (includes lettuce), *Chycorium* (include endive), *Carthamus* (includes safflower), or *Helianthus* (includes sunflower). It includes species rich in secondary compounds, used for medicinal and industrial/chemical modification. *Calendula officinalis* L. is used for ornamental, food, medicinal and industrial purpose.

4. The subfamily Asteroideae

Diversity and distribution

The subfamily *Asteroideae* comprises approximately 15 500 species, placed in 1 229 genera, and 20 tribes (Pelser and Watson 2009). It is one of the largest subfamilies of the Asteraceae (over 65% of the species in the family) found worldwide, except in Antarctica.

The subfamily is divided into two large groups (Figure 3). The *Heliantheae Alliance* group comprises 12 tribes (Helenieae, Coreopsideae, Neurolaeneae, Tageteae, Chaenactideae, Bahieae, Polymnieae, Heliantheae, Millerieae, Madieae, Perityleae and Eupatorieae). The rest of the subfamily includes eight tribes (Senecioneae, Calenduleae, Gnaphalieae, Astereae, Anthemideae, Inuleae, Athroismeae and Feddeeae). Most species included in the subfamily belong to Astereae (3 100 species), and Senecioneae (3 000 species) (Pelser and Watson 2009).

Classification and phylogeny

Carlquist (1976) and Wagenitz (1976) defined the concepts of subfamilies *Cichorioideae* and *Asteroideae*. Both used morphological characteristics (corolla, anther and style). Carlquist (1976) considered *Eupatorieae* as a member of *Cichorioideae*, however, Wagenitz (1976) placed *Eupatorieae* as a member of *Asteroideae*.

Since then, morphological, chemical and molecular data analyses have changed the phylogeny and classification of the Asteraceae. Various hypotheses of intertribal phylogenetic relationships of *Asteroideae* have been proposed (Pelser and Watson 2009).

Although it is one of the largest subfamilies within the Asteraceae, most of the studies are dedicated to individual genera and tribes (e.g. *Senecionideae* and *Heliantheae* alliance), and not to the subfamily as a whole.

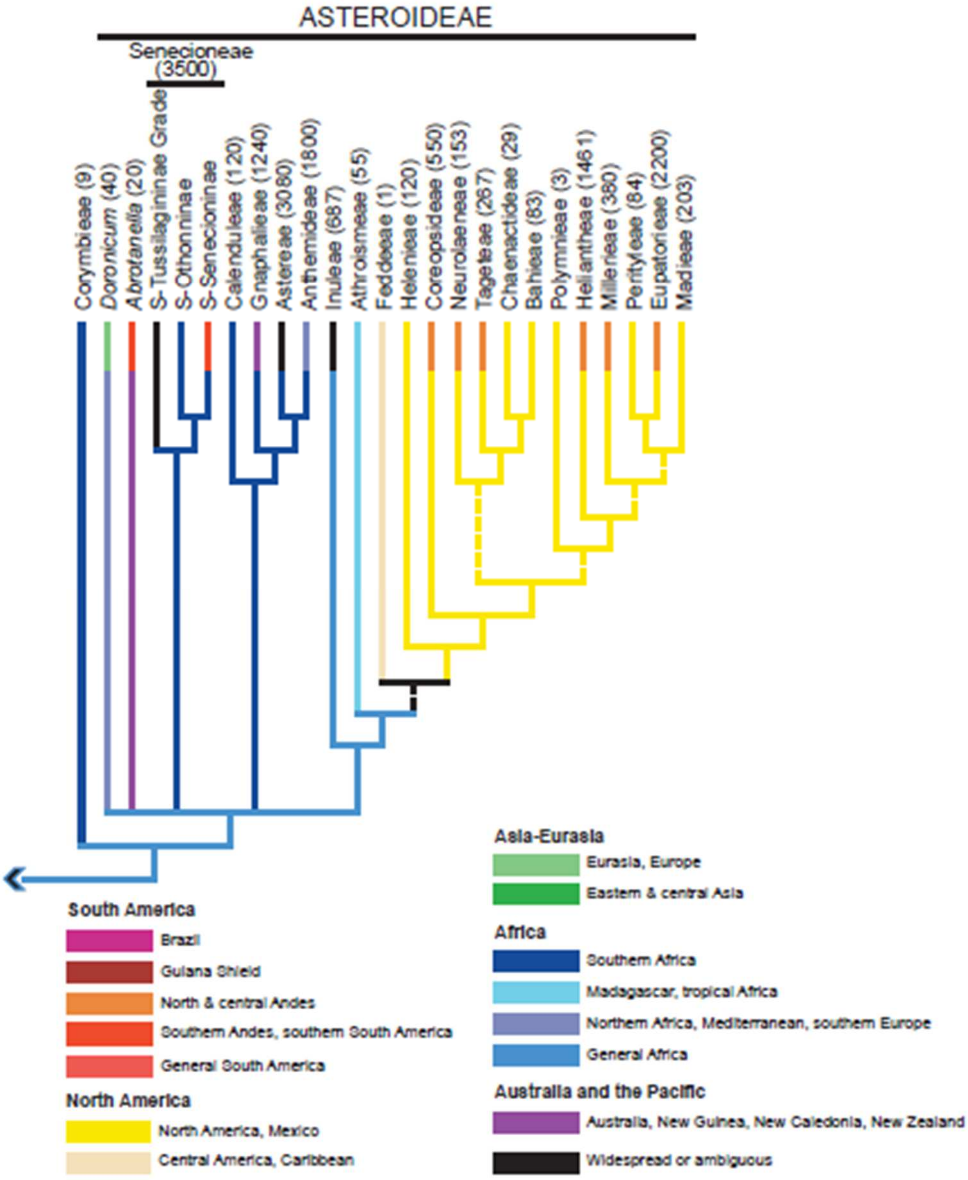


Figure 3 – *Phylogenetic supertree of Asteraceae, subfamily* Asteroideae (Funk *et al.* 2009b)

Morphology

The subfamily is characterised by (1) the presence of ray florets, commonly female; (2) disc florets usually actinomorphic, hermaphrodite or rarely male, with short lobes; (3) anthers without spurs (ecalcarate), and often lacking tail of sterile cells at their base (ecaudate); (4) style branches with two marginal stigmatic surfaces divided into two lines in the faces of the stigmas (marginal stigmatic lines); (5) pollen caveate and echinate with spines regularly or somewhat irregularly spaced; and (6) leaves alternate (except for members of *Heliantheae Alliance* group) (Bremer *et al.* 1994; Jeffrey 2007; Pelser and Watson 2009). These morphological characteristics are rarely seen in *Cichorioideae*.

5. The tribe Calenduleae

Diversity and distribution

The tribe Calenduleae contains about 120 species, assigned to 12 genera (Nordenstam and Källersjö 2009). Calenduleae is concentrated essentially in southern Africa, except the genus *Calendula*, which is confined to the northern hemisphere, with an, essentially, circum-Mediterranean distribution (Norlindh 1946, 1977a; Nordenstam 1994a; Nordenstam and Källersjö 2009). The distribution pattern of all genera could be found in Norlindh (1946) and Nordenstam (2007).

The tribe is commonly characterised by (1) the lack of paleae on the receptacle; (2) lack of pappus; (3) sterile styles in many *taxa*; (4) pollen-sweeping hairs; (5) widespread heterocarpy; and (6) unusual fruit structures such as fenestrate cavities, and a fleshy exocarp (Norlindh 1943, 1977a; Nordenstam 1994a).

Calenduleae is considered one of the smallest tribes in Asteraceae (Nordenstam 1994a; Nordenstam and Källersjö 2009). The tribe was first defined by Cassini (1816a) and placed in *Astereae* and *Cynareae* by Lessing (1832) and in *Senecioneae* by Harvey (1868). Much of the systematic knowledge of the tribe Calenduleae was contributed by Norlindh (1943, 1946, 1960, 1962, 1963, 1977a, b), later changed by Nordenstam (1994a; 1994b; 1996; 2006; 2007), and Nordenstam and Källersjö (2009). The various placements are given below. The tribe Calenduleae comprises an unusual number of polymorphous species, which involved difficulties on its classification.

Origin and diversification

The tribe Calenduleae contains two well-delimited centres of diversity:

(1) The Mediterranean centre. *Calendula* has its centre of distribution in Northern Africa, extending to the South and Central Europe, West to the Macaronesia, and eastwards as far as Turkey, and Iran (Norlindh 1946; Nordenstam and Källersjö 2009).

(2) The South African centre. This distinctive centre in South Africa comprises most genera (*Chrysanthemoides*, *Dimorphotheca*, *Garuleum*, *Gibbaria*, *Inuloides*, *Monoculus*, *Nephrotheca*, *Norlindhia*, *Oligocarpus*, *Osteospermum and Tripteris*), but with some more widespread distributed representatives (Norlindh 1946; Nordenstam and Källersjö 2009).

The South African centre is thought to be the first centre of diversity of the tribe. However, the '*primitive*' forms of the tribe do not necessarily originate from this area (Norlindh 1977a). The '*primitive*' Calenduleae had a wide and continuous distribution in Africa during the Tertiary (Norlindh 1946, 1977a).

Dimorphotheca is supposed to be the most '*primitive*' genus, with both ray and disc florets fertile, paleae on the receptacle, simple achene morphology, and pappus (Norlindh 1977a). According to Norlindh (1946, 1977a), two evolutionary lines have developed, from this '*primitive*' ancestor: (1) the pistils of the ray florets, or (2) the pistils of the disc florets, have through reduction, become sterile. Only *Castalis* (= *Dimorphotheca*) presents sterile ray florets. In the remaining genera, sterile disc florets prevail (Norlindh 1977a). The most advanced genera, or more distant from the '*primitive*' ancestor, are *Calendula*, *Oligocarpus*, *Tripteris* and *Osteospermum.*

An additional evolutive character was the development of heterocarpy in the tribe Calenduleae. It is believed that the polygamous *'primitive'* ancestor was heterocarpic, which lacks in *Tripteris* and *Osteospermum*, and is well developed in *Calendula* and *Oligocarpus.*

Concerning the morphology and position of the achenes, Calenduleae is the most diverse and complex tribe of Asteraceae (Norlindh 1946, 1977a). It has two different morphologies (morphs) of achenes: (1) disc achenes, or (2) ray achenes. Disc achenes are found only in the South African genus *Castalis* (= *Dimorphotheca*) (Norlindh 1946, 1977a). *Dimorphotheca* produced both achenes: those from the ray florets triangular, rugose or tuberculate, rarely winged; and those from the disc florets bilateral flattened, smooth and winged. Ray achenes are produced in all genera of the tribe. Normally, the achenes exhibit great variation in shapes. Most genera are homo-, di-, tri- or polymorphous. *Calendula* is extremely polymorphous, which makes it particularly difficult to classify (Norlindh 1946).

Calendula represents a special lineage within the tribe. The distribution pattern suggested that the Mediterranean centre has gradually evolved from the '*primitive*' ancestor in the Moroccan Atlas region (Norlindh 1946). Seven species were known to link these two centres [*Osteospermum muricatum* E. Mey. ex DC., *Tripteris volkensii* O. Hoffm., *Tripteris afromontana* (Norl.) B. Nord., *Tripteris monocephala* Oliv. and Hiern, *Tripteris nyikensis* (Norl.) B. Nord., *Tripteris vaillantii* Decne., and *Chrysanthemoides monilifera* (L.) Norl.]. These connecting species are rich in biotypes and succeed under quite a wide range of different ecological conditions (Norlindh 1946).

Most species belonging to Calenduleae in South Africa usually grow in a wide variety of habitats, in fynbos, and karoo (semi-deserts) to deserts vegetation types, from sea level, to moderate, and high altitudes (Norlindh 1946). Some genera extend northwards into woodlands next to the coastal areas of Angola, into savannahs, and deciduous woods of Congo, and Zimbabwe. *Calendula* species appear in all altitudes, from sea level up to 2500 m elevation.

Classification and phylogeny

A limited number of interesting, and influential treatments of the tribe Calenduleae were produced during the last 200 years. The genera are extremely polymorphous and have been subjected to many changes in its delimitation. Approximately 20 genera have been included in the tribe, but only 12 genera are considered valid. Below it is summarised the major advances in literature in the last 200 years:

1816a. The tribe Calenduleae was first defined by Cassini, as a natural group. He described the first two genera *Calendula* and *Osteospermum* of the '*Tribu. Les Calendulacées*' (Figure 4), one of 17 tribes divided in the family Asteraceae. He emphasised the outgrowths on the surface and the lack of a pappus of the fruits as characteristic of this tribe. Cassini pointed out the heteromorphous achenes of the genus *Calendula.*

1824. Cassini used the characters of corollas, stamens, styles, achenes and pappus, obtained from numerous representatives, to divide the family Asteraceae into 20 tribes. He recognised the tribe Calenduleae as a natural group, comprising nine genera: *Calendula*, *Osteospermum* and *Meteorina,* with six genera described/established: *Arnoldia*, *Blaxium*, *Castalis*, *Eriocline*, *Garuleum* and *Gibbaria.* Only four are recognised today. The remaining genera were later synonymised either with *Chrysanthemoides* or *Dimorphotheca* (Nordenstam and Källersjö 2009).

Première Section

Calendulées-Prototypes (*Calenduleae – Archetypae*)

Caractères : Calathide ordinairement grande : Pericline supérieur aux fleurs du disque, forme de squames subunisériées, à peu près égales, longues, étroites.

I. Ovaires de la couronne très-arque den dedans ; faux-ovaires du disque point comprimés ni bordes ; corolles du disque à tube long environ comme le tiers du limbe ; bourrelets stigmatiques papilles.

1. ***Calendula***. = *Caltha sp.* Tourn. . – Adans. – *Caltha* Vaill. (1720). – Moench – *Calendulae sp.* Lin. Juss.

Figure 4 – Key section of tribe Calenduleae, adapted from Cassini (1834)

1832. Lessing classified the family Asteraceae into eight tribes and 45 subfamilies. His classification was the most artificial/unnatural, breaking up all Cassini's natural tribe. He reduced the tribe Calenduleae to a small subtribe under *Cynareae*, retaining only *Calendula.* At the same time, this author included two new genera: *Oligocarpus* and *Tripteris*. *Osteospermum* and *Eriocline* were transferred to subtribe *Othonninae* ('*Othonneae*'). *Meteorina* and *Blaxium* were synonymised with *Dimorphotheca* and placed in a subtribe *Chrysantheminae* ('*Chrysanthemeae*') of the tribe *Senecioneae* ('*Senecionideae*'). Furthermore, *Garuleum* was moved to still another tribe *Astereae* ('*Asteroideae*'), and *Gibbaria* among the '*genera minus cognita*' (less recognised genera).

1837. De Candolle used the same characters as Lessing, keeping Calenduleae (including *Othonninae*) as a small subtribe of *Cynareae*, maintaining the same three genera. He enlarged the genus *Dimorphotheca* to include *Arnoldia* and *Castalis*. He added three new genera: *Acanthotheca*, *Xenismia and Xerothamnus*, but none of these are recognised today. Furthermore, all South African species were excluded from *Calendula*, transferring most of them to *Dimorphotheca*, *Tripteris* or *Osteospermum.* De Candolle was the first to circumscribe *Calendula* to become a strictly Mediterranean genus, based on a single character: vermiculate achenes.

1868. Harvey placed the Calenduleae as a subtribe of *Senecionideae*, a most closely related tribe than *Cynareae*, sharing the same style morphology. His classification was based, essentially, on the shape of the achenes. He recognised five genera: *Calendula*, *Dimorphotheca*, *Osteospermum*, *Tripteris* and *Xenismia.*

1873. Bentham adopted most of Cassini's system, reducing the family to 13 tribes. His system is largely still in use today. He raised up the Calenduleae to tribal rank, calling the group '*Calendulaceae*' instead. The tribe consisted of De Candolle's subtribes (*Calendula*, *Dimorphotheca*, *Osteospermum*, *Oligocarpus* and *Tripteris*). He added three more genera: *Ruckeria*, *Dipterocome* and *Eriachaenium.* The genus *Ruckeria* was synonymised to genus *Euryops* in *Senecioneae. Dipterocome* and *Eriachaenium* are both monotypic genera, later excluded from the tribe (Norlindh 1977a; Nordenstam 1994a).

1886. Baillon gave a wide circumscription to the tribe Calenduleae. He included several genera from the tribe *Arctotideae* (*Arctotis*, *Ursinia*, *Gorteria,* and *Berkheya*), among others.

1894. Hoffmann's treatment of the tribe Calenduleae essentially followed Bentham's classification. His separation of genera within Calenduleae was based on the ray florets, and shape of the achenes. All genera included in the tribe formed a natural group. Hoffmann transferred the genus *Ruckeria* to the tribe *Senecioneae*. According to Hoffmann, the achenes of the ray florets of *Calendula*, and *Oligocarpus* are polymorphous, although, *Dimorphotheca*, *Garuleum*, *Osteospermum* and *Tripteris* are homomorphous. Despite his effort to organise the tribe, the characters used may not have been suitable to distinguish the genera.

1919. Lanza published a monograph of *Calendula*, including morphology, *ex situ* experiments and hybridization, to assess the variability of the genus. He recognised 10 species and various '*forma carpica*'.

1943. Norlindh redefined the tribe Calenduleae, including nine genera (*Calendula*, *Castalis*, *Chrysanthemoides*, *Dimorphotheca*, *Dipterocome*, *Eriachaenium*, *Garuleum*, *Gibbaria* and *Osteospermum*). The occurrence of a relatively large number of polymorphic *taxa* led him to divide *Osteospermum* into two subgenera *Euosteospermum*, and *Tripteris*. Norlindh describes 12 sections within the subgenera, which previously corresponded to the genera *Acanthotheca*, *Blaxium*, *Xenismia*, *Oligocarpus* and *Tripteris*. He re-established the genera *Chrysanthemoides*, *Gibbaria and Castalis*.

1946. Norlindh discussed the probable interrelation of the genera and suggested the main lines of evolution in the tribe.

1963. Norlindh extended the knowledge of the tribe including chromosome numbers, and embryology. This author determined the chromosome number of 15 species and three genera of the tribe Calenduleae for the first time.

1977. Norlindh reconsidered that Calenduleae is composed by eight genera, excluding the genus *Eriachaenium*, but its placement remains uncertain.

1994a; b. Nordenstam made the first major attempt for a phylogenetic classification on the tribe Calenduleae, based on morphology, cytology and chemistry. This author made generic arrangements, including 12 new combinations in *Dimorphotheca*, and *Tripteris*. The Asiatic monotypic genus *Dipterocome* was excluded. The tribe was divided into two clades: (1) *Garuleum*, *Gibbaria*, and *Dimorphotheca* (including *Castalis*, *Osteospermum* sect. *Blaxium*); and (2) *Chrysanthemoides, Tripteris*, *Osteospermum*, *Oligocarpus and Calendula.*

1996. Nordenstam revised the generic limits of the tribe Calenduleae, and *Senecioneae*. In this study, nuclear ribosomal DNA (ITS1 and ITS2), and chloroplast *ndhF*, were used to delimit these two tribes. For a long time, the tribe Calenduleae was considered as closely related to Senecioneae (Bremer 1987; Nordenstam 1994a, 1996). Later, molecular evidence indicates affinities to *Astereae*, *Anthemideae and Gnaphalieae*, supported by Bayesian (95%), and Parsimonious (100%) analysis (Panero and Funk 2008; Nordenstam and Källersjö 2009).

2003. Wood and Nordenstam described a new species of *Osteospermum* from western Cape Province, South Africa, which was considered as intermediate between *Osteospermum* and *Chrysanthemoides* by its authors, and by Manning and Goldblatt (2008).

2006. Nordenstam *et al.* presented a first phylogenetic tree of the Calenduleae, based on molecular data, which revealed a monophyletic origin for *Garuleum* and *Dimorphoteca*, and polyphyletic for the remainder genera.

2008. Manning and Goldblatt, based on the phylogenetic tree published by Nordenstam *et al.* (2006), proposed that all the genera of Calenduleae, except *Garuleum* and *Dimorphoteca*, should be included in *Osteospermum.*

2009. Nordenstam and Källersjö provided further details on their phylogenetic analyses that have been conducted to investigate the relationships within the tribe (Figure 5). The analysis retrieves *Garuleum*, and *Dimorphotheca* as two early-branching monophyletic lineages (Nordenstam and Källersjö 2009), and polyphyletic character of the remainder genera. The position of *Garuleum* within the tribe remains uncertain, since it can be distinguished from other genera in the tribe by its unique style (Swelankomo 2013), deeply bifurcate, with linear lobes covered with papillae-hirsute branches in the disc florets, whereas in *Calendula* the style of disc florets are undivided (Nordenstam 2007). Similar to *Calendula*, *Garuleum* develops achenes from the ray florets. Only *G. bipinnatum*, have bisexual disc florets producing winged achenes (Swelankomo 2013).

2009. Barker *et al.* investigated the relationships between the *Chrysanthemoides* species, using both chloroplasts and nuclear non-coding DNA sequence data. The molecular results reveal extensive incongruence; neither *Chrysanthemoides* nor *Osteospermum* was resolved as monophyletic groups, which suggests evidence of past or ongoing hybridization within and possibly between these two lineages.

2012. Manning and Goldblatt described two new species from the semi-arid parts of the Greater Cape Floristic Region, including them in section *Trifenestrata* of the genus *Osteospermum*, which corresponds to *Tripteris sensu* Nordenstam (1994a, b, 1996), further supporting the inclusion of all the genera of Calenduleae, except for *Garuleum* and *Dimorphoteca*, in the genus *Osteospermum.* This option was explained by the interpretation of the molecular studies by Nordenstam and Källersjö 2009, which, for example, still locates *taxa* with other kinds of achenes (among them the genus *Monoculus*) among typical *Tripteris* species, and suggest a polyphyletic origin of most of the genus of Calenduleae, except for *Garuleum* and *Dimorphotheca.*

Up to the present, there has been no agreement among authors regarding the exact composition and phylogenetic relationships among the Calenduleae. Nordenstam (2006; 2009), proposed the segregation of the genus *Osteospermum* until then constituted by 12 sections, and one subgenus *Tripteris* (Norlindh 1943). After these adjustments, the tribe Calenduleae comprised 12 genera (*Calendula, Chrysanthemoides, Dimorphotheca, Garuleum, Gibbaria, Inuloides, Nephrotheca, Norlindhia, Monoculus, Oligocarpus, Osteospermum and Tripteris*). However, Manning and Goldblatt (2008; 2012), based on the phylogenetic results of Nordenstam (2006; 2009) argued that all genera, except *Garuleum* and *Dimorphotheca*, should be considered under the genus *Osteospermum*, including the Mediterranean genus *Calendula.* These disagreements confirm that the tribe is still not well resolved and that more studies should be performed.

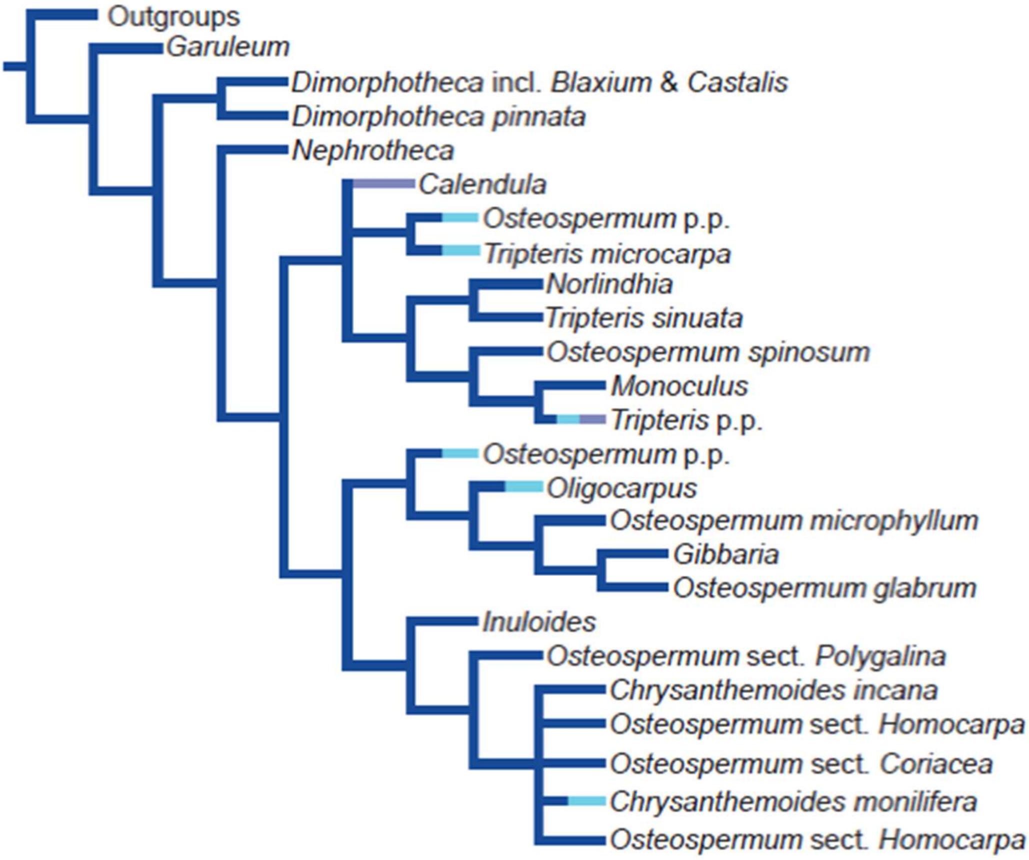


Figure 5 – Phylogenetic relationships within tribe Calenduleae (Nordenstam and Källersjö 2009)

Morphology

The descriptions that follow are based on the characteristics of the most diverse genera belonging to the tribe Calenduleae adapted from Nordenstam (Nordenstam 2007) and Nordenstam and Källersjö (2009):

Annual or perennial herbs, rarely sub-shrubs or shrubs (occasionally *Chrysanthemoides* form small trees). Leaves alternate, rarely opposite (*Tripteris* and *Osteospermum*), petiolate or sessile, lanceolate to obovate, entire or serrate, dentate to variously lobed or dissected, glabrous, glandular or pubescent. Capitula solitary or corymbose, pedunculated or rarely sessile, radiate and heterogamous. Involucre campanulate, hemispheric or rarely conic-shaped; sub-equal, phyllaries 1–3 (4) rows, usually with scarious margins. Receptacle flat or convex, naked (epaleate). Ray florets female or rarely neuter (some

Dimorphotheca species), fertile (pistillate) or sterile (some *Dimorphotheca* species), with short cylindrical tube; corolla ligulate, 3-denticulate, yellow to orange, white, sometimes pink, mauve purple or blue. Disc florets perfect or functionally male (staminate), actinomorphic, 5-lobed; corolla tubular to narrowly campanulate, yellow to orange or reddish. Anthers' apical appendage ovate or triangular-ovate, base sagittate ± tailed (short-caudate), flat. Style fertile or sterile, entire or shortly bilobed (some *Dimorphotheca* species), rarely bifurcate (some *Garuleum* species). Achenes homo-, di- or heteromorphic, dry or rarely drupaceous (*Chrysanthemoides* species), terete or flattened, straight or curved, sometimes rostrate, winged or fenestrate, exocarp sometimes fleshy and coloured, pappus absent (epappose).

Main morphological differences between genera of the tribe Calenduleae are presented in Table 1.

Table 1 – Main morphological characteristics of the tribe Calenduleae adapted from Nordenstam (Nordenstam 2007)

Genera	Habit	Leaves	Leaf Margin	Capitula	Involucre	Ray florets	Disc florets	Anthers	Style	Achenes (ray florets)	Achenes (disc florets)	Chromosome number (2n)	Number of species
Calendula	herbs (annual, perennial)	alternate, sessile	entire or dentate, rarely pinnatifid	solitary, radiate	1-2 seriate	female-fertile, yellow to orange	functionally male	-	undivided style	heteromorphic - rostrate, winged, vermiculate	-	14, 18,30, 32, 44, 88	10 to 25
Chrysanthemoides	Shrubs or small trees	alternate, shortly petiolate	entire or dentate	solitary or corymbose, radiate, shortly pedunculated	-	female-fertile, yellow	functionally male	-	minutely bifid	drupaceous with a fleshy red, orange, blue, purple or black exocarp, globose to	-	18, 20, 36	2 to 6
Dimorphotheca	herbs (annual, perennial), subshrubs or shrubs	alternate	entire or divided	solitary or corymbose, pedunculated, radiate	1 seriate	female-fertile or female-sterile or neuter, yellow or orange, purple or	perfect or functionally male	-	shortly bilobed	(when present) triquetrous or sub terete, sometimes winged or	(when present) flattened with thickened margin	18, 20	20
Garuleum	herbs (perennial) or shrubs	alternate, sessile	dentate to pinnatisect	solitary or corymbose, pedunculated, radiate	2-3 seriate	female-fertile, blue or purple to mauve or white or yellow	perfect or functionally male, yellow	-	deeply bifurcate with linear papillate-hirsute branches	straight, triquetrous or angular, glabrous	(when present) flattened, winged with a thickened rim, glabrous	-	8
Gibbaria	shrublet	alternate, linear-subulate	entire	solitary, pedunculated, radiate	2-3 seriate	female, fertile - yellow to orange	functionally male, yellow or orange	-	shortly bilobed	homomorphic, reniform with a ventral cavity, glabrous	-	-	1
Inuloides	shrublet or small subshrub	rosulate, petiolate	entire or spathulate-orbiculate	solitary, radiate	1 seriate	female, fertile - yellow (turning blue upon drying)	functionally male - yellow	appendage margined, blackish	bilobed	homomorphic (without fenestra), 3-winged	-	-	1
Monoculus	herbs (annual)	alternate	dentate or sinuate lobed	corymbose, radiate	2 seriate	female, fertile - yellow to orange	functionally male - blackish-purplish	-	shortly bifid	homomorphic (unifenestrate), 3-winged	-	16	2
Nephrotheca	shrub or shrublet	alternate	ovate-oblong, flat; margins dentate, scabrid	solitary, shortly pedunculated	1 seriate	female-fertile, yellow, sometimes reddish-purple	-	-	minutely bilobed	homomorphic, reniform, with ventral cavity	-	20	1
Norlindhia	herbs (annual)	alternate, petiolate or sessile	sinuate-dentate or denticulate	corymbose, radiate	1 seriate	female, fertile - yellow to orange	functionally male - yellow	-	apex minutely bifid	homo- or dimorphic (without fenestra), 3-winged or wingless	-	-	3
Oligocarpus	herbs (annual)	alternate	entire or dentate to lobate	solitary, radiate	1 seriate	female, fertile - yellow	functionally male - yellow	-	shortly bilobed	polymorphic - curve, rostrate, winged or wingless	-	-	2
Osteospermum	herbs (annual, perennial), subshrubs or shrubs	alternate or opposite	entire or divided	radiate	1-3 seriate	female, fertile - yellow to orange	-	-	-	homo- or heteromorphic, straight	-	-	45
Tripteris	herbs (annual, perennial), subshrubs or shrubs	alternate or opposite	entire or variously lobed	solitary or corymbose, radiate	1-2 seriate	female, fertile - yellow to orange	functionally male	-	shortly bifid	homomorphic (trifenestrate), 3-winged	-	18, 33	20

Palynology

There are a few studies describing the pollen grains of the genera belonging to the Calenduleae. The most important pollen morphological study was made by Stix (1960), who distinguished four pollen types: *Calendula*, *Dimorphotheca*, *Castalis* and *Osteospermum*. Later, Praglowski and Grafström (1980) studied eight genera of the tribe, recognising five pollen types: *Calendula*, *Dipterocome*, *Garuleum*, *Gibbaria* and *Osteospermum*. They found out that pollen grains of *Calendula* possesses several unique easily recognisable features: (1) largest equatorial diameter; (2) high caveae; (3) slender spines; and (4) thick endexine. By contrast, it was difficult to separate the pollen grains of *Dimorphotheca*, *Castalis*, *Chrysanthemoides* and *Osteospermum* (Praglowski and Grafström 1980). *Dipterocome* did not exhibit affinities within the tribe, by displaying an absence of caveae and reduced supratectal spines. Based on this, they proposed the exclusion of *Dipterocome* from the tribe (Praglowski and Grafström 1980). More recent studies of pollen morphology were performed in *Calendula* (Meo and Khan 2006; Zafar *et al.* 2007; Punt and Hoen 2009; Ahmad *et al.* 2010; Blackmore *et al.* 2010) and *Dimorphotheca* (Blackmore *et al.* 2010).

Pollen grains of the Calenduleae are stenopalynous, differing only in size, sculpture (number of spines) and caveae (Praglowski and Grafström 1980). Like the pollen of most Asteraceae, Calenduleae was found to be 3-colporate, oblate-spheroidal, tectate and caveate, the polar axis varies between 24-48 µm, and the equatorial diameter 24-49 µm (Praglowski and Grafström 1980).

Chromosome number

Little is known about the chromosome numbers of the genera included in Calenduleae. Apart from *Calendula*, only a few genera have been studied. Most chromosome counts were made by Norlindh (1963), who determined the chromosome number of *Chrysanthemoides* ($2n$ = 20), *Dimorphotheca* ($2n$ = 20), *Gibbaria* ($2n$ = 20), and *Osteospermum* ($2n$ = 16, 18, 20, 36). These chromosome numbers were confirmed by Gupta (1969), Mehra and Remanandan (1976), Löve (1979, 1988), Strother (1983), Podlech (1986), and Nordenstam (2009). Chromosome numbers in *Calendula* were the most studied, ranging from $2n$ = 14 to 88 (see Nora *et al.* 2013).

Phytochemistry

Species of the Calenduleae are indeed rich in secondary metabolites, which have interesting applications in pharmacology, chemical industry, agriculture and as chemotaxonomical markers. These metabolites are predominantly terpenes, mainly pimarane-type, and flavonoids (Bohm and Stuessy 2001; Alvarenga *et al.* 2005; Nordenstam and Källersjö 2009). Phytochemical data has been reported for *Calendula*, *Chrysanthemoides*, *Dimorphotheca*, *Garuleum* and *Osteospermum* (Bohm and Stuessy 2001; Alvarenga *et al.* 2005).

Many *taxa* of Calenduleae store fatty oils in the achenes, such as calendic acid (*Calendula, Chrysanthemoides* and *Osteospermum*) or dimorphecolic acid (*Dimorphotheca*), which have commercial potential in the technical industry (Smith *et al.* 1960; Earle *et al.* 1964; Barclay and Earle 1965; Kadereit and Jeffrey 2007; Nordenstam and Källersjö 2009). Achenes of *C. officinalis* are rich in fatty acids (Dulf *et al.* 2013).

Further studies including other genera, based on accurate identification and following the most recent data and techniques that contribute to the current classification approaches (Nordenstam 2007; Nordenstam and Källersjö 2009) are still lacking.

Economic importance

The tribe is economically important, as mentioned above. Some species are used in traditional medicine, pharmacology, chemical industry, agriculture or for ornamental purposes. *Chrysanthemoides* has been introduced in Tasmania, South Australia and Victoria for stabilising sand dunes. Today it is considered an invasive weed, and some actions have been developed to control it in Australia (Lindenmayer *et al.* 2015). *Garuleum* produces several chemical compounds, traditionally used as chest ailments, expectorant, diaphoretic, a diuretic in gout and dropsy, the antidote for snakebite, weak stomach, and for haemorrhoids (Van Wyk 2008). Other genera from Calenduleae are used as ornamentals, including *Calendula*, *Dimorphotheca*, *Chrysanthemoides* and *Osteospermum.*

6. The genus *Calendula*

Diversity and distribution

The genus *Calendula* comprises 16 species, although distinct taxonomic treatments have attributed to the genus 10 to around 25 species (Norlindh 1977a; Nordenstam 1994a).

It is the only genus of the tribe Calenduleae occurring essentially around the Mediterranean region and a region in the Middle East (Figure 6). *Calendula* extends to the south and central Europe, in the west to the Macaronesia islands, and east into Turkey, Iraq, Iran, Saudi Arabia and Yemen (Norlindh 1946, 1977a; Heyn *et al.* 1974; Nordenstam 2007; Nordenstam and Källersjö 2009).

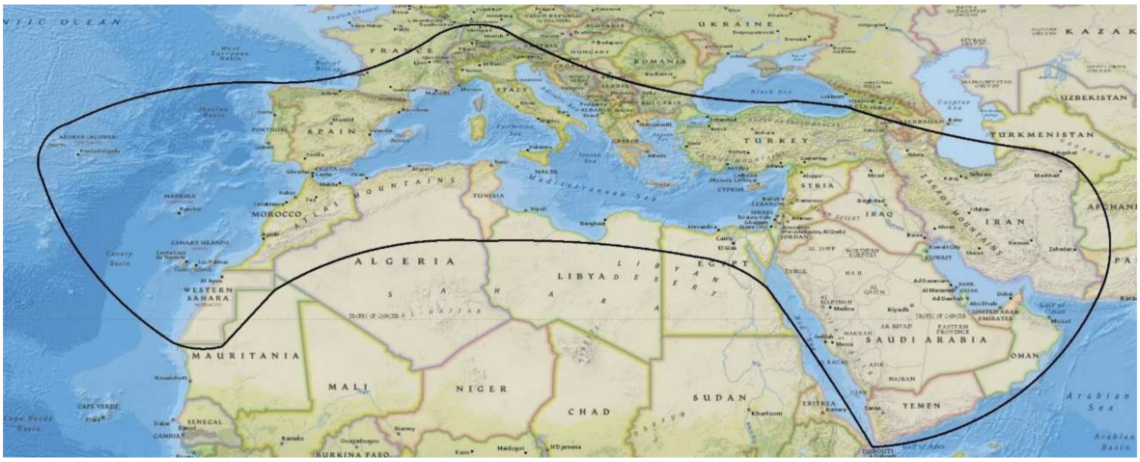


Figure 6 – Distribution of the genus *Calendula* adapted from Norlindh (1946)

Despite its large distribution in the Mediterranean region and few species, the number of endemic *taxa* is rather high. Some examples are the *C. meuselii* in the mountains of Zerhoun (Morocco), *C. eckerleinii* in the Ifrane region (Morocco), or *C. maroccana* confined to the High Atlas Mountains (Morocco). Some of the rarest species, such as *C. maderensis* from Madeira (Portugal) or *C. maritima* from Sicily (Italy), appear in the red list of the IUCN.

Calendula taxa present restricted geographical ranges in the Macaronesia archipelagos (Azores, Madeira, Canary Islands, and Cape Verde), only three species (*C.*

arvensis, *C. officinalis* and C. *suffruticosa*) were cited in the checklists or regional floras (Lowe 1857; Hansen 1971; Press and Short 1994; Schaefer 2002).

The taxonomy of this genus is extremely difficult (Norlindh 1977a). The main difficulty arises from the diversity and variability of achenes produced. On one hand, it is difficult to interpret the function of different types of achenes produced, and its intricate heredity (Heyn and Joel 1983). On the other hand, capitula within *Calendula* can produce 2-6 types of achenes, but more often 3-4 types, with different combinations (this is designated as heterocarpy). The value of some characters used to discriminate species has been questioned, such as life cycles (annual, biannual or perennial), habit (e.g.: erect or diffuse), leaf shape, indumentum, the diameter of the capitula, and colour of the florets (Battandier 1921). Despite important works on perennial (Ohle 1974, 1975a, b), and annual (Heyn *et al.* 1974) species, a recent monograph including the entire genus, is still lacking.

Origin and diversification

Norlindh (1946) suggested that the Mediterranean centre of distribution of the genus *Calendula* has evolved from the '*primitive*' genus *Dimorphotheca.* In the process of speciation, *Dimorphotheca* presented both ray and disc florets hermaphrodite, and fertile. *Calendula* presents a reduction of the pistils of the disc florets, and the development of heterocarpy, especially the vermiculate achenes, the most advanced morph of achenes (Norlindh 1946).

The centre of origin of *Calendula* is located in the SW-Mediterranean (Norlindh 1946; Meusel and Ohle 1966; Ehrendorfer 1970; Heyn *et al.* 1974), where species with low chromosome number occur, as in the species of *C. maroccana* group ($2n$ =18) (Heyn *et al.* 1974). From this area, the genus has successfully diversified, and radiated naturally toward the Macaronesia, throughout the middle of Europe (central France, and southern Germany), north of Africa (Morocco, Algeria, Tunisia, Libya, and Egypt), to the south-west of Asia (Turkey, Iraq, Iran, Saudi Arabia and Yemen) (Norlindh 1977a).

Classification and phylogeny

The etymology of *Calendula* comes from Latin *Kalendae*, which means the first day of the new moon. Linnaeus (1753) originally published *Calendula* as a genus, but several pre-Linnaean authors have described species using this epithet. For a long time, only two species, one *hortensis* (*C. officinalis*) and one *sylvestris* (*C. arvensis*), were known. Linnaeus, in 1753, described five species of *Calendula*, but only one is nowadays included in the genus, *C. officinalis*, since four are now placed in *Dimorphotheca*. Later, three more species were added, but only *C. arvensis* and *C. sancta* belong presently to the genus. The following works on *Calendula*: Cavanilles 1791; Forsskal and Vahl 1791; Desfontaines 1798; Willdenow 1803, included merely descriptions of new single species. A brief taxonomic history of the genus can be found in Lanza (1919) and Ohle (1974).

Lanza (1919) presented an incomplete monograph of the genus *Calendula*, using morphological characters, particularly the achenes, as differentiation of his '*forma carpica*' or varieties. Lanza divided the genus into two groups, that he called "sections"[1]: I - annual, and II – perennial species. In the first "section", three annual species (*C. aegyptiaca*, *C. arvensis*, and *C. bicolor*) were recognised, while the perennial "section", which comprised eight species, was divided into three "sub-sections": (1) *Fulgidea – C. fulgida* and *C. noeana*; (2) *Suffruticosae – C. suffruticosa*, *C. algarbiensis,* and *C. tomentosa*; (3) *Crassifoliae – C. monardii*, *C. maritima* and *C. maderensis*. Since then, numerous studies of the genus *Calendula* conducted by Battandier (1921), Norlindh (1943, 1946, 1962, 1963), Nègre (1958), and Meusel and Ohle (1966), have been published.

Ohle (1974, 1975a, b) reorganised the perennial species and updated the taxonomy of the genus. This author based is classification mainly on morphological characters (growth form, leaf and achenes's morphology), and chromosome numbers. Ohle proposed 24 *taxa* in seven species: the cultivated *C. officinalis*, and the wild species *C. suffruticosa*, *C. incana*, C. *meuselii*, *C. eckerleinii*, *C. maroccana* and *C. lanzae* (Table 2).

Heyn *et al* (1974) studied the hybridization and chromosome number of the annual species from Israel. They proposed that all forms with 2n = 44 chromosomes, should be considered as a single polymorphic species, *C. arvensis*. Heyn recognised five annual species: *C. stellata*, *C. tripterocarpa*, *C. arvensis*, *C. palaestina*, and *C. pachysperma* (Table 2).

---

[1] They do not seem to have been validly published.

Meikle (1976a) in his treatment for *Flora Europeae,* transferred all *C. incana* species into *C. suffruticosa*. This author did not recognise some of the intraspecific *taxa* (e.g.: *C. suffruticosa* subsp. *greuteri* Ohle) described by Ohle (1974).

These studies encompass both (annual and perennial) species, but they have dealt with the genus on a regional basis (e.g.: Meikle 1976), or separately with the annual (e.g.: Heyn *et al* 1974) or the perennial species (e.g.: Ohle 1974; 1975a; 1975b) (Table 2).

Little is known about the molecular phylogeny and genetic variability assessment of the genus. Molecular investigations in the genus *Calendula*, have been conducted using nuclear ribosomal internal transcribed spacer region (ITS), and different markers (*atpI–atpH*, *rbcL*, *5'trnK-matK*, *psbA-trnH*, and *trnL-trnF*) by Plume *et al* (2013) and Schmiderer *et al* (2015).

Plume *et al* (2013) used palynological and molecular data (ITS1, ITS2, and *atpI–atpH*) to study the impact of hybridization between the endemic *C. maritima*, and *C. suffruticosa* subsp. *fulgida* from Sicily. They found that has clear evidence for hybridization, and backcrossing between hybrids and parents.

Recent phylogenetic studies used molecular data to distinguish *C. officinalis* as an adulterant of saffron samples from other species of the genus and other Asteraceae genera (Schmiderer *et al.* 2015). Despite the genetic differences in the chloroplast set, and ITS were relatively small, these authors succeeded in separate *C. officinalis* from others species.

However, despite all these efforts on using molecular data, one study including all species and their relationships is still lacking.

Table 2 – *Taxa* of *Calendula* according to different taxonomic treatments

Heyn et al. (1974)[1]	**Ohle (1974)**[2]	**Meikle (1976)**[3]
C. arvensis		*C. arvensis*
C. pachysperma		
C. palaestina		
C. stellata		*C. stellata*
C. tripterocarpa		*C. tripterocarpa*
	C. officinalis	*C. officinalis*
	C. incana and suffruticosa group	
	C. incana subsp. *algarbiensis* var. *algarbiensis*	*C. suffruticosa* subsp. *algarbiensis*
	C. incana subsp. *algarbiensis* var. *cinerea*	
	C. incana subsp. *algarbiensis* var. *prostrata*	
	C. incana subsp. *incana* var. *incana*	*C. suffruticosa* subsp. *tomentosa*
	C. incana subsp. *maderensis*	
	C. incana subsp. *maritima*	*C. suffruticosa* subsp. *maritima*
	C. incana subsp. *microphylla*	
	C. suffruticosa subsp. *balansae*	
	C. suffruticosa subsp. *boissierii*	
	C. suffruticosa subsp. *carbonelli*	
	C. suffruticosa subsp. *fulgida* var. *fulgida*	*C. suffruticosa* subsp. *fulgida*
	C. suffruticosa subsp. *fulgida*var. *gussonei*	
	C. suffruticosa subsp. *greuterii*	
	C. suffruticosa subsp. *lusitanica*	*C. suffruticosa* subsp. *lusitanica*
	C. suffruticosa subsp. m*onardii*	
	C. suffruticosa subsp. *suffruticosa*	
	C. suffruticosa subsp. *suffruticosa* var. *tunetana*	
	C. suffruticosa subsp. *tlemcensis*	
	C. eckerleinii	
	C. lanzae	
	C. maroccana subsp. *maroccana*	
	C. maroccana subsp. *murbeckii*	
	C. meuselii	

[1] Heyn *et al* (1974) studied only annual *taxa*; [2] Ohle (1974, 1975a, b) studied only perennial *taxa*; [3] Meikle (1976a) studied both (annual and perennial) for Flora Europaea

Morphology

The *taxa* belonging to the genus *Calendula* are annual, biannual or perennial herbs, sometimes woody at the base. The habit is rather diverse, but most commonly ascending, erect, diffuse or prostrate.

The leaves are alternate, undivided – pinnatisect in Morocco; presenting a broad variation in shape, among and within *taxa*, being spatulate, sub-spatulate, obovate, oblanceolate, oblong, lanceolate or linear; the apex varies from obtuse to acuminate; the base can be attenuate, truncate or auriculate; the margins are entire, dentate, repand-dentate or rarely pinnatisect; the middle and upper cauline progressively smaller and shortly stalked toward the apex, oblanceolate to lanceolate, usually auriculate.

The indumentum type and density varies among and within *taxa*, most species have glandular (aromatic), and non-glandular hairs in variable proportions, but occasionally with a more or less dense white-arachnoid-pubescence.

The capitula are usually solitary, pedunculated and radiated; the involucres are campanulate or hemispherical, with one to two rows of bracts, sub-equal, linear-lanceolate, with apex acute and margin hyaline; the receptacle is flat-convex, nude, without inter-floral bracts. The ray florets are ligulate, yellow or orange, female and fertile. The disc florets are tubular, yellow, orange, brown or violet-purple, functionally male.

The achenes are heteromorphic: (1) rostrate achenes, from straight to strongly incurved, with or without dorsal spines; (2) bialate achenes, with two lateral wide wings; (3) trialate achenes, with three wings (two lateral, and one ventral); (4) cymbiform achenes, with two lateral wings, concave, bent to the ventral face, with, or without ventral wing; (5) sub-cymbiform (semi-cymbiform) achenes, with two large lateral wings less curved than the cymbiform (6) vermiculate-alate, with two lateral incurved wings, dorsally rugose or tuberculate; and (7) vermiculate-exalate, without wings, dorsally rugose or tuberculate; all achenes without pappus (Figure 7) (Gonçalves *et al.*, in press; Gonçalves *et al* 2014).

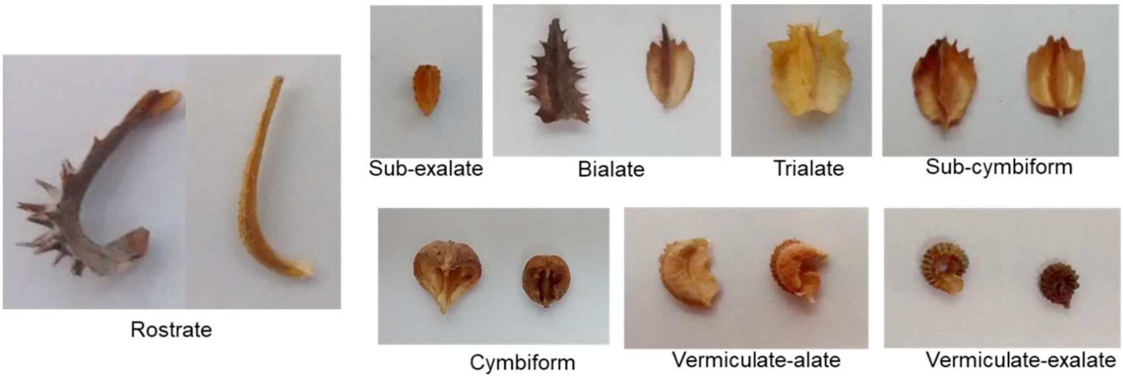

Rostrate
Sub-exalate
Bialate
Trialate
Sub-cymbiform
Cymbiform
Vermiculate-alate
Vermiculate-exalate

Figure 7 – Different morphs of achenes in *Calendula* L.

Palynology

The pollen morphology of *Calendula* was studied by a number of authors (Stix 1960; Praglowski and Grafström 1980; Noor *et al.* 2004; Meo and Khan 2006; El-Garf and Osman 2007; Punt and Hoen 2009; Ahmad *et al.* 2010), but only the first two involved more than one or two species (*C. arvensis* and *C. officinalis*).

Stix (1960) described the pollen morphology of five *taxa* belonging to *Calendula*, which was *C. algeriensis*, *C. arvensis*, *C. fulgida*, *C. lusitanica*, *C. officinalis* and *C. persica*. Praglowski and Grafström (1980) described the pollen grains of five species of *Calendula, C. arvensis*, *C. echinata* (= *C. arvensis*), *C. fulgida, C. maritima* and *C. suffruticosa* and, among other genera of the tribe Calenduleae. According to them, *Calendula* possesses some unique characteristic features, such as (1) largest equatorial diameter; (2) high caveae; (3) slender spines; and (4) thick endexine. These features are easily distinguishable when comparing with other genera. They pointed out that the pollen wall of *Calendula*-type consists of a large number of many slender spines (100–130). Meo and Khan (2006) examined pollen morphology of *C. arvensis* and *C. officinalis* from Pakistan. According to them, pollen grains are prolate-spheroidal in equatorial view, semi-angular in polar view, 3- to 4-zonocolporate, caveate, and echinate. Variations between the two species were found: the exine is thicker in *C. arvensis* (6.3 µm) as compared to *C. officinalis* (3.3 µm). Antunes *et al* (2007) made a major attempt to study pollen morphology of the genus from the Iberian Peninsula, including all the *taxa* known in this territory (*C. arvensis*, *C. officinalis*, *C tripterocarpa* and *C. suffruticosa*). The inter-sample variation of pollen grains is higher, which does not allow a clear separation of *taxa* (Antunes *et al.* 2007). Punt and Hoen (2009) further contributed to the knowledge of pollen of the Asteraceae. Besides their effort to compile pollen morphology of this family, they described only the pollen type of one species of *Calendula*, *C. arvensis*. The pollen grains were similar to the described in the literature.

Pollen grains in *Calendula* are 3- to 4-zonocolporate, rarely 4-pantocolporate or 4-loxocolporate, radially symmetrical, oblate-spheroidal to prolate-spheroidal, polar axis 23.5-32 (27.7 ± 1.95) µm, equatorial axis 25.2-34.3 (29.4 ± 2.01) µm, echinate, and caveate (Figure 8) (description adapted from Meo and Khan 2006; Punt and Hoen 2009; Antunes *et al.* 2007). Comparing with another Calenduleae, the distinctive features of the pollen of *Calendula* include its largest size (up to 50 µm), longer solid spines and thicker endexine (Meo and Khan 2006)[2]. Different sizes of pollen grains and 3- 4-zonocolporate pollen grains occur together within a single flower (Heyn and Joel 1983), and the number of apertures is correlated to the ploidy level and may be an evident obstacle for self-compatibility (Ohle 1974; Heyn and Joel 1983).

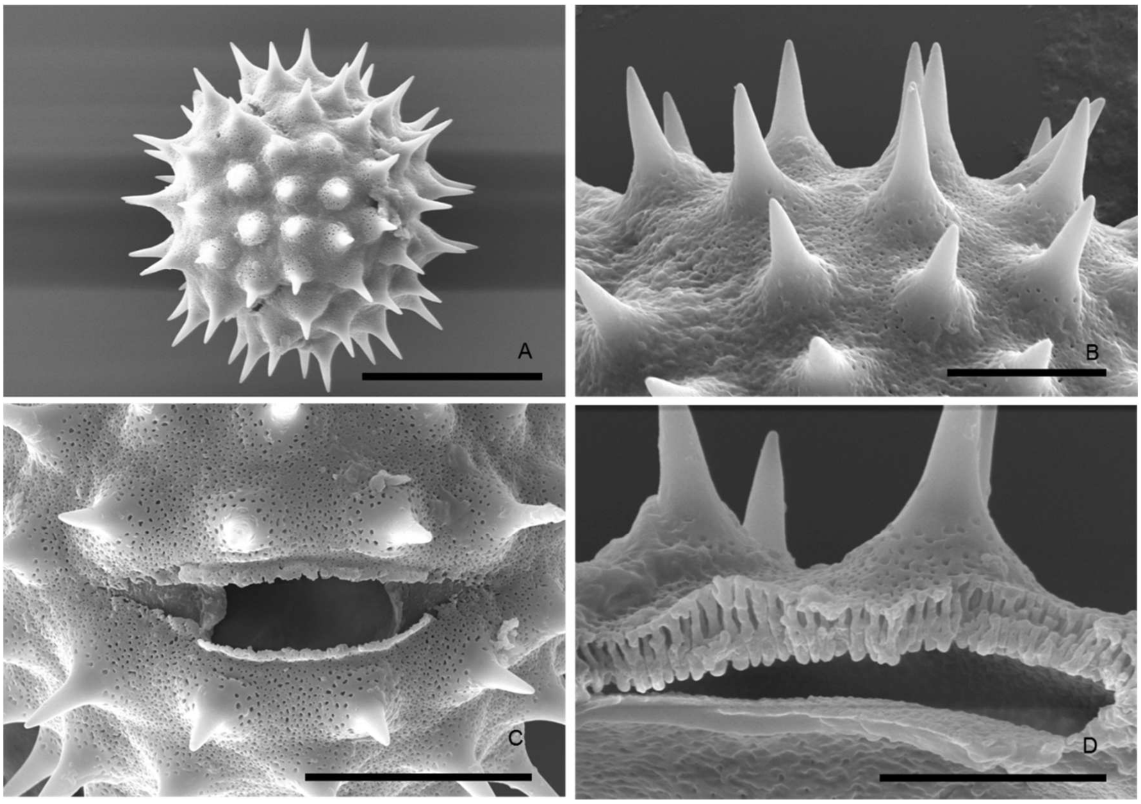


Figure 8 – Pollen of *Calendula* L. A. Polar view; B. Detail of spines; C. Detail of the aperture; D. Detail of exine. Scale bars: A. 20.0 µm; B. 5.0 µm; C. 10.0 µm; D. 5.0 µm.

Heyn and Snir (1986), studied pollen allocation of *C. arvensis* from Israel. They found that only 36% of the pollen grains produced in each flowering head has a known

---

[2] The spine are not solid (personal communication)

function, or is allocated. The remaining pollen (64%) is distributed according to fluctuating ecological factors, biotic (insects, etc.), and abiotic (the wind, rain, etc.). This study was confirmed by Orueta (2002), which pointed that less than 20% of pollen liberated by anthers germinates.

Reproduction and pollination

C. arvensis was found to be essentially self-compatible, although cross-fertilisation has been noted in the ray florets (Heyn and Joel 1983; Heyn and Snir 1986). Pollen is automatically transferred from disc to ray florets (Ruiz de Clavijo 2005). The self-compatibility provides advantages such as independence from pollinators and preservation of well-adapted genotypes. Normally, the annuals colonise unpredictable environments and possibly evolved to ensure the production of achenes in absence of pollinators, before dying. In contrast, the perennials, that have more time to reproduce, are obligated to outcross (self-incompatibility), which increases genetic diversity and ability to adapt to changing conditions.

Calendula is mainly visited by *Systropha* spp. (*Hymenoptera*), *Usia* spp. (*Diptera*), *Melanothrips* spp. (*Thysanoptera*), *Eurydema ornata* L. (*Hemiptera*), and *Psilothrix nobilis* Redtenbacher (*Coleoptera*) (Ruiz de Clavijo 2005). One of its most assiduous flower visitors is the bombyliid fly *Usia* spp. (Lanza 1919; Orueta 2002), but there are probably other pollinating agents (Figure 9).

The proportion of visitors seems to be temperature dependent (Heyn and Snir 1986; Orueta 2002; Ruiz de Clavijo 2005). Temperature can act as a limiting factor, especially for the annual plants, who start flowering with cold air temperature, and the number of other pollinators is still scarce. Pollinators are attracted by the inflorescence colour, due to the light reflection of incident solar radiation on the ray-floret petals. Experimental evidence showed that floral surface warms up with solar radiation and inflorescences with the brightest ‘ray florets’ has the highest temperatures on their disc florets (Orueta 2002).

Figure 9 – Pollinators of *Calendula* L.

Heterocarpy

Seed polymorphism– heterocarpy- is the production of different types (or morphs) of seeds by a single individual plant (Venable 1985; Imbert 2002). The seeds produced normally have a different shape, structure (pappus or wings), size (mass or length) and colour. This phenomenon has been reported for several plant families (*Chenopodiaceae*, *Brassicaceae*, *Poaceae*, *Fabaceae*, and others), but it is mainly concentrated in the Asteraceae (Tanowitz *et al.* 1987; Imbert 2002; Ruiz de Clavijo 2005; Afonso *et al.* 2014).

Heterocarpy is a mixed reproductive strategy, associated with flower polymorphism, to increase fitness (Imbert *et al.* 1996; Imbert 2001). It is an adaptation to unpredictable habitats, such as arid and semi-arid areas or saline soils. Various mechanisms are involved in adapting to these environments. Some studies associated the evolution of annual plants and development of the heterocarpy as an adaptive response to unpredictable environments (Venable 1985; Cruz-Mazo *et al.* 2009). However, in *Calendula* this association does not appear, as both annuals and perennials are heterocarpic.

Morphologically distinct achenes (morphs) differ in respect to dispersal, dormancy, germination and seedling competitive ability. Many species of Asteraceae are known to present two morphologies of achenes (dimorphism), between the peripheral and the central achenes. Normally, one morph has high or relatively high dispersal ability, and lack of dormancy, while the other one has low dispersal ability, and high or relatively high dormancy (Venable 1985).

In *Calendula,* heterocarpy is unusual, because all the achenes are produced from ray florets, and one capitulum can produce 2-6 different achene morphs, but more often 3-4 morphs. Achenes assume different positions within the head. In general, vermiculate, cymbiform and/or bi-trialate, and rostrate achenes migrate to an inner, median or external position on the capitulum, during maturation. In some species, different combinations of achenes might be found mixed in the same population.

Dispersion

Since *Calendula* produces different types of fruits in the same capitulum, it uses several dispersion strategies. A single head may comprise five or more shapes, with different size, colour, and ornamentation/adorning. The inner achenes (vermiculate) are fit to short-range dispersal, responsible for the maintenance of a seed bank, ensuring the survival of the species onsite, even under unfavourable conditions. The external and middle achenes are adapted for long-range dispersal (exozoochory or anemochory) responsible for colonising new ranges and habitats (Heyn 1988; Ruiz de Clavijo 2005).

Chromosome number

Calendula has been considered particularly complex, in terms of karyology, since several basic numbers, $x = 7, 8, 9, 11$ and 15, have been reported (Norlindh 1977a; Heyn and Joel 1983; Nordenstam and Källersjö 2009), although the main basic number was considered to be $x = 9$ (Norlindh 1977a). Moreover, the chromosomes are small, and numerous, sometimes resulting in erroneous counts, and due to the large morphological variability, many chromosome numbers have been recorded in misidentified *taxa*. For example, Carr *et al.* (Carr *et al.* 1999) determined $2n = 7_{II}$ for *C. officinalis*, differing from previous reports of $2n = 32$ for this species.

The most important studies on chromosome counts were reported by Ohle (Meusel and Ohle 1966; Ohle 1974, 1975a; b) who studied polymorphism of perennial *Calendula taxa*, and by Heyn's group (Heyn *et al.* 1974; Heyn and Joel 1983), who proposed the mechanisms of evolution between *Calendula* species.

Chromosome numbers in *Calendula* range from $2n = 14, 18, 30, 32, 44$, to ±88 (Nora *et al.* 2013). The lowest chromosome numbers ($2n = 14$ and 18) are found in North West Africa, the proposed centre of origin of the genus, while *taxa* with probably recent origin and polyploid ($2n = \pm88$) are found in the Palestine. B-chromosomes have been reported for *C. suffruticosa* subsp. *algarbiensis* (Fernandes and Queirós 1971), and *C. tripterocarpa* (Oberprieler and Vogt 1993; Vogt and Oberprieler 2008). B-chromosomes can play an important role in genome evolution and may be useful for studying molecular evolutionary processes (Camacho *et al.* 2000).

Genome size

In the Iberian Peninsula, holoploid genome sizes (2C values) range from 2.9 pg (*C. officinalis* L.) to 5.4 pg (*C. arvensis* L.). The mean value of the available data from Iberian Peninsula is 3.4 pg (Garnatje *et al.* 2011; Nora *et al.* 2013). Nora *et al.* (2013), reported a gradient in genome size between two species known to have the same chromosome number, *C. suffruticosa,* and *C. incana*, which suggested that these should better be treated as only one species.

Phytochemistry

Phytochemical studies identified the presence of various compounds (Table 3), mainly terpenes, flavonoids, carotenoids, and polysaccharides (Ruszkowski *et al.* 2006; Muley *et al.* 2009; Paolini *et al.* 2010; Paim *et al.* 2010; Raal and Kirsipuu 2011; Arora *et al.* 2013; Dulf *et al.* 2013). Among them, terpenes, particularly triterpene alcohols, and glycosides, are diversified and abundant. These secondary metabolites have multiple pharmacological application such as anti-HIV, cytotoxic, anti-inflammatory, hepatoprotective, spasmolytic and spasmogenic, among others (Muley *et al.* 2009).

Economic importance

Calendula has been used medicinally since long times. Several works on ethnobotany, pharmacology, medicinal plants, biochemistry, focused on *C. officinalis*, and its secondary compounds.

Traditionally, it is used in the treatment of various skin tumours, dermatological lesions (anti-bacterial, anti-fungal), ulcers, swellings and nervous disorders, blood purification and 200 cosmetic formulations, i.e. creams, lotions, shampoos (Arora *et al.* 2013). *Calendula* is used for human and animal food (e.g.: tea, salads, saffron substitute, and vinegar). For example, *C. arvensis* is used to feed animals such as cows (in ruderal conditions). Besides medicinal and food, *Calendula* is used as ornamental.

Table 3 – Phytoconstituents of *Calendula*, adapted from Arora (2013)

Phytoconstituents	
Carotenoids	Lutein, zeaxanthin, flavoxanthin, auroxanthin, β-carotene, luteoxanthin, violaxanthin, β-cryptoxanthin, mutaxanthin;
Fatty acids	Calendic acid, dimorphecolic acid, palmitic acid, linoleic acid;
Flavonoids	Quercetin, isorhamnetin, kaempferol, rutin, hyperoside, isoquercitrin, astragalin, quercetin 3-O-glucoside, quercetin 3-O-rutinoside, quercetin 3-O-neohesperidose, quercetin 3-O-2G-rhamno-sylrutinoside, isorhamnetin 3-O-glucoside, isorhamnetin 3-O-rutinoside, isorhamnetin 3-O-neohesperidoside, iso-rhamnetin 3-O-2G-rhamnosylrutinoside;
Hydroxycoumarins	Scopoletin, umbelliferone, esculetin;
Ionone glucosides	Officinosides A and B;
Phenolic acids	Chlorogenic acid, caffeic acid, coumaric acid, vanillic acid;
Quinones	α-tocopherol, phylloquinone;
Sesquiterpene glycosides	Arvoside A and B, Officinosides C and D;
Triterpene alcohols	Free and esterified (with fatty acids) monols, diols and triols of ψ-taraxastane-type including ψ-taraxasterol, faradiol, heliantriol B0, heliantriol C, taraxastane-type including taraxasterol, arnidiol, heliantriol B1, lupine-type including lupeol, calenduladiol, heliantriol B2, ursane-type including α-amyrin, brein, ursadiol, ursatriol, oleanane-type including β-amyrin, maniladiol, erythrodiol, longispinogenin, heliantriol A1;
Triterpene glycosides	Calendulaglycoside A, calendulaglycoside A 6’-O-methyl ester, calendulaglycoside A 6’-O-n-butyl ester, calendulaglycoside B, calendulaglycoside B 6’-O-n-butyl ester, calendulaglycoside C, calendula glycoside C 6’-O-methyl ester, calendulaglycoside C 6’-O-n-butyl ester, calendulaglycoside D, calendulaglycoside D2, calendulaglycoside F, calendulaglycoside F 6’-O-butyl ester, calendulaglycoside G 6’-O-methyl ester, calendasaponins A-D;
Triterpenoid saponins	Arvensoside A and B, arvensoside C, calenduloside C and D, calendulaoside G and H;
Volatile oils	δ-cadinene, α-cadinol; T-cadinol; α-cadinene, limonene, 1,8-cineol, linalool, linalyl acetate;
Others	Amino acids, alkaloids, tannins, malic acid, salicylic acid, mucilages, sterols, carbohydrates, resin, bitter principle calendin;

7. Species delimitation

'No one definition has satisfied all naturalists; yet every naturalist knows vaguely what he means when he speaks of a species.' – Darwin (1859)

Species is the basic unit of biogeography, ecology, evolution and conservation (Sites and Marshall 2004; De Queiroz 2007). However, identifying and delimiting species has always been a complex effort in taxonomy. The major problems in species delimitation are the definition of the species concepts, the methods for inferring the boundaries and numbers of species (De Queiroz 2007; Wiens 2007). Several definitions proposed, are still under debate. Nevertheless, the methodologies and disciplines used to define species are extremely varied, such as morphological, ecological, reproductive barriers, molecular, among others (Mayden 2002; Sites and Marshall 2004; Duminil and Di Michele 2009). It has been argued that species concepts are not definitions but rather tools/methods or criteria for distinguishing them (Sites and Marshall 2004; Duminil and Di Michele 2009; Tobias *et al.* 2010).

Species concepts

Aproximately 35 different species 'concepts' have been proposed (see. Zachos 2016 and Table 4), but no one, when applied to nature, is free from ambiguities (Mayden 1997, 2002; Hey 2006; Wilkins 2011; Zachos 2016). Furthermore, all species concepts have its strengths and limitations (Mallet 1995; Coyne and Orr 2004; Sites and Marshall 2004) and result in a significantly different number of species recognised.

It is not my intent to review all species concept, but two concepts have been particularly influential and should be remarked: the biological, and the phylogenetic species concepts:

The *biological species concept* (BSC), defined species as '*groups of interbreeding natural populations reproductively (geographical) isolated from other such groups*' Mayr (1963). The BSC, is based on reproductive incompatibility and associated with the theory of allopatric speciation, prevailed for more than a half century. This definition implies that species constitute reproductively isolated communities occupying distinct environments, representing distinct gene pools.

The *phylogenetic species concept* (PSC), and its different forms (diagnosable, monophyletic or genealogical concept), defined species as a '*grouping of living organisms in distinct lineages which share a common ancestor*' (Cracraft 1983). This concept is based on one or more synapomorphic characters that identify a monophyly of a group of individuals. The PSC does not state that gene flow occurs but implies that interbreeding between populations of different species does not occur.

However, these concepts did not consider hybrid speciation. In plants, hybridization and polyploidisation between species occur frequently (Whitney *et al.* 2010), affecting both, genotype and phenotype and may drive speciation. Natural hybridization occurs in around 25% of plant species, although in many families, such as Asteraceae, hybridization rates are greater (Mallet 2005; Whitney *et al.* 2010).

Table 4 – Various species concept and their abbreviations

- Agamospecies concept (ASC)
- Biological Species Concept (BSC)
- Biosemiotic Species Concept
- Biossimilarity
- Cladistic Species Concept (ClSC)
- Cohesion Species Concept (CSC)
- Compilospecies
- Composite Species Concept (CpCS)
- Consolidated Species Concept
- Diferrential fitness
- Ecological Species Concept (EcSC)
- Evolutionary Significant Unit (ESU)
- Evolutionary Species Concept (ESC)
- Genealogical Concordance Concept (GCC)
- General Lineage
- Genetic Species Concept (GSC)
- Genotypic Cluster Definition (GCD)
- Hennigian Species Concept (HSC)
- Internodal Species Concept (ISC)
- Least inclusive taxonomic unit
- Morphological Species Concept (MSC)
- Nondimensional Species Concept (NdSC)
- Nothospecies
- Phenetic Species Concept (PhSC)
- Phylogenetic Species Concepts (PSC):
  - Diagnosable Version (PSC1)
  - Monophyly Version (PSC2)
  - Diagnosable/Monophyly Version (PSC3)
- Phylo-phenetic
- Polythetic Species Concept (PtSC)
- Pragmatic
- Recognition Species Concept (RSC)
- Reproductive Competition Concept (RCC)
- Successional Species Concept (SSC)
- Taxonomic Species Concept (TSC)
- Unified Species Concept

Most species concepts are based on a single character, e.g. phenotype, genotype, ecotype, etc. Modern points of view agree that it is not possible to define a species using only one character, e.g. genotype, without considering the phenotype (De Queiroz 2007; Winker 2009). Subsequently, various attempts to combine a variety of species concepts have been done (Mallet 1995; Coyne and Orr 2004; De Queiroz 2005; De Queiroz 2007; Wiens 2007; Quaedvlieg *et al.* 2014).

Mallet (1995) suggested that species could be defined as '*groups of individuals which have few or no intermediates when in contact*'. This concept demands the existence of discontinuous variation or gaps on morphological or genetic variation between putative species. The most important feature of the genotypic cluster definition is that species can be affected by gene flow, selection and history, rather than being defined by these processes (Mallet 2007). Research that integrates rigorous analysis of genotypic and phenotypic data are clearly an improvement for biodiversity of what a species and subspecies are (Winker 2009).

Recently, De Queiroz (2007) proposed the unified species concept, as an alternative approach to BSC and PSC. This author attempted to separate the concept from the criteria used to delimit species (Sites and Marshall 2004; Hey 2006; Wiens 2007). The unified species concept, defined species as 'separately evolving metapopulation lineages', i.e. an inclusive population made up of connected subpopulations (De Queiroz 2007). According to this author, several kinds of criteria arise at different times during the process of speciation: (1) morphological; (2) phylogenetic (diagnosable, gene flow, monophyly); (3) reproductive; and finally, (4) geographical or ecological.

Subspecies concepts

Subspecies represents a lower basic unit or taxonomic rank of biological organisation. The subspecies concept was proposed to designate phenotypic variations within populations geographically isolated, but reproductively fertile from one another (O'Brien and Mayr 1991; Mallet 2013; Sackett *et al.* 2014; Patten 2015). However, as in the species concept, the criteria and methods adopted to circumscribe a subspecies, was controversial and inconsistent (Sackett *et al.* 2014; Patten 2015). The subspecies concept will depend on which species concept and criteria are used. Under some species concepts, such as PSC based only on molecular characters, it is not possible to distinguish subspecies, because these proprieties will consider *taxa* as full species. However, any subspecies have a set of genes that determine the phenotype of individuals in a particular geographic area (Patten and Unitt 2002).

The usual criterion used to discriminate subspecies is the '75% rule' (Amadon 1949; Patten and Unitt 2002; Patten 2015). According to this criterion, to be a valid subspecies, 75% of a population effectively must lie outside 99% of the range of other populations for a given defining character or set of characters (Figure 10).

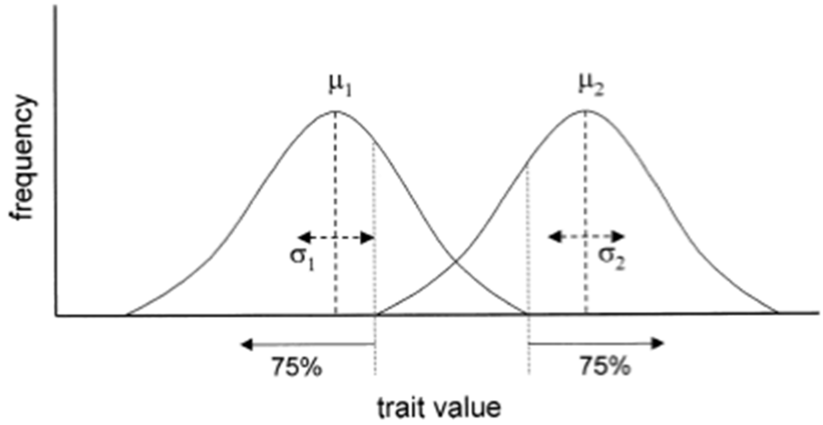


Figure 10 – The seventy-five per cent rule proposed by Amadon (1949)

Runemark (1961) suggested the following criteria to be used for recognition of subspecies: (1) if the gene-exchange between them is restricted on genetic grounds or is limited or made impossible by external means; and (2) if they are separated by a strong hereditary discontinuity in one or several basic morphological characters or a combination of such characters. By using these criteria, morphologically well-distinguishing ecotypes, geographic and biological races, morphologically distinct types (are ± genetically isolated), are regarded as subspecies (Runemark 1961).

Species and subspecies in Calendula

In *Calendula*, it has been difficult to reach a consensus in species and subspecies' delimitation. There is a great polymorphism, due the hybridization and polyploidy, this genus seems to be under active evolution, which brings great difficulties to the process of classification. Moreover, different characters were given taxonomic value depending on the taxonomic treatment (Lanza 1919; Jahandiez and Maire 1934; Heyn *et al.* 1974; Ohle 1974; Ohle 1975a; Ohle 1975b; Meikle 1976).

The species concept used in this thesis will be the Genotypic Cluster or Genomic Cluster Species Concept (GCSC), where genetic (chromosome numbers, ploidy level, and genome size) and morphological clusters will define species boundaries (Mallet 1995). Furthermore, some species of *Calendula* will be separated into several subspecies based on distinct morphologies and geographical distributions, following the 75% rule for quantitative characters.

8. Aims and objectives

The main aim of this present thesis is to contribute to a taxonomic revision of the genus *Calendula* L. in the SW-Mediterranean region.

For that purpose, the specific objectives were established:

- access the morphological variability of the genus, by reviewing the pertinent taxonomical literature and herbarium material, and performing morphometric analysis of qualitative and quantitative characters measured on wild plants and herbarium specimens;
- determine/confirm the chromosome numbers of the different *taxa*;
- increase the number of nuclear DNA content estimations, preferably, including plant material from all over the natural distribution area of the genus;
- improve the knowledge about species' ecological preferences, and distribution;
- check the taxonomic value of the different characters displayed on wild populations, namely by observing their variability in the wild and under cultivation;
- reassess, and redefine, the descriptions and characters used to distinguish *taxa* and the relationships between them.

This thesis is composed of six chapters. The chapter 1 consists of the contextualisation of this study and a brief revision, framing the Mediterranean region, family Asteraceae, subfamily Asteroideae, tribe Calenduleae, genus *Calendula* and species delimitation. The main objectives, as well as the structure are presented in the first chapter.

Besides the introduction and objectives, the thesis is composed by a compilation of scientific papers Chapter 2 to 5 (one published, one submitted and the other two in preparation to be submitted in international peer reviewed scientific journals). These publications expand the data provided in the monographs produced for the Flore Pratique du Maroc (Gonçalves *et al.* 2014) and *Flora iberica* (Silveira & Gonçalves *in press*). The last chapters include the final conclusions and future perspectives. Additional specimens examined are listed in two supplementary material on a CD accompanying the thesis.

9. References

Afonso A, Castro S, Loureiro J, Mota L, Cerca de Oliveira J, Torices R (2014) The effects of achene type and germination time on plant performance in the heterocarpic *Anacyclus clavatus* (Asteraceae). Am J Bot 101:892–898. doi: 10.3732/ajb.1400030

Ahmad K, Khan MA, Shaheen N (2010) Palyonological studies of the semi-desert plant species from Pakistan. African J Biotechnol 9:3527–3535

Alavi SA (1983) Asteraceae. *Calendula* L. In: Jafri S, El-Gadi A (eds) Flora of Libya. Al Faateh University, Dept. of Botany, Tripoli, pp 193–198

Alvarenga SA V., Ferreira MJP, Rodrigues GV., Emerenciano V (2005) A general survey and some taxonomic implications of diterpenes in the Asteraceae. *Bot J Linn Soc* 147:291–308. doi: 10.1111/j.1095-8339.2005.00357.x

Amadon D (1949) The seventy-five per cent rule for subspecies. Condor 51:250–258. doi: 10.2307/1364805

Anderberg AA, Baldwin BG, Bayer RG, Breitwieser J, Jeffrey C, Dillon MO, Eldenäs P, Funk VA, Garcia-Jacas N, Hind DJN, Karis PO, Lack HW, Nesom G, Nordenstam B, Oberprieler C, Panero JL, Puttock C, Robinson H, Stuessy TF, Susanna A, Urtubey E, Vogt R, Ward J, Watson LE (2007) Compositae. In: Kadereit JW, Jeffrey C (eds) Flowering Plants * Eudicots. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 61–588

Antunes P, Coutinho AXP, Silveira P (2007) Palinologia do género *Calendula* na região Mediterrânica Ocidental. Internship Report. Department of Biology. University of Aveiro.

Aparicio A (1989) Números cromosomáticos de plantas occidentales: 487-507. An del Jardín Botánico Madrid 45:483–494

Applequist WL (2014) Report of the nomenclature committee for vascular plants: 66. Taxon 63:1358–1371. doi: 10.12705/636.20

Arcet J, Cuvier G, Daubenton G, Lemonnier L, L'Heritier C (1802) Recueil des éloges historiques prononcés à l'Institut national. Baudouin, Paris (France). doi: 10.5962/bhl.title.1973

Arora D, Rani A, Sharma A (2013) A review on phytochemistry and ethnopharmacological aspects of genus *Calendula*. Pharmacogn Rev 7:179. doi: 10.4103/0973-7847.120520

Baillon H-E (1882) Histoire des plantes. Monographie des Composées. Librairie de Hachette et C.ie, Paris (France)

Ball J (1878) Spicilegium florae Maroccanae. Taylor and Francis, London (United Kingdom)

Ball J (1873) Description of some new species, subspecies and varieties of plants collected in Morocco by J. D. Hooker, G. Maw, and J. Ball. J Bot 11:364–374

Baltisberger M, Widmer A (2006) Chromosome numbers of plant species from the Canary Islands. Bot Helv 116:9–30. doi: 10.1007/s00035-006-0739-x

Barclay AS, Earle FR (1965) The search for new industrial crops. V. The South African Calenduleae (Compositae) as a source of new oil seeds. Econ Bot 19:33–43

Barker NP, Howis S, Nordenstam B, Källersjö M, Eldenäs P, Griffioen C, Linder HP (2009) Nuclear and chloroplast DNA-based phylogenies of *Chrysanthemoides* Tourn. ex Medik. (Calenduleae; Asteraceae) reveal extensive incongruence and generic paraphyly, but support the recognition of infraspecific taxa in *C. monilifera*. South African J Bot 75:560–572. doi: 10.1016/j.sajb.2009.05.006

Barreda V, Palazzesi L, Tellería MC (2008) Fossil pollen grains of Asteraceae from the Miocene of Patagonia: Nassauviinae affinity. Rev Palaeobot Palynol 151:51–58. doi: 10.1016/j.revpalbo.2008.02.002

Barreda V, Palazzesi L, Tellería MC, et al (2010a) Fossil pollen indicates an explosive radiation of basal Asteracean lineages and allied families during Oligocene and Miocene times in the Southern Hemisphere. Rev Palaeobot Palynol 160:102–110. doi: 10.1016/j.revpalbo.2010.02.004

Barreda V, Palazzesi L, Tellería MC, et al (2010b) Eocene Patagonia fossils of the daisy family. Science (80-) 329:1621–1621. doi: 10.1126/science.1193108

Battandier J-A (1921) De l'espèce dans le genre *Calendula*. Bull de la Soc Bot Fr 68:527–531. doi: 10.1080/00378941.1921.10836231

Battandier J-A (1910) Flore de l'Algérie. Supplement aux phanérogames. Librairie des

Sciences Naturelles, Paris (France)

Battandier J-A (1919) Contribution à la Flore atlantique. Bull la Société Bot Fr 56:51. doi: 10.1080/00378941.1909.10832130

Battandier J-A, Trabut L-C (1890) Flore de l'Algérie [...] Dicotylédones [...] (1888-1890). Libraire F. Savy, Paris (France)

Battandier J-A, Trabut L-C (1902) Flore analytique et synoptique de l'Algérie et de la Tunisie. Giralt, Alger (Algeria)

Battandier J-A, Trabut L-C (1888) Flore de l'Algérie [...] Dicotylédones. Typographie Adolphe Jourdan, Alger (Algeria)

Beaulieu JM, Moles AT, Leitch IJ, et al (2006) Correlated evolution of genome size and seed mass. New Phytol 173:422–437. doi: 10.1111/j.1469-8137.2006.01919.x

Bennett MD (1972) Nuclear DNA content and minimum generation time in herbaceous plants. Proc R Soc London B Biol Sci 181: doi: 10.1098/rspb.1972.0042

Bennett MD, Leitch IJ (2011) Nuclear DNA amounts in angiosperms: Targets, trends and tomorrow. Ann Bot 107:467–590. doi: 10.1093/aob/mcq258

Bennetzen JL, Ma J, Devos KM (2005) Mechanisms of recent genome size variation in flowering plants. Ann Bot 95:127–132. doi: 10.1093/aob/mci008

Bentham G (1873) Notes on the classification, history and geographical distribution of Compositae. J Linn Soc London, Bot 13:335–577. doi: 10.1111/j.1095-8339.1873.tb02575.x

Bentham G, Hooker JD (1873) IX. Tribu Calenduleae. In: Genera plantarum: ad exemplaria imprimis in Herberiis Kewensibus servata definita. A. Black, London (United Kingdom), pp 453–456

Blackmore S, Wortley AH, Skvarla JJ, et al (2010) Developmental origins of structural diversity in pollen walls of Compositae. Plant Syst Evol 284:17–32. doi: 10.1007/s00606-009-0232-2

Blanca G, Cabezudo B, Cueto M, et al (2011) Flora Vascular de Andalucía Oriental, 2nd edition. Consejería de Medio Ambiente (Junta de Andalucía), Sevilla (Spain)

Bohm BA, Stuessy TF (2001) Flavonoids of the sunflower family (Asteraceae). Springer, Vienna (Austria)

Boissier P-E (1849) Diagnoses plantarum Orientalium novarum. Series secunda. Ramboz et Schuchardt, Geneve (Switzerland)

Boissier P-E (1882) Flora orientalis sive enumeration plantarum in Oriente a Graecia et Aegypto ad Indiae hucus que observatarum 5(1). H. Georg, Basel, Geneve, 428 pp.

Boissier P-E (1859) Diagnoses plantarum orientalium novarum. Series secunda. Ramboz et Schuchardt, Lipsiae & Parisiis, 146 pp.

Boissier P-E (1839) Voyage botanique dans le midi de l'Espagne. Gide Cie, Paris (France)

Boissier P-E, Reuter G-F (1852) Pugillus plantarum novarum Africae borealis Hispaniaeque Australis. Ex-typographia Ferd. Ramboz et socii, Geneve

Bonifacino JM, Robinson H, Funk VA, et al (2009) A history of research in Compositae: early beginnings to the Reading Meeting (1975). In: Funk, Susanna, Stuessy & Bayer (ed.s) Systematics, Evolution, and Biogeography of Compositae. IAPT, Vienna, pp 3–38

Bonnet E, Barratte J-F-G (1896) Exploration scientifique de la Tunisie. Catalogue raisonné des plantes vasculaires de la Tunisie. Imprimerie nationale, Paris

Bougoutaia Y, Garcia S, Garnatje T, et al (2016) Genome size, chromosome number, and rDNA organisation in Algerian populations of Artemisia herba-alba (Asteraceae), a basic plant for animal feeding facing overgrazing erosion. Ann del Jardín Botánico Madrid 73:1–8

Brandt W, Gürke M, Köhler FE, et al (1890) Köhler's Medizinal-Pflanzen in naturgetreuen Abbildungen mit kurz erläuterndem [...]. Fr. Eugen Köhler, Gera-Untermhaus :

Bremer K (1994) Asteraceae: Cladistics and Classification. Timber Press, Oregon

Bremer K (1987) Tribal interrelationships of the Asteraceae. Cladistics 3:210–253. doi: 10.1111/j.1096-0031.1987.tb00509.x

Bremer K, Jansen RK, Karis PO, et al (1992) A review of the phylogeny and classification of the Asteraceae. Nord J Bot 12: 141–148. doi: 10.1111/j.1756-1051.1992.tb01281.x

Brotero FA (1804) Flora Lusitanica, seu plantarum, quae in Lusitania vel sponte crescunt, vel frequentius coluntur, ex florum praesertim sexubus systematice distributarum, synopsis 1. Typographia Regia, Olisipone [Lisbon], 607 pp.

Brummitt RK (2008) Proposal to add the 1754-1756 german translation of S. Vaillant, établissement de nouveaux caractères de trois familles, 1719-1725, to the “Opera Utique Oppressa.” Taxon 57:663. doi: 10.2307/25066051

Burdet H, Charpin A, Jacquemoud F (1983) Types nomenclaturaux des taxa ibériques décrits par Boissier ou Reuter. IV. Cistacées à Composées. Candollea 38:751–802

Bureš P, Wang Y-F, Horová L, Suda J (2004) Genome size variation in central European species of *Cirsium* (Compositae) and their natural hybrids. Ann Bot 94:353–363. doi: 10.1093/aob/mch151

Calabria L, Emerenciano V, Ferreira MJP, et al (2007) A phylogenetic analysis of tribes of the Asteraceae based on phytochemical data. Nat Prod Commun 2:277–285

Camacho JPM, Sharbel TF, Beukeboom LW (2000) B-chromosome evolution. Philos Trans R Soc B Biol Sci 355:163–178. doi: 10.1098/rstb.2000.0556

Carlquist S (1976) Tribal interrelationships and phylogeny of the Asteraceae. Aliso 8:465–492

Carr GD, King RM, Powell AM, Robinson H (1999) Chromosome numbers in Compositae. XVIII. Am J Bot 86:1003. doi: 10.2307/2656618

Cassini AHG (1816a) Troisième mémoire sur les Synanthérées. J Phys Chim d’Histoire Nat des Arts 82:116–146

Cassini AHG (1816b) De l’influence que l’avortement des étamines paroît avoir sur les Périanthes. J Phys Chim d’Histoire Nat des Arts 82:335–342

Cassini AHG (1817) Quatrième mémoire sur la famille des Synanthérées, contenant l’analyse de l’ovaire et de ses accessoires. J Phys Chim d’Histoire Nat des Arts 85:5–21

Cassini AHG (1819) Sixième mémoire sur la famille des Synanthérées, contenant les caractères des tribus. J Phys Chim d’Histoire Nat des Arts 88:150–163

Cassini H (1834) Opuscules Phytologiques, vol. 3. Levrault, Paris

Castro S, Loureiro J, Procházka T, Münzbergová Z (2012) Cytotype distribution at a diploidhexaploid contact zone in *Aster amellus* (Asteraceae). Ann Bot 110:1047–1055. doi: 10.1093/aob/mcs177

Castroviejo S (1986 - ongoing) Flora Iberica 1-8, 10-15, 17-18, 21. Real Jardín Botánico, CSIC, Madrid

Cavanilles AJ (1791) Icones et descriptiones plantarum [...]. Ex Regia Typographia (Eius operas dirigente Lazaro Gayguer), Madrid (Spain)

Čertner M, Fenclová E, Kúr P, et al (2017) Evolutionary dynamics of mixed-ploidy populations in an annual herb: dispersal, local persistence and recurrent origins of polyploids. Ann Bot 120:303–315. doi: 10.1093/aob/mcx032

Cosson E (1882) Répertoire alphabétique des principales localités mentionnées dans le Compendium et le Conspectus floræ atlanticæ, avec deux cartes botaniques de l'Algérie et des notices sur ces cartes. G. Masson, Paris (France)

Cosson ME (1856) Itinéraire d'un voyage botanique en Algérie, Entrepris en 1856 sous le Patronage du Ministère de la Guerre. Bull la Société Bot Fr 3:697–703. doi: 10.1080/00378941.1856.10839633

Cosson ME, Kralik L (1857) Notes sur quelques plantes rares ou nouvelles de la régence de Tunis. Bull de la Soc Bot Fr 4: 55–63. doi: 10.1080/00378941.1857.10826163

Coutinho A.X.P. (1913) A Flora de Portugal (Plantas Vasculares) disposta em chaves dichotomicas. Aillaud, Alves, Paris.

Coyne JA, Orr HA (2004) Speciation. Sinauer Associates, Sunderland

Cracraft J (1983) Species Concepts and Speciation Analysis. In: Current Ornithology. Springer US, Boston, pp 159–187

Cronquist A (1981) An integrated system of classification of flowering plants. Columbia University Press, New York

Cruz-Mazo G, Buide ML, Samuel R, Narbona E (2009) Molecular phylogeny of *Scorzoneroides* (Asteraceae): Evolution of heterocarpy and annual habit in unpredictable environments. Mol Phylogenet Evol 53:835–847. doi: 10.1016/j.ympev.2009.08.001

Darlington CD, Wylie AP (1955) Chromosome atlas of flowering plants. Chromosome atlas of flower plants 308–317: 308–501

Darwin C (1859) On the origin of species by means of natural selection, or, The preservation of favoured races in the struggle for life. John Murray, London (United Kingdom)

Darwin C, Hooker JD, Jackson BD (1893) Index Kewensis plantarum phanerogamarum: nomina et synonym omnium generum et specierum [...]. 1:383

De Candolle A-L-P-P (1838) Prodromus systematis naturalis regni vegetabilis [...]. Sumptibus Sociorum Treuttel et Würtz, Paris (France)

De Queiroz K (2007) Species Concepts and Species Delimitation. Syst Biol 56:879–886. doi: 10.1080/10635150701701083

De Queiroz K (2005) Ernst Mayr and the modern concept of species. Proc Natl Acad Sci 102:6600–6607. doi: 10.1073/pnas.0502030102

De Storme N, Mason A (2014) Plant speciation through chromosome instability and ploidy change: Cellular mechanisms, molecular factors and evolutionary relevance. Curr Plant Biol 1:10–33. doi: 10.1016/j.cpb.2014.09.002

Desfontaines RL (1799) Flora atlantica: sive historia plantarum quae in Atlante, agro tunetano et algeriensi crescunt. L.G. Desgranges, Paris (France)

Devesa J, Quintanar A (2015) Compositae. In: S. Castroviejo (Gen. Coord.) Flora Iberica XVI (1). CSIC, Madrid, pp 3–5

Diaz Linfante Z, Luque T, Santa Barbara C (1992) Chromosome numbers of plants collected during Iter Mediterraneum II in Israel. Bocconea 3:229–450

Dobignard, A, Chatelain, C (2011). Index synonymique de la flore d'Afrique du Nord, vol. 3: 449. Éditions des Conservatoire et Jardin botaniques de la Ville de Genève.

Doležel J, Bartos J, Voglmayr H, Greilhuber J (2003) Nuclear DNA content and genome size of trout and human. Cytometry 51A:127–128. doi: 10.1002/cyto.a.10013

Doležel J, Greilhuber J, Lucretti S, et al (1998) Plant Genome Size Estimation by Flow Cytometry: Inter-laboratory Comparison. Ann Bot 82:17–26. doi: 10.1093/oxfordjournals.aob.a010312

Dulf F V., Pamfil D, Baciu AD, Pintea A (2013) Fatty acid composition of lipids in pot marigold (*Calendula officinalis* L.) seed genotypes. Chem Cent J 7:8. doi: 10.1186/1752-153X-7-8

Duminil J, Di Michele M (2009) Plant species delimitation: A comparison of morphological and molecular markers. Plant Biosyst - An Int J Deal with all Asp Plant Biol 143:528–542. doi: 10.1080/11263500902722964

Earle FR, Mikolajczak KL, Wolff IA, Barclay AS (1964) Search for new industrial oils. X. Seed oils of the Calenduleae. J Am Oil Chem Soc 41:345–347. doi: 10.1007/BF02654810

Ehrendorfer F (1970) Mediterran-mitteleuropäische Florenbeziehungen im Lichte cytotaxonomischer Befunde. Feddes Repert 81:3–32. doi: 10.1002/fedr.19700810103

El-Garf IIA, Osman AK (2007) Palynological studies on Egyptian species of subfamily Asteroideae- Compositae (tribes Astereae, Calenduleae and Eupatorieae). Feddes Repert 118:192–205. doi: 10.1002/fedr.200711133

Fedorov AA (1969) Chromosome numbers of flowering plants. Acad. Sci. of the U. S. S. R., Leningard

Fennane M, Ibn Tattou M (1998) Catalogue del plantes vasculaires rares, menacées ou endémiques du Maroc. Bocconea 8:23–24

Fennane M, Ibn Tattou M (2005) Flore vasculaire du Maroc: inventaire et chorologie. Asteraceae et Monocotyledones. Université Mohammed V, Rabat

Fernandes A, Queirós M (1971) Contribution à la connaissance cytotaxonomique des spermatophytes du Portugal II. Bol de la Soc Broteriana 45:5–121

Font Quer P (1936) L'Alzina (Quercus ilex) en el limit meridionl de la seva area. Memories L'Academia Ciencies I Arts Barcelona XXV:779–798

Forsskal P, Vahl M (1791) Symbolae botanicae, sive plantarum, tam earum, quas in itinere, imprimis orientali. imprensis auctoris, excudebant N. Möller et filius, Copenhagen

Franco J. do A. (1998). Nova flora de Portugal (continente e Açores). Sociedade Astória (Portugal)

Funk VA, Anderberg AA, Baldwin BG, et al (2009a) Compositae metatrees: the next generation. In: Funk, Susanna, Stuessy & Bayer (ed.s) *Systematics, evolution, and biogeography of Compositae*. IAPT, Vienna, pp 747–777

Funk VA, Bayer RJ, Keeley SC, et al (2005) Everywhere but Antarctica: Using a supertree to understand the diversity and distribution of the Compositae

Funk VA, Susanna A, Stuessy TF, Robinson H (2009b) Classification of Compositae. In: Funk, Susanna, Stuessy & Bayer (ed.s) *Systematics, Evolution, and Biogeography of Compositae*. IAPT, Vienna, pp 171–189

Gallego MJ, Talavera S (1983) Nota sobre las especies Españolas del genero *Calendula* L. Lagascalia 11:100–104

Garcia S, Garnatje T, Gras A, et al (2013) GSAD: A genome size in the Asteraceae database. Release 2.0. http://www.etnobiofic.cat/gsad_v2/index.php

Garcia S, Sanz M, Garnatje T, et al (2004) Variation of DNA amount in 47 populations of the subtribe Artemisiinae and related taxa (Asteraceae, Anthemideae): karyological, ecological, and systematic implications. Genome 47:1004–1014. doi: 10.1139/g04-061

Gardocki ME, Zablocki H, El-Keblawy A, Freeman DC (2000) Heterocarpy in *Calendula* micrantha (Asteraceae): The effects of competition and availability of water on the performance of offspring from different fruit morphs. Evol Ecol Res 2:701–718

Garnatje T, Canela MÁ, Garcia S, et al (2011) GSAD: A genome size in the Asteraceae database. Cytom Part A 79A:401–404. doi: 10.1002/cyto.a.21056

Gonçalves AC, Ouhammou A, Jury S, Silveira P (2014) *Calendula* L. In: Mohammed Fennane, Mohammed Ibn Tattou JEO (ed.s) Flore Pratique du Maroc. Université Mohammed V, Rabat, pp 270–275

Greilhuber J, Doležel J, Lysák MA, Bennett MD (2005) The origin, evolution and proposed stabilization of the terms “genome size” and “C-value” to describe nuclear DNA contents. Ann Bot 95:255–260. doi: 10.1093/aob/mci019

Greuter W (2008) Damn Vaillant? A reply to Brummitt in Taxon 57: 663. 2008, and some alternative proposals, including adding all Steinwehr’s translations in Königl. Akad. Wiss. Paris Phys. Abh. 5–9. 1754–1760 to the “Opera utique oppressa.” Taxon 57:1015–1016

Greuter W, Aghababian M, Wagenitz G (2005) Vaillant on Compositae: Systematic Concepts and Nomenclatural Impact. Taxon 54:149. doi: 10.2307/25065315

Grover CE, Wendel JF (2010) Recent insights into mechanisms of genome size change in plants. J Bot 2010:1–8. doi: 10.1155/2010/382732

Guadagno M (1922) Nuovo Giornale botanico italiano. Nuova serie. Volume ventinovesimo

Gupta PK (1969) Cytological investigations in some Indian Compositae. Cytologia (Tokyo) 35:429–438. doi: 10.1508/cytologia.34.429

Gussone G (1825) Index seminum anni 1825 quae ab Horto Regio in Boccadifalco pro mutua commutatione exhibentur, Napoli (Italy)

Hansen A (1971) Contribution to the Flora of the Azores (especialy S. Maria and S. Miguel). Ann Soc Broteriana 37:

Harvey WH, Hooker JD (1868) The genera of South African plants. Longman, Green, Reader, & Dyer, London (United Kingdom)

Hey J (2006) On the failure of modern species concepts. Trends Ecol Evol 21:447–450. doi: 10.1016/j.tree.2006.05.011

Heyn CC (1988) Investing adaptations to rare events - a reproductive strategy. Lagascalia 15:29–36

Heyn CC, Dagan O, Nachman B (1974) The annual *Calendula* species: taxonomy and relationships. Isr J Bot 23:1201–1969

Heyn CC, Joel A (1983) Reproductive relationships between annual species of *Calendula*. Plant Syst Evol 143:311–329. doi: 10.1007/BF00986612

Heyn CC, Snir S (1986) Selfing and pollen allocation in some Asteraceae. Proc R Soc Edinburgh, Sect B Biol Sci 89:181–192

Heywood VH (2009) The recent history of Compositae systematics: from daisies to deep achenes, sister groups and matatrees. In: Funk, Susanna, Stuessy & Bayer (ed.s) Systematics, Evolution, and Biogeography of Compositae. IAPT, Vienna, pp 39–44

Heywood VH, Brummitt RK, Culham A, Seberg O (2007) Flowering plant families of the world. Royal Botanic Gardens, Kew

Hobbs CR, Baldwin BG (2013) Asian origin and upslope migration of Hawaiian Artemisia (Compositae-Anthemideae). J Biogeogr 40:442–454. doi: 10.1111/jbi.12046

Hoffmann O (1894) Compositae. In: Engler, Heinrich Gustav Adolf & Prantl KAE (ed.s) Die natürlichen Pflanzenfamilien [...] IV. Teil. 5. Abteilung. Leipzig, pp 87–387

Humphries CJ, Murray BG, Bocquet G, Vasudevan K (1978) Chromosome Numbers of Phanerogams from Morocco and Algeria. Bot Not 131:391–406

Imbert E (2002) Ecological consequences and ontogeny of seed heteromorphism. Perspect Plant Ecol Evol Syst 5:13–36. doi: 10.1078/1433-8319-00021

Imbert E (2001) Capitulum characters in a seed heteromorphic plant, Crepis sancta (Asteraceae): variance partitioning and inference for the evolution of dispersal rate. Heredity (Edinb) 86:78–86. doi: 10.1046/j.1365-2540.2001.00812.x

Imbert E, Escarré J, Lepart J (1996) Achene Dimorphism and Among-Population Variation in Crepis sancta (Asteraceae). Int J Plant Sci 157:309–315. doi: 10.1086/297350

IUCN (2012) The IUCN Red List Categories and Criteria. In: IUCN. http://www.iucnredlist.org/

Jahandiez É, Maire R-C (1934) Catalogue des plantes du Maroc. Dicotylédones et Supplément aux volumes I & II. Imprimerie Minerva. Alger (Algeria)

Jahandiez É, Maire R-C (1941) Catalogue des plantes du Maroc. Supplément aux volumes I, II et III. Imprimerie Minerva, Alger (Algeria)

Jeffrey C (2007) Compositae: introduction with key to tribes. In: Bremer K (ed) The Families and Genera of Vascular Plants. Springer, Berlin, pp 61–87

Kadereit JW, Jeffrey C (2007) The Families and Genera of Vascular Plants. Springer, Berlin

Katinas L, Crisci J V., Hoch P, et al (2013) Trans-oceanic dispersal and evolution of early composites (Asteraceae). Perspect Plant Ecol Evol Syst 15:269–280. doi: 10.1016/j.ppees.2013.07.003

Knight CA, Ackerly DD (2002) Variation in nuclear DNA content across environmental gradients: a quantile regression analysis. Ecol Lett 5:66–76. doi: DOI

10.1046/j.1461-0248.2002.00283.x

Knight CA, Beaulieu JM (2008) Genome size scaling through phenotype space. Annals of Botany 101(6): 759–766.

Knight CA, Molinari N, Petrov D (2005) The Large Genome Constraint Hypothesis: Evolution, Ecology and Phenotype. Ann Bot 95:177–190. doi: 10.1093/aob/mci011

Knoche HW (1921) Flora balearica, étude phytogéographique sur les íles Baléares. Imp. Roumégous et Déhan, Montpellier (France)

Krahulcová A, Trávníček P, Krahulec F, Rejmánek M (2017) Small genomes and large seeds: chromosome numbers, genome size and seed mass in diploid *Aesculus* species (Sapindaceae). Ann Bot 119: 957-964. doi: 10.1093/aob/mcw261

Lanza D (1919) Monografia del genero *Calendula.* Atti R. Acad. Sci., Palermo

Lavagnini A, Sempreviva AM, Transerici C, et al (2006) Offshore wind climatology over the Mediterranean basin. Wind Energy 9:251–266

Le Floc'h E, Boulos L (2008) Flore de Tunisie : Catalogue Synonymique Commenté. Montpellier (France)

Leitch IJ, Bennett MD (2007) Genome Size and its Uses: The Impact of Flow Cytometry. In: Flow Cytometry with Plant Cells: Analysis of Genes, Chromosomes and Genomes. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, pp 153–176

Lessing CF (1832) Synopsis generum compositarum earumque dispositionis novae tentamen, monographis multarum Capensium interjectis. Sumtibus Dunckeri et Humblotii, Berlin

Levin DA (1975) Minority cytotype exclusion in local plant populations. Taxon 24:35–43

Levin DA (2004) The Role of Chromosomal Change in Plant Evolution by Donald A. Levin. Syst Bot 29:460–461. doi: 10.1600/036364404774195656

Lindenmayer DB, Wood J, MacGregor C, Buckley YM, Dexter N, Fortescue M, et al. (2015) A Long-Term Experimental Case Study of the Ecological Effectiveness and Cost Effectiveness of Invasive Plant Management in Achieving Conservation Goals: Bitou Bush Control in Booderee National Park in Eastern Australia. PLoS ONE

10(6): e0128482. https://doi.org/10.1371/journal.pone.0128482

Linnaeus C von (1753) Species plantarum [...] Tomus II, 1th edition, 1st edn. Impensis Laurentii Salvii, Stockholm

Linnaeus C von (1763) Species plantarum [...] Tomus II, 2nd edition, 2nd edn. Impensis Laurentii Salvii, Stockholm

Linnaeus C von (1781) Supplementum plantarum. Systematis vegetabilium editionis decimae tertiae, Generum plantarum editionis sextae, et Specierum plantarum editionis secunda. Impensis Orphanotrophei, Brunsvigae [Braunschweig]

Linné C von (1753) Caroli Linnaei ... Species plantarum :exhibentes plantas rite cognitas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas... Impensis Laurentii Salvii, Holmiae :

Loureiro J, Rodriguez E, Doležel J, Santos C (2007) Two New Nuclear Isolation Buffers for Plant DNA Flow Cytometry: A Test with 37 Species. Ann Bot 100:875–888. doi: 10.1093/aob/mcm152

Löve Á (1979) Chromosome Number Reports LXIII. Taxon 28:265–279

Löve Á (1988) Chromosome Number Reports XCIX. Taxon 37:396–399

Lowe RT (1857) A manual flora of Madeira and the adjacent Islands of Porto Santo and the Dezertas. London

Maire R-C (1928a) Contributions à l'étude de la flore de l'Afrique du Nord - 12 (1). Bull la Société d'histoire Nat l'Afrique du Nord 19:56–57

Maire R-C (1928b) Contributions à l'étude de la flore de l'Afrique du Nord - 8. Bull la Société des Sci Nat du Maroc 8:138–139

Maire R-C (1938) Contributions à l'étude de la Flore de l'Afrique du Nord - 26. Bull la Société d'histoire Nat l'Afrique du Nord 29:424

Mallet J (1995) A species definition for the modern synthesis. Trends Ecol Evol 10:294–299. doi: 10.1016/0169-5347(95)90031-4

Mallet J (2005) Hybridization as an invasion of the genome. Trends Ecol Evol 20:229–237. doi: 10.1016/j.tree.2005.02.010

Mallet J (2007) Hybrid speciation. Nature 446:279–283. doi: 10.1038/nature05706

Mallet J (2013) Subspecies, Semispecies, Superspecies. In: Encyclopedia of Biodiversity. Elsevier, pp 45–48

Manning JC, Goldblatt P (2008) Tripteris calcicola, a new calciphilous species from Western Cape, South Africa. Bothalia 1:85–88

Manning JC, Goldblatt P, Helme NA (2012) Osteospermum norlindhianum and O. nordenstamii, two new species of Osteospermum sect. Trefenestratae (Calenduleae) from the greater Cape floristic region. Bothalia 42:59–66

Maranon T, Grubb PJ (1993) Physiological Basis and Ecological Significance of the Seed Size and Relative Growth Rate Relationship in Mediterranean Annuals. Funct Ecol 7:591. doi: 10.2307/2390136

Marchi P, Capineri R, D'Amato G (1974) Numeri cromosomici per la Flore Italiana. Inf Bot Ital 6:303–319

Marques I, Loureiro J, Draper D, et al (2017) How much do we know about the frequency of hybridization and polyploidy in the Mediterranean region? Plant Biol. doi: 10.1111/plb.12639

Mayden RL (2002) On biological species, species concepts and individuation in the natural world. Fish Fish 3:171–196. doi: 10.1046/j.1467-2979.2002.00086.x

Mayden RL (1997) A hierarchy of species concepts: the denoument in the saga of the species problem. In: Claridge MF, Dawah HA, Wilson MR (eds) Species: the units of diversity. Chapman and Hall, Netherlands, pp 381–423

Mayr E (1963) Animal species and evolution. Belknap Press of Harvard University, Cambridge

McNeill J, International Association for Plant Taxonomy., International Botanical Congress (18th : 2011 : Melbourne V. (2012) International code of nomenclature for algae, fungi and plants (Melbourne code) : adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011. Koeltz Scientific Books

Mehra PNN, Remanandan P (1976) Cytological Investigations on Indian Compositae. V. Tribes: Artotideae, Cynareae, Calenduleae and Mutiseae. Nucl 19:8–12

Meikle RD (1976a) *Calendula* L. In: Flora Europaea: Notulae systematicae ad floram

Europaeam spectantes. pp 319–320

Meikle, R.D. (1976) *Calendula* L. In: Tutin T. G., Heywood V. H., Burges N. A., Valentine D. H., Walters S. M., and Webb D. A. (Eds.), Flora Europaea Notulae Systematicae ad Floram Europaeam Spectantes. Cambridge University Press, Cambridge, pp. 206–207.

Meo AA, Khan MA (2006) Palynological observations on the genus *Calendula* (Calenduleae-Compositae) from Pakistan. Pakistan J Bot 38:511–520

Merino y Román B (1906) Flora descriptiva e ilustrada de Galicia. Santiago : Tipografía Galaica, Hórreo, 1, Santiago de Compostela (España)

Meusel H, Ohle H (1966) Zur Taxonomie und Cytologie der Gattung *Calendula*. Österreichische Bot Zeitschrift 113:191–210. doi: 10.1007/BF01441032

Muley BP, Khadabadi SS, Banarase NB (2009) Phytochemical Constituents and Pharmacological Activities of *Calendula* officinalis L. (Asteraceae): A Review. Trop J Pharm Res 8:455–465. doi: 10.4314/tjpr.v8i5.48090

Muñoz-Pajares AJ, Perfectti F, Loureiro J, et al (2017) Niche differences may explain the geographic distribution of cytotypes in Erysimum mediohispanicum. Plant Biol (Stuttg) 20 Suppl 1:139-147. doi: 10.1111/plb.12605.

Murbeck S (1923) Contributions à la connaissance de la flore du Maroc. Géraniacées-Composées. C. W. K. Gleerup, Lund (Sweden)

Myers N, Mittermeier RA, Mittermeier CG, et al (2000) Biodiversity hotspots for conservation priorities. Nature 403:853–858. doi: 10.1038/35002501

Negodi G (1937) Caratteri nucleari e morfologici in alcune Calenduleae. (Compositae-Tubuliflorae). Ann Bot 21:510–518

Nègre R (1958) A propos de quelques *Calendula* marocains. Bull la Société des Sci Nat Phys du Maroc XXXVIII:1–7

Nègre R (1961) Petite flore des régions arides du Maroc occidental. Centre national de la recherche scientifique, 982 pp.

Noor MJ, Ahmad M, Asghar R, et al (2004) Palynological Studies of Cultivated Plant Species at University of Arid Agriculture, Rawalpindi, Pakistan. Asian J. Plant Sci. 3:476–479

Nora SV, Castro S, Loureiro J, et al (2013) Flow cytometric and karyological analyses of *Calendula* species from Iberian Peninsula. Plant Syst Evol 299:853–864. doi: 10.1007/s00606-013-0767-0

Nordenstam B (2007) Calenduleae. In: Bremer K (ed) The Families and Genera of Vascular Plants. Springer, Berlin, pp 241–245

Nordenstam B (1994a) Tribe Calenduleae. In: Bremer K (ed) Asteraceae, Cladistics & Classification. Timber Press Portland, Oregon, pp 365–376

Nordenstam B (1994b) New combinations in the Calenduleae. Compos Newsl 25:46–49

Nordenstam B (1996) Recent revision of Senecioneae and Calenduleae systematics. In: Hind DJN, Beentje HJ (eds) Compositae: Systematics. Proceedings of the International Compositae Conference. Royal Botanic Gardens, Kew, pp 591–596

Nordenstam B (2006) Generic revisions in the tribe Calenduleae (Compositae). Compos Newsl 44:38–49

Nordenstam B (1994c) Calenduleae. In: Bremer K (ed) Asteraceae: Cladistics & Classification. Timber Press, Portland, pp 365–376

Nordenstam B, Funk VA (2009) Corymbieae. In: Funk, Susanna, Stuessy & Bayer (ed.s) Systematics, Evolution, and Biogeography of Compositae. IAPT, Vienna

Nordenstam B, Källersjö M (2009) Calenduleae. In: Funk, Susanna, Stuessy & Bayer (ed.s) Systematics, Evolution, and Biogeography of Compositae. IAPT, Vienna, pp 527–538

Norlindh T (1946) Studies in the Calenduleae. II. Phytogeography and interrelation. Bot Not 1946:471–506

Norlindh T (1977a) Calenduleae - Systematic review. In: Heywood VH, Harborne JB, Turner BL (eds) The Biology and Chemistry of the Compositae. Academic Press, London (United Kingdom), pp 961–987

Norlindh T (1943) Studies in the *Calendula*deae. I. Monograph of the genera Dimorphotheca, Castalis, Osteospermum, Gibbaria and Chrysanthemoides. C. W. K. Gleerup, Lund

Norlindh T (1960) Additions to the monograph on Osteosperum. Bot Not 113:385–399

Norlindh T (1962) Studies in *Calendula* maderensis DC. With a discussion on the delimitation of *Calendula* L. from Gibbaria Cass. and Osteospermum L. Bot Not 115:437–445

Norlindh T (1963) Chromosome numbers in the Calenduleae I. With discussions on relationships, hypridization and phytogeography. Bot Not 116:193–209

Norlindh T (1977b) Garuleum subgen. Rutidocarpaea, a monotypic subgenus with achene dimporphism. Bot Not 130:377–380

Nyman, C.F. (1878) Conspectus florae Europaeae. Vol. 2. Typis Bohlinianae, Örebro Sueciae, 858 pp.O'Brien S, Mayr E (1991) Bureaucratic mischief: recognizing endangered species and subspecies. Science (80-) 251:1187–1188. doi: 10.1126/science.251.4998.1187

Oberprieler C, Vogt R (1993) Chromosome Numbers of North African Phanerogams. II. Willdenowia 23:211–238

Ohle H (1974) Beiträge zur Taxonomie der Gattung *Calendula* II. Taxonomische Revision der südeuropäischen perennierenden *Calendula*-Sippen. Feddes Repert 85:245–283. doi: 10.1002/fedr.19740850402

Ohle H (1975a) Beiträge zur Taxonomie und Evolution der Gattung *Calendula* L. III. Revision der marokkanischen perennierenden Sippen unter Berücksichtigung einiger marokkanischer Annueller. Feddes Repert 86:1–17. doi: 10.1002/fedr.19750860102

Ohle H (1975b) Beiträge zur Taxonomie und Evolution der Gattung *Calendula* L. IV. Revision der algerisch-tunesischen perennierenden *Calendula*-Sippen unter Berücksichtigung einiger marokkanisch-algerischer Annueller und der marokkanischen und südeuropäischen perennierende. Feddes Repert 86:525–541. doi: 10.1002/fedr.19750860908

Ohri D (2005) Climate and growth form: The consequences for genome size in plants. Plant Biol 7:449–458. doi: 10.1055/s-2005-865878

Orueta D (2002) Thermal relationships between *Calendula* arvensis inflorescences and Usia aurata bombyliid flies. Ecology 83:3073–3085. doi: 10.1890/0012-9658(2002)083[3073:TRBCAI]2.0.CO;2

Otto SP (2007) The Evolutionary Consequences of Polyploidy. Cell 131:452–462. doi: 10.1016/j.cell.2007.10.022

Oualidi JEL, Khamar H, Fennane M, Ibn Tattou M, Chauvet S & Taleb MS (2012) Checklist des endémiques et spécimens types de la flore vasculaire de l'Afrique du Nord. Document de l’Institut Scientifique nº25, Universite Mohamed V-Agdal, Rabat.

Paim LFNA, Fontana M, Winckler M, et al (2010) Assessment of plant development, morphology and flavonoid content in different cultivation treatments of *Calendula* officinalis L., Asteraceae. Rev Bras Farmacogn 20:974–980. doi: 10.1590/S0102-695X2010005000050

Palazzesi L, Barreda V, Tellería MC (2010) First fossil record of Calyceraceae (Asterales): Pollen evidence from southern South America. Rev Palaeobot Palynol 158:236–239. doi: 10.1016/j.revpalbo.2009.09.003

Panero JL, Crozier BS (2016) Macroevolutionary dynamics in the early diversification of Asteraceae. Mol Phylogenet Evol 99:116–132. doi: 10.1016/j.ympev.2016.03.007

Panero JL, Funk VA (2002) Toward A Phylogenetic Subfamilial Classification For The Compositae. Proc Biol Soc Washingt 115:760–773

Panero JL, Funk VA (2008) The value of sampling anomalous taxa in phylogenetic studies: Major clades of the Asteraceae revealed. Mol Phylogenet Evol 47:757–782. doi: 10.1016/j.ympev.2008.02.011

Paolini J, Barboni T, Desjobert J-M, et al (2010) Chemical composition, intraspecies variation and seasonal variation in essential oils of *Calendula* arvensis L. Biochem Syst Ecol 38:865–874. doi: 10.1016/j.bse.2010.07.009

Patten MA (2015) Subspecies and the philosophy of science. Auk 132:481–485. doi: 10.1642/AUK-15-1.1

Patten MA, Unitt P (2002) Diagnosability versus mean differences of sage sparrow subspecies. Auk 119:26. doi: 10.1642/0004-8038(2002)119[0026:DVMDOS]2.0.CO;2

Pau y Español C (1924) Plantas del norte de Yebala (Marruecos). Mem de la R Soc Esp de Hist Nat 12: 264 - 401.

Pazy B (2000) Flora Palaestina: chromosome numbers. Isr J Plant Sci 48:7–32

Pelser PB & Watson LE (2009) Introduction to Asteroideae. In: Funk, Susanna, Stuessy & Bayer (ed.s) Systematics, evolution, and biogeography of Compositae. IAPT, Vienna.

Persoon CH (1807) Synopsis plantarum [...] Pars secunda. C.F. Cramerum, Paris (France)

Plume O (2015) Hybridization, genome duplication, and chemical diversification in the evolution of *Calendula* L. (Compositae). PhD thesis. Cornell University, Ithaca, New York

Plume O, Raimondo FM, Troia A (2015) Hybridization and competition between the endangered sea marigold (*Calendula* maritima, Asteraceae) and a more common congener. Plant Biosyst - An Int J Deal with all Asp Plant Biol 149:68–77. doi: 10.1080/11263504.2013.810182

Podlech ED (1986) Chromosomenstudien an Pflanzen des Saharo-sindischen Trockengebietes. Mitt Bot München 22:5–20

Pomel A-N (1875) Nouveaux matériaux pour la flore atlantique, Savy, Paris

Pottier-Alapetite G (1981) Flore de la Tunisie. Angiospermes-Dicotyledones. Ministère de l'Enseignement Supérieur et de la Recherche Scientifique et le Ministère de l'Agriculture. [Tunis]

Praglowski J, Grafström E (1980) The pollen morphology of the tribe *Calendula*e with reference to taxonomy. Bot Not 177–188

Press JR, Short MJ (1994) Flora of Madeira, Natural History Museum (London).

Price HJ, Crawford DJ, Bayer RJ (1984) Nuclear DNA contents of Coreopsis nuecensoides and C. nuecensis (Asteraceae), a progenitor-derivative species pair. Bot Gaz 145:240–245. doi: 10.2307/2474345

Punt W, Hoen PP (2009) The Northwest European Pollen Flora, 70. Rev Palaeobot Palynol 157:22–183. doi: 10.1016/j.revpalbo.2008.12.003

QGIS DT (2017) QGIS Geographic Information System 2.18. http://www.qgis.org/

Quaedvlieg W, Binder M, Groenewald JZ, et al (2014) Introducing the consolidated species concept to resolve species in the teratosphaeriaceae. Persoonia Mol

Phylogeny Evol Fungi 33:1–40. doi: 10.3767/003158514X681981

Quezel P, Santa S (1963) Nouvelle Flore de l'Algérie et des régions désertiques méridionales, CNRS – Paris, 1170 pp.

Raal A, Kirsipuu K (2011) Total flavonoid content in varieties of *Calendula* officinalis L. originating from different countries and cultivated in Estonia. Nat Prod Res 25:658–662. doi: 10.1080/14786419.2010.528417

R Development Core Team R (2010) R: a language and environment for statistical computing. Foundation for Statistical Computing, Vienna, Austria

Rafinesque CS, Costanza B, Costanza L, Vaincher P (1810) Caratteri di alcuni nuovi generi e nuove specie di animali e piante della Sicilia: con varie osservazioni sopra i medesimi. Per le stampe di Sanfilippo, Palermo

Raven PH (1966) Flora of Turkey. Science (80-) 151:319–320

Rouy G (1903) Flore de France ou Description des plantes qui croissent spontanément en France, en Corse et en Alsace-Lorraine. Asnières (Seine)

Ruiz de Clavijo E (2005) The reproductive strategies of the heterocarpic annual *Calendula* arvensis (Asteraceae). Acta Oecologica 28:119–126. doi: 10.1016/j.actao.2005.03.004

Ruiz de Clavijo E (1990) Números cromosomáticos de plantas occidentales, 619-630. An del Jardín Botánico Madrid 47:431–437

Runemark (1961) The species and subspecies concepts in flowering plants. Bot Not 114:22–32

Ruprecht FJ (1856) Index Seminum. ex Bull Phys-Math Acad Petersb 14:231

Ruszkowski D, Szakiel A, Auguciska E, Janiszowska W (2006) Biosynthesis of oleanolic acid glycosides in protoplasts isolated from *Calendula* officinalis L. roots. Acta Physiol Plant 28:217–223. doi: 10.1007/BF02706533

Ryan WBF (2009) Decoding the mediterranean salinity crisis. Sedimentology 56:95–136. doi: 10.1111/j.1365-3091.2008.01031.x

Sackett LC, Seglund A, Guralnick RP, et al (2014) Evidence for two subspecies of Gunnison's prairie dogs (Cynomys gunnisoni), and the general importance of the subspecies concept. Biol Conserv 174:1–11. doi: 10.1016/j.biocon.2014.03.010

Schaefer H (2002) Chorology and Diversity of the Azorean Flora. Part II. University of Regensburg

Schmiderer C, Lukas B, Ruzicka J, Novak J (2015) DNA-Based Identification of *Calendula* officinalis (Asteraceae). Appl Plant Sci 3:1500069. doi: 10.3732/apps.1500069

Sennikov A (2010) (089) Proposal to discard the nomenclatural value of reprints and translations of botanical publications first printed before the relevant nomenclatural starting-point date. Taxon 59:307–308

Sennikov A (2015) (038–039) Two proposals to deal with reprints and translations of publications first printed before the relevant nomenclatural starting-point date, and with recent posthumous publications of pre-Linnaean authors. Taxon 64:392–393. doi: 10.12705/642.13

Silveira P, Gonçalves AC (in press) *Calendula* L. In: S. Castroviejo (G.Coord.) Flora Iberica XVI (2). CSIC, Madrid

Silveira P, Gonçalves AC, Santos C, Paiva J (2013) Two lectotypifications and a new combination in *Calendula* (Asteraceae) for Flora Iberica. Phytotaxa 145:47–53. doi: 10.11646/phytotaxa.145.1.5

Sites JW, Marshall JC (2004) Operational Criteria for Delimiting Species. Annu Rev Ecol Evol Syst 35:199–227. doi: 10.1146/annurev.ecolsys.35.112202.130128

Smith CR, Wilson TL, Melvin EH, Wolff IA (1960) Dimorphecolic Acid—A Unique Hydroxydienoid Fatty Acid 2. J Am Chem Soc 82:1417–1421. doi: 10.1021/ja01491a034

Soltis PS, Marchant DB, Van de Peer Y, et al (2015) Polyploidy and genome evolution in plants. Curr Opin Genet Dev 35:119–125. doi: 10.1016/j.gde.2015.11.003

Soltis PS, Soltis DE (2009) The Role of Hybridization in Plant Speciation. Annu Rev Plant Biol 60:561–588. doi: 10.1146/annurev.arplant.043008.092039

Stix E (1960) Pollenmorphologische Untersuchungen an Compositen. Grana Palynol 2:41–104. doi: 10.1080/00173136009429443

Strother JL (1983) More Chromosome Studies in Compositae. Am J Bot 70:1217. doi:

10.2307/2443291

Swelankomo N (2013) FSA Contribution 22: Asteraceae: Calenduleae: *Garuleum.* Bothalia 43:167–178. doi: 10.4102/abc.v43i2.93

Talavera S, Devesa J, Fernández-Galiano E (1984) Notas cariosistemáticas sobre plantas Norteafricanas. I. Compositae. Candollea 39:271–280

Tanowitz BD, Salopek PF, Mahall BE (1987) Differential Germination of Ray and Disc Achenes in Hemizonia increscens (Asteraceae). Am J Bot 74:303–312. doi: 10.2307/2443806

Thompson JD (2005) Plant Evolution in the Mediterranean. Oxford University Press Inc., New York

Thunberg CP (1800) Prodromus plantarum Capensium [...] Pars prior. J. Edman, Upsalia

Tobias J, Seddon N, Spottiswoode C, et al (2010) Quantitative criteria for species delimitation. Ibis (Lond 1859) 152: 724–746. doi: 10.1111/j.1474-919X.2010.01051.x

Torrell M, Vallès J (2001) Genome size in 21 *Artemisia* L. species (Asteraceae, Anthemideae): Systematic, evolutionary, and ecological implications. Genome 44:231–238. doi: 10.1139/gen-44-2-231

Vaillant S (1720) Fortsetzung der Corymbiferarum, oder der zweyten Classe der Pflanzen mit zusammengesetzeten Blumen. In: Steinwehr WBA von (Ed.) Der Königliche Akademie der Wissenschaften in Paris anatomische, chymische und botanische Abhandlungen, aus dem Französischen übersetzt. Breslau, pp 545–613

Valdés B (2002) Catalogue des plantes vasculaires du Nord du Maroc, incluant des clés d'identification = Checklist of vascular plants of N Morocco with identification keys. Consejo Superior de Investigaciones Científicas, Madrid

Valdés B, Parra R (1997) Numeros cromossomaticos de plantas de Marruecos, I. Lagascalia 20:161–166

Valdés B, Talavera S, Fernández-Galiano E (1987a) Flora Vascular de Andalucía Occidental. Vol. 1. Ketres editora S.A., Barcelona

Valdés B, Talavera S, Fernández-Galiano E (1987b) Flora Vascular de Andalucía Occidental. Vol. 2. Ketres editora S.A., Barcelona

Valdés B, Talavera S, Fernández-Galiano E (1987c) Flora Vascular de Andalucía Occidental. Vol. 3. Ketres editora S.A., Barcelona

Vallès J, Canela MÁ, Garcia S, et al (2013) Genome size variation and evolution in the family Asteraceae. Caryologia 66:221–235. doi: 10.1080/00087114.2013.829690

Van Wyk B-EE (2008) A review of Khoi-San and Cape Dutch medical ethnobotany. J Ethnopharmacol 119:331–341. doi: 10.1016/j.jep.2008.07.021

Venable DL (1985) The Evolutionary Ecology of Seed Heteromorphism. Am Nat 126:577–595. doi: 10.1086/284440

Vogt R, Oberprieler C (2008) Chromosome Numbers of North African Phanerogams. VIII. More counts in Compositae. Willdenowia 38:497–519. doi: 10.3372/wi.38.38210

Vogt R, Oberprieler C (2012) Chromosome Numbers of North African Phanerogams. X. Plants collected during Iter Mediterraneum V of OPTIMA in Morocco. Ann des Naturhistorischen Museums Wien Ser B für Bot und Zool 193–221

Vogt R, Oberprieler C (1993) Chromosome Numbers of North African Phanerogams. I. Flora Mediterr 3:187–210

Wagenitz G (1976) Systematics and phylogeny of the Compositae (Asteraceae). Plant Syst Evol 125:29–46. doi: 10.1007/BF00986129

Weiss-Schneeweiss H, Emadzade K, Jang TS, Schneeweiss GM (2013) Evolutionary consequences, constraints and potential of polyploidy in plants. Cytogenet Genome Res 140:137–150. doi: 10.1159/000351727

Whitney KD, Ahern JR, Campbell LG, et al (2010) Patterns of hybridization in plants. Perspect Plant Ecol Evol Syst 12:175–182. doi: 10.1016/j.ppees.2010.02.002

Wiens JJ (2007) Species Delimitation: New Approaches for Discovering Diversity. Syst Biol 56:875–878. doi: 10.1080/10635150701748506

Wilkins JS (2011) Philosophically speaking, how many species concepts are there? Zootaxa 2765: 58–60

Willdenow CL Von (1803a) Species plantarum [...] Tomus III. Pars III, 4th edn. Impensis G. C. Nauk, Berlin

Willdenow CL Von (1803b) Caroli a Linné species plantarum exhibentes plantas rite cognitas ad genera relatas cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus secundum systema sexuale digestas, 4th editio. Berolini : Impensis G. C. Nauk, be

Willdenow CL Von (1809) Enumeratio plantarum Horti Regii Berolinensis. In Taberna libraria Scholae Realis, Berlin

Willkomm HM, Lange JMC (1865) Prodromus Florae Hispanicae. Stuttgartiae : Sumtibus E. Schweizerbart (E. Koch), Stuttgart (Alemania)

Winker K (2009) Reuniting Phenotype and Genotype in Biodiversity Research. Bioscience 59: 657–665. doi: 10.1525/bio.2009.59.8.7

Wood AR, Nordenstam B, Manning JC (2003) An interesting new species of Osteospermum (Asteraceae–Calenduleae) from the Western Cape Province, South Africa, providing a link to the genus Chrysanthemoides. South African J Bot 69:572–578. doi: 10.1016/S0254-6299(15)30297-0

Zachos FE (2016) Species Concepts in Biology. Springer International Publishing, Cham

Zafar M, Ahmad M, Khan MA (2007) Palynology of Family Asteraceae from Flora of Rawalpindi- Pakistan. Int J Agric Biol 9:156–161

Zahradníček J, Chrtek J (2015) Cytotype distribution and phylogeography of Hieracium intybaceum (Asteraceae). Bot J Linn Soc 179:487–498. doi: 10.1111/boj.12335

Zavada M, de Villiers S (2000) Pollen of the Asteraceae from the Paleocene-Eocene of South Africa. Grana 39:39–45. doi: 10.1080/00173130150503795

Zohary M (1950) Evolutionary Trends in the Fruiting Head of Compositae. Evolution (N Y) 4:103–109. doi: 10.2307/2405387

Zohary M (1941) Taxonomical studies in the Flora of Palestine and neighbouring countries. Palest J Bot Jerus. 1–2:151-184

Chapter 2 - Publication 1

Two lectotypifications and a new combination in *Calendula* (Asteraceae) for Flora Iberica

Two lectotypifications and a new combination in *Calendula* (Asteraceae) for Flora Iberica

PAULO SILVEIRA[1]*, ANA CARLA GONÇALVES[1], CONCEIÇÃO SANTOS[1] & JORGE PAIVA[2]

[1]Departamento de Biologia e CESAM, Universidade de Aveiro, 3810-193, Aveiro, Portugal.

Departamento de Ciências da Vida e Centro de Ecologia Funcional, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, Apartado 3046, 3001-401 Coimbra, Portugal.

**Author for correspondence. E–mail:* psilveira@ua.pt

This chapter has been published as an original paper in:

Phytotaxa: http://dx.doi.org/10.11646/phytotaxa.145.1.5

Abstract

This paper presents a new combination, *Calendula suffruticosa* subsp. *cinerea*, and two lectotypifications, for *Calendula suffruticosa* subsp. *tomentosa* and *Calendula suffruticosa* subsp. *cinerea*, in the course of a taxonomic revision for *Flora Iberica*. Explanations about the taxonomic concept here adopted are also provided.

Key words: lectotypifications, new combination, *Calendula suffruticosa*, *Calendula incana*

Introduction

The genus *Calendula* Linnaeus (1753: 921) (Calenduleae, Asteraceae) includes 10 to 27 species, depending on the taxonomic concept. It is native to the Mediterranean basin and has been considered as a taxonomically difficult genus (Norlindh 1977; Heyn & Joel 1983). So far, no taxonomic revision including the entire genus has been done and the most recent monographic studies have been conducted by Heyn *et al.* (1974) for the annual species and by Ohle (1974, 1975a, b) for the perennial ones.

Mainly on the basis of leaf morphology and life span, the latter author has split the group of wild perennials with 2n= 32 into two species, *Calendula suffruticosa* Vahl (1791: 94) and *C. incana* Willdenow (1803: 2341), both with several subspecies.

However, this reclassification was not consensual and later works (e.g. Meikle 1976), besides not recognizing some of the infraspecific taxa accepted by Ohle (1974), continued to include all the accepted subspecies under *C. suffruticosa*.

In the course of a taxonomic revision for *Flora Iberica* (Silveira & Gonçalves, *in press*), we revised the Iberian representatives of the genus and decided to follow Heyn *et al.* (1974) for the annual plants and a mixed approach for the perennials, i.e. to include most of the taxa recognised by Ohle (1974) but exclusively under *C. suffruticosa*, instead of sorting them between *C. incana* and *C. suffruticosa*.

This option was based on our observation that there are some patterns of morphological variation (especially regarding the achenes) correlated with geographical distribution and ecology that deserve taxonomic recognition. However, there is a gradient of morphological variation (and also of DNA content as demonstrated by Nora *et al.* 2013) between plants treated by Ohle as *C. incana* and its subspecies, towards others treated as *C. suffruticosa* and its subspecies, and we could not find a clear and consistent morphological separation between these two groups. In fact, life span and leaf morphology are sometimes variable within the same taxon or population and dependent upon microsite ecological conditions encountered by the individual plants.

Furthermore, it was not possible to find any closer phylogenetic relationship between the taxa included in any of these groups than with the other using molecular markers (Olofron Plume, personal communication).

When we consider morphological characters alone, the same conclusion can frequently be reached since, for example, achene morphology of *C. suffruticosa* subsp. *lusitanica* (Boissier 1849: 83) Ohle (1974: 270) is much more similar to the one observed in the plants treated by Ohle as *C. incana* subsp. *algarbiensis* Ohle (1974: 274) than in the other subspecies included under *C. suffruticosa*.

While the results of extended and appropriate molecular studies and an overall morphological revision of the genus are not available, we consider that the best option is to include all these plants under *C. suffruticosa* than to sort them between some more, possibly unnatural, groups.

Pursuing with this classification scheme we found out that for its application one typification and a new combination are required. In fact, most of the names that become in use have already been adequately typified, but one of them—*C. suffruticosa* subsp. *tomentosa* (Ball 1878: 517) Murbeck (1905: 9)—has not. Moreover, we agree with Ohle (1974) that the plants bearing a white-floccose tomentum from "Cabo de São Vicente", SW Portugal, are very distinct from the plants that share this same character from the southern Spain and northern Morocco. For this reason, there is a need to transfer *C. incana* subsp. *algarbiensis* var. *cinerea* Ohle (1974: 277) to *C. suffruticosa*.

New typification

Calendula tomentosa was initially described by Desfontaines (1799: 305). In his publication, Desfontaines provided a brief diagnostic description of C. tomentosa and cited its occurrence to be at '*regno Marocano*' fide Broussonet. However, he did not mention any type specimen, as the type concept had not been invented yet.

The specimen of *Calendula tomentosa* held in Desfontaines' Flora Atlantica's herbarium at P (Fig. 1 and 2) includes a label that states "*Tarifa juxta monia Urbis ad mare*" (Fig. 2-B), which seems to suggest that it might not be the type of this name. Nonetheless, the same specimen includes a label comprising the manuscript of the original diagnosis of this taxon (fig. 2-A and C) and is corroborated by similar labels, stating that the collecting locality is Tarifa, in duplicates held at MPU and MAF. Therefore, the original collecting site of this taxon should be correctly attributed to Tarifa Town in Cádiz, Spain and not to Morocco, as originally stated by Desfontaines. This is not surprising, since Broussonet was known to have been somewhat careless in what concerns the original localities whence his specimens came (Ball 1878: 283).

Willdenow (1803: 2341), aware of the previous use of the name '*Calendula tomentosa*' for a taxon from South Africa by Linnaeus filius (1782: 384), proposed the new name *Calendula incana* Willd. for the material of Desfontaines, maintaining the original location and expanding it to "*maritimis Lusitaniae*".

Since Willdenow indicated no type material, Ohle (1974: 272) lectotypified this name as "*Habitat in Barbaria*, leg. Willdenow, Nr. 16691 (B)". However, after careful examination of the specimen 16 691 in Willdenow's herbarium at B and the literature, we concluded that this specimen should, in fact, be a duplicate of the same Broussonet's collection used by Desfontaines to describe *C. tomentosa*, that was distributed to other botanists of the time like Gouan at Montpellier, Cavanilles at Madrid and Willdenow at Berlin (Ball 1878: 283). What it cannot be is that the specimen was collected by

Willdenow, since his own collections are from Berlin, Halle and Venice (Urban 1917: 415).

Later authors, however, accepted the name *C. tomentosa* and placed *C. incana* into its synonymy. This was done e.g. by Willkomm & Lange (1870: 130), who also suggested new localities for the species in the southern Spain ("*in agro Malacitano*" [*supra Campo Santo*], "*Gibraltariae*", "*prov. Gadit.*" [*ad fretum Hercul., pr. Tarifa*]), besides the localities mentioned by previous authors ("*Lusitaniae et Barbaria*").

Ball (1878: 517) proposed to treat *C. tomentosa* as a mere variety of *C. suffruticosa*, including *C. incana* as a synonym, and specified precise localities of the species in the northern Morocco ("*Tetuan in rupibus*" and "*In monte Beni Hosmar*").

Murbeck (1905: 9) was the first to use the epithet '*tomentosa*' at the rank of subspecies and applied it to plants found in the mountains of Bargou, Tunisia. Although he referred to the illegitimate name proposed by Desfontaines, recent authors (Greuter 2006–2009) correctly treated the combination *C. suffruticosa* subsp. *tomentosa* as based on Ball's first legitimate use of the epithet '*tomentosa*' at the rank of variety, but considered it as a synonym of *C. incana.*

The instability continued to affect these names until today, since, for example, Lanza (1919), in his revision of the genus *Calendula*, still accepted *C. tomentosa*, while Ohle (1974), revising the perennial representatives of this genus, correctly considered it as illegitimate and synonymised it with *C. incana.*

We accept these plants back at the rank of subspecies, now under *C. suffruticosa*, and the typification becomes as follows.

Calendula suffruticosa Vahl subsp. ***tomentosa*** (Ball) Murbeck (1905: 9). *Calendula suffruticosa* var. *tomentosa* Ball (1878: 517). *Calendula tomentosa* Desfontaines (1799: 305), nom. illeg., non Linnaeus *filius* (1782: 384). *Calendula incana* Willdenow (1803: 2341). **Type**:—SPAIN. "*Calendula tomentosa*", "*Tarifa juxta monia Urbis ad mare*", s.d. [1795 in MPU!], Broussonet s.n. (lectotype P! barcode no. P00680154, designated here; isolectotypes: MPU! barcode no. MPU022962, MAF! no. 3 613, B-Willd.! barcode no. 16691-01-0) - Figure 11 and 12.

New combination

The perennial representatives of *Calendula* bearing a white-floccose tomentum from "Cabo de São Vicente" (SW Portugal) were usually aggregated with plants presenting the same set of characters from the southern Spain and northern Morocco, mostly under C. tomentosa, *C. incana*, or *C. suffruticosa* subsp. *tomentosa*.

Ohle (1974) was the first to notice that these Portuguese plants are different from those bearing the same kind of indumentum from the southern Spain and northern Africa, and that they show a high affinity with *C. incana* subsp. *algarbiensis*. Based on these observations, Ohle (1974) described a new taxon at the rank of variety, *C. incana* subsp. *algarbiensis* var. *cinerea*.

We recognise the distinctiveness of this taxon and that it shows more affinities with *C. incana* subsp. *algarbiensis* (= *C. suffruticosa* subsp. *algarbiensis*) than with *C. incana* (= *C. suffruticosa* subsp. *tomentosa*). This conclusion is mostly based on the morphology of the achenes, since these plants have shorter outer rostrate achenes [(6–)8.8–15(–19) mm long] than those of the plants from Tarifa [(7–)13–17(–28) mm long], and produce cymbiform achenes which dimensions are (4–)5.5–7(–8.8) × (3–)3.8–5(–6.5) mm, with a ventral wing surpassing the lateral wings, while the Spanish plants, instead of the cymbiform ones, usually have trialate achenes which dimensions are (6.5–)9.2–11.2(14.2) × (4.3–)6.8–8.7(–13) mm. Furthermore, the leaves of the plants from "*Cabo de São Vicente*" are thicker [(0.5–)1.2–2.1(–3.3) mm thick] than in the plants from Tarifa [(0.3)0.5–0.7(–1.1) mm thick], while the latter have a more repand-dentate margin and a denser whitish-floccose tomentum than the former.

Calendula suffruticosa subsp. *algarbiensis*, endemic to the coast of Portugal and Galicia (Spain), also produces cymbiform or sub-cymbiform achenes which dimensions are (4.7–)6.5–7.7(–9.7) × (2.8–)4.5–6.8(– 8.5) mm, but its leaves are thinner [(0.3–)0.5–0.7(–1.8) mm thick] and with a predominantly glandular pubescence instead of a dense whitish-floccose indumentum.

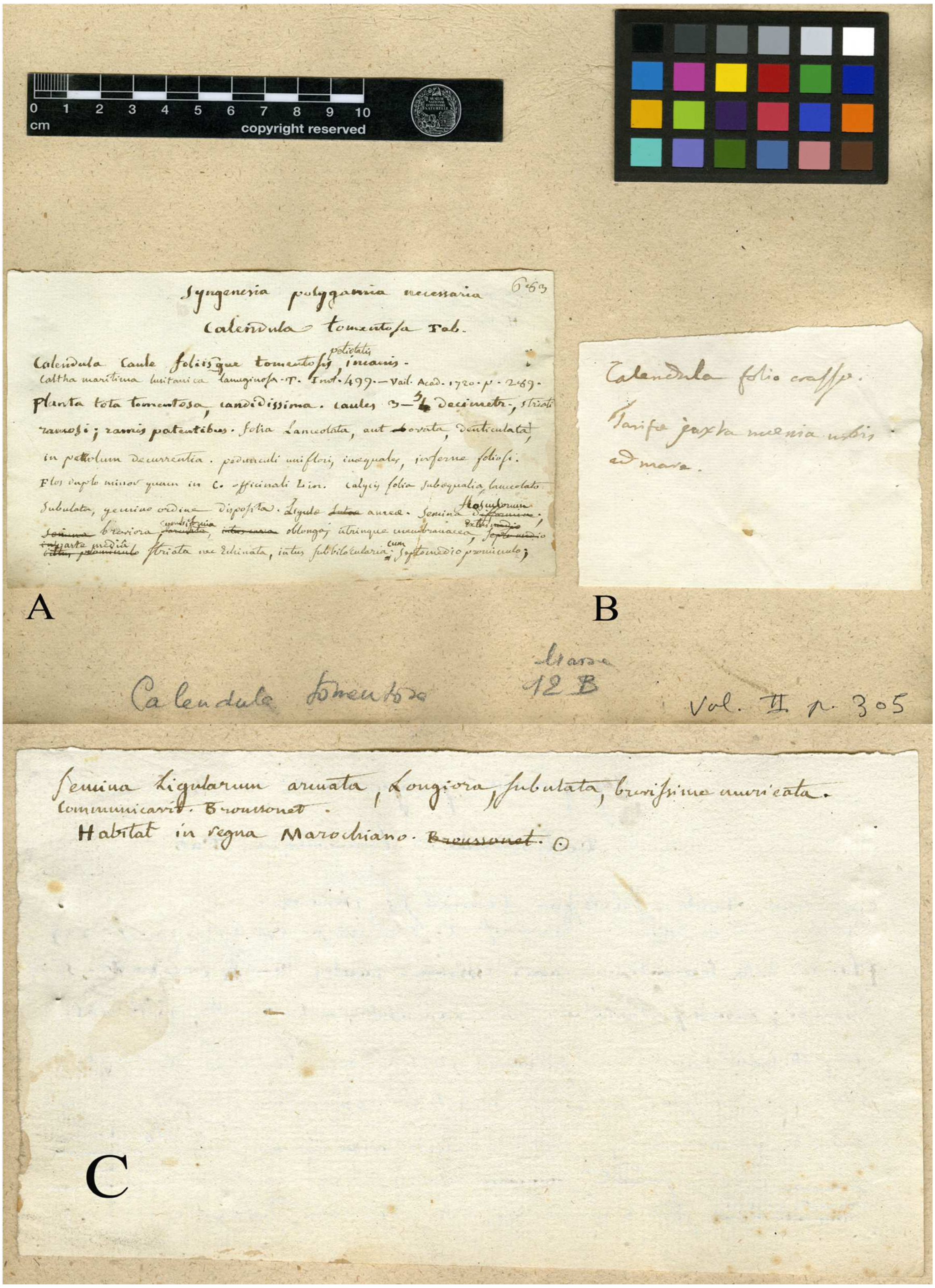
We have reasons to believe that achene morphology reflects the phylogenetic relationships among species of *Calendula* better than indumentum. For example, some variants of *C. arvensis* Linnaeus (1763: 1303) also display the dominance of whitish-floccose hairs, although their achenes remain the same as the typical plants. This fact and the geographic proximity between *C. suffruticosa* subsp. *cinerea* and *C. suffruticosa* subsp. *algarbiensis* are the reasons why we consider these taxa more closely related than the first one with *C. suffruticosa* subsp. *tomentosa*.

Since the sole population of *Calendula suffruticosa* subsp. *cinerea* occupies a very specific habitat, mostly limestone rocks facing the sea, and is morphologically homogeneous, we do not agree with the rank of variety and propose its classification at the rank of subspecies under *C. suffruticosa*.

Ohle (1974: 277) indicated two duplicates of the specimen 'Rechinger 0-2588', supposedly held at HAL and W herbaria, as syntypes. Nevertheless, there is no such collection held at HAL, while the existence of the W duplicate was confirmed. Consequently, we designate the syntype at W as the lectotype of *Calendula incana* var. *cinerea*.

Calendula suffruticosa Vahl subsp. ***cinerea*** (Ohle) P. Silveira & A.C.Gonç., *comb. & stat. nov.* Basionym:—*Calendula incana* subsp. *algarbiensis* var. *cinerea* Ohle (1974: 277). **Type**:—PORTUGAL. Algarve, Cabo de São Vicente, Rechinger 0-2588 (lectotype W!, designated here).

Figure 11 – Lectotype of *Calendula* suffruticosa subsp. tomentosa (Ball) Murb., based on the material used by Desfontaines to describe *Calendula* tomentosa Desf. in his Flora Atlantica (1799), deposited at P-Desf. (P00680154) [reproduced with permission of the Museum national d'Histoire Naturelle (MNHN), Paris Herbarium (P)].



663

Syngenesia polygamia necessaria

Calendula tomentosa Tab.

Calendula caule foliisque tomentosis petiolatis incanis.
Caltha maritima lusitanica lanuginosa. T. Inst. 499. — Vail. Acad. 1720. p. 269.
Planta tota tomentosa, candidissima. caules 3–5 decimetr., striati ramosi; ramis patentibus. folia lanceolata, aut ovata, denticulata, in petiolum decurrentia. pedunculi uniflori, inaequales, inferne foliosi. Flos duplo minor quam in C. officinali Lin. calycis folia subaequalia lanceolato subulata, gemino ordine disposita. Ligulae ~~luteae~~ aureae. Semina flosculorum ~~difformia~~. ~~Semina~~ breviora cymbiformia, ~~[illegible]~~, ~~[illegible]~~ oblonga, utrinque membranacea, externe ~~medio~~ ~~septo medio~~ in parte media ~~[illegible]~~ striata nec echinata, intus subbilocularia cum septo medio prominulo;

Calendula folio crasso.
Tarifa juxta moenia urbis ad mare.

Calendula tomentosa

Marse
12 B

Vol. II p. 305

Semina ligularum armata, longiora, subulata, brevissime muricata.
Communicavit. Broussonet.
Habitat in regno Marochiano. ~~Broussonet.~~ ⊙

Figure 1 – Additional labels attached to the lectotype (P00680154) of *Calendula suffruticosa* subsp. *tomentosa* (Ball) Murb. A. Manuscript including a draft of the diagnosis used for the publication of *Calendula tomentosa* by Desfontaines (his own handwriting); B. Indication of the collecting locality (handwriting of Broussonet); C. Back of the label A. [reproduced with permission of the Museum National d'Histoire Naturelle (MNHN), Paris Herbarium (P)].

Acknowledgements

We thank the consulted herbaria (B, C, HAL, MAF, MPU, P, W), and its staff. On this regard, we especially thank Robert Vogt (B), Uwe Braun (HAL), José Pizarro (MAF), Marc Jeanson (MPU), Cécile Aupic (P), Vanessa Damianthe (P), Gérard Aymonin (also from P, for confirming the identity of Desfontaines and Broussonet's handwritings), Armin Löckher (W) and Ernst Vitek (W). We should further thank Carlos Aedo and Félix Muñoz Garmendia for some fruitful discussions and advice and Alexander Sennikov for reviewing the manuscript. FCT supported the PhD fellowship of A.C. Gonçalves (SFRH / BD / 51 464 / 2011).

References

Ball, J. (1878) Spicilegium florae Maroccanae. Journal of the Linnean Society 16: 516–517. http://dx.doi.org/10.1111/j.1095-8339.1878.tb00104.x

Boissier, E. (1849) Diagnoses plantarum orientalium novarum 10. A. Ascher & Co., Paris, 122 pp.

Desfontaines, R.L. (1799) Flora Atlantica 2. C. Panckoucke, Paris, 458 pp.

Greuter, W. (2006+) Compositae (pro parte majore). In: Greuter, W. & Raab-Straube, E. von (eds.) Compositae. Euro+Med Plantbase – the information resource for Euro-Mediterranean plant diversity. Available from: http:// www.emplantbase.org (accessed: 09 April 2013).

Heyn, C.C., Dagan, O. & Nachman, B. (1974) The annual *Calendula* species: Taxonomy and Relationships. Israel Journal of Botany 23: 169–201.

Heyn, C.C. & Joel, A. (1983) Reproductive relationships between annual species of Calendula (Compositae). Plant Systematics and Evolution 143: 311–329. http://dx.doi.org/10.1007/bf00986612

Lanza, D. (1919) Monografia del genere *Calendula* L. Scuola Tip. Boccone del Povero, Palermo, 166 pp.

Linnaeus, C. (1753) Species Plantarum. Laurentii Salvii, Holmiae, 1200 pp.

Linnaeus, C. (1763) Species Plantarum (ed. 2). Laurentii Salvii, Holmiae, 1684 pp.

Linnaeus filius, C. von (1782) Supplementum plantarum. Impensis Orphanotrophei, Brunsvigae, 467 pp.

Meikle, R.D. (1976) *Calendula* L. In: Tutin, T.G., Heywood, V.H., Burges, N.A., Valentine, D.H., Walters, S.M. & Webb, D.A. (eds.) Flora Europaea 4. Cambridge University Press, Cambridge, pp. 206–207.

Murbeck, S.S. (1905) Contributions à la connaissance de la flore du nord-ouest de l'Afrique et plus spécialement de la Tunisie. Lunds universitets årsskrift (ser. 2) 1(4): 1–83.

Nora, S., Castro, S., Loureiro, J., Gonçalves, A.C., Oliveira, H., Castro, M., Santos, C. & Silveira, P. (2013) Flow cytometric and karyological analyses of *Calendula* species from Iberian Peninsula. Plant Systematics and Evolution, 299 (5): 853–864. http://dx.doi.org/10.1007/s00606-013-0767-0

Ohle, H. (1974) Beiträge zur Taxonomie der Gattung *Calendula*. II. Taxonomische Revision der südeuropäischen perennierenden *Calendula*-Sippen. Feddes Repertorium 85: 245–283. http://dx.doi.org/10.1002/fedr.19740850402

Ohle, H. (1975a) Beiträge zur Taxonomie und Evolution der Gattung *Calendula*. III. Revision der marokkanischen perennierenden Sippen unter Berücksichtigung einiger marokkanischer Annueller. Feddes Repertorium 86: 1–17. http://dx.doi.org/10.1002/fedr.19750860102

Ohle, H. (1975b) Beiträge zur Taxonomie der Gattung *Calendula*. IV. Revision der algerisch-tunesischen perennierenden *Calendula*-Sippen unter Berücksichtigung einiger marokkanisch-algerischer Annueller und der marokkanischen und südeuropäischen perennierenden Taxa. Feddes Repertorium 86: 525–541. http://dx.doi.org/10.1002/fedr.19750860908

Silveira, P. & Gonçalves, A.C. (in press) *Calendula* L. In: Castroviejo et al. (eds.) Flora iberica. Plantas vasculares de la Península Ibérica e Islas Baleares 16. Real Jardín Botánico, CSIC, Madrid.

Urban, I. (1917) Geschichte des Königlichen Botanischen Museums zu Berlin-Dahlem (1815–1913) nebst Aufzählung seiner Sammlungen. Beihefte zum Botanischen Centralblatt. Erste Abteilung: Anatomie, Histologie, Morphologie und Physiologie der Pflanzen 34: 1–457.

Vahl, M. (1791) Symbolae Botanicae 2. Möller & filius, Copenhagen, 108 pp.

Willdenow, C.L. von (1803) Species plantarum (ed. 4) 3(3). G.C. Nauk, Berlin, pp. 1477–2409.

Willkomm, H.M. & Lange, J.M.Ch. (1870) Prodromus florae hispanicae 2. Sumtibus E. Schweizerbart (E. Koch), Stuttgart, 680 pp.

Chapter 3 - Publication 2

Taxonomic revision of the genus *Calendula* (Asteraceae) in the Iberian Peninsula and the Balearic Islands

Taxonomic revision of the genus *Calendula* (Asteraceae) in the Iberian Peninsula and the Balearic Islands

ANA CARLA GONÇALVES[1], SÍLVIA CASTRO[2,3], JORGE PAIVA[2], CONCEIÇÃO SANTOS[4], PAULO SILVEIRA[1*]

[1]*Departamento de Biologia e CESAM, Universidade de Aveiro, 3810–193, Aveiro, Portugal.*

[2]*Centro de Ecologia Funcional, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, Calçada Martim de Freitas, 3000–456 Coimbra, Portugal.*

[3]*Jardim Botânico da Universidade de Coimbra, Calçada Martim de Freitas, 3000–456 Coimbra, Portugal.*

[4]*Faculdade de Ciências, Universidade do Porto, Requinte/LAQV, s/n, 4169–007 Porto, Portugal.*

*Author for correspondence. E–mail: psilveira@ua.pt

This chapter has been submitted as an original monograph in:

Phytotaxa

Table of contents

Abstract

A taxonomic revision of the genus *Calendula* is presented, based on an extensive analysis of its morphological variation, which allowed a re-evaluation of the species delimitations in the Iberian Peninsula and the Balearic Islands. Morphometric data based on field and herbarium material were gathered and analysed. Characters traditionally used to delimit taxa in the genus were re-evaluated, and the taxonomic value of new characters was explored. The variation between and within taxa was explored statistically. Morphological patterns were compared with chromosome numbers and genome size estimates. The results revealed that the achenes are particularly important to distinguish taxa, although, due to their variability, they should be used carefully. Four species are recognised in the area (*C. arvensis, C. officinalis*, *C. tripterocarpa* and *C. suffruticosa*), including nine subspecies of *C. suffruticosa.* Among these, two new subspecies (*C. suffruticosa* subsp. *trialata* and *C. suffruticosa* subsp. *vejerensis*) are described. Identification keys, descriptions, geographical distributions and conservation assessments are also provided for each taxon.

Resumen

Se presenta una revisión taxonómica del género *Calendula*, a partir de un extenso análisis de su variación morfológica, que permitió una reevaluación de las delimitaciones dentro del género en la Península Ibérica y Baleares. Se recogieron y analizaron datos morfométricos basados en material de campo y de herbario. Los caracteres tradicionalmente utilizados para delimitar taxones del género fueron reevaluados y se exploró el valor taxonómico de nuevos caracteres. La variación entre y dentro de cada taxon se exploró estadísticamente. Se compararon los patrones morfológicos con el número de cromosomas y las estimaciones del tamaño del genoma. Los resultados revelaron que los aquenios son particularmente importantes para distinguir taxones, aunque, debido a su variabilidad, deben usarse con cuidado. Se reconocen cuatro especies (*C. arvensis*, *C. officinalis*, *C. tripterocarpa* y *C. suffruticosa*), incluyendo nueve subespecies de *C. suffruticosa*. Entre estas últimas, se describen dos nuevas subespecies (*C. suffruticosa* subsp. *trialata* y *C. suffruticosa* subsp. *vejerensis*). Claves de identificación, descripciones, distribuciones geográficas y evaluaciones de conservación también se proporcionan para cada taxon.

Keywords Calenduleae, Compositae, Mediterranean, Portugal, Spain, systematic

Introduction

Calendula Linnaeus (1753) is the only genus of the tribe *Calenduleae* (Asteraceae) occurring in the Mediterranean region, extending to the Middle East. It is distributed from Macaronesia (Azores, Madeira, and the Canary Islands) through South and Central Europe (central France and Southern Germany), and North Africa (Morocco, Algeria, Tunisia, Libya, and Egypt), to SW Asia (Turkey, Iraq, Iran, Saudi Arabia and Yemen) (Norlindh 1946, Heyn *et al.* 1974, Norlindh 1977, Nordenstam 2007, Nordenstam and Källersjö 2009).

The distribution pattern of *Calendula* suggests that the genus originated in the SW Mediterranean region (Ehrendorfer 1970; Heyn, Dagan, and Nachman 1974; Meusel and Ohle 1966; Norlindh 1946), where most of the species with low chromosome number occur (Heyn, Dagan, and Nachman 1974). From this area, it has successfully diversified and radiated naturally toward the current distribution area. It has also been introduced in other parts of the world. The most common species are *C. officinalis* Linnaeus (1753: 921), which is frequently cultivated for ornamental or medicinal purposes, and the ruderal *C. arvensis* Linnaeus (1763: 1303), which is usually introduced accidentally.

Different treatments have considered 10 to around 25 *Calendula* species (Willdenow 1803, Norlindh 1977, Nordenstam 1994) but, after the completion of this revision, 16 species will be recognised in the genus. Despite being composed of such low number of species, the taxonomy of this genus has been considered extremely difficult (Norlindh 1943, 1977). The genus is ill-defined, due to the wide range of morphological and cytological variability, hybridization events and occurrence of intermediate forms. Several partial taxonomic treatments, using different approaches, have been done (e.g. Heyn *et al.* 1974, Lanza 1919, Meikle 1976, Ohle 1974, 1975a, 1975b), but no agreement among authors on the classification, and characters used to discriminate species has been reached.

The relevance of the characters used to distinguish the species of *Calendula* (e.g. life form: annual or perennial; habit: erect or diffuse; shape of the leaves; indumentum; diameter of the capitula; and colour of disc or ray florets) has been questioned (Battandier 1921; Nègre 1958), but main complexity of the genus arises from the still incompletely comprehended phenomenon of heterocarpy (Heyn & Joel 1983; Ruíz De Clavijo 2005), i.e. the diversity and variability of achenes produced. This phenomenon is common among Asteraceae, although it has evolved independently also in other families (Tanowitz *et al.* 1987; Imbert 2002; Ruíz De Clavijo 2005; Afonso *et al.* 2014). Heterocarpy is a mixed reproductive strategy, associated with floret polymorphism, and an adaptation to colonise unpredictable environments (e.g. arid and semi-arid areas, or saline soils) (Imbert *et al.* 1996, Gardocki *et al.* 2000). In *Calendula*, each capitulum can produce 2 to 6 different morphologies (morphs) of achenes, and in some species, specimens presenting different combinations of achene morphs can co-exist. It has been difficult to interpret the function of the different morphs of achenes produced and its intricate heredity. Indeed, achenes with identical morphology may occur in different species while different achene morphologies or its combinations can occur in the same species. The lack of agreement on the taxonomy of the genus also reflects differences among authors in the interpretation of existing number of species, and different taxonomic categories (Heyn and Joel 1983).

Hybridization and polyploidisation events were proposed by Heyn & Joel (1983) as the main mechanism of evolution of *Calendula* species. Furthermore, annuals seem to have evolved from perennial species, which is corroborated by the pattern of distribution. Perennials are partially out-breeders, growing in primary plant associations, while annuals are predominantly self-fertile, typical weeds or presenting a weed tendency (Heyn, Dagan, and Nachman 1974).

In the Iberian Peninsula, the taxonomic knowledge of this genus is restricted to treatments published in regional floras (Aizpuru Oiarbide et al. 1999; Blanca et al. 2011; Coutinho 1913; Franco 1984; Meikle 1976a; Merino y Román 1906; Valdés, Talavera, and Fernández-Galiano 1987a; Willkomm 1893; Willkomm and Lange 1865) all of them proposing different taxonomic concepts for the genus. Wider approaches also failed to include the entire genus. The monograph by Lanza (1919), for example, did not include many taxa from Morocco, and other Mediterranean areas. Heyn (1974) studied only the annuals, and Ohle (1974, 1975a, 1975b) studied mostly the perennials. Both annuals and perennials were studied by Meikle (1976b), but only for the European region. Thus, a recent monograph, including the entire genus and covering its full distribution area, is still lacking.

Prior taxonomic works and their restrictions

Before 1753. For a long time, only two species were known in *Calendula*, the cultivated *C. officinalis*, used for ornamental and medicinal purposes, and the ruderal *C. arvensis*, common on cultivated and waste grounds. However, several pre-Linnaean authors described *Calendula* species under the name *Caltha* (Vaillant 1720, 557–58). At that time, the descriptions were brief and dubious/unclear, and could simply refer to mere variations of the two known species. A brief historical survey of the taxonomy of *Calendula* before 1753 can be found in Lanza (1919) and Ohle (1974).

One of these pre-Linnaean works (Vaillant 1720), however, deserves special reference, because of a recent nomenclatural controversy. In that work, four taxa were listed under the name *Caltha*: *Caltha hortensis* (= *Calendula officinalis*), *Caltha arvensis* and *Caltha sylvestris* (= *Calendula arvensis*), and *Caltha maritima* (= *Calendula maritima* Gussone (1825: 3) (Greuter, Aghababian, and Wagenitz 2005). Since Vaillant's work was published 33 years before the publication of the first edition of Species Plantarum (Linnaeus 1753), it is not regarded as a source of validly published names (Brummitt 2008; Greuter, Aghababian, and Wagenitz 2005). However, Steinwehr published a translation of this work to German in Königl. Akad. Wiss. Paris Phys. Abh. 5–9 (1754–1760). Greuter *et al.* (2005) proposed to accept some of the names published in Steinwehr's translation as validly published species, while others consider that this publication should be suppressed under the '*Opera Utique Oppressa*' (see Brummitt 2008, Greuter 2008a, Sennikov 2010, Applequist 2014, Sennikov 2015). Presently, none of the *Caltha* names described by Vaillant is considered validly published (Applequist 2014).

1753 and afterwards. Linnaeus (1753, 921) described five species under *Calendula*: *Calendula officinalis* from Europe, and four African species (= *Calendula graminifolia* Linnaeus (1753: 922), *Calendula hybrida* Linnaeus (1753: 921), *Calendula nudicaulis* Linnaeus (1753: 922), and *Calendula pluvialis* Linnaeus (1753: 921)), that have subsequently been moved to *Dimorphotheca* Moench (1794: 585). Later, three more species have been added by Linnaeus (1763, 1303), of which only *C. arvensis* and *C. sancta* Linnaeus (1763: 1304) belong to the genus.

Vahl (1791) described *C. suffruticosa* Vahl (1791: 94), from Tunisia. Until then, the genus was known only in Europe. In the same year, Cavanilles (1791) described *C. stellata* Cavanilles (1791: 3) from plants cultivated from seeds sent by D. Lemonier (Louis G. Le Monnier 1717–1799), certainly collected by Desfontaines in the North of Africa (Desfontaines 1799, Cuvier 1819).

Desfontaines (1799) published *C. tomentosa* Desfontaines (1799: 305) from '*regno Marocano*' *fide* Broussonet, but the name was previously published by Linnaeus f. (1781) for a Southern African plant, so the name given by Desfontaines is illegitimate. In addition, Willdenow (1803), proposed *C. incana* Willdenow (1803: 2341) for the material of Desfontaines, maintaining the original location and expanding it to '*maritimis Lusitaniae*' (*vide* Silveira *et al.* 2013). Willdenow (1803) also produced a revision of *Calendula*, recognising 25 species.

After that, some new species of *Calendula* where described, included in several floristic accounts, such as, for example, *Calendula fulgida* Rafinesque (1810: 83) and *Calendula maritima* from Italy, and *Calendula maderensis* Candolle (1837: 454) from Madeira.

Lanza (1919) presented a monograph of the genus, based on morphological characters, like the shape and pubescence of the leaves, the length of the ray florets in relation to the bracts, the size and colour of the capitula, as well as achene morphology. He divided the achenes occurring in *Calendula* into four basic morphs, and classified them in different combinations within the capitulum to distinguish taxa. For example, in *C. arvensis* Lanza described six '*forma carpica*': I. *exalata rostrata*, II. *exalata longirostris*, III. *exalata erostris*, IV. *alata rostrata*, V. *alata longirostris*, and VI *alata erostris*.

Lanza (1919) recognised 10 species in two groups: '*Annuae'* and '*Perennes vel perennantes'*. The first included three annual species: *C. aegyptiaca* Desfontaines (1804: 100), *C. arvensis* and *C. bicolor* Rafinesque (1810: 82)); while the latter consisted of seven perennial species: *C. fulgida*, *C. noeana* Boissier (1875: 416), *C. suffruticosa*, *C. tomentosa*, *C. monardii* Boissier & Reuter (1859: 108), *C. maritima* and *C. maderensis*. Since the genus presents a great variability, Lanza (1919) maintained experimental cultures, to verify the stability/persistence of the characters throughout successive generations (3 to 10 years). He also crossed artificially various *Calendula* species, in order to assess the possible impact of hybridization and transmission, dominance or recession, in a Mendelian sense, of some characters. The experiences have demonstrated, among other things, the efficacy of cross-pollination among *Calendula* taxa with the same life cycle, annual or perennial, which resulted in fertile hybrids, while the crosses between annual and perennial forms, which we known now have different chromosome numbers, were not seed-producing and the few seeds obtained were sterile. This is important from a systematic point of view because there is obviously a genetic barrier, which led Lanza to divide the genus into two groups: annuals versus perennials. However, we presently known other annual and perennial species with chromosome numbers that are different from those presented by the species tested by Lanza (1919), which reduces the interest in this simple division.

Battandier (1921) questioned the characters used until then to distinguish the *Calendula* species, such as life form, habit, leaf shape, indumentum and the size and colour of the capitula. This author concluded that these characters are subject to ecological variation, and deserve no taxonomical value.

Nègre (1958) also pointed out that the size of basal leaves, the diameter and colour of the capitula are variable characters, changing with environmental variables such as temperature or dryness. Instead, he proposed the length of hairs at the base of ligules as a differential character. According to Nègre (1958), *C. arvensis* and *C. algeriensis* Boissier & Reuter (1856: 109) (= *C. stellata*) have bi-cellular hairs up to the apex, while *C. aegyptiaca* Persoon (1807: 492) (= *C. arvensis*) and *C. murbeckii* Lanza ex Murbeck (1923: 59) have unicellular hairs.

Heyn *et al.* (1974) proposed a taxonomic treatment for the annual *Calendula* taxa. These authors recognised the following five species: *C. stellata*, *C. tripterocarpa* Ruprecht (1856: 231), *C. arvensis*, *C. palaestina* Boissier (1849: 10), and *C. pachysperma* Zohary (1941: 172), based on morphological and cytological characters. Despite the different combinations of morphological characters, all the annual *Calendula* plants with $2n = 44$ chromosomes were included in *C. arvensis*. However, the same criterion was not followed for *C. palaestina*, and *C. pachysperma*, probably because the authors were not sure about the chromosome numbers of these plants, which were reported by them to be $2n = \pm 85$ for both taxa (Heyn *et al.* 1974).

In a major review, Ohle (1974, 1975a, 1975b) changed the taxonomy of the perennial species, recognising 24 taxa. This was preceded by morphological and cytological studies conducted by Meusel & Ohle (1966) in a first attempt to classify these taxa. Later, Ohle (1974) proposed the segregation of the wild perennial taxa as *C. incana* and *C. suffruticosa* groups. The *C. incana* group included plants with wide spatulate or obovate, obtuse and thick leaves, while the *C. suffruticosa* group, comprised plants with narrowly lanceolate or spatulate, acute and thin leaves. Moreover, Ohle (1975a) studied the *C. maroccana* group from Morocco, describing two species, *C. eckerleinii* Ohle (1975a: 8) and *C. meuselii* Ohle (1975a: 4). However, the work lacks key and species descriptions of the *C. suffruticosa* taxa from Morocco. Finally, Ohle (1975b) recognised six taxa under *C. suffruticosa* group from Algeria and Tunisia.

Afterwards, Meikle (1976b) revised *Calendula* for *Flora Europaea*. This author retained *C. incana* under *C. suffruticosa*, and recognised a few intraspecific taxa, giving low relevance to the variation of achene morphology.

Heyn & Joel (1983) proposed that hybridization and polyploidisation events were the main mechanisms of evolution of *Calendula* species. These authors also presented a hypothesis for the speciation process in the genus. According to them, *C. maroccana* (Ball) B.D. Jackson (1873: 367) and *C. stellata* belong to the region considered as the primary centre of the evolution of the genus (Norlindh 1946), where these species were essential and played a central role in the origin of several taxa. More details on the evolutionary steps of the genus were discussed in Nora *et al.* (2013), after gathering additional cytogenetic data.

Recently, molecular phylogenetic (Plume *et al.* 2015, Plume 2015, Schmiderer *et al.* 2015), and cytological approaches (Nora et al. 2013) were explored, but many issues remain unresolved. The latter study does not support the segregation of *C. suffruticosa* from *C. incana* because both have the same chromosome number and a gradual transition in genome size was observed (Nora et al. 2013). That study also presented, for the first time, genome size estimations for *Calendula* and supported polyploidisation and dysploidy as important phenomena contributing to the cytogenetic complexity of the genus (Nora *et al.* 2013).

Plume *et al.* (Plume, Raimondo, and Troia 2015) studied the impact of hybridization between the Sicilian endemic, *C. maritima* and *C. suffruticosa* subsp. *fulgida* (Raf.) Guadagno (1922: 64). Independent tests of hybridism were conducted using palynological and molecular (ITS1, ITS2, and *atpI–atpH*) data. The results provided evidence for hybridization between *C. maritima* and *C. suffruticosa* subsp. *fulgida*, and suggest that although the fertility of hybrids seems to be reduced they are apparently capable of back-crossing (Plume, Raimondo, and Troia 2015).

Plume (2015) also tested hypotheses for the origin of *Calendula* species using non-coding chloroplast regions (*atpI, atpH, petL, psbE, and ndhF-rpl32*), nuclear ribosomal DNA (ITS), and two low-copy nuclear markers (*Chs* and *A39*). Analyses of these markers provided support for a division of the genus into annual and perennial complexes, multiple origins of polyploid taxa, and a single origin of *C. officinalis* (Plume 2015). Molecular approaches using other markers were also able to distinguish *C. officinalis* from 10 other species of the genus (Schmiderer et al. 2015).

Considering the complexity of this genus, and deep divergences among taxonomic treatments proposed by different authors, a throughout revision were started for the *Flora iberica* project (Castroviejo 1986–), and later expanded to other Mediterranean regions. With this objective, some studies were performed on pollen morphology of *Calendula* (Antunes, Coutinho, and Silveira 2007), on karyology and nuclear DNA content (Nora et al. 2013), typification of Iberian *Calendula* taxa (Silveira et al. 2013), and a floristic treatment for the 'Flore Pratique du Maroc' (Gonçalves et al. 2014). The present study includes a revision of *Calendula* for the Iberian Peninsula and the Balearic Islands, based on the collections deposited in several herbaria and new field collections, aiming at (1) analysing the variation between and within taxa, and (2) re-evaluating taxa delimitations.

Materials and methods

Plant material

This revision is based on herbarium material and new field collections. Approximately 5000 herbarium specimens from Iberian Peninsula and the entire native range of the genus, including type specimens, were examined from the following herbaria: ABH, AL (ENSA), ARAN, B, BC (IBB), BCN, BM, BONN, BR, C, COI, E, ELVE, FI, G, GAT, GDA (GDAC), HAL, JACA, JAEN, JE, K, LD, LEB, LISI, M, MA, MACB, MAF, MARK, MGC, MPU, O, P, PO, RAB, RNG, SALA, SANT, SEV, TFMC, US, VAL and W. Field work by A. C. Gonçalves and P. Silveira was conducted on 45 field locations in the Iberian Peninsula (APPENDIX I) and other regions where *Calendula* is native, namely Morocco, Algeria, Tunisia and Macaronesia. These specimens are kept at AVE herbarium. Live collections were maintained for some time at the Department of Biology, University of Aveiro, to check the persistence of morphological characters.

Morphometric database compilation

In each visited population, 10 specimens were examined (except for those represented by smaller numbers of plants). Morphological characters were measured with a ruler, and/or a digital calliper. For each individual plant, the length of the longest branch, basal leaf thickness, and head diameter (measured after flattening the capitulum) were measured *in situ*. These specimens were collected and press dried for further study. Other measurements were performed on these specimens and/or other herbarium vouchers from the herbaria referred above, in the laboratory. Based on previous work in this genus (Heyn *et al.* 1974, Ohle 1974, 1975a, 1975b, Meikle 1976), and the observation of characters that appeared to vary between taxa, 29 quantitative and 26 qualitative characters (presented in Table 5 and 6, respectively) were defined and measured for each specimen.

Each specimen represents an Operational Taxonomic Unit (OTU) in the multivariate analysis (see below).

Data analysis

Differences among taxa were analysed separately for: (1) species and (2) subspecies of *C. suffruticosa*. Furthermore, for the convenience of the analysis, the subspecies of *C. suffruticosa* were divided into two subgroups, based on indumentum: (1) plants with white-arachnoid pubescence, including subsp. *cinerea* (Ohle) P.Silveira & A.C.Gonç. (2013: 52), subsp. *tomentosa* (Ball) Murbeck (1905: 9), subsp. *marginata* (Willd.) Maire (1934: 789) and subsp. *vejerensis*; and (2) plants without white-arachnoid pubescence, including subsp. *trialata,* subsp. *carbonelli* Ohle (1974: 268), subsp. *algarbiensis* (Boiss.) Nyman (1878: 398), subsp. *greuteri* Ohle (1974: 269) and subsp. *lusitanica* (Boiss.) Ohle (1974: 270). Depending on the group analysed, some characters were excluded due to their non-applicability to that group.

Normality and homoscedasticity were tested for all the variables. For normally distributed and homoscedastic variables, *t*-test (between two groups) or One-way ANOVA (between more than two groups) were used to evaluate for significant differences in quantitative traits. When normality and homoscedasticity were not achieved, Mann- Whitney test (between two groups) and Kruskal-Wallis One-way ANOVA on ranks (between more than two groups) were used instead. One-way ANOVA and Kruskal-Wallis One-way ANOVA on ranks were followed by Tukey tests or Dunn's tests, respectively. The main statistical parameters such as mean, percentiles (25 and 75), standard deviations (SD) and standard error (SE) were calculated. To represent the variation of each character among and within taxa, boxplots were prepared. Principal component analysis (PCA) was used to examine the variation within the genus and to estimate the contribution of each character to the analysis. The characters with the highest factor loadings on the first three components were determined. All statistical analysis was performed using R (R Development Core Team 2010), except for PCA, which was done with StatistiXL (Broadway – Nedlands, AUS).

Mapping, measurement of species richness and assessment of conservation status

Geographic distribution maps were produced for the Iberian Peninsula and the Balearic Islands, using latitude and longitude data for 1522 specimen's localities (field collections and herbarium specimens whose locality coordinates could be determined), with QGIS 2.18.4 (http://www.qgis.org/).

The taxa richness map was elaborated using the same datasets as for the distribution maps with the use of the "Analysis/Point to Grid" function in DIVA-GIS, v. 7.5 (http://www.diva-gis.org) in a 0.3 by 0.3-degree cell size. Altogether, 5969 grid cells were sampled, and these included 1207 occurrence records for 12 taxa.

The conservation status of each recognised taxa was assessed by applying the IUCN Red List Categories and Criteria (http://www.iucnredlist.org/). Georeferenced specimen data were imported into GeoCAT (http://geocat.kew.org/) to calculate the area of occupancy (AOO) and extent of occurrence (EOO).

Species delimitation

In the revision presented here, species are distinguished based on Genotypic Cluster or Genomic Cluster Species Concept (GCSC), where genetic (chromosome number, ploidy, and genome size) and morphological clusters were used to define species boundaries (Mallet 1995)). Furthermore, species were separated into subspecies based on distinct morphologies and geographical distributions, following the 75% rule for quantitative characters

Table 5 – List of quantitative characters used in this study. Characters are listed following the order used in the descriptions.

Characters	**Abb.**	**Unit/Scale**
Length of the longest branch	LB	cm
Basal leaf length	LL	cm
Basal leaf width	LW	cm
Basal leaf distance from base to point of maximum width	LD	cm
Ratio leaf length/point of maximum width	R1	ratio
Ratio leaf length/width	R2	ratio
Basal leaf thickness	LT	mm
Head diameter	HD	cm
Involucre length	IL	mm
Ligule length	LG	mm
Ratio ligule/involucre	R3	ratio
Sub-exalate achene length	SEL	mm
Sub-exalate achene width	SEW	mm
Rostrate achene length	RL	mm
Rostrate achene width	RW	mm
Rostrate achene angle	RA	mm
Rostrate achene dorsal spine	RS	mm
Bialate achene length	BL	mm
Bialate achene width	BW	mm
Bialate achene rostrum length	BR	mm
Trialate achene length	TL	mm
Trialate achene width	TW	mm
Trialate achene rostrum length	TR	mm
Cymbiform achene length	CL	mm
Cymbiform achene width	CW	mm
Sub-cymbiform achene length	SCL	mm
Sub-cymbiform achene width	SCW	mm
Sub-cymbiform achene rostrum length	SCR	mm
Vermiform-alate achene length	VAL	mm
Vermiform-alate achene width	VAW	mm
Vermiform-exalate achene length	VEL	mm
Vermiform-exalate achene width	VEW	mm

Table 6 – List of qualitative characters used in this study. Characters are listed following the order used in the descriptions.

Characters	Characters states	Abb.
Life cycle	(1) annual; (2) annual to perennial (3) perennial	LF
Stem pubescence	(1) mostly glandular; (2) glandular pubescent; (3) mostly white-arachnoid	SP
Leaf shape	(1) oblanceolate; (2) oblanceolate to spatulate; (3) ovate to oblong; (4) linear-oblong	LS
Leaf apex	(1) acuminate; (2) acute; (3) obtuse	LA
Leaf margins	(1) entire to sub-entire; (2) repand-dentate proximally; (3) repand-dentate distally; (4) repand-dentate totally	LM
Leaf lamina pubescence	(1) mostly glandular; (2) glandular pubescent; (3) mostly white-arachnoid	LP
Ray florets	(1) yellow; (2) yellow-orange; (3) orange	RF
Disk florets	(1) yellow; (2) yellow-orange; (3) orange; (4) purplish; (5) brown	DF
Sub-exalate achene		
ventral wings	(0) absent; (1) < than lateral; (2) sub-equal; (3) > than lateral	SEvw
lateral wings	(0) absent; (1) sub-entire; (2) sinuate-dentate; (3) incise; (4) deeply pinnately cut	SElw
Rostrate achene		
ventral tooth	(0) absent; (1) apical; (2) basal; (3) both	Rvt
Bialate achene		
lateral wings	(0) absent; (1) sub-entire; (2) sinuate-dentate; (3) incise; (4) deeply pinnately cut	Blw
dorsal wings	(0) absent; (1) < 2 mm straight; (2) > 2 mm bent toward the ventral face	Bdw
Trialate achene		
ventral wings	(0) absent; (1) < than lateral; (2) sub-equal; (3) > than lateral	Tvw
lateral wings	(0) absent; (1) sub-entire; (2) sinuate-dentate; (3) incise; (4) deeply pinnately cut	Tlw
dorsal wings	(0) absent; (1) < 2 mm straight; (2) > 2 mm bent toward the ventral face	Tdw
Cymbiform achene		
ventral wings	(0) absent; (1) < than lateral; (2) sub-equal; (3) > than lateral	Cvw
dorsal wings	(0) absent; (1) smaller and straight; (2) bigger and bent toward the ventral face	Cdw
ventral tooth	(0) absent; (1) apical; (2) basal; (3) both	Cvt
Curvature	(0) absent; (1) curved; (2) strongly curved	Cc
Sub-cymbiform achene		
ventral wings	(0) absent; (1) < than lateral; (2) sub-equal; (3) > than lateral	SCvw
lateral wings	(0) absent; (1) sub-entire; (2) sinuate-dentate; (3) incise; (4) deeply pinnately cut	SClw
dorsal wings	(0) absent; (1) < 2 mm straight; (2) > 2 mm bent toward the ventral face	SCdw
Vermiform-alate achene		
Shape	(0) absent; (1) circular; (2) falcate; (3) hook-shape	Vas
ventral tooth	(0) absent; (1) apical; (2) basal; (3) both	VAvt
Vermiform-exalate achene		
Shape	(0) absent; (1) circular; (2) falcate; (3) hook-shape	VEs
ventral tooth	(0) absent; (1) apical; (2) basal; (3) both	VEvt

Taxonomic characters

Some morphological characters that are important for species delimitation in *Calendula* have been described by previous authors (e.g. Lanza 1919, Ohle 1974, 1975a, 1975b). However, several selected traits used in this study are discussed below in more detail.

Lifecycle. The species of *Calendula* are either annual or perennial herbs, sometimes the latter with a relatively woody base. However, some taxa, such as *C. officinalis*, *C. suffruticosa* subsp. *greuteri* or *C. suffruticosa* subsp. *fulgida* sometimes behave as annual, biannual or perennial. This variability is most probably an adaptation to variable environmental conditions, bringing more difficulties to the process of taxonomical classification. Annual species are usually found with flowers and achenes simultaneously during winter and spring, while perennial species flourish more frequently in spring, and achenes are found in late spring-summer. This could be an explanation for why so many specimens, especially of perennials in Northern Africa, have been collected without achenes.

Habit. Species are rather diverse regarding habit, but most commonly, *Calendula* species are prostrate, decumbent, ascending, diffuse or erect. It is not always easy to determine which is the typical habit of a given taxon, because sometimes it is variable and/or intermediate and, sometimes, it varies somewhat with the habitat where the plant is growing. For example, species that live inland, in habitats sheltered from winds, usually are ascending to erect, while those living on the coast are somewhat prostrate to decumbent. Additionally, plants with identical achene morphology, displaying contrasting habits according to microhabitat adaptation, were found a few dozen meters from each other. Furthermore, most of the taxa display the equivalent wild habit under cultivation but, sometimes, it changes rapidly after transplantation into a new location with different environmental conditions. Thus, this character must be used carefully for taxonomical purposes, preferably after some cultivation trials. Additionally, in annual species, there is an unclear distinction between vegetative and reproductive portions, whereas the perennial species, usually present a basal rosette or vegetative zone, and the reproductive branches die after the fructification.

Leaves shape. The shape of the basal leaves presents a broad variation among and within taxa, ranging from lanceolate to linear-oblong, oblanceolate-spatulate or broadly spatulate. Their base can be attenuated in a ± large petiole or ± amplexicaul, especially on the transition to the cauline leaves, which are progressively smaller toward the apex, oblanceolate to lanceolate, usually auriculate. Normally, lanceolate leaves have acute apices, while the spatulate leaves are more obtuse. The leaf margins of the annual species are usually slightly repand-dentate — rarely pinnatisect (in one taxon from Morocco), while in the perennials, they are entire, repand-dentate or undulate-dentate. All leaves have small hydathodes in the margin, usually green or reddish.

Leaf thickness. This character may be correlated with environmental conditions (Ohle 1974). Species occurring in the coastal environments normally have shorter (broadly spatulate or obovate) and thicker leaves, while the longest (lanceolate) and thinner leaves are found in the mountain plants. Additionally, this character can only be correctly evaluated in fresh material, while it is difficult to assess in dried herbarium specimens. Comparisons between field and cultivated collection reveal the general persistence of this character.

Indumentum. The pubescence type and density are extremely variable among and within the species of *Calendula*. Most species are covered by a combination of glandular and non-glandular hairs. The latter may end up prolonged by a long fibre parallel to the surface of the leaf or stem, forming the white-arachnoid indumentum (Figure 13).

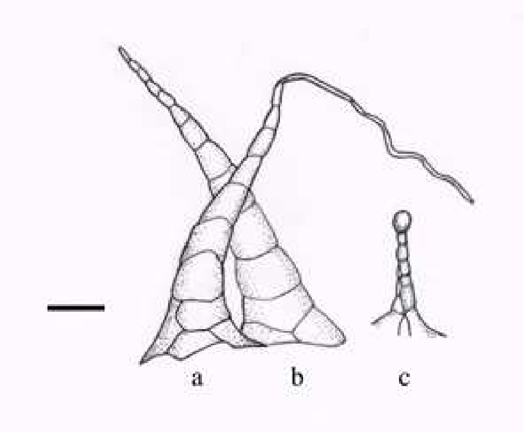


Figure 13 – Detail of the indumentum in Calendula. a) hair responsible for the white-arachnoid indumentum; b) non-glandular hair; c) glandular hair (scale bar = 0.1 mm). Drawn by P. Silveira from *C. suffruticosa* subsp. *maritima* (Guss.) R.D. Meikle, ITALY, Sicily, Ronciglio (Trapani), 10-Jun-1993, NCRPIS (USDA) PI597596 in *Silveira P.* 3101, AVE).

The pubescence is an important character to delimit species. We can find species with glandular and non-glandular hairs in variable proportions, or species, completely covered with white-arachnoid, non-glandular, hairs. However, this character must be used carefully, since in some species, individual plants of the same population or entire populations can be found with a different indumentum, or the indumentum can change with the season, or upon cultivation. In the leaves, this character was evaluated on the apical half of the lamina. In most species, the branches have glandular and aromatic hairs, although non-glandular hairs are also present. Less frequently, they are entirely covered by dense white-arachnoid hairs.

Capitula. All species of *Calendula* have terminal, solitary and radiate capitula. Each capitulum is subtended by an involucre of small modified leaves (bracts/phyllaries). The involucre is campanulate or hemispheric, with bracts arranged in 1–2 rows, lanceolate to linear-lanceolate, acuminate, and covered with glandular and non-glandular hairs, with scarious margins, which, in some species, are somewhat violet-purple. The receptacle is flat, or slightly convex, naked (without scales/paleae). The capitula include ray and disc florets the ray florets are always female, yellow or orange, arranged in 1–2 rows, rarely 3 or more, with a basal short tube and with an oblanceolate ligule, with 3 tiny apical teeth. The disc florets are functionally male, tubular, with five lobes, not sacciform. The capitula can be classified as concolorous, when ray and disc florets are of the same colour, yellow to orange; or discolorous, when ray florets are yellow or orange, while disc florets are ± purple. Normally, the perennial species are concolorous. *C. officinalis* is the exception, since it can be annual or perennial, and, concolorous or discolorous. On the other hand, annual species can be concolorous, discolorous or both.

Achenes. Calendula is characterised by the production of dissimilar morphs of achenes in a single head, all without a pappus (Heyn & Joel 1983). These are all produced from the ray florets, although assuming distinct positions within the head. The production of fruit morphs with different sizes, mass, shape, colour, and presence or absence of dispersal structures (e.g. wings, spines, and teeth) is called heterocarpy (Venable 1985, Imbert *et al.* 1996, Ruíz De Clavijo 2005). A single head produces, more frequently, 3–4 morphs of achenes, but this number can be higher.

In spite of the general knowledge of heterocarpy in *Calendula*, only a few authors described the morphology of the achenes. Lanza (1919) was the first to distinguish four morphs of achenes: rostrate, tripteroid, cymbiform and vermiform. Previously, some pre-Linnaean authors pointed out the heterocarpy of the genus and described vaguely the achenes (Lanza 1919). Later, Ohle (1974) distinguished seven distinct achene morphs in *Calendula*: rostrate-exalate, rostrate-bialate, rostrate-trialate, cymbiform-bialate, cymbiform-trialate, vermiform-exalate and vermiform-alate. Norlindh (1962) suggested that the presence and shape of the vermiform achenes is an evolutionary characteristic.

Additionally, the achenes of *Calendula* have three morphologically different structures: teeth, wings and spines. Ruíz De Clavijo (2005) argued that these structures in *Calendula* may favour the dispersion. The teeth are small, flat, triangular protrusions, projecting beyond the surface of the apical and/or basal margins of the achene, while the wings are ± flat membranous expansions, running along the length of the achene. According to Ohle (1974), the ventral wing development can be assumed as a connation of the apical and basal ventral teeth. Achenes with similar structures are found in other *Calenduleae* genera (e.g. *Garuleum* Cassini (1819: 172), *Norlindhia* Nordenstam (2006: 41), *Tripteris* Lessing (1831: 95), and *Osteospermum* Linnaeus (1753: 923)).

Within a capitulum, achenes may have different size, shape, mass, colour, and presence or absence of dispersal structures (e.g. wings, spines, teeth). Fruit size is positively correlated with embryo size and both with the competitive ability of the resulting plantlet. For example, in arid regions it has been observed that reductions in the mother plant size correlate with a reduction in fruit size. However, if resources are available, the more peripheral achenes of the capitulum may have especially enlarged embryos, functioning as an advantage in colonising new habitats (Heyn & Joel 1983). Furthermore, achene morphology sometimes changes with the season, i.e. from spring to summer, particularly in some subspecies of *C. suffruticosa*. These changes in achene morphology through the season seem to be epigenetic effects, probably driven by changes in temperature. Although this could discourage us to use achene morphology for taxonomic purposes, we believe this should not be the case, since if comparisons are made between achene morphology in the same season, preferably spring, clear patterns of variation, correlated with differential geographical distributions and habitats, can be observed and are an indication that different subspecies should be recognised. Therefore, the morphological analysis and identification keys included in this study are based on observations and specimens collected in spring.

Altogether, seven basic morph types of achenes can be found in the capitula of *Calendula*, although intermediate forms can also appear. The description of each achene morph is presented below (Figure 7, 39 and 42):

Rostrate achenes can be straight, slightly curved or strongly curved, always with an apical narrow rostrum, ± longer, from which derives its designation. In spite of this, the length of the rostrum of the rostrate achenes is not provided, because, in this kind of achenes, it is often difficult to distinguish the limits of this structure and the embryo itself. Some taxa present numerous dorsal spines, but usually, the dorsal surface of the achene is muricate or smooth. When present, the spines are arranged in 2–4 rows, on the backside of the achene. Furthermore, the rostrate achenes can present ventral teeth, one at the base, and another in the apex, or both. The combination of all these structures (rostrum, spines, and teeth) varies between taxa.

Bialate achenes, generally, show similar features (rostrum, curves, and teeth) as in the rostrate achenes. These achenes are characterised by two wide lateral wings, which can be entire, dentate, incise or more deeply divided. The lateral wings are generally larger at the base and tend to attenuate to the apex, forming a short rostrum. The ventral basal tooth, when present, can reach a considerable height, up to the middle of the achene (they never present a ventral wing). As in the rostrate achenes, bialate achenes may present dorsal spines or, less frequently, two undeveloped dorsal wings.

Trialate achenes, are normally straight to slightly curved, with 3 wings (2 lateral, and 1 ventral), with entire or sinuate-dentate margins, less commonly incise-dentate. More frequently, the wings are ± identical (sub-equal). The lateral wings usually slightly point toward the dorsal surface of the achene (Figure 42 – C1). The backside of the achene is smooth, or slightly muricate. The rostrum is relatively short or absent in most of the cases.

Cymbiform achenes (or navicular) are also broad-winged. The lateral wings are concave, bent toward the ventral face of the achene. The curvature of the achene will determine its shape, like a boat or a balloon. The ventral wings are important to distinguish taxa. They can be ± identical (sub-equal), surpassing the lateral wings or missing. The backside of the achene may be unprotected, muricate, presenting dorsal spines, or with two, ± broad, dorsal wings.

Intermediate forms between trialate and cymbiform achenes can be found in different degrees. So, we propose the designation **sub-cymbiform** for those achenes with three wide wings, whose two lateral wings are not straight like in the trialate achenes but are less ventrally curved than the cymbiform achenes. Furthermore, in *C. suffruticosa* subsp. *lusitanica*, **sub-exalate achene** (i.e. without wings or with narrow wings) is the typical morph occupying the median position, where other *C. suffruticosa* subspecies usually present trialate or cymbiform morphs. We propose this designation to describe them.

The innermost achenes in the fruiting capitulum are also the smallest ones and are designated **vermiform** or **annular** achenes. The first term refers to their worm-like appearance, because of their shape and rugose or tuberculate surface, while the second refers to their generally ± circular shape. Since their shape is not always circular, but in many species, they are ± falcate or hook-shaped, we prefer to use the first term. These achenes are further subdivided into **vermiform-alate**, and **vermiform-exalate** achenes. The vermiform-alate achenes have two lateral wings, small and curved toward the ventral face, whereas the vermiform-exalate achenes have no lateral wings. Its shape can be circular, falcate or hook-shaped. At least one of these achenes is always produced in every capitulum.

As mentioned, a single head may comprise four or more morphs, with different size, colour and ornamentation. During maturation, the achenes develop toward an inner, median or outer position on the capitulum, according to its morph type, which seems to favour their differential dispersion. The inner achenes (vermiform achenes) have none or short-range dispersal and fall to the ground quickly after maturation. Furthermore, they present dormancy, being responsible for the maintenance of a seed bank, which ensures the local survival of the species under unfavourable conditions. The middle and outer achenes (cymbiform and/or bi-trialate, and rostrate achenes) are adapted for mid- to long-range dispersal (anemochory or epizoochory), being responsible for the colonisation of new habitats (Ruiz De Clavijo 2005). The rostrate achenes are usually more tightly attached to the fruiting capitulum than the other achene morphs, probably to increase the chances to get in contact and transported in the fur of passing animals.

Chromosome number and genome size

Karyologically, the genus is characterised by a basic chromosome number of $x = 9$, although several other basic numbers, namely $x = 7$, 8, 11 and 15, have been reported (Norlindh 1977; Heyn & Joel 1983; Nordenstam & Källersjö 2009). Chromosome numbers vary from $2n = 14$ to 88 chromosomes. Most of the species are tetraploid, with only a few diploid exceptions, namely *C. tripterocarpa*, *C. maroccana,* and *C. stellata*. The two latter are thought to have played a central role in the origin of the other *Calendula* taxa by hybridization and polyploidisation (Heyn & Joel 1983). These latter two species are native to the SW Mediterranean region, which is considered as the primary centre of the evolution of *Calendula* (Norlindh 1946).

Table 7 – Chromosome numbers and genome size in *Calendula* taxa from Iberian Peninsula.

Heyn *et al.* (1974)	Ohle (1974)	Meikle (1976)	This study	Acronym	*2n*	Genome size (2C/pg)		
						Mean	**SD**	CV
C. arvensis		*C. arvensis*	*C. arvensis*	Carv	44	5.41	0.11	2.03%
C. tripterocarpa		*C. tripterocarpa*	*C. tripterocarpa*	Ctrip	30	3.44	0.06	1.74%
	C. officinalis	*C. officinalis*	*C. officinalis*	Coff	32	2.98	0.08	2.72%
	***C. suffruticosa* group**	***C. suffruticosa* group**	***C. suffruticosa* group**	Csuff				
		C. suffruticosa subsp. *suffruticosa*						
	C. suffruticosa subsp. *carbonelli*		*C. suffruticosa* subsp. *carbonelli*	Cs_car	32	3.18	0.08	2.45%
	C. suffruticosa subsp. *greuteri*		*C. suffruticosa* subsp. *greuteri*	Cs_greu	32	3.36	0.10	3.02%
	C. suffruticosa subsp. *lusitanica*	*C. suffruticosa* subsp. *lusitanica*	*C. suffruticosa* subsp. *lusitanica*	Cs_lus	32	3.38	0.08	2.37%
			C. suffruticosa subsp. *trialata* **	Cs_tri	32	3.23	0.10	2.99%
			C. suffruticosa subsp. *marginata**	Cs_mar	32	3.09	0.05	1.52%
			C. suffruticosa subsp. *vejerensis***	Cs_vej	32	3.22	0.07	2,.24%
	***C. incana* group**							
	C. incana subsp. *incana* var. *incana*	*C. suffruticosa* subsp. *tomentosa*	*C. suffruticosa* subsp. *tomentosa**	Cs_tom	32	3.31	0.08	2.42%
	C. incana subsp. *algarbiensis* var. *cinerea*		*C. suffruticosa* subsp. *cinerea**	Cs_cin	32	3.09	0.11	3.42%
	C. incana subsp. *algarbiensis* var. *algarbiensis*	*C. suffruticosa* subsp. *algarbiensis*	*C. suffruticosa* subsp. *algarbiensis*	Cs_alg	32	3.18	0.14	4.40%
	C. incana subsp. *algarbiensis* var. *prostrata*							
	C. incana subsp. *microphylla*							

* nomenclatural changes; ** new taxa described.

The most relevant and complete studies on chromosome numbers were made by Ohle (1966, 1974, 1975a, 1975b), and Heyn *et al.* (1974). Different taxonomic treatments, chromosome numbers and genome size in *Calendula* taxa from Iberian Peninsula are summarised in Table 7.

In a more recent contribution, Nora *et al.* (2013) confirmed the chromosome number of 11 taxa occurring in the Iberian Peninsula. This karyological data on *Calendula* agrees with previous studies in showing $2n$ = 44 for *C. arvensis*, $2n$ = 30 for *C. tripterocarpa* and $2n$ = 32 for the remaining wild Iberian taxa. This study assessed for the first time the genome size of *Calendula* species, ranging from 1.75 pg for *C. maroccana* subsp. *maroccana* to 5.41 pg for *C. arvensis*. The mean value of the available data were 3.20 pg (Garnatje *et al.* 2011, Nora *et al.* 2013).

Genome size and chromosome number are positively correlated, and the finding of a fairly continuous gradient in genome size among all taxa with $2n$ = 32 chromosomes, previously split between *C. incana* and *C. suffruticosa* by Ohle (1974, 1975a, 1975b), further supported our decision to join these groups (Nora et al. 2013), as previously proposed by Meikle (1976b). However, morphological characters, especially of the achenes, support the recognition of some of the subspecies proposed by Ohle (1974, 1975a, 1975b), and the description of two new subspecies.

Geographic distribution, endemism and ecology

In the Iberian Peninsula, *Calendula* comprises four species: *C. arvensis*, *C. officinalis*, *C. suffruticosa* and *C. tripterocarpa*, which grow in a variety of habitats, from sea level to above 1400 m of elevation (Figure 15). *C. arvensis* and *C. officinalis* are fairly dispersed throughout the territory, while *C. suffruticosa* is present in the littoral of the North-West, West and South Iberian Peninsula. *C. tripterocarpa* is restricted to Almeria, Murcia and Alicante (Spain) (Figure 14 and 16).

C. arvensis is a rather ubiquitous species, found in waste grounds and ruderal places. *C. officinalis* is an ornamental and medicinal plant, of unknown origin, usually cultivated, but sometimes escaped and/or naturalised in several regions. Both species are found in a wide elevation range from sea level to *ca.* 1400 m. The higher elevations of populations of *C. officinalis* when compared to *C. arvensis*, is probably due to a sampling artefact, because, since the former species is cultivated, it is often under collected. *C.*

tripterocarpa grows in grasslands, in semi-desert to desert regions, mostly at low altitudes (bellow 400 m elev.).

C. suffruticosa comprises nine infraspecific taxa, three from Portugal (subsp. *algarbiensis*, subsp. *cinerea*, and subsp. *lusitanica*), and six from Spain (subsp. *carbonelli*, subsp. *greuteri*, subsp. *marginata*, subsp. *tomentosa*, subsp. *trialata*, and subsp. *vejerensis*). These plants occur in limestone soils, sandy loams and dunes, near the sea, from 0 to 200 m of elevation (e.g. subsp. *algarbiensis* and subsp. *trialata*), but also in limestone cliffs, hills and coastal canyons, up to 1400 m of elevation (e.g. subsp. *greuteri* and subsp. *lusitanica*). Two taxa showed low elevation range because they are restricted, endemic and poorly represented taxa (namely, subsp. *cinerea* and subsp. *vejerensis*).

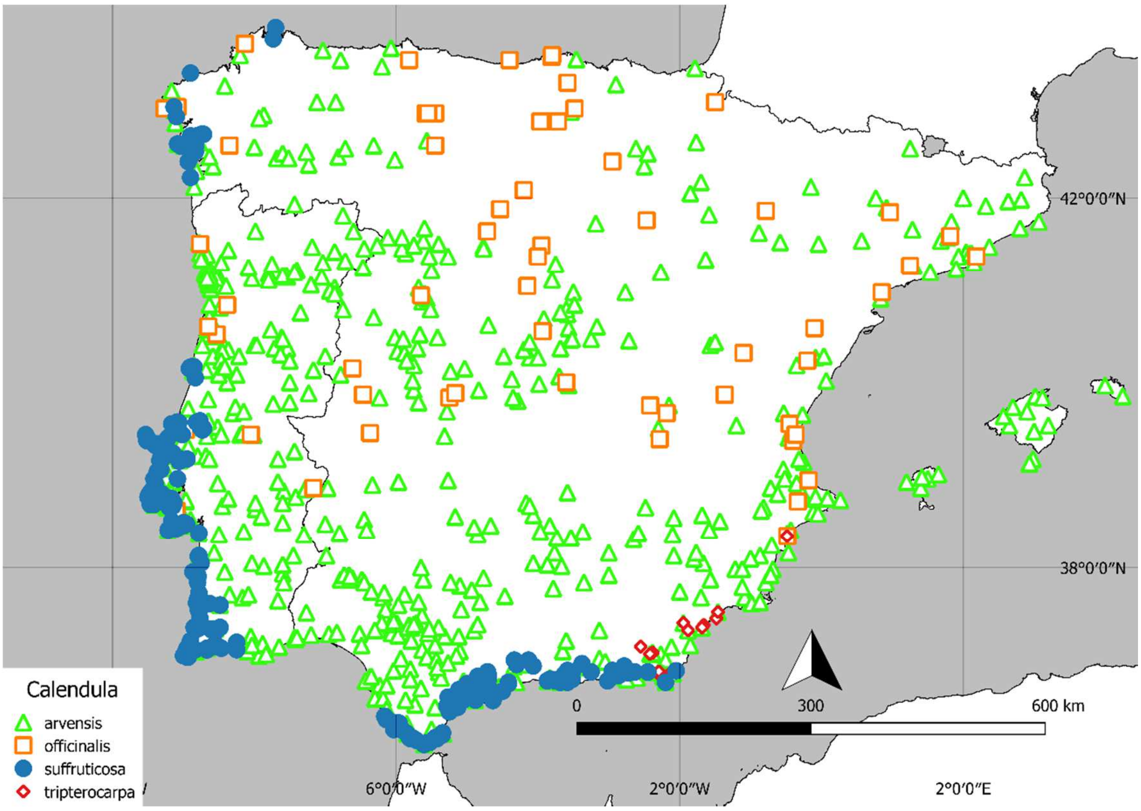


Figure 14 – Geographic distribution of *Calendula* specimens in the Iberian Peninsula and the Balearic Islands.

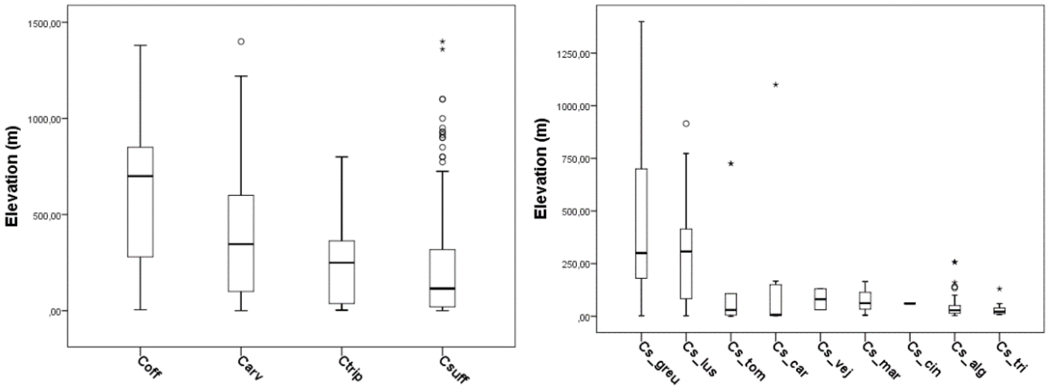


Figure 15 – Boxplot representing the elevation breadth of *Calendula* in the Iberian Peninsula and the Balearic Islands; A) *Calendula* species; B) *C. suffruticosa* subspecies.

Species richness

The South of Spain (Cádiz province) seems to represent the centre of diversity of the genus in the Iberian Peninsula since it presents the highest number of taxa per grid cell, i.e. five of the 12 recognised taxa occur in this region (Figure 14). This area corresponds well with the Northern section of *Calendula*'s 'centre of plant diversity and evolution' as mapped by Norlindh (1946) and agrees with a possible migration from the area where the genus has been proposed to have originated, in the SW Mediterranean (Heyn *et al.* 1974), via the area where the Strait of Gibraltar is nowadays located, during the Messinian salinity crisis (Ryan 2009)

Across most of the Iberian Peninsula, *Calendula* is represented by one or two taxa, only, corresponding to the widespread *C. arvensis* and/or *C. officinalis*. These distribution patterns also indicate that most of the subspecies of *C. suffruticosa* are distributed along the Western littoral of Galicia, Portugal and Southern Spain, where grid cells with three or more taxa are detected.

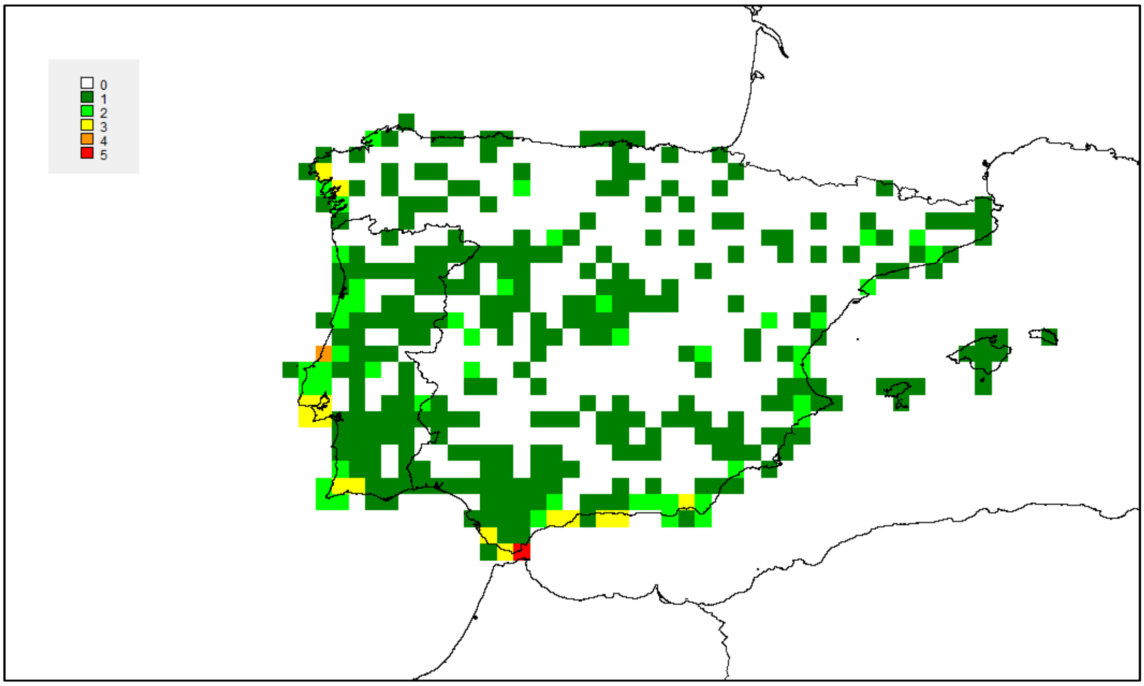


Figure 16 – Taxa richness (nº of taxa/grid cell) of *Calendula* in the Iberian Peninsula and Balearic Islands (mapped in 0.3 × 0.3° grid cells).

Conservation assessments

The only two species of this genus known to have been previously assessed under the IUCN criteria are *C. maderensis* and *C. maritima* (v. www.iucnredlist.org). The first one was assessed as Least Concern (LC), while the latter was assessed as Critically Endangered (CR). We now present conservation assessments for the Iberian taxa, including the subspecies of *C. suffruticosa*, since according with the IUCN Red List Guidelines (IUCN 2017), their criteria may be applied to any taxonomic unit at or below the species level. Altogether, six taxa were assessed as threatened (EN: five taxa; VU: one taxa) or of least concern (LC: five taxa) based on their distribution ranges (the EOO and AOO) and population trends. The cultivated *C. officinalis* was not evaluated. The proportion of threatened taxa was particularly high (50% of the assessed taxa studied). Among those, three taxa deserve special attention since they are restricted to one or two populations. On the contrary, *C. arvensis*, for example, is commonly found in ruderal and waste grounds and is widely distributed across the circum-Mediterranean region. Therefore, it was assessed as LC.

Morphometric analysis

A morphometric study was conducted including 12 taxa from the Iberian Peninsula and the Balearic Islands, whose collection sites are given in APPENDIX I. To interpret the results and re-access their taxonomic positions, several groups of taxa were formed for species and for *C. suffruticosa* subspecies. As described above, quantitative and qualitative characters were used in conjunction to perform the Principal Component Analysis (see below) and were used to build the keys for species and subspecies.

Variability of *Calendula* species

The analysis of variance performed on 23 quantitative characters showed significant variation among species ($P < 0.001$), except for bialate achene length, trialate achene length and trialate achene width (Table 8). However, in many cases, when there are no significant differences in the dimensions of these achenes, there are differences in shape, i.e. in qualitative characters, which can support the differentiation between taxa in the dichotomous keys. The dimensions of the sub-exalate and sub-cymbiform achenes were only determined for those taxa that produce such achenes, i.e. the subspecies of *C. suffruticosa*. Boxplots showing the variability of 23 quantitative characters for the *Calendula* species are presented in Figure 17, 18, 19 and 20. Characters that appear in a single taxon were not represented in a boxplot, but are provided APPENDIX III.

In the PCA for the species, 120 *Calendula* specimens of Iberian Peninsula were analysed (Figure 21 and 22). The first three components accounted for 44.31% of the total variation (APPENDIX II–A). The first principal component represented 20.77% of the variance, followed by 13.59% for the second component and 9.95% for the third. The characters vermiform-alate achene length (VAL), cymbiform achene length (CL), vermiform-alate achene width (VAW), trialate achene length (TL), trialate achene width (TW) and trialate achene ventral wing (Tvw) were the most influential for the first component. Therefore, PC1 distinguished taxa mainly based on distinct achene characters (Figure 21 and 22). For the second component, the highest loadings included ray florets colour (RF), rostrate achene dorsal spines (RS), ratio leaf length/width (R2), life cycle (LF), head diameter (HD) and ligule length (LG), which are important for the distinction between annual and perennial plants. For the third component, the highest loadings included sub-exalate achene length (SEL), sub-exalate achene width (SEW), sub-exalate achene lateral wing (SElw), bialate achene length (BL), bialate achene width (BW) and bialate achene lateral wing (Blw), once again giving relevance to achene morphology. Complete component loadings are provided in APPENDIX II.

Table 8 – Univariate statistical analysis of *Calendula* species from Iberian Peninsula

Morphological trait	**d.f.**	**statistical test**	***P***
Length of the longest branch	3	$H = 62.875$	0.001
Basal leaf length	3	$H = 68.457$	0.001
Basal leaf width	3	$H = 88.839$	0.001
Basal leaf distance from base to point of maximum width	3	$H = 77.932$	0.001
Ratio leaf length/point of maximum width	3	$H = 52.289$	0.001
Ratio leaf length/width	3	$H = 47.814$	0.001
Basal leaf thickness	3	$H = 29.203$	0.001
Head diameter	3	$H = 78.49$	0.001
Involucre length	3	$H = 47.244$	0.001
Ligule length	3	$H = 51.198$	0.001
Ratio ligule/involucre	3	$F = 19.038$	0.001
Rostrate achene length	2	$H = 28.825$	0.001
Rostrate achene width	2	$H = 50.838$	0.001
Bialate achene length	12*74	$T = 385.50$	0.089
Bialate achene width	84	$t = -5.429$	0.001
Trialate achene length	12*50	$T = 277.000$	0.070
Trialate achene width	60	$t = -0.117$	0.907
Cymbiform achene length	3	$H = 55.141$	0.001
Cymbiform achene width	3	$H = 34.169$	0.001
Vermiform-alate achene length	2	$H = 56.138$	0.001
Vermiform-alate achene width	2	$H = 26.971$	0.001
Vermiform-exalate achene length	3	$H = 72.568$	0.001
Vermiform-exalate achene width	3	$H = 78.208$	0.001

One-way ANOVA and t-test: F and t, respectively, for characters with normal distributions. Kruskal-Wallis One-way ANOVA on ranks and Mann-Whitney test: H and T, respectively, for characters with non-normal distributions. d.f. – degrees of freedom.

Table 9 – Univariate statistical analysis of *C. suffruticosa* subspecies in the Iberian Peninsula.

Morphological trait	**d.f.**	**statistical test**	***P***
Length of the longest branch	8	$H = 21.483$	0.006
Basal leaf length	8	$H = 214.059$	0.001
Basal leaf width	8	$H = 158.128$	0.001
Basal leaf distance from base to point of maximum width	8	$H = 196.947$	0.001
Ratio leaf length/point of maximum width	8	$H = 88.179$	0.001
Ratio leaf length/width	8	$H = 168.43$	0.001
Basal leaf thickness	8	$H = 174.008$	0.001
Head diameter	8	$H = 90.026$	0.001
Involucre length	8	$F = 38.56$	0.001
Ligule length	8	$H = 54.975$	0.001
Ratio ligule length/involucre length	8	$H = 14.237$	0.076
Rostrate achene length	8	$H = 88.215$	0.001
Rostrate achene width	8	$H = 96.683$	0.001
Bialate achene length	3	$F = 9.254$	0.001
Bialate achene width	3	$F = 8.342$	0.001
Bialate achene rostrum	4	$H = 18.736$	0.001
Cymbiform achene length	6	$H = 88.007$	0.001
Cymbiform achene width	6	$H = 70.668$	0.001
Sub-cymbiform achene length	5	$H = 77.154$	0.001
Sub-cymbiform achene width	5	$H = 57.106$	0.001
Vermiform-alate achene length	5	$H = 23.152$	0.001
Vermiform-alate achene width	5	$H = 71.187$	0.001
Vermiform-exalate achene length	8	$H = 93.497$	0.001
Vermiform-exalate achene width	8	$H = 69.225$	0.001

One-way ANOVA: F for characters with normal distributions. Kruskal-Wallis One-way ANOVA on ranks: H for characters with non-normal distributions. d.f. – degrees of freedom.

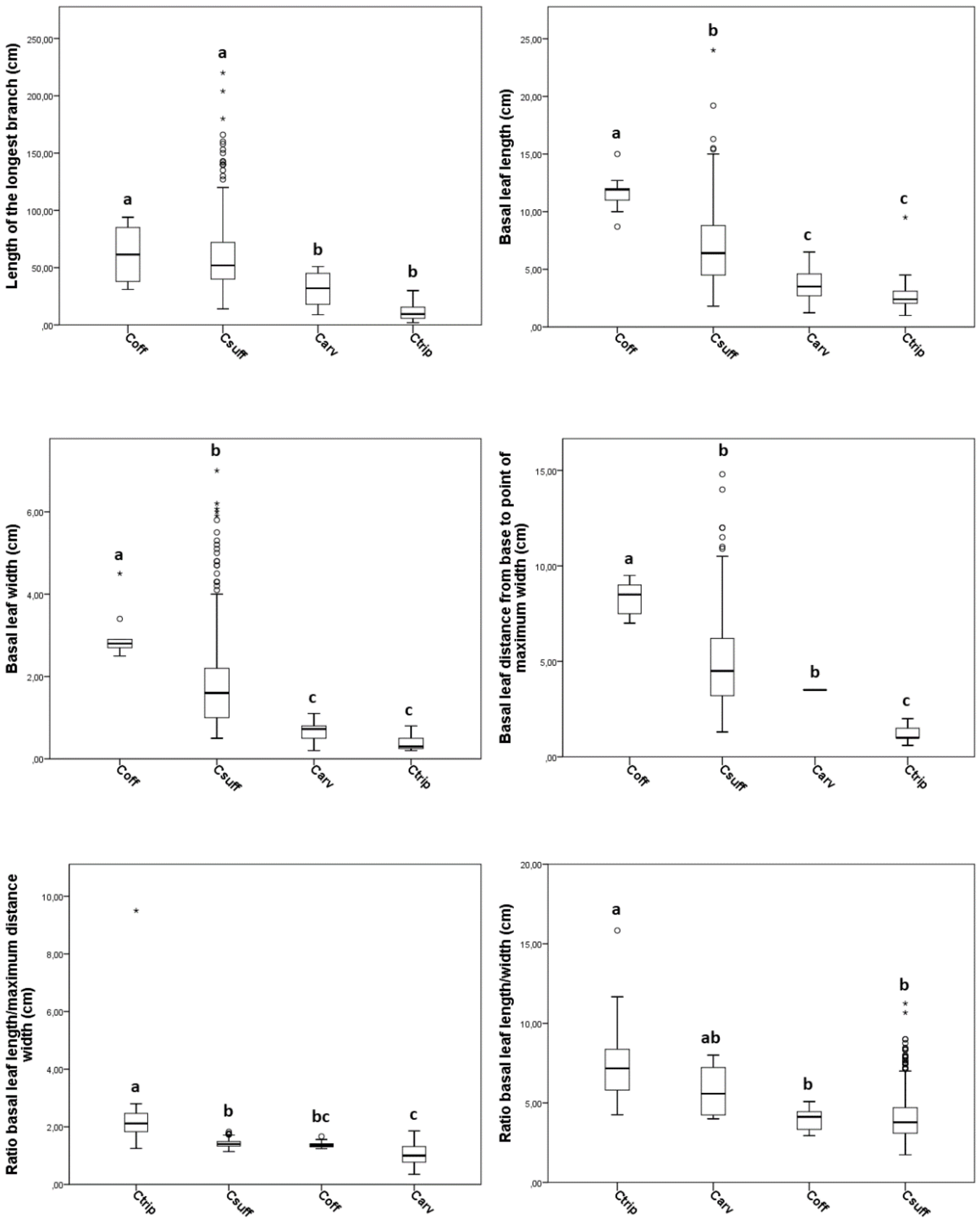


Figure 17 – Boxplots representing the variability of quantitative characters in *Calendula* species. Arithmetic median (lines), 25%--75% (boxes), minimum and maximum values (whiskers). Different letters reveal statistical differences at $P < 0.05$.

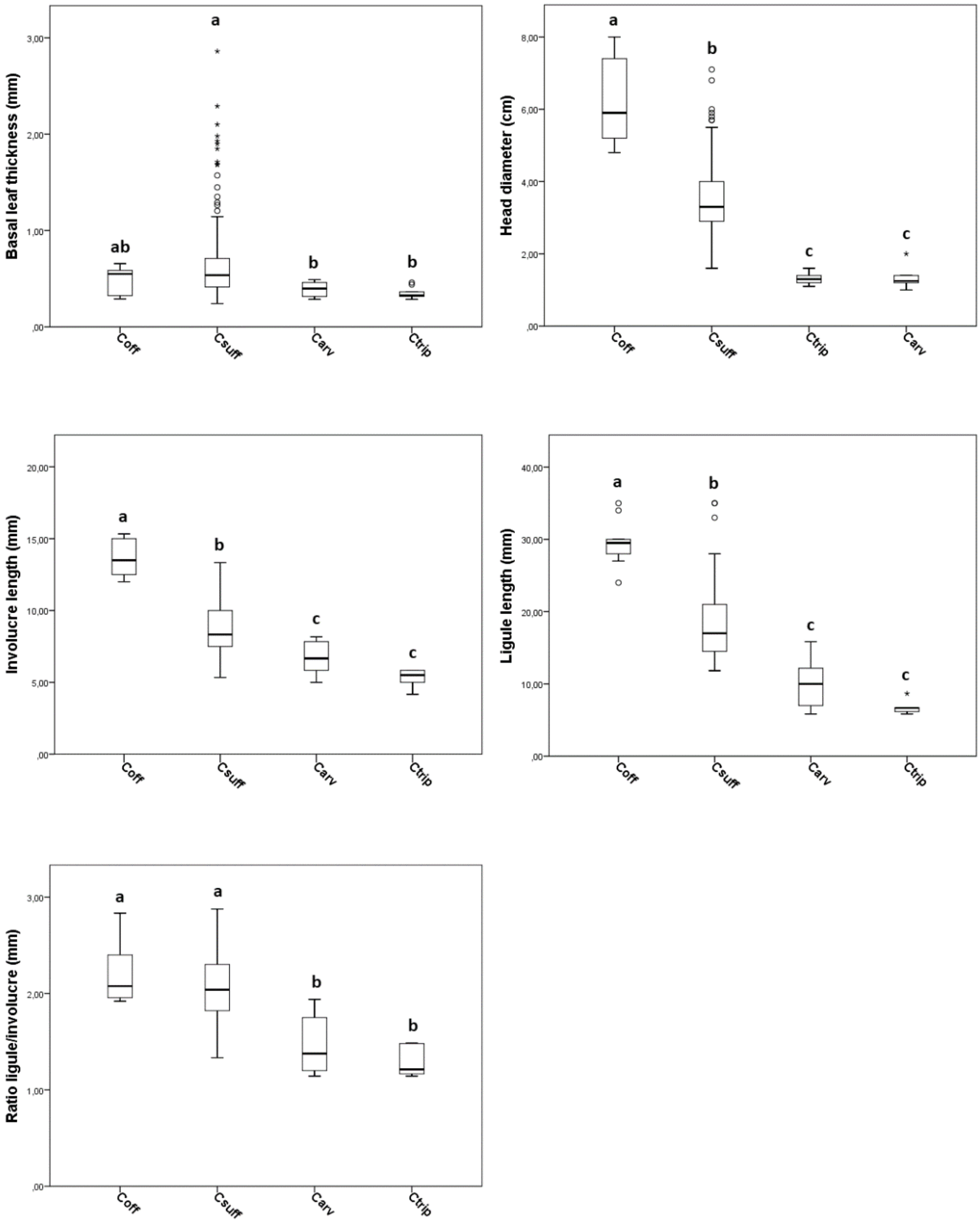


Figure 18 – Boxplots representing the variability of quantitative characters in *Calendula* species. Arithmetic median (lines), 25%--75% (boxes), minimum and maximum values (whiskers). Different letters reveal statistical differences at $P < 0.05$.

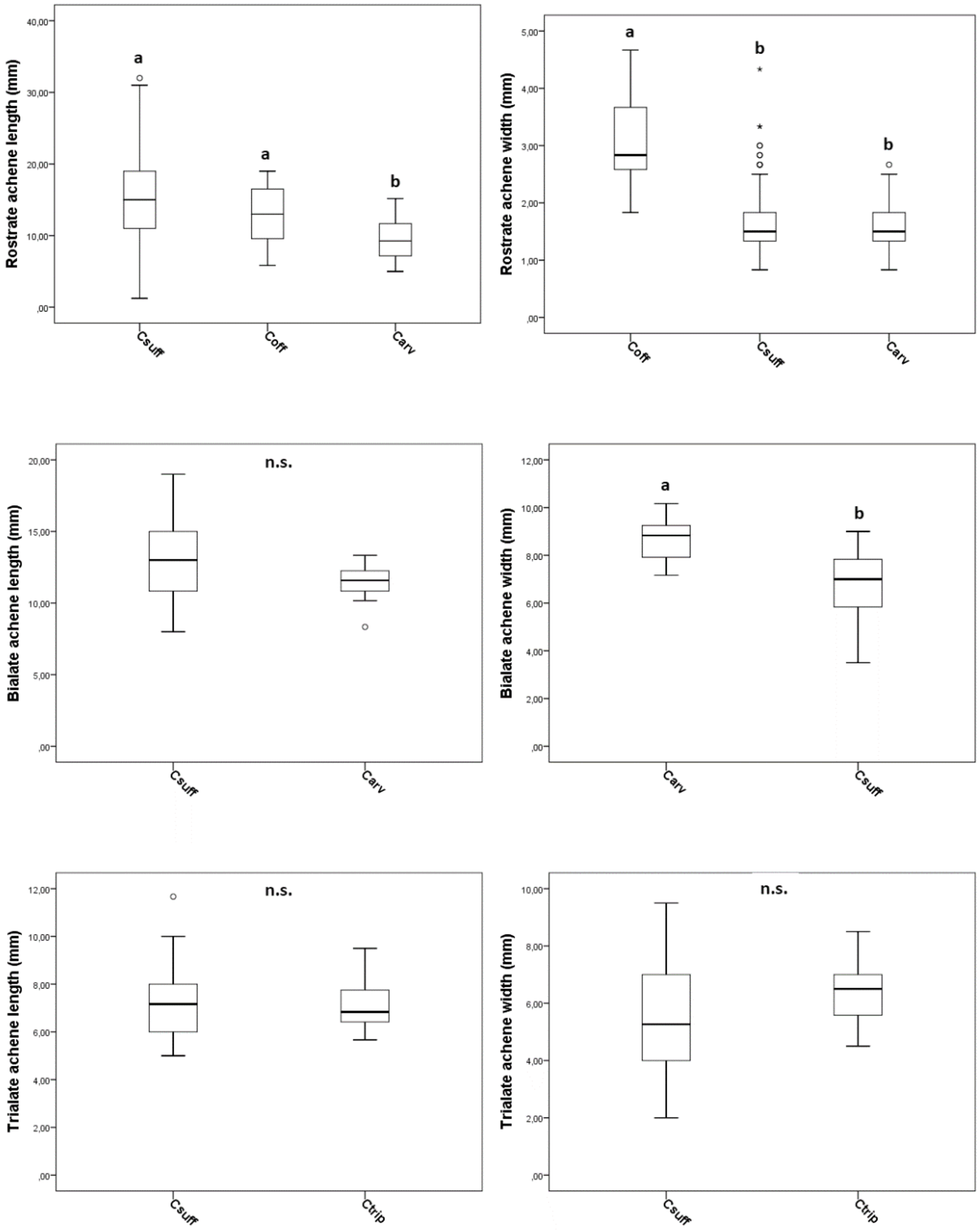


Figure 19 – Boxplots representing the fruit variability of quantitative characters in *Calendula* species. Arithmetic median (lines), 25%--75% (boxes), minimum and maximum values (whiskers). Different letters reveal statistical differences at $P < 0.05$; n.s. denotes non-significant differences.

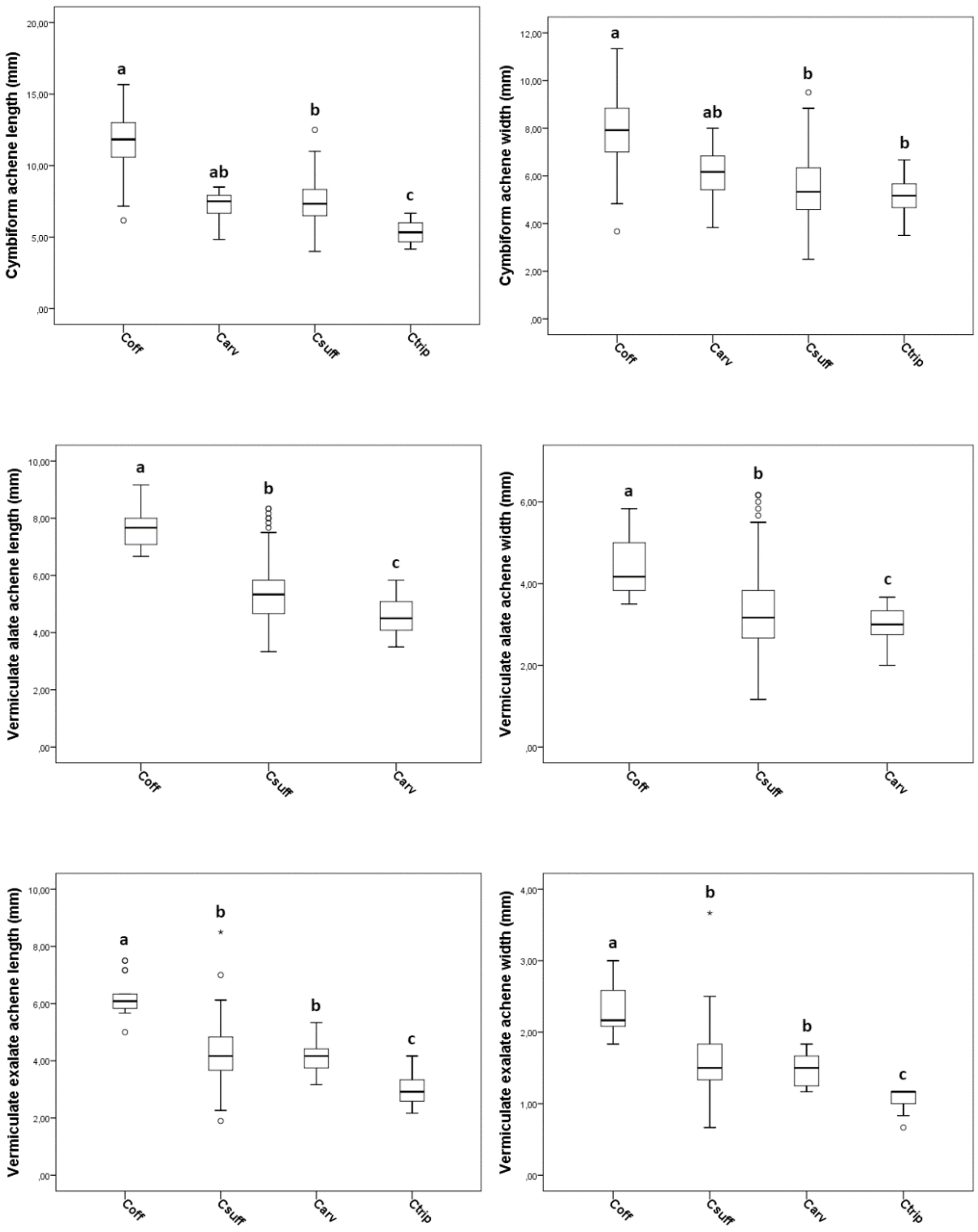


Figure 20 – Boxplots representing the fruit variability of quantitative characters in *Calendula* species. Arithmetic median (lines), 25%--75% (boxes), minimum and maximum values (whiskers). Different letters reveal statistical differences at $P < 0.05$.

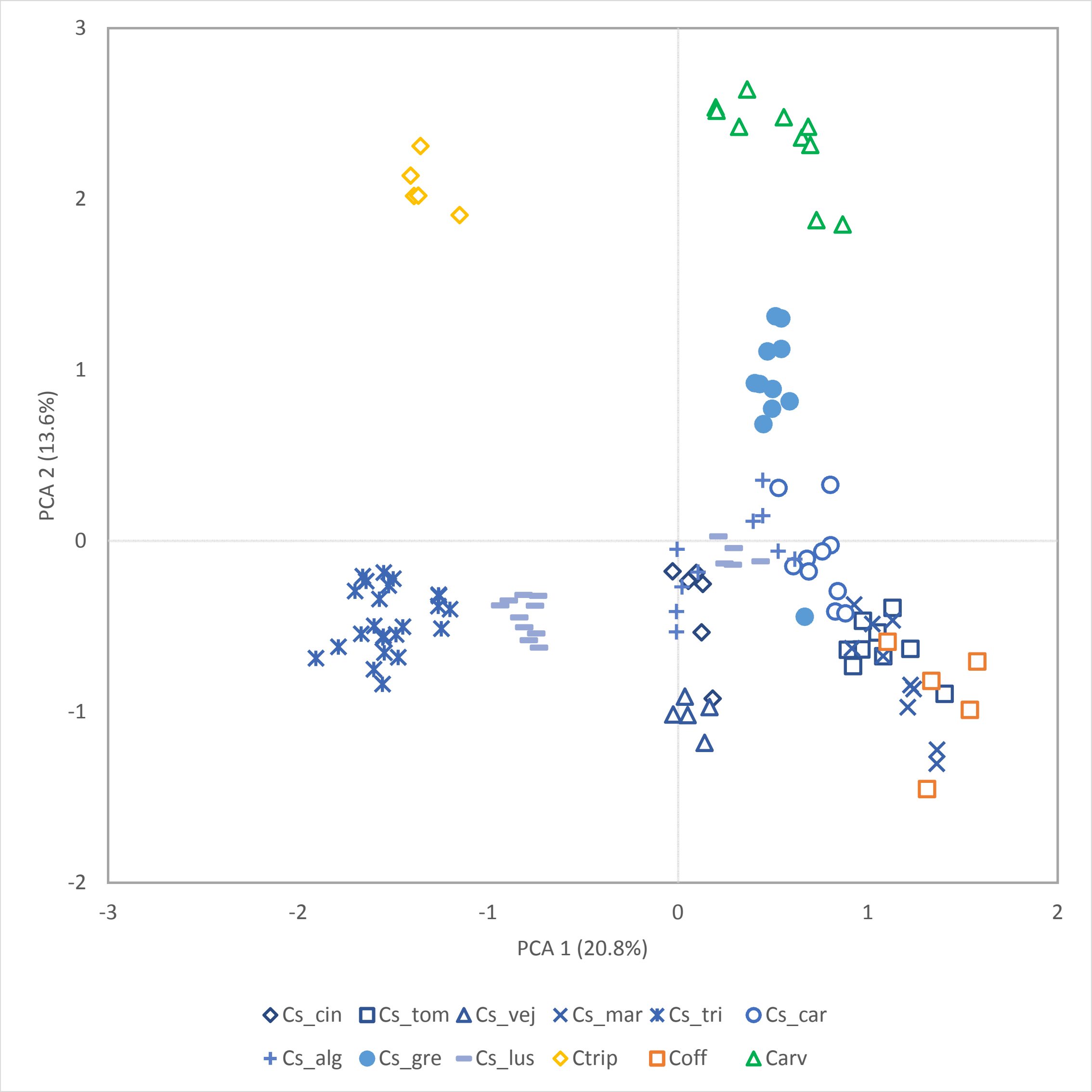


Figure 21 – Two-dimensional ordination diagram of PC 1x2 based on 57 morphological characters of 120 *Calendula* specimens.

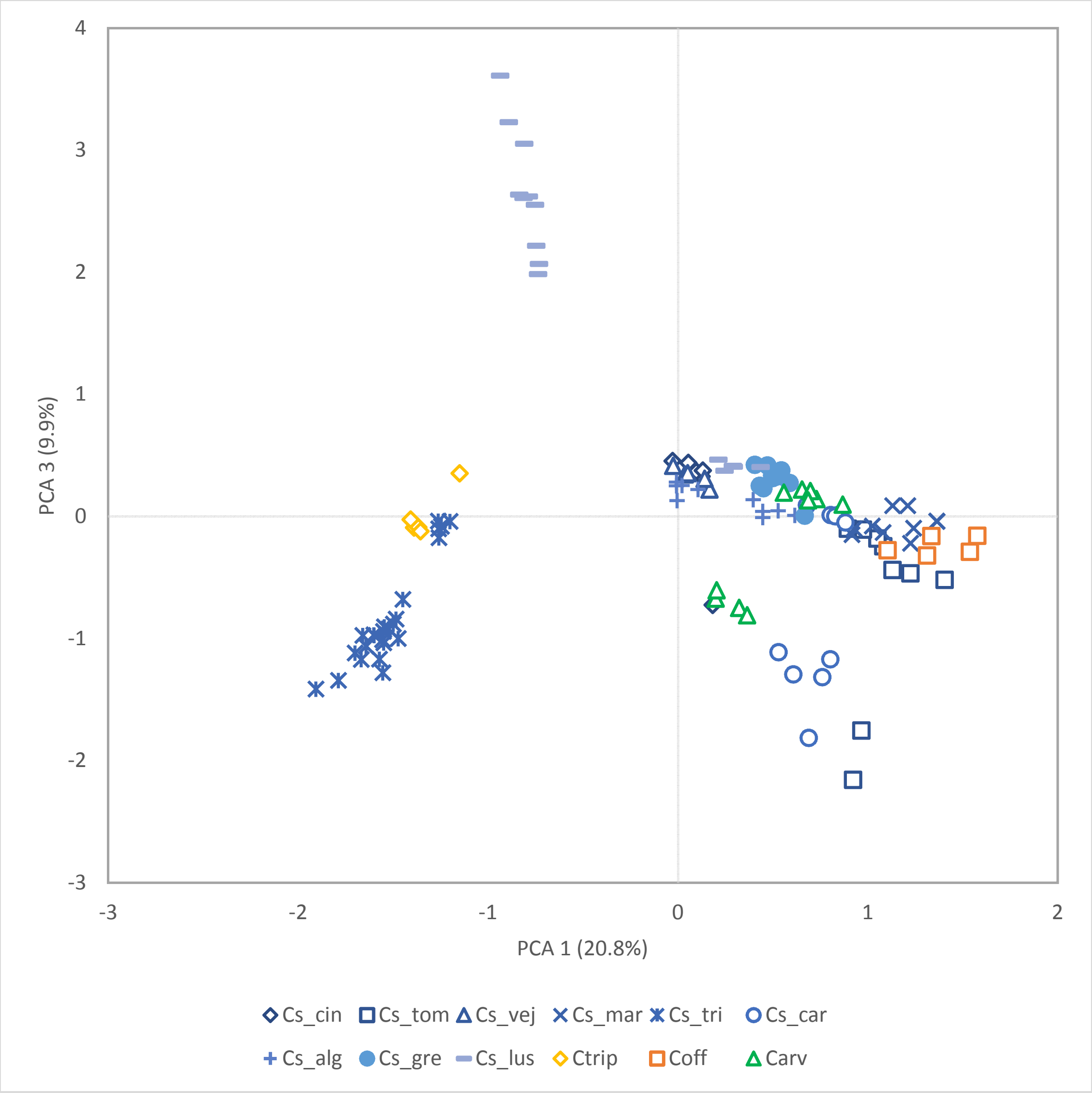


Figure 22 – Two-dimensional ordination diagram of PC 1x3 based on 57 morphological characters of 120 *Calendula* specimens.

Taxonomical implications for *Calendula* species

The character ratio ligule length/involucre length was significantly different at 99% in the ANOVA (Table 8) and was used in step 1 of the identification key for the species of *Calendula* provided in the taxonomic treatment section. This step is also supported by the qualitative character life cycle. Both characters were used by previous authors (Meikle 1976, Ohle 1974), but our data suggest that the relationship between ligule length and involucre length is 1.8 times, instead of twice. Steps 2 and 3, of the same key, were based on qualitative characters, in addition to the quantitative character ligule length, which was significantly different in the Kruskal-Wallis One-way ANOVA on ranks at 99%.

The PCA for species shows four major groups within *Calendula* (Figure 21 and 22). The major important characters for this separation were related to achene morphology. *C. arvensis* and *C. tripterocarpa* form two distinct clusters separated from the remaining species. They can be differentiated from each other by the presence of rostrate/bialate and vermiform-alate versus trialate achenes, respectively. Both can be distinguished from *C. suffruticosa* and *C. officinalis* by life form and traits related to the capitula (ray florets' colour - RF, head diameter - HD, and ligule length - LG). A partial overlap occurs between *C. suffruticosa* and *C. officinalis*. The latter is a cultivated species, presenting the same chromosome number ($2n = 32$) as *C. suffruticosa*, from which, however, it can be easily distinguished by higher inflorescence and flower dimensions, like head diameter (HD), involucre length (IL), ligule length (LG), all significantly bigger in *C. officinalis* when compared with *C. suffruticosa* ($P < 0.001$), and by various qualitative characters as depicted in step 3 of the key.

For the annuals, the classification proposed by Heyn *et al.* (1974) was followed in this study, i.e. all *Calendula* plants with 44 chromosomes and different morphologies (e.g. with concolorous and discolorous capitula, or rostrate and bialate achenes) were included in *C. arvensis*. On the contrary, plants with 30 chromosomes and trialate achenes were included in *C. tripterocarpa.*

C. arvensis is an extremely polymorphic species, which has a broad spectrum of intermediate forms (Heyn, Dagan, and Nachman 1974). For example, in the Iberian Peninsula, two distinct subspecies within *C. arvensis*, subsp. *arvensis* (with the external achenes rostrate with long beaks and exalate), and subsp. *macroptera* (with the external achenes bialate with shorter rostrum and wide toothed lateral wings) have been considered by some authors (Rouy 1903, Knoche 1922, Gallego & Talavera 1983; Blanca *et al.* 2011). The PCA with all taxa (Figure 21 and 22) distinguishes these morphs as two distinct groups. However, Heyn *et al.* (1974) suggested that these kinds of forms should not be taxonomically recognized, since *C. arvensis* is a variable species. Baltisberger & Widmer (2006), also confirmed that this species is variable and that this variability has no taxonomic value. Furthermore, we observed that both morphs frequently co-exist in the same population.

For the perennials, a mixed approach was used. Ohle (1974) recognised three perennial species of *Calendula* in the Southern Europe. According to this author, the *C. suffruticosa* group is characterised by having basal leaves narrowly lanceolate or ±

narrowly spatulate, mostly acuminate, ± thin, while the *C. incana* group is characterised by having basal leaves broad-spatulate to oblanceolate, edgeless, and dull or emarginated, mostly thick or thick-fleshy. These two groups (*C. incana* and *C. suffruticosa*) described by Ohle (1974) correspond in our opinion to one single taxon, which, due to nomenclatural priority, should be assigned to *C. suffruticosa*.

Results from our morphometric analyses show that taxa previously included in *C. incana* and *C. suffruticosa* are not consistently separated in the PCA with all taxa nor on the PCAs dedicated to *C. suffruticosa* specimens alone. The genome size analyses (Nora *et al.*, 20013) also do not support the segregation of these groups. Therefore, we agree with Meikle's (1976b) treatment in subsuming all wild *Calendula* taxa with $2n = 32$ under *C. suffruticosa* Vahl. However, we consider that the morphological variability of this species, especially of the achenes, is greater than that described by this author, and several subspecies deserve recognition.

Variability of *C. suffruticosa* subspecies

The univariate statistical analyses performed on 24 quantitative characters showed significant variation among subspecies of *C. suffruticosa* for all characters, except for ratio ligule length /involucre length (Table 9). However, this character was not important within *C. suffruticosa*, being used only to distinguish species. Furthermore, some achene traits were not included in this analysis because they occurred only in one subspecies, namely, sub-exalate achenes only occur in *C. suffruticosa* subsp. *lusitanica*, trialate achenes only occur in *C. suffruticosa* subsp. *trialata*, and sub-cymbiform achenes rostrum length only in *C. suffruticosa* subsp. *greuteri*. Boxplots showing the variability for subspecies are shown in Figure 23, 24, 25 and 26.

To make the multivariate analysis using PCA easier, *C. suffruticosa* taxa were grouped in: (1) plants with white-arachnoid indumentum and (2) plants without white-arachnoid indumentum.

1. **Plants with white-arachnoid indumentum**. This PCA was performed with 41 variables of 30 specimens of *C. suffruticosa* with white-arachnoid pubescence (Figure 27 and 28). The first three principal components accounted for 62.49% of the total variation (APPENDIX II–B). The first principal component represented 26.82% of the variance, followed by 23.58% for the second component and

12.09% for the third. The first principal component presented higher positive loadings for VAL, VAs and LG, contrasting with stronger negative loadings for R2, LT and SCvw. The second component had higher positive loadings for Cvt, SCdw and SCL, while stronger negative loadings for Cvw, LD and RW. The third component presented higher positive loadings for VEs, CW and Cc, while most negative loadings for SCvw, SClw and IL. Component loadings are presented in APPENDIX II–B.

2. **Plants with non-white-arachnoid indumentum**. This PCA was performed with 54 variables of 70 specimens of *C. suffruticosa* with non-white-arachnoid pubescence (Figure 30). The first three principal components accounted for 52.83% of the total variation (APPENDIX II–C). The first principal component represented 27.11% of the variance, followed by 14.14% for the second component and 11.59% for the third component. The first principal component has higher positive loadings for VAvt, Cvw and VAs, contrasting with higher negative loadings for Tvw, TL and TW. The second component had higher loadings for SEL, SEW and SElw, while higher negative loadings for BL, Blw and BW. The third component presented higher positive loadings for LF, VEL and VEW, while higher negative loadings for all characters related with sub-cymbiform achene's morphology. Component loadings are presented in APPENDIX II–C .

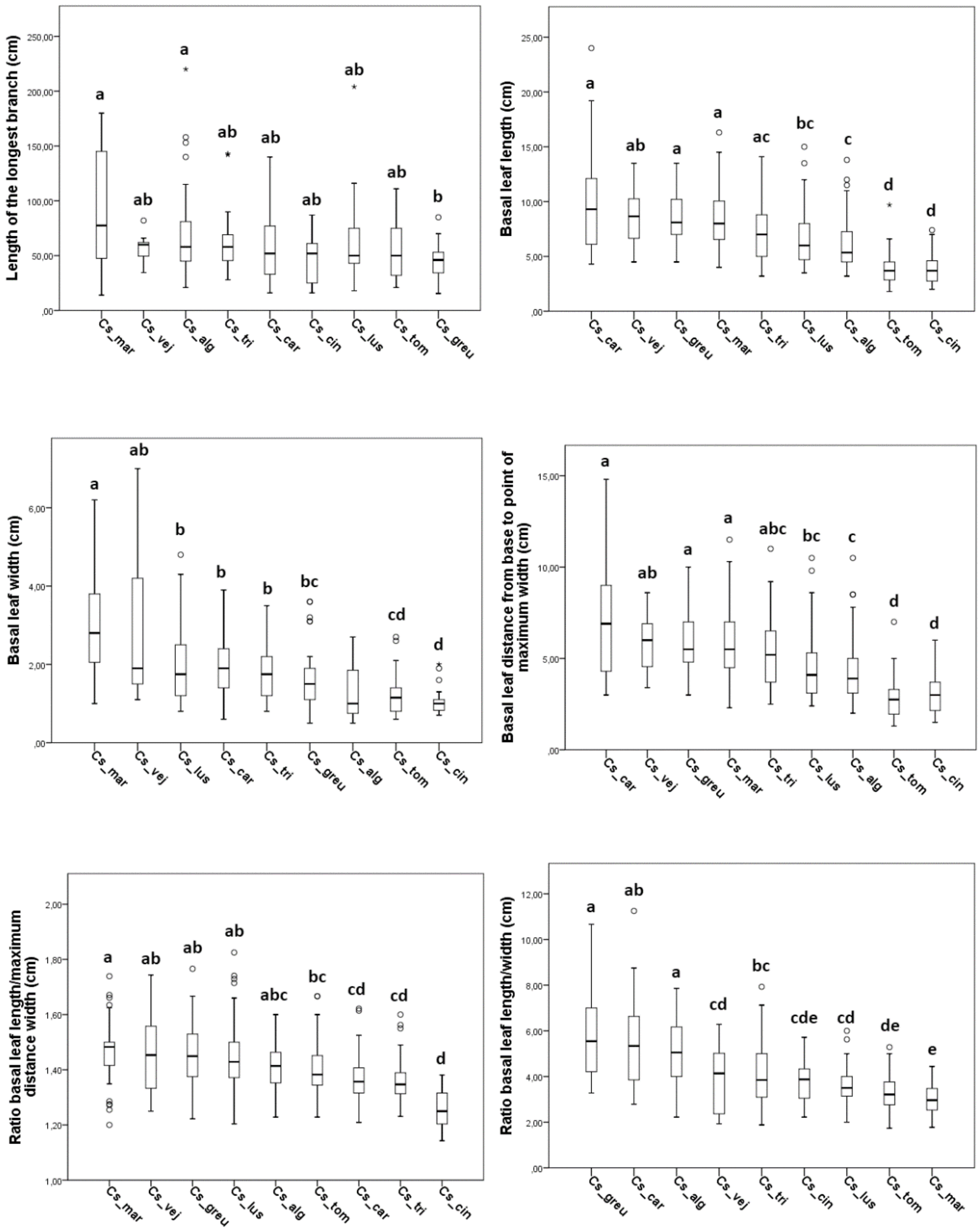


Figure 23 – Boxplots representing the variability of quantitative characters in *C. suffruticosa* subspecies. Arithmetic means (lines), 25%--75% (boxes), minimum and maximum values (whiskers). Different letters reveal statistical differences at $P < 0.05$.

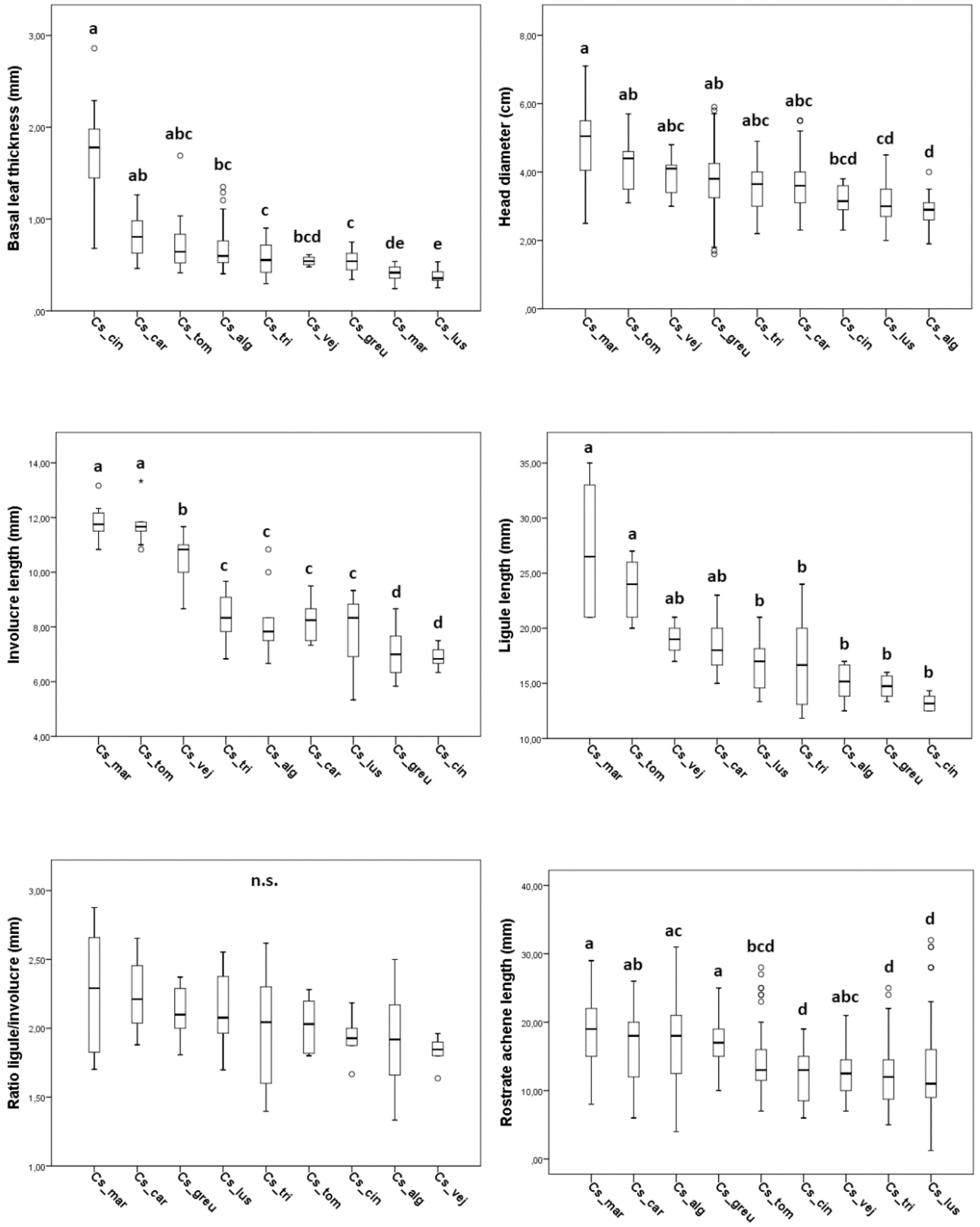


Figure 24 – Boxplots representing the variability of quantitative characters in *C. suffruticosa* subspecies. Arithmetic median (lines), 25%--75% (boxes), minimum and maximum values (whiskers). Different letters reveal statistical differences at $P < 0.05$; n.s. denotes non-significant differences.

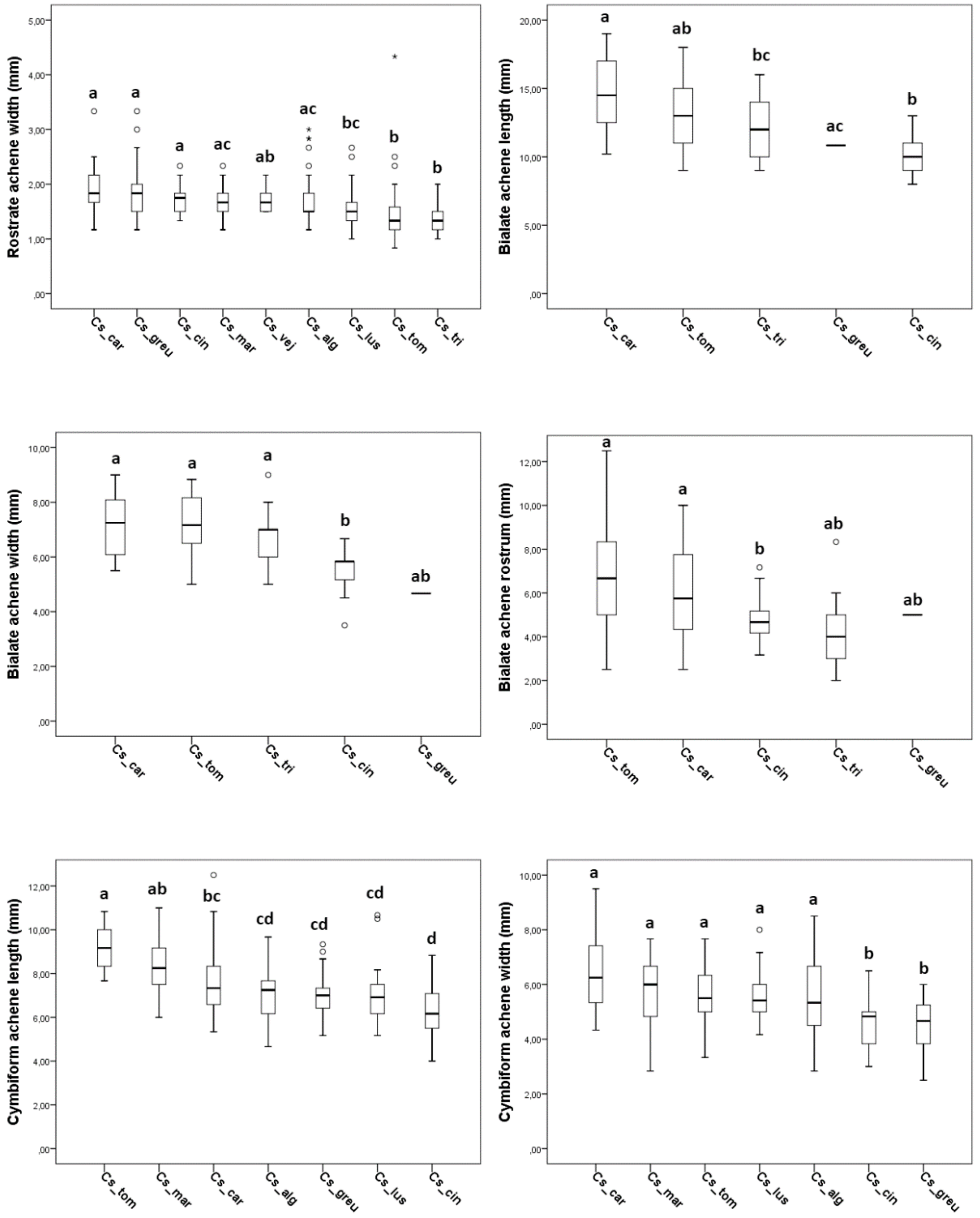


Figure 25 – Boxplots representing the variability of quantitative characters in *C. suffruticosa* subspecies. Arithmetic median (lines), 25%--75% (boxes), minimum and maximum values (whiskers). Different letters reveal statistical differences at $P < 0.05$.

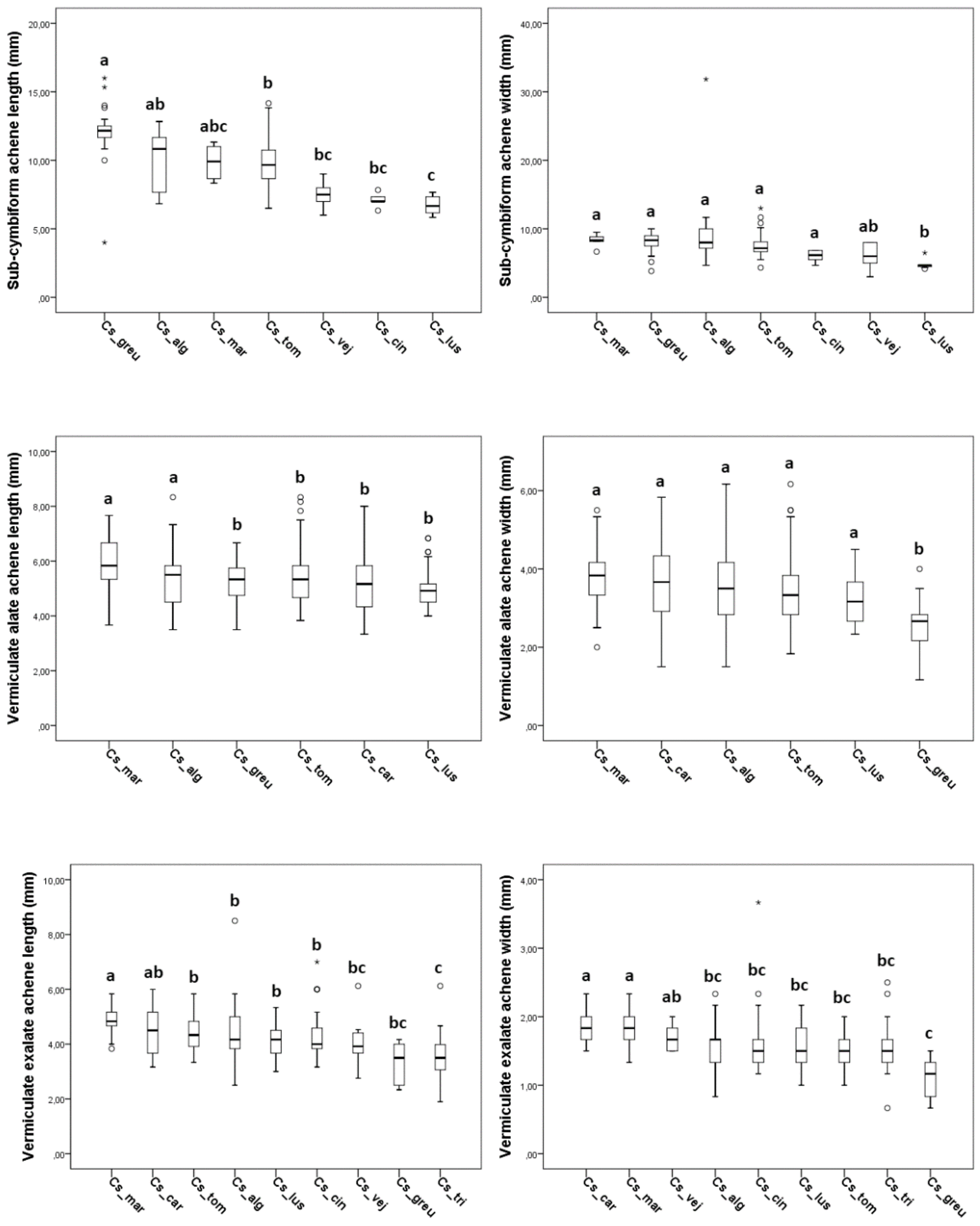


Figure 26 – Boxplots representing the variability of quantitative characters in *C. suffruticosa* subspecies. Arithmetic median (lines), 25%--75% (boxes), minimum and maximum values (whiskers). Different letters reveal statistical differences at $P < 0.05$.

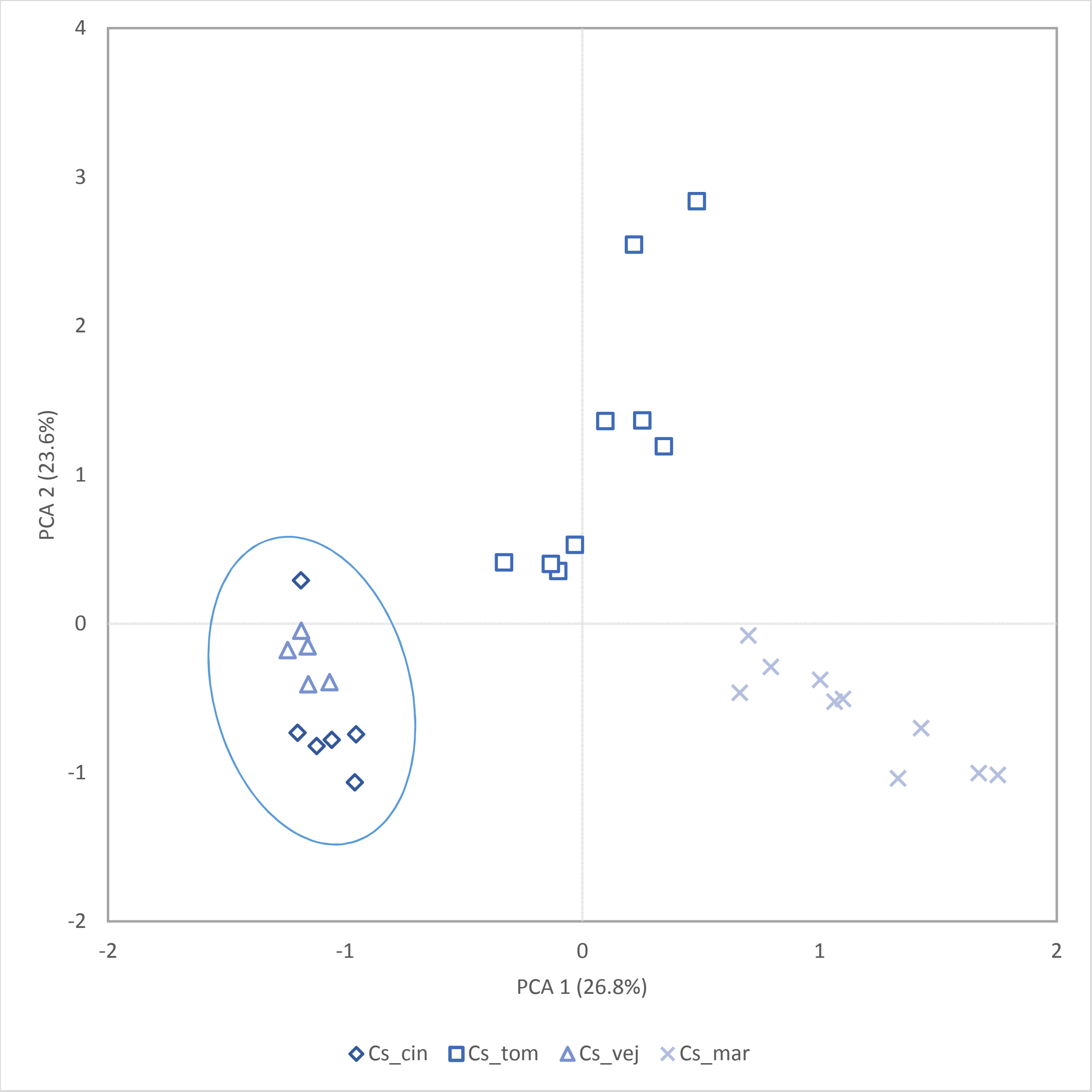


Figure 27 – Two-dimensional ordination diagram of PC 1x2 based on 41 morphological characters of 30 *C. suffruticosa* specimens with white-arachnoid indumentum.

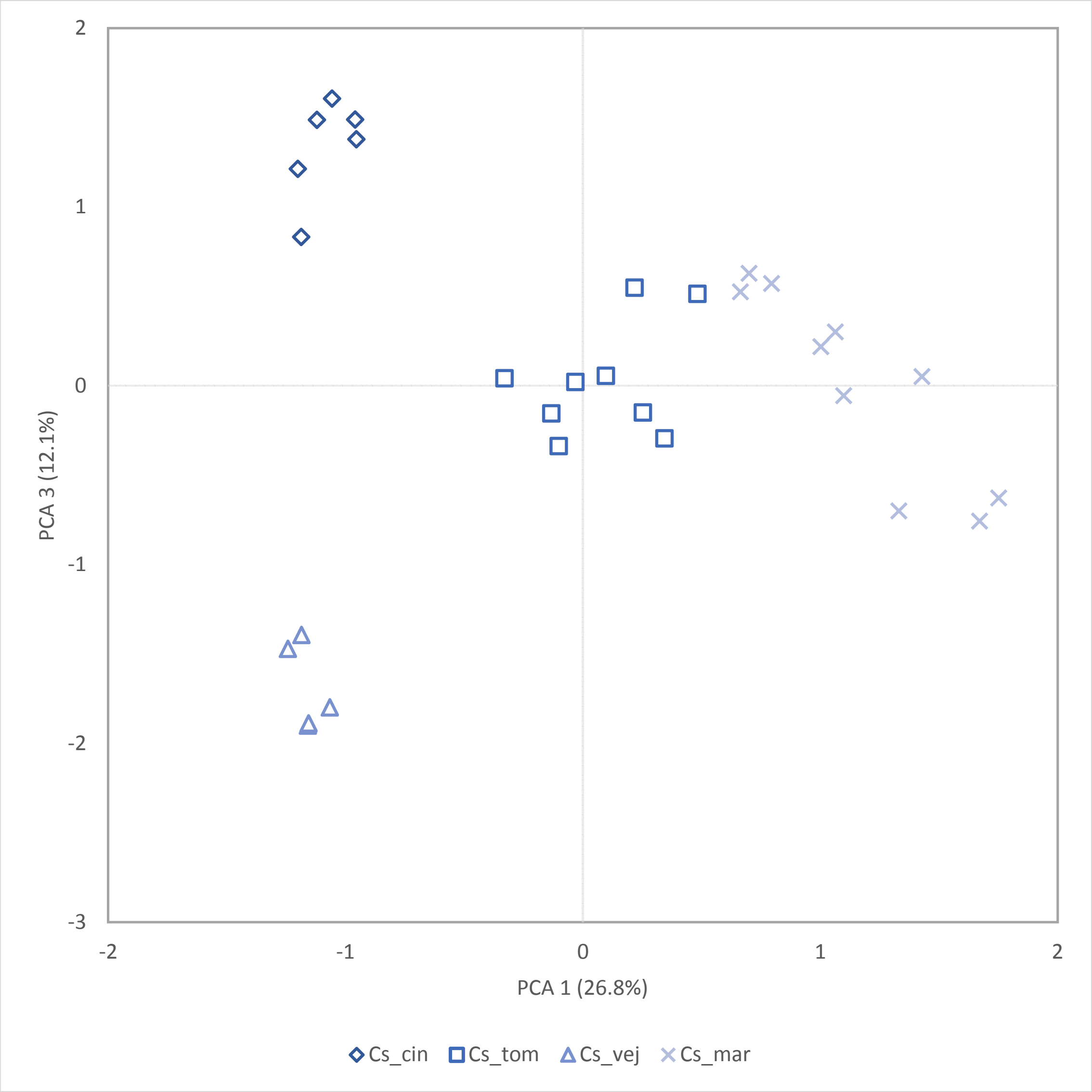


Figure 28 – Two-dimensional ordination diagram of PCA 1x3 based on 41 morphological characters of 30 *C. suffruticosa* specimens with white-arachnoid indumentum.

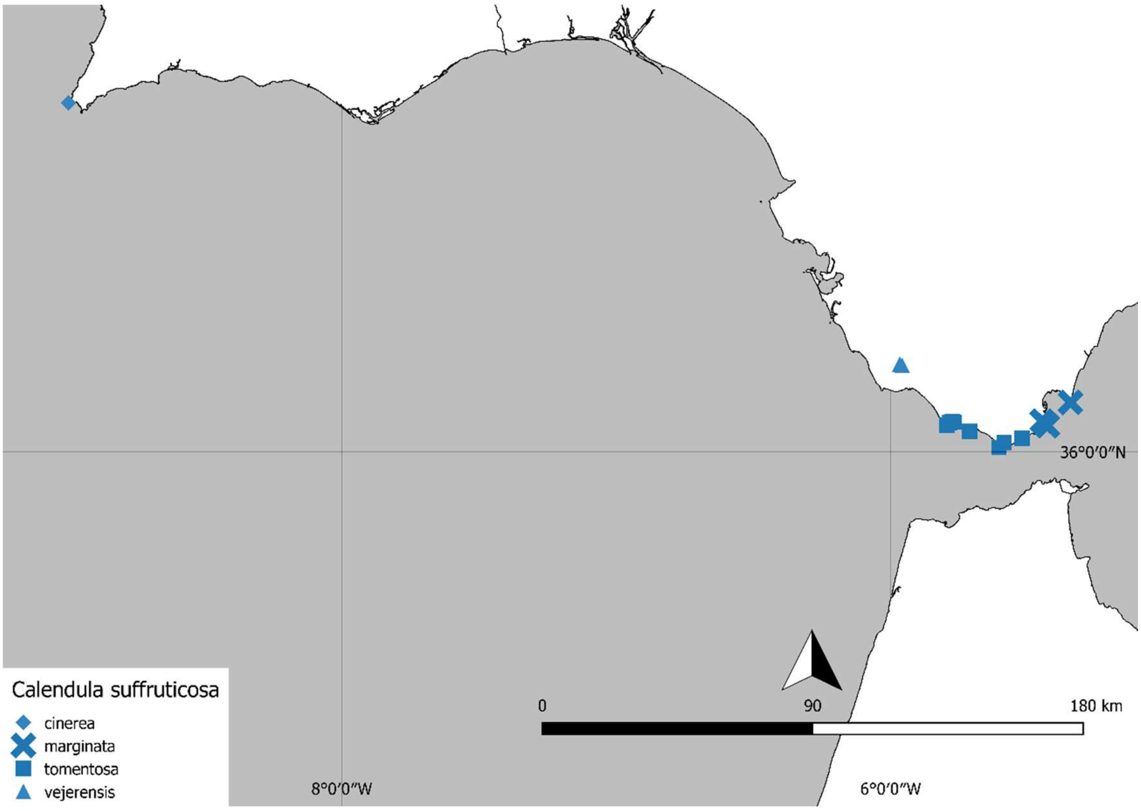


Figure 29 – Geographic distribution of *C. suffruticosa* subspecies (subsp. *cinerea*, *tomentosa*, *marginata* and *vejerensis*) in the South-West of the Iberian Peninsula.

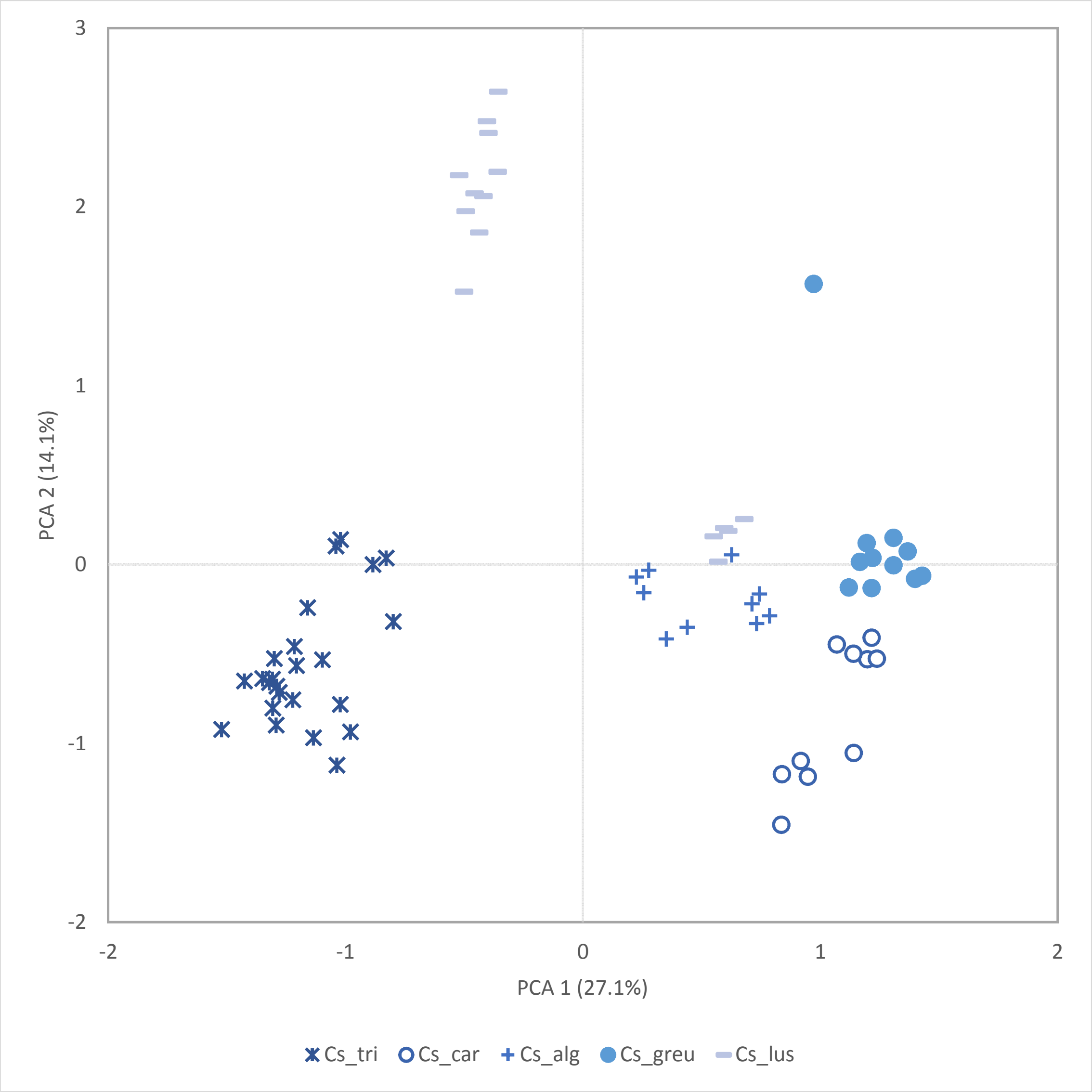
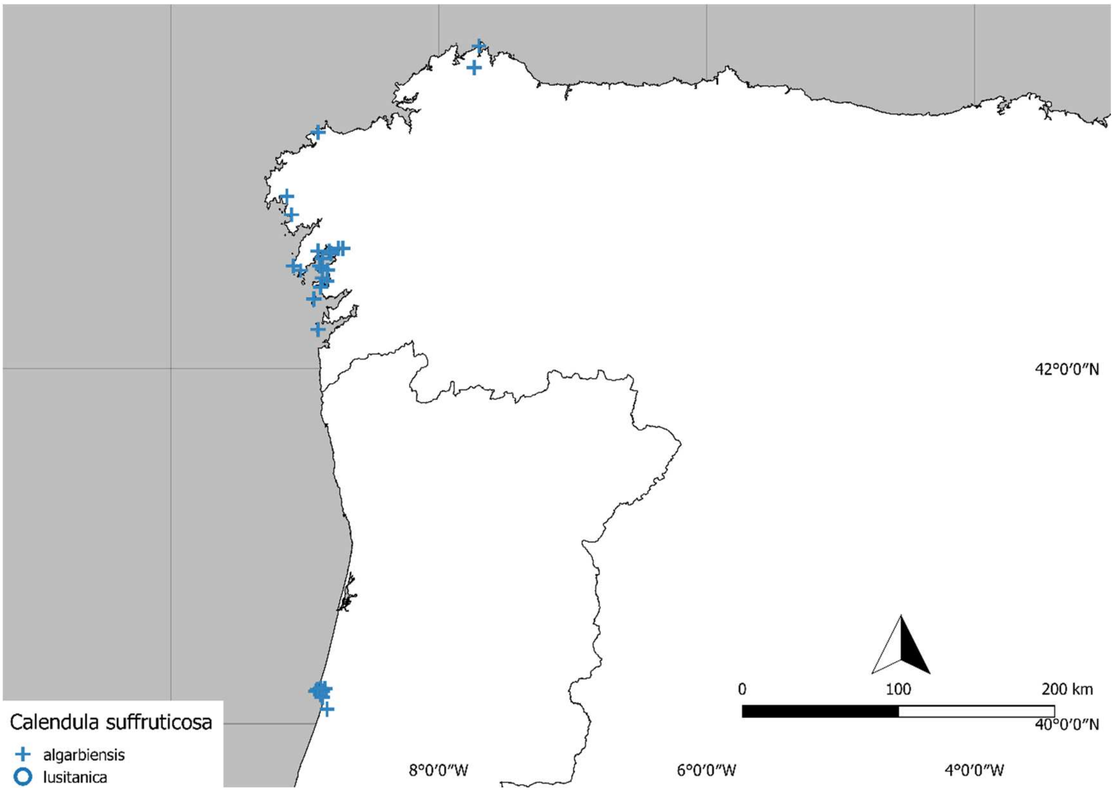
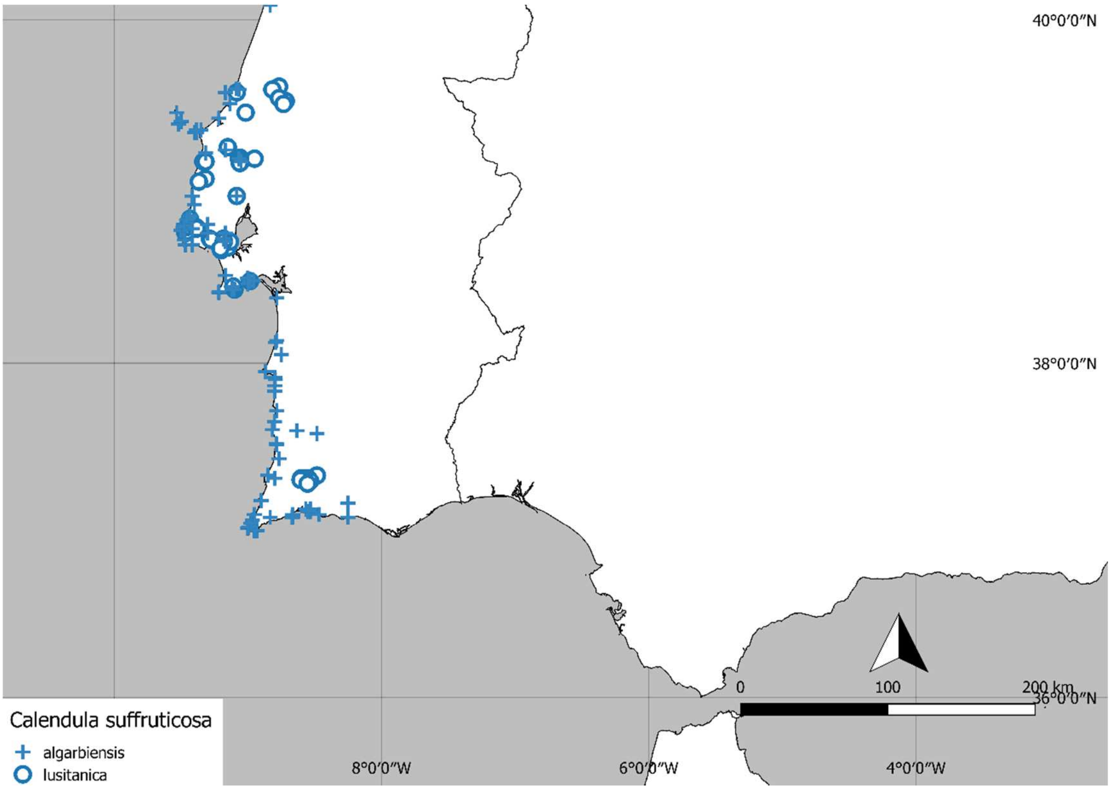


Figure 30 – Two-dimensional ordination diagram of PC 1x2 based on 54 morphological characters of 70 *C. suffruticosa* specimens without white-arachnoid indumentum.


42°0′0″N
0
100
200 km
40°0′0″N
Calendula suffruticosa
algarbiensis
lusitanica
8°0′0″W
6°0′0″W
4°0′0″W

A


40°0′0″N
38°0′0″N
0
100
200 km
36°0′0″N
Calendula suffruticosa
algarbiensis
lusitanica
8°0′0″W
6°0′0″W
4°0′0″W

B

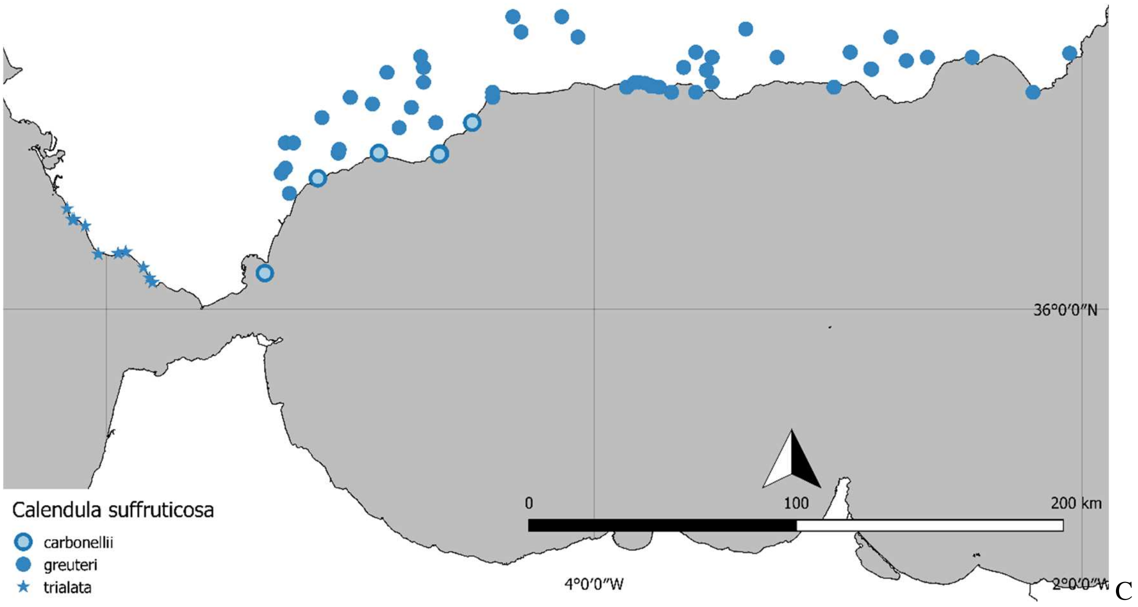


Figure 31 – Geographic distribution of *C. suffruticosa* taxa (subsp. *algarbiensis*, *carbonelli*, *greuteri*, *lusitanica,* and *trialata*) in the Iberian Peninsula. A) North of Galicia and Portugal, B) Centre and South of Portugal, C) South of Spain.

Taxonomical implications for *C. suffruticosa* subspecies

The key to the subspecies of *C. suffruticosa,* provided in the taxonomic treatment section, was mostly based on qualitative characters. In step 1, the *C. suffruticosa* taxa were divided into two groups, based on the indumentum. In the first group, four taxa with predominantly white-arachnoid indumentum in the stems and leaves were included, while in the second group the taxa included are generally more viscous, and rarely slightly white-arachnoid. This division is merely operational since most probably this character has appeared independently in several *Calendula* taxa, through selective forces driven by similar ecological conditions. In step 2, the presence/absence of cymbiform achenes was used. In step 3, the character basal leaf length and cymbiform achenes length. In step 4, the character basal leaf length was statistically significant, as mentioned. This step was also supported by two qualitative characters: the growth habit and indumentum. For the steps 5 to 7, qualitative characters were used. In step 8, the basal leave thickness.

PCAs were performed on the groups comprising *C. suffruticosa* taxa with different indumentum to enhance the resolution. The projection of PC1 versus PC2 for specimens with white-arachnoid indumentum showed a good separation between subsp. *tomentosa*, subsp. *marginata* and a group formed by subsp. *cinerea* and subsp. *vejerensis* (Figure 27 and 28). The major delimiting characters were vermiform-alate achenes, ligule length, and sub-cymbiform achenes. The second group consists of subsp. *cinerea* and *vejerensis*, which overlap considerably in morphological characters important for PC1, except for leaf thickness. Although these two taxa group together, they appear as distinct in the projection of PC1 *versus* PC3. The subsp. *cinerea* differs, mostly, by having small and thick leaves and cymbiform achenes, instead of large and thinner leaves and sub-cymbiform achenes as in subsp. *vejerensis*. Furthermore, their populations are geographically distant (Figure 29).

The PCA for specimens without white-arachnoid pubescence shows four main subgroups: subsp. *trialata*, which presents trialate achenes; specimens of subsp. *lusitanica*, with sub-exalate achenes; and the remaining subspecies with cymbiform achenes. This last subgroup includes some specimens identified as subsp. *lusitanica*, due to its erect habit and thin leaves, but that display cymbiform achenes, similar to those of subsp. *algarbiensis*, and we, therefore, consider being intermediate forms between these two subspecies. The subsp. *carbonelli* differs, mainly by having cymbiform achenes with large dorsal wings bent toward the ventral face, thicker leaves and vermiform-exalate achenes, while subsp. *greuteri* has strongly curved cymbiform achenes, thinner leaves and no vermiform-exalate achenes.

As previously discussed, the segregation of the two groups recognised by Ohle (1974) was not supported by our observations, since plants from the same populations, presenting the same achene morphology, sometimes display leaves with the shape attributed to the *incana* group together with leaves with the shape attributed to the *suffruticosa* group. Furthermore, plants that seem closely related, which hybridise easily and frequently, were included by Ohle (1974) in different species, as for example *C. incana* subsp. *algarbiensis* and *C. suffruticosa* subsp. *lusitanica.* On the contrary, subsp. *lusitanica* would be in the same species as the more distant, geographically and morphologically, subsp. *carbonelli* and subsp. *greuteri.* Finally, a continuous variation in 2C genome size values was found between *incana* and *suffruticosa* groups (Nora *et al.* 2013). Consequently, nine subspecies are recognised within *C. suffruticosa*. *C. incana* subsp. *algarbiensis* and *C. incana* subsp. *microphylla* are considered as synonyms of *C. suffruticosa* subsp. *algarbiensis*; while *C. incana* subsp. *incana* corresponds to *C. suffruticosa* subsp. *tomentosa.* Two new subspecies are described: *C. suffruticosa* subsp. *vejerensis* and *C. suffruticosa* subsp. *trialata*, both endemic to Spain.

Taxonomic treatment

A taxonomic revision of the genus *Calendula* in the Iberian Peninsula and the Balearic Islands is presented. Treatments for other areas are under preparation and will be published elsewhere.

Calendula Linnaeus (1753: 921). Lectotype (designated by Green in Hitchcock & Green 1929: 183):- *C. officinalis* L.

Annual or perennial herbs, sometimes woody at the base, unarmed, with glandular and non-glandular hairs, or, sometimes, white-arachnoid pubescent. Stems prostrate, decumbent, ascending, diffuse or erect, ± branched, leafy, cylindrical, slightly striated, often glandular and aromatic. Basal leaves alternate, undivided — pinnatifid in one *taxon* in Morocco — mainly in a basal rosette, spatulate, sub-spatulate, obovate, oblanceolate, oblong, lanceolate or linear-oblong, one-nerved, apex acute to obtuse, base attenuate, truncate or auriculate, margins entire, repand-dentate, undulate-dentate or ± irregularly dentate; petiole ± winged; the middle and upper leaves smaller and shortly stalked toward the apex, oblanceolate to lanceolate, usually auriculate, with glandular and non-glandular hairs. Capitula solitary, radiate and heterogamous. Involucre campanulate, with 1–2 rows of bracts, sub-equal, herbaceous, linear-lanceolate, acute, with a narrow hyaline margin, with glandular and non-glandular hairs. Receptacle flat-convex (without palea), glabrous. Ray florets ligulate, female, fertile, usually in 1 row, rarely 2 or more; corolla with a tubular base, hairy (non-glandular) at the base, yellow or orange, with an oblanceolate limb, obtuse, with 3 small teeth in the apex. Disc florets hermaphrodite, functionally male; corolla tubular, 5–lobed, hairy (non-glandular) at the base, yellow, orange, brown or violet-purple. Anthers sagittate, with a caudate base. Style with 2 linear stigmatic branches in ray florets and 2 triangular papillose stigmas in the disc florets. Achenes heteromorphic, ± mucronate; the outer achenes rostrate, straight to sharply curved, with or without dorsal spines, or sometimes bi- or trialate, with entire or toothed wings; the middle achenes usually cymbiform, sometimes bi- or trialate, sub-cymbiform or sub-exalate; the innermost achenes vermiform, usually smaller, slightly falcate, hook-shaped or almost annulate, transversely rugose-tuberculate on dorsal surface, wingless or with 2 narrow lateral wings. Pappus absent.

Key to the species of *Calendula* in the Iberian Peninsula and the Balearic Islands

1. Ligules, generally less than 1.8 times the length of the involucre; annual plants .2

   Ligules, generally more than 1.8 times the length of the involucre; perennial or sometimes, annual plants 3

2. Outer achenes trialate, with entire or inconspicuously dentate wings, erostrate and without dorsal spines, although they might be muricate-crested in the back, never bialate .... ***C. tripterocarpa***

   Outer achenes not as above, rostrate, generally strongly curved with dorsal spines; or sometimes bialate, with dentate lateral wings and dorsal spines ..... ***C. arvensis***

3. Involucre length (12.0) 12.4–15.0 (15.3) mm; disc florets yellow, orange or violet-purple, outer rostrate achenes generally curved .... ***C. officinalis***

   Involucre length (5.3) 7.5–10.0 (13.3) mm; disc florets yellow; outer rostrate achenes generally ± straight or slightly curved .... ***C. suffruticosa***

C. suffruticosa Vahl (1791: 94). Willkomm & Lange (1865 : 126); Willkomm (1893: 88); Merino y Roman (1906: 401); Coutinho (1913: 642, 1939: 758); Meikle (1976: 207); Franco (1984: 432); Valdés *et al.* 1987: 78); Bolós & Vigo (1995: 856); Aizpuru Oiarbide *et al.* (1999: 550); Greuter (2008b: 56); Greuter (2006+[2017]); Blanca (2011: 1610). Lectotype (designated by Ohle 1975b)**:—**TUNISIA. '*Legi in montosis circa Portum Farinam Tuneti*', *Forskål s.n.* [not Vahl *fide* Ohle 1975b] (C! [10000327]).

Perennial herbs, ± woody at the base, rarely annual. Stems (14) 40–72 (220) cm long, prostrate, decumbent, diffuse to erect, ± branched, ± glandular pubescent or ± white-arachnoid-tomentose. Basal leaves (1.8) 4.5–8.8 (24) × (0.5) 1–2.2 (7) cm, (0.2) 0.4–0.7 (2.9) mm thick; oblanceolate, spatulate or sub-spatulate; acute or obtuse; margins entire to repand-dentate or undulate-dentate, base attenuated in a ± longer petiole; with glandular and non-glandular hairs in variable proportions, sometimes predominantly white-arachnoid pubescent; the middle and upper leaves progressively smaller and shortly petiolate to sessile toward the apex, oblanceolate to lanceolate, usually auriculate. Capitula (1.6) 2.9–4 (7.1) cm in diameter. Involucre (5.3) 7.5–10 (13.3) × (0.8) 1.1–1.6 (2) mm, with 1–2 rows of bracts, sub-equal, linear-lanceolate, acute, narrowly hyaline, with scarious margins, apex usually reddish, glandular pubescent. Ray florets (13) 18–22 (36), in 1 row; (11.8) 14.5–21 (35) × (1.4) 2.1–3.8 (4.7) mm, usually more than twice the length of the involucre, yellow. Disc florets (21) 40–70 (100); 3.4–4.4 (6.1) × (1.4) 1.6–2.2 (2.6) mm, yellow. Anthers (1.6) 2.1–2.6 mm long. Styles 2.6–3.7 mm long. Outer achenes rostrate (1.2) 11–19 (32) × (0.8) 1.3–1.8 (4.3) mm, generally straight or slightly curved, without dorsal spines, or with them few and small, usually with one tooth at the base and another at the apex; middle achenes bialate (8) 10.7–15 (19) × (3.5) 5.8–7.8 (9) mm, with a rostrum (2) 4–6.7 (12.5) mm long; trialate (5) 7–9 (11.7) × (2) 5–7.5 (9.5) mm; cymbiform (4) 6.5–8.3 (12.5) × (2.5) 4.5–6.3 (9.5) mm, sub-cymbiform (4) 8–11.8 (16) × (3) 6.3–8.7 (31.8) mm, or sub-exalate (5.5) 5.5–6.1 (9.3) × (2) 2.5–3.2 (3.8) mm; inner achenes vermiform-alate (3.3) 4.7–5.8 (8.3) × (1.2) 2.7–3.8 (6.2) mm, ± circular, with 2 narrow lateral wings, and/or vermiform-exalate (1.9) 3.7–4.8 (8.5) × (0.7) 1.3–1.8 (3.7) mm, annular or hook-shaped.

Illustration: Ohle (1975b): tab. 28.

Habitat and distribution: Sandy beaches and dunes, cliffs, hills and coastal mountains, at 0–1100 m elev. From Madeira, through West and South coast and littoral mountains of the Iberian Peninsula, Sicily, South of Italy, Greece, to Turkey and Northern coast and mountains of Morocco and Algeria to Tunisia.

Chromosome number: $2n = 32$, 32+2B.

Notes: All citations of *C. suffruticosa* subsp. *suffruticosa* in the Iberian Peninsula should be assigned to other subspecies since the typical plants display a unique achene morphology and as far as we know, are exclusive of Tunisia. This will be more deeply examined in a future publication.

Key to the subspecies of *C. suffruticosa* in the Iberian Peninsula

1. Stems and leaves predominantly white-arachnoid pubescent and not viscous 2

   Stems and leaves predominantly glabrescent or glandular pubescent and ± viscous, without or with few white-arachnoid pubescence 5

2. Middle achenes predominantly cymbiform...3

   Middle achenes predominantly not cymbiform...4

3. Basal leaves length (4) 6.5–10.1 (16.3) cm; cymbiform achenes length (6) 7.5–9.2 (11) mm, mostly with 2 well-developed dorsal wings .... subsp. ***marginata***

   Basal leaves length (2) 2.5–3.9 (7.4) cm; cymbiform achenes length (4) 5–7.1 (8.8) mm, without 2 well-developed dorsal wings .... subsp. ***cinerea***

4. Herbs predominantly ± erect; basal leaves length (4.5) 6.6–10.3 (13.5) cm, sparsely white-arachnoid pubescent to glabrescent.................... subsp. ***vejerensis***

   Herbs prostrate to diffuse; basal leaves length (1.8) 2.8–4.5 (9.7) cm, densely white-arachnoid pubescent ....subsp. ***tomentosa***

5. Middle achenes predominantly trialate, never cymbiform, with lateral wings entire to sinuate-dentate at the apex, ventral wings sub-equal to the lateral wings; vermiform-alate achenes usually absent.. subsp. ***trialata***

   Middle achenes cymbiform to sub-cymbiform, or with lateral and ventral wings reduced (sub-exalate); vermiform-alate achenes usually present..........................6

6. Middle achenes predominantly cymbiform, with 2 well-developed dorsal wings, usually entire, and bent toward the ventral face .... subsp. ***carbonelli***

   Middle achenes not as above 7

7. Middle achenes generally cymbiform, strongly curved, with a ventral wing longer than the lateral wings, inner vermiform-exalate achenes generally absent .... subsp. ***greuteri***

   Middle achenes, not cymbiform, or, if cymbiform, slightly curved, with ventral wings absent or these smaller than the lateral wings, inner vermiform-exalate achenes always present 8

8. Herbs predominantly prostrate to decumbent; basal leaves thickness (0.4) 0.5–0.8 (1.4) mm; middle achenes predominantly cymbiform to sub-cymbiform, both with lateral and ventral wings well-developed .... subsp. ***algarbiensis***

   Herbs predominantly diffuse to erect; basal leaves thickness (0.3) 0.3–0.4 (0.5) mm; middle achenes predominantly with reduced to absent wings (sub-exalate), more rarely cymbiform .... subsp. ***lusitanica***

subsp. ***cinerea*** (Ohle) P.Silveira & A.C.Gonç. in Silveira *et al.* (2013: 52). Basionym: *C. incana* subsp. *algarbiensis* var. *cinerea* Ohle (1974: 277). Lectotype (designated by Silveira *et al.* 2013)**:—**PORTUGAL. Algarve: Cabo de São Vicente. *Rechinger* 0–2 588 (W! [1996–0007504]).

C. tomentosa auctt. (Willkomm & Lange 1865: 126*;* Coutinho 1939: 758; Sampaio 1947: 580) *p.p. quoad spec*. Lusit. *non* Desfontaines (1799: 305) *nom. illeg*.

C. incana auct. Greuter (2008b: 56) *p.p. quoad distr.* Lu non Willdenow (1803: 22341);

C. suffruticosa subsp. *tomentosa auctt*. (Meikle 1976: 206 *p.p. quoad distr.* SW Portugal; Franco 1984: 432; Greuter 2006+[2017] *p.p. quoad distr.* Lu) *non* (Ball) Murbeck (1905: 9).

Perennial herbs, ± woody at the base. Stems (16) 24–53.3 (87) cm long, prostrate to decumbent, ± branched, white-arachnoid pubescent, not viscous. Basal leaves (2) 2.5–3.9 (7.4) × (0.7) 0.8–1 (2) cm, (0.7) 1.7–2.1 (2.9) mm thick, oblanceolate to spatulate, apex obtuse or sometimes ± acute, margins sub-entire to repand-dentate, attenuated in a ± large petiole, with white-arachnoid pubescence. Capitula solitary, (2.3) 2.9–3.5 (3.8) cm in diameter. Outer achenes rostrate (6) 8.3–15 (19) × (1.3) 1.5–1.8 (2.3) mm, generally straight or slightly curved, without dorsal spines, sometimes with 1 tooth at the base and/or another at the apex; middle achenes usually cymbiform (4) 5.5–7.1 (8.8) × (3) 3.8–5 (6.5) mm, with ventral wings surpassing the lateral ones; inner achenes vermiform-exalate (3.2) 3.8–4.6 (7) × (1.2) 1.3–1.7 (3.7) mm, generally hook-shaped.

Illustration: Ohle (1974): tab. 30 (achenes) – Figure 32.

Habitat and distribution: Limestone cliffs exposed to N, directly above the sea, at 30–100 m elev. Endemic to "Cabo de São Vicente" in Algarve (PORTUGAL) – Figure 29.

Conservation status: IUCN Red List Category Global level: **Endangered (EN) D**. Although this taxon is known from a single population/location, with and EOO=AOO of 4 km^2, which meets the area requirements under criterion B for Critically Endangered (CR), there is no evidence of any population reduction, and it is located within a protected area (Parque Natural do Sudoeste Alentejano e Costa Vicentina – PNSACV). However, since the number of mature individuals is estimated to be less than 250, we assess it as Endangered (EN), according to the criterion D of the IUCN Red List Categories and Criteria (2012). Nevertheless, it should be kept under careful periodical observation, to identify possible threats and/or population reductions, which might justify an upgrade to a category of higher risk of extinction.

Chromosome number: $2n = 32$

Genome size: 3.09 ± 0.11 pg

subsp. ***tomentosa*** (Ball) Murbeck (1905: 9); Meikle (1976: 206) *p.p. distr.* SW Spain; Valdés *et al.* (1987: 80), Greuter (2006+[2017]) *p.p. quoad distr.* Hs. Basionym: *C. suffruticosa* var. *tomentosa* Ball (1878: 517); Willkomm (1893: 88). Lectotype (designated by Silveira et al. 2013)**:—** SPAIN. "*Calendula* tomentosa", "Tarifa juxta monia urbis ad mare", s.d. [1795 in MPU!], *Broussonet s.n.* (lectotype P! [P00680154]; isolectotypes: MPU! [MPU022962], MAF! [3613], B-Willd.! [16691-01-0]).

Homotypic synonyms:

C. tomentosa Desfontaines (1799: 305) *nom. illeg. non* Linnaeus (1782: 384); Willkomm & Lange (1865: 126).

C. incana Willdenow (1803: 2341); Ohle (1974: 272); Greuter (2008b: 56).

Perennial herbs, woody at base. Stems (21) 31.8–75 (111) cm tall, prostrate to diffuse, ± branched, densely white-arachnoid pubescent, not viscous. Basal leaves (1.8) 2.8–4.5 (9.7) × (0.6) 0.8–1.4 (2.7) cm, (0.4) 0.5–0.8 (1.7) mm thick, oblanceolate to spatulate, apex obtuse to slightly acute, margins conspicuously repand-dentate to undulate-dentate, densely white-arachnoid pubescent. Capitula solitary, (3.1) 3.4–4.6 (5.7) cm in diameter. Outer achenes rostrate (7) 11.3–16 (28) × (0.8) 1.2–1.6 (4.3) mm, usually slightly curved, without dorsal spines, sometimes, with 1 tooth at the base, rarely with another at the apex; middle achenes generally sub-cymbiform (6.5) 8.7–11 (14.2) × (4.3) 6.7–8.1 (13) mm, with lateral wings entire or sinuate-dentate, ventral wings sub-equal to the lateral ones, sometimes with 2 small dorsal wings; bialate (9) 11–15 (18) × (5) 6.3–8.3 (8.8) mm, with a rostrum (2.5) 5–8.3 (12.5) mm long, with lateral wings sinuate-dentate and ventral wing sometimes rudimentary; or cymbiform (7.7) 8.3–10.1 (10.8) × (3.3) 5–6.4 (7.7) mm, usually without dorsal wings; inner achenes vermiform-alate (3.8) 4.7–5.8 (8.3) × (1.8) 2.8–3.8 (6.2) mm, falcate, and vermiform-exalate (3.3) 3.9–4.8 (5.8) × (1) 1.3–1.7 (2) mm, hook-shaped or incompletely-circular, all with a small tooth at the base.

Illustration: Desfontaines (1799): tab. 245 – Figure 33.

Habitat and distribution: Sandy loams, not far from the sea, at 0–100 m elev. Endemic to the South of Cádiz (SPAIN), from Punta Camarinal to the coastline E of the mouth of the Guadalmesí River – Figure 29.

Conservation status: IUCN Red List Global Category: **Endangered (EN) B2ab (ii, iv)**. The taxon meets the criteria for the area under Criterion B for CR, based on EOO (71.51 km^2), or for EN, based on AOO (24 km^2). Since, at least the most typical plants inhabiting in the vicinities of Tarifa, seem to be threatened, due to risk of urban expansion, and the number of locations (assuming that the Western subpopulations are a single location, due to proximity and equal threats) equal to 4 or, at most 5, we propose this taxon to be assessed as Endangered (EN), according to the IUCN Red List Categories and Criteria IUCN categories and criteria (2012).

Chromosome number: *2n* = 32

Genome size: 3.31 ± 0.08 pg

Notes: The density of the indumentum, habit, achenes and leaf morphology is variable. Populations from Punta Paloma are prostate with thick leaves, but these characters do not persist in culture. The populations of Tarifa are the most typical, as they the type material for the original description and classification of this *taxon*. We have not found, so far, any population with similar morphology in North Africa.

subsp. ***marginata*** (Willd.) Maire (1934: 789). Basionym: *C. marginata* Willdenow (1809: 935). Lectotype (designated here):—SPAIN. Cádiz: '*ad Giberaltariam*', *Willdenow* 64 (B! [B-W 16688–01–0]).

C. tomentosa auct. Willkomm & Lange (1865: 126) *p.p. spec.* Gibraltariae *non* Desfontaines (1799: 305) *nom. illeg.*

C. suffruticosa subsp. *tomentosa auctt.* (Valdés *et al.* 1987: 80 *p.p. quoad loc.* Gibraltar *et prope urbe* Algeciras, Greuter 2006+[2017] *p.p. quoad loc.* Gibraltar) *non* (Ball) Murbeck (1905: 9).

Perennial herbs, ± woody at the base. Stems (14) 44.3–147.5 (180) cm long, prostrate to decumbent, ± branched, white-arachnoid pubescent, not viscous. Basal leaves (4) 6.5–10.1 (16.3) × (1) 2–3.8 (6.2) cm, (0.2) 0.4–0.5 (0.5) mm thick, oblanceolate to broadly spatulate, apex ± obtuse, margins sub-entire to slightly repand-dentate, attenuated in a ± large petiole, with white-arachnoid pubescence. Capitula solitary, (2.5) 3.9–5.5 (7.1) cm in diameter. Outer achenes rostrate (8) 15–22 (29) × (1.2) 1.4–1.8 (2.3) mm, generally straight or slightly curved, without dorsal; middle achenes cymbiform (6) 7.5–9.2 (11) × (2.8) 4.8–6.7 (7.7) mm, with the ventral wing surpassing the laterals, usually with 2 dorsal wings well-developed, curved toward the ventral face; rarely sub-cymbiform (8.3) 8.6–11.1 (11.3) × (6.7) 7.8–9 (9.5); inner achenes vermiform-alate (3.7) 5.3–6.7 (7.7) × (2) 3.3–4.2 (5.5) mm, and vermiform-exalate (3.8) 4.7–5.2 (5.8) × (1.3) 1.7–2 (2.3) mm, usually hook-shaped, with a small ventral wing always present.

Illustration: Lanza (1919): tab. II (achenes). – Figure 34.

Habitat and Distribution: Limestone cliffs exposed to ± North, or sandy-clay cliffs exposed to ± East, near the sea, 0–170 m elevation. Endemic to Cádiz (Spain), from Punta Carnero to Gibraltar (SPAIN) – Figure 29.

Conservation status: IUCN Red List Global Category: **Endangered (EN) B2ab (i, ii, iii)**. The taxon meets the criteria for the area under Criterion B for CR, based on EOO (12.4 km^2), or for EN based on AOO (12 km^2). Since the number of locations can be estimated to be between 2 and 3 (which falls within the limits for Endangered), and we infer that a reduction of EOO, AOO, and habitat has occurred and might continue, due to urban expansion in the Algeciras area, we suggest this taxon should be assessed as EN, according to the IUCN Red List Categories and Criteria (2012).

Chromosome number: $2n = 32$

Genome size: 3.09 ± 0.05 pg

Notes: Although these populations present an white-arachnoid pubescence similar to *C. suffruticosa* subsp. *tomentosa*, they display cymbiform achenes as the only type of achene occupying a median position in the capitula, while in the subsp. *tomentosa* this position is occupied by ± trialate achenes. Furthermore, the two well-developed dorsal wings displayed by its classical population in Gibraltar, indicate a probable origin related to *C. suffruticosa* subsp. *carbonelli*, its neighbour in S–SW of the same rocky slopes of Gibraltar. Moreover, it is further distinguishable from *C. suffruticosa* subsp. *tomentosa*, by having larger capitula and leaves, with margins only slightly repand-dentate. Willdenow's description of *C. marginata* was based on a form growing in shade, with white-arachnoid indumentum only at the veins and the margin of leaves. However, there is no reason to describe a new *taxon* for these plants from Gibraltar with white-arachnoid indumentum, as there is no doubt that the type specimen of *C. marginata* matches perfectly its original description and displays the same achene morphology that can still be found nowadays in its classical *locus*. We have seen some specimens recorded for San Roque (3 km SE of San Roque, 21 April 1951, *Alston, A.H.G. 10478*; San Roque, 21 April 1951, *Simpson, N.D. 51282*) which, are held at BM. We have searched, unsuccessfully, for any *Calendula* with the morphology displayed in these specimens in the mentioned area (3km SE of San Roque), so we have not included these in the following list of additional specimens examined.

subsp. ***vejerensis*** P.Silveira & A.C.Gonç., ***subsp. nov.*** **Type**:—SPAIN. Cádiz: Vejer de la Frontera, *Silveira & Gonçalves 3186* (holotype MA! [MA884480]; isotypes AVE!, MA! [MA884481]).

C. stellata auct. Willkomm & Lange (1865: 126) *p.p. quoad spec.* Vejer *non* Cavanilles (1791: 3).

C. suffruticosa subsp. *lusitanica auct.* Valdés *et al.* (1987: 80) *p.p. quoad loc.* Vejer de la Frontera *non* Boissier (1849: 83).

This subspecies presents similarities with *C. suffruticosa* subsp. *trialata* but it is distinguishable from it by its white-arachnoid indumentum and the sub-cimbiform middle achenes.

Perennial herbs, ± woody at the base. Stems (34.5) 44.8–63.5 (82) cm long, ± erect, ± branched, sparsely white-arachnoid pubescent, generally not viscous. Basal leaves (4.5) 6.6–10.3 (13.5) × (1.1) 1.5–4.3 (7) cm, 0.5–0.6 mm thick, oblanceolate to broadly spatulate, apex generally obtuse, margins entire to repand-dentate or undulate-dentate, attenuated in a ± large petiole, sparsely white-arachnoid pubescent, frequently with some leaves or parts of the leaves with a reddish colouration. Capitula solitary, (3) 3.4–4.2 (4.8) cm in diameter. Outer achenes rostrate (7) 10–15.4 (21) × (1.5) 1.5–1.8 (2.2) mm, straight or slightly curved, without dorsal spines, sometimes with 1 tooth at the base; middle achenes sub-cymbiform (6) 7–8.1 (9) × (3) 5–8 (8) mm, with lateral wings entire and sinuate-dentate at the apex, ventral wings sub-equal or slightly surpassing the lateral ones; inner achenes vermiform-exalate (2.8) 3.5–4.4 (6.1) × (1.5) 1.5–1.9 (2) mm, circular, with 1 small basal tooth.

Illustration: Figure 34

Habitat and Distribution: On ± stratified calcareous sandstones slopes (sandstones of silica sands cemented with carbonates), 30–130 m. Endemic to Vejer de la Frontera in Cádiz (SPAIN) – Figure 29.

Conservation status: IUCN Red List Global Category: **Endangered (EN) B1ab (i, ii, iii, iv) + 2ab (i, ii, iii, iv)**. This taxon is known from only two subpopulations, in the vicinities of Vejer de La Frontera (SPAIN), which meets the criteria for the area under Criterion B for CR (EOO = AOO = 8 km^2). Since one of the subpopulations has higher probability of been affected by urban expansion than the other, the number of locations is two, which justifies a downgrade from CR to EN, according to the IUCN Red List Categories and Criteria (2012)

Chromosome number: $2n = 32$

Genome size: 3.22 ± 0.07 pg

subsp. ***trialata*** P.Silveira & A.C.Gonç., ***subsp. nov***. **Type**:— SPAIN. Cádiz: Conil de la Frontera, [36°16'35" N, 6°05'15" W], 25 May 1981, *P. Cambó et al. 421/81* (holotype MA! [465711]); isotype SEV! [123133]).

C. suffruticosa subsp. *lusitanica auct.* Valdés *et al.* (1987b, 80) *p.p. non* Boissier (1849: 83)

C. suffruticosa subsp. *suffruticosa auct.* Valdés *et al.* (1987: 79*) p.p. non* Vahl (1791: 94).

Some plants present a ± decumbent habit similar to *C. suffruticosa* subsp. *algarbiensis*, while others are ± erect, like *C. suffruticosa* subsp. *lusitanica*. They differ from both subspecies by presenting trialate middle achenes, with margins entire and sinuate-dentate at the apex.

Perennial herbs, ± woody at the base. Stems (28) 44–69 (143) cm, decumbent to diffuse, ± branched, with glandular and *non*-glandular hairs in variable proportions, viscous. Basal leaves (3.2) 5–8.9 (14.1) × (0.8) 1.2–2.3 (3.5) cm, (0.3) 0.4–0.7 (0.9) mm thick, oblanceolate to spatulate, apex generally obtuse, margins entire to repand-dentate or slightly undulate-dentate, attenuated in a ± large petiole, with glandular and *non*-glandular hairs in variable proportions, viscous. Capitula (2.2) 3–4 (4.9) cm in diameter. Outer achenes rostrate (5) 8.5–15 (25) × (1) 1.2–1.5 (2) mm, generally straight or slightly curved, without dorsal spines, at times with one tooth at the base; middle achenes, usually, trialate (6) 7–9 (11.7) × (3) 5–7.5 (9.5) mm, wings entire and sinuate-dentate at the apex, sub-equal, or bialate (9) 10–14 (16) × (5) 6–7.3 (9) mm, with a rostrum (2) 3–5.3 (8.3) mm, with lateral wings sinuate-dentate; inner achenes vermiform-exalate (1.9) 3–4 (6.1) × (0.7) 1.3–1.7 (2.5) mm, generally hook-shaped to falcate.

Illustration: Figure 36.

Habitat and distribution: Cliffs, dunes, sandy soils and hills not far from the sea, 0–130 m. SW coast of Cádiz (SPAIN), from Playa de La Barrosa to Playa de la Atlantera, and one population at Cape Cires in Tanger (Morocco) – Figure 31.

Conservation status: IUCN Red List Regional Category for the Iberian Peninsula: **Vulnerable (VU) B1ab (ii, iii, iv) + 2ab (ii, iii, iv)**. The taxon meets the criteria for the area under criterion B for EN, both based on EOO (164.84 km^2) and AOO (36 km^2). Due to its coastal habitat, where some of its subpopulations are under threat due to urban expansion, among other threats, we estimate that the number of locations will be between 5 and 10 (depending on how the urban expansion progresses), which justifies a downgrade to VU, according to the IUCN Red List Categories and Criteria (2012). Since the Iberian populations do not seem to benefit from any immigration of propagules from the nearest neighbour subpopulations, in Northern Morocco, there is no change of category that should be applied.

Chromosome number: $2n = 32$

Genome size: 3.23 ± 0.10 pg

Notes: Its distinct location, in a region where the winds are frequent and intense, may explain the origin and prevalence of the trialate achenes, a character that persists under cultivation.

subsp. ***carbonellii*** Ohle (1974: 268); Greuter (2008b: 57); Greuter (2006+[2017]). **Type:—**SPAIN. Cádiz: ‘*Südostspanien, Gibraltar, Málaga, Küstenfelsen, collin/litoral*’, *J. Borja-Carbonell s.n* (holotype HAL!).

C. suffruticosa subsp. *suffruticosa auct.* Willkomm & Lange (1865: 126) *p.p. quoad spec.* Gibraltar *non* Vahl (1791: 94).

C. stellata auctt. (Willkomm & Lange 1865: 126; Willkomm 1893: 88) *p.p. quoad spec.* Estepona et Torremolinos *non* Cavanilles (1791: 3).

C. suffruticosa subsp. *algarbiensis auct.* Valdés *et al.* (1987: 80) *p.p. non* (Boiss.) Nyman (1878: 398).

Perennial herbs, ± woody at the base. Stems (16) 33–78 (140) cm long, generally decumbent, ± branched, with glandular and non-glandular hairs in variable proportions, ± viscous. Basal leaves (4.3) 6.1–12.2 (24) × (0.6) 1.4–2.5 (3.9) cm, (0.5) 0.6–1 (1.3) mm thick, usually oblanceolate, sometimes sub-spatulate, apex ± acute, margins slightly repand-dentate, attenuated in a ± large petiole, with non-glandular hairs more abundant than glandular hairs. Capitula solitary, (2.3) 3.1–4.1 (5.5) cm in diameter. Outer achenes rostrate (6) 12–20 (26) × (1.2) 1.7–2.2 (3.3) mm, generally straight or slightly curved, without or with small dorsal spines, sometimes with 1 tooth at the base and another at the apex; middle achenes are, usually, cymbiform (5.3) 6.5–8.3 (12.5) × (4.3) 5.3–7.5 (9.5) mm, with a ventral wing sub-equal or greater than the laterals, with 2 well-developed dorsal wings, entire or dentate and bent toward the ventral face, more rarely bialate (10.2) 12.3–17 (19) × (5.5) 6–8.1 (9) mm, with a rostrum (2.5) 4.3–7.9 (10) mm, with 2 well-developed dorsal wings, dentate; inner achenes vermiform-alate (3.3) 4.3–5.8 (8) × (1.5) 2.9–4.3 (5.8) mm, falcate, sometimes with a small ventral wing, and vermiform-exalate (3.2) 3.7–5.2 (6) × (1.5) 1.7–2 (2.3) mm, generally falcate to hook-shaped.

Illustration: Ohle (1974, 268): tab. 24, 25 -- Figure 36.

Habitat and Distribution: Rocky cliffs, not far from the sea, at 0–150 m elev. Endemic to Gibraltar and Malaga (SPAIN) – Figure 31.

Conservation status: IUCN Red List Category Global level: **Endangered (EN) B1ab (iii, iv) + 2ab (iii, iv)**. This taxon meets the area requirements under criterion B for EN due to both EOO (929 km^2) and AOO (24 km^2). Since its known subpopulations are restricted to 5, severely fragmented, and most of them highly menaced by urban expansion which has been the cause for recent reduction in extent of habitat and subpopulations number, we propose to assess it as Endangered (EN), according with the IUCN Red List Categories and Criteria (2012). The Northern subpopulations are restricted to small patches enclosed within urban areas, highly menaced by urban expansion. Only the subpopulation of Gibraltar seems to be less threatened since it is enclosed within a protected area.

Chromosome number: *2n* = 32

Genome size: 3.18 ± 0.08 pg

subsp. ***algarbiensis*** (Boiss.) Nyman (1878: 398); Coutinho (1913: 643, 1939: 759); Meikle (1976: 206) *p.p. excl. distr.* Spain; Franco (1984: 431); Greuter (2006+[2017]). Basionym: *C. algarbiensis* Boissier (1859: 106). Lectotype (designated by Burdet *et al.* 1983):**—**PORTUGAL. Algarve: '*in rupibus maritimis Algarbiae prope Lagos in Lusitania*', *Bourgeau 2080* (lectotype G! [00074227]; isolectotypes P!, MPU!, K!).

Homotypic synonym:

C. incana subsp. *algarbiensis* (Boiss.) Ohle (1974: 274); Greuter (2008b: 56).

Heterotypic synonyms:

C. microphylla Lange ex Nyman (1889: 178); Merino y Roman (1906: 400); Coutinho (1913: 643, 1939: 759). Type**:—**PORTUGAL. Crop of seeds from Portugal, September 1882, *Lange s.n.* (syntypes C! [C10013034] & C! [C10013033]).

C. incana subsp. *microphylla* (Lange ex Nyman) Ohle (1974: 278); Greuter (2008b: 56).

C. suffruticosa var. *gallaecica* Pau & Merino in Merino (1906: 402). Lectotype (designated here)**:—** SPAIN, Pontevedra: Isla Ons-Loveira, *Merino s.n.* (LOU [*1234/1*] photo!).

Other synonyms:

C. suffruticosa subsp. *algarbiensis auct.* Valdés *et al.* (1987: 81) *non* (Boiss.) Nyman (1878: 398).

Perennial herbs, ± woody at the base. Stems (21) 45–82 (220) cm long, predominantly prostrate to decumbent, ± branched, with glandular and non-glandular hairs in variable proportions, ± viscous. Basal leaves (3.2) 4.5–7.3 (13.8) × (0.5) 0.7–1.9 (2.7) cm, (0.4) 0.5–0.8 (1.4) mm thick, oblanceolate to spatulate, apex obtuse, at times ± acute, margins sub-entire or slightly repand-dentate, attenuated in a ± large petiole, with non-glandular hairs more abundant than glandular hairs. Capitula (1.9) 2.6–3.1 (4) cm in diameter. Outer achenes rostrate (4) 12–21 (31) × (1.2) 1.5–1.8 (3) mm, generally straight or slightly curved, without dorsal spines or with small ones, sometimes with 1 tooth at the base; middle achenes predominantly cymbiform (4.7) 6.1–7.7 (9.7) × (2.8) 4.5–6.7 (8.5) mm, or at times sub-cymbiform (6.8) 7.6–11.8 (12.8) × (4.7) 7–10.2 (31.8) mm, with ventral wings sub-equal to the laterals, both entire or dentate, sometimes with 2 small dorsal wings, ± dentate and plane; inner achenes vermiform-exalate (2.5) 3.8–5 (8.5) × (0.8) 1.3–1.7 (2.3) mm, always present; rarely vermiform-alate (3.5) 4.4–5.8 (8.3) × (1.5) 2.8–4.2 (6.2) mm, both falcate to circular.

Illustration: Figure 37.

Habitat and distribution: Cliffs and sand dunes, not far from the sea; 0–150 m elev. Endemic to the West coast of the Iberian Peninsula from La Coruña (SPAIN) to Algarve (PORTUGAL), absent in Douro Litoral and Minho – Figure 31.

Conservation status: IUCN Red List Category Global level: **Least Concern (LC)**. This subspecies of *C. suffruticosa* is relatively widespread in the West coast of the Iberian Peninsula, from Galicia to Algarve, on sands and rocky cliffs near the sea. Its EOO reaches 79.85 km^2, while its AOO averages 432 km^2. Although its AOO meets the area requirements under criterion B for EN, and some of its subpopulations have suffered some habitat lost, most of its current populations are relatively stable and healthy, most of them relatively well interconnected (not fragmented), and the number of locations is much higher than 10. Therefore, we propose for this subspecies to assess it as LC, according to the IUCN Red List Categories and Criteria (2012).

Notes: It is probably the most variable subspecies, especially in the shape and size of leaves, habit and morphology of the achenes. Near the sea, plants have a prostrate habit, thick-fleshy leaves and predominantly cymbiform achenes, with large lateral wings. The achenes of Galician populations show some affinities to the subsp. *lusitanica*. Their prostrate habit, thicker leaves and habitat have led us to include them in subsp. *algarbiensis*.

Chromosome number: *2n* = 32

Genome size: 3.18 ± 0.14 pg

subsp. ***greuteri*** Ohle (1974: 269); Greuter (2008b: 57); Greuter (2006+[2017]). **Type:—** SPAIN. Granada: '*Südostspanien, Motril*', *Greuter S.7133* (holotype G!).

C. suffruticosa subsp. *suffruticosa auct.* (Valdés *et al.* 1987: 79; Blanca *et al.* 2011: 1610) *non* Vahl (1791: 94).

C. stellata auct. Willkomm & Lange (1865: 126) *p.p. quoad spec.* Puerta de Zafarraya *et* Sierra de Gador *non* Cavanilles (1791: 3).

C. suffruticosa subsp. *lusitanica auctt.* (Meikle 1976: 206 *p.p. quoad distr.* Spain, Valdés *et al.* 1987: 80) *non* (Boiss.) Ohle (1974: 270).

Annual to perennial herbs, ± woody at the base. Stems (15.5) 34–54 (85) cm long, diffuse to erect, ± branched, with glandular and non-glandular hairs in variable proportions, ± viscous. Basal leaves (4.5) 7–10.2 (13.5) × (0.5) 1.1–1.9 (3.6) cm, (0.3) 0.4–0.6 (0.8) mm thick, oblanceolate, apex acute, margins slightly repand-dentate to conspicuously undulate-dentate, attenuated in a ± large petiole, with non-glandular hairs more abundant than glandular hairs. Capitula (1.6) 3.2–4.3 (5.9) cm in diameter. Outer achenes rostrate (10) 15–19 (25) × (1.2) 1.5–2 (3.3) mm, generally straight to slightly curved, without dorsal spines or with them small, sometimes with 1 tooth at the base and/or another at the apex; middle achenes; generally cymbiform (5.2) 6.3–7.3 (9.3) × (2.5) 3.8–5.3 (6) mm, ventrally sharply curved and with ventral wing slightly surpassing the laterals, sometimes with 2 straight small dorsal wings, rarely sub-cymbiform (4) 11.7–12.5 (16) × (3.8) 7.5–9 (10) mm, with a rostrum (1.7) 2.5–3.3 (7) mm, with lateral wings entire to sinuate-dentate at the apex, and ventral wing sub-equal to the laterals; inner achenes vermiform-alate (3.5) 4.7–5.8 (6.7) × (1.2) 2.2–2.8 (4) mm, with 2 lateral wings and one ventral, all of them narrow, generally hook-shaped, always present; vermiform-exalate (2.3) 2.5–4 (4.2) × (0.7) 0.8–1.4 (1.5) mm, circular, rare, or, generally, absent.

Illustration: Boissier (1839): tab. 99; Ohle (1974): tab. 27 [achenes]; – Figure 38.

Habitat and Distribution: Rocky slopes and coastal canyons, plants growing in shade; 0–1000 m elev. Endemic to SPAIN. SE of Andalucía: Almeria, Granada, and Malaga: between Berja and Cabo de Gata – Figure 31.

Conservation status: IUCN Red List Category Global level: **Least Concern (LC)**. Although the subspecies meet the area requirements under criterion B for VU, based on its EOO (9,991 km^2), or for EN, based on its AOO (200 km^2), the number of locations is relatively high and there is no evidence of significant recent population reduction. Therefore, it is assessed as Least Concern (LC), according to the IUCN Red List Categories and Criteria (2012). Since it prefers rocky environments and grows inland than

C. suffruticosa subsp. *carbonelli* Ohle, for example, it is less prone to be affected by the urban expansion. However, future monitoring should be conducted to evaluate the possible need to upgrade to a category of higher risk of extinction.

Chromosome number: *2n* = 32

Genome size: 3.36 ± 0.10 pg

Notes: This *taxon* presents some variability throughout its distribution area, sometimes it resembles *C. suffruticosa* subsp. *carbonelli*, others *C. suffruticosa* subsp. *trialata*, probably resulting from hybridization or permanence of recessive genes in the populations. Nevertheless, it is one of the better-defined subspecies in the Iberian Peninsula, with is distinctive leaf and achene morphology, correlated with geographical distribution and ecology.

subsp. ***lusitanica*** (Boiss.) Ohle (1974: 270); Meikle (1976: 206); Franco (1984: 431); Greuter (2008b: 57); Greuter (2006+[2017]). Basionym: *C. lusitanica* Boissier (1849: 83). Lectotype (designated by Burdet *et al.* 1983)**:—**PORTUGAL. Estremadura: '*prope Cintra Lusitaniae in petrosis*', *Guthnik* s.n. (G!).

Homotypic synonym:

C. lusitanica var. *genuina* Coutinho (1913: 642, 1939: 759) *p.p. excl. spec.* Berlengas, Cabo Carvoeiro, not validly published according to the ICBN (McNeill *et al.* 2012, article 24.3).

Heterotypic synonyms:

C. lusitanica var. *transtagana* Mariz (1891: 240) *p.p. excl. spec.* Peniche, Cabo Carvoeiro *et* Ilha Berlenga; Coutinho (1913: 642, 1939: 759) *p.p. excl. spec.* Praia da Rocha; Sampaio (1947: 579). Lectotype (designated here):—PORTUGAL. Estremadura, Rampa de l'estuaire du Tage près Almada, April 1883, *Daveau 979* (COI00023378 photo!).

C. lusitanica var. *microcephala* Lange ex Henriques (1883: 51). **Type:—**unknown

C. lusitanica var. *microcephala* (Lange) Mariz (1891: 240) *nom. illeg. superfl.*; Coutinho (1913: 642, 1939: 759); Sampaio (1947: 579).

Other synonyms:

C. suffruticosa subsp. *lusitanica auct.* Valdés (1987: 80) *p.p. non* (Boiss.) Ohle (1974: 270).

Perennial or annual herbs, ± woody at the base. Stems (18) 42–75 (204) cm tall, predominantly diffuse to erect, ± branched, with glandular and non-glandular hairs in variable proportions, ± viscous. Basal leaves (3.5) 4.7–8 (15) × (0.8) 1.2–2.5 (4.8) cm, (0.3) 0.3–0.4 (0.5) mm thick, narrowly oblanceolate to spatulate, apex acute to obtuse, margins sub-entire to repand-dentate, attenuate into a ± large petiole, with non-glandular hairs more abundant than glandular hairs. Capitula (2) 2.7–3.5 (4.5) cm in diameter. Outer achenes rostrate (1.2) 9–16.3 (32) × (1) 1.3–1.7 (2.7) mm, generally slightly curved to straight, without dorsal spines or with them small, sometimes with 1 tooth at the base and/or another at the apex; middle achenes typically sub-exalate (5) 5.5–6.7 (9.3) × (2) 2.5–3.5 (5.5) mm, lateral wings non-existent or narrow and sinuate-dentate, and ventral wing also non-existent or narrow and entire, sometimes cymbiform (5.2) 6.1–7.5 (10.7) × (4.2) 5–6.1 (8) mm, with the ventral wing smallest to sub-equal to the laterals; inner achenes vermiform-alate (4) 4.5–5.2 (6.8) × (2.3) 2.7–3.7 (4.5) mm, rarely present, and vermiform-exalate (3) 3.7–4.5 (5.3) × (1) 1.3–1.8 (2.2) mm, always present, both generally circular or almost.

Illustration: Figure 41.

Habitat and distribution: Clearings of forests and shrub vegetation, on granitic, loamy, limestone soils, hills and mountains of the coast; 0–500 m elev. Centre of PORTUGAL, plus Serra de Monchique in Algarve, and one population in Northern Morocco – Figure 31.

Conservation status: IUCN Red List Category for the Iberian Peninsula: **Least Concern (LC)**. The taxon meets the criteria for the area under criterion B for VU (EOO = 11,752 km^2) or EN (AOO = 160 km^2), but its locations are much more than 10 and it cannot be considered to be severely fragmented since more than 50% of its individuals are in the bigger subpopulations. Furthermore, although some subpopulations are small and threatened, most are doing well, and there are no known extreme fluctuations, and neither a significant and general population reduction. Al this considered, we assess it as LC, according to the IUCN Red List Categories and Criteria (2012).

Chromosome number: $2n = 32$

Genome size: 3.38 ± 0.08 pg

C. tripterocarpa Ruprecht (1856: 231); Meikle (1976: 207); Greuter (2008: 57); Blanca (2011: 1610); Greuter (2006+[2017]). Lectotype (designated by Heyn *et al.* 1974)**:—** IRAQ. Mesopotamia: '*Ad ripas Tigridis raram*', *Noë* 515, 407 (lectotype LE!).

Annual herbs. Stems (2) 5.6–15.8 (30) cm long, decumbent to diffuse, generally branched, with glandular and non-glandular hairs, in variable proportions. Basal leaves (1) 2–3.2 (9.5) × (0.2) 0.3–0.5 (0.8) cm, (0.3) 0.3–0.4 (0.5) mm thick, linear-oblong, acute, margin repand-dentate, rarely sub-entire, base attenuated in a ± longer petiole, the middle and upper cauline leaves, are smaller, oblanceolate to lanceolate, usually auriculate. Capitula solitary, (1.1) 1.2–1.4 (1.6) cm in diameter. Ray florets 10–22, arranged in 1 row, (5.8) 6–7.7 (8.7) × 0.6–1.2 mm, slightly surpassing the involucre, yellow or orange. Disc florets (4) 6–20, 1.8–2.4 × 0.6–1.2 mm, yellow. Anthers 1–1.5 mm long. Styles 1.4–1.6 mm. Outer achenes trialate (5.7) 6.3–7.9 (9.5) × (4.5) 5.5–7.1 (8.5) mm, with wings entire to inconspicuously dentate, flat or muricate in the back, without dorsal spines; middle achenes cymbiform (4.2) 4.5–6.1 (6.7) × (3.5) 4.6–5.7 (6.7) mm, with ventral wings sub-equal or slightly surpassing the laterals; inner achenes vermiform-exalate (2.2) 2.5–3.3 (4.2) × (0.7) 1–1.2 (1.2) mm, circular or falcate; with a small, apical or basal, ventral tooth like wing.

Illustration: Lanza (1919): tab. 4; Heyn *et al.* (1974, 179): Figure 43.

Habitat and Distribution: In grasslands, semi-desert to desert regions, sand, sandstone and gravel, rarely in ruderal/wasteland sites, 0–450 m elev. In the South of Europe from Almeria to Murcia and Alicante (SPAIN) to the South of France (occasional) and in all the North of Africa to the Middle East. Some literature records from the Balearic Islands could not be confirmed since forms of *C. arvensis* with bialate achenes are often confounded with *C. tripterocarpa*, which has trialate external achenes – Figure 14. **Conservation status:** IUCN Red List Category for the Iberian Peninsula: **Vulnerable (VU) B1ab (ii, iii, iv) +2ab (ii, iii, iv)**. In the Iberian Peninsula, the species meets the area requirements under criterion B for VU based on the calculation of EOO, which is estimated to be 6,074 km^2. Based on the AOO, which is estimated to be 60 km^2, the species meets the area requirements under criterion B for EN. However, since the number of locations seem to be more than 5 and less than 10 (±7) and that most of its subpopulations seem to be relatively well interconnected, and not very far one from another, except for the single known population in Alicante and there are no extreme fluctuations, added to the recent habitat reduction caused by greenhouse construction and urban expansion in the region, which are expected to continue in the coming years, we propose the assessment as Vulnerable (VU), according with the IUCN Red List Categories and Criteria (2012). Since the Iberian populations do not seem to benefit from any immigration of propagules from the nearest neighbour subpopulations in North Africa, there is no change of category that should be applied.

Chromosome number: *2n* = 30

Genome size: 3.44 ± 0.06 pg

Notes: It has been frequently confused with dwarf forms of *C. arvensis*, especially of var. *macroptera*, although its trialate achenes are unmistakable.

C. arvensis Linnaeus (1763: 1303); Willkomm & Lange (1865: 125); Willkomm (1893: 88); Merino y Roman (1906: 400); Coutinho (1913: 642, 1939: 758); Meikle (1976: 207); Franco (1984: 432); Valdés *et al.* 1987: 78); Bolós & Vigo (1995: 856); Aizpuru Oiarbide *et al.* (1999: 550); Greuter (2008b: 54); Blanca (2011: 1610); Greuter 2006+[2017]). Lectotype (designated by Heyn *et al.* 1974)**:—**EUROPE: '*in Europae arvis*', *Löfling s.n* (LINN! [1035.1]).

Heterotypic synonyms:

C. aegyptiaca Persoon (1807: 492); Blanca (2011: 1609). **Type**:—EGYPT. Bords des champs, Matarych près de Caire, *Kralik L. s.n.* (holotype FI! 000477])

C. parviflora Rafinesque (1810: 83) *nom. illeg. non* Thunberg (1800: 163); Willkomm & Lange (1865: 125); Willkomm (1893: 88). **Type**: —unknown.

C. bicolor Rafinesque (1810: 82). *C. arvensis* var. *bicolor* (Rafinesque) Candolle (1837: 452) **Type**: — unknown.

C. malacitana Boissier & Reuter (1852: 61); Willkomm & Lange (1865: 125) ; Willkomm (1893: 88). *C. arvensis* subsp. *malacitana* (Boissier & Reuter) Coutinho (1913: 642; 1939: 758). *C. arvensis* var. *malacitana* (Boissier & Reuter) Coutinho *ex* Bolós & Vigo (1995: 857). Syntypes:—PORTUGAL : '*Lusitania*', *Welwitsch, F. 252* (syntype G! [00074224]); —SPAIN: '*Malacae arvis*', 1838, *Boissier s.n.* (syntype G! [G00472061])

C. arvensis subsp. *macroptera* Rouy (1903: 355). *C. arvensis* var. *macroptera* (Rouy) Bolós & Vigo (1995: 857). **Type:—**unknown.

Annual herbs. Stems (2) 17.3–45.5 (80) cm long, ascending to erect, sometimes decumbent, branched at the base, with glandular and non-glandular hairs, in varying proportions, ± viscous. Basal leaves (1.2) 2.4–4.8 (6.5) × (0.2) 0.4–0.8 (1.1) cm, (0.3) 0.3–0.5 (0.5) mm thick, oblanceolate, acute, or obtuse, margin sub-entire to sinuate-dentate, base attenuated in a ± longer petiole, with glandular and non-glandular hairs; the middle and upper cauline leaves progressively smaller toward the apex, oblanceolate to lanceolate, sessile and usually auriculate. Capitula solitary, (1) 1.2–1.4 (2) cm of in diameter. Bracts (4.1) 6.7–7.9 (10.4) × (0.6) 1–2.4 mm, arranged in 1–2 rows, sub-equal, linear-lanceolate, usually acute, green and reddish at the apex, with glandular and non-glandular hairs, margin hyaline. Ray florets (13) 17–21, (4.5) 8.2–10.2 (13) × (1) 1.3–2.7 mm, usually shorter than twice the involucre, arranged in 1–2 rows, with 3 teeth at the apex, yellow or orange. Disc florets (14) 23–31 (38), 2.7–3.4 × 1.1–1.8 mm, yellow or purple-brown. Anthers 1.1–1.9 mm long. Styles 2.2–2.7 mm long. Outer achenes rostrate (5) 7.1–11.7 (15.2) × (0.8) 1.3–1.9 (2.7) mm, usually strongly curved and aculeate at back, usually with 2 small teeth, one at the base and another at the apex, sometimes bialate (8.3) 10.8–12.4 (13.3) × (7.2) 7.7–9.3 (10.2) mm, with 2 wings, with irregularly incise margins, extended along both sides up to the apex; middle achenes cymbiform (4.8) 6.7–8 (8.5) × (3.8) 5.3–7 (8) mm, sometimes lacking; inner achenes vermiform-alate (3.5) 3.8–5.2 (5.8) × (2) 2.7–3.3 (3.7) mm, hook-shaped to circular, with 2 narrow lateral wings, and/or vermiform-exalate (3.2) 3.7–4.5 (5.3) × (1.2) 1.2–1.7 (1.8) mm, circular.

Illustration: Heyn *et al.* (1974, 181, 183, 184, 188, 189): Figures 4-10; Valdés *et al.* (1987: 78). Figure 43.

Habitat and distribution: Dispersed throughout the Iberian Peninsula and the Balearic Islands in waste grounds, cultivated fields and grasslands or in the margin of roads and ditches, up to 1300 m. The remainder of its native range embraces also Central and South Europe, North Africa, SW Asia and Macaronesia. Introduced in other parts of the globe such as Australia and California.

Conservation status: IUCN Red List Category for the Iberian Peninsula: **Least Concern (LC)**. *C. arvensis* is a widely-spread plant in the Iberian Peninsula (Figure 14), and in most of the circum-Mediterranean region, inhabiting ruderal and waste grounds. Currently, it does not face any major threat in the region. Therefore, according to the IUCN Red List Categories and Criteria (2012), this species is assessed as Least Concern (LC) for the Iberian Peninsula.

Chromosome number: $2n = 44$.

Genome size: 5.69 ± 0.65 pg

C. officinalis Linnaeus (1753: 921); Willkomm & Lange (1865: 126); Merino y Roman (1906: 399); Coutinho (1913. 641, 1939: 758); Sampaio (1947: 580); Meikle (1976: 207); Franco (1984: 432); Bolós & Vigo (1996: 858); Aizpuru Oiarbide *et al.* (1999: 550); Greuter (2008b: 56); Blanca (2011: 1610); Greuter (2006+[2017]). Lectotype (designated by Alavi 1983)**:—**EUROPE: '*in Europae arvis*' *Löfling s.n.* (LINN! [1035.4])

Annual or perennial herbs, ± woody at the base. Stems (31) 37.9–86 (94) cm long, erect, diffuse or prostrate, ± branched, leafy almost to the apex, with glandular and non-glandular hairs in varying proportions, ± viscous. Basal leaves (8.7) 10.8–12.2 (15) × (2.5) 2.7–3 (4.5) cm, (0.3) 0.3–0.6 (0.7) mm thick, oblanceolate, narrowly obovate, oblong or spatulate, shortly acute or obtuse, margin sub-entire to repand-dentate, base attenuated in a ± longer petiole, with glandular and non-glandular hairs in varying proportions; the middle and upper cauline oblanceolate to lanceolate, usually auriculate, progressively smaller and sessile toward the apex. Capitula solitary (4.8) 5.2–7.6 (8) cm in diameter. Bracts 4.8–15.5 × 1–2.9 mm, arranged in 2–(3) or more rows, sub-equal, linear-lanceolate, acute, green with hyaline margin, frequently reddish at the apex, with glandular and non-glandular hairs. Ray florets 20–60, 17–32 × 3–5.1 mm, more than twice the length of the involucre, arranged in 2 or more rows, with 3 teeth at the apex of the limb, yellow or orange. Florets (60) 80–130 (300), 3.2–5.8 × 1.8–3.2 mm, yellow or dark purple. Anthers 2–2.7 mm long. Styles 2.9–4.8 mm long. Outer achenes rostrate, (5.8) 9.4–16.8 (19) × (1.8) 2.8–3.8 (4.7) mm, usually ± curved, sometimes with small dorsal spines and with 1 or 2 ventral teeth, one at the base and another at the apex; middle achenes cymbiform (6.2) 10.5–13 (15.7) × (3.7) 7–8.8 (11.3) mm; inner achenes vermiform-alate (6.7) 7–8 (9.2) × (3.5) 3.8–5 (5.8) mm, hook-shaped, with two narrow lateral wings, and/or vermiform-exalate (5) 5.8–6.3 (7.5) × (1.8) 2–2.6 (3) mm, ± circular or hook-shaped.

Illustration: Brandt *et al.* (1890) plate 61 -- Figure 43.

Habitat and distribution: Cultivated as an ornamental and medicinal plant, up to 1380 m of elev., sometimes escaped to waste ground or other ruderal vegetation. Widely dispersed throughout the world by humans – Figure 14.

Conservation status: IUCN Red List Category: **Not Applicable**. The origin of this species is unknown but as it is widely cultivated since ancient times, and only sometimes escaping to waste ground and roadsides, it has been treated here as a domesticated taxon and was not assessed. Only wild taxa were assessed following the IUCN Red List Categories and Criteria (2012).

Chromosome number: $2n = 28$[1], 32.

Genome size: 2.98 ± 0.08 pg

---

[1] A miscount, see Nora *et al.* (2013)

Figure 32 – *C. suffruticosa* subsp. *cinerea* (Silveira & Gonçalves 3256a, AVE). A) Habit; B) flowering capitulum; C) fruiting capitulum.

Figure 33 – *C. suffruticosa* subsp. *tomentosa* (Silveira 3043, AVE). A) woody base of the stem; B) flowering capitulum; C) Habit; D) immature fruiting capitulum; E) mature fruiting capitulum.

Figure 34 – A –D) *C. suffruticosa* subsp. *marginata* (A–C. Silveira & Gonçalves 3302, AVE; D. Silveira & Gonçalves 3306, AVE). A) habit; B) habit of a shade form; C) fruiting capitulum of the typical form from Gibraltar; D) fruiting capitulum of the form from Punta Carnero; E-F); *C. suffruticosa* subsp. *vejerensis* (Silveira & Gonçalves 3186, AVE); E) habit; F) fruiting capitulum.

Figure 35 – Variability of leaf morphology in *Calendula*. A) *C. suffruticosa* subsp. *cinerea* (Silveira & Gonçalves 3256a, AVE); B) *C. suffruticosa* subsp. *tomentosa* (Silveira & Gonçalves 3270, AVE); C) *C. suffruticosa* subsp. *marginata* (Silveira & Gonçalves 3302, AVE); D) *C. suffruticosa* subsp. *vejerensis* (Silveira & Gonçalves 3186, AVE); E) *C. suffruticosa* subsp. *trialata* (Silveira & Gonçalves 3185, AVE); scale bars = 1 cm.

Figure 36 – A–D) *C. suffruticosa* subsp. *trialata* (A–B Silveira & Gonçalves 3310, AVE; C–D. Silveira 3041, AVE). A) habit of a shade form in spring; B) fruiting capitulum; C) habit of a form growing near the sea in June; D) fruiting capitulum; E–F) *C. suffruticosa* subsp. *carbonelli*; E) habit (Silveira & Gonçalves 3223, AVE); F) fruiting capitulum (Silveira & Gonçalves 3222, AVE);

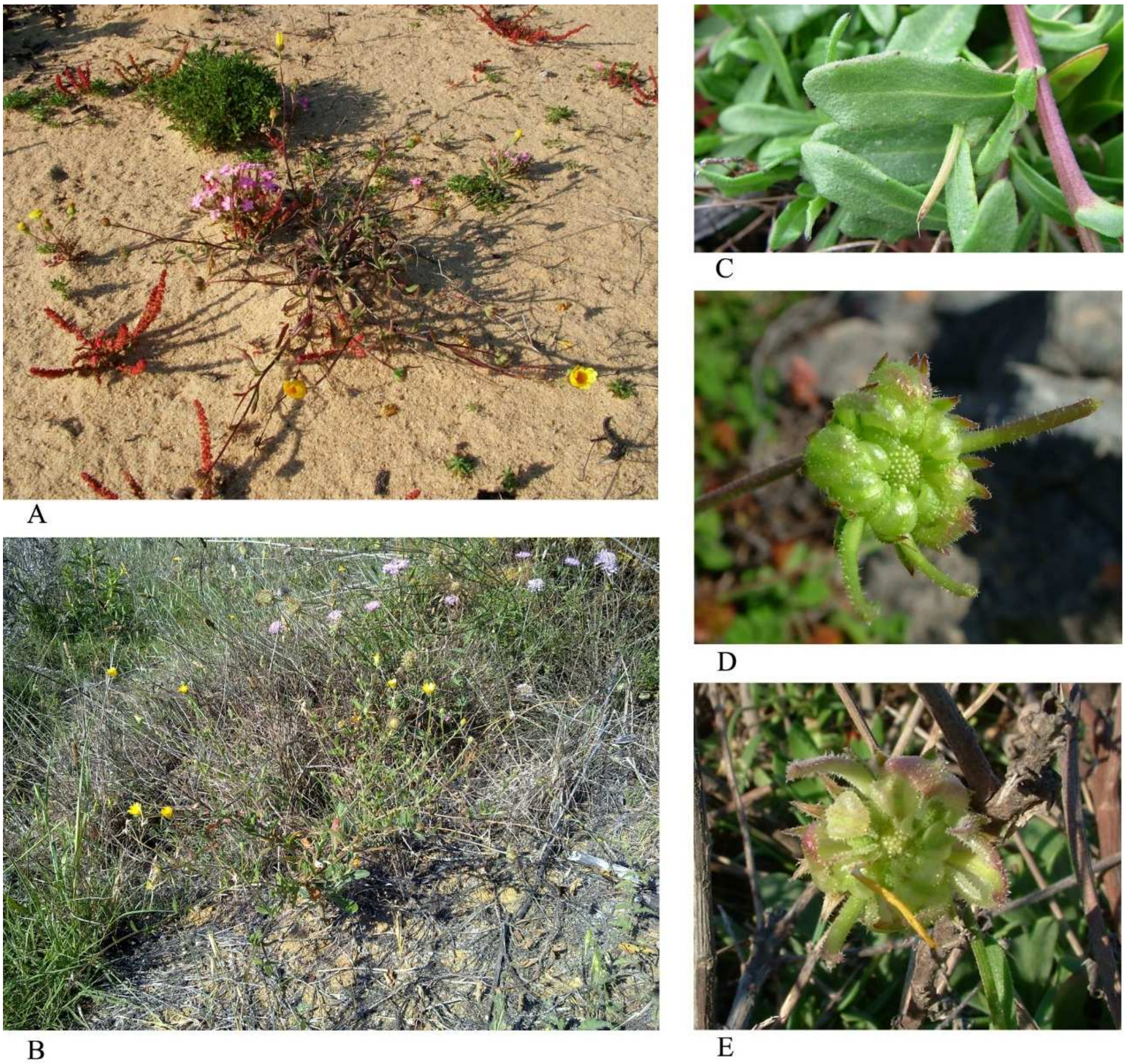


Figure 37 – *C. suffruticosa* subsp. *algarbiensis*. A) habit of a prostrate form (Silveira 2891, AVE); B) habit of a diffuse form (Silveira & Gonçalves 3255, AVE); C) detail of leaves (Silveira & Nora 3029, AVE); D) fruiting capitulum (Silveira & Gonçalves 3238, AVE); E) fruiting capitulum (Silveira & Nora 3029, AVE);

Figure 38 – *C. suffruticosa* subsp. *greuteri*. (A–C. Silveira & Gonçalves 3195, AVE); A) habit; B) detail of leaves; C) flowering capitulum; D) fruiting capitulum (Silveira & Gonçalves 3200, AVE); E) flowering capitulum with red strips on the back of the ligules (Silveira & Gonçalves 3196, AVE).

Figure 39 – Variability of achene morphology of *Calendula*. A) *C. suffruticosa* subsp. *cinerea* (Silveira & Gonçalves 3256, AVE); B) *C. suffruticosa* subsp. *tomentosa* (Silveira 2937b, AVE); C) *C. suffruticosa* subsp. *marginata* (Silveira & Gonçalves 3302, AVE); D) *C. suffruticosa* subsp. *marginata* (Silveira & Gonçalves 3305, AVE); E) *C. suffruticosa* subsp. *vejerensis* (Silveira & Gonçalves 3186, AVE); F) *C.*

suffruticosa subsp. *trialata* (Silveira 3041, AVE); G) *C. suffruticosa* subsp. *carbonelli* (Silveira & Gonçalves 3222, AVE); H) *C. suffruticosa* subsp. *algarbiensis* (Silveira & Gonçalves 3255, AVE); I) *C. suffruticosa* subsp. *algarbiensis* (Silveira 2977, AVE); J) *C. suffruticosa* subsp. *algarbiensis* (Silveira 3022, AVE); K) *C. suffruticosa* subsp. *lusitanica* (Silveira 3017b, AVE). Rostrate achenes: A1, A2, B1, C1, D1, D2, E1, E2, E3, F1, F2, G1, G2, H1, H2, I1, I2, J1, J2, K1, K2; bialate achenes: B2, F3; cymbiform achenes: A3, A4, B5, B6, C2, C3, D3, D4, G3, G4, H3, I3, I4; sub-cymbiform achenes: B4, E4, E5, J3, J4; trialate achenes: B3, F4, F5; sub-exalate achenes: K3, K4; vermiform-alate achenes: C4, D5, G5, H4; vermiform-exalate: A5, B7, B8, C5, D6, E6, E7, G6, H5, I6, J5, K5. All achenes in side view, except: A3, B4, C2, D3, E4, F3, F4, G3, H3, J3, J4 and K3 in ventral face view, and C3, E5, F5 and G4 in ± dorsal face view. Scale bars = 1 cm

Figure 40 – Variability of leaf morphology in *Calendula*. A) *C. suffruticosa* subsp. *carbonelli* (1. Silveira & Gonçalves 3222, AVE; 2–4. Silveira & Gonçalves 3223, AVE); B) *C. suffruticosa* subsp. *algarbiensis* (1. Silveira & Gonçalves 3249, AVE; 2. Silveira & Gonçalves 3254, AVE; Silveira & Gonçalves 3255, AVE); C) *C. suffruticosa* subsp. *greuteri* (Silveira & Gonçalves 3197, AVE); D) *C. suffruticosa* subsp. *lusitanica* (1. Silveira & Gonçalves 3245, AVE; 2. Silveira & Gonçalves 3252, AVE; 3–4. Silveira & Gonçalves 3237, AVE); Scale bars = 1cm.

Figure 41 – A–E) *C. suffruticosa* subsp. *lusitanica* (A. Silveira & Gonçalves 3237, AVE; C–E. Silveira & Gonçalves 3234, AVE). A) habitat on mountain top of Serra de Montejunto; B) habit of form growing on shade of trees; C) fruiting capitulum; D) fruiting capitulum; E) flowering capitulum; F–I) *C. tripterocarpa* (F–G. Silveira 2982, AVE; H–I. Silveira & Gonçalves 3215, AVE); F) habitat on the Tabernas region, South of Spain; G) habit; H) fruiting capitulum; I) flowering capitulum.

Figure 42 – Variability of achene morphology of *Calendula*. A) *C. suffruticosa* subsp. *greuteri* – typical form (Silveira 2983, AVE); B) *C. suffruticosa* subsp. *greuteri* – less frequent form (Silveira & Gonçalves 3200, AVE); C) *C. tripterocarpa* (Silveira 2982, AVE); D) C. *arvensis* – typical form (Nora in Silveira 3054, AVE); E) C. *arvensis* – '*macroptera*' form (Silveira 2985, AVE); F) *C. officinalis* (Silveira 3058, AVE). Rostrate achenes: A1, A2, B1, B2, D1, E1, F1, F2; cymbiform achenes: A3, C3, C4, D2, D3, E4, F3; sub-cymbiform achenes: B3, B4, F4; bialate achenes: E2, E3; trialate achenes: C1, C2; vermiform-alate achenes: A4, B5, D4, E5, F5; vermiform-exalate: C5, D5, E6, F6, F7, F8. All achenes in lateral view, except B3, C2, C3, D2, E2 and F3 in ventral face view, and B4, C4 and D3 in ± dorsal face view. Scale bars = 1 cm.

Figure 43 – A–E) *C. arvensis*. A). habit (Silveira & Gonçalves 3208, AVE); B) form with purple disc florets (Silveira 2978, AVE). C) form with strait rostrate achenes and slightly whitish indumentum (Silveira & Gonçalves 3209a, AVE); D) fruiting capitulum of a “*macroptera*” form (Silveira 2980, AVE); E) fruiting capitulum with less spiny and shorter rostrate achenes (Silveira & Gonçalves 3206, AVE); F–G) *C. officinalis* (cultivated); F) flowering and fruiting capitulum; G) flowering capitulum of a double-flowered form.

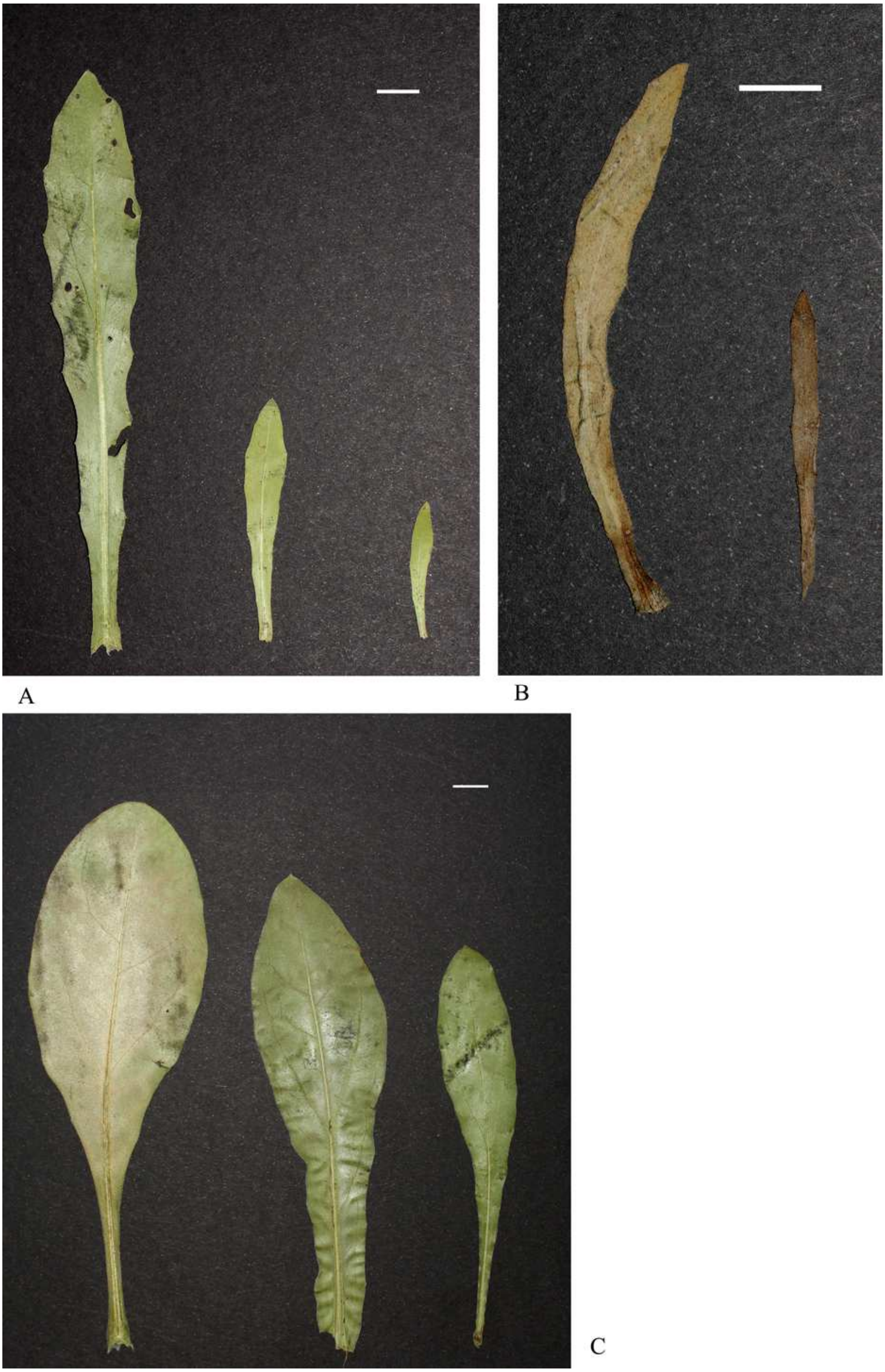


Figure 44 – Variability of leaf morphology in *Calendula*. A) *C. arvensis* (Silveira 3345, AVE); B) *C. tripterocarpa* (Silveira & Gonçalves 3215, AVE); C) *C. officinalis* (Silveira 3345, AVE). Scale bars = 1cm.

Conclusions

Calendula is an extremely complex and poorly understood genus. However, our results indicate that most of the taxa can be sufficiently well characterised from a morphological perspective. This study was mostly focused on the morphological variation of the taxa, but also comparing with chromosome numbers and genome size data. Our treatment for *Calendula* in the Iberian Peninsula and the Balearic Islands includes 12 taxa, most of them endemic to the region.

The high diversity of combinations of various morphs of achenes and the high number of characters required for its analysis were the major challenges to distinguish taxa. The morphology of the achenes cannot be neglected as has been done, for example, by Meikle (1976). For instance, the achenes are the best characters to distinguish small sized forms of *C. arvensis*, which may present several morphs of achene morphologies, from *C. tripterocarpa*, which presents a lower variation and always trialate achenes, which do not occur in *C arvensis*. The combination of different morphs of achenes is an important character to distinguish taxa, although it is sometimes difficult to interpret since a morph can appear in more than one species, and one species may present more than one combination of morphs. *C. suffruticosa* includes all morphs of achenes, while other species are distinguished by having a lack of one or more morphs of achenes. For example, *C. tripterocarpa* never presents rostrate or bialate achenes.

In addition, we cannot admit as correct the inclusion in the same taxon of plants with similar indumentum, but that have achenes with different morphologies, such as those of Tarifa, versus Gibraltar or Cabo de São Vicente. Furthermore, we have not seen in the study area any population of the *C. suffruticosa* group with achenes similar to those occurring in Tunisia (Porto Farina, Hamam Lif, Cap Bon), from where the species has been described. Therefore, in our opinion, all records of *C. suffruticosa* subsp. *suffruticosa*, in the Iberian Peninsula, should be assigned to another subspecies. It could be argued that some of these subspecies should be raised to the rank of species. But, all have $2n$ = 32, they hybridise easily, in many cases populations with intermediate characteristics occur, and the great variability and little constancy of some characteristics, associated with the absence of reliable detailed studies of the genetic variability that can clarify the degree of affinity between the different entities, have led us to maintain, for the moment, the inclusion of all these taxa in a single species, with its various subspecies. Further studies should use molecular methods to corroborate the results obtained and assess evolutionary relationships among taxa. A chemical characterisation, including analysis of the intra and inter-population variability, could also aid in the enlightenment of the diversity and relationships of the *Calendula* taxa.

Acknowledgments

The authors are grateful to several herbaria (ABH, AL (ENSA), ARAN, B, BC (IBB), BCN, BM, BONN, BR, C, COI, E, ELVE, FI, G, GAT, GDA (GDAC), HAL, JACA, JAEN, JE, K, LD, LEB, LISI, M, MA, MACB, MAF, MARK, MGC, MPU, O, P, PO, RAB, RNG, SALA, SANT, SEV, TFMC, US, VAL and W) which allowed the study and loaned specimens of *Calendula*. We are very grateful to several institutions that have authorized collections of *Calendula* specimens, namely: Instituto da Conservação da Natureza e das Florestas (Portugal), Parque Nacional de las Islas Atlanticas de Galicia y Parque Natural de la Sierra de las Nieves (Spain). Violeta Barrios kindly revised the IUCN assessments. This study was partly funded by the Portuguese Foundation for Science and Technology and UNESCO Fund for financing the work of ACG with the scholarship SFRH/BD/51464/2011 and by the University of Aveiro and FCT/MEC for the financial support to CESAM RU (UID/AMB/50017), through national founds and where applicable co-financed by the FEDER, within the PT2020 Partnership Agreement. Additional support was provided by SYNTHESYS, grant GB-TAF-2900, to Paulo Silveira, allowing the study of BM and K's *Calendula* collections.

References

Afonso, A., Castro, S., Loureiro, J., Mota, L., Cerca de Oliveira, J. & Torices, R. (2014) The effects of achene type and germination time on plant performance in the heterocarpic *Anacyclus clavatus* (Asteraceae). *American Journal of Botany* 101: 892–898. http://dx.doi.org/10.3732/ajb.1400030

Aizpuru Oiarbide, I., C. Aseginolaza Iparragirre, P.M. Uribe-Echebarría Díaz, P. Urrutia & I. Zorrakin (eds.) (1999) *Claves ilustradas de la flora del País Vasco y territorios limítrofes*. Gobierno Vasco. Vitoria. 831 pp.

Alavi, S.A. (1983) Asteraceae. *Calendula* L. *In*: Jafri S.M.H. and El-Gadi A. (Eds.), *Flora of Libya*. Al Faateh University, Dept. of Botany, Tripoli, pp. 193–198.

Antunes, P., Coutinho, A.X.P. & Silveira, P. (2007) Palinologia do género *Calendula* na região Mediterrânica Ocidental. Unpublished internship report. University of Aveiro, Aveiro. 65 pp.

Applequist, W.L. (2014) Report of the Nomenclature Committee for Vascular Plants: 66. *Taxon* 63: 1358–1371. http://dx.doi.org/ 10.12705/636.20

Ball, J. (1878) Spicilegium florae Maroccanae. *The Journal of the Linnean Society, Botany* 16: 281–772. Available from:

Baltisberger, M. & Widmer, A. (2006) Chromosome numbers of plant species from the Canary Islands. *Botanica Helvetica* 116 : 9–30. http://dx.doi.org/10.1007/s00035-006-0739-x

Battandier, J.-A. (1921) De l'espèce dans le genre *Calendula*. *Bulletin de la Société Botanique de France* 68 : 527–531.http://dx.doi.org/10.1080/00378941.1921.10836231

Blanca, G., Cabezudo, B., Cueto, M., Salazar, C. & Morales Torres, C. (2011) *Flora Vascular de Andalucía Oriental*. 2nd Ed. Consejería de Medio Ambiente (Junta de Andalucía), Sevilla, 1751 pp.

Boissier, P.-E. (1839) *Voyage botanique dans le midi de l'Espagne pendant l'année 1837,* vol 1. Gide & Cie, Paris, 469 pp.

Boissier, P.-E. (1849) *Diagnoses plantarum orientalium novarum. Series secunda*, vol. 2. No. 10. Ramboz & Schuchardt, Genève, 122 pp.

Boissier, P.-E. (1859) *Diagnoses plantarum orientalium novarum. Series secunda,* vol. 2. No. 6. Ramboz & Schuchardt, Genève, 148 pp.

Boissier, P.-E. & Reuter, G.-F. (1852) *Pugillus plantarum novarum Africae borealis Hispaniaeque australis*. Ramboz & Schuchardt, Genève, 134 pp.

Bolós, O. & Vigo, J. (1995) *Flora dels Països Catalans*. Vol. 3. Editorial Barcino. Barcelona.

Brandt, W., Gürke, M., Köhler, F.E., Pabst, G., Schellenberg, G. & Vogtherr, M. (1890) *Köhler's Medizinal-Pflanzen in naturgetreuen Abbildungen mit kurz erläuterndem, [*...]. Fr. Eugen Köhler, Gera-Untermhaus

Brummitt, R.K. (2008) Proposal to Add the 1754-1756 German Translation of S. Vaillant, Établissement de nouveaux caractères de trois familles, 1719-1725, to the "Opera Utique Oppressa." *Taxon* 57: 663. http://dx.doi.org/10.2307/25066051

Burdet, H., Charpin, A. & Jacquemoud, F. (1983) Types nomenclaturaux des taxa ibériques décrits par Boissier ou Reuter. IV. Cistacées à Composées. *Candollea* 38: 751–802.

Candolle, A. P. de (1837) *Prodromus systematis naturalis regni vegetabilis* [...]. vol. 6. Treuttel & Würtz, Paris, 687 pp.

Castroviejo, S. (Ed.) (1986 - ongoing) *Flora Iberica.* Vol. 1-21. Real Jardín Botánico, CSIC, Madrid.

Cassini AHG (1819) Sixième mémoire sur la famille des Synanthérées, contenant les caractères des tribus. J Phys Chim d'Histoire Nat des Arts 88:150–163.

Cavanilles, A.J. (1791) *Icones et descriptiones plantarum, [*...], vol. 1. Ex Regia Typographia, Madrid, 67 pp.

Coutinho, A.X.P. (1913) *A Flora de Portugal (Plantas vasculares) Disposta em chaves dicotómicas*. 1. Ed. Aillaud, Alves & Cia, Lisboa. 767 pp.

Coutinho, A.X.P. (1939) *Flora de Portugal (Plantas vasculares) Disposta em chaves dicotómicas*. 2. Ed. Bertrand, Ltd., Lisboa. 938 pp.

Cuvier, C. (1819) *Recueil des éloges historiques de Louis G. Lemonnier*. F. G. Levrault (Ed). Strasbourg, Paris.

Desfontaines, R.L. (1799) *Flora atlantica: sive historia plantarum quae in Atlante, agro tunetano et algeriensi crescunt. [...] Tomus secundus*, L.G. Desgranges, Paris, 458 pp.

Desfontaines R.L. (1804) Tableau de l'École de botanique du Muséum d'histoire naturelle. J. A. Brosson, Paris, 238 p.

Ehrendorfer, F. (1970) Mediterran-mitteleuropäische Florenbeziehungen im Lichte cytotaxonomischer Befunde. *Feddes Repertorium* 81: 3–32. http://dx.doi.org/10.1002/fedr.19700810103

Franco, J. do A. (1984) *Nova Flora de Portugal. Clethraceae-Compositae.* vol. II. Sociedade Astória, Lisboa, 660 pp.

Gallego, M.J. & Talavera, S. (1983) Nota sobre las especies Españolas del genero *Calendula* L. *Lagascalia* 11: 100–104.

Gardocki, M.E., Zablocki, H., El-Keblawy, A. & Freeman, D.C. (2000) Heterocarpy in *Calendula micrantha* (Asteraceae): The effects of competition and availability of water on the performance of offspring from different fruit morphs. *Evolutionary Ecology Research* 2: 701–718.

Garnatje, T., Canela, M.Á., García, S., Hidalgo, O., Pellicer, J., Sánchez-Jiménez, I., Siljak-Yakovlev, S., Vitales, D. & Vallès, J. (2011) GSAD: A genome size in the Asteraceae database. *Cytometry Part A* 79A: 401–404.. http://dx.doi.org/10.1002/cyto.a.21056

Gonçalves, A.C., Ouhammou, A., Jury, S. & Silveira, P. (2014) *Calendula* L. *In*: Oualidi J., Fennane M., Ibn Tattou, M. (Eds.), *Flore Pratique du Maroc.* Université Mohammed V, Rabat, pp. 270–275.

Greuter, W. (2006+[2017]): Compositae (pro parte majore). – In: Greuter, W. & Raab-Straube, E. von (ed.): Compositae. *Euro+Med Plantbase - the information resource for Euro-Mediterranean plant diversity.* Available from: http://ww2.bgbm.org/EuroPlusMed/query.asp (assessed: 19 october 2017).

Greuter, W. (2008a) Damn Vaillant? A reply to Brummitt in Taxon 57: 663. 2008, and some alternative proposals, including adding all Steinwehr's translations in Königl. Akad. Wiss. Paris Phys. Abh. 5–9. 1754–1760 to the "Opera utique oppressa". *Taxon* 57: 1015–1016.

Greuter, W. (2008b) *Med-Checklist. A critical inventory of vascular plants of the circum-mediterranean countries*, 2. Dicotyledones (Compositae). - Palermo, Genève & Berlin. 798 pp.

Greuter, W., Aghababian, M. & Wagenitz, G. (2005) Vaillant on Compositae: Systematic Concepts and Nomenclatural Impact. *Taxon* 54: 149–174. http://dx.doi.org/10.2307/25065315

Gussone, G. (1825) *Index seminum anni 1825 quae ab Horto Regio in Boccadifalco pro mutua commutatione exhibentur.* 12 pp.

Heyn, C.C. (1988) Investing adaptations to rare events - a reproductive strategy. *Lagascalia* 15: 29–36.

Heyn, C.C., Dagan, O. & Nachman, B. (1974) The annual *Calendula* species: taxonomy and relationships. *Israel Journal of Botany* 23: 1201–1969.

Heyn, C.C. & Joel, A. (1983) Reproductive relationships between annual species of *Calendula. Plant Systematics and Evolution* 143: 311–329. http://dx.doi.org/10.1007/BF00986612

Hitchcock, A.S. & Green, M.L. (1929) Standard-species of Linnaeus genera of Phanerogamae (1753-1754). In *International Botanical Congress, Cambridge (England), 1930: Nomenclature. Proposals by British botanists*. His Majesty's Stationery Office. London. pp. 110-199.

Imbert, E. (2002) Ecological consequences and ontogeny of seed heteromorphism. *Perspectives in Plant Ecology, Evolution and Systematics* 5: 13–36. http://dx.doi.org/10.1078/1433-8319-00021

Imbert, E., Escarré, J. & Lepart, J. (1996) Achene Dimorphism and Among-Population Variation in *Crepis sancta* (Asteraceae). *International Journal of Plant Sciences* 157: 309–315. http://dx.doi.org/10.1086/297350

IPNI [The International Plant Names Index] (2017). Published on the Internet http://www.ipni.org [accessed 21 October 2017]

IUCN (2012) *The IUCN Red List Categories and Criteria*. IUCN. Available from: http://www.iucnredlist.org/.

IUCN (2017) *Guidelines for using the IUCN Red List Categories and Criteria*. Version 13. Prepared by the Standards and Petitions Subcommittee. Downloadable from http://www.iucnredllist.org/documents/RedListGuidelines.pdf.

Jahandiez, É. & Maire, R.-C. (1934) *Catalogue des plantes du Maroc. Dicotylédones et Supplément aux volumes I & II.* Imprimerie Minerva, Alger, 913 pp.

Knoche, H.W. (1922) *Flora balearica, étude phytogéographique sur les îles Baléares.* Imp. Roumègous et Dèhan, Montpellier.

Lange, J. (1883). In : *I Boletim annual. Sociedade Broteriana.* Tomo I. Imprensa da Universidade, Coimbra.

Lanza, D. (1919) Monografia del genere *Calendula. Atti del Reale Istituto Veneto di Scienze.*, 12: 1–166.

Lessing CF (1832) Synopsis generum compositarum earumque dispositionis novae tentamen, monographis multarum Capensium interjectis. Sumtibus Dunckeri et Humblotii, Berlin

Linnaeus, C. von (1753) *Species plantarum, [...] Tomus II*, 1st ed. Laurentius Salvius, Stockholm

Linnaeus, C. von (1763) *Species plantarum, [...] Tomus II*, 2nd ed. Laurentius Salvius, Stockholm

Linnaeus, C. von (1781) *Supplementum plantarum, [...].* Orphanotrophei, Brunsvigae [Braunschweig].

Mallet J (1995) A species definition for the modern synthesis. Trends Ecol Evol 10:294–299. doi: 10.1016/0169-5347(95)90031-4

Mariz, J. (1891) Subsídios para o estudo da flora Portuguesa – Compositae L. *Boletim da Sociedade Broteriana, série I* 9: 144 – 243.

Mcneill, J., Barrie, F.R., Buck, W.R., Demoulin, W., Greuter, D.L., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Marhold, K., Prado, J., Prud'Homme van Reine, W.F., Smith, G.F., Wiersema, J.H., Turland, N.J. (2012) *International Code of Nomenclature for algae, fungi, and plants (Melbourne Code).* Regnum Vegetabile 154. Koeltz Scientific Books.

Meikle, R.D. (1976) *Calendula* L. *In*: Tutin T. G., Heywood V. H., Burges N. A., Valentine D. H., Walters S. M., and Webb D. A. (Eds.), *Flora Europaea Notulae*

Systematicae ad Floram Europaeam Spectantes. Cambridge University Press, Cambridge, pp. 206–207.

Merino & Román, B. (1906) *Flora descriptiva e ilustrada de Galicia.* vol. 2 Galicia, Santiago de Compostela, 635 pp.

Meusel, H. & Ohle, H. (1966) Zur Taxonomie und Cytologie der Gattung *Calendula. Österreichische botanische Zeitschrift* 113 : 191–210. http://dx.doi.org/10.1007/BF01441032

Murbeck, S. (1923) *Contributions à la connaissance de la flore du Maroc. Géraniacées-Composées.* C. W. K. Gleerup, Lund, 68 pp.

Nègre, R. (1958) A propos de quelques *Calendula* marocains. *Bulletin de la Société des Sciences Naturelles et Physiques du Maroc* XXXVIII: 1–7.

Nora, S.V., Castro, S., Loureiro, J., Gonçalves, A.C., Oliveira, H., Castro, M., Santos, C. & Silveira, P. (2013) Flow cytometric and karyological analyses of *Calendula* species from Iberian Peninsula. *Plant Systematics and Evolution* 299: 853–864. http://dx.doi.org/10.1007/s00606-013-0767-0

Nordenstam, B. (1994) Tribe Calenduleae. *In*: Bremer K. (Ed.) *Asteraceae, Cladistics & Classification.* Timber Press Portland, Oregon, pp. 365–376.

Nordenstam B., Källersjö M., Eldenäs P. (2006) Nephrotheca, a new monotypic genus of the Compositae-Calenduleae from the Southwestern Cape Province. Compos Newsl 44:32–37.

Nordenstam, B. (2007) Calenduleae. *In*: Bremer K. (Ed.), *The Families and Genera of Vascular Plants*. Springer, Berlin, pp. 241–245.

Nordenstam, B. & Källersjö, M. (2009) Calenduleae. *In*: *Systematics, Evolution, and Biogeography of Compositae.* IAPT, Vienna, pp. 527–538.

Norlindh, T. (1943) *Studies in the Calenduleae. I. Monograph of the genera Dimorphotheca, Castalis, Osteospermum, Gibbaria and Chrysanthemoides*. C. W. K. Gleerup, Lund, 432 pp.

Norlindh, T. (1946) Studies in the Calenduleae. II. Phytogeography and interrelation. *Botaniska Notiser* 1946: 471–506.

Norlindh, T. (1962) Studies in *Calendula* maderensis DC. With a discussion on the delimitation of *Calendula* L. from Gibbaria Cass. and Osteospermum L. *Botaniska Notiser* 115: 437–445.

Norlindh, T. (1977) Calenduleae - Systematic review. *In*: Heywood V. H., Harborne J. B., and Turner B. L. (Eds.), *The Biology and Chemistry of the Compositae*. Academic Press, London, pp. 961–987.

Nyman, C.F. (1878) *Conspectus florae Europaeae*. Vol. 2. Typis Bohlinianae, Örebro Sueciae.

Ohle, H. (1974) Beiträge zur Taxonomie der Gattung *Calendula* II. Taxonomische Revision der südeuropäischen perennierenden *Calendula*-Sippen. *Feddes Repertorium* 85: 245–283. http://dx.doi.org/10.1002/fedr.19740850402

Ohle, H. (1975a) Beiträge zur Taxonomie und Evolution der Gattung *Calendula* L. III. Revision der marokkanischen perennierenden Sippen unter Berücksichtigung einiger marokkanischer Annueller. *Feddes Repertorium* 86: 1–17. http://dx.doi.org/10.1002/fedr.19750860102

Ohle, H. (1975b) Beiträge zur Taxonomie und Evolution der Gattung *Calendula* L. IV. Revision der algerisch-tunesischen perennierenden *Calendula*-Sippen unter Berücksichtigung einiger marokkanisch-algerischer Annueller und der marokkanischen und südeuropäischen perennierende. *Feddes Repertorium* 86: 525–541. http://dx.doi.org/10.1002/fedr.19750860908

Persoon, C.H. (1807*) Synopsis plantarum, [...*] Pars secunda. C.F. Cramerum, Paris. 656 pp.

Plume, O. (2015) *Hybridization, Genome Duplication, And Chemical Diversification in the Evolution of Calendula L. (Compositae)*. Cornell University, Ithaca, NY.

Plume, O., Raimondo, F.M. & Troia, A. (2015) Hybridization and competition between the endangered sea marigold (*Calendula maritima*, Asteraceae) and a more common congener. *Plant Biosystems* 149: 68–77. http://dx.doi.org/10.1080/11263504.2013.810182

QGIS, D.T. (2017) QGIS Geographic Information System 2.18. Available from: http://www.qgis.org/.

R Development Core Team, R. (2010) R: a language and environment for statistical computing. Foundation for Statistical Computing, Vienna, Austria. Available from: http://www.r-project.org.

Rafinesque, C.S. (1810) *Caratteri di alcuni nuovi generi e nuove specie di animali e piante della Sicilia: con varie osservazioni sopra i medesimi.* Sanfilippo, Palermo.

Reinchenbach, H.G.L. (1853) *Icones florae germanicae et helveticae […]. Cynarocephalae et Calendulaceae*. Vol. 15(2). Ambrosii Abel, Leipzig, 427 pp.

Rouy, G. (1903) *Flore de France ou Description des plantes qui croissent spontanément en France, en Corse et en Alsace-Lorraine*. Vol. 8. Asnières. 406 pp.

Ruíz De Clavijo, E. (2005) The reproductive strategies of the heterocarpic annual *Calendula arvensis* (Asteraceae). *Acta Oecologica* 28: 119–126. http://dx.doi.org/10.1016/j.actao.2005.03.004

Ruprecht, F.J. (1856) Animadversiones in Plantas Nonnullas Horti Imp. Botanici Petropolitani (1851 – 1854). *Bulletin de la Classe physico-mathématique de l'Académie impériale des sciences de Saint-Pétersbourg* 14: 229 - 240.

Ryan, W.B.F. (2009) Decoding the Mediterranean salinity crisis. *Sedimentology* 56: 95–136. http://dx.doi.org/10.1111/j.1365-3091.2008.01031.x

Sampaio, G. (1947) Flora Portuguesa. 2ª ed. por Américo Pires de Lima. Imprensa Moderna, Lda. Porto. 792 pp.

Schmiderer, C., Lukas, B., Ruzicka, J. & Novak, J. (2015) DNA-Based Identification of *Calendula officinalis* (Asteraceae). *Applications in Plant Sciences* 3: 1500069. http://dx.doi.org/10.3732/apps.1500069

Sennikov, A. (2010) (089) Proposal to discard the nomenclatural value of reprints and translations of botanical publications first printed before the relevant nomenclatural starting-point date. *Taxon* 59: 307–308.

Sennikov, A. (2015) (038–039) Two proposals to deal with reprints and translations of publications first printed before the relevant nomenclatural starting-point date, and with recent posthumous publications of pre-Linnaean authors. *Taxon* 64: 392–393. http://dx.doi.org/10.12705/642.13

Silveira, P., Gonçalves, A.C., Santos, C. & Paiva, J. (2013) Two lectotypifications and a new combination in *Calendula* (Asteraceae) for Flora Iberica. *Phytotaxa* 145: 47–53. http://dx.doi.org/10.11646/phytotaxa.145.1.5

Tanowitz, B.D., Salopek, P.F. & Mahall, B.E. (1987) Differential Germination of Ray and Disc Achenes in *Hemizonia increscens* (Asteraceae). *American Journal of Botany* 74: 303–312.

Thunberg, C.P. (1800) 2 *Prodromus plantarum Capensium, [...*] Pars prior. J. Edman, Upsalia.

Vahl, M. (1791) *Symbolae botanicae [...] Pars Secunda*. N. Möller & filius, Copenhagen, 105 pp.

Vaillant, S. (1720) Fortsetzung der Corymbiferarum, oder der zweyten Classe der Pflanzen mit zusammengesetzeten Blumen. In: W. B. A. von Steinwehr (Ed*), Der Königliche Akademie der Wissenschaften in Paris anatomische, chymische und botanische Abhandlungen, aus dem Französischen übersetzt.* Breslau, pp. 545–613.

Valdés, B., Talavera, S. & Fernández-Galiano, E. (1987) *Flora Vascular de Andalucía Occidental.* Vol. III. Ketres editora S.A., Barcelona. 555 pp.

Venable, D.L. (1985) The Evolutionary Ecology of Seed Heteromorphism. *The American Naturalist* 126: 577–595.

Willdenow, C.L. Von (1803) *Species plantarum, [...*] Tomus III. Pars III. 4th ed. Impensis G. C. Nauk, Berlin, [1477] –2409 pp.

Willdenow, C.L. Von (1809) *Enumeratio plantarum Horti Regii Berolinensis.* Scholae Realis, Berlin. 1099 pp.

Willkomm, H.M. (1893) *Supplementum Prodromi Florae Hispanícae sive enumeratio et descriptio omnium plantarum inde ab anno 1862 usque ad annum 1893 in Hispania detectarum quae innotuerunt auctori, adjectis locis novis specierum jam nolarum, auctore...* Schweizerbart (E. Koch), Stuttgart. 370 pp.

Willkomm, H.M. & Lange, J.M.C. (1865) *Prodromus Florae Hispanicae.* E. Schweizerbart (E. Koch), Stuttgart. 680 pp.

APPENDIX I

Herbarium specimens used in the statistical analysis and PCA. This list comprises *Calendula* specimens collected in the field from the Iberian Peninsula.

Calendula suffruticosa Vahl subsp. algarbiensis (Boiss.) Nyman

PORTUGAL **E**: Sesimbra a W da ETAR, 18 m, 26 May 2012, [38°26'04" N, 9°07'02" W] *Silveira, P.* 3248 (AVE); **E**: entre Portinho da Arrábida e Setúbal, 14 m, 27 May 2012, [38°29'06" N, 8°57'22" W] *Silveira, P.* 3249 (AVE); **Ag**: Ponta da Piedade, 27 m, 28 May 2012, [37°04'53" N, 8°40'09" W] *Silveira, P.* 3254 (AVE); **Ag**: Praia da Rocha, 30 m, 28 May 2012, [37°07'08" N, 8°33'02" W] *Silveira, P.* 3255 (AVE); **BAl**: Praia de Zambujeira do Mar, 16 m, 28 May 2012, [37°31'20" N, 8°47'17" W] *Silveira, P.* 3258 (AVE); **BAl**: a N de Porto Covo, 24 m, 29 May 2012, [37°54'11" N, 8°47'41" W] *Silveira, P.* 3260 (AVE); SPAIN **Po**: Playa das Patiñas junto a ponte para a Isla de Arousa, 5 m, 9 July 2012, [42°32'38" N, 8°49'43" W] *Silveira, P.* 3272 (AVE); **C**: Playa dos Carragueiros, 5 m, 10 July 2012, [42°36'30" N, 8°52'02" W] *Silveira, P.* 3276 (AVE);

Calendula suffruticosa Vahl subsp. carbonelli Ohle

SPAIN **Ma**: El Faro, 3 8 24 March 2012, [36°30'55" N, 4°37'56" W] *Silveira, P.* 3190 b (AVE); **Ma**: El Faro, 10–20 m, 5 April 2012, [36°30'44" N, 4°38'08" W] *Silveira, P.* 3222 (AVE); **Ca**: Gibraltar p. Europa Point, 12 m, 5 April 2012, [36°07'11" N, 5°21'03" W] *Silveira, P.* 3223 (AVE);

Calendula suffruticosa Vahl subsp. cinerea (Ohle) P. Silveira & A.C. Gonç.

PORTUGAL **Ag**: Vila do Bispo, Cabo de S. Vicente, m, m, 4 August 2003, [37°01'25" N, 8°59'41" W] *Silveira, P.* 2902 (AVE); **Ag**: Vila do Bispo, Cabo de São Vicente, 60 m, 28 May 2012, [37°01'25" N, 8°59'41" W] *Silveira, P.* 3256 (AVE);

Calendula suffruticosa Vahl subsp. greuteri Ohle

SPAIN **Ma**: Benahavis à saída da povoação para Norte pelo caminho de la Molinilla, 162 m, 24 March 2012, [36°31'42" N, 5°02'47" W] *Silveira, P.* 3190 a (AVE); **Al**: a caminho de El Tartel base da Serra de Gador, 365 m, 25 March 2012, [36°49'20" N, 2°43'11" W] *Silveira, P.* 3195 (AVE); **Gr**: Cañon del Rio Guadalfeo entre Salobreña e Velez de Benaudalla, 160 m, 26 March 2012, [36°47'27" N, 3°32'23" W] *Silveira, P.* 3200 (AVE); **Gr**: Pampaneira a 3–4 km de Pampaneira, ao descer para argiva, 930 m, 26 March 2012, [36°55'34" N, 3°22'42" W] *Silveira, P.* 3201 (AVE); **Gr**: Cerro Gordo, 224 m, 4 April 2012, [36°44'12" N, 3°46'02" W] *Silveira, P.* 3218 (AVE); **Gr**: 500 m a E-NE de Torre del Pino na arriba e margens da N340, 127 m, 4 April 2012, [36°44'53" N, 3°47'33" W] *Silveira, P.* 3219 (AVE);

Calendula suffruticosa Vahl subsp. lusitanica (Boiss.) Ohle

PORTUGAL **E**: Charco na saída para Pragança, 196 m, 25 May 2012, [39°12'18" N, 9°03'29" W] *Silveira, P.* 3234 (AVE); **E**: MONTEJUNTO SERRADE junto à capela Nª Srª Neves, 661 m, 25 May 2012, [39°10'31" N, 9°03'36" W] *Silveira, P.* 3237 (AVE); **E**: São Pedro de Sintra, 266 m, 26 May 2012, [38°47'39" N, 9°22'57" W] *Silveira, P.* 3241 (AVE); **E**: Barcarena, 51 m, 26 May 2012, [38°43'43" N, 9°16'36" W] *Silveira, P.* 3243 (AVE); **E**: Monsanto ao descer de Alvito para Alcântara, 50 m, 26 May 2012, [38°43'03" N, 9°10'48" W] *Silveira, P.* 3244 (AVE); **E**: Porto Brandão ao descer de Alvito para Alcântara, 15 m, 26 May 2012, [38°40'40" N, 9°12'19" W] *Silveira, P.* 3245 (AVE); **E**: Sesimbra junto ao Castelo, 155 m, 26 May 2012, [38°27'04" N, 9°06'32" W] *Silveira, P.* 3247 (AVE); **Ag**: Fóia, 915 m, 27 May 2012, [37°18'58" N, 8°36'09" W] *Silveira, P.* 3250 (AVE); **Ag**: Miradouro da Serra de Monchique, 773 m, 27 May 2012, [37°18'27" N, 8°36'20" W] *Silveira, P.* 3251 (AVE); SPAIN **R**: Benzú, 5 20 14 June 2012, [35°54'06" N, 5°20'44" W] *Silveira, P.* 3269 (AVE);

Calendula suffruticosa Vahl subsp. marginata (Willd.) Maire

SPAIN **Ca**: Gibraltar p. Great Siege Tunnels, 165 m, 5 April 2012, [36°08'43" N, 5°20'42" W] *Silveira, P.* 3224 (AVE); **Ca**: Gibraltar por cima dos "Great Sieje Tunnels", 165 m, 20 April 2013, *Silveira, P.* 3302 (AVE); **Ca**: Punta Carnero bermas da CA–224 entre Guetares e Punta Carneiro, 5 m, 21 April 2013, [36°05'25" N, 5°26'53" W] *Silveira, P.* 3305 (AVE); **Ca**: Punta Carnero, 62 m, 21 April 2013, [36°04'35" N, 5°25'45" W] *Silveira, P.* 3306 (AVE);

Calendula suffruticosa Vahl subsp. tomentosa Murb.

SPAIN **Ca**: Tarifa, 107 m, 5 July 2005, [36°00'45" N, 5°36'20" W] *Silveira, P.* 2937 b (AVE); **Ca**: Bolónia, nos muros de uma ponte ao chegar à Praia, 5 m, 20 June 2009, [36°05'13" N, 5°46'06" W] *Silveira, P.* 3042 (AVE); **Ca**: Tarifa, lado esquerdo da N340 ao sair da povoação em direcção a Algeciras, 107 m, 20 June 2009, [36°01'38" N, 5°35'12" W] *Silveira, P.* 3043 (AVE); **Ca**: Tarifa lado esquerdo da N340 ao sair da povoação em direcção a Algeciras, 107 m, 14 June 2012, [36°01'38" N, 5°35'12" W] *Silveira, P.* 3270 (AVE); **Ca**: Punta Paloma, 5 m, 21 April 2013, [36°03'34" N, 5°42'45" W] *Silveira, P.* 3307 (AVE); **Ca**: Punta Camarinal, 8 m, 22 April 2013, [36°05'10" N, 5°47'05" W] *Silveira, P.* 3309 (AVE);

Calendula suffruticosa Vahl subsp. trialata P. Silveira & A.C. Gonç.

SPAIN **Ca**: Cabo Roche, 17 m, 24 March 2012, [36°17'48" N, 6°08'24" W] *Silveira, P.* 3185 (AVE); **Ca**: Atlantera, 24 m, 6 April 2012, [36°06'13" N, 5°49'26" W] *Silveira, P.* 3225 (AVE); **Ca**: Zahara de los Atunes, 8 m, 6 April 2012, [36°08'19" N, 5°50'56" W] *Silveira, P.* 3226 (AVE); **Ca**: Cabo de Trafalgar, 13 m, 6 April 2012, [36°10'59" N, 6°02'04" W] *Silveira, P.* 3227 (AVE); **Ca**: Playa de La Barrosa, 12 m, 22 April 2013, [36°19'59" N, 6°09'43" W] *Silveira, P.* 3308 (AVE); **Ca**: Barbate, entre Barbate e Los Caños de Meca, 39 m, 22 April 2013, [36°11'04" N, 5°57'11" W] *Silveira, P.* 3310 (AVE); ***idem***, 6 May 2014, *Silveira, P.* 3310 b (AVE);

Calendula suffruticosa Vahl subsp. vejerensis P. Silveira & A.C. Gonç.

SPAIN **Ca**: Vejer de la Frontera nos taludes da A–2229 junto ao miradouro antes da povoação, 130 m, 24 March 2012, [36°15'29" N, 5°58'02" W] *Silveira, P.* 3186 (AVE); **Ca**: Vejer de la Frontera margem da A–314, à entrada de La Barca de Vejer, 31 m, 21 April 2013, [36°15'18" N, 5°57'30" W] *Silveira, P.* 3304 (AVE);

Calendula tripterocarpa Rupr.

SPAIN **Al**: 4 km SW Tabernas a c. 4 km a SW de Tabernas, 346 m, 3 April 2007, [37°02'10" N, 2°25'06" W] *Silveira, P.* 2982 (AVE); **Al**: 3 km SW Tabernas a c. 3 km a SW de Tabernas, 346 m, 30 March 2009, [37°02'10" N, 2°25'06" W] *Silveira, P.* 2982 b (AVE); **Mu**: 5,3 km W-SW de Calnegre bermas da D–20, 338 m, 3 April 2012, [37°30'22" N, 1°27'41" W] *Silveira, P.* 3215 (AVE);

Calendula arvensis L.

SPAIN. **Se**: entre Mairena del Alcor e Alcal de Guadaira, 92– [37°21'41" N, 5°49'01" W], 5 April 2007, *Silveira, P.;& Carqueja MJM 2985* (AVE!); **Ma**: na saída para Carratraca, 254– [36°50'04" N, 4°42'33" W], 27 March 2012, *Silveira, P.;Gonçalves, A.C.R.S. 3202 b* (AVE!); Alora, na saída para Carratraca, 254– [36°50'04" N, 4°42'33" W], 27 March 2012, *Silveira, P.;Gonçalves, A.C.R.S. 3202 a* (AVE!);

Calendula officinalis L.

PORTUGAL **BL**: Oliveira do Bairro, Oiã, m, m, 12 April 2007, [40°33' N, 8°32' W] *Silveira, P.* 2986 b (AVE); **AAl**: Abrantes, Alvega, Areia de Cima, m, m, 29 April 2007, [39°28' N, 8°03' W] *Silveira, P.* 2986 c (AVE);

APPENDIX II

Results of principal component analysis (PCA) of *Calendula* from Iberian Peninsula. A – Component loadings for *Calendula* species in Iberian Peninsula, B – Component loadings for *C. suffruticosa* taxa with white-arachnoid indumentum, C – Component loadings for *C. suffruticosa* taxa with non-with-arachnoid indumentum. Morphological characters showing the highest component loadings on the first three axes in bold type.

Character	A			B			C		
	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3
LF	0.028	**-0.729**	0.099	--	--	--	0.257	0.014	**0.611**
LB	0.059	-0.348	-0.035	0.192	-0.123	0.054	-0.025	-0.096	0.067
SP	0.733	-0.121	0.205	--	--	--	0.850	0.410	0.175
LL	0.157	-0.354	-0.100	0.382	-0.565	-0.461	0.219	-0.286	-0.304
LW	0.187	-0.538	-0.085	0.658	-0.474	-0.349	-0.083	-0.180	-0.365
LD	0.135	-0.368	-0.119	0.234	**-0.591**	-0.480	0.197	-0.336	-0.279
R1	-0.096	0.292	0.063	0.763	0.475	-0.057	0.055	0.465	-0.089
R2	-0.041	**0.571**	-0.010	**-0.654**	-0.178	0.037	0.472	-0.161	-0.038
LT	0.078	-0.227	-0.099	**-0.516**	-0.197	0.609	0.164	-0.455	0.082
LS	-0.504	-0.077	0.054	--	--	--	-0.811	0.177	0.176
LA	0.047	-0.682	-0.124	--	--	--	-0.557	-0.116	0.200
LM	0.152	0.092	-0.248	0.189	0.566	-0.075	0.558	-0.428	-0.064
LP	0.683	-0.113	-0.117	--	--	--	0.851	-0.253	-0.045
HD	0.305	**-0.710**	-0.034	0.730	-0.089	-0.248	-0.156	0.107	-0.310
IL	0.433	-0.630	-0.117	0.718	0.375	**-0.488**	-0.354	0.098	-0.212
LG	0.437	**-0.701**	-0.052	**0.856**	0.166	-0.302	-0.131	0.178	-0.418
R3	0.215	-0.540	0.095	0.651	-0.122	0.037	0.175	0.111	-0.246
RF	0.160	**0.710**	-0.055	--	--	--	--	--	--
DF	0.400	0.239	0.336	0.662	-0.388	-0.189	0.594	0.449	0.023
SEL	-0.243	-0.132	**0.814**	--	--	--	-0.176	**0.886**	0.252
SEW	-0.235	-0.122	**0.801**	--	--	--	-0.163	**0.864**	0.251
SEvw	-0.187	-0.089	0.667	--	--	--	-0.120	0.687	0.214
SElw	-0.238	-0.135	**0.797**	--	--	--	-0.174	**0.865**	0.245
RL	0.245	-0.356	-0.047	0.343	-0.431	0.078	0.198	-0.156	0.138
RW	0.399	-0.420	0.046	-0.049	**-0.567**	-0.031	0.291	-0.011	0.095

RA	0.052	-0.660	-0.105	0.358	-0.194	0.093	-0.090	-0.477	0.105
RS	0.182	**0.632**	-0.027	--	--	--	--	--	--
Rvt	0.431	0.346	-0.123	0.451	0.308	-0.428	0.229	-0.083	0.098
BL	-0.421	-0.039	**-0.659**	-0.003	0.698	0.199	-0.520	**-0.594**	-0.009
BW	-0.398	0.041	**-0.635**	-0.027	0.678	0.207	-0.542	**-0.587**	-0.018
BR	-0.326	-0.206	-0.610	0.032	0.699	0.177	-0.435	-0.571	0.005
Blw	-0.410	0.070	**-0.623**	-0.055	0.642	0.215	-0.570	**-0.589**	-0.026
Bdw	0.172	-0.037	-0.358	0.095	0.732	0.145	0.238	-0.322	0.147
TL	**-0.841**	-0.041	-0.404	--	--	--	**-0.846**	-0.414	-0.180
TW	**-0.832**	-0.035	-0.397	--	--	--	**-0.836**	-0.411	-0.177
Tvw	**-0.832**	-0.052	-0.404	--	--	--	**-0.850**	-0.410	-0.175
Tlw	-0.779	0.026	-0.369	--	--	--	-0.795	-0.390	-0.161
Tdw	-0.241	-0.085	-0.180	--	--	--	-0.255	-0.136	-0.007
CL	**0.850**	0.168	-0.214	0.665	0.307	0.570	0.877	-0.308	0.213
CW	0.766	0.308	-0.211	0.520	0.151	**0.669**	0.814	-0.329	0.240
Cvw	0.586	0.150	-0.050	0.318	**-0.586**	0.558	**0.885**	-0.305	0.156
Cdw	0.397	-0.161	-0.147	0.620	-0.303	-0.204	0.504	-0.355	0.149
Cvt	0.318	0.512	-0.152	0.127	**0.847**	0.062	--	--	--
Cc	0.579	0.453	-0.085	0.474	-0.040	**0.656**	0.813	-0.204	0.075
SCL	0.427	-0.345	-0.158	-0.258	**0.821**	-0.348	0.118	0.190	-0.891
SCW	0.414	-0.357	-0.160	-0.304	0.789	-0.391	0.118	0.190	-0.891
SCvw	0.238	-0.301	-0.106	**-0.494**	0.572	**-0.551**	0.118	0.190	**-0.891**
SClw	0.345	-0.346	-0.135	-0.420	0.687	**-0.498**	0.118	0.190	**-0.891**
SCdw	0.242	-0.143	-0.204	0.127	**0.847**	0.062	0.118	0.190	**-0.891**
VAL	**0.855**	0.134	-0.191	**0.907**	0.206	-0.008	0.865	-0.216	-0.092
VAW	**0.820**	0.121	-0.215	0.823	0.287	0.061	0.783	-0.245	0.006
Vas	0.764	0.273	-0.146	**0.865**	0.353	0.031	**0.880**	-0.159	-0.217
VAvt	0.775	0.395	-0.139	0.854	0.311	0.056	**0.895**	-0.145	-0.197
VEL	0.223	-0.357	0.025	0.075	-0.059	0.182	-0.316	0.103	**0.434**
VEW	0.052	-0.450	0.007	0.463	-0.506	-0.164	-0.399	0.025	**0.385**
VEs	0.219	-0.426	-0.320	0.214	0.258	**0.850**	-0.265	-0.391	0.257
VEvt	0.433	0.233	-0.224	0.790	0.145	-0.135	0.473	-0.310	0.186
% of Var.	20.77	13.59	9.95	26.82	23.58	12.09	27.11	14.13	11.59

APPENDIX III

Results of morphometric analysis of *Calendula* from Iberian Peninsula. A – 1 and 2 species; B – 1 and 2 subspecies *algarbiensis*, *carbonelli* and *cinerea*. C – 1 and 2 subspecies *greuteri*, *lusitanica* and *marginata*. D – 1 and 2 subspecies *tomentosa*, *trialata* and *vejerensis*.

A-1

Characters	***C. suffruticosa***						***C. tripterocarpa***						***C. officinalis***						***C. arvensis***					
	N	Min.	25%	Mean	75%	Max.	N	Min.	25%	Mean	75%	Max.	N	Min.	25%	Mean	75%	Max.	N	Min.	25%	Mean	75%	Max.
LB	281	14.0	40.0	60.6	72.0	220.0	20	2.0	5.6	11.7	15.8	30.0	10	31.0	37.9	62.0	86.0	94.0	10	9.0	17.3	31.2	45.5	51.0
LL	452	1.8	4.5	6.8	8.8	24.0	20	1.0	2.0	2.8	3.2	9.5	10	8.7	10.8	11.7	12.2	15.0	10	1.2	2.4	3.6	4.8	6.5
LW	452	0.5	1.0	1.8	2.2	7.0	20	0.2	0.3	0.4	0.5	0.8	10	2.5	2.7	3.0	3.0	4.5	10	0.2	0.4	0.7	0.8	1.1
LD	452	1.3	3.2	4.8	6.2	14.8	20	0.6	1.0	1.2	1.5	2.0	10	7.0	7.5	8.4	9.1	9.5	10	3.5	3.5	3.5	3.5	3.5
R1	452	1.1	1.3	1.4	1.5	1.8	20	1.3	1.8	2.5	2.5	9.5	10	1.2	1.3	1.4	1.4	1.7	10	0.4	0.7	1.0	1.4	1.9
R2	452	1.7	3.1	4.1	4.7	11.3	20	4.3	5.7	7.6	8.4	15.8	10	2.9	3.3	4.0	4.5	5.1	10	4.0	4.2	5.7	7.3	8.0
LT	310	0.2	0.4	0.6	0.7	2.9	12	0.3	0.3	0.3	0.4	0.5	13	0.3	0.3	5.0	0.6	0.7	10	0.3	0.3	0.4	0.5	0.5
HD	292	1.6	2.9	3.5	4.0	7.1	10	1.1	1.2	1.3	1.4	1.6	10	4.8	5.2	6.2	7.6	8.0	10	1.0	1.2	1.3	1.4	2.0
IL	98	5.3	7.5	8.8	10.0	13.3	5	4.2	4.6	5.3	5.8	5.8	10	12.0	12.4	13.5	15.0	15.3	10	5.0	5.8	6.7	7.8	8.2
LG	98	11.8	14.5	18.1	21.0	35.0	5	5.8	6.0	6.8	7.7	8.7	10	24.0	27.8	29.5	31.0	35.0	10	5.8	6.9	10.2	12.9	15.8
R3	98	1.3	1.8	2.1	2.3	2.9	5	1.1	1.2	1.3	1.5	1.5	10	1.9	1.9	2.2	2.4	2.8	10	1.1	1.2	1.5	1.8	1.9

A-2

Characters	*C. suffruticosa*						*C. tripterocarpa*						*C. officinalis*						*C. arvensis*					
	N	**Min.**	**25%**	**Mean**	**75%**	**Max.**	**N**	**Min.**	**25%**	**Mean**	**75%**	**Max.**	**N**	**Min.**	**25%**	**Mean**	**75%**	**Max.**	**N**	**Min.**	**25%**	**Mean**	**75%**	**Max.**
SEL	13	5.5	5.5	6.1	6.1	9.3																		
SEW	13	2.0	2.5	2.9	3.2	3.8																		
RL	436	1.2	11.0	15.0	19.0	32.0							20	5.8	9.4	13.0	16.8	19.0	26	5.0	7.1	9.6	11.7	15.2
RW	436	0.8	1.3	1.6	1.8	4.3							20	1.8	2.8	3.1	3.8	4.7	26	0.8	1.3	1.6	1.9	2.7
BL	74	8.0	10.7	12.9	15.0	19.0													12	8.3	10.8	11.4	12.4	13.3
BW	74	3.5	5.8	6.8	7.8	9.0													12	7.2	7.7	8.7	9.3	10.2
BR	74	2.0	4.0	5.4	6.7	12.5																		
TL	50	5.0	7.0	7.4	9.0	11.7	12	5.7	6.3	7.1	7.9	9.5												
TW	50	2.0	5.0	5.5	7.5	9.5	12	4.5	5.5	6.4	7.1	8.5												
CL	263	4.0	6.5	7.4	8.3	12.5	12	4.2	4.5	5.3	6.1	6.7	20	6.2	10.5	11.4	13.0	15.7	27	4.8	6.7	7.2	8.0	8.5
CW	263	2.5	4.5	5.4	6.3	9.5	12	3.5	4.6	5.2	5.7	6.7	20	3.7	7.0	7.9	8.8	11.3	27	3.8	5.3	6.0	7.0	8.0
SCL	131	4.0	8.0	9.9	11.8	16.0																		
SCW	131	3.0	6.3	7.4	8.7	31.8																		
VAL	266	3.3	4.7	5.4	5.8	8.3							20	6.7	7.0	7.6	8.0	9.2	15	3.5	3.8	4.6	5.2	5.8
VAW	266	1.2	2.7	3.3	3.8	6.2							20	3.5	3.8	4.4	5.0	5.8	15	2.0	2.7	2.9	3.3	3.7
VEL	336	1.9	3.7	4.2	4.8	8.5	12	2.2	2.5	3.0	3.3	4.2	20	5.0	5.8	6.2	6.3	7.5	16	3.2	3.7	4.2	4.5	5.3
VEW	336	0.7	1.3	1.5	1.8	3.7	12	0.7	1.0	1.2	1.2	1.2	20	1.8	2.0	2.3	2.6	3.0	16	1.2	1.2	1.5	1.7	1.8

B-1

Characters	**subsp. *algarbiensis***						**subsp. *carbonelli***						**subsp. *cinerea***					
	N	**Min.**	**25%**	**Mean**	**75%**	**Max.**	**N**	**Min.**	**25%**	**Mean**	**75%**	**Max.**	**N**	**Min.**	**25%**	**Mean**	**75%**	**Max.**
LB	55.0	21.0	45.0	67.7	82.0	220.0	34.0	16.0	33.0	60.1	78.0	140.0	14.0	16.0	24.0	48.6	53.3	87.0
LL	44.0	3.2	4.5	6.2	7.3	13.8	40.0	4.3	6.1	9.6	12.2	24.0	27.0	2.0	2.5	3.9	3.9	7.4
LW	44.0	0.5	0.7	1.3	1.9	2.7	40.0	0.6	1.4	1.9	2.5	3.9	27.0	0.7	0.8	1.0	1.0	2.0
LD	44.0	2.0	3.1	4.4	5.0	10.5	40.0	3.0	4.3	7.0	9.0	14.8	27.0	1.5	1.9	3.1	3.0	6.0
R1	44.0	1.2	1.4	1.4	1.5	1.6	40.0	1.2	1.3	1.4	1.4	1.6	27.0	1.1	1.2	1.3	1.3	1.4
R2	44.0	2.2	4.0	5.1	6.3	7.9	40.0	2.8	3.8	5.4	6.7	11.3	27.0	2.2	2.7	3.8	4.3	5.7
LT	59.0	0.4	0.5	0.7	0.8	1.4	35.0	0.5	0.6	0.8	1.0	1.3	14.0	0.7	1.7	1.7	2.1	2.9
HD	55.0	1.9	2.6	2.8	3.1	4.0	37.0	2.3	3.1	3.7	4.1	5.5	14.0	2.3	2.9	3.2	3.5	3.8
IL	10.0	6.7		8.2		10.8	10.0	7.3		8.2		9.5	6.0	6.3		6.9		7.5
LG	10.0	12.5		15.1		17.0	10.0	15.0		18.2		23.0	6.0	12.5		13.3		14.3
R3	10.0	1.3		1.9		2.5	10.0	1.9		2.2		2.7	6.0	1.7		1.9		2.2

B-2

Characters	subsp. *algarbiensis*						subsp. *carbonelli*						subsp. *cinerea*					
	N	**Min.**	**25%**	**Mean**	**75%**	**Max.**	**N**	**Min.**	**25%**	**Mean**	**75%**	**Max.**	**N**	**Min.**	**25%**	**Mean**	**75%**	**Max.**
SEL																		
SEW																		
RL	51.0	4.0	12.0	16.9	21.0	31.0	40.0	6.0	12.0	16.9	20.0	26.0	40.0	6.0	8.3	12.1	15.0	19.0
RW	51.0	1.2	1.5	1.7	1.8	3.0	40.0	1.2	1.7	1.9	2.2	3.3	40.0	1.3	1.5	1.7	1.8	2.3
BL							20.0	10.2	12.3	14.8	17.0	19.0						
BW							20.0	5.5	6.0	7.2	8.1	9.0						
BR							20.0	2.5	4.3	5.9	7.9	10.0						
TL																		
TW																		
TR																		
CL	34.0	4.7	6.1	7.0	7.7	9.7	40.0	5.3	6.5	7.6	8.3	12.5	40.0	4.0	5.5	6.2	7.1	8.8
CW	34.0	2.8	4.5	5.5	6.7	8.5	40.0	4.3	5.3	6.4	7.5	9.5	40.0	3.0	3.8	4.5	5.0	6.5
SCL	17.0	6.8	7.6	10.1	11.8	12.8												
SCW	17.0	4.7	7.0	9.5	10.2	31.8												
SCR																		
VAL	25.0	3.5	4.4	5.4	5.8	8.3	40.0	3.3	4.3	5.2	5.8	8.0						
VAW	25.0	1.5	2.8	3.7	4.2	6.2	40.0	1.5	2.9	3.7	4.3	5.8						
VEL	41.0	2.5	3.8	4.3	5.0	8.5	23.0	3.2	3.7	4.5	5.2	6.0	40.0	3.2	3.8	4.3	4.6	7.0
VEW	41.0	0.8	1.3	1.6	1.7	2.3	23.0	1.5	1.7	1.8	2.0	2.3	40.0	1.2	1.3	1.6	1.7	3.7

C-1

Characters	**subsp. *greuteri***						**subsp. *lusitanica***						**subsp. *marginata***					
	N	**Min.**	**25%**	**Mean**	**75%**	**Max.**	**N**	**Min.**	**25%**	**Mean**	**75%**	**Max.**	**N**	**Min.**	**25%**	**Mean**	**75%**	**Max.**
LB	35.0	15.5	34.0	45.1	54.0	85.0	61.0	18.0	42.0	60.0	75.0	204.0	16.0	14.0	44.3	93.9	147.5	180.0
LL	50.0	4.5	7.0	8.5	10.2	13.5	74.0	3.5	4.7	6.0	8.0	15.0	59.0	4.0	6.5	8.6	10.1	16.3
LW	50.0	0.5	1.1	1.6	1.9	3.6	74.0	0.8	1.2	1.9	2.5	4.8	59.0	1.0	2.0	3.1	3.8	6.2
LD	50.0	3.0	4.8	5.9	7.0	10.0	74.0	2.4	3.1	4.6	5.3	10.5	59.0	2.3	4.5	5.9	7.0	11.5
R1	50.0	1.2	1.4	1.5	1.5	1.8	74.0	1.2	1.4	1.5	1.5	1.8	59.0	1.2	1.4	1.5	1.5	1.7
R2	50.0	3.3	4.2	5.8	7.1	10.7	74.0	2.0	3.1	3.6	4.0	6.0	59.0	1.8	2.5	3.0	3.5	4.4
LT	40.0	0.3	0.4	0.5	0.6	0.8	61.0	0.3	0.3	0.4	0.4	0.5	26.0	0.2	0.4	0.4	0.5	0.5
HD	47.0	1.6	3.2	3.7	4.3	5.9	61.0	2.0	2.7	3.1	3.5	4.5	16.0	2.5	3.9	4.9	5.5	7.1
IL	10.0	5.8		7.0		8.7	15.0	5.3		7.9		9.3	10.0	10.8		11.9		13.2
LG	10.0	13.3		14.8		16.0	15.0	13.3		16.8		21.0	10.0	21.0		27.2		35.0
R3	10.0	1.8		2.1		2.4	15.0	1.7		2.1		2.6	10.0	1.7		2.3		2.9

C-2

Characters	**subsp. *greuteri***						**subsp. *lusitanica***						**subsp. *marginata***					
	N	**Min.**	**25%**	**Mean**	**75%**	**Max.**	**N**	**Min.**	**25%**	**Mean**	**75%**	**Max.**	**N**	**Min.**	**25%**	**Mean**	**75%**	**Max.**
SEL							33.0	5.0	5.5	6.1	6.7	9.3						
SEW							33.0	2.0	2.5	3.2	3.5	5.5						
RL	65.0	10.0	15.0	17.2	19.0	25.0	70.0	1.2	9.0	11.0	16.3	32.0	58.0	8.0	15.0	18.5	22.0	29.0
RW	65.0	1.2	1.5	1.8	2.0	3.3	70.0	1.0	1.3	1.5	1.7	2.7	58.0	1.2	1.4	1.6	1.8	2.3
BL	1.0	10.8	10.8	10.8	10.8	10.8												
BW																		
BR																		
TL																		
TW																		
TR																		
CL	43.0	5.2	6.3	7.0	7.3	9.3	30.0	5.2	6.1	6.9	7.5	10.7	58.0	6.0	7.5	8.2	9.2	11.0
CW	43.0	2.5	3.8	4.5	5.3	6.0	30.0	4.2	5.0	5.4	6.1	8.0	58.0	2.8	4.8	5.8	6.7	7.7
SCL	31.0	4.0	11.7	12.1	12.5	16.0	14.0	5.7	6.0	6.7	7.5	8.8	6.0	8.3	8.6	9.9	11.1	11.3
SCW	31.0	3.8	7.5	8.1	9.0	10.0	14.0	3.5	3.5	4.6	5.0	6.5	6.0	6.7	7.8	8.3	9.0	9.5
SCR	31.0	1.7	2.5	3.1	3.3	7.0												
VAL	60.0	3.5	4.7	5.2	5.8	6.7	30.0	4.0	4.5	4.9	5.2	6.8	43.0	3.7	5.3	5.9	6.7	7.7
VAW	60.0	1.2	2.2	2.5	2.8	4.0	30.0	2.3	2.7	3.2	3.7	4.5	43.0	2.0	3.3	3.8	4.2	5.5
VEL	6.0	2.3	2.5	3.3	4.0	4.2	62.0	3.0	3.7	4.2	4.5	5.3	44.0	3.8	4.7	4.9	5.2	5.8
VEW	6.0	0.7	0.8	1.1	1.4	1.5	62.0	1.0	1.3	1.5	1.8	2.2	44.0	1.3	1.7	1.8	2.0	2.3

D-1

Characters	**subsp. *tomentosa***						**subsp. *trialata***						**subsp. *vejerensis***					
	N	**Min.**	**25%**	**Mean**	**75%**	**Max.**	**N**	**Min.**	**25%**	**Mean**	**75%**	**Max.**	**N**	**Min.**	**25%**	**Mean**	**75%**	**Max.**
LB	14.0	21.0	31.8	54.8	75.0	111.0	39.0	28.0	44.0	60.1	69.0	143.0	13.0	34.5	44.8	56.1	63.5	82.0
LL	80.0	1.8	2.8	3.8	4.5	9.7	50.0	3.2	5.0	7.3	8.9	14.1	28.0	4.5	6.6	8.5	10.3	13.5
LW	80.0	0.6	0.8	1.2	1.4	2.7	50.0	0.8	1.2	1.8	2.3	3.5	28.0	1.1	1.5	2.7	4.3	7.0
LD	80.0	1.3	1.9	2.8	3.3	7.0	50.0	2.5	3.7	5.4	6.6	11.0	28.0	3.4	4.5	5.8	7.0	8.6
R1	80.0	1.2	1.3	1.4	1.5	1.7	50.0	1.2	1.3	1.4	1.4	1.6	28.0	1.3	1.3	1.5	1.6	1.7
R2	80.0	1.7	2.8	3.3	3.8	5.3	50.0	1.9	3.1	4.2	5.1	7.9	28.0	1.9	2.4	3.9	5.2	6.3
LT	16.0	0.4	0.5	0.7	0.8	1.7	49.0	0.3	0.4	0.6	0.7	0.9	10.0	0.5	0.5	0.5	0.6	0.6
HD	14.0	3.1	3.4	4.2	4.6	5.7	38.0	2.2	3.0	3.6	4.0	4.9	10.0	3.0	3.4	3.9	4.2	4.8
IL	9.0	10.8		11.7		13.3	23.0	6.8		8.4		9.7	5.0	8.7		10.4		11.7
LG	9.0	20.0		23.7		27.0	23.0	11.8		16.8		24.0	5.0	17.0		19.0		21.0
R3	9.0	1.8		2.0		2.3	23.0	1.4		2.0		2.6	5.0	1.6		1.8		2.0

D-2

Characters	subsp. *tomentosa*						subsp. *trialata*						subsp. *vejerensis*					
	N	**Min.**	**25%**	**Mean**	**75%**	**Max.**	**N**	**Min.**	**25%**	**Mean**	**75%**	**Max.**	**N**	**Min.**	**25%**	**Mean**	**75%**	**Max.**
SEL																		
SEW																		
RL	73.0	7.0	11.3	14.4	16.0	28.0	55.0	5.0	8.5	12.1	15.0	25.0	10.0	7.0	10.0	13.0	15.4	21.0
RW	73.0	0.8	1.2	1.4	1.6	4.3	55.0	1.0	1.2	1.4	1.5	2.0	10.0	1.5	1.5	1.7	1.8	2.2
BL	19.0	9.0	11.0	13.1	15.0	18.0	25.0	9.0	10.0	12.2	14.0	16.0						
BW	19.0	5.0	6.3	7.2	8.3	8.8	25.0	5.0	6.0	6.7	7.3	9.0						
BR	19.0	2.5	5.0	6.7	8.3	12.5	25.0	2.0	3.0	4.1	5.3	8.3						
TL							50.0	6.0	7.0	7.8	9.0	11.7						
TW							50.0	3.0	5.0	6.3	7.5	9.5						
TR							3.0	0.8	0.8	1.2		1.3						
CL	18.0	7.7	8.3	9.2	10.1	10.8												
CW	18.0	3.3	5.0	5.6	6.4	7.7												
SCL	55.0	6.5	8.7	9.7	11.0	14.2							10.0	6.0	7.0	7.6	8.1	9.0
SCW	55.0	4.3	6.7	7.6	8.2	13.0							10.0	3.0	5.0	6.1	8.0	8.0
SCR																		
VAL	68.0	3.8	4.7	5.4	5.8	8.3												
VAW	68.0	1.8	2.8	3.4	3.8	6.2												
VEL	60.0	3.3	3.9	4.4	4.8	5.8	50.0	1.9	3.0	3.5	4.0	6.1	10.0	2.8	3.5	4.0	4.4	6.1
VEW	60.0	1.0	1.3	1.5	1.7	2.0	50.0	0.7	1.3	1.5	1.7	2.5	10.0	1.5	1.5	1.7	1.9	2.0

Chapter 4 - Publication 3

Contribution to the knowledge of genome size variation in *Calendula* L. (Asteraceae) with special focus on the SW Mediterranean region

Contribution to the knowledge of genome size variation in *Calendula* L. (Asteraceae) with special focus on the SW Mediterranean region

Ana Carla Gonçalves[1], Helena Oliveira[1], João Loureiro[2], Sílvia Castro[2], Teresa Ribeiro[3], Ahmed Ouhammoud[4], Rachid Amirouche[5], Leonor Morais-Cecílio[3], Conceição Santos[6], Paulo Silveira[1]*

[1]*Departamento de Biologia e CESAM, Universidade de Aveiro, 3810–193, Aveiro, Portugal.*

[2]*Departamento de Ciências da Vida e Centro de Ecologia Funcional, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal.*

[3]*Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal.*

[4]*Herbier Régional 'MARK', Laboratoire d'Ecologie et Environnement, Faculté des Science Semlalia, Université Cadi Ayyad, Avenue Prince Moulay Abdallah, B.P. 2390, Marrakech, 40 001, Maroc*

[5]*Laboratoire de Biologie et Physiologie des Organismes, Université des Sciences et de la Technologie Houari Boumediene, USTHB, BP 32 El-Alia, 16111 Bab-Ezzouar, Alger, Algeria*

[6]*Departamento de Biologia & LAQV/REQUIMTE, Faculdade de Ciências, Universidade do Porto, s/n, 4169–007 Porto, Portugal*

**Author for correspondence. E–mail: psilveira@ua.pt*

This chapter is in preparation to be submitted as an original article in:

Plant Systematic and Evolution

Abstract

Calendula is one of the most taxonomically complex genera within the Asteraceae family, due to hybridization and polyploidisation events and production of a highly variable morphology of the achenes. Considering the complexity of *Calendula*, this study was conducted to extend the understanding of the relationships between taxa. The aim of this study was to extend the knowledge of *Calendula* taxa in the Mediterranean and assess the relationships between genome size and chromosome number, ploidy level, life cycle, and eco-geographic variables (altitude, latitude, longitude) in 82 populations covering 14 taxa. The mean 2C values differed up to 6-fold among different species (from 1.37 pg in diploid to 8.26 pg in octoploid accessions). The 1Cx values varied 2.37-fold (between 0.77 pg and 1.83 pg). *C. tripterocarpa* populations harboured diploid and tetraploid individuals that based on genome size values seem of autopolyploid origin. Mean 1Cx genome size revealed significant differences between different ploidy levels (from 1.02 pg in diploids to 0.93 pg in tetraploids and octoploids) and suggested the occurrence of genome downsizing in some polyploids. Correlation of genome size with altitude and longitude was not significant. However, a latitudinal correlation was found, suggesting migrations/evolution of polyploid taxa from south to north Morocco. These results strongly support the SW Mediterranean as the main centre of origin of the *Calendula* genus. Genome size variation is a significant factor for explaining the relationships within *Calendula*, and individuals not fitting the current classification were found and should be analysed in detail in future studies.

Keywords Calenduleae, Compositae, Chromosome numbers, hybridization, nuclear DNA content, eco-geographic variables, polyploids

Introduction

The Asteraceae family presents a large variation in nuclear DNA content, ranging from 1C = 0.4 pg (in *Leontodon longirostris*; Maranon and Grubb 1993; Garcia et al. 2013) to 1C = 28.3 pg (in *Coreopsis nuecensis*; Price et al. 1984; Garcia et al. 2013), totalling around a 70.75-fold variation (Vallès et al. 2013). Variation in genome size among genera and even within a genus can reflect important evolutionary processes, involving several mechanisms that operate to add and/or remove DNA (Bennetzen et al. 2005; Grover and Wendel 2010). These mechanisms include interspecific hybridization and/or whole genome duplications (homoploid hybrids, allopolyploids and autopolyploids), as well as aneuploidisation and dysploidy (De Storme and Mason 2014). From these, polyploidisation (Leitch and Bennett 2007; Otto 2007; Soltis et al. 2015) and hybridization (Soltis and Soltis 2009; Whitney et al. 2010) have been considered the most important evolutionary forces driving the diversification of flowering plants (Soltis and Soltis 2009; Weiss-Schneeweiss et al. 2013; Soltis et al. 2015; Marques et al. 2017).

Correlations between genome size and morphological characters or with relevant factors related with breeding systems and ecology have been documented in the literature (Greilhuber and Leitch 2013). Over the years, there have been several attempts to explain the impact of genome size in the phenotypes (Knight and Beaulieu 2008). Genome size has been associated with variation in cell size (Bennett 1972; Knight et al. 2005; Leitch and Bennett 2007), cell cycle duration (Bennett 1972; Knight et al. 2005; Leitch and Bennett 2007), cell cycle (Bennett and Leitch 2011), seed size and seed mass (Knight et al. 2005; Beaulieu et al. 2006), leaf mass and growth rate and/or photosynthetic rate (Knight and Beaulieu 2008; Krahulcová et al. 2017), growth form and distribution (Ohri 2005). In addition, positive relationships between genome size and temperature, water availability, latitude/longitude and altitude of the habitats have been reported (Knight and Ackerly 2002).

The genus *Calendula* L. (Asteraceae, Calenduleae) is native to the Mediterranean basin, occurring from the Macaronesia islands to the Middle East regions (Norlindh 1946, 1977; Heyn et al. 1974; Nordenstam 1994; Nordenstam and Källersjö 2009). The SW Mediterranean region is considered the primary centre of evolution of the genus (Norlindh 1946; Heyn et al. 1974), but the knowledge and patterns of evolution in the genus are largely based on the analysis of European taxa. Although comprising only nearly 16 species, it is considered as one of the most complex and taxonomically difficult genera within the Asteraceae family (Norlindh 1977; Heyn and Joel 1983; Nordenstam and Källersjö 2009). The taxonomic complexity results from a high morphological variability in some of its taxa, which are under active evolution. The high morphological variability results from high levels of hybridization frequently leading to occurrence of intermediate forms (Lanza 1919; Heyn and Joel 1983) and from the occurrence of different achene morphologies within the same taxon (e.g. *C. arvensis*), along with similar achene morphologies in different taxa (e.g. *C. arvensis* vs. *C. stellata*). The production of more than one type of fruit per plant, known as heterocarpy (Zohary 1950), and its intricate heredity, introduces further difficulties in the process of taxonomical classification of the genus. Moreover, cytogenetically, the genus is also highly variable, with chromosome numbers ranging drastically from $2n = 14$ to ± 88 (Nora et al. 2013), and with several base numbers being assumed (namely $x = 7, 8, 9, 11$ and 15; Darlington and Wylie 1955; Norlindh 1977; Heyn and Joel 1983; Nordenstam 1994). Hybridization and polyploidisation events were proposed as the main mechanisms giving rise to new entities (Heyn et al. 1974; Nora et al. 2013). Hence, a wide range of intermediate forms arises from allopolyploidization, genome duplication or dysploidy (Nora et al. 2013), and most taxa are recognised by several names under different taxonomic categories (Nègre 1958; Heyn and Joel 1983).

Traditionally, *Calendula* is divided into annual and perennial herbs. The annual herbs, *sensu* Heyn et al. (1974), includes five species: *C. stellata* Cav. (2*n* = 14 chromosomes), *C. tripterocarpa* Rupr. (2*n* = 30), *C. arvensis* L. (2*n* = 44), *C. palaestina* Boiss. (2*n* = ±88), and *C. pachysperma* Zoh. (2*n* = ±88). In contrast, the perennial taxa were divided into two groups (i) the *C. maroccana* group, and (ii) *C. incana* and *C. suffruticosa* (Ohle 1974, 1975a, b). The *C. maroccana* group comprises four species (*C. eckerleinii* Ohle, *C. maroccana* Ball, *C. meuselii* Ohle, and *C. lanzae* Maire), with 2*n* = 18 chromosomes (Ohle 1975a). The *C. incana* and *C. suffruticosa* groups are composed by 7 and 11 taxa, respectively, all with 2*n* = 32 chromosomes (Ohle 1974; 1975b). However, it is accepted that *C. incana* and *C. suffruticosa* groups are artificial and very difficult to distinguish (Nora et al. 2013), henceforth we followed the nomenclature proposed by Meikle (1976) and Silveira et al. (2013), and included all the *C. incana* taxa under *C. suffruticosa*.

Since the publications of Heyn et al. (1974) on annual taxa of *Calendula*, and of Ohle (1974, 1975a, b) on perennial taxa, few studies have followed. Recently, Nora et al. (2013) analysed the chromosome number and genome size of 11 *Calendula* taxa. A gradient in genome size between *C. incana* and *C. suffruticosa* groups, along with considerations regarding morphological relationships, led these authors to aggregate both species. Nora and co-authors (2013) also discussed the main mechanisms of evolution, considering that, in agreement with previous authors (Norlindh 1977; Heyn and Joel 1983), *C. maroccana* (2*n* = 18) and *C. stellata* (2*n* = 14) played a central role in the origin of several taxa. These species also belong to the main centre of diversity and evolution of the genus. Although Nora et al. (2013) provided information on the taxonomy and evolution of *Calendula*, the study involved a limited number of taxa. Later, Plume et al. (2015) used chloroplast markers (*atpI-atpH*) and the nuclear ribosomal internal transcribed spacer region (ITS), together with palynological data to assess hybridization events between *C. maritima* and *C. suffruticosa* subsp. *fulgida*. This study, of a limited sample set, suggested that the hybrids are capable to back-cross with their parents and that hybridization may be placing *C. maritima* at risk of extinction via introgression (Plume et al. 2015). Plume (2015) also studied the phylogenetic relationships within *Calendula* using molecular markers, providing support for a division of the genus into annual and perennial polyploid complexes, multiple origins of most polyploid taxa, and a single origin of *C. officinalis*. Although these studies strongly support the idea that hybridization and polyploidisation have been important in the speciation of *Calendula*, the relationships within each taxon are still unclear.

This study was conducted to extend the understanding of the relationships between *Calendula* taxa, by sampling largely unexplored areas of the SW Mediterranean basin, that are important centres of diversification of the genus, namely Morocco, but including also taxa from Algeria, Tunisia, Sicily and Israel. The following specific questions were formulated: (a) how does genome size relates with chromosome number, ploidy levels, life cycle and eco-geographic variables such as altitude, latitude and longitude. And (b) how does the level of intraspecific variation in holoploid and monoploid genome sizes relates with the circumscription of the *Calendula* genus?

Material and methods

Plant material

An intensive field survey was conducted between 2009 and 2014, focusing in Morocco and in the most important/accessible taxa from Algeria and Tunisia. Collection sites were selected from the literature, and by inspecting the labels of ca. 5 000 herbarium specimens. In total, achenes and/or fresh leaves from 78 populations were collected in the field, supplemented with four *ex situ* germinated seedlings from Italy and Israel-Palestine (see Supplementary Table SD1), totalling 82 populations. At each site, we aimed to collect leaves from five individual plants. However, in some cases, this was not possible, due to the small number of individuals available. Taxa were identified according to Heyn et al. (1974) for annuals and Ohle (1975a, b) for perennials, except that *C. incana* and its subspecies were included in *C. suffruticosa* (Meikle 1976; Silveira et al. 2013). Populations from known taxa that showed distinct morphologies were treated in this work as distinct entities (e.g. *Calendula* sp.1, *Calendula* sp.2 and *Calendula* sp.3), whose description and typification will be published elsewhere. Vouchers were prepared and deposited in the Herbarium of the University of Aveiro (AVE).

To determine the circumscription and the patterns of genome size evolution in *Calendula*, a complete analysis comprising all the recognised taxa from Iberian Peninsula and Morocco, and the most important/accessible taxa from Algeria, Tunisia, Italy and Israel-Palestine were used to explore correlations between monoploid genome size and eco-geographic variables. The widely distributed taxa *C. arvensis* and the cultivated *C. officinalis* were not included in the analyses. QGIS 2.18.4 (Quantum GIS Development Team, 2017) was used to represent geographically all the studied accession/populations.

Genome size assessments

Fresh leaves were collected in the field and stored at 4ºC until processed. Nuclear suspension was obtained by chopping approximately 100 mg of *Calendula* spp. and 50 mg of *Pisum sativum* 'Ctirad' (internal reference standard), using a razor blade in a Petri dish containing 1 mL of ice-cold WPB buffer [200 mM Tris.HCl, 4 mM $MgCl_2.H_2O$, 2 mM EDTA $Na_2O.2H_2O$, 86 mM NaCl, 10 mM sodium metabisulfite, 1% PVP–10 and 1% (v/v) Triton X–100, pH adjusted to 7.5, and stored at 4ºC, following Loureiro et al. (2007)]. The nuclear suspension was filtered through a 50 µm nylon cloth and 50 µg mL^{-1} of propidium iodide (Fluka, Buchs, Switzerland) and 50 µg mL^{-1} of RNAse (Fluka, Buchs, Switzerland) were added, to stain DNA and prevent staining of double-stranded RNA, respectively. The samples were incubated for 5-20 min. on ice before being analysed on a Beckman-Coulter EPICS XL flow cytometer (Beckman-Coulter, Hialeah, FL, USA) operating at 488 nm air-cooled argon-ion laser at 25 mW power; one run per preparation was performed, in which 5 000 particles were measured/recorded. Results were acquired using the SYSTEM II (v.2.5) software in the form of five graphics: fluorescence pulse integral in linear scale (FL); forward light scatter (FS) vs. side light scatter (SS) in logarithmic (log) scales; FL vs. time; FL vs. fluorescence pulse height; FL vs. SS in log scale. FL vs. fluorescence pulse height was used eliminate partial nuclei and other debris, nuclei with associated cytoplasm and doublets, while in FL vs. SS (log) a polygonal region was defined to include only intact nuclei. These regions were used to gate all the other graphics. As a quality standard, only histograms with a coefficient of variation (CVs) lower than 5% for G_1 peaks of both the sample and the standard species were considered. Five randomly selected individuals represented each population, and at least two replicates per individual were obtained, being selected the replicate with higher quality. The holoploid genome size in mass values (2C in pg; *sensu* Greilhuber et al. 2005) was obtained by the ratio between G_1 mean peaks of *Calendula* spp. and *P. sativum*, multiplied by the genome size of the reference standard (2C = 9.09 pg; Doležel et al. 1998). The holoploid genome size in Mbp was also calculated using the conversion rate, 1 pg = 980 Mbp (Doležel et al. 2003). The monoploid genome size (1Cx; *sensu* Greilhuber et al. 2005) of each sample was calculated by dividing the 2C-value by the ploidy level of the corresponding sample (Greilhuber et al. 2005). Because genome sizes were obtained for several individuals that were also characterised karyologically (below), DNA ploidy levels could be inferred for all individuals analysed.

Chromosome counts

To confirm the ploidy levels estimated based on nuclear DNA content results, chromosome counts were made using the squashing methods described in Nora et al. (2013), with some modifications. In brief, seeds were germinated on wet filter paper in Petri dishes at room temperature (20–25 °C). One week later, seedlings were potted in Jiffy-7 pots (www.jiffy.com) and maintained in homogeneous conditions (20 ± 2 °C, with a light intensity of 60 ± 5 mol m^{-2} s^{-1}) in the Department of Biology, University of Aveiro.

Young root tips were pre-treated with ice-cold water for 12 h, fixed in a cold mixture of absolute ethanol and glacial acetic acid (3:1, v/v) fixative at room temperature for 24 h, and were then kept in the same fixative at 4°C. The fixed material was thoroughly washed with distilled water (twice for 5 min.), then transferred into a 1N HCl and digested at 37°C until the material was soft (10–15 min). The root tips were stained with 2% alcoholic hydrochloric acid–Carmine for a minimum of 48 h. Temporary slides were made using the squash method on a drop of 45% acetic acid: glycerol (9:1). Chromosome spreads were analysed using Nikon Eclipse 80i microscope (Nikon Instruments, NY, USA), and images of chromosome spreads were acquired with a Leica digital camera DC200 (Gmbtt Leica Microsystems, Wetzlar, Germany) incorporated in the referred microscope and processed using the Leica IM1000 v.1.1 software (Leica Microsystems AG, Heerbrugg, Switzerland). At least three plants per accession were studied karyologically.

Statistical analysis

Descriptive statistics were calculated for genome size data (mean, standard deviation of the mean (SD), coefficient of variation (CV), and minimum and maximum values of the holoploid (2C, pg) and monoploid (1Cx) genome sizes, in pg and Mbp). Boxplots of genome size variations were analysed separately for chromosome number, ploidy level, life cycle and *C. maroccana* vs. *C. suffruticosa* group.

Due to the non-normal distribution of the data (as assessed by Shapiro-Wilk test), Kruskal-Wallis One-way ANOVA on ranks (among more than two groups) or Mann-Whitney *U* test (between two groups) were implemented to assesses the differences in holoploid (2C) and monoploid (1Cx) genome sizes considering chromosome number, ploidy level, life cycle and *C. maroccana* vs. *C. suffruticosa* group. Multiple comparisons tests were calculated using Dunn's method to find significantly different groups of taxa or accessions. Correlations between mean holoploid or monoploid genome sizes and chromosome number, ploidy level and life cycle were examined using a linear regression analysis. The relationship between monoploid nuclear DNA content and preferred ecological variables (altitude, latitude and longitude) were also tested. Statistical analyses were performed using SPSS (SPSS for Windows, 1999. Chicago: SPSS Inc.) or R (R Development Core Team, 2010).

Results

Chromosome counts, ploidy level and life cycle

The chromosome numbers analysed in the present study are summarised in Table 10 and illustrated in Figure 43, while chromosome numbers published elsewhere are summarised in the supplementary data (Table SD2). The most common chromosome number was $2n = 2x = 18$, which was present in eight of the taxa analysed. Three ploidy levels were detected among the 14 taxa (Table 10), namely: diploidy, which was the most common ploidy level and was present in *C. stellata* ($2n$ = 14 chromosomes), *C. eckerleinii*, *C. maroccana* subsp. *maroccana*, *C. maroccana* subsp. *murbeckii*, *C. meuselii*, *C. lanzae*, *Calendula* sp.1, *Calendula* sp.2, *Calendula* sp.3 ($2n$ = 18) and *C. tripterocarpa* 1 ($2n$ = 30); tetraploidy in *C. suffruticosa* ($2n$ = 32), *C. arvensis* ($2n$ = 44) and *C. tripterocarpa* 2 ($2n$ = 60?); and octoploidy in *C. palaestina* and *C. pachysperma* ($2n$ = ±88). One taxa, *C. tripterocarpa*, presents two ploidy levels, diploidy ($2n = 2x$ = 30) and tetraploidy ($2n = 4x$ = 60?), in three mixed-ploidy populations (3067, 3133 and 3140).

The studied *Calendula* taxa were divided according with their usual life cycle. While *C. stellata*, *C. lanzae*, *C. maroccana* subsp. *murbeckii, Calendula* sp.3, *C. tripterocarpa*, *C. arvensis*, *C. pachysperma* and *C. palaestina* usually present an annual life cycle, *C. eckerleinii*, *C. maroccana* subsp. *maroccana*, *C. meuselii*, *Calendula* sp.1, *Calendula* sp.2 and *C. suffruticosa* are mostly perennial.

Inter- and intraspecific variation in holoploid genome size

A total of 329 individuals of 82 populations were analysed by flow cytometry (Supplementary Table SD1). Fluorescence histograms of genome size assessments yielded well-defined peaks for both *Calendula* samples and the internal reference standard. Coefficients of variance (CVs) of most of the studied taxa were lower than 5% (mean CV = 4.43%).

The mean 2C-values varied 6-fold from 1.37 pg in diploid *C. maroccana* subsp. *maroccana* to 8.26 pg in octoploid *C. pachysperma*, with an overall mean of 2.87 ± 1.41 pg (Table 10). Significant differences in 2C-value (H_{14} = 314.078; P <0.001) were obtained when comparing all the taxa studied (Figure 464A). The most variable taxon with respect to genome size was the annual *C. tripterocarpa*, which presented two discrete groups of holoploid genome sizes growing together in three out of the seven populations studied (3067, 3133 and 3140). Indeed, individuals displaying 3.52 ± 0.14 pg or 7.32 ± 0.03 pg (a difference of 2.08-fold), with no significant morphological distinction, were detected in those three populations. This pattern of variation leads us to label the typical diploid plants as *C. tripterocarpa* 1, and the tetraploid plants as *C. tripterocarpa* 2. The octoploid *C. pachysperma* presented the highest 2C-value (8.19 ± 0.09 pg) among the taxa studied. By contrast, the lowest 2C-value was detected in populations of *C. maroccana* subsp. *maroccana* (1.59 ± 0.14 pg) (Table 10; Figure 464A).

As expected, mean 2C-values were found to be significantly correlated with chromosome numbers ($R^2 = 0.878$, $P < 0.001$). However, it is interesting to note that despite presenting a lower number of chromosomes, *C. stellata* ($2n = 14$) and *C. tripterocarpa* ($2n = 30$) showed slightly higher holoploid genome sizes than the *C. maroccana* group ($2n = 18$) and *C. suffruticosa* group ($2n = 32$), respectively (Figure 45A). The analysis of variance also supported significant differences ($H_6 = 295.035$, $P < 0.001$) between holoploid genome sizes and different chromosome numbers. Mean 2C-value increased with ploidy levels, ranging from 2.04 ± 0.54 pg in diploids, 3.72 ± 1.18 pg in tetraploids to 7.41 ± 0.56 pg in octoploids. Mean 2C-value revealed significant differences ($H_2 = 193.134$; $P < 0.001$) between different ploidy levels (**Error! Reference source not found.**C). A significant correlation between 2C-value and different ploidy levels could also be detected ($R^2 = 0.624$, $P < 0.001$).

Perennial taxa presented significantly ($T_{132/204} = 27\ 360$; $P < 0.001$) lower 2C-value (2.43 ± 0.71 pg) than the annual ones (3.54 ± 1.87 pg) (Figure 45A). Therefore, a significant correlation ($R^2 = 0.144$, $P < 0.001$) between holoploid genome size and life cycle was detected.

Interspecific variation in holoploid genome size ($T_{108/124} = 19\ 278$; $P < 0.001$) was observed between the *C. maroccana* and *C. suffruticosa* groups (Figure 45C). Mean 2C-value varied from 1.80 ± 0.23 pg in *C. maroccana* group to 3.09 ± 0.21 pg in *C. suffruticosa* group, differing by ~1.72-fold. Mean 2C-value of *C. maroccana* group ranged from 1.37 pg in *C. maroccana* subsp. *maroccana* to 2.33 pg in *Calendula* sp.3. Significant differences ($H_7 = 90.496$; $P < 0.001$) within *C. maroccana* group were obtained. Mean 2C-value in the *C. suffruticosa* group varied from 2.71 to 3.62 pg, with an overall mean of 3.09 ± 0.21 pg (Table 11). Interpopulation variation in 2C-value ($H_{21} = 91.006$; $P < 0.001$) was also observed in the *C. suffruticosa* group.

Inter- and intraspecific variation in monoploid genome size

The mean 1Cx-values varied 2.37-fold from 0.68 pg in *C. suffruticosa* to 1.87 pg in *C. tripterocarpa* 1, with an overall mean of 0.98 ± 0.28 pg (Table 10). The mean 1Cx-values of *C. tripterocarpa* 2 was calculated based on a hypothetical ploidy level (tetraploidy), due to the lack of exact chromosome counts. Differences in 1Cx-values between different taxa are significant, either without (H_{13} = 280.768, P < 0.001) or with (H_{14} = 288.430, P <0.001) the inclusion of the hypothetical mean 1Cx-value for *C. tripterocarpa* 2 (Figure 44B).

Significant differences (H_6 = 245.658, P < 0.001) between 1Cx-value and different chromosome numbers were obtained (Figure 44B). A significant correlation (R^2 = 0.0409, P < 0.001) between 1Cx-value and chromosome number was found. Still, *C. tripterocarpa* 1 (2*n* = 30) and *C. tripterocarpa* 2 (2*n* = 60?) presented similar monoploid DNA content.

The 1Cx-values varied among ploidy levels, with diploids presented mean values of 1.02 pg and tetraploids and octoploids average values of approximately 0.93 pg (Figure 44D). The 1Cx-values of diploids varied 2.22-fold between 0.80 pg in *C. maroccana* subsp. *maroccana* to 1.76 pg in *C. tripterocarpa* 1, including taxa with 2*n* = 14, 18 and 30 chromosomes; in tetraploids, 1Cx values varied 2.37-fold between 0.77 pg in *C. suffruticosa* to 1.83 pg in *C. tripterocarpa* 2, including taxa with 2*n* = 32, 44 and 60? chromosomes; and in the octoploids 1Cx values varied 1.17-fold between 0.88 pg in *C. palaestina* to 1.02 pg in *C. pachysperma* (both with 2*n* = ±88). Significant differences in 1Cx-values were found between the different ploidy levels (H_2 = 42.193, P < 0.001) (Figure 44D). However, no significant correlation (R^2 = 0.0225, P = 0.036) was found.

Annuals presented significantly ($T_{132/204}$ = 34 836, P <0.001) higher (1.22 ± 0.30 pg) 1Cx-values than perennials (0.82 ± 0.09 pg) (Figure 45B). A significant correlation (R^2 = 0.502, P <0.001) was also obtained. The 1Cx-values of annuals varied 2.08-fold from 0.88 pg in *C. palaestina* to 1.83 pg in *C. tripterocarpa* 2, while for perennials this value varied 1.34-fold from 0.77 pg in *C. suffruticosa* to 1.03 pg in *C. maroccana* subsp. *murbeckii.*

Interspecific variation in monoploid genome size ($T_{108/124}$ = 7 859.5; P < 0,001) was observed between *C. maroccana* and *C. suffruticosa* groups (Figure 45D). Mean 1Cx-value differed by approximately 0.13-fold, from 0.77 pg in *C. suffruticosa* group to 0.90 pg in *C. maroccana* group. Interpopulation variation in 1Cx-value were also found in the *C. maroccana* group (H_7 = 90.496; $P < 0.001$). and the *C. suffruticosa* group (H_{21} = 87.864; $P < 0.001$).

Correlation between monoploid genome size and eco-geographic variables

No significant correlation between genome size and altitude (R^2 = 3E^{-06}, P =0.986) (Figure 46A) nor with longitude (R^2 = 0.00286, P = 0.572) (Figure 46C) were found. However, the 1Cx-value was positively correlated with latitude (R^2 = 0.176, $P < 0.001$) (Figure 46B), with the 1Cx-value increasing from lower to higher latitudes. Diploid and tetraploid populations of different taxa occur mainly in the southern and northern parts of the SW Mediterranean region, respectively, whereas the octoploids populations exhibit a more restricted distribution in Israel and Palestine (Figure 47A).

Cytogeographic patterns of Calendula taxa

The most widespread taxon is the tetraploid *C. arvensis* (not represented in the map), which is ruderal. In some cases, it grows together with the diploids *C. tripterocarpa* and *C. stellata* in ruderal areas. The tetraploid *C. suffruticosa* was found essentially near the coastal range, extending from Morocco to Tunisia and south of Italy (Figure 47A-B), whereas some populations occurred inland at 1 000 to 2 000 m a.s.l. It is in Morocco, where diploids harbour a greater taxonomic diversity. In addition, eight narrow endemics, are also found in Morocco, namely the *C. eckerleinii*, *C. meuselii,* and *Calendula* sp.2 (restricted to the Middle Atlas), the *C. maroccana* subsp. *maroccana* and *C. maroccana* subsp. *murbeckii* (in the High Atlas), *C. lanzae* and *Calendula* sp.3 (in the Anti-Atlas) and *Calendula* sp.1 (in the Rif Mountains) (Figure 47B). The octoploid taxa *C. pachysperma* and *C. palaestina* seem restricted to Israel and Palestine.

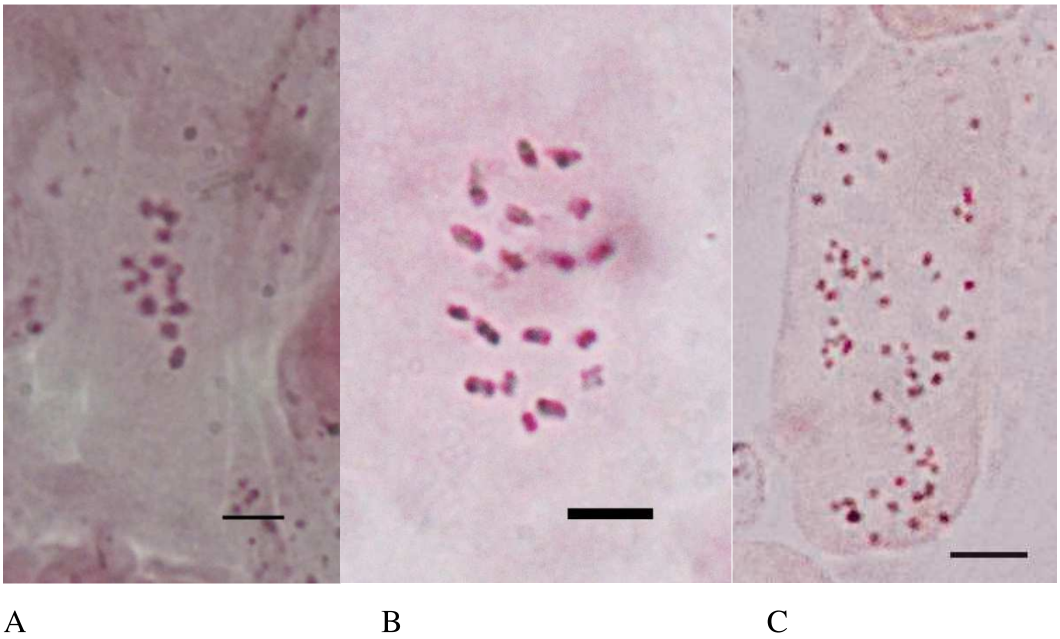


Figure 1 – Somatic cells with C-metaphase chromosomes. A. *C. stellata* with 2*n* = 14 chromosomes (accession 3061); B. *Calendula* sp.1 with 2*n* = 18 chromosomes (accession 3339). C. *C. tripterocarpa* 2 with 2*n* = ± 60 chromosomes (accession 3133). Scale bars 10 µm

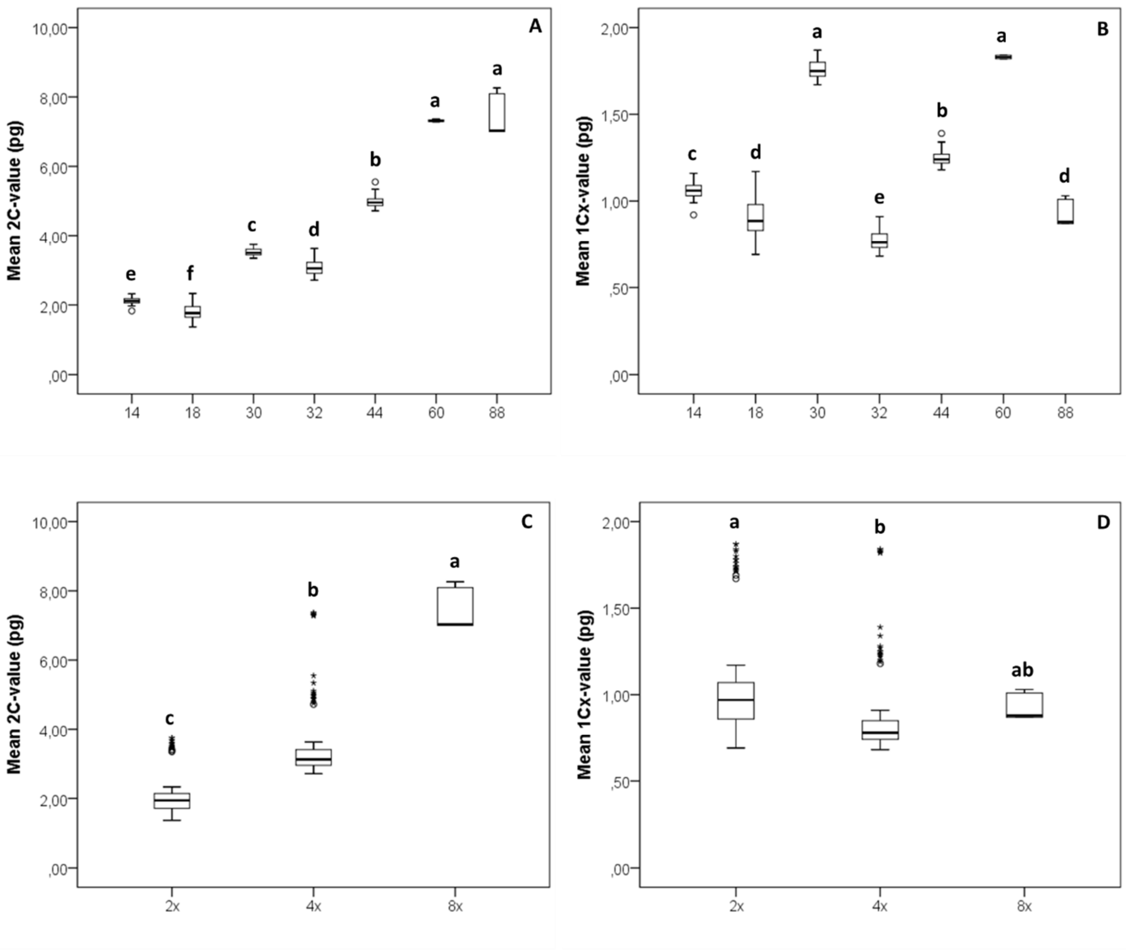


Figure 44 – Box-and-whisker plots showing holoploid (2C-values) and monoploid (1Cx-values) genome size variation in the *Calendula* taxa studied. A-B) chromosome numbers 2*n* = 14, 18, 30, 32, 60?, and ±88; C-D) ploidy categories are marked as "2*x*" for diploids, "4*x*" for tetraploids and "8*x*" for octoploids; Median (lines), 25%-75% (boxes), minimum and maximum values (whiskers), outliers (small circles). Different letters reveal statistically significant differences at $\alpha > 0.05$ after a Dunn's test.

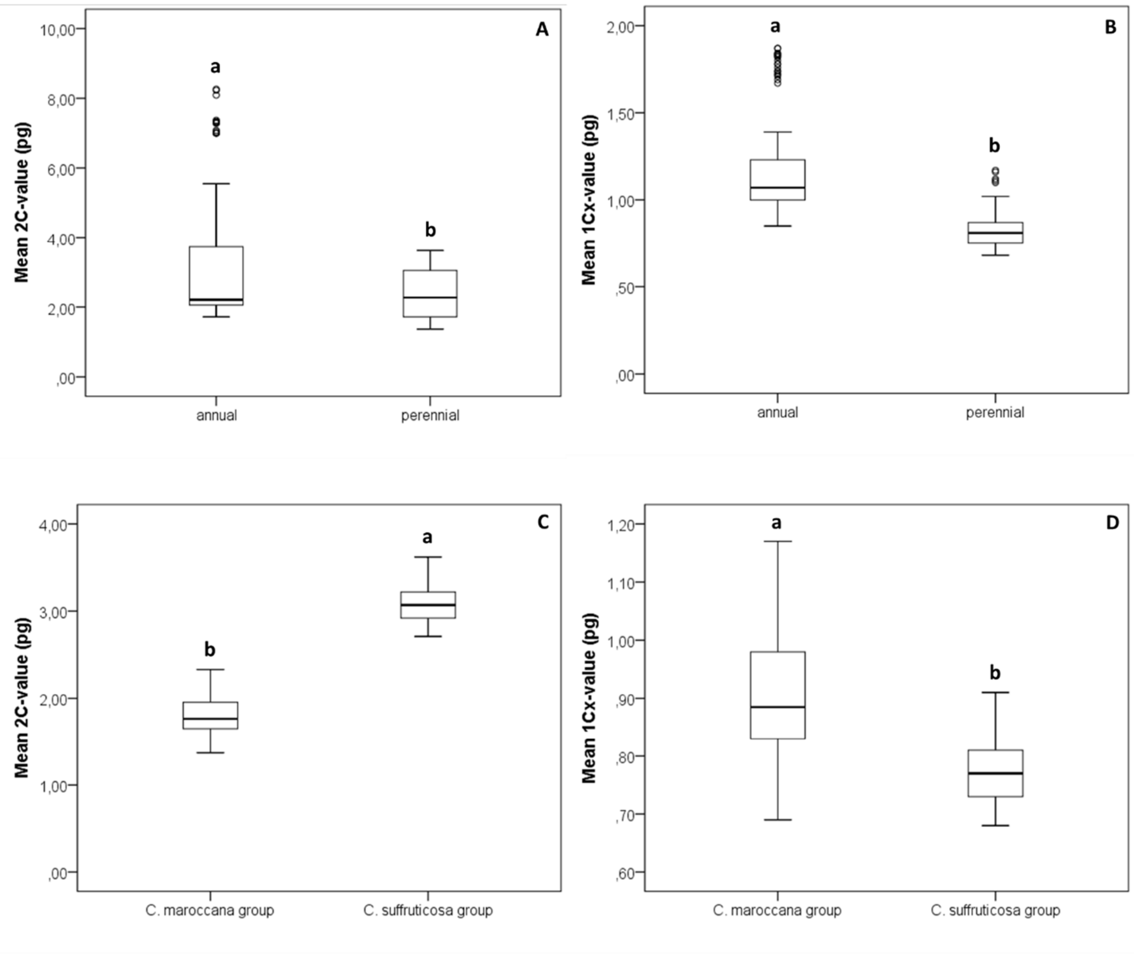


Figure 45 – Box-and-whisker plots showing holoploid (2C-values) and monoploid (1Cx-values) genome size variation in *Calendula* taxa. A-B) life cycle: “annual” and “perennial”; C-D) *C. maroccana* and *C. suffruticosa* groups representing different taxa and cytotypes of *Calendula*. Median (lines), 25%-75% (boxes), minimum and maximum values (whiskers), outliers (small circles). Different letters reveal statistically significant differences at $\alpha > 0.05$ after a Dunn’s test.

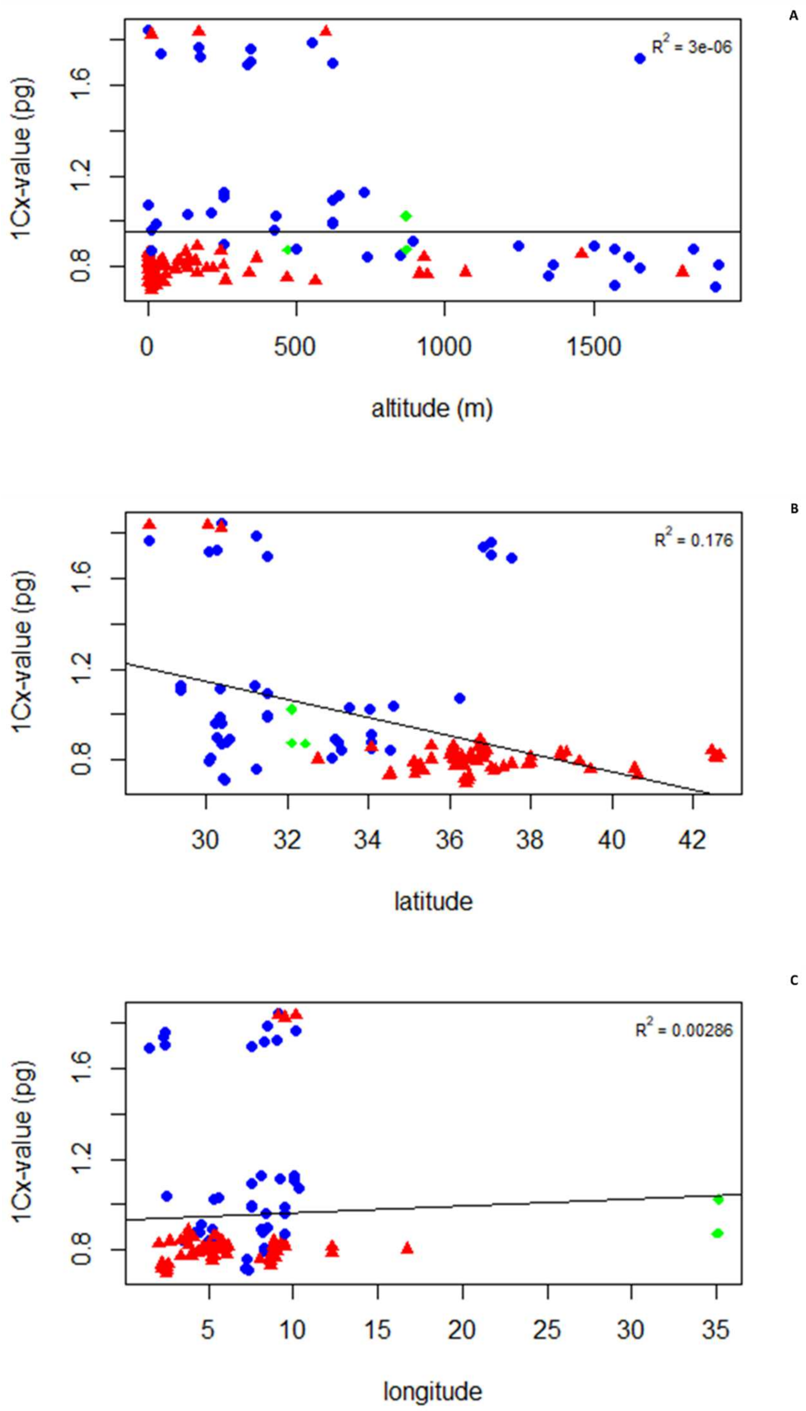


Figure 46 – Distribution of 1Cx-values versus eco-geographic variables based on a subset after excluding the widely distributed taxa *C. arvensis*. (A) altitude position; (B) latitudinal position; (C) longitudinal position. Different colours represent ploidy level of each population: ● diploids = blue; ▲ tetraploids = red; ◆ octoploids = green.

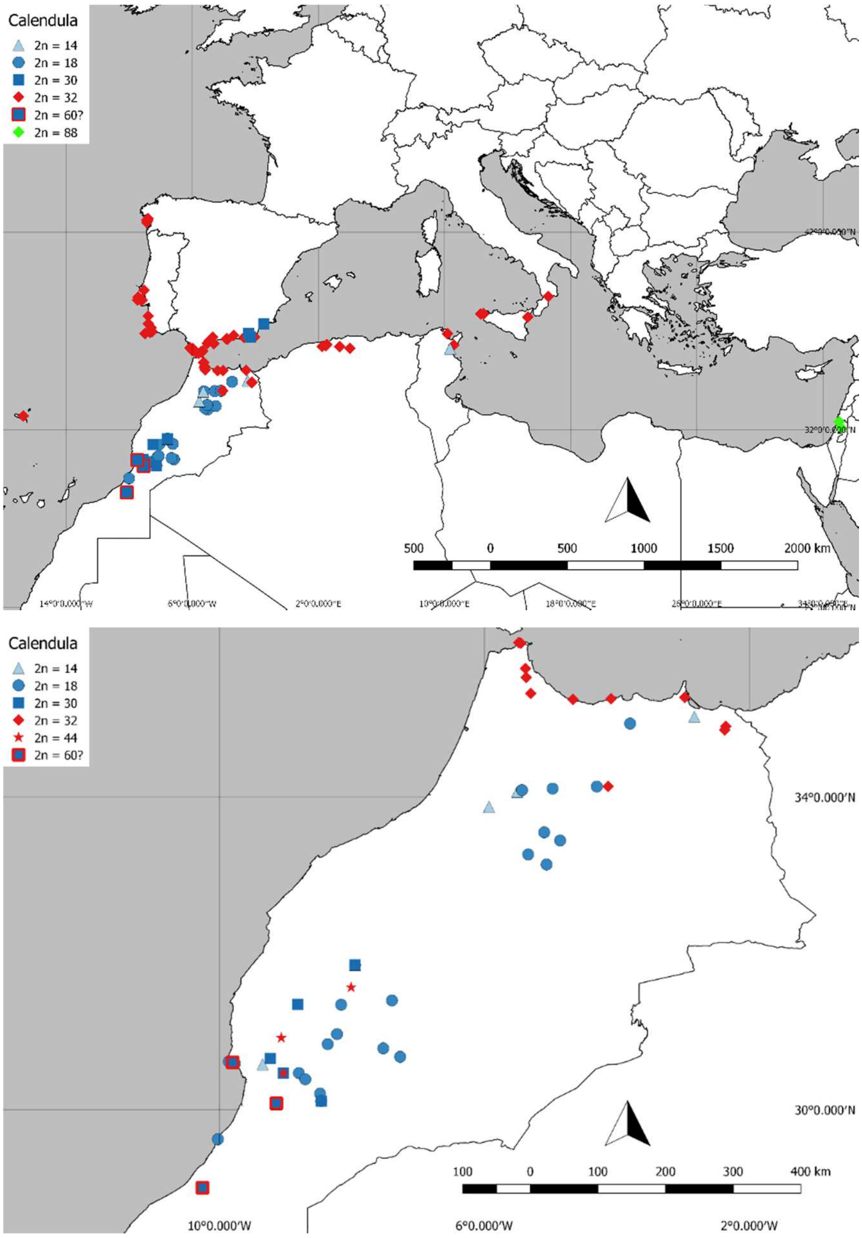


Figure 47 – Geographical distribution of all studied *Calendula* populations in the study area (Morocco, Algeria, Tunisia, Italy and Israel-Palestine), including accessions from Nora et al. (2013), except the widespread *C. arvensis* and the cultivated *C. officinalis*. Different symbols represent different chromosome numbers. Different colours represent ploidy level of each population: diploids = blue; tetraploids = red; octoploids = green.

Table 1 – Genome size variation among the *Calendula* taxa studied (2C-values and 1Cx-values, both given in mass values (pg) and in Mbp). Information about life cycle, number of analysed individuals, number of analysed populations, chromosome number (2*n*) and ploidy levels, is also given for each taxon.

Taxa	**Life cycle**	***n*ind.**	***n*pop.**	**2*n***	**Ploidy**	**2C-value (pg)**				**2C (Mbp)**	**1Cx-value (pg)**				**1C (Mbp)**
						Mean ± SD	**Min.**	**Max.**	**CV%**		**Mean ± SD**	**Min**	**Max**	**CV%**	
C. arvensis	A	28	17	44	*4x*	5.23 ± 0.29	4.72	5.65	5.59	5 111	1.31 ± 0.07	1.18	1.41	5.69	1 278
C. eckerleinii	P	24	5	18	*2x*	1.74 ± 0.08	1.62	1.94	4.65	1 700	0.87 ± 0.04	0.81	0.97	4.75	850
C. lanzae	A	8	2	18	*2x*	1.85 ± 0.08	1.72	1.99	4.08	1 809	0.93 ± 0.04	0.86	1.00	4.36	905
C. maroccana subsp. *maroccana*	P	37	8	18	*2x*	1.59 ± 0.14	1.37	1.83	8.68	1 555	0.80 ± 0.07	0.69	0.92	8.8	778
C. maroccana subsp. *murbeckii*	A	16	2	18	*2x*	2.07 ± 0.14	1.88	2.33	6.59	2023	1.03 ± 0.07	0.94	1.17	6.81	1 012
C. meuselii	P	9	1	18	*2x*	1.71 ± 0.03	1.66	1.74	1.51	1 670	0.85 ± 0.01	0.83	0.87	1.6	835
C. pachysperma	A	3	1	±88	*8x*	8.19 ± 0.07	8.09	8.26	0.89	8 014	1.02 ± 0.01	1.01	1.03	1.09	1 002
C. palaestina	A	6	2	±88	*8x*	7.02 ± 0.03	7.00	7.08	0.45	6 863	0.88 ± 0.00	0.87	0.89	0.5	858
C. stellata	A	43	8	14	*2x*	2.11 ± 0.10	1.83	2.32	4.61	2 066	1.06 ± 0.05	0.92	1.16	4.67	1 033
C. suffruticosa	P	108	22	32	*4x*	3.10 ± 0.21	2.71	3.62	6.62	3 038	0.77 ± 0.05	0.68	0.91	6.65	757
C. tripterocarpa 1	A	18	7	30	*2x*	3.52 ± 0.12	3.34	3.74	3.4	3 445	1.76 ± 0.06	1.67	1.87	3.5	1 723
C. tripterocarpa 2	A	6	3	60?	*4x*	7.32 ± 0.03	7.28	7.37	0.42	7 156	1.83 ± 0.01*	1.82*	1.84*	0.46*	1 789*
Calendula sp.1	P	5	1	18	*2x*	1.69 ± 0.10	1.57	1.83	5.99	1 651	0.84 ± 0.06	0.79	0.92	6.69	825
Calendula sp.2	P	5	1	18	*2x*	1.76 ± 0.05	1.67	1.81	3.10	1 723	0.88 ± 0.03	0.84	0.91	3.46	862
Calendula sp.3	A	20	4	18	*2x*	2.09 ± 0.15	1.83	2.33	7.25	2045	1.05 ± 0.08	0.92	1.17	7.44	1 022
Total		**329**	**82**			**2.87 ± 1.41**	**1.37**	**8.26**		**2 812**	**0.98 ± 0.28**	**0.68**	**1.87**		**958**

Life cycle (A= annual; P = perennial); Number of individuals (*n*ind.), Number of populations (*n*pop.); Chromosome number (2*n*); ploidy level; mean holoploid genome size (2C, pg) and monoploid genome size (1Cx, pg), standard deviation (SD), minimum (min.) and maximum (max.), the coefficient variation (CV, %) and 2C and 1Cx-value in megabase pairs (Mbp). * Accurate Mean 1Cx-value in the high polyploidy *C. tripterocarpa* 2 could not be determined due to the lack of exact chromosome counts.

Table 2 – Genome size variation of the *Calendula suffruticosa* accessions/populations studied (2C-values and 1Cx-values, both given in mass values (pg) and in Mbp). All accessions are perennial, tetraploid and with 2n = 32 chromosomes.

Taxa	**accession/population**	**2C-value (pg)**					**2C (Mbp)**	**1Cx-value (pg)**					**1C (Mbp)**
		Mean ± SD		**Min.**	**Max.**	**CV%**		**Mean ± SD**		**Min**	**Max**	**CV%**	
C. suffruticosa s.l.	AL3320	3.08 ± 0.04	0..04	3..04	3..13	0..01	3 014	0.77 ± 0.01	0.01	0.76	0.78	0.01	754
	MO3261	3.10 ± 0.10	0.10	3.04	3.25	0.03	3 032	0.78 ± 0.03	0.03	0.76	0.81	0.03	758
	MO3262	3.00 ± 0.07	0.07	2.89	3.07	0.02	2 938	0.75 ± 0.02	0.02	0.72	0.77	0.02	734
	MO3265	2.96 ± 0.05	0.05	2.92	2.99	0.02	2 890	0.74 ± 0.01	0.01	0.73	0.75	0.02	722
	MO3266	3.16 ± 0.07	0.07	3.05	3.22	0.02	3 086	0.79 ± 0.02	0.02	0.76	0.81	0.02	771
	MO3267	3.21 ± 0.05	0.05	3.16	3.27	0.01	3 143	0.80 ± 0.01	0.01	0.79	0.82	0.01	786
	MO3268	3.44 ± 0.12	0.12	3.27	3.62	0.04	3 366	0.86 ± 0.03	0.03	0.82	0.91	0.04	842
	MO3269	3.36 ± 0.15	0.15	3.21	3.60	0.04	3 286	0.84 ± 0.04	0.04	0.80	0.90	0.04	822
	MO3334	3.42 ± 0.07	0.07	3.35	3.51	0.02	3 347	0.86 ± 0.02	0.02	0.84	0.88	0.02	837
	MO3340	3.18 ± 0.06	0.06	3.10	3.25	0.02	3 107	0.79 ± 0.01	0.01	0.78	0.81	0.02	777
	MO3341	3.06 ± 0.10	0.10	2.94	3.23	0.03	2 993	0.77 ± 0.03	0.03	0.74	0.81	0.03	748
	AL3321	3.10 ± 0.14	0.14	2.91	3.33	0.04	3 029	0.77 ± 0.03	0.03	0.73	0.83	0.04	757
C. suffruticosa subsp. *fulgida*	IT3109	3.25 ± 0.04	0.04	3.21	3.31	0.01	3 181	0.81 ± 0.01	0.01	0.80	0.83	0.01	795
	MO3335	2.92 ± 0.20	0.20	2.71	3.15	0.07	2 851	0.73 ± 0.05	0.05	0.68	0.79	0.07	713
	MO3336	2.97 ± 0.11	0.11	2.89	3.04	0.04	2 900	0.74 ± 0.03	0.03	0.72	0.76	0.04	725
C. suffruticosa subsp. *maritima*	IT3101	3.14 ± 0.04	0.04	3.10	3.20	0.01	3 068	0.78 ± 0.01	0.01	0.78	0.80	0.01	767
C. suffruticosa subsp. *monardii*	AL3316	2.90 ± 0.04	0.04	2.83	2.96	0.01	2 838	0.73 ± 0.01	0.01	0.71	0.74	0.01	709
	AL3317	2.85 ± 0.11	0.11	2.71	3.00	0.04	2 782	0.71 ± 0.03	0.03	0.68	0.75	0.04	696
	AL3318	2.79 ± 0.03	0.03	2.75	2.83	0.01	2 733	0.70 ± 0.01	0.01	0.69	0.71	0.01	683
	AL3319	2.87 ± 0.06	0.06	2.80	2.95	0.02	2 803	0.72 ± 0.01	0.01	0.70	0.74	0.02	701
C. suffruticosa subsp. *suffruticosa*	TU3038	3.28 ± 0.07	0.07	3.21	3.40	0.02	3 210	0.80 ± 0.01	0.01	0.79	0.82	0.01	787
C. suffruticosa subsp. *tunetana*	TU3039d	3.39 ± 0.08	0.08	3.33	3.44	0.02	3 320	0.74 ± 0.02	0.02	0.72	0.75	0.02	720

Mean holoploid (2C, pg) and monoploid (1Cx, pg) genome sizes and standard deviation (SD), minimum (min.) and maximum (max.); the coefficient variation (CV, %) of the holoploid genome size; the monoploid genome size in megabase pairs (1Cx; Mbp).

Table 12 – The relationship between basic chromosome numbers and 1Cx-value

Taxa	**Basic chromosome number (x)**	**Ploidy**	**Chromosome number (2n)**	**1Cx**	**Alternative 1Cx**
C. suffruticosa	8	4x	32	0.77	-
C. maroccana group	9	2x	18	0.90	-
C. pachysperma / C. palaestina	11	8x	±88	0.93	-
C. stellata	7	2x	14	1.06	-
C. arvensis	11	4x	44	1.31	-
C. tripterocarpa 1	15	2x	30	1.76	**0.88***
C. tripterocarpa 2	15	4x	?60	1.83	**0.92****

*with a hypothetical tetraploid origin, ** with a hypothetical octoploid origin

Discussion

Our study provided novel insights into the cytogenetics of *Calendula* from Morocco, including also some taxa from Algeria, Tunisia, Italy and Israel-Palestine, adding to the first contribution provided for the Iberian Peninsula by Nora et al. (2013).

Chromosome numbers in *Calendula* vary from *2n* = 14 to ±88, and numerous counts, for many *Calendula* taxa, have been published to date (see supplementary data). Some of these counts, however, are contradictory, because of the taxonomic complexity of the genus. For example, Humphries et al. (1978) reported 2*n* = 14 and 2*n* = 18 chromosomes for *C. arvensis* for Morocco, but the most frequent number is 2*n* = 44 (Meusel and Ohle 1966; Oberprieler and Vogt 1993; Vogt and Oberprieler 2008, 2012). These contradictory counts resulted, most certainly, from misidentifications since two taxa occurring in Morocco present these chromosome numbers: *C. stellata* (*2n* = 14) and *C. maroccana* group (*2n* = 18). Valdés and Parra (1997) reported *2n* = 32 for *C. maroccana*, however, the material deposited in SEV (140 824) herbaria from 'Beni Snassen' (Morocco) was later identified as *C. suffruticosa*, which possess 2*n* = 32 chromosomes. This is in accordance with former reports by Vogt and Oberprielier (2008) of *C. suffruticosa* from 'Beni Snassen' (Morocco). Meusel and Ohle (1966) reported 2*n* = 18 for *C. suffruticosa* from High Atlas (Morocco), but later this taxon was included by the authors in the *C. maroccana* group. The remaining chromosome numbers agree with previously published counts for the genus (see Supplementary Table SD2).

High cytogenetic diversity has been detected in this study. Three ploidy levels harbouring different basic chromosome numbers were detected among the 14 taxa studied (Table 10): diploidy ($2n = 2x = 14$, 18, and 30), tetraploidy ($2n = 4x = 32$, and 44) or octoploidy ($2n = 8x = \pm 88$) (ploidy according to Pazy 2000). Among the obtained results, a new ploidy level, tetraploidy, was reported for *C. tripterocarpa* ($2n = 4x = 60$? besides the $2n = 2x = 30$). Indeed, two discrete genome sizes groups were detected in the same populations (differing by 2-fold), indicating that DNA tetraploids (*C. tripterocarpa* 2) were growing in sympatry with diploid plants (*C. tripterocarpa* 1). Intraspecific ploidy variation and mixed-ploidy populations have been documented in many plant groups including several Asteraceae genera (Castro et al. 2012; Bougoutaia et al. 2016; Čertner et al. 2017). The patterns of variation within mixed-ploidy taxa can provide insights into the early stages of polyploid evolution (Čertner et al. 2017). Interestingly, the differences in genome size and chromosome number observed between *C. tripterocarpa* and other *Calendula* species places it a distinct evolutionary line apart from other species (Heyn and Joel 1983; Nora et al. 2013), and at the same time the chromosome number and the lack of differences in monoploid genome size values between diploid and tetraploid *C. tripterocarpa* suggest that these tetraploids might have arisen from the diploid *C. tripterocarpa* through autopolyploidy by the fusion of unreduced gametes. The small number of individuals detected in nature suggest that tetraploid plants might be emerging in the diploid populations and their fate will depend on ecological factors promoting their fitness under a scenario of strong frequency dependent selection (Levin 1975). Further studies are still needed to evaluate the importance of these polyploids in the evolution of *Calendula*.

Calendula is one of the smallest genera of Asteraceae, presenting only 14 species, usually divided into annual and perennial plants. However, this seems to be an entirely artificial arrangement, since these groups seem to include plants from distinct evolutionary lines, like the diploids *C. stellata* ($2n = 14$), *C. lanzae* ($2n = 18$) and *C. tripterocarpa* ($2n = 30$); the tetraploid *C. arvensis* ($2n = 44$); and the octoploid *C. pachysperma* ($2n = \pm 88$), in the annuals. The same happens in the perennials, which include the *C. maroccana* diploid group of taxa (with $2n = 18$), and the tetraploid *C. suffruticosa* ($2n = 32$).

The estimates of genome size, including new assessments for *C. eckerleinii*, *C. lanzae*, *C. maroccana* subsp. *murbeckii, C. meuselii*, *C. pachysperma, C. palaestina*, and some subspecies of *C. suffruticosa* are presented. The results of this study probably cover all the genome size variation present in the genus, since we included genome size estimates - in this study and Nora et al. (2013) - of all the 11 currently recognised *Calendula* species plus three new entities yet undescribed. Holoploid genome size in *Calendula* taxa included in the present study ranged 6-fold, while monoploid ranged 2.37-fold. Variation in 2C-value among taxa was relatively lower than that reported in previous studies in the genus (Garcia et al. 2013; Nora et al. 2013). However, the estimates we obtained for *C. arvensis*, *C. stellata*, *C. suffruticosa*, and *C. tripterocarpa* were consistent with previous reports. These results may indicate that the genome could provide information for taxonomic and evolutionary studies.

The monoploid genome size across the species suggest that the basic number is 8 or 9 with 1Cx = 0.77 pg and 0.90 pg, respectively. Considering that species with basic chromosome number $x = 9$ are predominant in *Calendula*, and present the most primitive morphological characters, especially the achenes (Norlindh 1946), we can suggest that all other basic chromosome numbers proposed for this genus are, probably, derived numbers. Considering the similarities in monoploid genome sizes and the presence of multiple basic chromosome numbers it seems that dysploidy is present in the genus (Heyn and Joel 1983; Nora et al. 2013).

Diploids differ significantly in their 1Cx-values from tetraploids. However, *post hoc* comparison revealed that the same is not true for the comparison of diploids with octoploids. Diploid and tetraploid cytotypes of *C. tripterocarpa* shared very similar monoploid genome sizes (1.66 and 1.83 pg, respectively), which as mentioned above provided support for inferring that the tetraploids have an autopolyploid origin. Exploring the monoploid genome sizes and chromosome numbers of *C. tripterocarpa* in comparison with the other *Calendula* species also provided interesting insights for this species. The monoploid genome size calculated for *C. tripterocarpa* assuming a diploid and tetraploid ploidy level resulted in significantly higher values in comparison with the other studied species. Considering that the basic chromosome number of the genus is proposed to be x = 8 or 9, and that *C. tripterocarpa* presents an x = 15, one could hypothesise that this species might have suffered a genome duplication event followed or preceded by chromosome losses/dysploidy phenomena, currently resulting in an individual with 30 chromosomes. This has already been proposed by Heyn and Joel 1983 and explored by Nora et al. (2013). If this is true, then the currently accepted diploid although behaving as a diploid could actually correspond to an ancient tetraploid, and likewise the newly detected tetraploids correspond to an ancient octoploid. Interestingly, the monoploid genome sizes calculated based on ploidies incorporating this evolutionary perspective are more similar to the typical values of the genus (0.88 and 0.92 pg) and the holoploid genome sizes fit with the ranges of tetraploid and octoploid species (Figure 45C-D, Table 12). Future studies are needed to confirm this hypothesis. Based on the chromosome numbers reported here and in the bibliography, an autopolyploid origin is also hypothesised for the octoploid taxa *C. palaestina* and *C. pachysperma* from the widespread tetraploid *C. arvensis*, an evolutionary route already proposed by previous authors (Heyn and Joel 1983; Nora et al. 2013). Under this scenario, genome size estimates suggest the occurrence of genome downsizing with the increase in the ploidy level.

Our data confirm previous reports that both annuals and perennials encompass several chromosome numbers and ploidy levels. However, annuals are more variable cytogenetically than perennials, as they include diploids, tetraploids and even two octoploid species. Correlations between genome size and life cycle have been widely discussed, although no general conclusions have emerged (Vallès et al. 2013). Plants with an annual life history are preferentially associated with inbreeding species characterised by low genome size than outbreeders (Bennett, 1972; Rejmanek and Richardson, 1996; Bennett et al., 1998; Garnatje et al., 2004; Grotkopp et al., 2004). However, annual plants from Asteraceae seem to show the opposite pattern (Vallès et al. 2013). In *Calendula*, a higher mean genome size was observed in annuals, agreeing with Torrell and Vallès (2001). However, in many cases, such correlation between life cycle and nuclear DNA content does not exist (Torrell and Vallès 2001). Furthermore, while Plume (2015) found evidence to support the division of the genus into annual and perennial polyploid complexes, our data were not able to completely confirm this division, since plants with the same chromosome number may behave as annual or perennial (e.g. *C. maroccana* subsp. maroccana – perennial, *C. maroccana* subsp. *murbeckii* - annual), and both groups include plants with different chromosome numbers that do not seem to interbreed naturally (e.g. species of the *C. maroccana* group with any of the *C. suffruticosa* subspecies).

According to Ohle (1975a), *Calendula* is divided in two groups: I. *C. maroccana* and II. *C. suffruticosa* and *C. incana* (designated as *C. suffruticosa* in this work). On one hand, a higher 2C-value of the *C. suffruticosa* group was confirmed when compared with the *C. maroccana* group (Figure 47C), which is expectable given the differences in ploidy levels and chromosome numbers. On the other hand, genome downsizing, i.e. a decrease in monoploid genome size was observed in the tetraploids *C. suffruticosa* (Figure 47D). The mechanism of evolution of *C. suffruticosa* is still poorly understood, however, it is hypothesised that its parental taxa originated an intermediate entity (through dysploidy from *C. maroccana* group) which then suffered a genome duplication event, originating an entity with $2n = 32$ chromosomes (Heyn et al. 1974; Nora et al. 2013). However, the monoploid genome size values might suggest that part of the *C. maroccana* group has evolved from perennial (with lower 1Cx values) to annual taxa (having higher 1Cx values). Interestingly, the pattern in monoploid genome size found within the genus is also found within the *C. maroccana* group, i.e. annual plants having high monoploid genome sizes than perennial. This study also confirms that taxa from *C. maroccana* group played a central role in the origin of several taxa, through hybridization and polyploidisation events (Nora et al. 2013).

Correlations between genome size and eco-geographic variables enabled to explore the variation in species distribution across altitudinal ranges and latitudinal and longitudinal gradients. Despite the limited sampling, this study covered a large region of the natural distribution of the genus. No significant correlation between 1Cx-value and geographical variables, such as altitude and longitude were found, similarly to the lack of patterns found in other Asteraceae species (Bureš et al. 2004; Garcia et al. 2004; Zahradníček and Chrtek 2015). However, monoploid genome size correlated with latitude, with values increasing from south to north (Figure 6B, 7A). This seem to be due to an effect of the taxa that grow in the Atlas Mountains (mostly *C. maroccana* group), which present smaller genome sizes than those inhabiting along northern Morocco and south and west Iberian Peninsula coastal ranges (*C. suffruticosa* group). The differences in geographic range between polyploids and diploids could also be explained through a variety of different ecological processes and morphological and physiologic adaptations (references). In general, the polyploids are more tolerant to cold, drought and/or or other environmental disturbances (Levin 2004; Muñoz-Pajares et al. 2017). For example, the octoploids *C. pachysperma* and *C. palaestina* produce larger achenes, as an adaptability to extreme environments (Judean desert).

The geographic distribution also reveals an interesting pattern, with most of the diploid taxa being limited to the Atlas Mountains from the latitude 3ºW to 10ºW, while the tetraploids have a longitudinal distribution occurring northwards in the coastal range; finally, octoploids mainly occur eastwards in Israel and Palestine. The contact zone between the diploids of the *C. maroccana* group and the tetraploids of the *C. suffruticosa* group is approximately in the Medium Atlas. Still, in some cases, both diploid *C. stellata* and *C. tripterocarpa*, and the tetraploid *C. arvensis*, partially overlap in their distributional ranges. These results strongly support the SW Mediterranean as the main centre of origin of the *Calendula* genus (Norlindh 1946; Meusel and Ohle 1966; Ehrendorfer 1970; Heyn et al. 1974).

Conclusion

The current study concludes the analysis of genome size variation in *Calendula* performed by the team encompassing all known species of the genus. Chromosome numbers are reported for the first time for three, yet undescribed, new species, while previous reports for already known species were confirmed. Diploid, tetraploid and octoploid populations, most of them occupying the SW Mediterranean region, compose the genus. Genome sizes were found to be correlated with chromosome number, ploidy level and life cycle. A significant positive correlation between genome size and longitude was also found. Evidences for genome downsizing with the increase in ploidy level were also detected. Knowledge of the geographical distribution and genome sizes was important to understand the distribution patterns. However, further research, namely using molecular techniques, is needed to explore the evolutionary relationships between taxa and unravel their origins.

Supplementary data

Supplementary data are available online at **www.xyz.com** and consist of the following. Table SD 1 -- *Calendula* specimens from SW Mediterranean (Morocco, Algeria, Tunisia, Italy and Israel) used in the analysis. Table SD 2 -- Chromosome numbers reported in the *Calendula* taxa of the SW Mediterranean.

Acknowledgements

We thank several herbaria (ABH, AL (ENSA), ARAN, B, BC (IBB), BCN, BM, BONN, BR, C, COI, E, ELVE, FI, G, GAT, GDA (GDAC), HAL, JACA, JAEN, JE, K, LD, LEB, LISI, M, MA, MACB, MAF, MARK, MGC, MPU, O, P, PO, RAB, RNG, SALA, SANT, SEV, TFMC, US, VAL and W) for allowing the study or loaning plant material of *Calendula*. We also thank for the achene samples provided by: Real Jardín Botanico de Madrid (CSIC), USDA, IPGN and Olofron Plume. This study was partly funded by the Portuguese Foundation for Science and Technology and UNESCO Fund for financing the work of ACG with the scholarship SFRH/BD/51464/2011 and in the form of institutional resources provided by the CESAM RU (UID/AMB/50017), through national funds and where applicable co-financed by the FEDER, within the PT2020 Partnership Agreement, POCI/01/0145/FEDER/007265, PT2020 UID/QUI/50006/2013. This research was also supported by POPH/FSE funds by the Portuguese Foundation for Science and Technology (FCT) with a starting grant to SC (IF/01267/2013), and Portuguese Foundation for Science and Technology and European Social Fund financed the work of HO with the scholarship H.O. (SFRH/BPD/111736/2015). Additional support was provided by SYNTHESYS, grant GB-TAF-2900, to PS, allowing the study of BM and K's *Calendula* collections.

References

Aparicio A (1989) Números cromosomáticos de plantas occidentales: 487-507. An del Jardín Botánico Madrid 45:483–494

Beaulieu JM, Moles AT, Leitch IJ, et al (2006) Correlated evolution of genome size and seed mass. New Phytol 173:422–437. doi: 10.1111/j.1469-8137.2006.01919.x

Bennett MD (1972) Nuclear DNA Content and Minimum Generation Time in Herbaceous Plants. Proc R Soc London B Biol Sci 181:. doi: 10.1098/rspb.1972.0042

Bennett MD, Leitch IJ (2011) Nuclear DNA amounts in angiosperms: Targets, trends and tomorrow. Ann Bot 107:467–590. doi: 10.1093/aob/mcq258

Bennetzen JL, Ma J, Devos KM (2005) Mechanisms of recent genome size variation in flowering plants. Ann Bot 95:127–132. doi: 10.1093/aob/mci008

Bougoutaia Y, Garcia S, Garnatje T, et al (2016) Genome size , chromosome number, and rDNA organisation in Algerian populations of Artemisia herba-alba (Asteraceae), a basic plant for animal feeding facing overgrazing erosion. An del Jardín Botánico Madrid 73:1–8

Bureš P, Wang YF, Horová L, Suda J (2004) Genome size variation in Central European species of Cirsium (Compositae) and their natural hybrids. Ann Bot 94:353–363. doi: 10.1093/aob/mch151

Castro S, Loureiro J, Procházka T, Münzbergová Z (2012) Cytotype distribution at a diploidhexaploid contact zone in Aster amellus (Asteraceae). Ann Bot 110:1047–1055. doi: 10.1093/aob/mcs177

Čertner M, Fenclová E, Kúr P, et al (2017) Evolutionary dynamics of mixed-ploidy populations in an annual herb: dispersal, local persistence and recurrent origins of polyploids. Ann Bot 120:303–315. doi: 10.1093/aob/mcx032

Darlington CD, Wylie AP (1955) Chromosome Atlas of Flowering Plants. Chromosom atlas Flower plants 308–317:308–501

De Storme N, Mason A (2014) Plant speciation through chromosome instability and ploidy change: Cellular mechanisms, molecular factors and evolutionary relevance. Curr Plant Biol 1:10–33. doi: 10.1016/j.cpb.2014.09.002

Diaz Linfante Z, Luque T, Santa Barbara C (1992) Chromosome numbers of plants collected during Iter Mediterraneum II in Israel. Bocconea 3:229–450

Doležel J, Bartos J, Voglmayr H, Greilhuber J (2003) Nuclear DNA content and genome size of trout and human. Cytometry 51A:127–128. doi: 10.1002/cyto.a.10013

Doležel J, Greilhuber J, Lucretti S, et al (1998) Plant Genome Size Estimation by Flow

Cytometry: Inter-laboratory Comparison. Ann Bot 82:17–26. doi: 10.1093/oxfordjournals.aob.a010312

Ehrendorfer F (1970) Mediterran-mitteleuropäische Florenbeziehungen im Lichte cytotaxonomischer Befunde. Feddes Repert 81:3–32. doi: 10.1002/fedr.19700810103

Fedorov AA (1969) Chromosome numbers of flowering plants. Acad. Sci. of the U. S. S. R., Leningard

Garcia S, Garnatje T, Gras A, et al (2013) GSAD: A genome size in the Asteraceae database. Release 2.0. http://www.etnobiofic.cat/gsad_v2/index.php

Garcia S, Sanz M, Garnatje T, et al (2004) Variation of DNA amount in 47 populations of the subtribe Artemisiinae and related taxa (Asteraceae, Anthemideae): karyological, ecological, and systematic implications. Genome 47:1004–1014. doi: 10.1139/g04-061

Greilhuber J, Doležel J, Lysák MA, Bennett MD (2005) The origin, evolution and proposed stabilization of the terms “genome size” and “C-value” to describe nuclear DNA contents. Ann Bot 95:255–260. doi: 10.1093/aob/mci019

Grover CE, Wendel JF (2010) Recent Insights into Mechanisms of Genome Size Change in Plants. J Bot 2010:1–8. doi: 10.1155/2010/382732

Heyn CC, Dagan O, Nachman B (1974) The annual Calendula species: taxonomy and relationships. Isr J Bot 23:1201–1969

Heyn CC, Joel A (1983) Reproductive relationships between annual species of Calendula. Plant Syst Evol 143:311–329. doi: 10.1007/BF00986612

Humphries CJ, Murray BG, Bocquet G, Vasudevan K (1978) Chromosome Numbers of Phanerogams from Morocco and Algeria. Bot Not 131:391–406

Knight CA, Ackerly DD (2002) Variation in nuclear DNA content across environmental gradients: a quantile regression analysis. Ecol Lett 5:66–76. doi: 10.1046/j.1461-0248.2002.00283.x

Knight CA, Beaulieu JM (2008) Genome size scaling through phenotype space. Annals of Botany 101(6): 759–766.

Knight CA, Molinari N, Petrov D (2005) The Large Genome Constraint Hypothesis: Evolution, Ecology and Phenotype. Ann Bot 95:177–190. doi: 10.1093/aob/mci011

Krahulcová A, Trávníček P, Krahulec F, Rejmánek M (2017) Small genomes and large seeds: chromosome numbers, genome size and seed mass in diploid *Aesculus* species (Sapindaceae). Ann Bot 119(6):957-964.. doi: 10.1093/aob/mcw261

Lanza D (1919) Monografia del genero Calendula. Atti R. Acad. Sci., Palermo

Leitch IJ, Bennett MD (2007) Genome Size and its Uses: The Impact of Flow Cytometry. In: Flow Cytometry with Plant Cells: Analysis of Genes, Chromosomes and Genomes. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, pp 153–176

Levin DA (1975) Minority cytotype exclusion in local plant populations. Taxon 24:35–43

Levin DA (2004) The Role of Chromosomal Change in Plant Evolution by Donald A. Levin. Syst Bot 29:460–461. doi: 10.1600/036364404774195656

Loureiro J, Rodriguez E, Doležel J, Santos C (2007) Two New Nuclear Isolation Buffers for Plant DNA Flow Cytometry: A Test with 37 Species. Ann Bot 100:875–888. doi: 10.1093/aob/mcm152

Maranon T, Grubb PJ (1993) Physiological Basis and Ecological Significance of the Seed Size and Relative Growth Rate Relationship in Mediterranean Annuals. Funct Ecol 7: 591-599. doi: 10.2307/2390136

Marchi P, Capineri R, D'Amato G (1974) Numeri cromosomici per la Flore Italiana. Inf Bot Ital 6:303–319

Marques I, Loureiro J, Draper D, *et al.* (2017) How much do we know about the frequency of hybridization and polyploidy in the Mediterranean region? Plant Biol. 20 Suppl 1:21-37. doi: 10.1111/plb.12639

Meikle RD (1976) Calendula L. In: Tutin TG, Heywood VH, Burges NA, et al. (eds) Flora Europaea Notulae Systematicae ad Floram Europaeam Spectantes. Cambridge University Press, Cambridge, pp 206–207

Meusel H, Ohle H (1966) Zur Taxonomie und Cytologie der Gattung Calendula. Österreichische Bot Zeitschrift 113:191–210. doi: 10.1007/BF01441032

Muñoz-Pajares AJ, Perfectti F, Loureiro J, *et al.* (2017) Niche differences may explain the geographic distribution of cytotypes in Erysimum mediohispanicum. Plant Biol. 20 Suppl 1:139-147. doi: 10.1111/plb.12605

Negodi G (1937) Caratteri nucleari e morfologici in alcune Calenduleae. (Compositae-Tubuliflorae). Ann Bot 21:510–518

Nègre R (1958) A propos de quelques Calendula marocains. Bull la Société des Sci Nat Phys du Maroc 38: 1–7

Nora SV, Castro S, Loureiro J, *et al.* (2013) Flow cytometric and karyological analyses of Calendula species from Iberian Peninsula. Plant Syst Evol 299:853–864. doi: 10.1007/s00606-013-0767-0

Nordenstam B (1994) Calenduleae. In: Bremer K (ed) Asteraceae: Cladistics & Classification. Timber Press, Portland, pp 365–376

Nordenstam B, Källersjö M (2009) Calenduleae. In: Systematics, Evolution, and Biogeography of Compositae. IAPT, Vienna, pp 527–538

Norlindh T (1946) Studies in the Calenduleae. II. Phytogeography and interrelation. Bot Not 1946:471–506

Norlindh T (1977) Calenduleae - Systematic review. In: Heywood VH, Harborne JB, Turner BL (eds) The Biology and Chemistry of the Compositae. Academic Press, London (United Kingdom), pp 961–987

Oberprieler C, Vogt R (1993) Chromosome Numbers of North African Phanerogams. II. Willdenowia 23:211–238

Ohle H (1974) Beiträge zur Taxonomie der Gattung Calendula II. Taxonomische Revision der südeuropäischen perennierenden Calendula-Sippen. Feddes Repert 85:245–283. doi: 10.1002/fedr.19740850402

Ohle H (1975a) Beiträge zur Taxonomie und Evolution der Gattung Calendula L. III. Revision der marokkanischen perennierenden Sippen unter Berücksichtigung einiger marokkanischer Annueller. Feddes Repert 86:1–17. doi: 10.1002/fedr.19750860102

Ohle H (1975b) Beiträge zur Taxonomie und Evolution der Gattung Calendula L. IV. Revision der algerisch-tunesischen perennierenden Calendula-Sippen unter

Berücksichtigung einiger marokkanisch-algerischer Annueller und der marokkanischen und südeuropäischen perennierende. Feddes Repert 86:525–541. doi: 10.1002/fedr.19750860908

Ohri D (2005) Climate and growth form: The consequences for genome size in plants. Plant Biol 7:449–458. doi: 10.1055/s-2005-865878

Otto SP (2007) The Evolutionary Consequences of Polyploidy. Cell 131:452–462. doi: 10.1016/j.cell.2007.10.022

Pazy B (2000) Flora Palaestina: chromosome numbers. Isr J Plant Sci 48:7–32

Plume O (2015) Hybridization, Genome Duplication, And Chemical Diversification In The Evolution Of Calendula L. (Compositae). PhD thesis. Cornell University, Ithaca, NewYork.

Plume O, Raimondo FM, Troia A (2015) Hybridization and competition between the endangered sea marigold (Calendula maritima, Asteraceae) and a more common congener. Plant Biosyst - An Int J Deal with all Asp Plant Biol 149:68–77. doi: 10.1080/11263504.2013.810182

Price HJ, Crawford DJ, Bayer RJ (1984) Nuclear DNA contents of Coreopsis nuecensoides and C. nuecensis (Asteraceae), a progenitor-derivative species pair. Bot Gaz 145:240–245. doi: 10.2307/2474345

Ruiz de Clavijo E (1990) Números cromosomáticos de plantas occidentales, 619-630. An del Jardín Botánico Madrid 47:431–437

Silveira P, Gonçalves AC, Santos C, Paiva J (2013) Two lectotypifications and a new combination in Calendula (Asteraceae) for Flora Iberica. Phytotaxa 145:47–53. doi: 10.11646/phytotaxa.145.1.5

Soltis PS, Marchant DB, Van de Peer Y, et al (2015) Polyploidy and genome evolution in plants. Curr Opin Genet Dev 35:119–125. doi: 10.1016/j.gde.2015.11.003

Soltis PS, Soltis DE (2009) The Role of Hybridization in Plant Speciation. Annu Rev Plant Biol 60:561–588. doi: 10.1146/annurev.arplant.043008.092039

Talavera S, Devesa J, Fernández-Galiano E (1984) Notas cariosistemáticas sobre plantas Norteafricanas. I. Compositae. Candollea 39:271–280

Torrell M, Vallès J (2001) Genome size in 21 *Artemisia* L. species (Asteraceae, Anthemideae): Systematic, evolutionary, and ecological implications. Genome 44:231–238. doi: 10.1139/gen-44-2-231

Valdés B, Parra R (1997) Numeros cromossomaticos de plantas de Marruecos, I. Lagascalia 20:161–166

Vallès J, Canela MÁ, Garcia S, *et al.* (2013) Genome size variation and evolution in the family Asteraceae. Caryologia 66:221–235. doi: 10.1080/00087114.2013.829690

Vogt R, Oberprieler C (2008) Chromosome Numbers of North African Phanerogams. VIII. More counts in Compositae. Willdenowia 38:497–519. doi: 10.3372/wi.38.38210

Vogt R, Oberprieler C (2012) Chromosome Numbers of North African Phanerogams. X. Plants collected during Iter Mediterraneum V of OPTIMA in Morocco. Ann des Naturhistorischen Museums Wien Ser B für Bot und Zool 193–221

Vogt R, Oberprieler C (1993) Chromosome Numbers of North African Phanerogams. I. Flora Mediterr 3:187–210

Weiss-Schneeweiss H, Emadzade K, Jang TS, Schneeweiss GM (2013) Evolutionary consequences, constraints and potential of polyploidy in plants. Cytogenet Genome Res 140:137–150. doi: 10.1159/000351727

Whitney KD, Ahern JR, Campbell LG, et al (2010) Patterns of hybridization in plants. Perspect Plant Ecol Evol Syst 12:175–182. doi: 10.1016/j.ppees.2010.02.002

Zahradníček J, Chrtek J (2015) Cytotype distribution and phylogeography of Hieracium intybaceum (Asteraceae). Bot J Linn Soc 179:487–498. doi: 10.1111/boj.12335

Zohary M (1950) Evolutionary Trends in the Fruiting Head of Compositae. Evolution (N Y) 4:103–109. doi: 10.2307/2405387

Table SD 1 -- *Calendula* specimens from Morocco, Algeria, Tunisia, Italy and Israel, used in the analysis.

Taxa	**Locality**	**Coordinates**	**Voucher. No.**
c. arvensis	TUNISIA: Sfax, the city of Möez, between Sfax and Thaenae; May 2009, 10 m, CA16164 in Silveira, P.	34°40'57" N, 10°42'06" E	3 072
	MOROCCO: NCRPIS (USDA), PI578099 in Silveira, P.		3 093
	MOROCCO: Agadir, road N8 between Agalgal and Argana (road Marrakesh - Agadir); April 2011, 1037 m, Silveira, P.	30°56' N, 9°04' W	3 129
	MOROCCO: Agadir, road N1 near Arhoud, between Agadir and Cap Rhir; April 2011, 11 m, Silveira, P.	30°36'53" N, 9°48'04" W	3 132
	MOROCCO: Marrakech, near to the Airport; March 2013, 0 m, Silveira, P. Gonçalves, ACRS; Ouhammou, A.	31°35'00" N, 8°00'44" W	3 284
	MOROCCO: Taroudant, 17 km W of Taroudant; March 2013, 176 m, Silveira, P. Gonçalves, ACRS; Ouhammou, A.	30°28'32" N, 9°02'05" W	3 290
	MOROCCO: Nador, El Kadia (Beni Said); May 2014, 397 m, Silveira, P. Gonçalves, ACRS	35°11'52" N, 3°03'30" W	3 338
C. eckerleinii	MOROCCO: Ifrane, limestone outcrop 3 km N of Ifrane on the N8 road to Fez; April 2010, 1621 m, Silveira, P.	33°33'26" N, 5°05'51" W	3 064
	MOROCCO: Meknes, Ain Leuh, road P7311, near l'Oued Er-Rbia; May 2014, 1505 m, Silveira, P. Gonçalves, ACRS	33°16'48" N, 5°20'23" W	3 330
	MOROCCO: Timahdite, Foung Kheneg; May 2014, 1920 m, Silveira, P. Gonçalves, ACRS	33°09'10" N, 5°03'43" W	3 331
	MOROCCO: Boulemane, 31 km from Ifrane, 48 km from Sefrou, 25 km of Boulemane, at the junction of Ifrane road; May 2014, 1572 m, Silveira, P. Gonçalves, ACRS	33°27'18" N, 4°51'25" W	3 332
	MOROCCO: Fes, Jebel Zalagh; May 2014, 895 m, Silveira, P. Gonçalves, ACRS	34°06'19" N, 4°58'11" W	3 333
C. lanzae	MOROCCO: Taroudant, S of Ait-Yazza; March 2013, 257 m, Silveira, P. Gonçalves, ACRS; Ouhammou, A.	30°28'35" N, 8°48'03" W	3 292

	MOROCCO: Taroudant, gravel on the margins of the river Tiout; March 2013, 424 m, Silveira, P. Gonçalves, ACRS; Ouhammou, A.	30°23'48" N, 8°42'17" W	3 293
C.maroccana* subsp. *maroccana	MOROCCO: NCRPIS (USDA), PI607416 in Silveira, P.		3 105
	MOROCCO: Asni, High Atlas, between Tizi-n-Test and Asni; April 2011, 1836 m, Silveira, P.	30°51'08" N, 8°21'56" W	3 142
	MOROCCO: Asni, High Atlas, near Tazalt, between Asni and Tizi-n-Test; April 2011, 1250 m, Silveira, P.	30°58'52" N, 8°13'33" W	3 143
	MOROCCO: Asni, High Atlas, ca. 7 km N of Asni, road to Marrakesh; April 2011, 1131 m, Silveira, P.	31°17'55" N, 7°57'52" W	3 144
	MOROCCO: Taroudant, between Tiout and Igherm; March 2013, 1365 m, Silveira, P. Gonçalves, ACRS; Ouhammou, A.	30°12'34" N, 8°28'51" W	3 294
	MOROCCO: Taroudant, between Tiout and Igherm, at 3 km from Igherm; March 2013, 1656 m, Silveira, P. Gonçalves, ACRS; Ouhammou, A.	30°06'41" N, 8°27'50" W	3 295
	MOROCCO: Taroudant, leaving Igherm to Taliouine; March 2013, 1569 m, Silveira, P. Gonçalves, ACRS; Ouhammou, A.	30°41'16" N, 7°16'18" W	3 298
	MOROCCO: Taroudant; March 2013, 1909 m, Silveira, P. Gonçalves, ACRS; Ouhammou, A.	30°47'48" N, 7°31'38" W	3 299
	MOROCCO: Tizi-n-Tichka, on the N9 almost at Tizi-n-Tichka, coming from Tachokchte; March 2013, 1351 m, Silveira, P. Gonçalves, ACRS; Ouhammou, A.	31°24'53" N, 7°23'43" W	3 300
C. maroccana* subsp. *murbeckii	MOROCCO: Marrakech, near to the house of the gazelle reserve; March 2013, 624 m, Silveira, P. Gonçalves, ACRS; Ouhammou, A.	31°52'09" N, 7°57'08" W	3 280
	MOROCCO: Marrakech, south of Barrage Lalla Takerhust; March 2013, 729 m, Silveira, P. Gonçalves, ACRS; Ouhammou, A.	31°21'36" N, 8°09'46" W	3 287
C. meuselii	MOROCCO: Meknes, between Moulay Idriss and N'zala des Beni-Ammar; April 2010, 852 m, Silveira, P. & Gonçalves, ACRS	34°05'09" N, 5°25'58" W	3 063

c pachysperma	ISRAEL: Judea and Samaria Area, Samaria mountains; 871 m, IPGN 20 562 in Silveira, P.	32°12'12" N, 35°16'24 E	3 121
C. palaestina	ISRAEL: Mount Carmel, 472 m, IPGN 21 124 in Silveira, P.	32°44'33.83" N, 35°02'54.21" E	3 120
	ISRAEL: Judea and Samaria Area, Samaria mountains; 871 m, IPGN 20 563 in Silveira, P.	32°12'12" N, 35°16'24 E	3 122
calendula sp1	MOROCCO: Al Hoceima, Bokkoya, 33 km from Imzouren; May 2014, 740 m, Silveira, P. Gonçalves, ACRS	34°54'46" N, 3°47'59" W	3 339
calendula sp2	MOROCCO: Taza; June 2012, 500 m, Silveira, P. Gonçalves, ACRS	34°07'50" N, 4°18'09" W	3 263
calendula sp3	MOROCCO: between Agadir and Cap Rhir, Ahroud; April 2011, 27 m, Silveira, P.	30°36' N, 9°46' W	3 130
	MOROCCO: Agadir, 2.6 km from the lighthouse of Cap Rhir; April 2011, 12 m, Silveira, P.	30°37'34" N, 9°51'27" W	3 134
	MOROCCO: Sidi Ifni, 35 km N of Sidi Ifni; April 2011, 256 m, Silveira, P.	29°36'50" N, 10°01'25" W	3 137
	MOROCCO: Sidi Ifni, 35 km N of Sidi Ifni; April 2011, 256 m, Silveira, P.	29°36'50" N, 10°01'25" W	3 138
C. stellata	TUNISIA: between Bir Bou Rekba and Hammamet; April 2009, 0 m, Silveira, P.	36°26' N, 10°35' E	3039a
	TUNISIA: between Bir Bou Rekba and Hammamet; April 2009, 0 m, Silveira, P.	36°26' N, 10°35' E	3039b
	MOROCCO: Meknes, vicinities of Moulay Idriss Zerhoun; April 2010, 429 m, Silveira, P.	34°03'54" N, 5°30'19" W	3 061
	MOROCCO: Meknes, vicinities of Moulay Idriss Zerhoun; April 2010, 429 m, Silveira, P.	34°03'54" N, 5°30'19" W	3 062

	MOROCCO: Agadir, road Agadir - Marrakech, before the Abdelmoumen water dam; April 2010, 644 m, Silveira, P.	30°35'21" N, 9°20'53" W	3 068
	MOROCCO: Marrakech, near to the house of the gazelle reserve; March 2013, 624 m, Silveira, P. Gonçalves, ACRS; Ouhammou, A.	31°52'09" N, 7°57'08" W	3 281
	MOROCCO: Khemisset, Oued Beht, 18 km of Khemisset, near Oued Beht bridge; May 2014, 132 m, Silveira, P. Gonçalves, ACRS	33°52'47" N, 5°55'49" W	3 329
	MOROCCO: Zaio, road N2, between Zaio and Nador; May 2014, 213 m, Silveira, P. Gonçalves, ACRS	34°59'52" N, 2°49'47" W	3 337
C. suffruticosa	ITALY: Sicily, Ronciglio (Trapani); NCRPIS (USDA), PI597596 in Silveira, P.	38°01' N, 12°29' E	3 101
	ITALY: Sicily, Mount Erice near Trapani; NCRPIS (USDA), PI613021 in Silveira, P.	38°01' N, 12°29' E	3 109
	MOROCCO: Tangier-Tetouan; June 2012, 1070 m, Silveira, P. Gonçalves, ACRS	35°28'50" N, 5°22'06" W	3 261
	MOROCCO: Tangier-Tetouan; June 2012, 468 m, Silveira, P. Gonçalves, ACRS	35°35'26" N, 5°22'45" W	3 262
	MOROCCO: Nador; June 2012, 562 m, Silveira, P. Gonçalves, ACRS	35°14'11" N, 2°58'29" W	3 265
	MOROCCO: Tangier-Tetouan; June 2012, 90 m, Silveira, P. Gonçalves, ACRS	35°12'39" N, 4°39'46" W	3 266
	MOROCCO: Tangier-Tetouan; June 2012, 10 m, Silveira, P. Gonçalves, ACRS	35°54'27" N, 5°28'54" W	3 267
	MOROCCO: Tangier-Tetouan; June 2012, 3 m, Silveira, P. Gonçalves, ACRS	35°54' N, 5°27' W	3 268
	ALGERIA: Algiers, Plage de La Madrague; June 2013, 13 m, Silveira, P. Gonçalves, ACRS; Amirouche, R.	36°47'25" N, 2°53'56" E	3 316
	ALGERIA: Algiers, near to the beach; June 2013, 13 m, Silveira, P. Gonçalves, ACRS; Amirouche, R.	36°43'42" N, 2°50'27" E	3 317
	ALGERIA: Algiers; June 2013, 13 m, Silveira, P. Gonçalves, ACRS; Amirouche, R.	36°41'25" N, 2°47'37" E	3 318
	ALGERIA: Tipasa; June 2013, 30 m, Silveira, P. Gonçalves, ACRS; Amirouche, R.	36°37'19" N, 2°24'27" E	3 319
	ALGERIA: Gorge des Palestro; June 2013, 340 m, Silveira, P. Gonçalves, ACRS; Amirouche, R.	36°36' N, 3°35' E	3 320

	ALGERIA: Djurdjura; June 2013, 1798 m, Silveira, P. Gonçalves, ACRS; Amirouche, R.	36°27'46" N, 4°00'00" E	3 321
	MOROCCO: Taza, Taza, Ras-El-Ma, Sidi Msbar; May 2014, 1460 m, Silveira, P. Gonçalves, ACRS	34°07'58" N, 4°07'58" W	3 334
	MOROCCO: Berkane, Beni Snassen, road between Zegzel - Tazarhine - Takerkoust; May 2014, 54 m, Silveira, P. Gonçalves, ACRS	34°50'01" N, 2°22'17" W	3 335
	MOROCCO: Berkane, Beni Snassen, near Oued Zegzel; May 2014, 260 m, Silveira, P. Gonçalves, ACRS	34°52'40" N, 2°21'20" W	3 336
	MOROCCO: Al Hoceima, Bokkoyas, Taoussarte; May 2014, 133 m, Silveira, P. Gonçalves, ACRS	35°13'08" N, 4°05'14" W	3 340
	MOROCCO: Tangier-Tetouan, Jebel Kelti, Arifane; May 2014, 943 m, Silveira, P. Gonçalves, ACRS	35°17'04" N, 5°18'00" W	3 341
	TUNISIA: between Bir Bou Rekba and Hammamet; April 2009, 0 m, Silveira, P.	36°26' N, 10°35' E	3 038
	TUNISIA: between Bir Bou Rekba and Hammamet; April 2009, 0 m, Silveira, P.	36°26' N, 10°35' E	3039b
C. tripterocarpa	MOROCCO: Agadir, near Abdelmoumen water dam, NE of Agadir; April 2010, Silveira, P.	30°39'48" N, 9°13'56" W	3 065
	MOROCCO: Guelmim, on the road to Plage Blanche; April 2010, 170 m, Silveira, P.	28°58'36" N, 10°15'25" W	3 066
	MOROCCO: Guelmim, on the road to Plage Blanche; April 2010, 170 m, Silveira, P.	28°58'36" N, 10°15'25" W	3 067
	MOROCCO: Agadir, between Marrakesh and Agadir, road N8 after Chichaoua; April 2011, 552 m, Silveira, P.	31°22' N, 8°49' W	3 128
	MOROCCO: Agadir, road N1 between Agadir and Cap Rhir; April 2011, 11 m, Silveira, P.	30°36'53" N, 9°48'04" W	3 133
	MOROCCO: Agadir, near Ait Baha; April 2011, 599 m, Silveira, P.	30°04'58" N, 9°08'25" W	3 140
	MOROCCO: Marrakech, near to the house of the gazelle reserve; March 2013, 624 m, Silveira, P. Gonçalves, ACRS; Ouhammou, A.	31°52'09" N, 7°57'08" W	3 282
	MOROCCO: Taroudant, 17 km W of Taroudant; March 2013, 176 m, Silveira, P. Gonçalves, ACRS; Ouhammou, A.	30°28'32" N, 9°02'05" W	3 289
	MOROCCO: Taroudant, between Tiout and Igherm, at 3 km from Igherm; March 2013, 1656 m, Silveira, P. Gonçalves, ACRS; Ouhammou, A.	30°06'41" N, 8°27'50" W	3 296

Table SD 2 -- Chromosome numbers reported in the *Calendula* taxa from SW Mediterranean

Taxa	**Country/region**	**Chromosome number report**		
		n	**2*n***	**Reference**
C. arvensis	Mediterranean basin	18[1*] 22[2]	14[3*] 18[3*] 44[4]	[1](Negodi 1937) [2](Meusel and Ohle 1966; Heyn and Joel 1983; Aparicio 1989) [3](Humphries et al. 1978) [4](Meusel and Ohle 1966; Marchi et al. 1974; Diaz Linfante et al. 1992; Vogt and Oberprieler 1993, 2008, 2012)
C. eckerleinii	Morocco		18	(Ohle 1975a; Vogt and Oberprieler 2008)
C. lanzae	Morocco		18	(Ohle 1975a)
C. maroccana* subsp. *maroccana	Morocco		18[1] 32[2*]	[1](Ohle 1975a, b; Oberprieler and Vogt 1993; Vogt and Oberprieler 2012; Nora et al. 2013) [2](Valdés and Parra 1997)
C. maroccana subsp. murbeckii	Morocco		18[1]	[1](Meusel and Ohle 1966; Ohle 1975a)

			32[2*]	(Fedorov 1969)
C. meuselii	Morocco		18	(Ohle 1975a)
C. pachysperma	Israel, Palestine	±43[1]	±85[2] ±88[3]	[1](Heyn and Joel 1983) [2](Heyn et al. 1974) [3](Pazy 2000)
C. palaestina	Israel, Palestine	±43[1]	±85[2]	[1](Heyn and Joel 1983) [2](Heyn et al. 1974)
C. stellata	Morocco, Algeria, Tunisia, Italy	7[1]	14[2] 44[3]	[1](Negodi 1937; Meusel and Ohle 1966; Humphries et al. 1978; Talavera et al. 1984; Aparicio 1989; Ruiz de Clavijo 1990) [2](Meusel and Ohle 1966; Heyn et al. 1974; Ohle 1975a; Humphries et al. 1978; Oberprieler and Vogt 1993; Vogt and Oberprieler 1993; Vogt and Oberprieler 2008; Vogt and Oberprieler 2012; Nora et al. 2013) [3](Meusel and Ohle 1966)

C. suffruticosa	Morocco, Algeria, Tunisia, Portugal, Spain	9[1] 14[2] 16[3]	32[4]	[1](Meusel and Ohle 1966) [2](Negodi 1937) [3](Meusel and Ohle 1966; Ohle 1974, 1975b; Aparicio 1989) (Talavera et al. 1984; Vogt and Oberprieler 2008)
C. tripterocarpa	Morocco, Algeria, Tunisia, Spain	7[1] 14[2] 15[3]	30[4] 30+2B[5]	[1](Negodi 1937) [2]Reese [3](Meusel and Ohle 1966; Heyn et al. 1974; Heyn and Joel 1983; Aparicio 1989) [4](Meusel and Ohle 1966; Heyn et al. 1974; Diaz Linfante et al. 1992) [5](Oberprieler and Vogt 1993; Vogt and Oberprieler 2008)

Chapter 5 - Publication 4

Taxonomic revision of the genus *Calendula* (Asteraceae) in Morocco, including taxa from Algeria and Tunisia

A taxonomic revision of *Calendula* (Asteraceae) in Morocco, including some taxa from Algeria and Tunisia

ANA CARLA GONÇALVES[1], ESTRELA FIGUEIREDO[2,3], AHMED OUHAMMOUD[4], RACHID AMIROUCHE[5], CONCEIÇÃO SANTOS[6], PAULO SILVEIRA[1*]

[1]*Departamento de Biologia e CESAM, Universidade de Aveiro, 3810–193, Aveiro, Portugal.*

[2]*Centro de Ecologia Funcional, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal.*

[3]*Jardim Botânico da Universidade de Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal.*

[4]*Herbier Régional 'MARK', Laboratoire d'Ecologie et Environnement, Faculté des Science Semlalia, Université Cadi Ayyad, Avenue Prince Moulay Abdallah, B.P. 2 390, Marrakech, 40 001, Maroc*

[5]*Laboratoire de Biologie et Physiologie des Organismes, Université des Sciences et de la Technologie Houari Boumediene, USTHB, BP 32 El-Alia, 16 111 Bab-Ezzouar, Alger, Algeria*

[6]*Faculdade de Ciências, Universidade do Porto, Requinte/LAQV, s/n, 4169–007 Porto, Portugal.*

**Author for correspondence. E–mail: psilveira@ua.pt*

This chapter is in preparation to be submitted as an original article in:
Phytotaxa

Table of contents

Abstract

This study examines morphological patterns within *Calendula* (Asteraceae) from Morocco, including some taxa from Algeria and Tunisia. This genus was inadequately studied in this territory, particularly the perennial plants with $2n = 32$ (classically included under *C. suffruticosa* and *C. incana*), whose specimens held in the herbaria generally lacked achenes, essential for proper classification/identification. Principal components analyses allowed us to reassess the classification, relationships, distribution and evolution of *Calendula* taxa, which was further supported by chromosome numbers and genome size estimations. Morphometric analyses among and between *C. maroccana* and *C. suffruticosa* groups found that the major delimiting characters were the achene's morphology. A taxonomic treatment for SW Mediterranean region (Morocco, Algeria and Tunisia) taxa is presented, based on field and herbarium specimens. Three new species and seven new subspecies of *C. suffruticosa* were described. Identification keys full generic descriptions, complete nomenclature including typification and notes on distribution and habitat, are also provided for this complex genus.

Introduction

The genus *Calendula* Linnaeus (1753: 921) is widely distributed in the Mediterranean region, occurring from Macaronesia throughout most of the north African countries, south of Europe and Middle East regions (Heyn et al. 1974; Nordenstam 2007; Nordenstam & Källersjö 2009; Norlindh 1946, 1977). However, most species are restricted to the SW Mediterranean region, the main centre of the evolution of the genus (Norlindh 1977). One widespread (*C. arvensis* Linnaeus (1763: 1303)) and another cultivated (*C. officinalis* Linnaeus (1753: 921)) species are found in other parts of the world. A brief history of the generic delimitation of the genus can be found in Norlindh (1946: 1977).

Calendula is one of the most challenging and least-known genus within the family Asteraceae, whose complexity derives from frequent hybridisation, and polyploidisation (Heyn and Joel 1983; Nora et al. 2013; Plume 2015), unusual diversity of fruits – heterocarpy (Heyn and Joel 1983; Ruíz De Clavijo 2005) and its intricate heredity (Heyn and Joel 1983). The achenes produced differ not only in types of morphology (morphs) but also in respect to dispersal mechanisms (Ruíz De Clavijo 2005). These variations lead to uncertainty in species classification and determination. Consequently, many species have been described within what should be one single taxon, including a wide spectrum of forms and their intermediates (Heyn and Joel 1983). For example, up to 46 names related to *C. arvensis* have been published, many of which misapplied in other taxa, like *C. tripterocarpa* Ruprecht (1856: 231) or *C. stellata* Cavanilles (1791: 3).

As presently redefined, *Calendula* is a genus of 16 species, usually annual or perennial herbs, characterised by having sessile leaves, arranged alternately, capitula solitary with internal tubular yellow, orange, brown or violet-purple flowers, functionally male and external yellow or orange ligulate female flowers and by its heteromorphic achenes, pappus absent (Nordenstam 2007; Gonçalves et al. 2014). According to Heyn et al. (1974), the annuals comprised five species: *C. stellata*, *C. tripterocarpa*, *C. arvensis*, *C. palaestina* Boissier (1849: 10), and *C. pachysperma* Zohary (1941: 172). The annual *C. lanzae* Maire (Maire 1928a: 138), which is from Morocco, was not included. While the perennials are grouped into: (1) *C. maroccana* (Ball) B.D. Jackson (1893: 383) group; (2) *C. suffruticosa* Vahl (1791: 94) and *C. incana* Willdenow (1803: 2341) group; and (3) the cultivated *C. officinalis* (Ohle 1974: 1975 a; b). Ohle (1974, 1975 a; b) recognised 24 perennial taxa, among these groups, most of them based on leaves' shape and thickness. Meikle's (1976) treatment included both, annual and perennial taxa, but only for the European flora. This author recognised few infraspecific taxa under *C. suffruticosa*, neglecting Ohle's work. However, the variability of the genus is greater than what was described by these authors. Gonçalves et al. (in press) took a deeper view into the genus and recognised the importance of some morphological characters, especially the variation of achene morphology and its combinations. Furthermore, new data obtained by means of fieldwork in the study area lead to a reassessment of the classification of the genus. A brief taxonomic history and the criteria used for the taxonomic treatment and characters were discussed in Gonçalves et al. (in press).

Prior taxonomic works on Calendula from North Africa

Since the description of the genus by Linnaeus (1753), *Calendula* was known only in Europe. Considering that the species of *Calendula* occur in the Mediterranean region and its immediate vicinities, soon it became the object of study by many botanists. Among which, works made by Candolle (1838), Boissier (1839, 1849, 1859, 1875), Boissier &

Reuter (1852), Cosson (1856, 1882), Cosson & Kralik (1857) or Battandier (1890, 1902, 1910, 1919, 1921) contributed greatly to the knowledge of the genus. Our intention in this section is not to describe in detail all works on *Calendula*, especially since excellent reviews exist (Lanza 1919; Ohle 1974; 1975 a; b; Gonçalves et al. 2017), but just to highlight here some significant/critical points for the discussion, concerning the study area.

The first species described for the north Africa was *C. suffruticosa* Vahl (1791)[1] from Tunisia. Since its description, the typical appearance of the real *C. suffruticosa* has been discussed quite intensively. Several authors mentioned its presence in other Mediterranean regions, e.g. Candolle (1837) reported the taxon for Portugal or Boissier (1839) for southern Spain. It was also misapplied in Moroccan taxa ('*C. suffruticosa* subsp. *C. maroccana*' Ball (1873: 367)). Only much later, Ohle (1975b) has considered that the range of the typical *C. suffruticosa* was limited to the coast of the Gulf of Tunis, in Tunisia.

For a long time, the morphology of the achenes went disregarded by many authors. Ball (1878) was the first to recognise *C. maroccana* as a distinct taxon based on the achenes, but originally it was described as '*C. suffruticosa* subsp. *C. maroccana*' Ball (1873: 367). This author used the first diagnosis to confirm it. Nevertheless, the name '*C. maroccana* (Ball) B.D. Jackson (1893: 383)' has been preserved as a new combination, which has generated some confusion. However, Jackson (1893: 383) only included the taxon in the Index Kewensis referring to Ball's works. Apart from the authorship of *C. maroccana*, for a long time, many of the SW Mediterranean taxa remain recognised under *C. suffruticosa* (Ohle 1975a). The segregation of these taxa was made by Ohle (1975a), a German botanist who worked with perennial taxa. Ohle (1974: 1975 a; b) compared morphology (e.g. growth habit, leaves, capitula, achenes, pollen), cytology and distribution in his revision. As mentioned, Ohle divided the genus into groups, the first group *C. maroccana* included four species, with $2n = 18$, diploid, all of them from Morocco; the second group included *C. incana* and *C. suffruticosa*, both with $2n = 32$, tetraploid, mainly distributed in the SW Mediterranean coast, and the cultivated *C. officinalis*, also with $2n = 32$. However, this classification has been questioned, since some species have the same/identical chromosome number and a gradual transition in

---

[1] Both, *C. stellata* and *C. suffruticosa* were described in 1791, but the origin of *C. stellata* is uncertain ('*Plant grow from seeds*' maybe from north Africa).

genome size (Nora et al. 2013). Silveira et al. (2013), proposed to include all *C. incana* under *C. suffruticosa* taxa because these groups are artificial and difficult to distinguish one from the other. These studies shed some light on this problem as well as helped to clarify the relationships among species, but many taxonomical problems remain unclear. More detailed and discussions on these issues and taxa are found in Gonçalves et al. in press.

Another controversy subject has been the recognition of *C. tomentosa* Desfontaines (1799: 305), subsequently treated as *C. suffruticosa* subsp. *tomentosa* (Ball) Murbeck (1905: 9), or *C. incana*, both erroneously described from North Africa, but based on a type collected in the south of Spain. A brief history about this nomenclatural problem is found in Silveira et al. (2013), who also proposed a new lectotype for *C. suffruticosa* subsp. *tomentosa*.

Compared with Algeria and Tunisia, the Moroccan *C. suffruticosa* taxa have received comparatively little attention from the taxonomists. In a major review, Lanza (1919) focused especially on the impact of hybridisation, transmission, and dominance or recession, in a Mendelian sense, of some morphological characters. This author recognised 10 species in two groups: '*Annuae*' (three species) and '*Perennes vel perennantes'* (seven species), many of which from North African countries. Later, this author described the annual *C. murbeckii* Lanza ex Murbeck (1923: 59), pointing out that it has two morphologies (morphs) of achenes (trialate and vermiculate-exalate achenes). Maire (Maire 1928b), however, distinguished the specimens of *C. murbeckii* from Marrakesh by having distinct morphs of the achenes (rostrate, trialate, cymbiform and vermiculate-exalate). He also described a new variety '*C. murbeckii* var. *pinnatiloba*' (Cosson) Maire (Maire 1928c: 57) [*C. suffruticosa* var. *pinnatiloba* Cosson (Lanza 1919: 134), *nom. nudum*.]. In the same year, Maire described *C. lanzae* Maire (1928a: 138), differing from *C. murbeckii* by having deeply sinuate-dentate to pinnatisect leaves with hydatodium dark-purple in the margin.

As previously discussed, Ohle (1975 a) recognised the *C. maroccana* group, which included four species, *C. maroccana*, *C. meuselii* Ohle (1975 a: 4), *C. eckerleinii* Ohle (1975 a: 8) and *C. lanzae*. However, in Ohle's (1975 a) revision, the *C. suffruticosa* taxa from Morocco were not monographed. Instead, the knowledge of this group in Morocco remained largely based on older and outdated works, like of Jahandiez & Maire

(1934). These authors made the first major treatment, as part of a revision of plants from Morocco. They recognised five *C. suffruticosa* taxa (subsp. *eusuffruticosa* Maire (Jahandiez and Maire 1934: 789), var. *maroccana* (Ball) Maire (Jahandiez and Maire 1934: 789), subsp. *tomentosa* (Desfontaines) Maire (Jahandiez and Maire 1934: 789), subsp. *marginata* (Willdenow) Maire (1934: 789), and var. *balansae* (Boissier & Reuter) Maire (Jahandiez and Maire 1934: 789)). However, it is difficult to interpret the taxonomic categories described. Besides the *C. suffruticosa* group, these authors also study other taxa from Morocco. The major inconsistency was that *C. murbeckii* and *C. lanzae* where treated as varieties of *C. echinata* Candolle (1838: 453). Later, Jahandiez & Maire (1938)warned that *C. echinata* is a synonym of *C. arvensis*. Maire (1938) further placed *C. murbeckii* as a distinct species based on the broader cauline leaves with less developed teeth, and by the indumentum.

Apart from partial (e.g. Heyn et al. 1974, Meikle 1976, Ohle 1974, 1975 a; 1975b) or regional revision's (Fennane and Ibn Tattou 1998, 2005; Valdés 2002; Dobignard and Chatelain 2011; Oualidi et al. 2012; Gonçalves et al. 2014), no significant revisions on the genus have been undertaken. Most of the earlier taxonomic studies were focused on the limited material available at that time, and shown recurrent discrepancies between the different classification attempts, since, most of the studies were based on the dried material, many times incomplete. Some characters that can be easily observed on living material become unclear in dried herbarium specimens. In particular, the habit, leaf thickness, diameter or colour of the capitula, and shape of the achenes. Moreover, many herbarium specimens, especially in North Africa, lack some important features, such as capitula and/or, most of all, achenes.

Finally, as part of an ongoing project to revise *Calendula*, two floristic treatments, one for the Iberian Peninsula (Silveira & Gonçalves, in press, Gonçalves et al. in press) and Morocco (Gonçalves et al. 2014) have been published. A revision of selected *Calendula* taxa from the SW Mediterranean region (Morocco, Algeria and Tunisia), aiming at (1) analyse the variation between and within taxa, and (2) re-evaluate taxa delimitations, is presented. Several taxonomical changes, including the description of new species/taxa, designation of new combinations, the establishment of new synonyms, and the exclusion of some taxa, are also presented.

Material and methods

Plant material

An intensive survey was conducted by A.C. Gonçalves and P. Silveira in the years 2012–2014, during the spring season, throughout most of the study area (Morocco, Algeria and Tunisia), to collect *Calendula* species and see their characteristics in the field. A total of 32 field collections resulted from this fieldwork, which are given in APPENDIX 1 and are held at the University of Aveiro (AVE). Specimens deposited at ABH, AL (ENSA), ARAN, B, BC (IBB), BCN, BM, BONN, BR, C, COI, E, ELVE, FI, G, GAT, GDA (GDAC), HAL, JACA, JAEN, JE, K, LD, LEB, LISI, M, MA, MACB, MAF, MARK, MGC, MPU, O, P, PO, RAB, RNG, SALA, SANT, SEV, TFMC, US, VAL and W herbaria were also examined. Over 5 000 specimens, including types of most taxa, have been examined. Specimens' information of all collections examined was entered in a BRAHMS (Version 7.9.6 – University of Oxford) database, available on request from the authors.

Morphological studies

Morphological characters of *Calendula* were examined *in situ* and in herbaria material. In each population, 10 randomly selected specimens were sampled. However, at some locations, this number was reduced due to the lower number of samples available. The morphological characters screened are summarised in Table 13 and 14, following Gonçalves et al. (in press), adapted for North African taxa. Each specimen represents an Operational Taxonomic Unit (OTU) in the multivariate analysis (see below).

Data analysis

Morphological data were subjected to one-way analysis of variance to determine the significant difference between taxa for each character measured. For normally distributed and homoscedastic variables, *t*-test (between two groups) or One-way ANOVA (between more than two groups) were performed. When normality and homoscedasticity were not achieved, Mann-Whitney test (between two groups) and Kruskal-Wallis One-way ANOVA on ranks (between more than two groups) were used instead. Some taxa were excluded from the analysis of variance because of the scarcity of available samples (e.g. leave thickness or head diameter). Boxplots containing medians and percentiles of each character within species and subspecies were prepared. The most discriminant characters were used to perform the multivariate analysis and to build the identification keys. Principal component analysis (PCA) was used to evaluate the morphological variation among taxa. All statistical analyses were performed using the R programme (R Development Core Team 2010), except for PCA, which was performed with StatistiXL (Broadway – Nedlands, AUS).

Mapping and conservation status assessment

Map distributions of each taxon were plotted using QGIS 2.18.4 (QGIS 2017), available in http://qgis.org, based on field and herbarium collection. Species richness distribution was assessed in a grid cell of 0.3º × 0.3º.

The conservation status assessments followed the IUCN Red List Categories and Criteria procedure (IUCN 2012), available in http://www.iucnredlist.org/, where any taxa can be classified in one of seven categories: Extinct (EX), Extinct in the Wild (EW), Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Near Threatened (NT), Least Concern (LC) or Data Deficient (DD). The extent of occurrence (EOO) and area of occupancy (AOO) were calculated for each taxon to evaluate their status based on geographic coordinates, using the software GeoCAT (Geospatial Conservation Assessment Tool), available in http://geocat.kew.org/.

Taxonomic concept

The species concept used in the present work is Genotypic Cluster or Genomic Cluster Species Concept (GCSC), in line with that previously employed in the Iberian Peninsula (Gonçalves et al. 2017). Species of *Calendula* are defined as groups of individuals that form genetic (chromosome numbers, ploidy level, and genome size) or morphological clusters with few or no intermediates (Mallet 1995). Hybridisation and/or introgression may occur when closely related species meet occasionally. Species will differ from each other in a few, distinct characters, but intergrade in areas where their distribution and habitats overlap. The “75% rule” that defines the criteria for subspecies classification was also applied (Amadon 1949).

Table 13 – Quantitative characters of both vegetative and reproductive structures used in this study.

Characters	Abbreviation	Unit/Scale
Length of the longest branch	LB	cm
Basal leaf length	LL	cm
Basal leaf width	LW	cm
Basal leaf distance from base to point of greatest width	LD	cm
Ratio leaf length/point of greatest width	R1	ratio
Ratio leaf length/width	R2	ratio
Basal leaf thickness	LT	mm
Head diameter	HD	cm
Involucre length	IL	mm
Ligule length	LG	mm
Ratio ligule/involucre	R3	ratio
Sub-exalate achene length	SEL	mm
Sub-exalate achene width	SEW	mm
Rostrate achene length	RL	mm
Rostrate achene width	RW	mm
Bialate achene length	BL	mm
Bialate achene width	BW	mm
Bialate achene rostrum length	BR	mm
Trialate achene length	TL	mm
Trialate achene width	TW	mm
Cymbiform achene length	CL	mm
Cymbiform achene width	CW	mm
Sub-cymbiform achene length	SCL	mm
Sub-cymbiform achene width	SCW	mm
Vermiculate-alate achene length	VAL	mm
Vermiculate-alate achene width	VAW	mm
Vermiculate-exalate achene length	VEL	mm
Vermiculate-exalate achene width	VEW	mm

Table 14 – Qualitative characters of both vegetative and reproductive structures used in this study.

Characters	Characters states	Abbreviation
Life cycle	(1) annual; (2) annual to short-lived perennial (3) perennial	LF
Stem pubescence	(1) mostly glandular; (2) glandular pubescent; (3) mostly white-arachnoid; (4) densely white-arachnoid	SP
Leaf shape	(1) linear to oblanceolate; (2) oblanceolate to spatulate (3) oblanceolate to obovate	LS
Leaf apex	(1) acuminate; (2) acute; (3) obtuse	LA
Leaf margins	(1) entire to sub-entire; (2) sinuate-dentate; (3) sinuate-dentate with acute teeth (4) sinuate-dentate with acute teeth to ± irregularly pinnatifid; (5) deeply sinuate-dentate to ± irregularly pinnatipartite	LM
Leaf lamina pubescence	(1) mostly glandular; (2) glandular pubescent; (3) mostly white-arachnoid; (4) densely white-arachnoid	LP
Sub-exalate achene		
ventral wings	(0) absent; (1) < than lateral; (2) sub-equal; (3) > than lateral	SEvw
lateral wings	(0) absent; (1) sub-entire; (2) sinuate-dentate; (3) incised; (4) deeply pinnately cut	SElw
Rostrate achene		
ventral tooth	(0) absent; (1) apical; (2) basal; (3) both	Rvt
Bialate achene		
lateral wings	(0) absent; (1) sub-entire; (2) sinuate-dentate; (3) incised; (4) deeply pinnately cut	Blw
dorsal wings	(0) absent; (1) < 2 mm straight; (2) > 2 mm bent towards the ventral face	Bdw
Trialate achene		
ventral wings	(0) absent; (1) < than lateral; (2) sub-equal; (3) > than lateral	Tvw
lateral wings	(0) absent; (1) sub-entire; (2) sinuate-dentate; (3) incised; (4) deeply pinnately cut	Tlw
dorsal wings	(0) absent; (1) < 2 mm straight; (2) > 2 mm bent towards the ventral face	Tdw
Cymbiform achene		
ventral wings	(0) absent; (1) < than lateral; (2) sub-equal; (3) > than lateral	Cvw
dorsal wings	(0) absent; (1) < 2 mm straight; (2) > 2 mm bent towards the ventral face	Cdw
ventral tooth	(0) absent; (1) apical; (2) basal; (3) both	Cvt
Sub-cymbiform achene		
ventral wings	(0) absent; (1) < than lateral; (2) sub-equal; (3) > than lateral	SCvw
lateral wings	(0) absent; (1) sub-entire; (2) sinuate-dentate; (3) incise; (4) deeply pinnately cut	SClw
dorsal wings	(0) absent; (1) < 2 mm straight; (2) > 2 mm bent towards the ventral face	SCdw
Vermiculate-alate achene		
Shape	(0) absent; (1) circular to hemicyclic; (2) hook-shaped; (3) falcate	Vas
ventral tooth	(0) absent; (1) apical; (2) basal; (3) both	VAvt
Vermiculate-exalate achene		
Shape	(0) absent; (1) circular to hemicyclic; (2) hook-shaped; (3) falcate	VEs
ventral tooth	(0) absent; (1) apical; (2) basal; (3) both	VEvt

Results

Analysis of variance

The analysis of variance performed on various quantitative data, for both species and subspecies, showed significant differences ($P < 0.001$) (Table 15 and 16). The highest variation estimated from the ratio basal leaves length/width for species, and rostrate achenes length for *C. suffruticosa* subspecies, result from the existence of a high degree of variability on these traits and the same can be used for differentiating some of the taxa. The dimensions of the sub-exalate and sub-cymbiform achenes were only determined for those taxa that produce such achenes, i.e. the subspecies of *C. suffruticosa*. Boxplots showing the variability of the quantitative characters used are presented in Figure 50 to 57. Characters that appear in a single taxon were not represented in a boxplot, but are provided in APPENDIX 2.

Table 15 – Univariate statistical analysis of *Calendula* species from Morocco

Morphological trait	**d.f.**	**statistical test**	***P***
Length of the longest branch	8	H = 31.807	0.001
Basal leaf length	8	H = 18.089	0.021
Basal leaf width	8	H = 115.664	0.001
Basal leaf distance from base to point of maximum width	8	H = 48.283	0.001
Ratio leaf length/point of maximum width	8	H = 106.276	0.001
Ratio leaf length/width	8	H = 164.821	0.001
Basal leaf thickness	7	H = 33.314	0.001
Head diameter	7	H = 19.924	0.001
Rostrate achene length	8	H = 117.545	0.001
Rostrate achene width	8	H = 94.078	0.001
Bialate achene length	4	H = 54.990	0.001
Bialate achene width	4	H = 23.224	0.001
Bialate achene rostrum	2	H = 42.066	0.001
Trialate achene length	5	H = 114.271	0.001
Trialate achene width	5	H = 97.790	0.001
Cymbiform achene length	3	H = 52.520	0.001
Cymbiform achene width	3	H = 55.599	0.001
Vermiculate-alate achene length	2	H = 8.085	0.018
Vermiculate-alate achene width	2	H = 10.378	0.006
Vermiculate-exalate achene length	8	H = 88.167	0.001
Vermiculate-exalate achene width	8	H = 101.541	0.001

Kruskal-Wallis One-way ANOVA *H* for characters with non-normal distributions. d.f. – degrees of freedom.

Table 16 – Univariate statistical analysis of *C. suffruticosa* subspecies from Morocco

Morphological trait	**d.f.**	**statistical test**	***P***
Length of the longest branch	14	H = 97.302	0.001
Basal leaf length	14	H = 103.940	0.001
Basal leaf width	14	H = 110.291	0.001
Basal leaf distance from base to point of maximum width	14	H = 102.958	0.001
Ratio leaf length/point of maximum width	14	F = 7.889	0.001
Ratio leaf length/width	14	H = 94.675	0.001
Basal leaf thickness	14	H = 115.970	0.001
Head diameter	14	H = 81.710	0.001
Rostrate achene length	14	H = 144.709	0.001
Rostrate achene width	14	H = 135.726	0.001
Bialate achene length	12	F = 27.738	0.001
Bialate achene width	14	H = 75.194	0.001
Bialate achene rostrum	14	H = 70.779	0.001
Trialate achene length	14	H = 69.559	0.001
Trialate achene width	14	H = 58.950	0.001
Cymbiform achene length	6	F = 49.791	0.001
Cymbiform achene width	13	H = 70.350	0.001
Sub-cymbiform achene length	14	H = 45.250	0.001
Sub-cymbiform achene width	2	F = 529.509	0.001
Vermiform alate achene length	14	H = 34.945	0.004
Vermiform alate achene width	14	H = 42.059	0.001
Vermiform exalate achene length	14	H = 87.807	0.001
Vermiform exalate achene width	14	H = 62.936	0.001

One-way ANOVA: *F* for characters with normal distributions. Kruskal-Wallis One-way ANOVA on ranks: *H* for characters with non-normal distributions. d.f. – degrees of freedom.

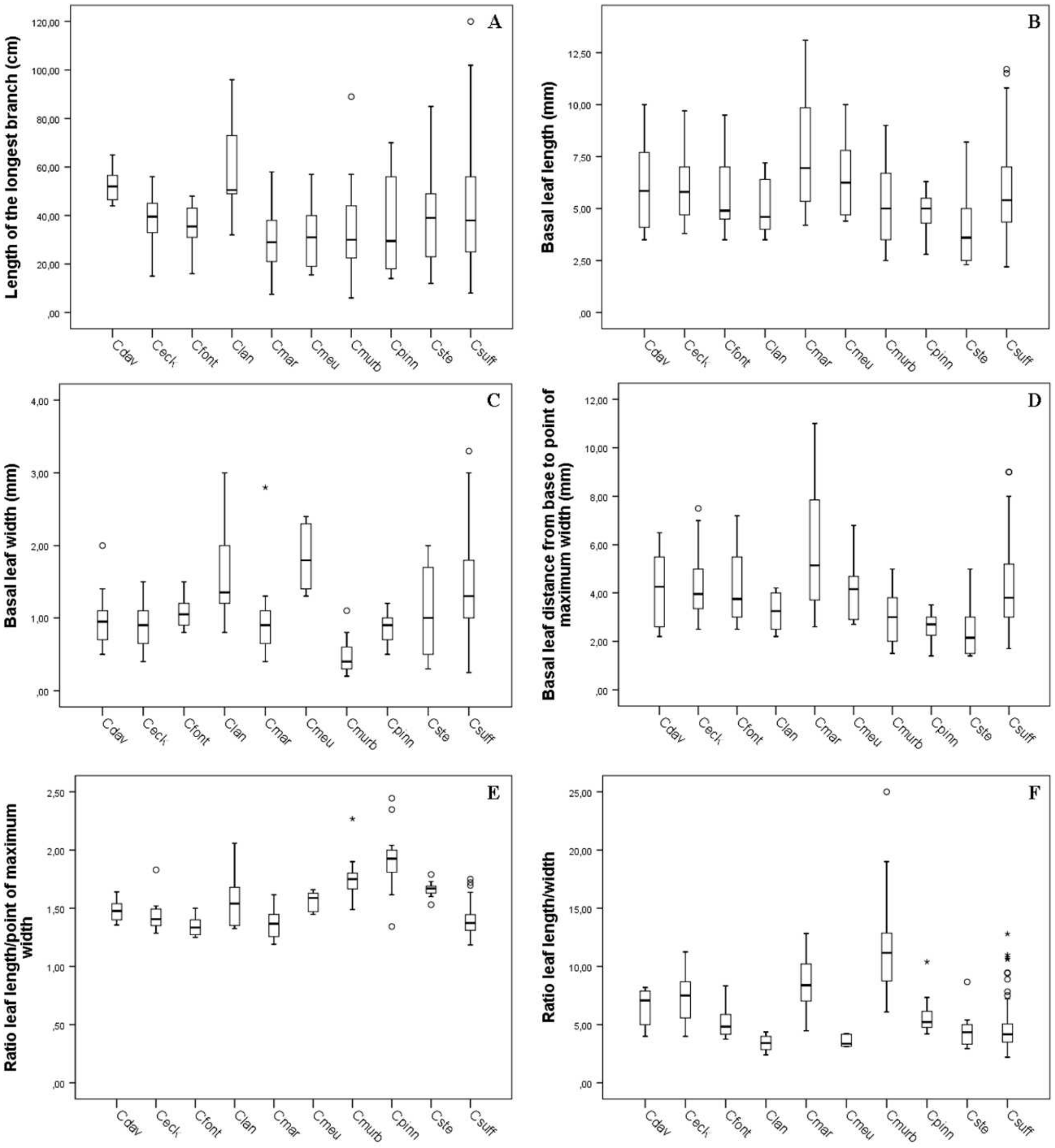


Figure 50 – Boxplots representing the variability of quantitative characters in *Calendula* species. Median (lines), 25%-75% (boxes), minimum and maximum values (whiskers), outliers (small circles). Different letters reveal statistical differences at $P < 0.05$.

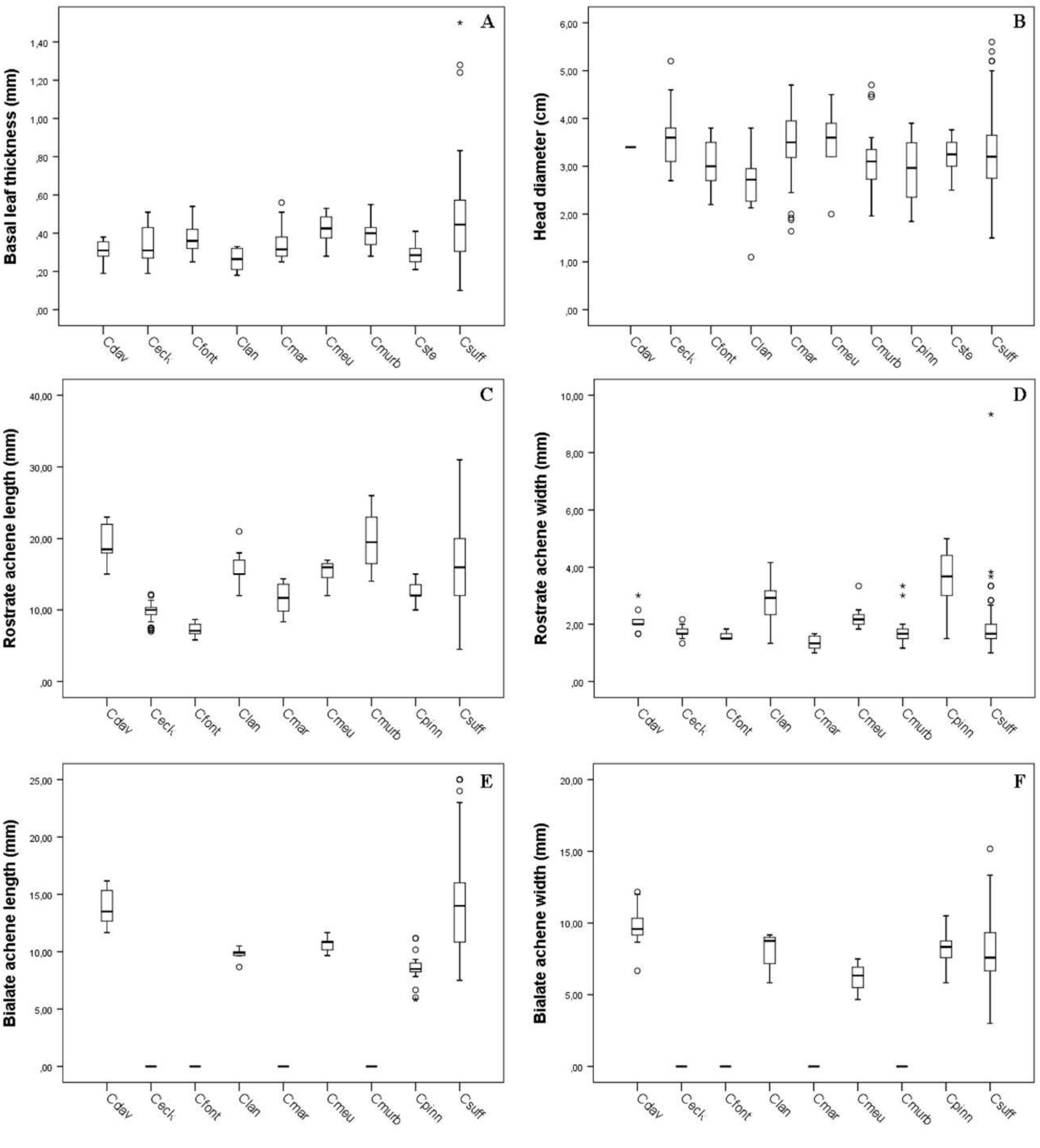


Figure 51 – Boxplots representing the variability of quantitative characters in *Calendula* species. Median (lines), 25%-75% (boxes), minimum and maximum values (whiskers), outliers (small circles). Different letters reveal statistical differences at $P < 0.05$.

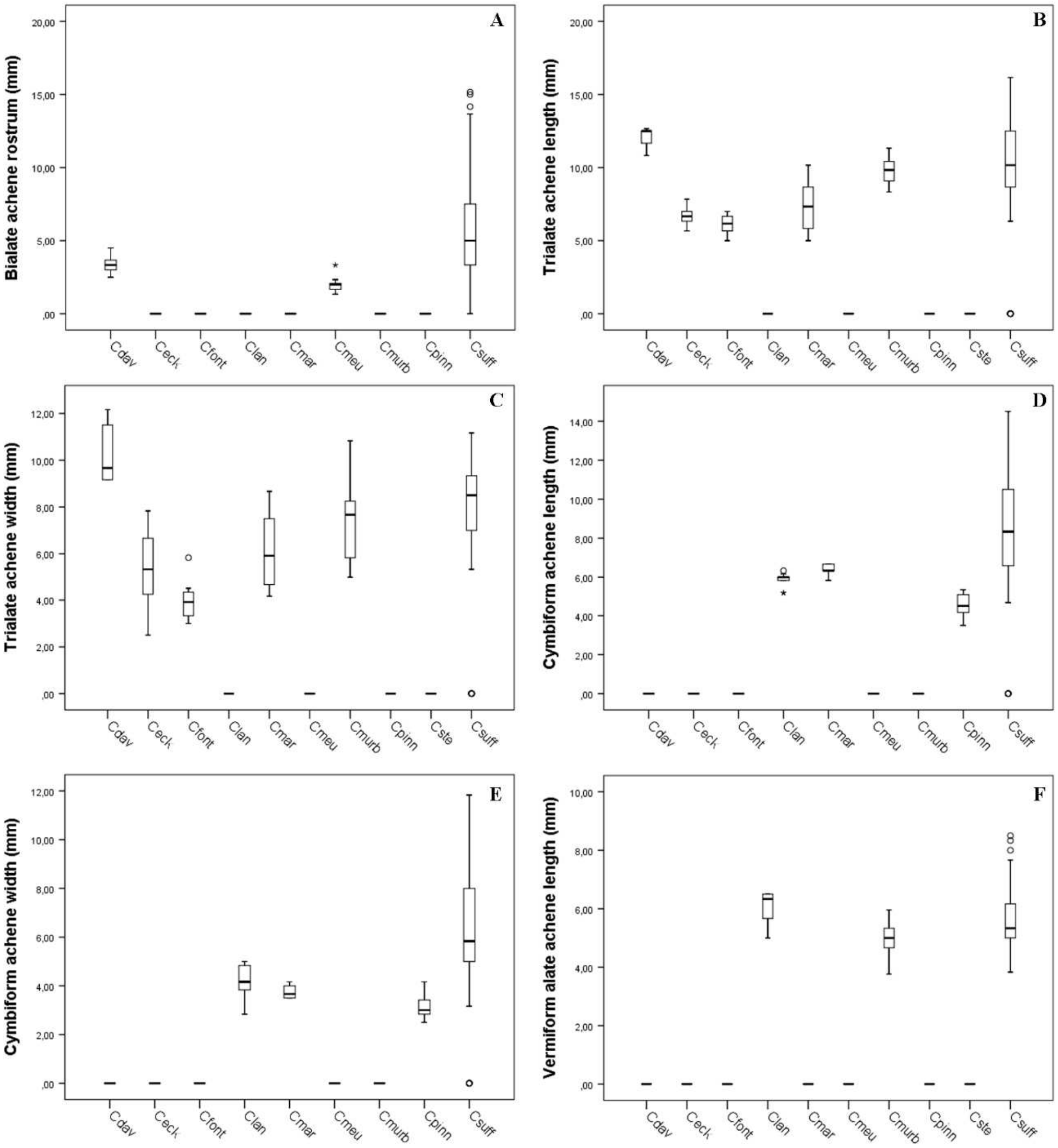


Figure 53 – Boxplots representing the variability of quantitative characters in *Calendula* species. Median (lines), 25%-75% (boxes), minimum and maximum values (whiskers), outliers (small circles). Different letters reveal statistical differences at $P < 0.05$.

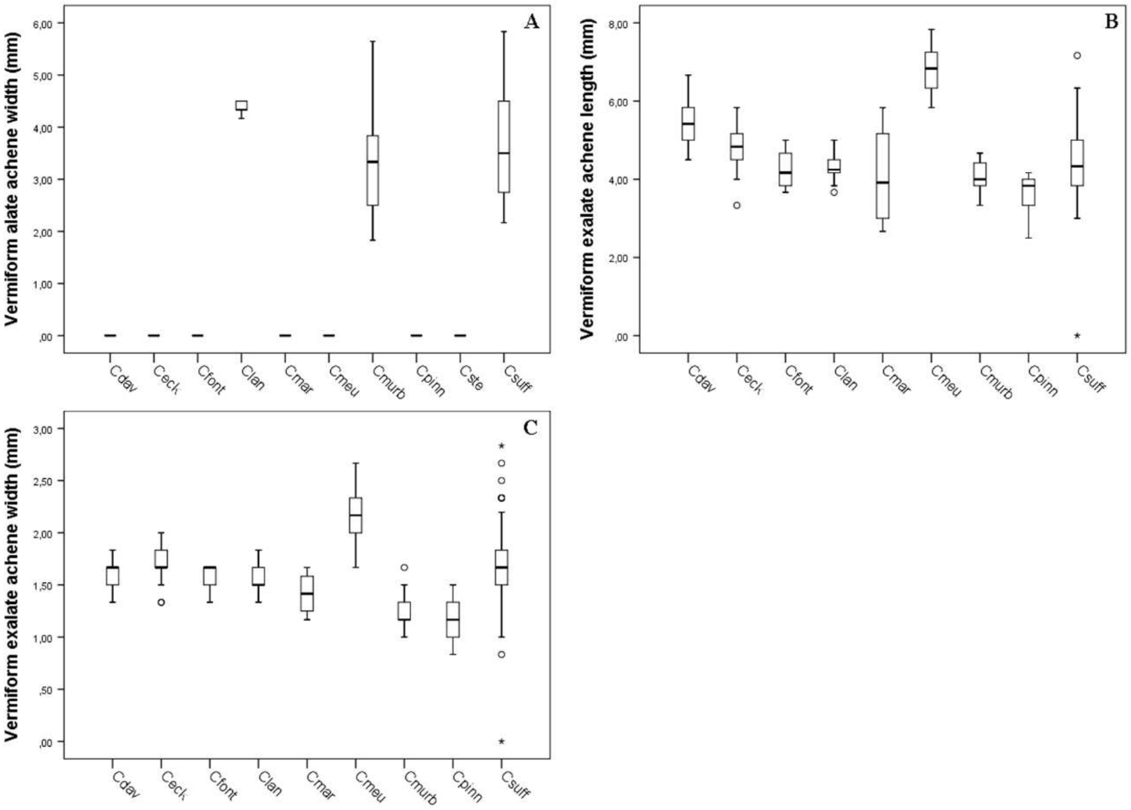


Figure 54 – Boxplots representing the variability of quantitative characters in *Calendula* species. Median (lines), 25%-75% (boxes), minimum and maximum values (whiskers), outliers (small circles). Different letters reveal statistical differences at $P < 0.05$.

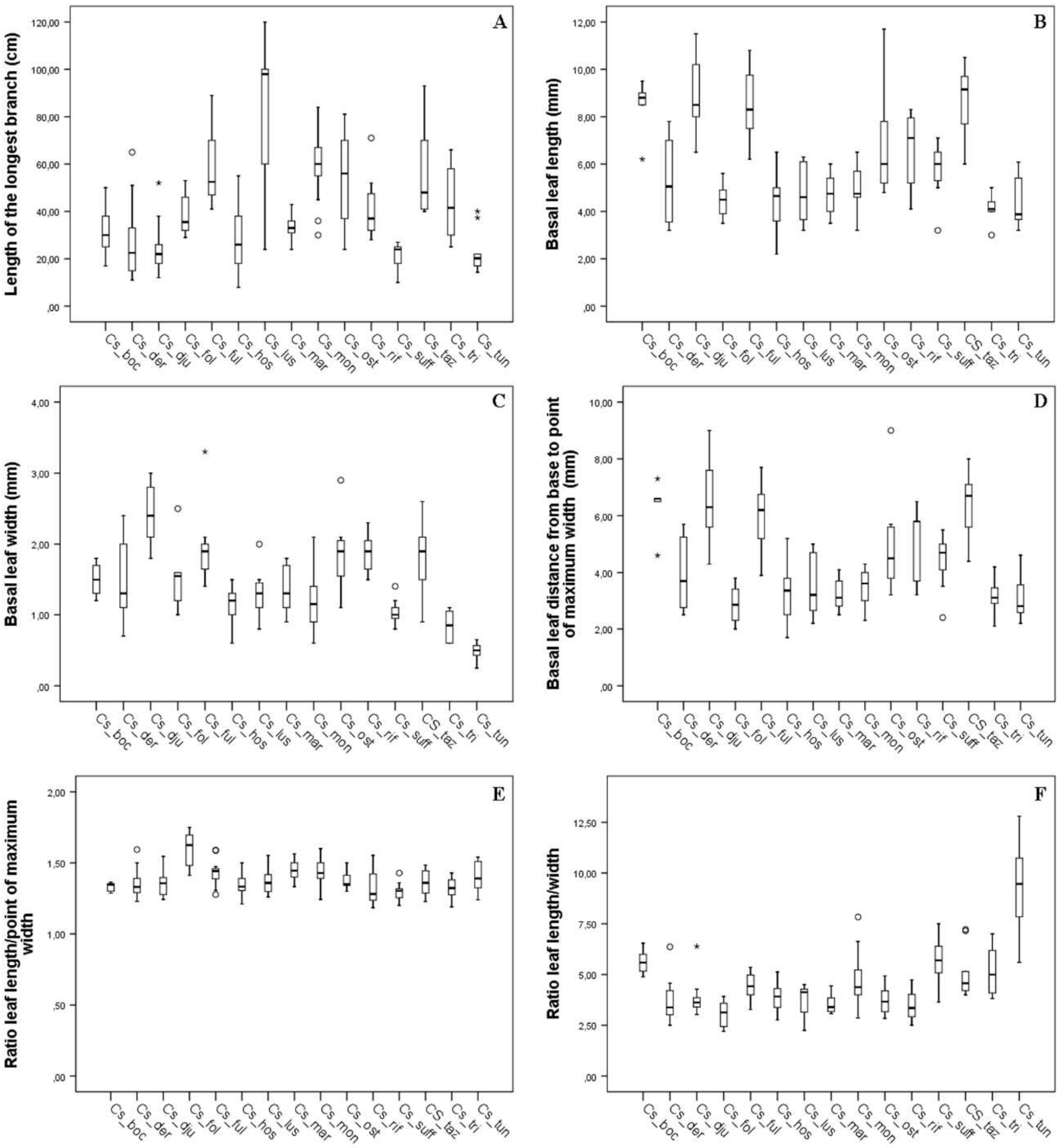


Figure 55 – Boxplots representing the variability of quantitative characters in *C. suffruticosa* taxa. Median (lines), 25%-75% (boxes), minimum and maximum values (whiskers), outliers (small circles). Different letters reveal statistical differences at $P < 0.05$.

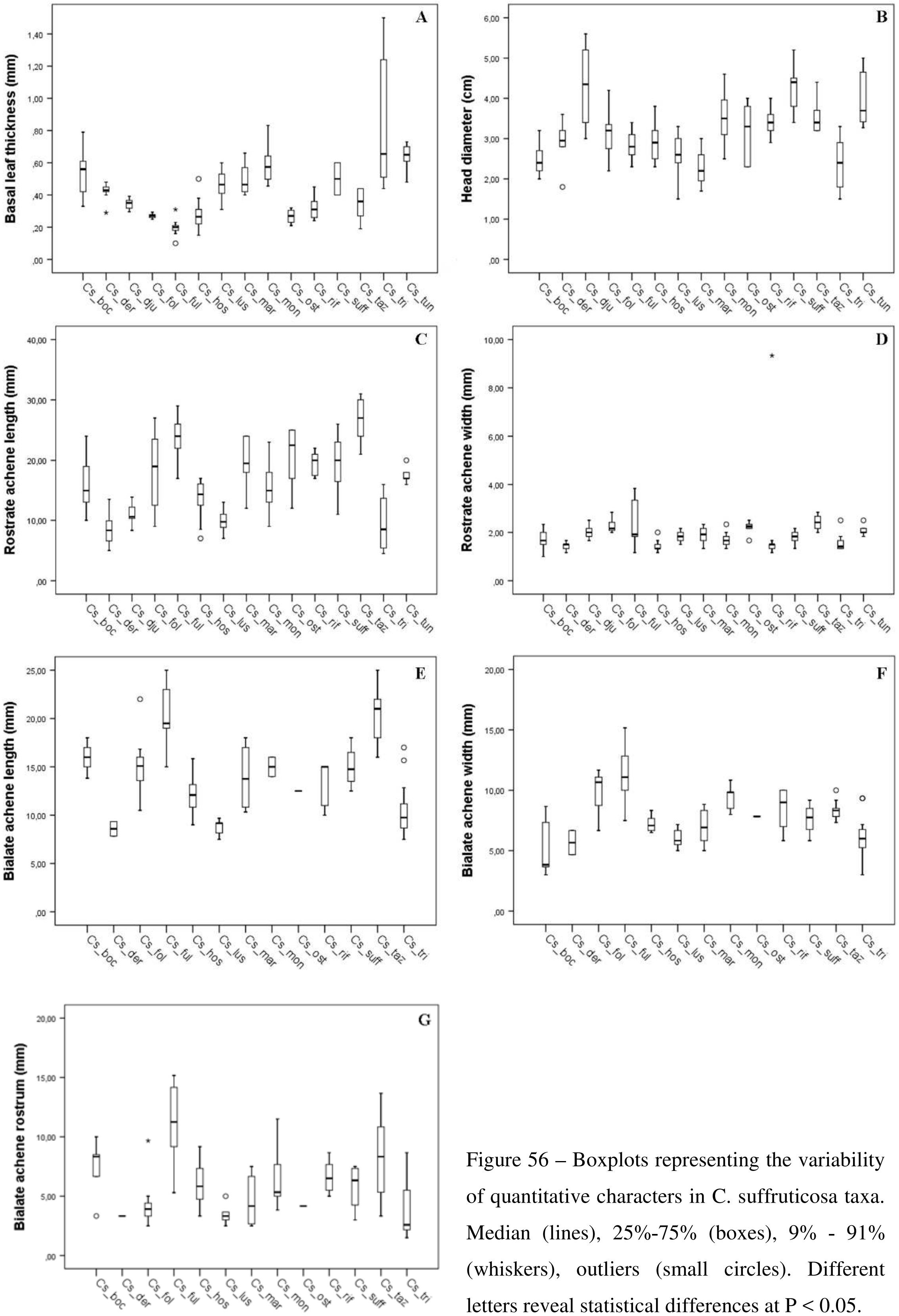


Figure 56 – Boxplots representing the variability of quantitative characters in C. suffruticosa taxa. Median (lines), 25%-75% (boxes), 9% - 91% (whiskers), outliers (small circles). Different letters reveal statistical differences at P < 0.05.

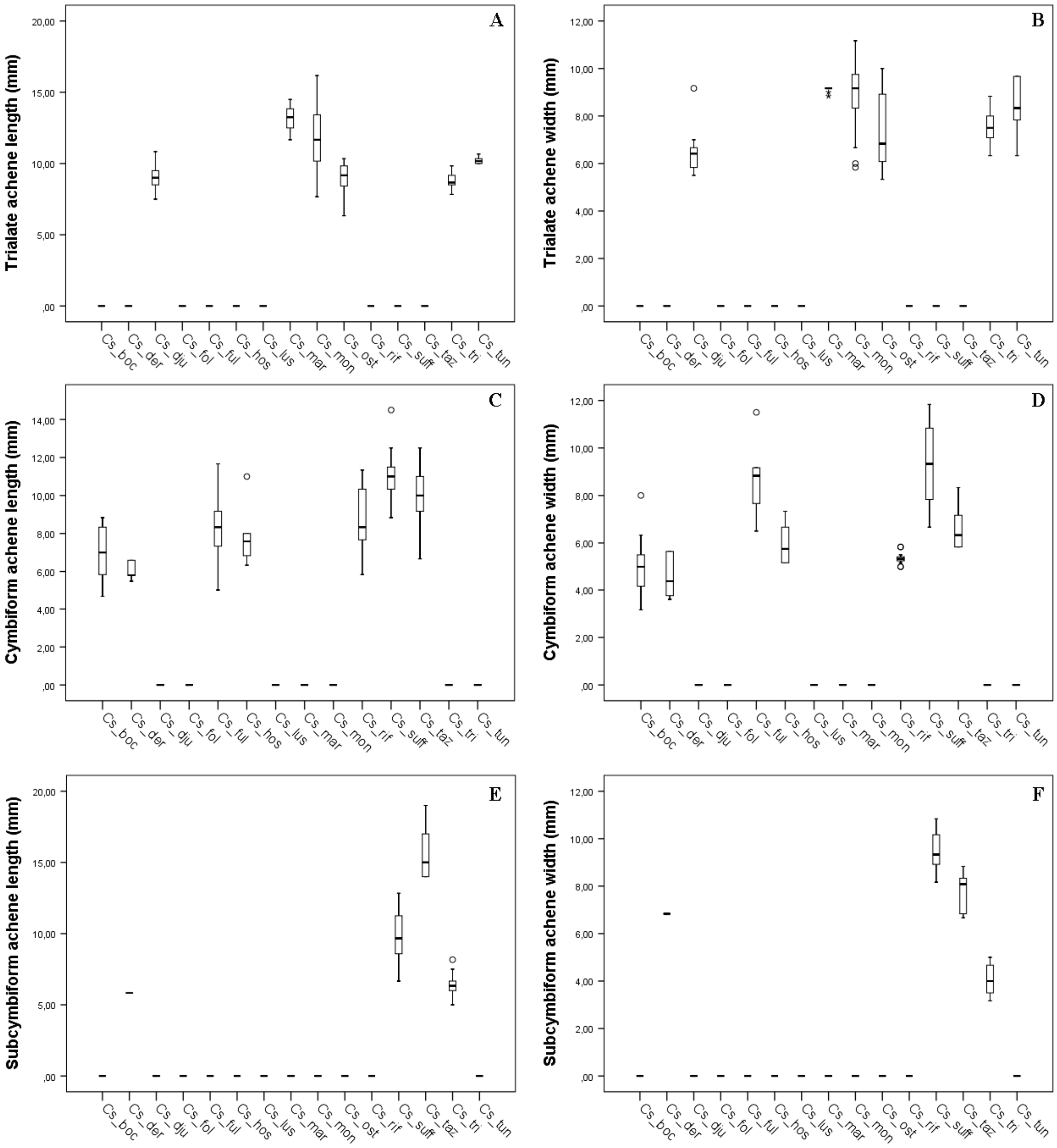


Figure 57 – Boxplots representing the variability of quantitative characters in *C. suffruticosa* taxa. Median (lines), 25%-75% (boxes), minimum and maximum values (whiskers), outliers (small circles). Different letters reveal statistical differences at $P < 0.05$.

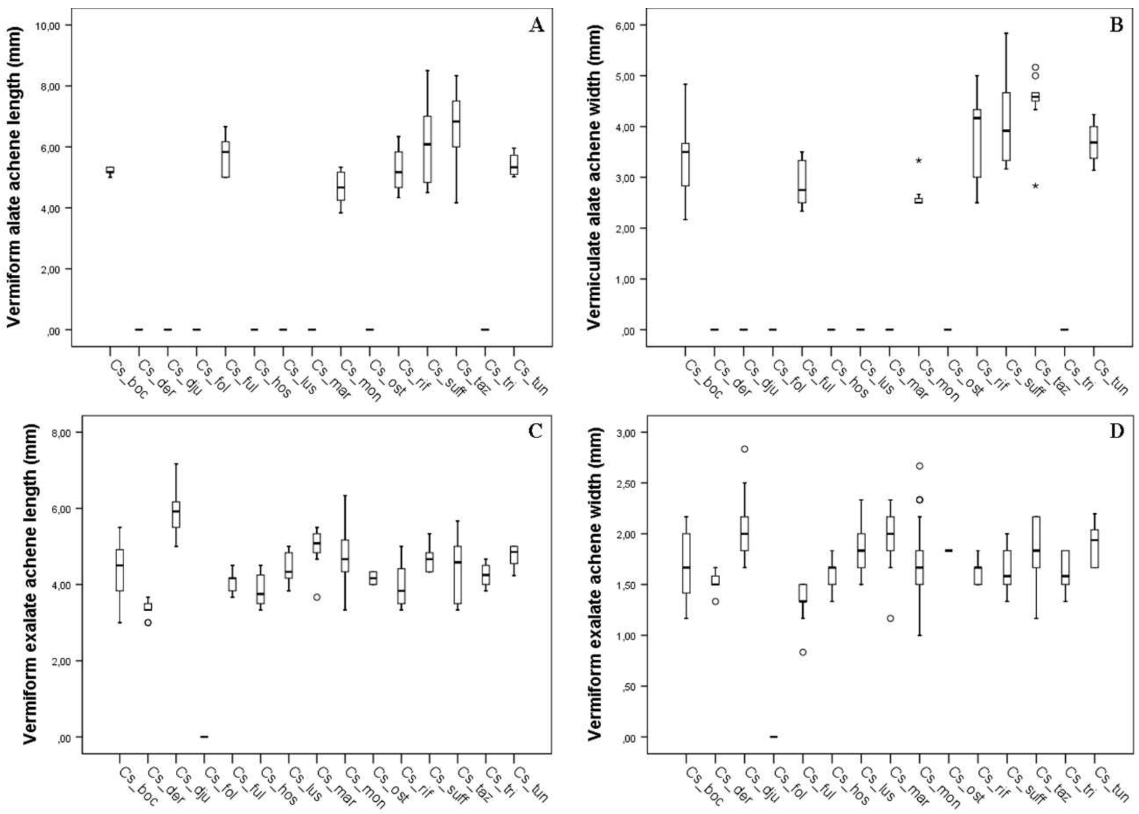


Figure 58 – Boxplots representing the variability of quantitative characters in *C. suffruticosa* taxa. Median (lines), 25%-75% (boxes), minimum and maximum values (whiskers), outliers (small circles). Different letters reveal statistical differences at $P < 0.05$.

Principal component analysis

The most complex and difficult to classify groups of *Calendula* taxa were analysed separately: (1) *Calendula maroccana* group, taxa with 2n = 18 and (2) *Calendula suffruticosa* group, taxa with 2n = 32.

1) In the PCA of *Calendula maroccana* group, 32.38% of the variance was accounted for by the first principal component, followed by 20.64% for the second and 8.85% for the third principal component (APPENDIX 3, Figure 59). The characters LF, VEs, TL, VAs, VAvt and Cvt, that significantly loaded on the first component. For the second component, the highest loadings included Tlw, R2, TL, LW, BL and Blw. The PCA of *Calendula maroccana* group (Figure 59) displays a good separation between eight species, mainly distinguished by different achene morphologies; *C. lanzae* and *C. pinnatiloba*, both annual to

short-lived perennial, form two distinct clusters separated from the remaining species. Although they are in very close geographic regions, they can be differentiated from each other by the basal leaf margin, bialate and cymbiform achenes. Furthermore, the nuclear DNA content among these taxa are different, 1.85 ± 0.08 pg and 2.09 ± 0.15 pg, respectively (Table 17); *C. murbeckii* has trialate achenes with wings deeply pinnately cut into segments with truncate apices, which is easy to distinguish from other species; the species *C. maroccana*, *C. eckerleinii*, *C. davisii, C. fontquerii* and *C. meuselii* partially grouped on the positive part of axis 2, which tends to be correlated with the life cycle, but also with vermiculate achenes; *C. meuselii* was well separated along the negative side of PC2, due to basal leaf, stem pubescence and bialate achenes. *C. maroccana* and C. murbeckii appear in the positive section of PC2 mostly due to the presence of trialate achenes.

2) In the PCA of *C. suffruticosa* taxa 48.3% of the variance was accounted for the three first axis, 23.6% for the first component, followed by 13.2% for de second component and 11.5% for the third principal component (APPENDIX 3, Figure 60). The characters TL, TW, Tvw, CL, CW and R3 were found influential for the first component. These characters allow us to separate the *C. suffruticosa* group in two subgroups: taxa with and without trialate achenes. Therefore, PCA2 distinguished taxa mainly by pubescence and vermiculate achenes, only a few taxa separate from the others. For the PCA of *C. suffruticosa* taxa with trialate achenes, the first component accounted for 29.08% of the variations observed, while the second component accounted for 21.4% of the variations (Figure 61). Thus, the three components show a cumulative of 66.42% of the variance. The characters that significantly loaded on the first component were LT, TW, VAW, LD, LA and Blw. For the second component, the characters which showed a high loading included LS, Tvw, Tlw, R1, R2 and SP. For the PCA of *C. suffruticosa* taxa without trialate achenes, the first principal component accounts for 25.65% of the variation, the second component for 17.56%, and the third component for 13.09% for a total of 56.3% (Figure 62). The following

variables load heavily on the first component variables: VAL, VAW, Vas, SEL, SElw and VEs.

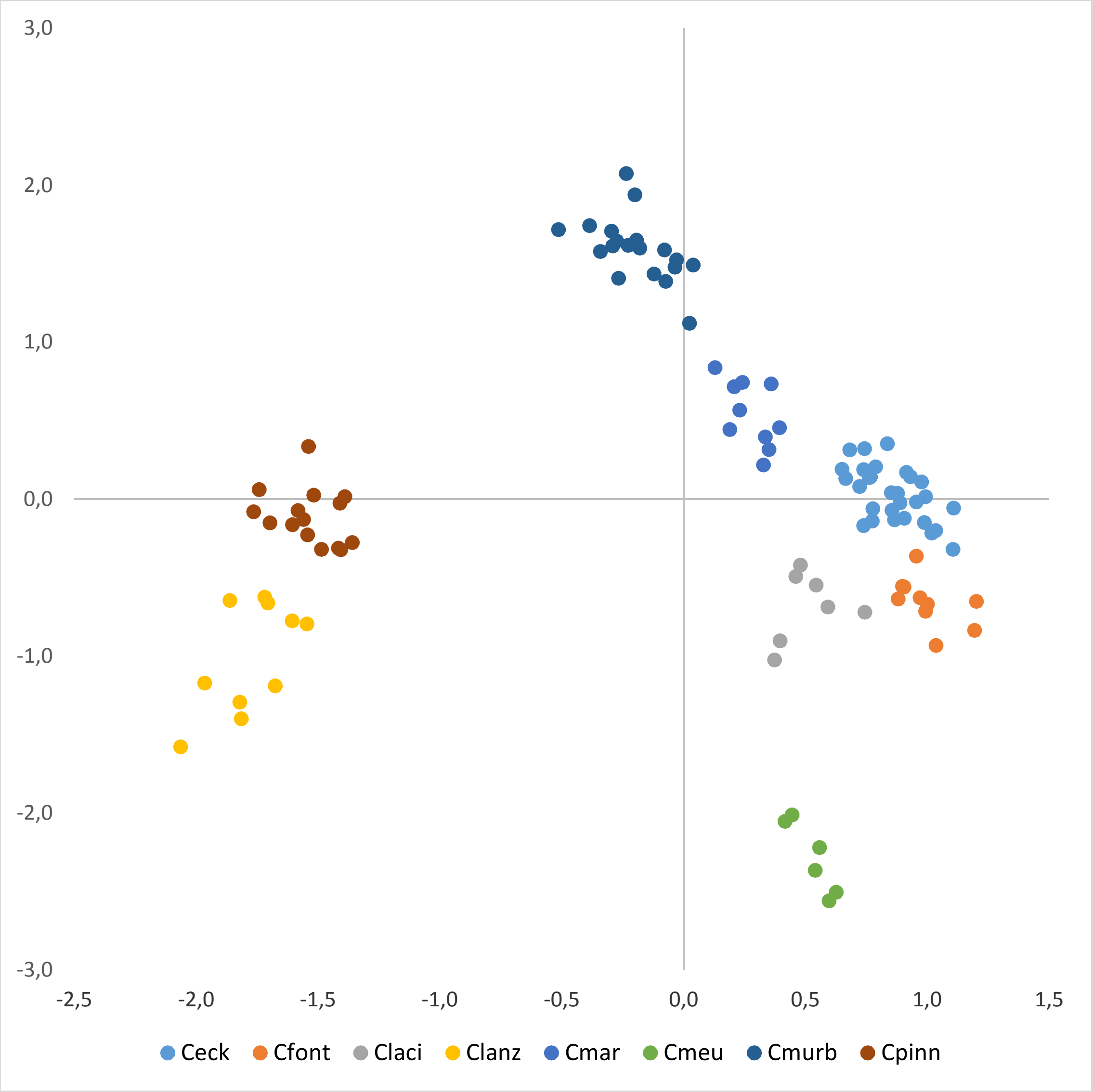


Figure 59 – Two-dimensional ordination diagram of PC 1x2 based on 34 morphological characters of 107 specimens from *Calendula maroccana* group.

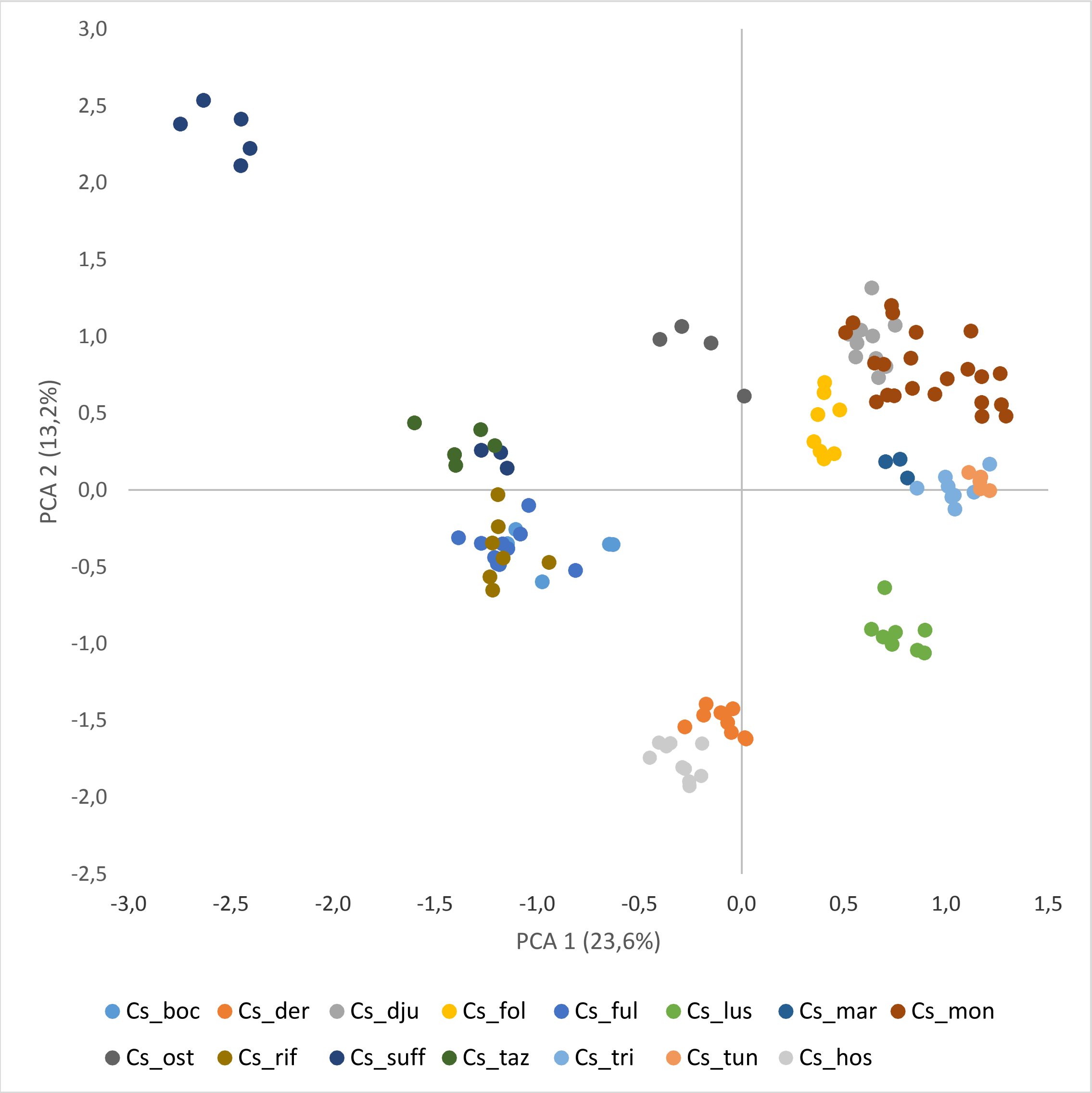


Figure 60 – Two-dimensional ordination diagram of PC 1x2 based on 49 morphological characters of 125 specimens from *C. suffruticosa* group.

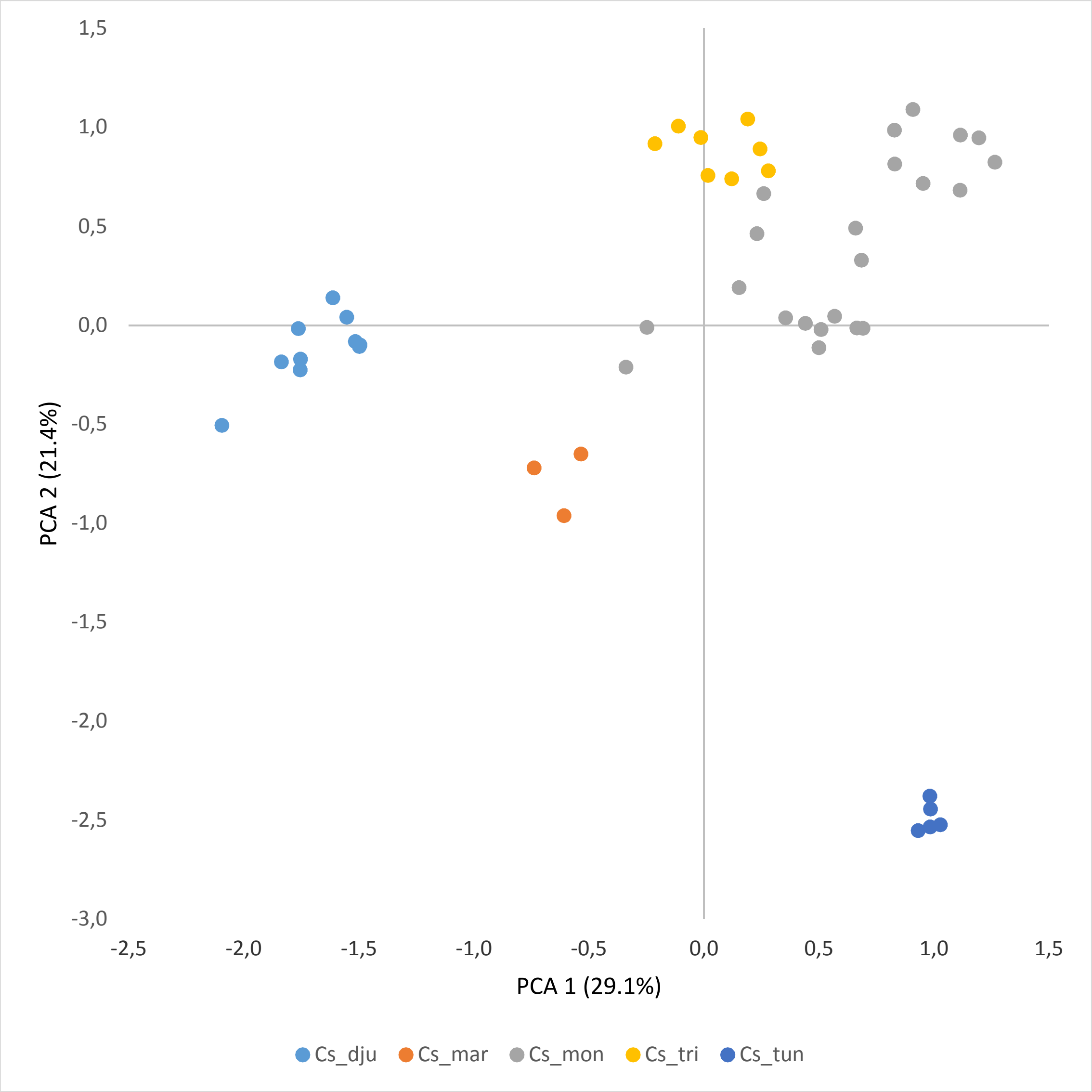


Figure 61 – Two-dimensional ordination diagram of PC 1x2 based on 31 morphological characters of 49 specimens from *C. suffruticosa* taxa with trialate achenes.

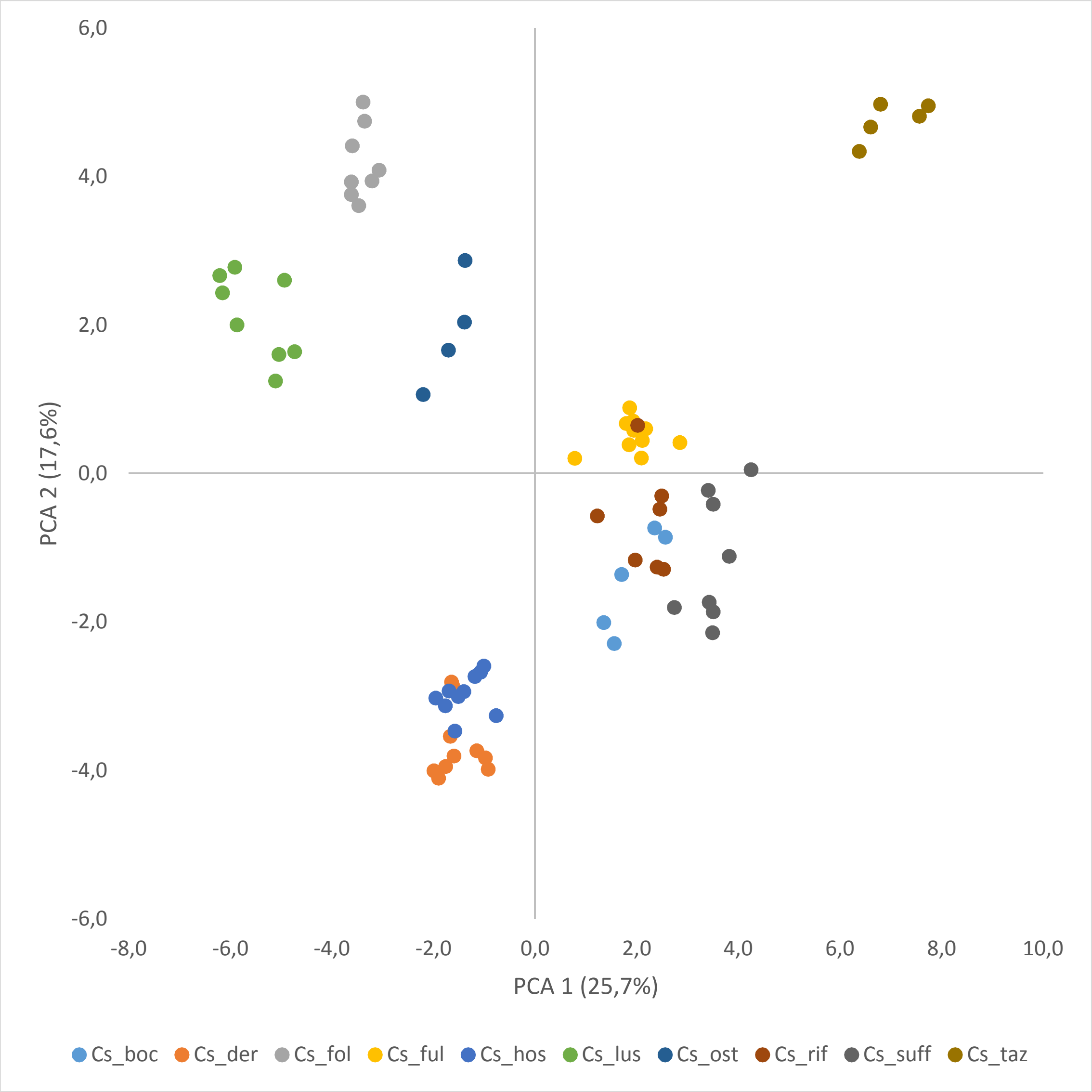


Figure 62 – Two-dimensional ordination diagram of PC 1x2 based on 44 morphological characters of 76 specimens from *C. suffruticosa* taxa with non-trialate achenes.

Taxonomical implications for Calendula species

The statistical analysis of quantitative characters showed significant differences among the *Calendula* studied, which allows identification of those quantitative characters that distinguish taxa. Thus, these characters can be taxonomically useful.

Our results showed that the morphological characters provide enough support to identify different taxa within the genus *Calendula.* Based on achene's morphology, especially the bialate and trialate achenes, the *Calendula maroccana* group can be divided into eight independent species. In addition, the life form and the basal leaf characters contributed significantly to the grouping. These characters were used in the identification key for the species of *Calendula* provided in the taxonomic treatment section. In the step 5, *C. murbeckii* is distinguished from the other *C. maroccana* annuals group, by having trialate achenes with lateral wings deeply pinnately cut into segments with truncate apices. The basal leaves of *C. lanzae* and *C. pinnatiloba* are distinct and unique, pinnatifid and not pinnatifid with the margin repand-dentate with the teeth more or less prominent, respectively. This character were used in step 6. The margin of the basal leaves (step 9 and 10) and the presence or absence of cymbiform achenes were also used to distinguish the perennial *C. maroccana* group.

For the *Calendula suffruticosa* group, the major delimiting characters for taxa were trialate and cymbiform achenes. The taxa with these characters were separated into two groups: trialate achenes and non-trialate achenes, to perform separate PCAs, which allowed a more detailed analysis. The *C. suffruticosa* taxa with trialate achenes separate very well the five taxa analysed. The subsp. *tunetana* is the only taxa from this group with linear to narrowly oblanceolate basal leaves (step 2 of key for *C. suffruticosa*). Normally, the rostrate achenes in *C. suffruticosa* taxa are straight, without dorsal spines, however in this group the rostrate achenes of subsp. *rirensis* are slightly curved, forming an angle of approx. 90º and with spines 0.5-1.5 mm on the dorsal face. The bialate and trialate achenes were also used in steps 5, 6 and 7. The *C. suffruticosa* subsp. *monardii* reveal to be the most variable taxon within this group. On the contrary, one of the subspecies of the *C. suffruticosa* taxa with non-trialate achenes; subsp. *riffiniensis* appeared with some overlapping with subsp. *fulgida* and subsp. *boccoyana.* Nonetheless, some qualitative morphological characters important to distinguish these subspecies did not have a significant impact on the analysis. Actually, in these cases, the plants are from geographically separated regions and characters allowing a clear distinction were identified based on a careful study of the specimens, as can be seen in the key prepared for the *C. suffruticosa* group. Results of the statistical analysis indicate that these taxa did not show more than 75% of overlap and we accepted the subspecies classification under the “75% rule”.

The achene’s morphology was found significant in the grouping of different taxa. Gonçalves et al. (2017) also made similar observations where they emphasised the importance of achene’s morphology. The present study also reflected the importance of the nuclear DNA content, which can be used as a supporting aid to morphological characters.

Geographic distribution, endemism and ecology

The SW Mediterranean is the most important centre of diversity and speciation of the genus C*alendula* (Norlindh 1977). Morocco host the highest diversity of species, with 8 of 15 recognised species, which grow in a variety of habitats, from sea level to above 3000 m of elevation in the High Atlas Mountains (Figure 63 to 65). The distribution of endemic species in the study area reveals that most of them are unique, particularly in Morocco. It is furthermore interesting that a high concentration of endemic species is found along the Atlas Mountains. *C. eckerleinii*, *C. davisii* and *C. meuselii* inhabiting the Medium Atlas, *C. maroccana* and *C. murbeckii* in the High Atlas, and the *C. lanzae* and *C. pinnatiloba* in the Anti-Atlas Mountains, while *C. fontquerii* occupying the Mediterranean littoral (Figure 63).

C. suffruticosa comprises 16 infraspecific taxa, eight from Morocco (subsp.: *boccoyana, dercana, fulgida, hosmarensis, lusitanica, marsea, osteni, riffiniensis, suffruticosa,* and *tazzea*), six from Algeria (subsp.: *balansae, boissieri, djurdjurensis, foliosa, monardii* and *tlemcensis*), and two from Tunisia (subsp.: *suffruticosa* and *tunetana*). Most of them occur near the sea from 0 to 200 m of elevation, only a few taxa occurs inland (e.g. subsp. *riffiniensis*, *tazzea* or *djurdjurensis*).

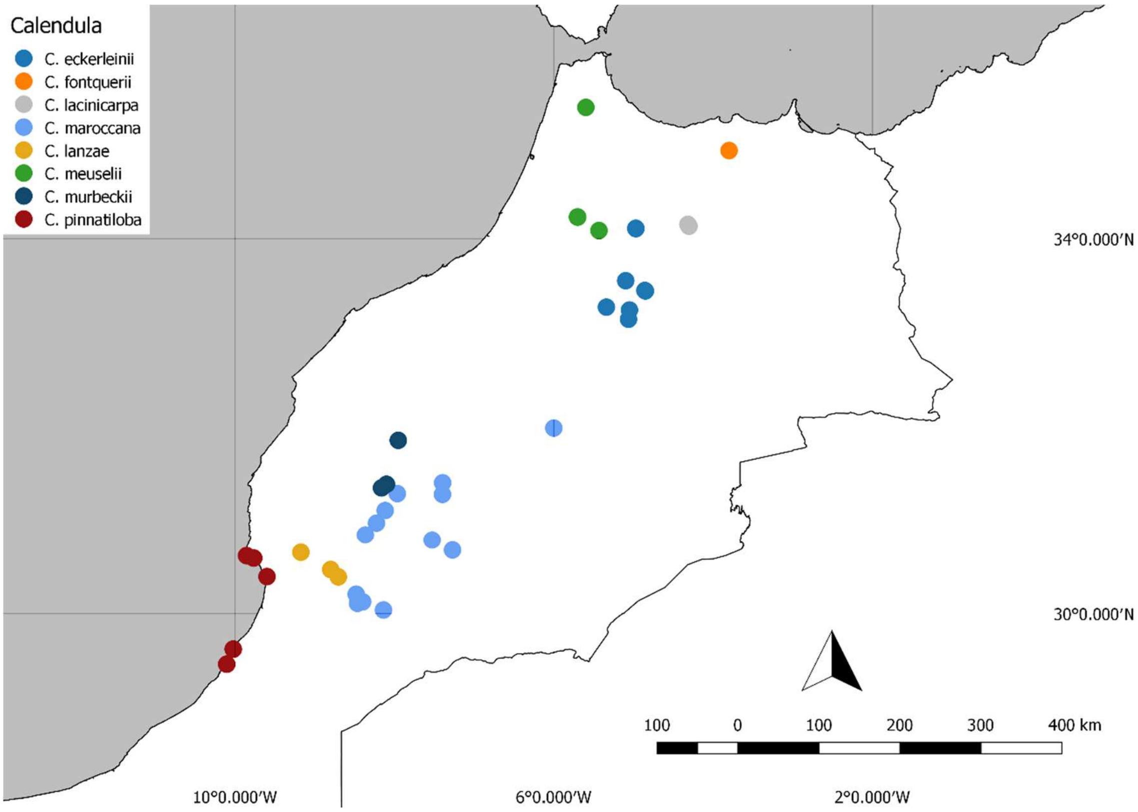
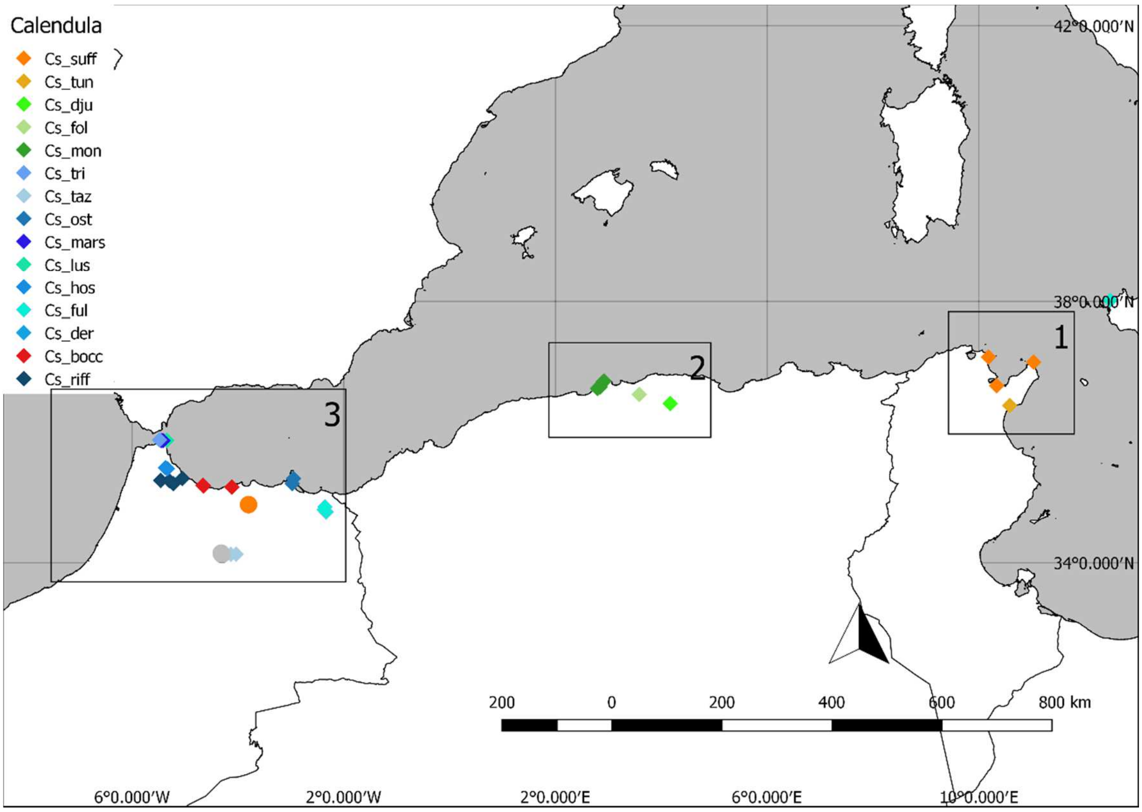
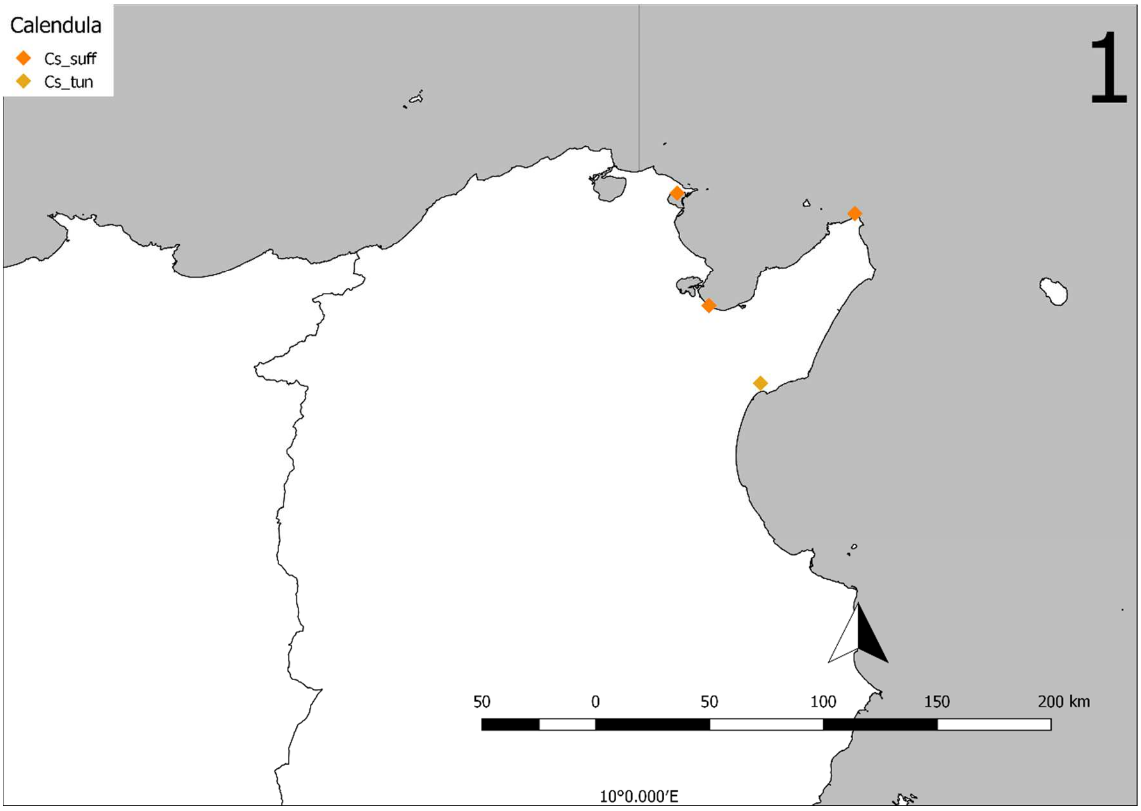
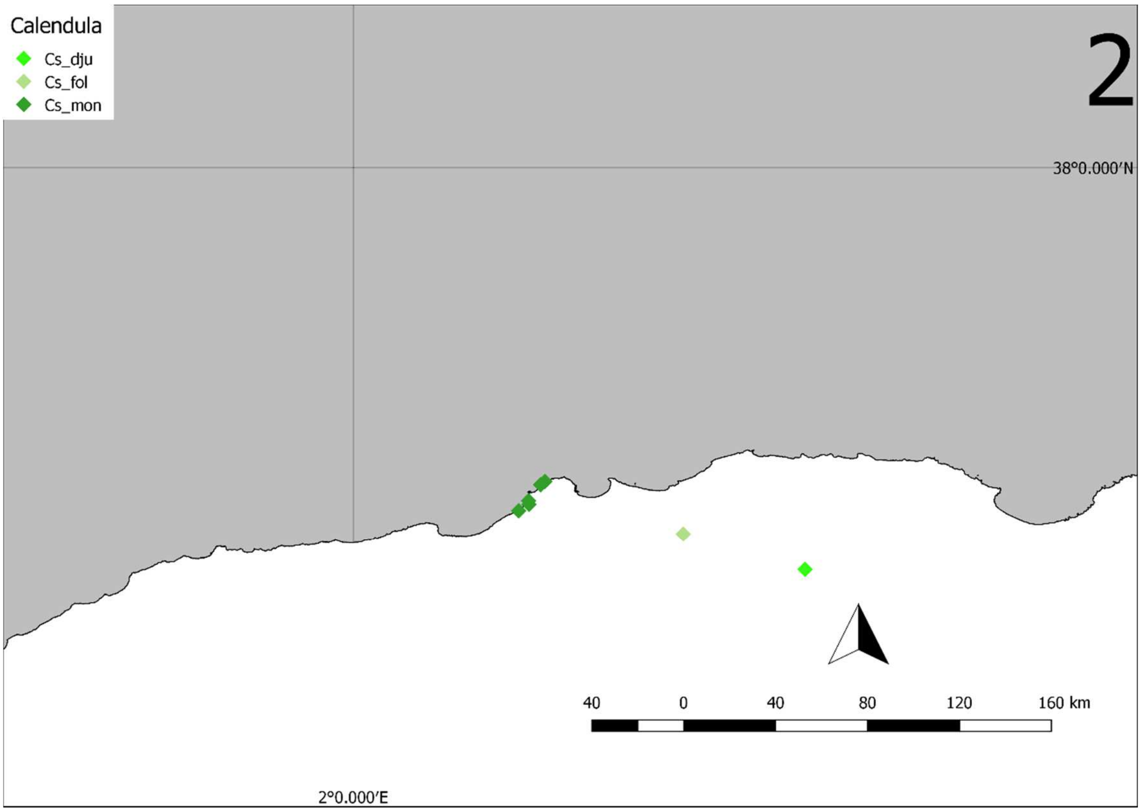


Figure 63 – Geographic distribution of the species included in the *C. maroccana* group.




Calendula
Cs_suff
Cs_tun
1
50
0
50
100
150
200 km
10°0.000′E


Calendula
Cs_dju
Cs_fol
Cs_mon
2
38°0.000′N
40
0
40
80
120
160 km
2°0.000′E

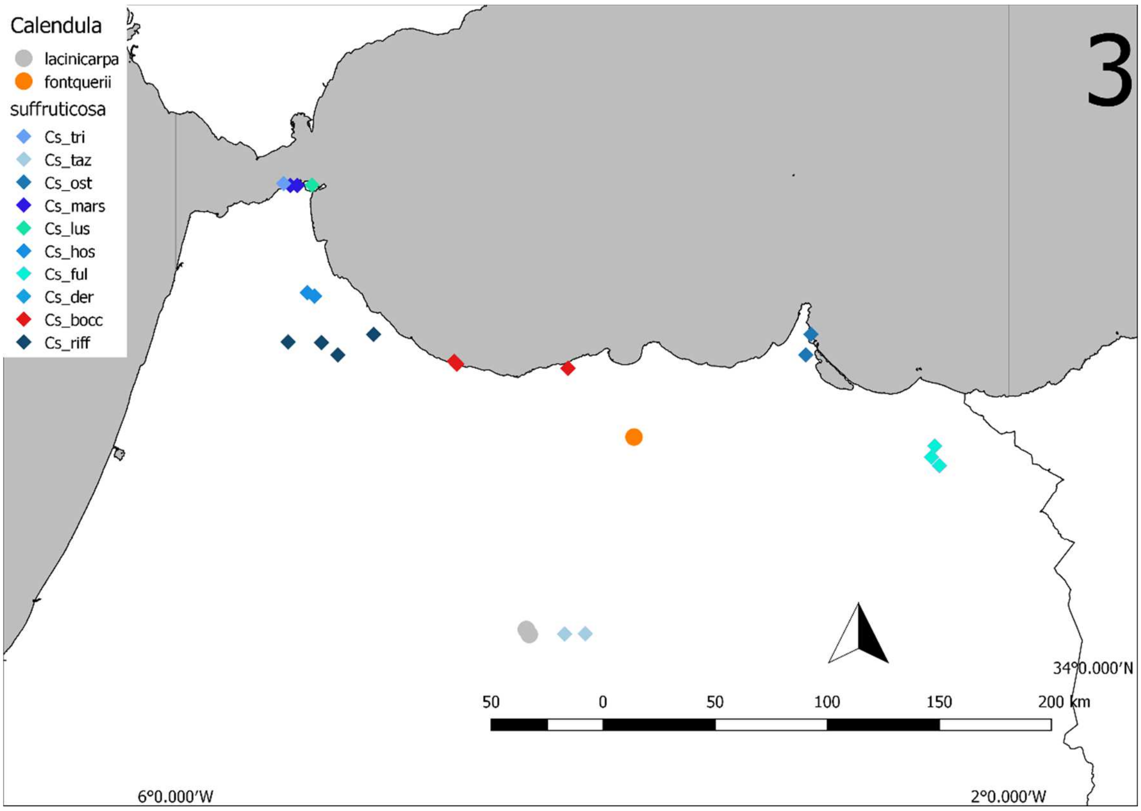


Figure 64 – Geographic distribution of *C. suffruticosa* in Morocco, including taxa from Algeria and Tunisia. 1. *C. suffruticosa* taxa in Tunisia. 2. *C. suffruticosa* taxa in Algeria. 3. *C. suffruticosa* taxa in Morocco.

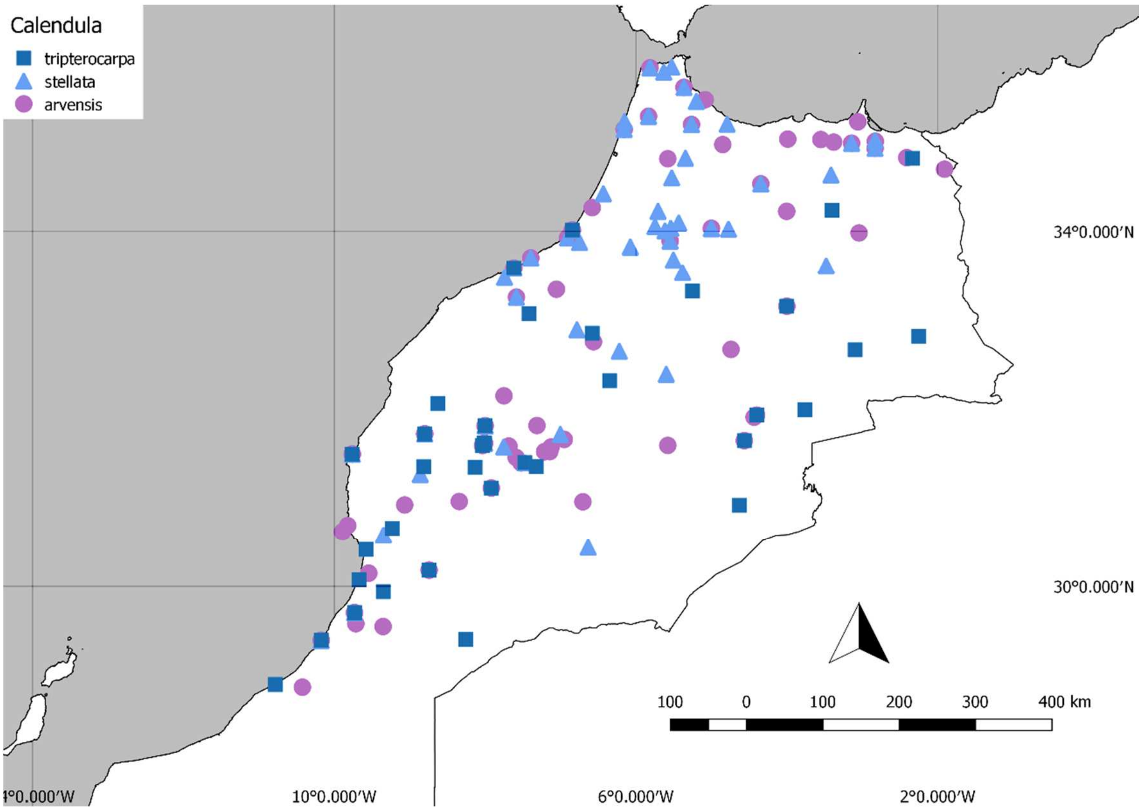


Figure 65 – Geographic distribution of *C. arvensis*, *C. stellata*, and *C. tripterocarpa* in Morocco.

Conservation assessments

Some of the *Calendula* species are widespread and co-occur, more or less, in the same areas, like *C. arvensis*, *C. stellata* and *C. tripterocarpa* (Figure 65), which are found in most of the North African countries. These species were assessed least concern (LC). The cultivated (*C. officinalis*) was not analysed in this study. Unfortunately, detailed information of endemism/conservation assessments of Algerian and Tunisian *Calendula* is not available, but we know that some taxa are restricted to some of these regions. For the three species with only one population (*C. fontquerii*, *C. davisii* and *C. meuselii*), the assessed category of threat was critically endangered (CR). For the three species with two or three known populations (*C. lanzae*, *C. pinnatiloba* and *C. murbeckii*), the assessed category of threat was endangered (EN). Only *C. eckerleinii* assessed as Vulnerable (VU), and *C. maroccana* widely distributed in High Atlas Mountains assessed as least concern (LC). For the majority of *C. suffruticosa* subspecies, Data Deficient or critical endangered (CR) were assessed, most of them from Algeria and Tunisia. The ones assessed as Data Deficient need a future careful analysis based on further field surveys.

Table 17 – Chromosome numbers and genome size in *Calendula* taxa from SW Mediterranean (Morocco, Algeria and Tunisia).

Heyn et al. (1974)	Ohle (1975 a)	Ohle (1975b)	This study	Acronym	*2n*	Genome size (2C/pg)
						Mean ± SD
C. arvensis			*C. arvensis*[1]	Carv	44	5.23 ± 0.29
C. stellata			*C. stellata*[1]	Cste	14	2.11 ± 0.10
C. tripterocarpa			*C. tripterocarpa*[1]	Ctrip1	30	3.52 ± 0.12
	C. maroccana group		*C. maroccana* group			
	C. eckerleinii		*C. eckerleinii*	Ceck	18	1.74 ± 0.08
			*C. fontquerii***	Cfont	18	1.69 ± 0.10
			*C. davisii***	Claci	18	1.76 ± 0.05
	C. lanzae		*C. lanzae*	Clan	18	1.85 ± 0.08
	C. maroccana subsp. *maroccana*		*C. maroccana*	Cmar	18	1.59 ± 0.14
	C. meuselii		*C. meuselii*	Cmeu	18	1.71 ± 0.03
	C. maroccana subsp. *murbeckii*		*C. murbeckii**	Cmurb	18	2.07 ± 0.14
			*C. pinnatiloba***	Cpinn	18	2.09 ± 0.15

*nomenclatural changes; **new taxa described.

Table 17 – Continued.

Heyn *et al.* (1974)	Ohle (1975 a)	Ohle (1975b)	This study	Acronym	2n	Genome size (2C/pg)
						Mean ± SD
		C. suffruticosa group	*C. suffruticosa* group	C suff		
		C. suffruticosa subsp. *balansae*	*C. suffruticosa* subsp. *balansae*[1]	Cs_bal	32	—
			C. suffruticosa subsp. *boccoyana***	Cs_boc	32	3.17 ± 0.06
		C. suffruticosa subsp. *boissieri*	*C. suffruticosa* subsp. *boissieri*[1]	Cs_boi	32	—
			C. suffruticosa subsp. *dercana*	Cs_der	32	3.00 ± 0.07
			C. suffruticosa subsp. *djurdjurensis*	Cs_dju	32	3.10 ± 0.14
			C. suffruticosa subsp. *foliosa**	Cs_fol	32	3.08 ± 0.04
			C. suffruticosa subsp. *hosmarensis***	Cs_hos	32	3.10 ± 0.10
		C. suffruticosa subsp. *fulgida*	*C. suffruticosa* subsp. *fulgida*	Cs_ful	32	3.06 ± 0.21
			C. suffruticosa subsp. *lusitanica*	Cs_lus	32	3.36 ± 0.15
		C. suffruticosa subsp. *monardii*	*C. suffruticosa* subsp. *monardii*	Cs_mon	32	2.86 ± 0.07
			C. suffruticosa subsp. *marsea***	Cs_mar	32	3.44 ± 0.12
			C. suffruticosa subsp. *osteni***	Cs_ost	32	2.96 ± 0.05
			C. suffruticosa subsp. *riffiniensis***	Cs_riff	32	3.06 ± 0.10
		C. suffruticosa subsp. *suffruticosa*	*C. suffruticosa* subsp. *suffruticosa*	Cs_suff	32	3.28 ± 0.07
			C. suffruticosa subsp. *tazzea***	Cs_taz	32	3.42 ± 0.07
		C. suffruticosa subsp. *tlemcensis*	*C. suffruticosa* subsp. *tlemcensis*[1]	Cs_tlem	32	—
			C. suffruticosa subsp. *trialata*	Cs_tri	32	3.21 ± 0.05
		C. suffruticosa subsp. *tunetana*	*C. suffruticosa* subsp. *tunetana*	Cs_tun	32	3.39 ± 0.08

*nomenclatural changes; **new taxa described; [1]taxa not included in this taxonomic treatment.

Taxonomic treatment

A taxonomic revision of the genus *Calendula* in Morocco including some taxa from Algeria and Tunisia is presented. Treatments for other areas are under preparation and planned to be published.

Calendula Linnaeus (1753: 921). **Lectotype** (designated by Green in Hitchcock & Green 1929: 183): — *C. officinalis* Linnaeus (*vide* M. L. Green in A. S. Hitchcock & M. L. Green, Prop. Brit. Bot. 183. Aug 1929).

Annual (to short-lived perennial) or perennial herbs, sometimes woody at the base, habit various but most commonly erect, or prostrate to ascending, often with glandular and aromatic, and non-glandular hairs. Leaves alternate, undivided, rarely pinnatisect; spatulate, obovate, oblanceolate, lanceolate, or linear-oblong, one nerved, apex acute to obtuse; base attenuate, truncate or auriculate; margins sub-entire, sinuate-dentate, or ± irregularly sinuate-dentate to pinnatisect; glandular or non-glandular pubescent; the middle and upper cauline leaves progressively smaller and small stalked towards the apex, oblanceolate to lanceolate, usually auriculate. Capitula terminal, solitary, radiate, heterogamous. Involucre bracts campanulate, 1–2 rows, sub-equal, herbaceous, linear-lanceolate, acute, with a narrow scarious-hyaline margin, glandular and non-glandular hairs. Receptacle flat-convex, glabrous. Florets dimorphic; ray florets ligulate, female, fertile, yellow or orange, 1–2 rows rarely more; disc florets tubular, male with a rudimentary ovary, yellow, orange, brown or violet-purple, 5–lobed; anthers sagittate-caudate. Achenes heteromorphic, the outer rostrate, less common bialate or trialate achenes; middle usually cymbiform, sometimes bialate, trialate or sub-cymbiform achenes; the internal vermiculate-alate or vermiculate-exalate.

Key to the species of *Calendula* in Morocco

1. Annual to short-lived perennial plants 2

   Perennial plants 7

2. Ligules, less than twice the length of the involucre 3

   Ligules, more than twice the length of the involucre 4

3. Outer achenes trialate, with the margin of the wings entire........ ***C. tripterocarpa***

   Outer achenes rostrate or bialate with the margin of the wings deeply incised .... ***C. arvensis***

4. Leaves with the margin entire; disc florets violet-purple; trialate achenes absent and bialate achenes, when present, with the margins of the wings deeply incise with triangular teeth.. ***C. stellata***

   Leaves with the margin repand-dentate with the teeth more or less prominent or pinnatifid; disc florets yellow-orange, more rarely, violet-purple; trialate achenes present, or not and bialate achenes, when present, with the margins entire or with teeth not deeper than half of the width of the wing...5

5. Trialate achenes with lateral wings deeply pinnately cut into segments with truncate apices, usually present; bialate achenes never present....... ***C. murbeckii***

   Trialate achenes, when present, with the margins entire or inconspicuously toothed; bialate achenes predominant..6

6. Leaves pinnatifid; capitula concolorous.. ***C. lanzae***

   Leaves not pinnatifid, with the margin repand-dentate with the teeth more or less prominent; capitula concolorous or, less frequently, discolorous .... ***C. pinnatiloba***

7. Capitula appearing on the apex of stems usually branched and bearing cauline leaves; cymbiform and sub-cymbiform achenes, when present, usually with ventral wings; plants with $2n = 32$ chromosomes .... ***C. suffruticosa***

   Capitula appearing on the apex of unbranched stems and not bearing cauline leaves for near half of its length, or with them very small; cymbiform achenes, when present, without ventral wings; plants with $2n = 18$ chromosomes 8

8. Leaves mostly densely white-arachnoid pubescent..9

Leaves mostly glabrous or glandular pubescent..10

9. Margin of the leaves repand-dentate; rostrate achenes (5.8) 6.5–8.1 (8.7); bialate achenes absent, trialate achenes with lateral wings entire................ ***C. fontquerii***

Margin of the leaves entire; rostrate achenes (12) 14.5–17; bialate achenes present, trialate achenes with lateral wings repand-dentate.............................. ***C. meuselii***

10. Margin of the leaves entire; trialate achenes with lateral wings deeply incise dentate .... ***C. davisii***

Margin of the leaves repand-dentate, trialate achenes with lateral wings entire to sinuate-dentate...11

11. Cymbiform achenes absent; vermicular achenes falcate or hook-shaped ....***C. eckerleinii***

Cymbiform achenes present; vermicular achenes circular to hemicyclic .... ***C. maroccana***

C. arvensis Linnaeus (1763: 1303); Desfontaines 1799; Battandier and Trabut 1902; Pitard 1913; Jahandiez and Maire (1934); Quezel and Santa (1963: 967); Heyn et al. 1974; Potteier-Alapetite 1981; Valdés 2002; Fennane and Ibn Tattou 2005; Dobignard and Chatelain 2011; Gonçalves et al. 2014. Lectotype (designated by Heyn et al. 1974)**:—** EUROPE: '*in Europae arvis*', *Löfling s.n* (LINN! [1035.1]).

Heterotypic synonyms*:*

C. arvensis var. *micrantha* Ball (1878: 516). **Type:** ITALY: Sicily, Palermo, *s. coll. s.d.*, (holotype: S-G-1106)

C. aegyptiaca Persoon (1807: 492). Quezel and Santa (1963: 966)

C. aegyptiaca var. *ceratosperma* (Viv.) Pamp. (1931: 450)

C. ceratosperma Viviani (1824: 59)

C. aegyptiaca subsp. *ceratosperma* (Viv.) Murbeck (1897: 102)

C. crista-galli Viviani (1824: 59)

C. aegyptiaca var. *crista-galli* (Viv.) Bég. & A.Vacc. (1912: 66)

C. sancta subsp. *crista-galli* (Viv.) Gallego & Talavera (1983: 103)

C. aegyptiaca var. *exalata-longirostris* Lanza (1919)

C. aegyptiaca var. microcephala Boissier (1875)

C. aegyptiaca var. suberostris Boissier (1875)

C. bicolor Raffiniesque (1810: 82)

C. arvensis var. bicolor (Rafinesque) Font Quer (1838)

C. officinalis var. hydruntina Fiori (1904)

C. hydruntina (Fiori) Lanza (1919)

C. arvensis subsp. hydruntina (Fiori) Lanza (1919)

C. arvensis subsp. macroptera Rouy (1903)

C. parviflora Raffiniesque

C. arvensis var. parviflora (Rafinesque) Battandier (1889)

C. malacitana Boissier & Reuter (1852)

C. malvaecarpa Pomel (1874)

C. platycarpa var. *malvaecarpa* (Pomel) Battandier (1889)

C. sancta L. (1763 : 1304). Lectotype (designated by Heyn *et al.* 1974: 170)**:—** PALAESTINA, s.d., *Hasselquist* & *Linnaeus* (LINN! [Herb. Linn. Nº 1035.2]).

C. arvensis Battandier

C. subinermis Pomel (1874)

C. sicula var. *hymenocarpa* Candolle

C. stellata var. *hymenocarpa* (Candolle) Cosson & Kralik (1857)

C. aegyptiaca var. *hymenocarpa* (Candolle) Pamp. (1914)

C. aegyptiaca var. *intermedia* (Cosson & Kralik) Pamp.

C. stellata var. *intermedia* Cosson & Kralik (1857)

C. arvensis var. *echinata* Ball (1878)

C. gracilis Candolle (1838). **Type:—**IRAN. Karabagh: circa castellum Schachlagh, 1832, *F. E. L. Fischer s.n.* (syntype G00472406!)

C. persica C.A. Mey. (1831)

Annual herbs. Stems (2) 17.3–45.5 (80) cm long, ascending to erect, sometimes decumbent, branched at the base, with glandular and non-glandular hairs, in varying proportions, ± viscous. Basal leaves (1.2) 2.4–4.8 (6.5) × (0.2) 0.4–0.8 (1.1) cm, (0.3) 0.3–0.5 (0.5) mm thick, oblanceolate, acute or obtuse, margin sub-entire to sinuate-dentate, base attenuated in a ± longer petiole, with glandular and non-glandular hairs; the middle and upper cauline leaves progressively smaller towards the apex, oblanceolate to lanceolate, sessile and usually auriculate. Capitula solitary, (1) 1.2–1.4 (2) cm of in diameter. Outer achenes rostrate (5) 7.1–11.7 (15.2) × (0.8) 1.3–1.9 (2.7) mm, usually strongly curved and with long spines at the back, usually with two small teeth, one at the base and another at the apex, sometimes bialate (8.3) 10.8–12.4 (13.3) × (7.2) 7.7–9.3 (10.2) mm, with two wings, with irregularly incise margins, extended along both sides up to the apex; middle achenes cymbiform (4.8) 6.7–8 (8.5) × (3.8) 5.3–7 (8) mm, sometimes lacking; inner achenes vermicular-alate (3.5) 3.8–5.2 (5.8) × (2) 2.7–3.3 (3.7) mm, hook-shaped to circular, with two narrow lateral wings, and/or vermicular-exalate (3.2) 3.7–4.5 (5.3) × (1.2) 1.2–1.7 (1.8) mm, circular.

Habitat and distribution: Dispersed throughout the North Africa in waste grounds, cultivated fields and grasslands or in the margin of roads and ditches, up to 1300 m. The remainder of its native range embraces also Central and South Europe, SW Asia and Macaronesia. Introduced in other parts of the globe such as Australia and California.

Conservation status: This species is a widely spread plant in the study area, and in the circum-Mediterranean region, including central Europe and the Middle East. It inhabits ruderal and wastes grounds and, currently, it does not face any major threat. Therefore, we propose to assess it as Least Concern (LC) for Morocco.

Chromosome number: $2n = 44$.

Genome size: 5.23 ± 0.29 pg.

C. davisii A.C. Gonç. & P. Silveira ***sp. nov.*** **Type:** —MOROCCO. NW foot of Jbel Tazzeka (W of Taza), 350–400 m, [34°09' N, 4°19' W], 14 April 1971, *Davis 51 257* (holotype BM! [813 587], isotypes RNG!, E! [245 753]).

"C. maroccana" auct. Vogt & Oberprielier (2012: 198) *non* (Ball) Ball (1878: 517).

C. davisii seems close to *C. eckerleinii* and some forms of *C. maroccana.* It differs from the first by presenting leaves with margins entire and by having trialate or bialate achenes with lateral wings deeply incised, and from the second by the margin of the leaves and by not presenting cymbiform achenes.

Perennial herbs, ± woody at the base. Stems (44) 45–57 (65) cm, erect to ascending, with glandular hairs. Basal leaves (3.5) 4.1–8 (10) × (0.5) 0.7–1.2 (2) cm, with (0.2) 0.3–0.4 mm thick, oblanceolate to spatulate, apex acute, margin entire to sub-entire, attenuated in a ± large petiole, with glandular hairs predominating in the lamina and white-aracnoid non-glandular hairs in the margin. Capitula solitary, ± 3.4 cm diameter, concolorous, yellow. Outer achenes rostrate (15) 18–22 (23) × (1.7) 1.9–2.3 (3) mm, ± straight or slightly curved, without dorsal spines, or with them very small; middle achenes bialate (11.7) 12.5–15.4 (16.2) × (6.7) 9–10.8 (12.2) mm, with a rostrum (2.5) 3–3.8 (4.5) mm, lateral wings incised, without dorsal wings but, sometimes, with small dorsal spines; or, less frequently, trialate 8.1–12.5 (12.7) × 6.9–11.7 (12.2) mm, with lateral and ventral wings incised, without dorsal wings but, sometimes, with small dorsal spines; inner achenes vermiculate-exalate (4.5) 4.9–6.0 (6.7) × (1.3) 1.5–1.7 (1.8) mm, hemicyclic to hook-shaped, with a small basal ventral tooth.

Habitat and distribution: Limestone cliffs with some intrusion of granites, at 350–530 m elevation and is found growing under a semi-arid to sub-humid Mediterranean bioclimate. Endemic to Morocco restricted to the eastern slope of Jbel Tazzeka in Taza (Morocco) – Figure 66

Conservation status: This species is under numerous threats, especially climate change (extreme weather and drought). It is rare and with a small-restricted range, since only one subpopulation is currently known (the two points on the map belong to the same subpopulation) and few herbarium specimens are known (from the same locality). The species lives in a Natural Park, which might result in some protection. However, the number of mature individuals is estimated to be <50 based on field observations. The estimated area of occupancy is 8 km^2, which qualifies for CR. We propose to assess it as CR B1ab(iii, v)+2ab(iii, v), due to the reduced size of the population and of its habitat, which confers a high risk of extinction due to climate changes, or other unexpected threats.

Chromosome number: $2n = 18$.

Genome size: 1.76 ± 0.05 pg.

Notes: Neighbour of *C. suffruticosa* subsp. *tazzea* in the same region, *C. davisii* inhabits the east face of Jbel Tazzeka Mountains. Only one head flower was found in the field. The specific epithet *davisii* is in honour of the collector of the type specimen P.H. Davis.

C. eckerleinii Ohle (1975 a: 8). Valdés (2002: 671); Fennane and & Ibn Tattou (2005: 27); Gonçalves et al. (2014: 273). **Type:** —MOROCCO. Middle Atlas, Ifrane, 15 May 1955, *Meusel s.n.* (holotype HAL! [29 895]).

Heterotypic synonym:

C. suffruticosa var. *maroccana* (Ball) Maire in Jahandiez & Maire (1934: 789) *p.p. quoad distr.* "Moyen Atlas".

Perennial herbs, ± woody at the base. Stems (15) 33–45.5 (56) cm long, ascending to decumbent, with glandular hairs. Basal leaves (3.8) 4.6–7.0 (9.7) × (0.4) 0.6–1.1 (1.5) cm, with (0.2) 0.3–0.4 (0.5) mm thick, linear to oblanceolate, apex obtuse, margins sinuate-dentate, with glandular hairs predominating in the lamina and white-aracnoid non-glandular hairs in the margin. Capitula solitary, (2.7) 3.1–3.8 (5.2) cm diameter, concolorous, yellow. Achenes heteromorphic: outer achenes rostrate (7) 9.3–10.3 (12.2) × (1.3) 1.7–1.8 (2.2) mm, ± straight or slightly curved, without dorsal spines and ventral tooth; middle achenes trialate (5.7) 6.3–7 (7.8) × (2.5) 4.2–6.7 (7.8) mm, slightly curved, lateral wings sinuate-dentate, with a narrow ventral wing, without dorsal wings or spines; inner achenes vermiculate-exalate (3.3) 4.5–5.2 (5.8) × (1.3) 1.7–1.8 (2) mm, falcate or hook-shaped, sometimes with a small ventral wing.

Habitat and distribution: Limestone rocks, slopes or gravel of low and medium mountains, at 895–1950 m elevation, and is found in humid to sub-humid cold Mediterranean bioclimates. Endemic to Medium Atlas (Ifrane, Ain Leuh near Oued Oum-er-Rbia; Timahdite; Massif du Kandar; and Jbel Zalagh); with one population in Kef-el-Ghar WSW de Tainaste (?) – Figure 66

Conservation status: Some populations of this species are threatened by climate change, due to reduced range of habitat and lack of connection between some of the populations. The species is currently known from six subpopulations (6 locations) in the Medium Atlas Mountains. The species is rare and local with a small-restricted range. Both the estimated extent of occurrence 17 871.722 km^2 and the estimated area of occupancy is 52 km^2, qualify for EN. Therefore, we propose its assessment as EN Bab(iii, v) +2ab(iii, v).

Chromosome number: $2n = 18$.

Genome size: 1.74 ± 0.08 pg.

Notes: Widely distributed in the moist region of Ifrane, *C. eckerleinii* inhabits near *C. meuselii* in the Middle Atlas Mountains. According to Ohle (1975 a), the limits of this taxon may extend further towards Tlemcen (Algeria), however, no specimen of *C. eckerleinii* was found beyond the above-mentioned area. Moreover, later Ohle (1975b) described *C. suffruticosa* subsp. *tlemcensis* from Tlemcen, but no mention about *C. eckerleinii* in this region was made. Some authors (Fennane & Ibn Tattou (2005: 27); Valdés (2002: 671) also cited the *C. eckerleinii* for Jbel Tazzeka, but a different plant morphology was found in this region (see *C. davisii* described for Jbel Tazzeka). As *C. maroccana*, it was identified as *C. suffruticosa*, as well as *C. maroccana*, due to some similar morphologies (e.g. habit, basal leaves). Nevertheless, it was supposed to be at the origin of the *C. suffruticosa* group ($2n = 32$), from the cross of *C. eckerleinii* ($2n = 18$) and *C. stellata* ($2n = 14$) (Heyn & Joel 1983; Ohle 1975 a). The basal leaves of the populations nearby Ifrane have the narrowest leaves, with margins sinuate-dentate, while those from Jbel Zalagh and Oued Oum-er-Rbia have oblanceolate leaves, with margins sub-entire. This variation of basal leaves may be linked to environmental conditions (temperature, humidity, shade). In contrast with the description made by Ohle (1975 a), sometimes they present achenes trialate with a narrow ventral wing or with it reduced, resembling a bialate achene.

C. fontquerii A.C. Gonç. & P. Silveira ***sp. nov.*** **Type:** —MOROCCO. Bokkoya, 33 km from Imzouren, 740 m [34°54'46" N, 3°47'59" W], 13 May 2014, *Silveira & Gonçalves 3339* (holotype G!, isotype AVE!)

C. fontquerii seems close to *C. eckerleinii* but differs from it by its white-arachnoid indumentum and trialate achenes with margins sub-entire.

Perennial herbs, ± woody at the base. Stems (16) 30.3–44 (48) cm long, erect to ascending, with a mixture of glandular and white-aracnoid non-glandular hairs. Basal leaves (3.5) 4.5–7.1 (9.5) × (0.8) 0.9–1.3 (1.5) cm, with 0.3–0.4 (0.5) mm thick, oblanceolate to spatulate, apex acute, margins sinuate-dentate, with some glandular hairs, but predominantly white-arachnoid pubescent. Capitula solitary, (2.2) 2.6–3.6 (3.8) cm diameter, concolorous, yellow. Achenes heteromorphic: outer achenes rostrate (5.8) 6.5–8.1 (8.7) × 1.5–1.7 (1.8) mm (brevirostrate), ± straight or slightly curved, without dorsal spines; middle achenes trialate (5) 5.6–6.7 (7) × (3) 3.3–4.4 (5.8) mm, lateral and ventral wings sub-equal and sub-entire; inner achenes vermiculate-exalate (3.7) 3.8–4.8 (5) × (1.3) 1.5–1.7 mm, falcate, without ventral wing.

Habitat and distribution: Limestone rocks, at ± 740 m elevation, and grows under semi-arid Mediterranean bioclimates. Endemic to Bokkoya Mountains in Al Hoceïma (Morocco) – Figure 66

Conservation status: This species is under numerous threats, especially grazing, climate change and drought. The species is rare and local with a small-restricted range; only one subpopulation is currently known, intersected by a road. The number of mature individuals is estimated to be <50 based on field observations. The estimated area of occupancy is 8 km^2, which qualifies for CR. Although it is not known for sure if there are more populations or not, this is not very probable, so we propose to assess this species as CR B1ab(iii, v)+2ab(iii, v).

Chromosome number: $2n = 18$.

Genome size: 1.69 ± 0.10 pg.

Notes: The specific epithet is in honour of the well-known botanist Font Quer, who did important collections in the North of Morocco.

C. lanzae Maire (1928a: 138). Fennane and Ibn Tattou (1998: 23, 2005: 28); Gonçalves et al. (2014). **Neotype** (designated here): —MOROCCO. Aït-Massi, rochers gréseux près de Tirkou, 500–600 m, 21 April 1922, MAIRE *s.n.* (neotype MPU! [001956], isoneotype P! [00084055]).

Homotypic synonyms:

C. echinata subsp. *lanzae* (Maire) Maire in Jahandiez and Maire (1934: 788)

C. murbeckii subsp. *lanzae* (Maire) Maire (1938: 424) *p.p.*Anti Atlas

Heterotypic synonyms:

C. echinata subsp. *murbeckii* (Lanza) Maire in Jahandiez and Maire (1934: 788) *p.p. quoad distr.* "*Maroc Atlantique Moyen*" (S), High Atlas, Anti Atlas

C. echinata subsp. *murbeckii* var. *pinnatiloba* Maire in Jahandiez and Maire (1934: 788) *p.p. quoad distr.* "*Maroc Atlantique Moyen*" (S), High Atlas, Anti Atlas

Annual herbs. Stems (32) 45.8–78.5 (96) cm, ascending, with glandular hairs. Basal leaves (3.5) 4–6.5 (7.2) × (0.8) 1.2–2.1 (3) cm, with 0.2–0.3 mm thick, outline oblanceolate to obovate, apex acute to obtuse, margin deeply sinuate-dentate to ± irregularly pinnatipartite, not wavy, glandular pubescent. Capitula solitary, (1.1) 2.2–3 (3.8) cm diameter, concolorous, yellow to orange. Achenes heteromorphic: outer achenes rostrate (12) 14.8–17.3 (21) × (1.3) 2.3–3.2 (4.2) mm, ± straight or slightly curved, with dorsal spines or muricate, sometimes with a apical ventral tooth; middle achenes bialate (8.7) 9.7–10 (10.5) × (5.8) 7.1–9 (9.2) mm, with lateral wings sinuate-dentate; and cymbiform (5.2) 5.7–6 (6.3) × (2.8) 3.8–4.9 (5) mm, with two ventral teeth, one basal and one apical; inner achenes vermiculate-alate (5) 5.3–6.5 × (4.2) 4.3–4.5 mm, circular to hemicyclic; with a small ventral wing; vermiculate-exalate (3.7) 4.1–4.6 (5) × (1.3) 1.5–1.7 (1.8) mm, circular to hemicyclic; both with small apical and/or basal teeth.

Habitat and distribution: Gravel riverbeds, mostly composed of limestone, at 200–600 m elevation and is found in semi-arid to arid Mediterranean bioclimates. Endemic to Morocco with a restricted distribution to the Anti-Atlas in Tirkou near Bigoudine; Aït-Yazza and Tiout – Figure 66.

Conservation status: This species is under numerous threats, especially climate change (extreme weather and drought) urbanization and water pollution. It is rare, with two currently known populations, while only Maire has collected in the classical population, at Tiout. The number of mature individuals is estimated to be <250 (100-150 individuals) based on field observations. The estimated extent of occurrence is 62.781 km^2, which qualifies for CR and the estimated area of occupancy is 12 km^2, which qualifies for EN. Therefore, we propose to assess this *taxon* as CR C2a(I)EN B1+2ab(iii).

Chromosome number: *2n* = 18.

Genome size: 1.85 ± 0.08 pg.

Notes: In the protologue, Maire mentioned that the holotype of *C. lanzae* is "*in rupestribus arenaceis et in arganietis clivi meridionalis Atlantis Majoris: in valle amnis Aït-Moussa prope Tirkou, 500–600 m, ubi martio et aprili floret*" housed at AL and RAB herbaria. Despite the several attempts, no type of *C. lanzae* was found in these herbaria. Most of Maire's collection was transferred to MPU herbarium, where a specimen collected in 1922 (duplicated at P herbarium) was found, which corresponds to the description of *C. lanzae*. The specimen with a label in Maire's hand that reads "*Aït-Massi, rochers gréseux près de Tirkou, 500–600 m, 21 April 1922*." Therefore, a neotype is designated here (Art. 9.7 of the ICBN, McNeill et al. 2012). Besides the characteristic of the achenes, *C. lanzae* differs from other taxa of the *C. maroccana* group by having pinnatisect leaves. Ohle (1974a) seemed to have included specimens of what we now call *C. pinnatiloba*, with discolorous capitula, under *C. lanzae*.

C. maroccana (Ball) Ball (1878: 517). Valdés (2002: 672); Fennane and Ibn Tattou (2005: 28). Basionym: *C. suffruticosa* subsp. *maroccana* Ball (1873: 367). **Lectotype** (corrected here): —MOROCCO. Greater Atlas, Seksaoua, May 1871, *Hooker s.n.* (K! [000307201]).

Homotypic synonyms:

C. maroccana subsp. *maroccana* (Ball) B.D. Jackson (1893: 383) *comb. illeg.*

C. suffruticosa var. *maroccana* (Ball) Maire *in* Jahandiez & Maire (1934: 788) *p.p. quoad distr.* "High Atlas" and "Anti Atlas".

Heterotypic synonym:

C. echinata subsp. *murbeckii* (Lanza ex Murb. 1923: 59) Maire in Jahandiez and Maire (1934: 788) *p.p. quoad distr.* High Atlas, Anti Atlas.

C. maroccana subsp. *murbeckii* (Lanza ex Murb. 1923: 59) Ohle (1975 a: 13) *p.p. quoad spec.* Tachokcht, 8 June 1936, *Balls 2691* (BM!, K!, RAB!, E!).

Perennial herbs. Stems (7.5) 21–38 (58) cm, erect to ascending, with glandular or, sometimes, white-aracnoid, hairs. Basal leaves (4.2) 5.3–10 (13.1) × (0.4) 0.6–1.1 (2.8) cm, with 0.3–0.4 (0.6) mm thick, oblanceolate to spatulate, apex acute, margin slightly sinuate-dentate, glandular pubescent in the lamina, and glandular or white-aracnoid pubescent in the margin. Capitula solitary, (1.6) 3.2–4 (4.7) cm diameter, concolorous, yellow. Achenes heteromorphic: outer achenes rostrate (8.3) 9.5–13.7 (14.3) × (1) 1.2–1.7 mm, ± straight or slightly curved, without dorsal spines or muricate; middle achenes usually trialate, (5) 5.8–8.7 (10.2) × (4.2) 4.6–7.5 (8.7) mm, with lateral wings entire to, more rarely, sinuate-dentate; cymbiform (5.8) 6.1–6.7 × 3.5–4.1 (4.2) mm, with basal and apical teeth; inner achenes vermiculate-exalate (2.7) 2.8–5.5 (5.8) × 1.2–1.6 (1.7) mm, circular to hemicyclic; with a small ventral wing and/or with apical and/or basal teeth.

Habitat and distribution: Various substrates from rocky to sandy especially in limestone cliffs, between 980?–3000 m elevation, and is found in sub-humid Mediterranean bioclimates; Endemic to High Atlas Mountains (Amizmiz, Asni, Igherm, Tachokcht, Taroudant, Tizi-n-Test, Tizi-n-Tichka, etc) – Figure 66.

Conservation status: This species is under numerous threats, especially agricultural intensification, grazing, climate change, development (tourism in the Atlas area, leisure activities), and human interference. The species is currently known from 13 subpopulations in the High Atlas Mountains. The number of mature individuals is estimated to be >10 000, based on field observations. The estimated extent of occurrence is 17 871.722 km^2, which qualifies for VU and the estimated area of occupancy is 52 km^2, which qualifies for EN. Considering the number of populations and that it occurs at different elevations, despite all the threats we propose to assess it as LC.

Chromosome number: $2n = 18$.

Genome size: 1.59 ± 0.14 pg.

Notes: Ohle (1975 a, 10) mentioned the lectotype as to be: *"Marokko, Marrakech, leg. Hooker 1871* (K)*"*, but it is an error to be corrected. In K herbarium, one folder with three sheets was found, one sheet with three specimens and two separate specimen's sheets with different labels, collector, date and location. Ohle (1975 a) "*clearly indicated the type element*" (Art. 7.10, before 2001 of the ICBN, McNeill et al. 2012) as to be the collection at K as the "*lectotype*," but the number and locality are not in agreement with the prologue (Ball 1873, 1878). Nevertheless, there is no *C. maroccana* in "*N of the city*" (Marrakech), but instead *C. murbeckii*. Our data suggest that *C. maroccana* and *C.*

murbeckii are distinct taxa. In this study, the correct lectotype of *C. maroccana* is presented, because in the last revision of the genus some confusion was made by Ohle (1975 a).

C. meuselii Ohle (1975 a: 6). Fennane & Ibn Tattou (1998: 23, 2005: 28); Valdés (2002: 672); Gonçalves et al. (2014: 271). **Neotype** (designated here): —MOROCCO. Meknès, Zerhoun, 11 April 1955, *Pailler 841* (RAB!).

Heterotypic synonym:

C. suffruticosa subsp. *marginata* var. *balansae* (Boiss. & Reut. in Boissier 1859: 107) Maire in Jahandiez & Maire (1934: 789) *p.p. quoad distr.* "*Maroc Central, partie septentrionale*" (Mont Zerhoun). Lectotype (designated here): — ALGERIA, *Oran, dans les sables avoisinant la Batterie espagnole, 20 April 1852*, *Balansa 515* (G! [G00386549]).

Perennial herbs. Stems (15.5) 18.1–44.3 (57) cm long, erect to ascending, densely white-arachnoid pubescent, not viscous. Basal leaves (4.4) 4.6–8.4 (10) × (1.3) 1.4–2.3 (2.4) cm, with (0.2) 0.3–0.5 (0.6) mm thick, oblanceolate to spatulate, apex acute to slightly acuminate, margins entire to sub-entire, densely white-arachnoid pubescent. Capitula solitary, (2) 3.3–3.8 (4.5) cm diameter, concolorous, yellow to orange. Achenes heteromorphic: outer achenes rostrate (12) 14–17 × (1.8) 2–2.3 (3.3) mm, ± straight or slightly curved, without dorsal spines or muricate; middle achenes bialate (9.7) 10.2–11 (11.7) × (4.7) 5.3–7 (7.5) mm, with a rostrum (1.3) 1.7–2.2 (3.3) mm, lateral wings sinuate-dentate, sometimes with a rudimentary ventral wing; inner achenes vermiculate-exalate (5.8) 6.3–7.3 (7.8) × (1.7) 2–2.3 (2.7) mm, falcate with a small ventral wing, or with an apical and/or a basal tooth

Habitat and distribution: Limestone cliffs at 650–800 m elevation and is found in sub-humid to humid Mediterranean bioclimates. Endemic to Massif du Zerhoun, Jbel El–Rherraf near Sidi Kdat, and Jbel Takerma (distribution that is, based on herbarium specimens and literature).

Conservation status: This species is threatened by climate change, due to its small-restricted range; the team currently knows only one population, but there may be others in the Massif of Zerhoun (based on herbarium specimens), although we have known, that, other researchers have recently searched for it, unsuccessfully. The number of mature individuals is estimated to be <50 based on field observations. The estimated area of occupancy, based in the only subpopulation known by the team, is 4 km^2, which qualifies for CR. This result in a categorisation of CR B1ab(i, ii, iv, v)+2ab(i, ii, iv,v) – Figure 66.

Chromosome number: *2n* = 18.

Genome size: 1.71 ± 0.03 pg.

Notes: Several unsuccessful attempts have been made to find the type. We know that the type was loaned to Ohle, but was never returned. Therefore, a neotype was selected to serve as a nomenclatural type of *C. meuselii* (Art. 9.6 of the ICNB, McNeill et al. 2012). Although the neotype has no capitula and achenes, it is the only duplicate at MPU herbarium. It is distinguishable from the remaining C. *maroccana* group species by having leaves with very dense white-arachnoid indumentum, and by the absence of cymbiform and vermiculate-alate achenes. The vermiculate-exalate achenes are usually large and falcate. There is a specimen collected in Zoumi (MGC), but the team failed to found this population on the field. Two gatherings were originally indicated as types of *C. balansae* Boiss. & Reut., one collected by Balansa (nº515) and other by Boiss. & Reut. themselves (v. Boissier 1859: 107). We prefer to select Balansa's collection as type, as it seem to have been the first collected and is more complete.

C. murbeckii Lanza ex Murb. (1923: 59). Maire (1928b: 56) *p.p. excl. spec.* Mogador; Jahandiez & Maire (1941: 1152); Nègre (1958:1-7, 1962: 300). **Type**: —MOROCCO. Environs de Marrakech: Aviation, champs arides, 1921, *Murbeck s.n.* (holotype LD! [1217767]; syntype MPU! [00819]).

Homotypic synonyms:

C. echinata subsp. *murbeckii* (Lanza ex Murb. 1923 : 59) Maire in Jahandiez & Maire (1934: 788) *p.p. quoad distr. "Maroc meridional steppique".*

C. maroccana subsp. *murbeckii* (Lanza ex Murb. 1923: 59) Ohle (1975 a: 13) *p.p. excl. distr.* Mogador, Agadir and Tachocht

Annual to short-lived perennial herbs. Stems (6) 22–46 (89) cm long, ascending to diffuse, with glandular hairs. Basal leaves (2.5) 3.5–6.7 (9) × (0.2) 0.3–0.6 (1.1) cm, with (0.2) 0.3–

0.4 (0.6) mm thick, linear to oblanceolate, apex acute to slightly acuminate, margins sinuate-dentate, with acute teeth, to ± irregularly pinnatifid, glandular pubescent. Capitula solitary, (2) 2.7–3.4 (4.7) cm diameter, concolorous, yellow to orange. Achenes heteromorphic: outer achenes rostrate (14) 16.3–23 (26) × (1.2) 1.5–1.8 (3.3) mm, ± straight or slightly curved, with dorsal spines or muricate; middle achenes trialate (8.3) 9–10.5 (11.3) × (5) 5.8–8.3 (10.8) mm, lateral wings deeply pinnately cut into segments with truncate apices; inner achenes vermiculate-alate (3.8) 4.3–5.6 (6) × (2.2) 3.1–5.6 mm, with basal and apical teeth; and vermiculate-exalate (3.3) 3.8–4.5 (4.7) × (1) 1.2–1.3 (1.7) mm, both circular to hemicyclic.

Habitat and distribution: Clay-limestone rocks, between 20–150 m a.s.l., and is found in arid Mediterranean bioclimates. Endemic to Morocco with a distribution restricted to Marrakech (Jbilet and Lalla Takerkoust) – Figure 66.

Conservation status: This species is under numerous threats, especially climate change, drought, development (infrastructures, urbanisation), human interference and grazing. The species is rare and local with a small-restricted range; at present, we only know two populations, one in a reserve of gazelles and another near the Lalla Takerkoust barrage. The classic population disappeared due to the development of the airport. The number of mature individuals is estimated to be <250 based on field observations. The estimated extent of occurrence is 124.946 km^2 and the estimated area of occupancy is 12 km^2, both qualifying for EN. There was an observed and is projected a future continuing decline in the extent of occurrence, the area of occupancy, area, extent and quality of the habitat, number of locations/subpopulations and number of mature individuals. Therefore, we propose to assess it as EN B1ab(i, ii, iii, iv, v) +2ab(i, ii, iii, iv, v), C2a(i), D.

Chromosome number: $2n = 18$.

Genome size: 2.07 ± 0.14 pg.

Notes: Lanza described this species has displaying only trialate and vermiculate achenes, without specifying which type of vermiculate achenes (alate or exalate). Later Maire confirmed, that they have found rostrate, trialate, cymbiform and vermiculate-exalate achenes. We have not seen any specimen (field or herbaria) with cymbiform achenes. So, two hypotheses are raised, either Maire considered as cymbiform the vermicular-alate achenes, or the specimen he observed was a hybrid. We observed in the field that two, or more, different combinations of achenes may appear in the fruiting capitula. For example, we observed plants with rostrate, trialate, vermiculate-alate and vermiculate-exalate achenes and others lacking the trialate achenes. Nègre (1958) made some observations about *C. murbeckii* under cultivation: the first generation came out with the same characteristics, the *second* generation displayed intermediate characteristics between *C. stellata* and *C. murbeckii*. However, we cannot exclude that this variability result from cross-pollination with some *C. stellata* that he had growing in the garden at the same time. Maire (Jahandiez and Maire 1934: 788) synonymised all *C. murbeckii* specimens collected in the coastal of the Anti-Atlas region (*environs of Mogador, collines des Haha, Agadir, Sous*) under *C. echinata*, without seeing the type (G00457529 = *C. arvensis*) from Mogador. Later the same author (Maire 1938: 424) revised his description and elevated this taxon to species. We agree with this last option, particularly when comparing with *C. maroccana*, the species where *C. murbeckii* has been included by recent authors (e.g. Ohle, 1975?), due to the different life form, achene morphology and 2C values.

C. pinnatiloba Maire (1928c: 57) A.C. Gonç. & P. Silveira ***comb. nov.*** Basionym: *C. murbeckii* var. *pinnatiloba* Maire (1928b: 57); Maire (1938: 424). **Lectotype** (designated here):— MOROCCO. Agadir, Sud-Ouest du Maroc '*Par les soins de M. Beaumier,*' *Mardochee s.n.* (P! [02413663]).

Homotypic synonym:

C. echinata subsp. *murbeckii* var. *pinnatiloba* (Maire) Maire (Jahandiez and Maire 1934: 788) *p.p. quoad distr.* "*Secteur macaronésien marocain*".

Heterotypic synonyms:

C. suffruticosa var. *pinnatiloba* Cosson *in schedulis* ex Lanza (1919: 134) *nom. nudum.*

C. suffruticosa subsp. *ifniensis* Font Quer (1936: 16); Maire (1941: 1152). Lectotype (designated here): —MOROCCO. *In montibus Sidi Tual et Bu-Mesguida, in rupibus arenaceis*, 1 000 a 1 250 m alt., 14 April 1935, *Font Quer s.n.* (holotype: BC! [812047! and 812050!]).

C. maroccana subsp. *murbeckii* (Lanza) Ohle (1975 a: 13) *p.p. quoad distr.* Mogador, Agadir.

Annual herbs. Stems (14) 18–57.5 (70) cm long, diffuse to erect, with glandular hairs. Basal leaves (2.8) 4.2–5.6 (6.3) × (0.5) 0.7–1 (1.2) cm, linear to oblanceolate, apex acute to acuminate, margins sinuate-dentate with acute teeth, with glandular hairs. Capitula solitary, (1.8) 2.3-3.5 (3.9) cm, concolorous or discolorous. Achenes heteromorphic: outer achenes rostrate (10) 12–14 (15) × (1.5) 2.8–4.5 (5) mm, ± straight or slightly curved, without, or with small, dorsal spines, occasionally with a apical ventral tooth; middle achenes bialate, or sometimes trialate with similar dimensions, (5.8) 8.2–9.2 (11.2) × (5.8) 7–8.8 (10.5) mm, lateral wings sub-entire to sinuate-dentate; cymbiform (3.5) 4.2–5.2 (5.3) × (2.5) 2.8–3.5 (4.2) cm; inner achenes vermiculate-exalate (2.5) 3.3–4 (4.2) × (0.8) 1–1.3 (1.5) mm, circular to hemicyclic, with a small ventral wing or with apical and/or basal teeth.

Habitat and distribution: Clay -limestone rocks; coastal plains and hills, at 20–150 m elevation and is found in arid Mediterranean bioclimates. Endemic to Agadir, Aït-Baha, Tamanar, Adar-Ou-Aman, Cap Rhir and Sidi Ifni – Figure 66.

Conservation status: This species is under numerous threats, especially climate changes (in some places rise in sea level, extreme weather events) and urban development (coastal development, urbanisation, tourism). The species is currently known from about five subpopulations. The number of mature individuals is estimated to be <2 500 based on field observations and declining. The estimated extent of occurrence is 1 994.687 km^2, which qualifies for EN and the estimated area of occupancy is 20 km^2, which also qualifies for EN. Therefore, we propose its assessment as EN B1ab(ii, iii, iv, v) +21ab(ii, iii, iv, v).

Chromosome number: $2n = 18$.

Genome size: 2.09 ± 0.15 pg.

Notes: Maire (1928: 57) indicated several specimens (syntypes) in the description of this taxon. Therefore, there is the need to select one as type (lectotype). We prefer Mardochée's specimen, which is the oldest. Since Font Quer (1936: 16) cites two collections as types of is *C. suffruticosa* subsp. *ifniensis*, there is also the need to select one as type (lectotype).

C. stellata Cavanilles (1791, 3). Desfontaines (1799: 3048); Fennane and Ibn Tattou (1998: 24, 2009: 29); Valdés (2002: 671). **Type: —**AFRICA?: '*Plant grow from seeds*', *Lemmonier s.n* ([*fide* Heyn et al. 1974]).

Homotypic synonym:

C. arvensis var. *stellata* (Cav.) Lanza

Heterotypic synonyms:

C. algeriensis Boissier & Reuter (1859: 109). Lectotype (designated by ?) – ALGERIA: circa Alger, 1849, Boissier & Reuter s.n. (G photo! [00022976]).

C. sicula Willdenow (1809: 934)

C. arvensis var. sicula (Willd.) Quézel & Santa (1963)

C. bicolor Rafinesque (1810: 82)

C. bicolor var. *cossonii* Quézel & Santa (1963)

C. bicolor var. *faurelii* Quézel & Santa (1963)

C. bicolor var. *odettei* Quézel & Santa (1963)

C. vidalii Pau (Pau y Español 1924)

C. denticulata Schousb. In Willdenow (1809: 935)

Annual herbs. Stems (2) 17.3–45.5 (80) cm long, ascending to erect, sometimes decumbent, branched at the base, with glandular hairs predominating over non-glandular hairs, ± viscous. Basal leaves (1.2) 3–6,5 (10) × (0.4) 1.5–3 (4) cm, (0.3) 0.3–0.5 (0.5) mm thick, oblanceolate, acute or, more frequenty, obtuse, margin sub-entire to sinuate-dentate, base attenuated in a ± longer petiole, with glandular hairs predominating over non-glandular hairs, ± viscous; the middle and upper cauline leaves progressively smaller towards the apex, oblanceolate to lanceolate, sessile and usually auriculate. Capitula solitary, (2,5) 3–4 (5) cm of in diameter. Achenes heteromorphic: outer achenes rostrate (5) 7.1–11.7 (15.2) × (0.8) 1.3–1.9 (2.7) mm, usually strongly curved and with long spines at the back, usually with two small teeth, one at the base and another at the apex; sometimes bialate (8.3) 10.8–12.4 (13.3) × (7.2) 7.7–9.3 (10.2) mm, wings with irregularly incised margins, extended along both sides up to the apex; middle achenes cymbiform (4.8) 6.7–8 (8.5) × (3.8) 5.3–7 (8) mm; inner achenes vermiculate-alate (3.5) 3.8–5.2 (5.8) × (2) 2.7–3.3 (3.7) mm, hook-shaped to circular, with two narrow lateral wings, and/or vermiculate-exalate (3.2) 3.7–4.5 (5.3) × (1.2) 1.2–1.7 (1.8) mm, circular.

Habitat and distribution: widespread taxon in Morocco, Algeria, Tunisia and Sicilia, occurring in ruderal and waste ground cultivated fields and grasslands or in the margin of roads and ditches, up to 1300 m.

Conservation status: Since this is a widespread taxon we assess it as least concern (LC), based on the IUCN criteria, for the study area.

Chromosome number: $2n = 44$.

Genome size: 2.11 ± 0.10 pg.

C. suffruticosa Vahl (1791: 94). Lectotype (designated by Ohle 1975b):—TUNISIA. '*Legi in montosis circa Portum Farinam Tuneti*', *Forskål s.n.* [not Vahl *fide* Ohle 1975b] (C! [100003279]).

Key to the subspecies of *C. suffruticosa*

1. Trialate achenes present 2

   Trialate achenes absent..7

2. Basal leaves linear to narrowly oblanceolate .... subsp. *tunetana*

   Basal leaves oblanceolate to spatulate..3

3. Rostrate achenes curved forming and angle of aprox. 90º and with spines 0.5-1.5 mm on the dorsal face.. subsp. *djurdjurensis*

   Rostrate achenes strait, or not so curved, usually without spines 4

4. Plants with a predominantly white-arachnoid indumentum.................................5

   Plants glabrous to glandulose 6

5. Trialate achenes (11.7) 12.4–13.8 (14.5) × (8.8) 9.1–9.2 mm, with length/width > 1.5..subsp. *marsea*

Trialate achenes (6.3) 8.4–9.8 (10.3) × (5.3) 6–9.1 (10) mm, with length/width approx. 1 .... subsp. *osteni*

6. Trialate achenes length (7.8) 8.5–9.2 (9.8) mm .... subsp. *trialata*

Trialate achenes length (7.7) 10.2–13.5 (16.2) mm .... subsp. *monardii*

7. Middle achenes exclusively bialate (10.5) 13.5–16.1 (22) × (6.7) 8.5–11.1 (11.7) mm, with a rostrum (2.5) 3.3–4.5 (9.7) mm, lateral wings sinuate-dentate to incise dentate; inner vermiculate achenes falcate .... subsp. *foliosa*

Middle achenes not exclusively bialate, with another more predominant type of achene (sub-cymbiform or cymbiform), and/or smaller and margins entire or almost entire; inner vermiculate achenes ±circular, not falcate 8

8. Middle achenes sub-exalate or, at least, predominantly sub-exalate .... subsp. *lusitanica*

Middle achenes, not sub-exalate.. 9

9. Outer rostrate achenes length (17) 21.5–26.5 (29) mm, with conspicuous dorsal spines averaging 2 mm .... subsp. *fulgida*

Outer rostrate achenes smaller, without spines or with them much smaller or less abundant 10

10. Indumentum of leaves and stems predominantly white-arachnoid 11

Indumentum of leaves and stems not predominantly white-arachnoid, ± glabrous to glandulose, or mostly glandulose with young parts ± white-arachnoid 12

11. Outer rostrate achenes length (5) 6–10.2 (13.5) mm, with 4-6 dorsal spines averaging 1 mm; middle achenes exclusively cymbiform .... subsp. *dercana*

Outer rostrate achenes length (7) 12.3–16.1 (17) mm, without dorsal spines; middle achenes cymbiform, sub-cymbiform and/or bialate ... subsp. *hosmarensis*

12. Indumentum of leaves and stems predominantly ± glabrous to glandulose....... 13

Indumentum of leaves and stems mostly ± glabrous to glandulose but with young parts ± white-arachnoid 14

13. Rostrate achenes length (21) 24–30 (31) mm; cymbiform achenes width 5.8–7.3 (8.3) mm .... subsp. *tazzea*

Rostrate achenes length (11) 16–23 (26) mm; cymbiform achenes width (6.7) 7.7–10.8 (11.8) mm ....subsp. *suffruticosa*

14. Ratio length/width of basal leaves (2.5) 2.7–4.2 (4.7); basal leaves thickness (0.2) 0.3–0.4 (0.5) mm .... subsp. *riffiniensis*

    Ratio length/width of basal leaves (4.9) 5.0–6.3 (6.5); basal leaves thickness (0.3) 0.4–0.6 (0.8) mm .... subsp. *boccoyana*

subsp. ***boccoyana*** A.C. Gonç. & P. Silveira ***subsp. nov.*** **Type:** —MOROCCO. Al Hoceïma, Bokkoya, Taoussarte, 133 m [35°13'08" N, 4°05'14" W], 14 May 2014, *Silveira & Gonçalves 3340* (holotype AVE!)

C. suffruticosa subsp. *boccoyana* seems close to *C. suffruticosa* subsp. *riffiniensis* but it has larger [(6.2) 8.5–9 (9.5) × (1.8) 2.1–2.8 (3) cm] and thicker [1.7–2.2] basal leaves.

Perennial herbs. Stems (17) 24–39 (50) cm long, prostrate to decumbent, with both glandulose and white-arachnoid pubescence, the latter predominant in younger parts. Basal leaves (6.2) 7.4–9.3 (9.5) × (1.2) 1.3–1.8 cm, (0.3) 0.4–0.6 (0.8) mm thick, oblanceolate to spatulate, apex obtuse, or sometimes ± acute, margins sub-entire to sinuate-dentate, with both glandulose and white-arachnoid pubescence, the latter predominant in younger parts. Capitula solitary, (2) 2.2–2.8 (3.2) cm diameter. Outer achenes rostrate (10) 13–19.5 (24) × (1) 1.5–2 (2.3) mm, straight or slightly curved, without dorsal spines, sometimes with one teeth at the base and/or at the apex; middle achenes bialate (13.8) 14.4–17.5 (18) × (3) 3.3–8 (8.7) mm, with a rostrum (3.3) 5–9.3 (10) mm; most frequently cymbiform (4.7) 5.8–8.3 (8.8) × (3.2) 4–5.5 (8) mm, with ventral wings surpassing the lateral ones; inner achenes vermiculate-alate (5) 5.2–5.3 × (2.2) 2.7–3.7 (4.8) mm, and vermiculate-exalate (3) 3.8–5 (5.5) × (1.2) 1.4–2 (2.2) mm, both circular.

Habitat and distribution: Limestone rocks/cliffs, at ± 133 m elevation, and is found growing under a semi-arid Mediterranean bioclimates. Endemic to Morocco with a restricted distribution in the National Park of Al Hoceïma (Bokkoya) coastal mountains to El-Yebha.

Conservation status: This taxon is under numerous threats, especially climate change (in some places rise in sea level, extreme weather events) and drought. The species is rare and local with a small-restricted range; the species is currently known from about two populations located in hillside slopes with difficult access. The number of mature individuals is estimated to be <50 in one of the populations, based on field observations, but we do not have accurate data for other localities whose access is difficult, in the coastal cliffs. The estimated extent of occupancy is 121 km^2, and the estimated area of occupancy is 16 km^2, both qualify for EN. This result in a categorisation of NT.

Chromosome number: $2n = 32$.

Genome size: 3.17 ± 0.06 pg.

subsp. ***dercana*** A.C. Gonç. & P. Silveira **subsp. *nov.* Type:** —MOROCCO: Tetouan, Jbel Dersa, 468 m [35°35'26" N, 5°22'45" W], 7 June 2012, *Silveira & Gonçalves 3262* (holotype: AVE!)

C. tomentosa Desf. (1799: 305) *p.p.* in regno Maroccano

C. suffruticosa subsp. *dercana* seems close to *C. suffruticosa* subsp. *hosmarensis* but it has smaller rostrate achenes (with 8-10 mm instead of 11-17 mm) and with dorsal spines, and its middle achenes are exclusively cymbiform, while *C. suffruticosa* subsp. *hosmarensis* presents other types of achenes

Perennial herbs. Stems (11) 17–32.5 (65) cm long, prostrate to decumbent, with white–arachnoid pubescence. Basal leaves (3.2) 3.5–7 (7.8) × (0.7) 1.1–2 (2.4) cm, (0.3) 0.4–0.4 (0.6) mm thick, oblanceolate to spatulate, apex obtuse, or sometimes ± acute, margins sub-entire to sinuate-dentate, with white–arachnoid pubescence. Capitula solitary, (1.8) 2.8–3.2 (3.6) cm diameter. Outer achenes rostrate (5) 6–10.2 (13.5) × (1.2) 1.3–1.5 (1.7) mm, straight or slightly curved, without dorsal spines, sometimes with 1–2 teeth, at the base or at the apex; middle achenes cymbiform (5,5) 5.8–6.6 × (3.6) 3.8–5.6 mm; inner achenes vermiculate-exalate (3) 3.3–3.5 (3.7) × (1.3) 1.5–1.7 mm.

Habitat and distribution: The species occurs in limestone rocks/cliffs, at ± 500 m elevation, and is found in per humid Mediterranean bioclimates. Endemic to Jbel Dersa in Tetouan (Morocco).

Conservation status: This taxon is under numerous threats, especially agricultural intensification (grazing), climate change (extreme weather), and human intervention. The species is rare and local with a small-restricted range; only one population is currently known close to abandoned pastures/fields. The number of mature individuals is estimated to be <50 based on field observations. The estimated area of occupancy is 8 km^2 and qualify for CR. The population is. This result in a categorisation of CR B2ab(ii, iii).

Chromosome number: 2*n* = 32.

Genome size: 3.00 ± 0.07 pg.

subsp. ***djurdjurensis*** Ohle ex A.C. Gonç. & P. Silveira **subsp. *nov.* Type:** —ALGERIA. Algier: versant septentrional des montagnes du Djurdjura, territoire des Beni Bou Addou, cercle de Dra el Mizan, July 1854, *Boissier, P. E. s.n.* (holotype G!).

Heterotypic synonyms:

C. suffruticosa subsp. *boissieri* Lanza (1919: 142, 143); Ohle (1975b: 539); *p.p. quoad distr.* Djurdjura.

C. suffruticosa subsp. *boissieri* Lanza (1919, 142); Ohle (1975b, 539);

"C. tomentosa" auct. Battandier & Trabut (1888, 479) *p.p. quoad distr.* Djurdjura *non* Desfontaines (1799: 305), *nom. illeg.*, *non* Linnaeus filius (1782: 384)

C. suffruticosa subsp. *djurdjurensis* seems close to *C. suffruticosa* subsp. *boissieri* but it differs by its leaves predominantly glandular pubescent and middle achenes sub-cymbiform to trialate with lateral wings with serrate margins.

Perennial herbs, ± woody at the base. Stems (12) 17.8–29 (52) cm long, erect to ascending, ± branched, predominantly glandular pubescent to slightly white-arachnoid pubescent. Basal leaves (6.5) 7.8–10.4 (11.5) × (1.8) 2–2.8 (3) cm, 0.3–0.4 mm thick, narrowly oblanceolate to obovate, apex acute, or sometimes ± obtuse, margins sinuate-dentate, with glandular and non-glandular white-arachnoid pubescence. Capitula solitary, (3) 3.4–5.3 (5.6) cm diameter. Outer achenes rostrate (8.3) 10.3–12.3 (13.8) × (1.7) 1.8–2.3 (2.5) mm, curved at aprox. 90º, with dorsal spines 0.5 – 1.5 mm long, normally with one basal tooth; middle achenes sub-cymbiform to trialate (7.5) 8.4–9.7 (10.8) × (5.5) 5.8–6.8 (9.2) mm, with ventral wings smaler than the lateral ones, and lateral wings with serrate margins; inner achenes vermiculate-exalate (5) 5.5–6.2 (7.2) × (1.7) 1.8–2.3 (2.8) mm, generally circular to hemicyclic.

Habitat and distribution: Limestone mountains at 1150–1800 m elevation and is found in humid Mediterranean bioclimates. Endemic to Algeria with a restricted distribution to the Djurdjura Mountains (National Park of Tikdja, Tizi n'Kouilal).

Conservation status: This taxon is under numerous threats, especially climate change, and development (uncontrolled tourism, leisure activities). The species is rare and local with a small-restricted range. However, although we found only one population, there seems to be more in other parts of the Djurdjura Mountains. The estimated area of occupancy is 4 km^2, which qualifies for CR. However, since there is no appropriate information to assess their extinction risk based on distribution and/or population we assess this taxon as Data Deficient (DD) on the IUCN Red List.

Chromosome number: $2n = 32$.

Genome size: 3.10 ± 0.14 pg.

Note: Although Ohle has not published this taxon, the achene’s morphology corresponds its "*C. suffruticosa* var. *djurdjurensis*" *in sched.* – CAL44 (D4766) from Djurdjura.

subsp. ***foliosa*** (Batt.) A.C. Gonç & P. Silveira, ***comb. nov.*** Basyonym: *C. foliosa* Batt. in Battandier & Trabut (1888: 479). Lectotype (designated here): — ALGERIA. Djebel Bou Zecza, *May 1882*, *Battandier s.n.* (MPU! [007674]).

Homotypic synonyms:

C. fulgida var. *foliosa* (Batt.) Quezel & Santa (1963: 968) *des. inval.*

C. tomentosa var. *foliosa* (Batt.) Battandier & Trabut (1902: 189).

Heterotypic synonyms:

C. suffruticosa subsp. *boissieri* Lanza (1919: 143) *p.p.* versante .settentrionale dei nionti del Djurdjura a Dra el Mizan

C. fulgida var. polymorphocarpa Lanza (1919, 137)

Perennial herbs. Stems (29) 32–46.3 (53) cm long, erect to ascending, with glandular and non-glandular pubescence, in some areas, sligtlhy white-aracnoid. Basal leaves (3.5) 3.8–5.1 (5.6) × (1) 1.2–1.6 (2.5) cm, (0.2) 0.3–0.3 (0.3) mm thick, obovate to oblong, apex acute to obtuse, margins sub-entire to slightly sinuate-dentate, with glandular and non-glandular pubescence, sligtlhy white-aracnoid in the margins. Capitula solitary, (2.2) 2.6–3.4 (4.2) cm diameter. Outer achenes rostrate (9) 12.3–23.8 (27) × 2–2.5 (2.8) mm, ± straight or slightly curved, without dorsal spines, with or without a ventral basal tooth; middle achenes bialate (10.5) 13.5–16.1 (22) × (6.7) 8.5–11.1 (11.7) mm, with a rostrum (2.5) 3.3–4.5 (9.7) mm, lateral wings sinuate-dentate to incise dentate; inner achenes vermiculate-exalate (3.3) 4.3–5.5 (7.2) × (1.3) 1.7–2 (2.8) mm, falcate.

Habitat and distribution: Limestone rocks/cliffs, at ± 200 m elevation, and is found in sub-humid Mediterranean bioclimates; Endemic to Gorges of Lakhdaria (ex Palestro), Zaccar, Jbel Bou Zecza.

Conservation status: The species is under numerous threats, especially climate change and drought. The species is rare and local with a small restricted range; only one population is currently known (classical population), but there may be others in this region. The number of mature individuals is estimated to be <250 based on field observations. The estimated area of occupancy is 4 km^2 and qualify for CR. This result in a categorisation of Data Deficient (DD) on the IUCN Red List, because there is inadequate information to assess their extinction risk based on distribution and/or population status.

Chromosome number: $2n = 32$.

Genome size: 3.08 ± 0.04 pg.

Notes: Since few herbarium materials are available; this taxon went unnoticed by many botanists. Even Lanza (1919), showed his concern about '*C. foliosa*,' placing it under *C. fulgida* var. *polymorphocarpa*. However, there is an evident consistence of characters on the available specimens, which as been also proved by us to be persistent under culture. Since Battandier (in Battandier & Trabut 1888: 479) listed two localities for is taxon (Djebel bou Zecza and Gorges de Palestro), there is the need to select one of is specimens as lectotype. We selected MPU007674, since it is clearly from one of the mentioned localities (Djebel bou Zecza), which is not possible to say from some of its other known collections (MPU007673 has been databased as “Gorges de "illisible"”, and P00084054 is from “Gorges de Kedara”).

subsp. ***fulgida*** (Raf.) Guadagno (1922: 64). Fennane & Ibn Tattou 1998, 2005; Gonçalves et al. 2014. Basionym: *C. fulgida* Raffiniesque (1810: 82). Neotype (designated here): — ITALY. Escarpments of Monte Pellegrino, Palermo, Sicily (loco Rafinesque), 04 June 1847, *Kralik s.n.* (P! [04277642]).

Perennial herbs. Stems (41) 46.8–71 (89) cm long, prostrate to decumbent, with glandular and non-glandular pubescence, mostly white-aracnoid towards the base. Basal leaves (6.2) 7.5–9.9 (10.8) × (1.4) 1.5–2 (3.3) cm, (0.1) 0.2–0.2 (0.3) mm thick, oblanceolate to spatulate, apex obtuse, or sometimes ± acute, margins sub-entire to sinuate-dentate, with glandular and non-glandular pubescence, white-aracnoid especially in the margins and midrib. Capitula solitary, (2.3) 2.6–3.2 (3.4) cm diameter. Outer achenes rostrate (17) 21.5–26.5 (29) × (1.2) 1.8–3.4 (3.8) mm, straight or slightly curved, with conspicuous dorsal spines averaging 2 mm long, sometimes with one teeth, at the base and/or at the apex; or bialate rostrate (15) 18.3–23.5 (29) × (7.5) 9.7–13 (15.2) mm, with a rostrum (5.3) 8.6–14.4 (15.2) mm, middle achenes cymbiform (5) 6.8–9.3 (11.7) × (6.5) 7.5–9.2 (11.5) mm, with ventral wings smaller than the lateral ones; inner achenes vermiculate-alate 5–6.2 (6.7) × (2.3) 2.5–3.4 (3.5) mm; or vermiculate-exalate (3.7) 3.8–4.2 (4.5) × (0.8) 1.3–1.5 mm, both circular.

Habitat and distribution: Limestone rocks/cliffs, c. 50-1200 m, in sub-humid Mediterranean bioclimate. This taxon in Morocco is distributed in the Béni Snassen Mountains, but there are other well-known native populations in Italy, Sicily and Malta (Greuter (2006+[2017]).

Conservation status: This taxon is under numerous threats, especially climate change (extreme weather), and development (tourism, leisure activities). The species is currently known from three populations in the Béni Snassen Mountains, but there may be others in this region. The number of mature individuals is estimated to be <250 based on field observations. The estimated extent of occupancy is 11.26 km^2 and qualify for CR, and the estimated area of occupancy is 12 km^2 and qualify for EN. This result in a categorisation for Morocco of Data Deficient (DD) on the IUCN Red List, because there is inadequate information to assess their extinction risk based on distribution and/or population status.

Chromosome number: *2n* = 32.

Genome size: 3.06 ± 0.21 pg.

Notes: Most of Rafinesque's herbarium was lost wen E.M.Durand (1794-1873) bought it, and when finding that it was in very bad shape he discarded a great part of the specimens (Pennell 1944-1945). Later, Durand sent his personal herbarium to Paris, but it is difficult to known which specimens came from Rafinesque's herbarium because they usually lack labels (Reveal 2013). The result of this is that no previous author could find the type of rafinesque's *C. fulgida.* Therefore, we have chosen one that seemed a god option for a neotype.

subsp. ***hosmarensis*** A.C. Gonç. & P. Silveira ***subsp. nov.*** Type: —MOROCCO. Tetouan: Jbel Gorghiz (Beni Hosmar) [35°28'50" N, 5°22'06" W], 6 June 2012, *Silveira & Gonçalves 3261* (holotype AVE!).

Heterotypic synonims:

C. tomentosa Desf. (1799: 305), nom. illeg., non Linnaeus filius (1782: 384) *p.p.* in regno Maroccano

C. incana subsp. *incana* Willd. (Willdenow 1803: 2342) *p.p. in regno Maroccano*

C. suffruticosa subsp. *tomentosa auct. non* (Desf. ex Ball) Murb. (1905: 9) *sensu* Ball (1878)

C. suffruticosa subsp. *hosmarensis* seems close to *C. suffruticosa* subsp. *dercana* but it has bigger rostrate achenes, with (7) 12.3 – 16.1 (17) mm instead of (5) 6 - 10.2 (13.5) mm, and without dorsal spines, and its middle achenes are of diverse types and not exclusively cymbiform like in subsp. *dercana.*

Perennial herbs. Stems (8) 15–42 (55) cm long, prostrate to decumbent, ± densely white–arachnoid pubescent. Basal leaves (2.2) 3.6–5 (6.5) × (0.6) 1–1.3 (1.5) cm, 0.2–0.3 (0.5) mm thick, oblanceolate to spatulate, apex obtuse, or sometimes ± acute, margins sub-entire to sinuate-dentate, white-arachnoid pubescent. Capitula solitary, (2.3) 2.5–3.4 (3.8) cm diameter. Outer achenes rostrate (7) 12.3–16.1 (17) × (1.2) 1.3–1.5 (2) mm; middle achenes bialate (9) 10.7–13.2 (15.8) × (4.7) 6.7–7.7 (8.3) mm, with a rostrum (3.3) 4.4–7.3 (9.2); trialate (6) 8–15 (17) × (1.3) 1.5–1.9 (2.3) mm; or cymbiform (6.3) 6.7–8.8 (11) × 5.2–6.8 (7.3) mm; inner achenes vermiculate-exalate (3.3) 3.5–4.3 (4.5) × (1.3) 1.5–1.7 (1.8) mm, circular.

Habitat and distribution: Limestone mountains, at ± 430-1070 m elevation, in pre-humid Mediterranean bioclimate. This taxon is endemic to Morocco with a restricted distribution to the southern Tetouan Mountains (Beni Hosmar).

Conservation status: This taxon is under numerous threats, especially climate change (extreme weather), and droughts. The species is rare and local with a small restricted range; only two populations are currently known and few herbarium specimens are known (from the same locality). The number of mature individuals is estimated to be <50 based on field observations. The estimated area of occupancy is 8 km2 and qualify for CR. This result in a categorisation of DD Data Deficient on the IUCN Red List, because there is inadequate information to make an assessment of their extinction risk based on distribution and/or population status.

Chromosome number: $2n = 32$.

Genome size: 3.10 ± 0.10 pg.

subsp. ***lusitanica*** (Boissier) Ohle (1974: 270); Fennane & Ibn Tattou (2005); Gonçalves et al. (2014 : 272). Basionym: *C. lusitanica* Boissier (1849: 83). Lectotype (designated by Burdet et al. 1983):—PORTUGAL. Estremadura: '*prope Cintra Lusitaniae in petrosis*', *Guthnik* s.n. (G!).

Perennial or annual herbs, ± woody at the base. Stems (24) 59–100.5 (120) cm long, diffuse to erect, ± branched, with glandular hairs predominating over non-glandular, sometimes white-aracnoid, hairs, ± viscous. Basal leaves (3.2) 3.5–6.2 (6.3) × (0.8) 1.1–1.5 (2) cm, (0.3) 0.4–0.5 (0.6) mm thick, narrowly oblanceolate to spatulate, apex acute to obtuse, margins sub-entire to sinuate-dentate, attenuate into a ± large petiole, with glandular hairs more abundant than non-glandular hairs, except in the margins, where white-aracnoid hairs predominate. Capitula solitary, (1.5) 2.4–3 (3.3) cm diameter. Outer achenes rostrate (7) 8.7–11 (13) × (1.5) 1.6–2 (2.2) mm, generally slightly curved to straight, without dorsal spines or with them small, sometimes with one tooth at the base and/or another at the apex; middle achenes typically sub-exalate (7.5) 7.8–9.4 (9.7) × (5) 5.3–6.9 (7.2) mm, lateral wings missing or narrow and sinuate-dentate, and ventral wing also missing or narrow and sub-entire, less frequently sub-cymbiform to cymbiform (5.2) 6.1–7.5 (10.7) × (4.2) 5–6.1 (8) mm, with the ventral wing smaller to sub-equal to the laterals; inner achenes vermiculate-exalate (3.8) 4.2–4.9 (5) × (1.5) 1.7–2 (2.3) mm, always present, hemicyclic to circular.

Habitat and distribution: Clearings of forests and shrub vegetation, on granitic, loamy, limestone soils, hills, and mountains of the coast; 0–500 m elevation and is found in sub-humid Mediterranean bioclimates. West and South of Portugal, in Morocco, it has a distribution restricted to Benzu (Ceuta)

Conservation status: This taxon is under numerous threats, especially climate change (extreme weather), and human intervention (gardening or embankment fixation). It is rare and local with a small-restricted range; only one population is currently known. The number of mature individuals is estimated to be <250 based on field observations. The estimated area of occupancy is 4 km^2, which qualifies for CR. We propose to assess this taxon for Morocco as CR C2a(ii).

Chromosome number: *2n* = 32

Genome size: 3.36 ± 0.15 pg.

subsp. ***marsea*** A.C. Gonç. & P. Silveira ***subsp. nov.*** **Type:** — MOROCCO. Tanger, Oued Marsa, 3–5 m, [35°54' N, 5°27' W], 13 June 2012, *Silveira, P. & Gonçalves, ACRS 3268* (holotype AVE!);

C. suffruticosa subsp. *marsea* seems close to *C. suffruticosa* subsp. *osteni* but its midlle achenes are bi-trialate (10.5) 13.6–16 (22) mm, with length/width > 1.5, instead of trialate (6.3) 8.5–9.8 (10.3) mm, with length/width approx. 1.

Perennial herbs. Stems (24) 30.3–36.3 (43) cm long, erect to ascending, with glandular hairs distaly and predominace of non-glandular white-aracnoid pubescence proximaly, not viscid/viscous. Basal leaves (3.5) 4–5.5 (6) × (0.9) 1.1–1.7 (1.8) cm, 0.4–0.6 (0.7) mm thick, obovate to oblong, apex obtuse, margins sub-entire to slightly sinuate-dentate, densely white-arachnoid pubescent, with progressively less white-aracnoid and more glandular pubescence towards the apice of the stems. Capitula solitary, 1.7–2.6 (3) cm diameter. Outer achenes rostrate (12) 17.8–24 × (1.3) 1.6–2.2 (2.3) mm, ± straight or slightly curved, without dorsal spines, without a ventral basal tooth; middle achenes bialate (10.3) 10.8–17 (18) × (5) 5.8–8.3 (8.8) mm, with a rostrum (2.5) 2.6–6.9 (7.5) mm, to trialate (11.7) 12.4–13.8 (14.5) × (8.8) 9.1–9.2 mm, lateral wings sinuate-dentate to incised; inner achenes vermiculate-exalate (3,7) 4,8–5,3 (5,5) × (1,2) 1,8–2,2 (2,3) mm, hemicyclic to circular.

Habitat and distribution: We could only found this taxon growing slightly above sea level, but there are older specimens, probably of the same taxon, collected at 400-500 m elevation. It is found in sub-humid Mediterranean bioclimate. Endemic to Morocco with distribution that seems restricted to the NW foot of Jbel Mousa, margins of Oued Marsa (Tétouan).

Conservation status: This taxon is under numerous threats, especially climate change (extreme weather, the rise in sea level), and human intervention. The species is rare and local with a small-restricted range; only one population is currently known and suffering from high habitat degradation due to human pressure. The number of mature individuals is estimated to be <50 based on field observations. The estimated area of occupancy is 4 km^2, which qualifies for CR. Although it is not known if there are more populations or not, this is not very probable, so we propose to assess this species as CR B2ab(iii, v), C2a(I, ii), D.

Chromosome number: $2n = 32$.

Genome size: 3.44 ± 0.12 pg.

subsp. ***monardii*** (Boissier & Reuter 1859: 108) Ohle (1975b). Le Floc'h & Boulos (2008). Basyonym: *C. monardii* Boissier & Reuter (1859: 108). **Type** (corrected here): —ALGERIA. Sidi Ferruch, July 1830, *Monard s.n.* (holotype G! [0386646]).

Perennial herbs. Stems (30) 55–67 (84) cm long, erect to ascending, with glandular pubescence predominating over non-glandular, sometimes white-aracnoid, pubescence. Basal leaves (3.2) 4.6–5.7 (6.5) × (0.6) 0.9–1.4 (2.1) cm, 0.5–0.6 (0.8) mm thick, obovate to oblong, apex acute to obtuse, margins sub-entire to slightly sinuate-dentate, lamina with few glandular and non-glandular hairs, and margins predominantly with white-aracnoid hairs. Capitula solitary, (2.5) 3.1–4 (4.6) cm diameter. Outer achenes rostrate (9) 13–18 (23) × (1.3) 1.5–1.8 (2.3) mm, ± straight or slightly curved, without dorsal spines, or with few and small, with or without a ventral basal tooth; middle achenes trialate (7.7) 10.2–13.5 (16.2) × (5.8) 8.3–9.8 (11.2) mm, margin of lateral wings slightly sinuate-dentate; inner achenes vermiculate-alate 3,8–5,3 x 2,5–2,7 (3,3); and vermiculate-exalate (3,3) 4,3–5,2 (6,3) x (1) 1,5–1,8 (2,7) mm, both circular.

Habitat and distribution: Coastal limestone rocks/cliffs and sandy dunes, near the sea, from see level to ± 300 m elevation, and is found in semi-arid Mediterranean bioclimates. Distributed between Alger (Algeria) and Cape Tabarka (Tunisia).

Conservation status: This taxon is under numerous threats, especially climate change (extreme weather), and development (infrastructures, urbanisation, tourism, leisure activities). The species is currently known from about five populations with a small-restricted range. The number of mature individuals is estimated to be <250 based on field observations. Based on our field collection, the estimated extent of occupancy is 16.264 km^2 and qualify for CR, the estimated area of occupancy is 20 km^2 and qualify for EN. Although not taking into account all populations, there is an inferred continuing decline in the area of occupancy, quality of habitat and number of mature individuals. This result in a categorisation of CR B2b(ii, iv, v).

Note: The type of *C. monardii* is not at MPU like is stated by Ohle (1975b: 532), but at G.

Chromosome number: *2n* = 32.

Genome size: 2.86 ± 0.07 pg.

subsp. ***osteni*** A.C. Gonç & P. Silveira ***subsp. nov.*** Type: MOROCCO. Al Hoceïma, Gurugú, 950 m, [35°19' N, 2°57' W], 19 May 1932, *Sennen, F. & Mauricio, Hno.* 8 438 (holotype BM! [000813672]; isotypes BC! [139240], G!).

C. suffruticosa subsp. *osteni* seems close to *C. suffruticosa* subsp. *marsea* but its midlle achenes are trialate (6.3) 8.5–9.8 (10.3) mm, with length/width approx. 1, instead of bi-trialate (10.5) 13.6–16 (22) mm, with length/width > 1.5.

Perennial herbs. Stems (37) 44.5–75.5 (81) cm long, prostrate to decumbent, with glandular hairs distaly and predominace of non-glandular white-aracnoid pubescence proximaly, not viscid/viscous. Basal leaves (4.8) 5–7.9 (11.7) × (1.1) 1.5–2.1 (2.9) cm, 0.2–0.3 mm thick, oblanceolate to spatulate, apex obtuse, or sometimes ± acute, margins sub-entire to sinuate-dentate, with white-arachnoid pubescence. Capitula solitary, (2,3) 3.1–3.9 (4.0) cm diameter. Outer achenes rostrate (12) 16.5–25 × (1.7) 2.2–2.4 (2.5) mm, straight or slightly curved, without dorsal spines, sometimes with 1–2 teeth, at the base or at the apex; middle achenes trialate (6.3) 8.4–9.8 (10.3) × (5.3) 6–9.1 (10) mm, with a rostrum 2–3.6 (3.7) mm; inner vermiculate-exalate 4–4.3 × 1.8–1.8 mm, hemicyclic-falcate.

Habitat and distribution: As far as we know, this taxon might grows on greywacke rocks, under semi-arid Mediterranean climate, from sea level to 800 m ? (our collection is from c. 580 m) elevation, in mounts Gurugú and Kebdana, near Mellila, and with another know specimen from near sea level at Al Hoceima.

Conservation status: The taxon is under numerous threats, especially climate change (extreme weather events) and drought. The species is rare and local with a small restricted range; only one population is currently known by the team. The number of mature individuals is estimated to be <10 based on field observations. The estimated area of occupancy is 4 km^2, which qualifies for CR. Considering the number of individuals we propose to assess it as CR B2ab(ii, iv, v), due to the reduced size of the population and of its habitat, which confers a high risk of extinction due to climate changes, or other unexpected threats.

Chromosome number: 2n = 32.

Genome size: 2.96 ± 0.05 pg.

Notes: This name is inspired on a designation autored, but not validly published (McNeill *et al.* 2012, article 38.1), by Sennen & Mauricio (1933: 62), which was also used in the label of the specimen we selected as type.

subsp. ***riffiniensis*** A.C. Gonç. & P. Silveira ***subsp. nov.*** **Type:** — MOROCCO. Tétouan, Jbel Kelti, Arifane, 943 m, [35°17'04" N, 5°18'00" W], 15 May 2014, *Silveira, P.; Gonçalves, ACRS 3341* (holotype AVE!);

C. suffruticosa subsp. *riffiniensis* seems close to *C. suffruticosa* subsp. *boccoyana* but its basal leaves have a ratio length/width of (2.2) 2.4–4.0 (4.4) and a thickness of (0.2) 0.3–0.4 (0.6) mm.

Perennial herbs. Stems (28) 32–49 (71) cm long, erect to ascending with glandular hairs distaly and predominace of non-glandular white-aracnoid pubescence proximaly and in young branches. Basal leaves (4.1) 4.5–8.2 (8.3) × 1.5–2.1 (2.3) cm, (0.2) 0.3–0.4 (0.6) mm thick, obovate to oblong, apex obtuse, margins sub-entire to slightly sinuate-dentate, lamina with more glandular than non-glandular hairs, except in margins or leaves of young branches where non-glandular white-arachnoid pubescence predominates. Capitula solitary, (2.9) 3.2–3.6 (4) cm diameter. Outer achenes rostrate 17–21 (22) × (1.2) 1.3–1.5 (2.8) mm, ± straight or slightly curved, without dorsal spines, without a ventral basal tooth; middle achenes bialate (10) 10.8–15 × (5.8) 6.7–10 mm, with a rostrum (5) 5.4–7.9 (8.7) mm, margin of lateral wings sinuate-dentate to incised; cymbiform (5,8) 7,5–10,5 (11,3) × (5,0) 5,2–5,5 (5,8) mm; inner achenes vermiculate-alate (4,3) 4,5–5,8 (6,3) × (2,5) 2,8–4,5 (5) mm; and vermiculate-exalate 3,3–4,7 (5) × 1,5–1,7 (1,8) mm, both circular.

Habitat and distribution: Limestone rocks/cliffs, from 30 to 1800 m elevation, and is found in perhumid Mediterranean bioclimate. Endemic to Morocco with a restricted distribution to the southern Tetouan Mountains (Arrifane - Jbel Kelti).

Conservation status: This taxon is under numerous threats, especially climate change (extreme weather), and droughts. The taxon is rare and local with a small restricted range; only one population is currently known by the team, and few herbarium specimens are known (from the same locality). The number of mature individuals, in the population known by the team, is estimated to be <250 based on field observations. The estimated area of occupancy is 4 km^2, which qualifies for CR. However, we propose its assessment as Data Deficient (DD), because there is inadequate information to make an assessment of their extinction risk based on distribution, since we think that there might be other subpopulations in the area.

Chromosome number: 2n = 32.

Genome size: 3.06 ± 0.10 pg.

subsp. ***suffruticosa*** Vahl (1791: 94). Bonnet and Barratte (1896: 231); Jahandiez and Maire (1934: 789); Quezel and Santa (1963: 969); Potteier-Alapetite (1981: 1024); Le Floc'h and Boulos (2008: 80); Fennane and Ibn Tattou (2005: 29). Lectotype (designated by Ohle 1975b): —TUNISIA. 'Legi in montosis circa Portum Farinam Tuneti', *Forskål s.n.* [not Vahl fide Ohle 1975b] (C! [100003277]).

Perennial herbs. Stems (10) 16.5–25 (27) cm long, ascending to erect, sparsely glandular and non-glandular pubescent, very slightly white-aracnoid towards the base. Basal leaves (3.2) 5.1–6.5 (7.1) × (0.8) 0.9–1.2 (1.4) cm, 0.4–0.6 mm thick, broadly lanceolate, apex obtuse to slightly acute, margins sinuate-dentate, sparsely glandular and non-glandular pubescence, slightly white-aracnoid in the margins. Capitula solitary, (3.4) 3.6–4.6 (5.2) cm diameter, concolorous, yellow. Outer achenes rostrate (11) 16–23 (26) × (1.3) 1.7–2 (2.2) mm, straight or slightly curved, normally without dorsal spines or muricate, rarely with a ventral basal tooth; middle achenes bialate (12.5) 13–17.3 (18) × (5.8) 6.3–8.8 (9.2) mm, lateral wings sub-entire to sinuate-dentate, with a rostrum (3) 3.6–7.4 (7.5); more frequently cymbiform (8.8) 10.3–11.5 (14.5) × (6.7) 7.7–10.8 (11.8) mm, muricated dorsaly, ventral wing smaler to subequal to lateral wings; sub-cymbiform (6.7) 8.5–11.7 (12.8) × (8.2) 8.7–10.3 (10.8) mm, lateral wings sub-entire to sinuate-dentate; inner achenes vermiculate-alate (4.5) 4.8–7.3 (8.5) × (3.2) 3.3–4.7 (5.8) mm, and vermiculate-exalate 4.3–4.9 (5.3) × (1.3) 1.5–1.8 (2) mm, both circular to hemicyclic.

Habitat and distribution: Limestone rocks at 10–20 m elevation, near the sea, in semi-arid Mediterranean bioclimate. Endemic from Tunisia, Porto Farina (Ghar el Melh), Hammam-Lif, Korbous near Cap Bon.

Conservation status: Despite the small geographic distribution, the team was only able to visit one population (Ghar el Melh). The number of mature individuals is estimated to be <250 based on field observations. However, since there is inadequate information to assess their extinction risk based on distribution and/or population status, we propose to assess it as Data Deficient (DD).

Chromosome number: *2n* = 32.

Genome size: 3.28 ± 0.07 pg.

Notes: Since its description, this taxon was often confused with other taxa. Several authors cited *C. suffruticosa* for other Mediterranean areas: Portugal (Brotero 1804), Spain (Boissier 1849), Morocco (Ball 1873). Only Ball (1878) pointed out that his *C. maroccana* had nothing to do with *C. suffruticosa.* It has certain morphological similarities with those taxa, but the cymbiform achenes, do not resemble with the typical *C. suffruticosa*. Effectively, as far as we know, *C. suffruticosa* subsp. *suffruticosa* is restricted to the coast of the Gulf of Tunis, in Tunisia.

subsp. ***tazzea*** A.C. Gonç. & P. Silveira ***subsp. nov.*** **Type:** —MOROCCO. Taza, Ras-El-Ma, Sidi Msbar, 1460 m, [34°07'58" N, 4°07'58" W], 11 May 2014, *Silveira, P.; Gonçalves, ACRS 3334* (holotype AVE!).

C. suffruticosa subsp. *tazzea* seems close to *C. suffruticosa* subsp. *suffruticosa*, but its rostrate achenes are longer, (21) 24–30.0 (31) mm, and its cymbiform achenes are narrower, with 5.8–7.3 (8.3) mm width.

Perennial herbs. *Stems* (40) 40.5–81.5 (93) cm long, decumbent to erect, predominatly glandular pubescent distaly, slightly white-aracnoid proximaly. Basal leaves (6) 7.4–9.9 (10.5) × (0.9) 1.5–2.1 (2.6) cm, (0.2) 0.3–0.4 mm thick, oblanceolate, apex obtuse, or sometimes ± acute, margins sub-entire to sinuate-dentate, with glandular and non-glandular hairs to glabrescent, slightly white-arachnoid pubescent in the margins, becoming light green when dry. Capitula solitary, 3.2–4 (4.4) cm diameter. Outer achenes rostrate (21) 24–30 (31) × (2) 2.2–2.7 (2.8) mm, straight or slightly curved, without dorsal spines, sometimes with 1–2 teeth, at the base or at the apex; middle achenes bialate (16) 17,8–22,5 (25) × (7,3) 7,8–8,7 (10) mm, with a rostrum (3,3) 5,3–11 (13,7) mm; most frequently cymbiform (6.7) 8.5–11 (12.5) × 5.8–7.3 (8.3) mm, with ventral wings, frequently, surpassing the laterals; inner achenes vermiculate-alate (4.2) 6–7.5 (8.3) × (2.8) 4.5–4.8 (5.2) mm; vermiculate-exalate (3.3) 3.5–5.1 (5.7) × (1.2) 1.6–2.2 mm, circular.

Habitat and distribution: limestone rocks/cliffs, at ± 300 m elevation, and is found in subhumid Mediterranean bioclimate. Endemic to Morocco with a restricted distribution to the western Taza Mountains (Jbel Tazzeka).

Conservation status: The taxon is under numerous threats, especially climate change (extreme weather), and droughts. The species is rare and local with a small restricted range; only one population is currently known by the team, and few herbarium specimens are known (from the same region). The number of mature individuals is estimated to be <50 based on field observations. The estimated area of occupancy is 4 km2, which qualifies for CR. However, we propose its assessment as Data Deficient (DD), because there is inadequate information to make an assessment of their extinction risk based on distribution, since we think that there might be other subpopulations in the area.

Chromosome number: *2n* = 32.

Genome size: 3.42 ± 0.07 pg.

subsp. ***trialata*** P. Silveira & A.C. Gonç. (in press). **Type:** — SPAIN. Cádiz: Conil de la Frontera, [36°16'35" N, 6°05'15" W], 25 May 1981, *P. Cambó et al. 421/81* (holotype MA!).

Perennial herbs, ± woody at the base. Stems (25) 29.8–59.5 (66) cm, decumbent to diffuse, ± branched, mostly with glandular hairs and occasional non-glandular hairs, viscous. Basal leaves (3) 4–4.5 (5) × 0.6–1.1 cm, (0.4) 0.5–1.2 (1.5) mm thick, oblanceolate, apex acute to obtuse, margins sub-entire to sinuate-dentate or slightly undulate-dentate, attenuated in a ± large petiole, lamina with glandular and *non*-glandular hairs, mostly white-aracnoid in the margins, viscous. Capitula solitary, (1.5) 1.8–2.9 (3.3) cm diameter. Outer achenes rostrate (4.5) 5.4–14.1 (16) × 1.3–1.7 (2.5) mm, generally straight or slightly curved, without dorsal spines, at times with one tooth at the base; middle achenes, usually, trialate (7.8) 8.5–9.2 (9.8) × (6.3) 7–8 (8.8) mm, wings sub-entire and sinuate-dentate at the apex, sub-equal, or bialate (9.2) 10.3–13.5 (17) × (3) 5–6.9 (9.3) mm, with a rostrum (1.5) 2–5.7 (8.7) mm, with lateral wings sinuate-dentate; inner achenes vermiculate-exalate (3.8) 4–4.5 (4.7) × (1.3) 1.5–1.8 mm, generally hook-shaped to falcate.

Habitat and distribution: Dunes, sandy soils and hills not far from the sea, 0–130 m, near the lighthouse at Cape Cires in Tanger (Morocco).

Chromosome number: *2n* = 32.

Genome size: 3.21 ± 0.05 pg.

subsp. ***tunetana*** (Cuénod) A.C.Gonç. & P.Silveira ***Comb. nov.*** Basionym: *C. tunetana* Cuénod (1910, ci). **Type:** —TUNISIA. 'Berges sablounneuses de l'Oued, entre Bir bou Rekba et Hamamet', *Cuneod. A. s.n.* (holotype G! [00022975]).

Homotypic synonyms:

C. suffruticosa var. *tunetana* (Cuénod) Ohle (1975b: 532)

"C. suffruticosa subsp. *tunetana"* (Cuénod) Pottier-Alapetite (1981: 1024), *des. inv.* not validly published (McNeill *et al.* 2012, article 41.5)

Perennial herbs. Stems (14,3) 16.5–25.8 (40) cm long, ascending to erect, with glandular and non-glandular, slightly predominant white-arachnoid, pubescence. Basal leaves (3.2) 3.6–5.4 (6.1) × (0.3) 0.4–0.6 (0.7) cm, 0.4–0.6 mm thick, linear to narrowly oblanceolate, apex acute, margins slightly sinuate-dentate, with glandular and non-glandular, slightly predominantly white-arachnoid, pubescence, drying into a light grey-greenish colour. Capitula solitary, (3.3) 3.4–4.7 (5.0) cm diameter, concolorous, yellow. Outer achenes rostrate (16) 16.5–19 (20) × (1.8) 1.9–2.3 (2.5) mm, straight or curved up to ± 110º, with small dorsal spines or muricated, with basal, and sometimes apical, ventral teeth; middle achenes trialate (10) 10–10.5 (10.7) × (6.3) 7.1–9.7 (9.7) mm, lateral wings sub-entire; inner achenes vermiculate-alate (5.0) 5.1–5.8 (6.0) × (3.1) 3.3–4.1 (4.2); vermiculate-exalate 4.2–4.7 × 2.0–2.2 mm, both circular and with apical and basal teeth.

Habitat and distribution: Riverine sandy banks at 10–20 m elevation, in semi-arid Mediterranean bioclimate. Endemic to Tunisia is restricted to near Bir bou Rekba and Hamamet.

Conservation status: Despite the small geographic distribution, the team was only able to visit one population (Hamamet). The number of mature individuals is estimated to be <250 based on field observations. However, since there is inadequate information to assess their extinction risk based on distribution and/or population status, we propose to assess it as Data Deficient (DD).

Chromosome number: $2n = 32$.

Genome size: 3.39 ± 0.08 pg.

Notes: With its narrow basal leaves and trialate achenes this taxon is unique. Furthermore, due to the difference in habitats, and edaphic conditions we do not agree with Ohle, which included this taxon under *C. suffruticosa* subsp. *suffruticosa* as a variety.

C. tripterocarpa Ruprecht (1856: 231). Fennane & Ibn Tattou 2005; Le Floc'h & Boulos 2008; Gonçalves et al. 2014; Heyn et al. 1974; Valdés 2002. Type (designated by Heyn et al. 1974)**:—**IRAQ. Mesopotamia: *'Ad ripas Tigridis raram', Noë 515, 407* (lectotype LE!).

Homotypic synonym:

C. aegyptiaca subsp. *tripterocarpa* (Ruprertch) Lanza (1919)

Heterotypic synonyms:

C. aegyptiaca var. *platycarpa* Battandier

C. thapsiaecarpa Pomel (1875). Type – ALGERIA: Mzab, Metlili, s.d., Pomel s.n. (holotype P photo ! [00084057], isotype MPU photo ! [004740]).

C. platycarpa Cosson ex Battandier & Trabut (1888)

C. sicula var. *hymenocarpa* Candolle (1838)

C. stellata var. *hymenocarpa* (Candolle) Cosson & Kralik (1857)

C. palaestina var. *intermedia* (Cosson & Kralik) Bonnet & Barratte (1896)

C. palaestina var. *hymenocarpa* (Candolle) Bonnet & Barratte (1896)

Annual herbs. Stems (2) 5.6–15.8 (30) cm long, decumbent to diffuse, generally branched, with glandular hairs geneally predominating over non-glandular hairs. Basal leaves (1) 2–3.2 (9.5) × (0.2) 0.3–0.5 (0.8) cm, (0.3) 0.3–0.4 (0.5) mm thick, linear-oblong, acute, margin repand-dentate, rarely sub-entire, base attenuated in a ± longer petiole, the middle and upper cauline leaves, are smaller, oblanceolate to lanceolate, usually auriculate, both with glandular hairs geneally predominating over non-glandular hairs. Capitula solitary, (1.1) 1.2–1.4 (1.6) cm in diameter. Outer achenes trialate (5.7) 6.3–7.9 (9.5) × (4.5) 5.5–7.1 (8.5) mm, with wings entire to inconspicuously dentate, flat or muricated in the back, without dorsal spines; middle achenes cymbiform (4.2) 4.5–6.1

(6.7) × (3.5) 4.6–5.7 (6.7) mm, with ventral wings sub-equal or slightly surpassing the laterals; inner achenes vermiculate-exalate (2.2) 2.5–3.3 (4.2) × (0.7) 1–1.2 mm, circular or falcate; with small, apical and/or basal, ventral teeth.

Habitat and Distribution: In grasslands, semi-desert to desert regions, sand, sandstone and gravel, rarely in ruderal/wasteland sites, 0–450 m elev. In the south of Europe from Almeria to Murcia and Alicante (SPAIN) to the south of France (occasional) and in all the north of Africa (Morocco, Algeria and Tunisia) to the Middle East. More details in Gonçalves et al. 2017.

Conservation status: This is a widespread taxon in sub-arid areas of the south Mediterranean region. Therefore, we assess it as Least Concern (LC) following the IUCN criteria (IUCN, 2012).

Chromosome number: 2n = 30.

Genome size: 3.52 ± 0.12 pg.

Figure 66 – (next page) Variability of achene morphology of *Calendula*. A) *C. meuselii* (Silveira 3063, AVE); B) *C. eckerleinii* (Silveira 3064, AVE); C) *C. fontquerii* (Silveira & Gonçalves 3339, AVE); D) *C. davisii* (Silveira & Gonçalves 3263, AVE); E) *C. maroccana* (Silveira 3144, AVE); F) *C. maroccana* (Silveira, Gonçalves, & Ouhammou 3295, AVE); G) *C. murbeckii* (Silveira, Gonçalves, & Ouhammou 3280, AVE); H) *C. lanzae* (Silveira, Gonçalves, & Ouhammou 3293, AVE); I) *C. pinnatilob*a (Silveira 3137, AVE); J) *C. stellata* (Silveira 3137, AVE); K) *C. stellata* (Silveira 3062, AVE); L) *C. tripterocarpa* (Silveira 3068, AVE). Rostrate achenes: A1, 2; B1, 2; C1, 2; D1, 2; E1-3; F1, 2; G1-4; H1-2; I1, 2; J1, 2; K1. Bialate achenes: A3, 4; B3, 4; E4, H4-6; K2. Cymbiform achenes: E7, 8; G7; H7, 8; I6; J3, 4; K4, 5; L2, 3. Trialate achenes: B5, 6; C3, 4; D4, 5; E5, 6; F3, 4; G5, 6; I3, 4; L1. Vermiculate-alate achenes: H9; J5; K6; L4. Vermiculate-exalate achenes: A5; B7; B8; C5, 6; D6, 7; E9, 10; F5, 6; G8; H10, I7, J6, K7; L5. All achenes in side view, except: A3, B4, B5, C3, D3, D5, E4, E5, F3, G5, H4, H5, H7, I3, I5, I6, J3, K2, K4, L1 and L2 in ventral face view, and A4, B3, B6, C4, D4, E6, F4, G6, H6, H8, I4, J4, K3, K5 and L3 in ± dorsal face view. Scale bars = 1 cm.

A

B

C

D

E

F

G

H

I

J

K

L

Figure 67 – (next page) Variability of achene morphology of *Calendula*. A) *C. suffruticosa* subsp. *trialata* (Silveira & Gonçalves 3267, AVE); B) *C. suffruticosa* subsp. *marsea* (Silveira & Gonçalves 3268, AVE); C) *C. suffruticosa* subsp. *lusitanica* (Silveira & Gonçalves 3269, AVE); D) *C. suffruticosa* subsp. *dercana* (Silveira & Gonçalves 3262, AVE); E) *C. suffruticosa* subsp. *hosmarensis* (Silveira & Gonçalves 3261, AVE); F) *C. suffruticosa* subsp. *riffiniensis* (Silveira 3341, AVE); G) *C. suffruticosa* subsp. *tazzea* (Silveira & Gonçalves 3334, AVE); H) *C. suffruticosa* subsp. *boccoyana* (Silveira & Gonçalves 3266, AVE); I) *C. suffruticosa* subsp. *boccoyana* (Silveira & Gonçalves 3340, AVE); J) *C. suffruticosa* subsp. *osteni* (Silveira & Gonçalves 3265, AVE); K) *C. suffruticosa* subsp. *fulgida* (Silveira & Gonçalves 3335, AVE). Rostrate achenes: A1, 2; B1, 2; C1, 2; D1, 2; E1, 2; F1-3; G1, 2; H1, 2; I1, 2; J1, 2; K1-4; bialate achenes: A3; B3, 4; C3, 4; D3; E3; F4, 5; G3-5; cymbiform achenes: D4-6; E4, 5; F6, 7; G6, 7; H3, 4; I3, 4; K5, 6; subcymbiform achenes: B4, E4, E5, J3, J4; trialate achenes: A4, 5; B5; J3, 4; exalate achenes: C5, 6; vermicular-alate achenes: F8; G8; I5; K7; vermicular-exalate achenes: A6, B6, 7; C7; D7, 8; E6; F9; G9, 10; H5; I6; J5; K8. All achenes in side view, except: A3-5, B3-5, C3-6, D3, D5, E3, E4, F4-7, G3-6, H3, I3, J3, K2, and K5 in ventral face view, and D6, G7, H4, I4, J4, K1, and K6 in ± dorsal face view. Scale bars = 1 cm.

A

B

C

D

E

F

G

H

I

J

K

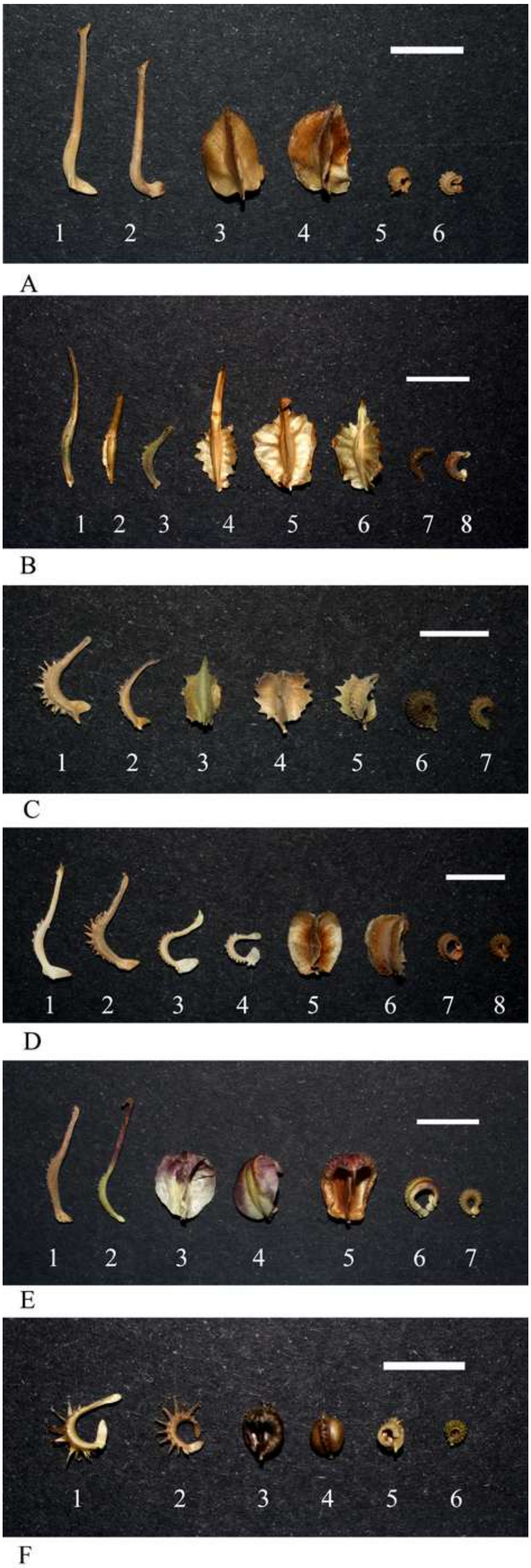


Figure 68 – Variability of achene morphology of Calendula. A) C. suffruticosa subsp. monardii (Silveira, Gonçalves & Amirouche 3316, AVE); B) C. suffruticosa subsp. foliosa (Silveira, Gonçalves & Amirouche 3320, AVE); C) C. suffruticosa subsp. djurdjurae (Silveira, Gonçalves & Amirouche 3321, AVE); D) C. suffruticosa subsp. tunetana (Silveira 3039d, AVE); E) C. suffruticosa subsp. suffruticosa (Silveira 3038, AVE); F) C. arvensis (S. Castro P35 in Silveira 3078, AVE). Rostrate achenes: A1, 2; B1-3; C1, 2; D1-4; E1, 2; F1, 2; bialate achenes: B4-6; trialate achenes: A3, 4; C3-5; D5, 6; ; cymbiform achenes: E3-5; F3, 4; vermicular-alate achenes: A5; D7; E6; F5; vermiculate-exalate achenes: A6, B7, 8; C6, 7; D8; E7 and F6. All achenes in side view, except: A3, B4, B5, C4, D5, E3, E5 and F3 in ventral face view, and B6, C3, C5, D6, E4 and F4 in ± dorsal face view. Scale bars = 1 cm.

Figure 69 – A – C) *C. fontquerii* (Silveira & Gonçalves 3339, AVE). A) habit; B) detail of leaves; C) fruiting capitulum; D-F) *C. maroccana* (D and E- Silveira 3142, AVE; F- Silveira, Gonçalves & Ouhammou 3299); D) habit; E) and F) fruiting capitula.

Figure 70 – A – D) *C. davisii* (C. Silveira & Gonçalves 3339, AVE). A) habit; B) detail of the base of stem; C) flowering capitulum; D) fruiting capitulum.

Figure 71 – A-B) *C. meuselii* (Silveira 3063, AVE). A) Habit and habitat; B) fruiting capitulum. C-F) *C. eckerleinii* (Silveira 3064, AVE). C) Habit; D) flowering capitulum; E) fruiting capitulum; F) detail of leaves.

Figure 72 – A – F) *C. murbeckii* (Silveira, Gonçalves & Ouhammou 3280, AVE). A) habit; B) habit and concolorous and discolorous forms; C) detail of leaves; D) detail of typical fruiting capitulum; E-F) detail of alternative fruiting capitula.

Figure 73 – A – C) *C. pinnatiloba* (A- Silveira 3137, AVE; B-C Silveira 3130, AVE). A) Habit of a discolorous form; B) flowering capitulum; C) fruiting capitulum. D-F) *C. lanzae* (Silveira, Gonçalves & Ouhammou 3293). D) Habit; E) flowering capitulum; F) fruiting capitulum.

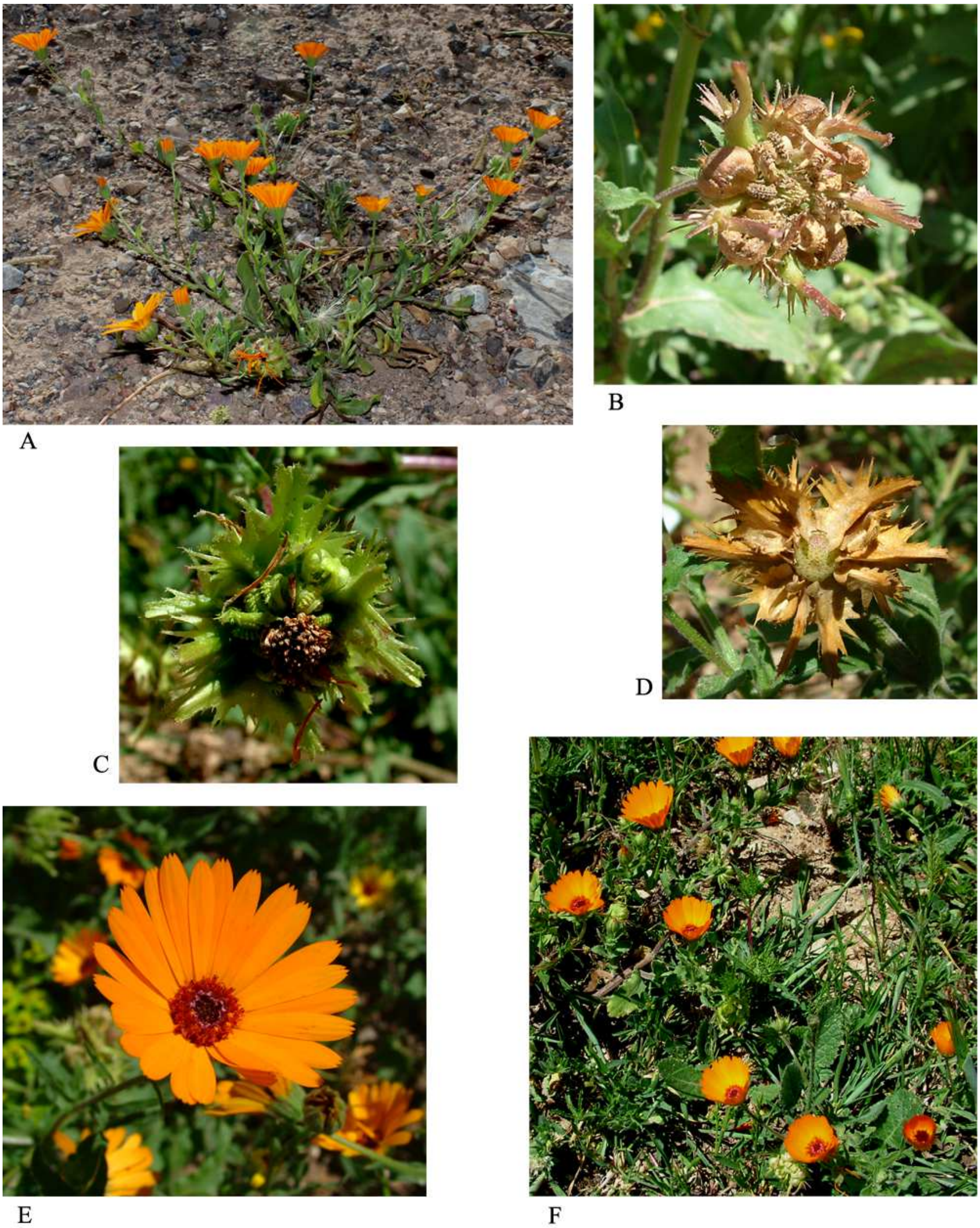


Figure 74 – A – F) *C. stellata* (A-Silveira & Gonçalves 3337, AVE; B) Silveira 3061, AVE; C-F) Silveira 3062). A) habit; B) fruiting capitulum; C) immature fruiting capitulum of the form with bialate achenes; D) mature fruiting capitulum of the form with bialate achenes; E) flowering capitulum; F) habit and habitat.

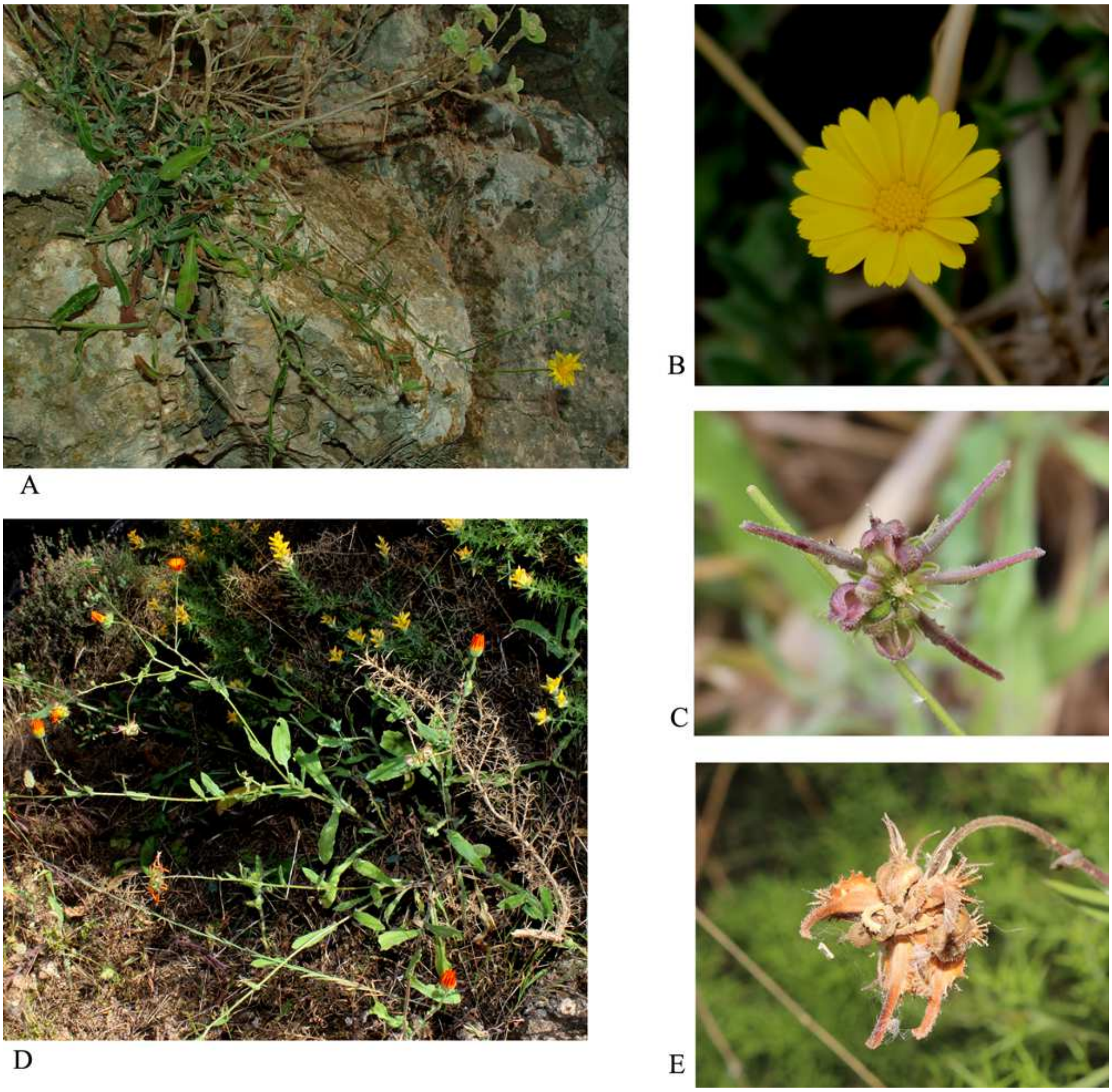


Figure 75 – A-C) *C. suffruticosa* subsp. *boccoyana* (A- Silveira & Gonçalves 3266, AVE; B-C. Silveira & Gonçalves 3340, AVE). A) Habit; B) flowering calitulum; C) fruiting capitulum. E-F) *C. suffruticosa* subsp. *fulgida* (Silveira & Gonçalves 3335, AVE); E) fruiting capitulum.

Figure 76 – A-C) *C. suffruticosa* subsp. *lusitanica* (Silveira & Gonçalves 3269, AVE). A) Habit; B) fruiting capitulum; C) flowering capitula. D-E) *C. suffruticosa* subsp. *marsea* (Silveira & Gonçalves 3268, AVE); D) habit; E) fruiting capitulum. F-G) *C. suffruticosa* subsp. *trialata* (Silveira & Gonçalves 3267, AVE); F) habit; G) fruiting capitulum.

Figure 77 – A-C) *C. suffruticosa* subsp. *ostenii* (Silveira & Gonçalves 3265, AVE). A) Habit; B) flowering capitulum; C) fruiting capitulum. D-E) *C. suffruticosa* subsp. *riffiniensis* (Silveira & Gonçalves 3341, AVE); D) habit; E) typical fruiting capitula; F) alternative fruiting capitula.

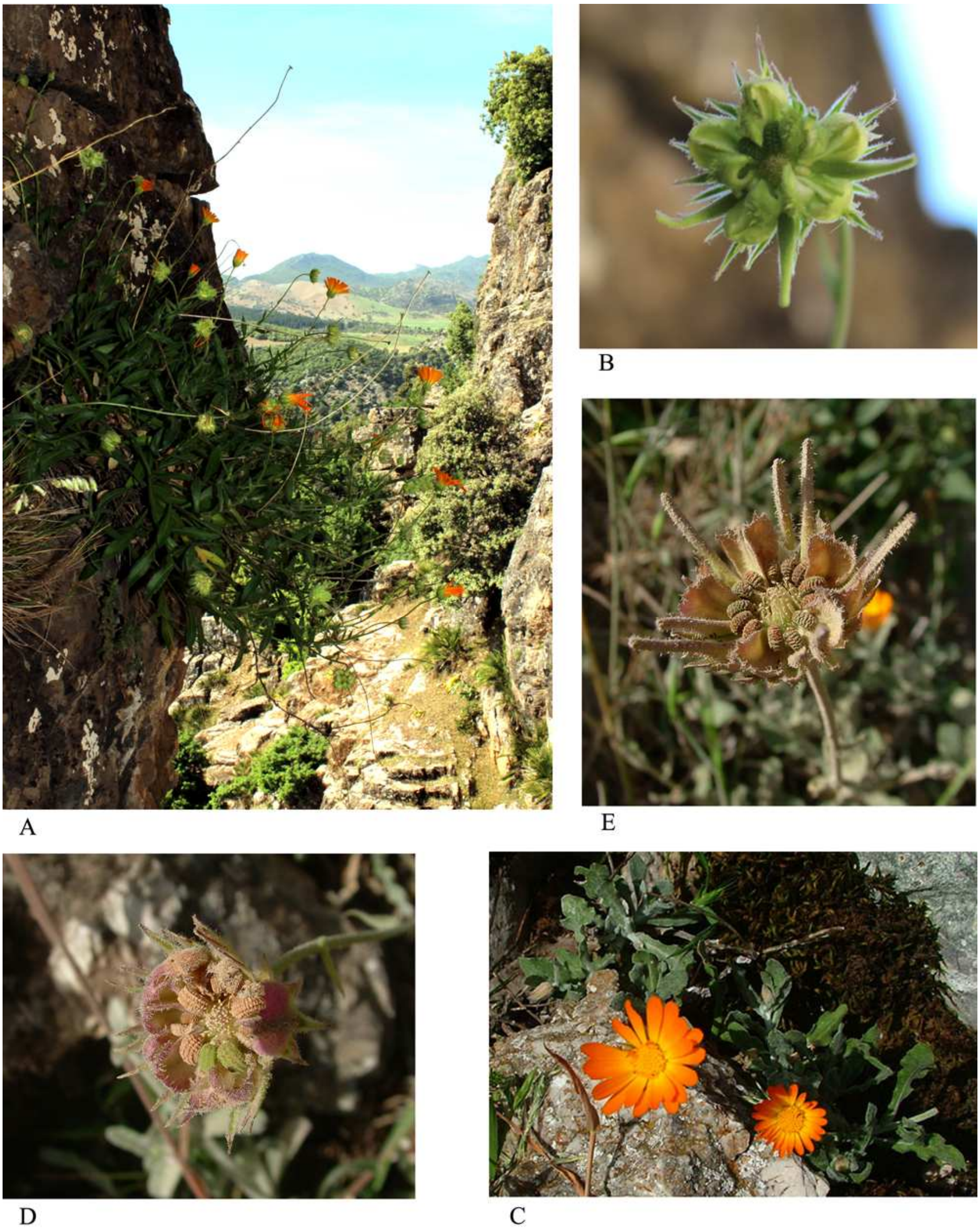


Figure 78 – A-B) *C. suffruticosa* subsp. *tazzea* (Silveira & Gonçalves 3334, AVE). A) Habit; B) fruiting capitulum. C-E) *C. suffruticosa* subsp. *hosmarensis* (Silveira & Gonçalves 3261, AVE); C) habit; D) fruiting capitula with cymbiform middle achenes; E) alternative fruiting capitula with sub-cymbiform middle achenes.

Figure 79– A-C) *C. suffruticosa* subsp. *dercana* (Silveira & Gonçalves 3262, AVE). A) Habit; B) fruiting capitulum; C) orange and yellow flowering capitula. D-F) *C. suffruticosa* subsp. *monardii* (Silveira, Gonçalves & Amirouche 3316, AVE); D) typical orange flowered form; E) alternative yellow flowered form; F) fruiting capitulum.

Figure 80 – A-C) *C. suffruticosa* subsp. *foliosa* (Silveira, Gonçalves & Amirouche 3320, AVE). A) Habit; B) fruiting capitulum; C) flowering capitula. D-F) *C. suffruticosa* subsp. *djurdjurensis* (Silveira, Gonçalves & Amirouche 3321, AVE); D) habit and habitat; E) fruiting capitulum; F) flowering capitulum.

Figure 81 – A-D) *C. suffruticosa* subsp. *suffruticosa* (Silveira 3038, AVE). A) Habit; B) fruiting capitulum; C) flowering capitulum; D) detail of leaves. E) *C. arvensis* (Silveira 3129, AVE); D) small sized form.

Figure 82 – Variability of basal leaf morphology in *Calendula*. A) *C eckerleinii* (Silveira & Gonçalves 3332, AVE); B) *C. fontquerii* (Silveira & Gonçalves 3339, AVE); C) *C. davisii* (Silveira & Gonçalves 3263, AVE); D) *C. maroccana* (Silveira & Gonçalves 3295, 3299, 3300, AVE); E) *C. pinnatiloba* (Silveira & Gonçalves 3138, AVE); F) *C. meuselii* (Silveira & Gonçalves 3063, AVE); G) *C. murbeckii* (Silveira & Gonçalves 3280, AVE); H) *C. lanzae* (Silveira & Gonçalves 3292, AVE).

Figure 83 – Variability of basal leaf morphology in *Calendula*. A) *C suffruticosa* subsp. *lusitanica* (Silveira & Gonçalves 3269, AVE); B) *C suffruticosa* subsp. *trialata* (Silveira & Gonçalves 3267); C) *C suffruticosa* subsp. *marsea* (Silveira & Gonçalves 3268, AVE); D) *C suffruticosa* subsp. *boccoyana* (Silveira & Gonçalves 3266, AVE); E) *C suffruticosa* subsp. *boccoyana* (Silveira & Gonçalves 3240, AVE); F) *C suffruticosa* subsp. *dercana* (Silveira & Gonçalves 3262, AVE)); G) *C suffruticosa* subsp. *riffiniensis* (Silveira & Gonçalves 3341, AVE); H) *C suffruticosa* subsp. *tazzea* (Silveira & Gonçalves 3334, AVE); I) *C suffruticosa* subsp. *hosmarensis* (Silveira & Gonçalves 3261, AVE); J) *C suffruticosa* subsp. *ostenii* (Silveira & Gonçalves 3265, AVE); K) *C suffruticosa* subsp. *fulgida* (Silveira & Gonçalves 3335, AVE).

Figure 84 – Variability of basal leaf morphology in *Calendula*. A) *C suffruticosa* subsp. *monardii* (1- Silveira & Gonçalves 3318, AVE, 2- Silveira & Gonçalves 3316, AVE); B) *C suffruticosa* subsp. *foliosa* (Silveira & Gonçalves 3320); C) *C suffruticosa* subsp. *djurdjurensis* (Silveira & Gonçalves 3321, AVE); D) *C suffruticosa* subsp. *tunetana* (Silveira 3029d, AVE); E) *C suffruticosa* subsp. *suffruticosa* (Silveira 3038, AVE).

Conclusions

The two subspecies of *C. maroccana* recognised by Ohle in 1975 a (*C. maroccana* and *C. maroccana* subsp. *murbeckii*) correspond, in our opinion, to clearly different taxa, which should have the rank of species. We consider that they have distinct morphologies, especially the achenes and duration of their life cycle. Furthermore, the type specimens for these taxa were not properly established and were corrected here. Several other problems with typification were also found and corrected. Maire's (1928b: 57) variety *C. murbeckii* var. *pinnatiloba* was raised to species, and two new species were described *C. fontquerii* and *C. davisii*. In the treatment of perennial *Calendula* taxa by Ohle (1975 a), no *C. suffruticosa* is cited for Morocco, but thanks to surveys conducted by the team, eight subspecies of *C. suffruticosa* are now recognised for this country, five of them described as new. Additional taxa from Algeria and Tunisia were also analysed, in order to check relationships with Moroccan taxa. For Algeria, two new subspecies of *C. suffruticosa* are described. In future studies, we hope to expand the knowledge on the Algerian and Tunisian taxa and this might have implications on the treatment for Morocco now presented because some of the *C. suffruticosa* taxa here described could be reassessed and/or synonymised, on the light of new data. However, this study contributed towards a significant increase in the knowledge of the genus in Morocco and in the relationships with some Algerian and Tunisian taxa, although a global revision, including a phylogenetic study, is still needed.

References

Amadon D (1949) The Seventy-Five Per Cent Rule for Subspecies. Condor 51:250–258. doi: 10.2307/1364805

Ball J (1873) Description of some new species, subspecies and varieties of plants collected in Morocco by J. D. Hooker, G. Maw, and J. Ball. J Bot 11:364–374

Ball J (1878) Spicilegium florae Maroccanae. Taylor and Francis, London (United Kingdom)

Battandier J-A (1910) Flore de l'Algérie. Supplement aux phanérogames. Librairie des Sciences Naturelles, Paris (France)

Battandier J-A (1919) Contribution à la Flore atlantique. Bull la Société Bot Fr 56:51. doi: 10.1080/00378941.1909.10832130

Battandier J-A (1921) De l'espèce dans le genre Calendula. Bull la Société Bot Fr 68:527–531. doi: 10.1080/00378941.1921.10836231

Battandier J-A, Trabut L-C (1890) Flore de l'Algérie [...] Dicotylédones [...] (1888-

1890). Libraire F. Savy, Paris (France)

Battandier J-A, Trabut L-C (1902) Flore analytique et synoptique de l'Algérie et de la Tunisie. Giralt, Alger (Algeria)

Battandier J-A, Trabut L-C (1888) Flore de l'Algérie [...] Dicotylédones. Typographie Adolphe Jourdan, Alger (Algeria)

Boissier P-E (1849) Diagnoses plantarum Orientalium novarum. Series secunda. Ramboz et Schuchardt, Geneve

Boissier P-E (1839) Voyage botanique dans le midi de l'Espagne. Gide Cie, Paris (France)

Boissier P-E (1859) Diagnoses plantarum Orientalium novarum. Series secunda. Ramboz et Schuchardt, Leipzig

Boissier P-E (1875) Flora Orientalis. H. Georg, Geneve

Boissier P-E, Reuter G-F (1852) Pugillus plantarum novarum Africae borealis Hispaniaeque australis. Ex-typographia Ferd. Ramboz et socii, Geneve

Bonnet E, Barratte J-F-G (1896) Exploration scientifique de la Tunisie. Catalogue raisonné des plantes vasculaires de la Tunisie. Imprimerie nationale, Paris

Brotero F de A (1804) Flora Lusitanica

Burdet H, Charpin A, Jacquemoud F (1983) Types nomenclaturaux des taxa ibériques décrits par Boissier ou Reuter. IV. Cistacées à Composées. Candollea 38:751–802

Cavanilles AJ (1791) Icones et descriptiones plantarum [...]. Ex Regia Typographia (Eius operas dirigente Lazaro Gayguer), Madrid

Cosson E (1882) Répertoire alphabétique des principales localités mentionnées dans le Compendium et le Conspectus floræ atlanticæ, avec deux cartes botaniques de l'Algérie et des notices sur ces cartes. G. Masson, Paris (France)

Cosson ME (1856) Itinéraire d'un Voyage Botanique en Algérie, Entrepris en 1856 sous le Patronage du Ministère de la Guerre. Bull la Société Bot Fr 3:697–703. doi: 10.1080/00378941.1856.10839633

Cosson ME, Kralik L (1857) Notes sur Quelques Plantes Rares ou Nouvelles de la

Régence de Tunis. Bull la Société Bot Fr 4:55–63. doi: 10.1080/00378941.1857.10826163

Darwin C, Hooker JD, Jackson BD (1893) Index Kewensis plantarum phanerogamarum: nomina et synonym omnium generum et specierum [...]. 1:383

De Candolle A-L-P-P (1838) Prodromus systematis naturalis regni vegetabilis [...]. Sumptibus Sociorum Treuttel et Würtz, Paris (France)

Desfontaines RL (1799) Flora atlantica: sive historia plantarum quae in Atlante, agro tunetano et algeriensi crescunt. L.G. Desgranges, Paris (France)

Dobignard A, Chatelain C (2011) Index synonymique de la flore d'Afrique du Nord

Fennane M, Ibn Tattou M (1998) Catalogue del plantes vasculaires rares, menacées ou endémiques du Maroc. Bocconea 8:23–24

Fennane M, Ibn Tattou M (2005) Flore vasculaire du Maroc: inventaire et chorologie. Asteraceae et Monocotyledones. Université Mohammed V, Rabat

Font Quer P (1936) L'Alzina (Quercus ilex) en el limit meridionl de la seva area. Memories L'Academia Ciencies I Arts Barcelona XXV:779–798

Forsskal P, Vahl M (1791) Symbolae botanicae, sive plantarum, tam earum, quas in itinere, imprimis orientali. imprensis auctoris, excudebant N. Möller et filius, Copenhagen

Gonçalves AC, Ouhammou A, Jury S, Silveira P (2014) Calendula L. In: Mohammed Fennane, Mohammed Ibn Tattou JEO (ed) Flore Pratique du Maroc. Université Mohammed V, Rabat, pp 270–275

Guadagno M (1922) Nuovo Giornale botanico italiano. Nuova serie. Volume ventinovesimo

Heyn CC, Dagan O, Nachman B (1974) The annual Calendula species: taxonomy and relationships. Isr J Bot 23:1201–1969

Heyn CC, Joel A (1983) Reproductive relationships between annual species of Calendula. Plant Syst Evol 143:311–329. doi: 10.1007/BF00986612

IUCN (2012) The IUCN Red List Categories and Criteria. In: IUCN. http://www.iucnredlist.org/

Jahandiez É, Maire R-C (1934) Catalogue des plantes du Maroc. Dicotylédones et Supplément aux volumes I & II

Jahandiez É, Maire R-C (1941) Catalogue des plantes du Maroc. Supplément aux volumes I, II et III. Imprimerie Minerva, Alger (Algeria)

Lanza D (1919) Monografia del genero Calendula. Atti R. Acad. Sci., Palermo

Le Floc'h E, Boulos L (2008) Flore de Tunisie : Catalogue Synonymique Commenté

Linnaeus C von (1753) Species plantarum [...] Tomus II, 1th edition, 1st edn. Impensis Laurentii Salvii, Stockholm

Linnaeus C von (1763) Species plantarum [...] Tomus II, 2nd edition, 2nd edn. Impensis Laurentii Salvii, Stockholm

Linné C von (1753) Caroli Linnaei ... Species plantarum :exhibentes plantas rite cognitas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas... Impensis Laurentii Salvii, Holmiae :

Maire R-C (1928a) Contributions à l'étude de la flore de l'Afrique du Nord - 8. Bull la Société des Sci Nat du Maroc 8:138–139

Maire R-C (1928b) Contributions à l'étude de la flore de l'Afrique du Nord - 12 (1). Bull la Société d'histoire Nat l'Afrique du Nord 19:56–57

Maire R-C (1938) Contributions à l'étude de la Flore de l'Afrique du Nord - 26. Bull la Société d'histoire Nat l'Afrique du Nord 29:424

Mallet J (1995) A species definition for the modern synthesis. Trends Ecol Evol 10:294–299. doi: 10.1016/0169-5347(95)90031-4

McNeill J, International Association for Plant Taxonomy., International Botanical Congress (18th : 2011 : Melbourne V. (2012) International code of nomenclature for algae, fungi and plants (Melbourne code) : adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011. Koeltz Scientific Books

Meikle RD (1976) Calendula L. In: Tutin TG, Heywood VH, Burges NA, et al. (eds) Flora Europaea Notulae Systematicae ad Floram Europaeam Spectantes.

Cambridge University Press, Cambridge, pp 206–207

Murbeck S (1923) Contributions à la connaissance de la flore du Maroc. Géraniacées-Composées. C. W. K. Gleerup, Lund (Sweden)

Nègre R (1958) A propos de quelques Calendula marocains. Bull la Société des Sci Nat Phys du Maroc XXXVIII:1–7

Nora SV, Castro S, Loureiro J, et al (2013) Flow cytometric and karyological analyses of Calendula species from Iberian Peninsula. Plant Syst Evol 299:853–864. doi: 10.1007/s00606-013-0767-0

Nordenstam B (2007) Calenduleae. In: Bremer K (ed) The Families and Genera of Vascular Plants. Springer, Berlin, pp 241–245

Nordenstam B, Källersjö M (2009) Calenduleae. In: Systematics, Evolution, and Biogeography of Compositae. IAPT, Vienna, pp 527–538

Norlindh T (1946) Studies in the Calenduleae. II. Phytogeography and interrelation. Bot Not 1946:471–506

Norlindh T (1977) Calenduleae - Systematic review. In: Heywood VH, Harborne JB, Turner BL (eds) The Biology and Chemistry of the Compositae. Academic Press, London (United Kingdom), pp 961–987

Ohle H (1974) Beiträge zur Taxonomie der Gattung Calendula II. Taxonomische Revision der südeuropäischen perennierenden Calendula-Sippen. Feddes Repert 85:245–283. doi: 10.1002/fedr.19740850402

Ohle H (1975a) Beiträge zur Taxonomie und Evolution der Gattung Calendula L. III. Revision der marokkanischen perennierenden Sippen unter Berücksichtigung einiger marokkanischer Annueller. Feddes Repert 86:1–17. doi: 10.1002/fedr.19750860102

Ohle H (1975b) Beiträge zur Taxonomie und Evolution der Gattung Calendula L. IV. Revision der algerisch-tunesischen perennierenden Calendula-Sippen unter Berücksichtigung einiger marokkanisch-algerischer Annueller und der marokkanischen und südeuropäischen perennierende. Feddes Repert 86:525–541. doi: 10.1002/fedr.19750860908

Oualidi JEL, Khamar H, Fennane M (2012) Checklist des endémiques et spécimens types de la flore vasculaire de l â€™ Afrique du Nord

Pau y Español C (1924) Plantas del norte de Yebala (Marruecos)... XII:345.

Pennel, F.W. (1944-1945) How Durand acquired Rafinesque's herbarium. *Bartonia* 23: 43-46.

Plume O (2015) Hybridization, Genome Duplication, And Chemical Diversification In The Evolution Of Calendula L. (Compositae)

Pomel A-N (1875) Nouveaux matériaux pour la flore atlantique

Potteier-Alapetite G (1981) Flore de la Tunisie. Angiospermes-Dicotyledones

QGIS DT (2017) QGIS Geographic Information System 2.18. http://www.qgis.org/

Quezel P, Santa S (1963) Nouvelle Flore de l'Algérie et des régions désertiques méridionales

R Development Core Team R (2010) R: a language and environment for statistical computing. Foundation for Statistical Computing, Vienna, Austria

Rafinesque CS, Costanza B, Costanza L, Vaincher P (1810) Caratteri di alcuni nuovi generi e nuove specie di animali e piante della Sicilia: con varie osservazioni sopra i medesimi. Per le stampe di Sanfilippo, Palermo.

Reveal JL (2013) *Constantine Samuel Rafinesque-Schmaltz (1783-1840)*. Department of Plant Biology, Cornell University, Ithaca, NY. Available from: http://www.plantsystematics.org/reveal/pbio/LnC/rafinesque.html (31-01-2018).

Ruiz de Clavijo E (2005) The reproductive strategies of the heterocarpic annual Calendula arvensis (Asteraceae). Acta Oecologica 28:119–126. doi: 10.1016/j.actao.2005.03.004

Ruprecht FJ (1856) Index Seminum. ex Bull Phys-Math Acad Petersb 14:231

Silveira P, Gonçalves AC, Santos C, Paiva J (2013) Two lectotypifications and a new combination in Calendula (Asteraceae) for Flora Iberica. Phytotaxa 145:47–53. doi: 10.11646/phytotaxa.145.1.5

Valdés B (2002) Catalogue des plantes vasculaires du Nord du Maroc, incluant des clés d'identification = Checklist of vascular plants of N Morocco with identification keys. Consejo Superior de Investigaciones Científicas, Madrid

Vogt R, Oberprieler C (2012) Chromosome Numbers of North African Phanerogams. X. Plants collected during Iter Mediterraneum V of OPTIMA in Morocco. Ann des Naturhistorischen Museums Wien Ser B für Bot und Zool 193–221

Willdenow CL Von (1803) Species plantarum [...] Tomus III. Pars III, 4th edn. Impensis G. C. Nauk, Berlin

Willdenow CL Von (1809) Enumeratio plantarum Horti Regii Berolinensis. In Taberna libraria Scholae Realis, Berlin

Zohary M (1941) Calendula. Palest J Bot 1–2:172

APPENDIX A

Herbarium specimens used in the statistical analysis and PCA. This list comprises *Calendula* specimens collected in the field from Morocco, Algeria and Tunisia.

Calendula davisii

Fès-Meknès: Taza, Jbel Tazzeka, 500- [34°20'13" N, 4°37'40" W], 9 June 2012, *Silveira, P. ; Gonçalves, A.C.R.S. 3263* (AVE!)

Calendula eckerleinii

Casablanca-Settat: Ain Leuh, estrada P7311, perto de l'Oued Oum-er-Rbia, 1505- [33°16'48" N, 5°20'23" W], 8 May 2014, *Silveira, P. ; Gonçalves, A.C.R.S. 3330* (AVE!); **Fès-Meknès**: Timahdite, rocas de Foung Kheneg, 1920- 1980m, [33°14'12" N, 5°03'34" W], 8 May 2014, *Silveira, P. ; Gonçalves, A.C.R.S. 3331* (AVE!); 31 km from Ifrane, 48 km from Sefrou, 25 km of Boulemane, at junction of Ifrane road, 1572- [33°27'18" N, 4°51'25" W], 8 May 2014, *Silveira, P. ; Gonçalves, A.C.R.S. 3332* (AVE!); Fes Jbel Zalagh, 895- [34°06'19" N, 4°58'11" W], 9 May 2014, *Silveira, P. ; Gonçalves, A.C.R.S. 3333* (AVE!);

Calendula fontquerii

Bokkoyas, estrada para Al Hoceima, a 33 km de Imzouren, 740- [34°54'46" N, 3°47'59" W], 13 May 2014, *Silveira, P. ; Gonçalves, A.C.R.S. 3339* (AVE!);

Calendula lanzae

Drâa-Tafilalet: Taroudant Ait-Yazza, S of Ait-Yazza, 257- [30°28'35" N, 8°48'03" W], 26 March 2013, *Silveira, P. ; Gonçalves, A.C.R.S.; Ouhammou, A. 3292* (AVE!); **Souss-Massa**: Taroudant gravel on the margins of the river Tiout, 424- [30°23'48" N, 8°42'17" W], 26 March 2013, *Silveira, P. ; Gonçalves, A.C.R.S.; Ouhammou, A. 3293 a* (AVE!);

Calendula maroccana

Marrakech-Safi: leaving Igherm to Taliouine, 1569- [30°41'16" N, 7°16'18" W], 27 March 2013, *Silveira, P. ; Gonçalves, A.C.R.S.; Ouhammou, A. 3298* (AVE!); on the N9 almost at Tizi-n-Tichka, coming from Tachokchte, 1351- [31°24'53" N, 7°23'43" W], 27 March 2013, *Silveira, P. ; Gonçalves, A.C.R.S.; Ouhammou, A. 3300* (AVE!); **Souss-Massa**: Taroudant between Tiout and Igherm, 1365- [30°12'34" N, 8°28'51" W], 26 March 2013, *Silveira, P. ; Gonçalves, A.C.R.S.; Ouhammou, A. 3294* (AVE!); Taroudant between Tiout and Igherm, at 3 km from Irherm, 1656- [30°06'41" N, 8°27'50" W], 26 March 2013, *Silveira, P. ; Gonçalves, A.C.R.S.; Ouhammou, A. 3295* (AVE!); Taroudant Tachokcht, 1909- [30°47'48" N, 7°31'38" W], 27 March 2013, *Silveira, P. ; Gonçalves, A.C.R.S.; Ouhammou, A. 3299* (AVE!);

Calendula meuselii

Fès-Meknès: Meknes between Moulay Idriss and N'Zalat-Beni-Ammar, 852- [34°05'40" N, 5°25'59" W], 26 April 2010, Silveira, P. 3063 (AVE!);

Calendula murbeckii

Marrakech-Safi: Jbilet, near to the house of the gazele reserve, 624- [31°52'09" N, 7°57'08" W], 25 March 2013, *Silveira, P. ; Gonçalves, A.C.R.S.; Ouhammou, A. 3280* (AVE!); S of Barrage Lalla Takerhust, 729- [31°21'36" N, 8°09'46" W], 25 March 2013, *Silveira, P. ; Gonçalves, A.C.R.S.; Ouhammou, A. 3287* (AVE!);

Calendula pinnatiloba

Souss-Massa: Agadir entre Agadir e Cap Rhir, Arround, 27- [30°37'36" N, 9°53'26" W], 10 April 2011, *Silveira, P. 3130* (AVE!); Agadir cerca de 2,6 km antes de chegar ao farol de Cap Rhir vindo de Agadir, 12- [30°37'36" N, 9°53'26" W], 10 April 2011, *Silveira, P. 3134* (AVE!);

Calendula suffruticosa subsp. ***boccoyana***

MOROCCO. **Tanger-Tétouan-Al Hoceïma**: El-Jebha (El-Yebha), 90- [35°12'39" N, 4°39'46" W], 11 June 2012, *Silveira, P. ; Gonçalves, A.C.R.S. 3266* (AVE!);

Calendula suffruticosa subsp. ***dercana***

MOROCCO. **Tanger-Tétouan-Al Hoceïma**: Tetouan Jbel Dersa, 468- [35°35'26" N, 5°22'45" W], 7 June 2012, *Silveira, P. ; Gonçalves, A.C.R.S. 3262* (AVE!);

Calendula suffruticosa subsp. ***djurdjurensis***

ALGERIA. **Djurdjura**: 1798- [36°27'46" N, 4°10'06" E], 7 June 2013, *Silveira, P. ; Gonçalves, A.C.R.S.; Amirouche, R. 3321* (AVE!); 1798- [36°27'46" N, 4°10'06" E], 8 August 2013, *Amirouche, R. & Amirouche, N. in Silveira, P. 3343* (AVE!);

Calendula suffruticosa subsp. ***foliosa***

ALGERIA. **Gorges de Palestro**: 100- [36°36' N, 3°35' E], 7 June 2013, *Silveira, P. ; Gonçalves, A.C.R.S.; Amirouche, R. 3320* (AVE!); AC + PS, Silveira, P., **3320**, 7 June 2013, Algeria,

Calendula suffruticosa subsp. ***fulgida***

MOROCCO. **Oriental**: Berkane, Montes de Beni Snassen, estrada Zegzel entre Tazarhine e Takerboust, 54- [34°50'01" N, 2°22'17" W], 11 May 2014, *Silveira, P. ; Gonçalves, A.C.R.S. 3335* (AVE!); Berkane, Montes de Beni Snassen, estrada Zegzel proximo do Oued Zegzel, 260- [34°52'40" N, 2°21'20" W], 11 May 2014, *Silveira, P. ; Gonçalves, A.C.R.S. 3336* (AVE!);

Calendula suffruticosa subsp. ***hosmarensis***

MOROCCO: **Tanger-Tétouan-Al Hoceïma**: Tetouan Jbel Gorghiz (Beni Hosmar), 1070- [35°28'50" N, 5°22'06" W], 6 June 2012, *Silveira, P. ; Gonçalves, A.C.R.S. 3261*

Calendula suffruticosa subsp. ***lusitanica***

SPAIN. **Ceuta**: 5- 20m, [35°54'06" N, 5°20'44" W], 14 June 2012, *Silveira, P. ; Gonçalves, A.C.R.S. 3269* (AVE!);

Calendula suffruticosa Vahl subsp. ***marsea***

MOROCCO. **Tanger-Tétouan-Al Hoceïma**: Tanger Oued Marsa, 3- 5m, [35°54' N, 5°27' W], 13 June 2012, *Silveira, P. ; Gonçalves, A.C.R.S. 3268* (AVE!);

Calendula suffruticosa subsp. ***monardii***

ALGERIA. **Alger**: Ain Benian (Guyotville), Plage de La Madrague, 13- [36°47'25" N, 2°53'56" E], 7 June 2013, *Silveira, P. ; Gonçalves, A.C.R.S.; Amirouche, R. 3316* (AVE!); near to the plage, 13- [36°43'42" N, 2°50'27" E], 7 June 2013, *Silveira, P. ; Gonçalves, A.C.R.S.; Amirouche, R. 3317* (AVE!); 13- [36°41'25" N, 2°47'37" E], 7 June 2013, *Silveira, P. ; Gonçalves, A.C.R.S.; Amirouche, R. 3318* (AVE!);

Calendula suffruticosa subsp. ***osteni***

MOROCCO. **Melilla**: Jbel Gurugú, 562- [35°14'11" N, 2°58'29" W], 11 June 2012, *Silveira, P. ; Gonçalves, A.C.R.S. 3265* (AVE!);

Calendula suffruticosa Vahl subsp. ***riffiniensis***

MOROCCO: **Tanger-Tétouan-Al Hoceïma**: Tetouan Jbel Kelti, Arifane, 943- [35°17'04" N, 5°18'00" W], 15 May 2014, *Silveira, P. ; Gonçalves, A.C.R.S. 3341* (AVE!);

Calendula suffruticosa subsp. ***suffruticosa***

TUNISIA. **Ariana**: Ghar el Melh [Porto Farina], 10- [37°10' N, 10°11' E], 11 April 2009, *Silveira, P. 3038* (AVE!);

Calendula suffruticosa subsp. ***tazzea***

MOROCCO. **Fès-Meknès**: Taza, Ras-El-Ma, Sidi Msbar, 1460- [34°07'58" N, 4°07'58" W], 11 May 2014, *Silveira, P. ; Gonçalves, A.C.R.S. 3334* (AVE!);

Calendula suffruticosa subsp. ***trialata***

MOROCCO: **Tanger-Tétouan-Al Hoceïma**: Tanger Cap Cires, 10- [35°54'27" N, 5°28'54" W], 12 June 2012, *Silveira, P. ; Gonçalves, A.C.R.S. 3267* (AVE!);

Calendula suffruticosa Vahl subsp. ***tunetana***

TUNISIA. **Nabeul**: entre Bir Bou Regba e Hammamet, [36°26' N, 10°35' E], 11 April 2009, *Silveira, P. 3039 d* (AVE!);

APPENDIX B

Results of principal component analysis (PCA) of *Calendula* from Morocco, Algeria and Tunisia.Iberian Peninsula. A – Component loadings for *C. maroccana group* and *C. suffruticosa* group in Morocco.

Variable	*C. maroccana* group			*C. suffruticosa* group		
	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3
LB	-0,091	-0,056	0,152	0,096	0,218	0,472
LL	0,104	-0,082	0,039	-0,466	0,351	0,045
LW	-0,032	-0,288	-0,016	-0,345	0,195	0,278
LD	0,157	-0,100	-0,013	-0,494	0,319	0,036
R1	-0,195	0,096	0,077	0,304	0,084	0,002
R2	0,052	0,268	0,118	0,119	0,150	-0,402
LT	-	-	-	0,472	0,184	-0,311
HD	0,128	0,014	0,061	-0,025	0,366	-0,215
SEL	-	-	-	0,205	-0,247	0,717
SEW	-	-	-	0,202	-0,242	0,711
RL	-0,102	0,102	0,437	-0,530	0,361	0,133
RW	-0,200	-0,083	-0,013	-0,210	0,250	0,203
BL	-0,162	-0,227	0,277	-0,451	0,088	0,496
BW	-0,202	-0,199	0,199	-0,356	0,004	0,567
BR	0,055	-0,167	0,444	-0,497	-0,005	0,367
TL	0,176	0,238	0,187	0,704	0,462	-0,358
TW	0,173	0,220	0,205	0,707	0,455	-0,372
TR	-	-	-	0,147	0,107	-0,037
CL	-0,220	0,157	-0,003	-0,886	-0,317	-0,216
CW	-0,238	0,129	-0,020	-0,860	-0,322	-0,202
R3	-	-	-	-0,821	-0,406	-0,228
SCL	-	-	-	-0,620	0,481	-0,011
SCW	-	-	-	-0,561	0,390	-0,089
SCR	-	-	-	-0,501	0,506	0,159
VAL	-0,125	-0,111	0,051	-0,730	0,267	-0,176
VAW	-0,125	-0,111	0,050	-0,749	0,256	-0,180
VEL	0,137	-0,172	0,266	0,254	0,536	0,273
VEW	0,159	-0,209	0,071	0,250	0,513	0,248
-	0,257	-0,134	-0,044	-	-	-
SP	0,097	-0,220	-0,065	0,084	-0,796	-0,360
LS	-0,069	-0,213	0,104	-0,201	0,101	0,230
LA	-	-	-	-0,377	-0,154	-0,147
LM	-0,219	0,138	-0,004	0,251	-0,399	-0,399
LP	0,097	-0,220	-0,065	0,112	-0,763	-0,338
SEvw	-	-	-	0,204	-0,246	0,716
SElw	-	-	-	0,204	-0,246	0,716
Rvt	-0,260	-0,121	-0,056	0,199	0,310	0,128
Blw	-0,122	-0,234	0,354	-0,126	-0,049	0,522
Bdw	-	-	-	-0,391	-0,130	0,093
Tvw	0,170	0,199	0,182	0,627	0,476	-0,253
Tlw	0,124	0,285	0,255	0,599	0,482	-0,205
Cvw	-	-	-	-0,718	-0,294	-0,175
Cdw	-	-	-	-0,562	0,403	0,061
Cvt	-0,264	-0,079	-0,133	-0,736	-0,423	-0,220
SCvw	-	-	-	-0,501	0,516	0,158
SClw	-	-	-	-0,568	0,413	-0,062
VAs	-0,264	0,083	-0,049	-0,738	0,395	-0,129
VAvt	-0,257	0,134	0,044	-0,502	0,258	-0,285
VEs	0,236	-0,160	-0,110	0,302	-0,009	0,633
VEvt	-0,108	0,097	0,078	0,363	0,548	-0,300

Results of principal component analysis (PCA) of *Calendula* from Morocco, Algeria and Tunisia. Component loadings for *C. suffruticosa* taxa with trialate achenesand with non-trialate achenes.

Variable	with trialate achenes			with non trialate achenes		
	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3
LB	0,454	0,554	0,173	-0,177	0,535	0,039
LL	-0,756	0,002	0,426	0,653	0,181	-0,150
LW	-0,759	0,212	0,461	0,242	0,275	-0,248
LD	-0,794	0,129	0,353	0,682	0,113	-0,047
R1	0,442	-0,731	0,122	-0,321	0,370	-0,387
R2	0,415	-0,672	-0,283	0,537	-0,107	0,162
LT	0,587	0,344	-0,190	-0,057	-0,203	0,616
HD	-0,203	-0,357	0,535	0,402	0,055	0,178
SEL	-	-	-	-0,570	0,267	0,319
SEW	-	-	-	-0,562	0,267	0,313
RL	0,222	-0,456	0,458	0,509	0,584	-0,288
RW	-0,340	-0,580	0,387	0,168	0,354	-0,171
BL	-0,784	0,059	-0,143	0,325	0,489	-0,639
BW	-0,662	0,149	-0,262	0,116	0,515	-0,648
BR	-0,585	-0,108	0,216	0,357	0,273	-0,687
TL	0,372	-0,063	0,268	-	-	-
TW	0,648	0,068	0,243	-	-	-
CL	-	-	-	0,806	-0,481	-0,055
CW	-	-	-	0,762	-0,454	-0,173
R3	-	-	-	0,672	-0,578	-0,056
SCL	-	-	-	0,621	0,346	0,608
SCW	-	-	-	0,541	0,216	0,624
SCR	-	-	-	0,482	0,492	0,412
VAL	0,493	0,439	0,652	0,870	0,108	-0,119
VAW	0,501	0,436	0,641	0,861	0,092	-0,045
VEL	-0,636	-0,412	0,172	-0,091	0,665	-0,089
VEW	-0,557	-0,184	0,186	-0,097	0,602	0,416
SP	0,171	-0,834	-0,125	-0,351	-0,826	-0,066
LS	-0,338	0,857	0,085	-0,482	-0,225	-0,034
LA	-0,889	-0,168	0,189	0,379	-0,436	-0,255
-	-	-	-	-0,082	-0,731	0,015
LP	-0,164	-0,203	-0,085	-0,343	-0,767	-0,138
SEvw	-	-	-	-0,569	0,266	0,318
SElw	-	-	-	-0,569	0,266	0,318
Rvt	-0,360	-0,420	0,489	-0,132	0,473	-0,690
Blw	-0,894	0,093	-0,224	-0,111	0,358	-0,566
Bdw	-	-	-	0,274	0,028	-0,646
Tvw	-0,262	0,899	0,114	-	-	-
Tlw	-0,711	0,630	0,198	-	-	-
Cvw	-	-	-	0,559	-0,341	-0,093
Cdw	-	-	-	0,588	0,359	0,313
Cvt	-	-	-	0,520	-0,565	0,020
SCvw	-	-	-	0,475	0,499	0,437
SClw	-	-	-	0,539	0,256	0,626
VAs	0,488	0,436	0,665	0,919	0,249	-0,009
VAvt	0,488	0,436	0,665	0,664	-0,023	0,193
VEs	0,029	0,400	-0,820	-0,600	0,618	-0,117
VEvt	0,096	-0,661	0,690	0,002	0,265	0,256

APPENDIX C

Results of morphometric analysis of *Calendula* from Morocco including some taxa from Algeria and Tunisia; A1 and A3 *C. maroccana* group; B1 to B5 subspecies of *C. suffruticosa.*

A1

	C. eckerleinii					*C. davisii*					*C. fontquerii*				
Characters	Min.	25%	Mean	75%	Max.	Min.	25%	Mean	75%	Max.	Min.	25%	Mean	75%	Max.
LB	15,0	33,0	38,4	45,5	56,0	44,0	45,0	52,4	57,0	65,0	16,0	30,3	35,7	44,0	48,0
LL	3,8	4,6	6,0	7,0	9,7	3,5	4,1	6,2	8,0	10,0	3,5	4,5	5,7	7,1	9,5
LW	0,4	0,6	0,9	1,1	1,5	0,5	0,7	1,0	1,2	2,0	0,8	0,9	1,1	1,3	1,5
LD	2,5	3,3	4,3	5,0	7,5	2,2	2,6	4,3	5,7	6,5	2,5	3,0	4,3	5,6	7,2
R1	1,3	1,3	1,4	1,5	1,8	1,4	1,4	1,5	1,6	1,6	1,3	1,3	1,4	1,4	1,5
R2	4,0	5,5	7,3	8,8	11,3	4,0	4,9	6,5	7,9	8,2	3,8	4,1	5,3	6,1	8,3
LT	0,2	0,3	0,3	0,4	0,5	0,2	0,3	0,3	0,4	0,4	0,3	0,3	0,4	0,4	0,5
HD	2,7	3,1	3,5	3,8	5,2						2,2	2,6	3,0	3,6	3,8
RL	7,0	9,3	9,7	10,3	12,2	15,0	18,0	19,4	22,0	23,0	5,8	6,5	7,2	8,1	8,7
RW	1,3	1,7	1,7	1,8	2,2	1,7	1,9	2,1	2,3	3,0	1,5	1,5	1,6	1,7	1,8
BL	-	-	-	-	-	11,7	12,5	13,7	15,4	16,2	-	-	-	-	-
BW	-	-	-	-	-	6,7	9,0	9,7	10,8	12,2	-	-	-	-	-
BR	-	-	-	-	-	2,5	3,0	3,4	3,8	4,5	-	-	-	-	-
TL	5,7	6,3	6,7	7,0	7,8	0,0	8,1	10,0	12,5	12,7	5,0	5,6	6,1	6,7	7,0
TW	2,5	4,2	5,3	6,7	7,8	0,0	6,9	8,6	11,7	12,2	3,0	3,3	4,0	4,4	5,8
CL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SCL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SCW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VAL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VAW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VEL	3,3	4,5	4,8	5,2	5,8	4,5	4,9	5,5	6,0	6,7	3,7	3,8	4,2	4,8	5,0
VEW	1,3	1,7	1,7	1,8	2,0	1,3	1,5	1,6	1,7	1,8	1,3	1,5	1,6	1,7	1,7

A2

	C. lanzae					*C. maroccana*					*C. meuselii*				
Characters	Min.	25%	Mean	75%	Max.	Min.	25%	Mean	75%	Max.	Min.	25%	Mean	75%	Max.
LB	32,0	45,8	60,2	78,5	96,0	7,5	21,0	29,8	38,0	58,0	15,5	18,1	32,3	44,3	57,0
LL	3,5	4,0	5,0	6,5	7,2	4,2	5,3	7,6	10,0	13,1	4,4	4,6	6,6	8,4	10,0
LW	0,8	1,2	1,6	2,1	3,0	0,4	0,6	1,0	1,1	2,8	1,3	1,4	1,8	2,3	2,4
LD	2,2	2,5	3,2	4,0	4,2	2,6	3,7	5,8	7,9	11,0	2,7	2,9	4,2	5,2	6,8
R1	1,3	1,3	1,6	1,7	2,1	1,2	1,3	1,4	1,4	1,6	1,4	1,5	1,6	1,6	1,7
R2	2,4	2,8	3,4	4,0	4,4	4,5	6,7	8,6	10,3	12,8	3,1	3,1	3,6	4,2	4,2
LT	0,2	0,2	0,3	0,3	0,3	0,3	0,3	0,3	0,4	0,6	0,3	0,4	0,4	0,5	0,5
HD	1,1	2,2	2,6	3,0	3,8	1,6	3,1	3,4	4,0	4,7	2,0	2,9	3,5	4,1	4,5
RL	12,0	14,8	15,8	17,3	21,0	8,3	9,5	11,5	13,7	14,3	12,0	14,0	15,3	17,0	17,0
RW	1,3	2,3	2,8	3,2	4,2	1,0	1,2	1,4	1,7	1,7	1,8	2,0	2,3	2,3	3,3
BL	8,7	9,7	9,8	10,0	10,5	-	-	-	-	-	9,7	10,2	10,6	11,0	11,7
BW	5,8	7,1	8,2	9,0	9,2	-	-	-	-	-	4,7	5,3	6,2	7,0	7,5
BR	0,0	0,0	0,0	0,0	0,0	-	-	-	-	-	1,3	1,7	2,0	2,2	3,3
TL	-	-	-	-	-	5,0	5,8	7,4	8,7	10,2	-	-	-	-	-
TW	-	-	-	-	-	4,2	4,6	6,1	7,5	8,7	-	-	-	-	-
CL	5,2	5,7	5,9	6,0	6,3	5,8	6,1	6,4	6,7	6,7	-	-	-	-	-
CW	2,8	3,8	4,2	4,9	5,0	3,5	3,5	3,8	4,1	4,2	-	-	-	-	-
SCL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SCW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VAL	5,0	5,3	6,0	6,5	6,5	-	-	-	-	-	-	-	-	-	-
VAW	4,2	4,3	4,4	4,5	4,5	-	-	-	-	-	-	-	-	-	-
VEL	3,7	4,1	4,3	4,6	5,0	2,7	2,8	4,1	5,5	5,8	5,8	6,3	6,8	7,3	7,8
VEW	1,3	1,5	1,5	1,7	1,8	1,2	1,2	1,4	1,6	1,7	1,7	2,0	2,2	2,3	2,7

A3

	C. murbeckii						*C. pinnatiloba*					*C. suffruticosa*				
Characters	Min.	25%	Mean	75%	Max.		Min.	25%	Mean	75%	Max.	Min.	25%	Mean	75%	Max.
LB	6,0	22,0	33,6	46,0	89,0		14,0	18,0	36,5	57,5	70,0	8,0	28,0	44,2	58,0	120,0
LL	2,5	3,5	5,1	6,7	9,0		2,8	4,2	4,8	5,6	6,3	2,2	4,5	5,9	7,5	11,7
LW	0,2	0,3	0,5	0,6	1,1		0,5	0,7	0,9	1,0	1,2	0,6	1,0	1,4	1,9	3,3
LD	1,5	2,0	3,0	3,9	5,0		1,4	2,2	2,6	3,0	3,5	1,7	3,0	4,3	5,5	9,0
R1	1,5	1,7	1,7	1,8	2,3		1,3	1,8	1,9	2,0	2,4	1,2	1,3	1,4	1,4	1,8
R2	6,1	8,5	11,6	13,4	25,0		4,2	4,7	5,8	6,3	10,4	2,2	3,5	4,3	5,0	7,8
LT	0,3	0,3	0,4	0,4	0,6							0,1	0,3	0,5	0,6	1,5
HD	2,0	2,7	3,1	3,4	4,7		1,8	2,3	2,9	3,5	3,9	1,5	2,7	3,2	3,6	5,6
RL	14,0	16,3	19,9	23,0	26,0	-	10,0	12,0	12,5	14,0	15,0	4,5	11,2	15,8	20,0	31,0
RW	1,2	1,5	1,8	1,8	3,3	-	1,5	2,8	3,6	4,5	5,0	1,0	1,5	1,8	2,0	9,3
BL	-	-	-	-	-	-	5,8	8,2	8,6	9,2	11,2	7,5	10,3	13,8	16,1	25,0
BW	-	-	-	-	-	-	5,8	7,0	8,2	8,8	10,5	3,0	6,2	7,8	9,3	15,2
BR	-	-	-	-	-	-	0,0	0,0	0,0	0,0	0,0	0,0	2,6	5,5	7,5	53,0
TL	8,3	9,0	9,8	10,5	11,3	-	-	-	-	-	-	7,5	8,9	11,0	13,0	16,2
TW	5,0	5,8	7,4	8,3	10,8	-	-	-	-	-	-	5,5	7,5	8,5	9,5	11,2
CL	-	-	-	-	-	-	3,5	4,2	4,6	5,2	5,3	4,7	7,2	8,7	10,5	14,5
CW	-	-	-	-	-	-	2,5	2,8	3,2	3,5	4,2	3,2	5,3	6,7	8,0	11,8
SCL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SCW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VAL	-	-	-	-	-	-	-	-	-	-	-	3,8	5,0	5,7	6,2	8,5
VAW	-	-	-	-	-	-	-	-	-	-	-	2,2	2,7	3,6	4,5	5,8
VEL	3,3	3,8	4,1	4,5	4,7	-	2,5	3,3	3,6	4,0	4,2	3,0	3,8	4,4	5,0	7,2
VEW	1,0	1,2	1,3	1,3	1,7	-	0,8	1,0	1,2	1,3	1,5	0,8	1,5	1,7	1,8	2,8

B1

Characters	subsp. *tunetana*					subsp. *boccoyana*					subsp. *dercana*				
	Min.	25%	Mean	75%	Max.	Min.	25%	Mean	75%	Max.	Min.	25%	Mean	75%	Max.
LB	14.3	16.5	22.7	25.8	40.0	17.0	24.0	31.8	39.0	50.0	11.0	17.0	27.7	32.5	65.0
LL	3.2	3.6	4.4	5.4	6.1	6.2	7.4	8.4	9.3	9.5	3.2	3.5	5.3	7.0	7.8
LW	0.3	0.4	0.5	0.6	0.7	1.2	1.3	1.5	1.8	1.8	0.7	1.1	1.5	2.0	2.4
LD	2.2	2.4	3.1	3.6	4.6	4.6	5.6	6.3	7.0	7.3	2.5	2.7	3.9	5.3	5.7
R1	1.2	1.3	1.4	1.5	1.5	1.3	1.3	1.3	1.4	1.4	1.2	1.3	1.4	1.4	1.6
R2	5.6	6.8	9.3	10.8	12.8	4.9	5.0	5.6	6.3	6.5	2.5	2.9	3.7	4.2	6.4
HD	3.3	3.4	4.0	4.7	5.0	2.0	2.2	2.5	2.8	3.2	1.8	2.8	2.9	3.2	3.6
LT						0,3	0,4	0,5	0,6	0,8	0.3	0,4	0,4	0,4	0.6
SEL															
SEW															
RL	16.0	16.5	17.6	19.0	20.0	10.0	13.0	16.4	19.5	24.0	5.0	6.0	8.4	10.2	13.5
RW	1.8	1.9	2.1	2.3	2.5	1.0	1.5	1.7	2.0	2.3	1.2	1.3	1.4	1.5	1.7
BL	-	-	-	-	-	13.8	14.4	16.0	17.5	18.0	-	-	-	-	-
BW	-	-	-	-	-	3.0	3.3	5.3	8.0	8.7	-	-	-	-	-
BR	-	-	-	-	-	3.3	5.0	7.4	9.3	10.0	-	-	-	-	-
TL	10.0	10.0	10.2	10.5	10.7	-	-	-	-	-	-	-	-	-	-
TW	6.3	7.1	8.4	9.7	9.7	-	-	-	-	-	-	-	-	-	-
TR	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-	-	-	-
CL	-	-	-	-	-	4.7	5.8	7.1	8.3	8.8	5.5	5.8	6.1	6.6	6.6
CW	-	-	-	-	-	3.2	4.0	4.9	5.5	8.0	3.6	3.8	4.6	5.6	5.6
SCL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SCW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SCR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VAL	5.0	5.1	5.4	5.8	6.0	5.0	5.2	5.2	5.3	5.3	-	-	-	-	-
VAW	3.1	3.3	3.7	4.1	4.2	2.2	2.7	3.4	3.7	4.8	-	-	-	-	-
VEL	4.2	4.2	4.5	4.7	4.7	3.0	3.8	4.4	5.0	5.5	3.0	3.3	3.4	3.5	3.7
VEW	2.0	2.0	2.1	2.2	2.2	1.2	1.4	1.7	2.0	2.2	1.3	1.5	1.5	1.7	1.7

B2

Characters	subsp. *djurdjurensis*					subsp. *foliosa*					subsp. *fulgida*				
	Min.	25%	Mean	75%	Max.	Min.	25%	Mean	75%	Max.	Min.	25%	Mean	75%	Max.
LB	12.0	17.8	25.0	29.0	52.0	29.0	32.0	38.3	46.3	53.0	41.0	46.8	58.1	71.0	89.0
LL	6.5	7.8	8.8	10.4	11.5	3.5	3.8	4.5	5.1	5.6	6.2	7.5	8.4	9.9	10.8
LW	1.8	2.0	2.4	2.8	3.0	1.0	1.2	1.5	1.6	2.5	1.4	1.5	1.9	2.0	3.3
LD	4.3	5.6	6.5	7.7	9.0	2.0	2.3	2.8	3.4	3.8	3.9	5.2	5.9	7.0	7.7
R1	1.2	1.3	1.4	1.4	1.5	1.4	1.5	1.6	1.7	1.8	1.3	1.4	1.4	1.5	1.6
R2	3.0	3.4	3.8	3.9	6.4	2.2	2.4	3.0	3.6	3.9	3.3	4.0	4.5	5.0	5.4
HD	3.0	3.4	4.3	5.3	5.6	2.2	2.6	3.1	3.4	4.2	2.3	2.6	2.9	3.2	3.4
LT	0,3	0,3	0,3	0,4	0,4	0,2	0,3	0,3	0,3	0,3	0,1	0,2	0,2	0,2	0,3
SEL															
SEW															
RL	8.3	10.3	11.0	12.3	13.8	9.0	12.3	18.1	23.8	27.0	17.0	21.5	23.9	26.5	29.0
RW	1.7	1.8	2.1	2.3	2.5	2.0	2.0	2.3	2.5	2.8	1.2	1.8	2.5	3.4	3.8
BL	-	-	-	-	-	10.5	13.5	15.0	16.1	22.0	15.0	18.3	20.1	23.5	25.0
BW	-	-	-	-	-	6.7	8.5	9.9	11.1	11.7	7.5	9.7	11.2	13.0	15.2
BR	-	-	-	-	-	2.5	3.3	4.1	4.5	9.7	5.3	8.6	11.1	14.4	15.2
TL	7.5	8.4	9.0	9.7	10.8	-	-	-	-	-	-	-	-	-	-
TW	5.5	5.8	6.6	6.8	9.2	-	-	-	-	-	-	-	-	-	-
TR	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-	-	-	-
CL	-	-	-	-	-	-	-	-	-	-	5.0	6.8	8.2	9.3	11.7
CW	-	-	-	-	-	-	-	-	-	-	6.5	7.5	8.6	9.2	11.5
SCL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SCW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SCR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VAL	-	-	-	-	-	-	-	-	-	-	5.0	5.0	5.7	6.2	6.7
VAW	-	-	-	-	-	-	-	-	-	-	2.3	2.5	2.9	3.4	3.5
VEL	5.0	5.5	5.9	6.2	7.2	3.3	4.3		5.5	7.2	3.7	3.8	4.1	4.2	4.5
VEW	1.7	1.8	2.1	2.3	2.8	1.3	1.7		2.0	2.8	0.8	1.3	1.3	1.5	1.5

B3

Characters	subsp. *hosmarensis*					subsp. *lusitanica*					subsp. *marsea*				
	Min.	25%	Mean	75%	Max.	Min.	25%	Mean	75%	Max.	Min.	25%	Mean	75%	Max.
LB	8.0	15.0	28.2	42.0	55.0	24.0	59.0	84.6	100.5	120.0	24.0	30.3	33.2	36.5	43.0
LL	2.2	3.6	4.3	5.0	6.5	3.2	3.5	4.8	6.2	6.3	3.5	4.0	4.7	5.5	6.0
LW	0.6	1.0	1.1	1.3	1.5	0.8	1.1	1.3	1.5	2.0	0.9	1.1	1.4	1.7	1.8
LD	1.7	2.5	3.2	3.8	5.2	2.2	2.5	3.5	4.7	5.0	2.5	2.8	3.2	3.7	4.1
R1	1.2	1.3	1.3	1.4	1.5	1.3	1.3	1.4	1.4	1.6	1.3	1.4	1.5	1.5	1.6
R2	2.8	3.4	3.9	4.3	5.1	2.3	2.9	3.7	4.3	4.5	3.1	3.2	3.5	3.9	4.4
HD	2.3	2.5	3.0	3.4	3.8	1.5	2.4	2.6	3.0	3.3	1.7	1.7	2.3	2.6	3.0
LT	0.2	0,2	0,3	0,3	0.5	0,3	0,4	0,5	0,5	0,6	0,4	0,4	0,5	0,6	0,7
SEL						7.5	7.8	8.7	9.4	9.7					
SEW						5.0	5.3	6.0	6.9	7.2					
RL	7.0	12.3	14.0	16.1	17.0	7.0	8.7	9.9	11.0	13.0	12.0	17.8	19.6	24.0	24.0
RW	1.2	1.3	1.4	1.5	2.0	1.5	1.6	1.8	2.0	2.2	1.3	1.6	1.9	2.2	2.3
BL	9.0	10.7	11.9	13.2	15.8	-	-	-	-	-	10.3	10.8	13.9	17.0	18.0
BW	4.7	6.7	7.1	7.7	8.3	-	-	-	-	-	5.0	5.8	7.0	8.3	8.8
BR	3.3	4.4	5.8	7.3	9.2	-	-	-	-	-	2.5	2.6	4.6	6.9	7.5
TL	-	-	-	-	-	-	-	-	-	-	11.7	12.4	13.1	13.8	14.5
TW	-	-	-	-	-	-	-	-	-	-	8.8	9.1	9.1	9.2	9.2
TR	-	-	-	-	-	-	-	-	-	-	0.0	0.0	0.0	0.0	0.0
CL	6.3	6.7	7.9	8.8	11.0	-	-	-	-	-	-	-	-	-	-
CW	5.2	5.2	6.0	6.8	7.3	-	-	-	-	-	-	-	-	-	-
SCL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SCW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SCR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VAL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VAW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VEL	3.3	3.5	3.9	4.3	4.5	3.8	4.2	4.5	4.9	5.0	3.7	4.8	5.0	5.3	5.5
VEW	1.3	1.5	1.6	1.7	1.8	1.5	1.7	1.9	2.0	2.3	1.2	1.8	1.9	2.2	2.3

B4

characters	subsp. *monardii*					subsp. *osteni*					subsp. *riffiniensis*				
	Min.	25%	Mean	75%	Max.	Min.	25%	Mean	75%	Max.	Min.	25%	Mean	75%	Max.
LB	30.0	55.0	59.7	67.0	84.0	37.0	44.5	60.0	75.5	81.0	28.0	32.0	40.8	49.0	71.0
LL	3.2	4.6	5.0	5.7	6.5	4.8	5.0	6.9	7.9	11.7	4.1	4.5	6.5	8.2	8.3
LW	0.6	0.9	1.1	1.4	2.1	1.1	1.5	1.9	2.1	2.9	1.5	1.5	1.9	2.1	2.3
LD	2.3	3.0	3.5	4.0	4.3	3.2	3.6	5.1	5.7	9.0	3.2	3.6	4.9	5.8	6.5
R1	1.2	1.4	1.4	1.5	1.6	1.3	1.3	1.4	1.4	1.5	1.2	1.2	1.3	1.4	1.6
R2	2.9	4.0	4.7	5.3	7.8	2.8	3.0	3.7	4.4	4.9	2.5	2.7	3.5	4.2	4.7
HD	2.5	3.1	3.6	4.0	4.6	2.3	3.1	3.4	3.9	4.0	2.9	3.2	3.4	3.6	4.0
LT	0,5	0,5	0,6	0,6	0,8	0,2	0,2	0,3	0,3	0,3	0,2	0,3	0,3	0,4	0,6
SEL															
SEW															
RL	9.0	13.0	15.5	18.0	23.0	12.0	16.5	20.6	25.0	25.0	17.0	17.0	19.3	21.0	22.0
RW	1.3	1.5	1.7	1.8	2.3	1.7	2.2	2.2	2.4	2.5	1.2	1.3	2.1	1.5	2.8
BL	-	-	-	-	-	-	-	-	-	-	10.0	10.8	13.5	15.0	15.0
BW	-	-	-	-	-	-	-	-	-	-	5.8	6.7	8.5	10.0	10.0
BR	-	-	-	-	-	-	-	-	-	-	5.0	5.4	6.6	7.9	8.7
TL	7.7	10.2	11.8	13.5	16.2	6.3	8.4	8.9	9.8	10.3	-	-	-	-	-
TW	5.8	8.3	9.0	9.8	11.2	5.3	6.0	7.4	9.1	10.0	-	-	-	-	-
TR	-	-	-	-	-	2.0	2.0	2.8	3.6	3.7	-	-	-	-	-
CL	-	-	-	-	-	-	-	-	-	-	5.8	7.5	8.7	10.5	11.3
CW	-	-	-	-	-	-	-	-	-	-	5.0	5.2	5.4	5.5	5.8
SCL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SCW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SCR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VAL	3.8	3.8	4.7	5.3	5.3	-	-	-	-	-	4.3	4.5	5.3	5.8	6.3
VAW	2.5	2.5	2.6	2.7	3.3	-	-	-	-	-	2.5	2.8	3.8	4.5	5.0
VEL	3.3	4.3	4.7	5.2	6.3	4.0	4.0	4.2	4.3	4.3	3.3	3.3	4.0	4.7	5.0
VEW	1.0	1.5	1.7	1.8	2.7	1.8	1.8	1.8	1.8	1.8	1.5	1.5	1.6	1.7	1.8

B5

characters	subsp. *suffruticosa*					subsp. *tazzea*					subsp. *trialata*				
	Min.	25%	Mean	75%	Max.	Min.	25%	Mean	75%	Max.	Min.	25%	Mean	75%	Max.
LB	10.0	16.5	21.4	25.0	27.0	40.0	40.5	58.4	81.5	93.0	25.0	29.8	43.5	59.5	66.0
LL	3.2	5.1	5.8	6.5	7.1	6.0	7.4	8.7	9.9	10.5	3.0	4.0	4.1	4.5	5.0
LW	0.8	0.9	1.0	1.2	1.4	0.9	1.5	1.8	2.1	2.6	0.6	0.6	0.8	1.1	1.1
LD	2.4	4.0	4.5	5.0	5.5	4.4	5.3	6.4	7.3	8.0	2.1	2.9	3.2	3.5	4.2
R1	1.2	1.3	1.3	1.3	1.4	1.2	1.3	1.4	1.5	1.5	1.2	1.3	1.3	1.4	1.4
R2	3.6	5.0	5.7	6.5	7.5	4.0	4.2	5.0	5.7	7.2	3.8	4.0	5.2	6.4	7.0
HD	3.4	3.6	4.2	4.6	5.2	3.2	3.2	3.6	4.0	4.4	1.5	1.8	2.3	2.9	3.3
LT	0,4	0,4	0,5	0,6	0,6	0,2	0,3	0,3	0,4	0,4	0,4	0,5	0,8	1,2	1,5
SEL															
SEW															
RL	11.0	16.0	19.7	23.0	26.0	21.0	24.0	26.7	30.0	31.0	4.5	5.4	9.6	14.1	16.0
RW	1.3	1.7	1.8	2.0	2.2	2.0	2.2	2.4	2.7	2.8	1.3	1.3	1.5	1.7	2.5
BL	12.5	13.0	15.0	17.3	18.0	16.0	17.8	20.5	22.5	25.0	9.2	10.3	12.0	13.5	17.0
BW	5.8	6.3	7.6	8.8	9.2	7.3	7.8	8.3	8.7	10.0	3.0	5.0	6.1	6.9	9.3
BR	3.0	3.6	5.8	7.4	7.5	3.3	5.3	8.4	11.0	13.7	1.5	2.0	3.7	5.7	8.7
TL	-	-	-	-	-	-	-	-	-	-	7.8	8.5	8.8	9.2	9.8
TW	-	-	-	-	-	-	-	-	-	-	6.3	7.0	7.6	8.0	8.8
TR	-	-	-	-	-	-	-	-	-	-	0.0	0.0	0.0	0.0	0.0
CL	8.8	10.3	11.0	11.5	14.5	6.7	8.5	9.7	11.0	12.5	-	-	-	-	-
CW	6.7	7.7	9.3	10.8	11.8	5.8	5.8	6.6	7.3	8.3	-	-	-	-	-
SCL	6.7	8.5	9.9	11.7	12.8	-	-	-	-	-	-	-	-	-	-
SCW	8.2	8.7	9.5	10.3	10.8	-	-	-	-	-	-	-	-	-	-
SCR	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-	-	-	-
VAL	4.5	4.8	6.1	7.3	8.5	4.2	6.0	6.7	7.5	8.3	-	-	-	-	-
VAW	3.2	3.3	4.1	4.7	5.8	2.8	4.5	4.5	4.8	5.2	-	-	-	-	-
VEL	4.3	4.3	4.7	4.9	5.3	3.3	3.5	4.4	5.1	5.7	3.8	4.0	4.2	4.5	4.7
VEW	1.3	1.5	1.7	1.8	2.0	1.2	1.6	1.8	2.2	2.2	1.3	1.5	1.6	1.8	1.8

Chapter 6 - Conclusion

6.1 Concluding remarks

This taxonomic study of the genus *Calendula* L. was conducted for the Iberian Peninsula and Morocco, including some taxa from Algeria, Tunisia, Sicily and Israel-Palestine, in order to improve the taxonomic core of the study. An exhaustive re-evaluation of the morphological characters used by previous authors, supported by chorological, karyological, and genome size approaches, allowed us to recognize 14 species in the study area.

Several morphological characters, both quantitative and qualitative, were found to be useful to distinguish *Calendula* taxa. Among these characters, some related with achene morphology revealed to be particularly important, although they must be used carefully, and in correlation with other types of characters, like growth form, genome size, chromosome number, and leaf morphology. The high diversity of combinations of various morphs of achenes and the high number of characters required were the major challenges to distinguish taxa.

The *C. suffruticosa* group revealed to be the most variable. Considering that all taxa from this group share the same chromosome number and it is known that they easily interbreed when in contact, we decided to keep them in the same species, although recognizing several subspecies, when enough morphological distinctiveness was observed, correlated with clear allopatry. *C. arvensis* is also a variable species, presenting concolours or discolours head flowers, with, at least, three main distinct combinations of achene morphologies. However, a correlation between these morphotypes and geographical distribution is much more difficult, and may of these froms are sympatric in this taxon, so we refrain from recognising any subespecific taxon under *C. arvensis*.

The karyology and genome size estimations of all taxa occurring in the studied region were studied. The results were generally consistent with previous investigations on the genus. We found that the chromosome number varies between 2n = 14 to ±88, but it is difficult to reach accurate counts for the taxa with higher number of chromosomes due to their small size. Our results also revealed evidence of autopolyploidy in *C. tripterocarpa*. We provided genome size estimations for the first time for the following taxa: *C. eckerleinii, C. fontquerii*, *C. davisii*, C. *lanzae*, *C. murbeckii, C. meuselii*, *C. pachysperma, C. palaestina*, *C. pinnatiloba* and 15 subspecies of *C. suffruticosa*.

Genome size estimation in *Calendula* taxa proved to be a valuable, quick and useful tool for taxonomy. It eases up the identification of probable chromosome numbers and ploidy levels, which, along with morphology, allows the detection of new taxa and its positioning in the proper taxonomic group.

The correct identification of *Calendula* taxa, together with, chromosome number, ploidy levels, genome size and eco-geographical variables, was imperative to understand the taxonomic relationships within the genus.

Finally, a description including all morphological characters is provided for each taxon, with additional information about distribution and habitat, conservation status, notes in the world and in the region of interest, comments on the variability, relationships between taxa, and full information about typification. In addition, dichotomous identification keys were elaborated. The new taxonomic treatments proposed for Iberian Peninsula and Morocco include:

For the Iberian Peninsula and the Balearic Islands, we recognize four species: *C. arvensis* L., *C. officinalis* L., *C. tripterocarpa* Rupr. and *C. suffruticosa* Vahl. For *C. suffruticosa*, nine subspecies are recognized in the Iberian Peninsula: subsp. *algarbiensis* (Boiss.) Nyman, subsp. *carbonelli* Ohle, subsp. *cinerea* (Ohle) P. Silveira & AC. Gonç. (nomenclatural change), subsp. *greuteri* Ohle, subsp. *lusitanica* (Boiss.) Ohle, subsp. *marginata* (Willd.) Maire (nomenclatural change) subsp. *tomentosa* (Desf. ex-Ball) Murb., subsp. *trialata* P. Silveira & AC. Gonç. (new subspecies described), and subsp. *vejerensis* P. Silveira & AC. Gonç. (new subspecies described), all of them endemic, or almost endemic, to the South of Spain

For Morocco, two new species were described, *C. fontquerii* AC. Gonç. & P. Silveira and, *C. davisii* AC. Gonç. & P. Silveira. Furthermore, one new combination *C. pinnatiloba* (Maire) AC. Gonç. & P. Silveira was made, and within the *C. suffruticosa* group 15 subspecies were recognized (eigth of them new), including taxa from Algeria and Tunisia.

6.2 Future perspectives

In spite of the great contribution here presented, we believe that the knowledge on Iberian Peninsula and Moroccan taxa need to be expanded. Studies integrating Macaronesia and/or more taxa from other Mediterranean regions, particularly Algeria and Tunisia, could also give insights into the relationships and evolution of the genus in the Mediterranean. Since herbarium specimens are, many times, not sufficiently representative of the diversity encountered in the populations where they come from, it's is very important to conduct expeditions in order to study *in loco* the morphological diversity and check if these patterns should be recognised taxonomically, or are not worthy of such changes.

Besides morphology, karyology, and genome size, it is important to use a combination of different methods and sources of information to prepare a global systematic treatment, such as palynology, chemotaxonomy and artificial hybridization. Furthermore, studies using different molecular methods, such ITS, microsatellites or other molecular markers, should be used to corroborate the results obtained and assess phylogenetic evolutionary relationships among taxa.

Furthermore, parallel studies using the 19 standard bioclimatic variables (Worldclim 1.4) and the monthly values for precipitation, maximum and minimum temperature could be used to check their different environmental preferences, hybrids' contact zones, and to determine the relationship between the distribution of the different taxa and environmental factors. Non-climatic variables (geology, aspect, elevation, solar radiation and slope) could also be included.

Another interesting study could be to try to understand the evolution of *Calendula* within the tribe Calenduleae.