

## *Carex camposii* subsp. *tejedensis* (Cyperaceae), a new taxon for Southern Iberian Peninsula based on molecular, morphological and ecological differentiation

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**Abstract.** *Carex camposii* Boiss. & Reut., endemic to high mountain ranges in Southern Iberian Peninsula (Sierra Nevada, Sierra de los Filabres and Sierra de Baza), is a morphologically and molecularly well-defined species included in *Carex* gr. *laevigata* (subg. *Carex*, sect. *Spirostachyae*). We have discovered a population of a morphologically similar species to *C. camposii* in a different mountain range (Sierra de Tejeda), that displayed some deviant morphological characters and was found in a different habitat from that typical of the species. In order to disentangle the taxonomic status of this population, we have conducted a phylogenetic analysis using five nuclear (*ITS*, *ETS*, *G3PDH*, *CATP* and *GZF*) and three plastid (*matK*, *rpS16* and *5 trnK* intron) DNA regions. In addition, a morphological analysis including the population from Sierra de Tejeda was carried out. The resulting phylogenetic trees show that the samples of the problematic population are closely related to *C. camposii*, while the morphological study revealed that a total of nine morphological features did not match those of typical *C. camposii*. Even though the samples from Sierra de Tejeda are genetically not well differentiated from *C. camposii*, the morphological and ecological differentiation supports its recognition as a new subspecies, *C. camposii* subsp. *tejedensis*. An assessment of its conservation status using IUCN categories and criteria suggests that it could be critically endangered (CR).

**Keywords:** Baetic mountain ranges, Sierra de Tejeda, *Carex camposii*, *Carex* gr. *laevigata*, DNA, phylogeny.

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### Introduction

*Carex* L. (Cyperaceae), with c. 2000 species, is one of the largest genera of angiosperms (WCSP, 2021; POWO, 2021; Larridon *et al.*, 2021). Most species occur in cold-temperate regions of the Northern Hemisphere (Reznicek, 1990; Martín-Bravo *et al.*, 2019). In Europe and the Mediterranean basin grow approximately 230 species of this genus (Jiménez-Mejías *et al.*, 2011), of which almost 100 (Luceño, 2008; Jiménez Mejías *et al.*, 2011a; Benítez-Benítez *et al.*, 2017; Maguilla & Escudero, 2017; Luceño *et al.*, in prep.) can be found in the Iberian Peninsula. Among the six recently recognized subgenera within *Carex* (Villaverde *et al.*, 2020: *Carex*, *Euthyceras* Peterm., *Psyllophorae* (Degl.) Peterm., *Siderosticta* Waterway, *Uncinia* (Pers.) Peterm., *Vignea* (P. Beauv. ex T. Lestib.) Heer), *Carex* (c. 1400 species) is the most species rich subgenus in the Iberian Peninsula, with 69 species recognized to date (Luceño,

2008; Jiménez Mejías *et al.*, 2011a; Benítez-Benítez *et al.*, 2017; Luceño *et al.*, in prep.).

Within the subgenus *Carex*, sect. *Spirostachyae* Drejer ex L.H. Bailey, with approximately 100 accepted species, is currently the second largest section of the genus (Global Carex Group, 2021). According to the most recent taxonomic and phylogenetic studies of the section (Escudero *et al.*, 2008, 2009; Escudero & Luceño, 2009, 2011; Global Carex Group, 2015, 2016, 2021; Martín-Bravo *et al.*, 2019) the species of sect. *Spirostachyae* should be classified in three different groups: (1) subsect. *Spirostachyae*, (2) subsect. *Elatae* (Kük) Luceño & M. Escudero and (3) the *Echinochlaenae* clade. Species within the section *Spirostachyae* present a worldwide disjunct distribution. According to Escudero *et al.* (2009), the ancestral area and the center of differentiation of this section is located in the Mediterranean-Eurasian region, from where it has colonized and diversified throughout the world through mechanisms of vicariance and long-

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distance dispersal. In the Iberian Peninsula a total of nine species of this section have been reported.

Within subsect. *Elatae*, *Carex camposii* Boiss. & Reut. is endemic to high mountain ranges in Southern Spain (hitherto known from Sierra Nevada, Sierra de los Filabres and Sierra de Baza). It is a morphologically (Luceño & Escudero, 2008) and phylogenetically (Escudero & Luceño, 2009) well-defined species that is grouped in the *C. gr. laevigata* clade (Escudero *et al.*, 2013), which is formed by *C. laevigata* Sm., *C. binervis* Sm., *C. paulo-vargasii* Luceño & J.M. Marín and *C. camposii*, a group of species occurring in the SW of the Palearctic region including the Iberian Peninsula. Concretely, *C. binervis* is found in heathland, stream edges, peat bogs margins and wet meadows of Western Europe (Luceño & Escudero, 2008). *Carex laevigata* shares almost the same geographical area as *C. binervis*, but it grows most often in dissimilar habitats, such as stream edges in riparian forests (Luceño & Escudero, 2008). Finally, *C. paulo-vargasii* is an endemic species

to the Middle Atlas and the Rif Mountain Range (Luceño & Marín, 2002), where it grows in edges of streams, bogs and wet meadows in open areas.

In the course of the numerous collecting campaigns and herbarium revisions performed in the Iberian Peninsula as part of different systematic studies of the genus *Carex*, we have detected a *Carex* population in Sierra de Tejada (Malaga, S Spain) formed by a few young, non-flowering individuals and an adult, mature one (Figure 1). The morphology of these individuals includes characters that do not match any of the taxa described so far for the Iberian territory (Luceño, 2008), although some of them indicate that this population should probably be included in sect. *Spirostachyae* and that it is a taxon morphologically close to *C. camposii*.

The aim of the present work is to carry out a morphological and a molecular study of the problematic population found in Sierra de Tejada that help us to elucidate its taxonomic status within sect. *Spirostachyae* and, more concretely, in relation with *Carex camposii*.

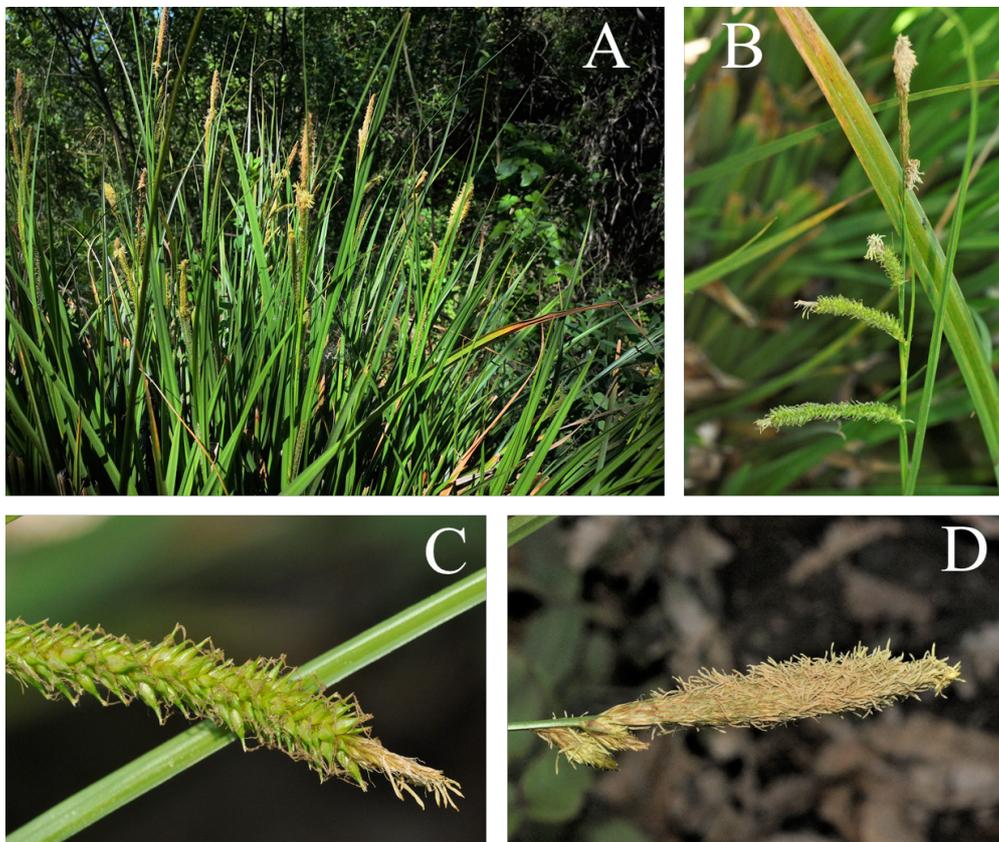


Figure 1. Morphological characters of *Carex* from Sierra de Tejada. A, general view of the plant; B, inflorescence; C, androgynous spike; D, male spikes.

## Materials and Methods

### Sampling

A total of 14 populations of *Carex gr. laevigata* have been sampled: five of *C. binervis* (three from the Iberian Peninsula and two from Southwestern Europe), two of *C. laevigata* (one from Southern Iberian Peninsula and other from the Northern Iberian Peninsula), two of *C. paulo-vargasii*, four of *C. camposii* (two from Sierra Nevada, one from Sierra de Los Filabres and one from

Sierra de Baza) and three individuals (one mature and two immature) of the problematic population found in Sierra de Tejada (Supplementary Material, Table S1). In addition, two populations of two species belonging to the subsect. *Elatae* of sect. *Spirostachyae* (*C. helodes* Link and *C. catharinensis* Boeck.) were included as outgroup. All samples were obtained from field collections, from herbarium materials belonging to the UPOS herbarium and from previous molecular studies (Global Carex Group, 2016; Martín-Bravo *et al.*, 2019) via the GenBank database (Supplementary Material, Table S1).

## Morphological study

We have examined a total of 20 quantitative and 28 qualitative morphological characters of the only two mature known individuals of the Sierra de Tejada plant, one collected by the authors in 2021 (UPOS-14126; see Supplementary Material, Table S2) and the other one collected by B. Cabezudo *et al.* in 2003 (MGC-58571). We have compared them with the already described characters of *Carex camposii*. These morphological characters were chosen based on the most important diagnostic characters described for section *Spirostachyae* (Luceño & Escudero, 2008; Escudero & Luceño, 2011; Supplementary Material, Table S4). We used a ruler for larger measurements, whereas an Olympus SZX16 stereo microscope was used for utricle, achene, and glume measurements.

## Cytogenetic study

One of the non-flowering specimens found in the Sierra de Tejada population was collected alive and cultivated in the research greenhouse of the Pablo de Olavide University. When it started flowering, anthers were fixed and stained following the protocol described by Luceño (1988), and chromosomes were observed in metaphase I (MI) of meiosis using a Nikon Eclipse E400 microscope equipped with a digital camera Nikon DXM1200F.

## DNA extraction, amplification and sequencing

From each sample 20–60 mg of dried leaf tissue was separated. DNA extraction was carried out using the CTAB method (Doyle & Doyle, 1990) with some minor modifications. A total of eight DNA regions were amplified and sequenced: two multi-copy nuclear regions (*ITS* and *ETS*), three single-copy nuclear genes (*G3PDH*, *CATP* and *GZF*), and three plastid regions (*matK* gene, *rps16* intron and *5 trnK* intron). Procedures for DNA amplification followed those in Escudero *et al.* (2009) for *ITS* and *5 trnK* intron, in Starr *et al.* (2003) for *ETS*, in Maguilla *et al.* (2018) for *G3PDH*, in Maguilla *et al.* (2015) for *CATP* and *GZF*, in Jiménez-Mejías *et al.* (2016) for *matK*, and in Shaw *et al.* (2005) for *rps16*. A BioRad T100TM Thermal Cycler was used to perform the Polymerase Chain Reactions. PCR products were sent to Macrogen (Seoul, South Korea) for sequencing.

## Phylogenetic analysis

The sequences were assembled automatically using Muscle v.3.8.425 (Edgar, 2004) and revised and edited with the software Geneious 11.1.2 (<https://www.geneious.com>). As a result, a total of three matrices were generated: (i) a nuclear matrix composed of 18 accessions and 2313 sites, including 18 *ITS* sequences (two out of them were from NCBI Genbank), 18 of *ETS* (two from NCBI Genbank), 15 of *G3PDH*, 16 of *CATP* and 12 of *GZF*; (ii) a plastid matrix composed of 18 accessions and 2157 sites, including 18 *matK* sequences (two from NCBI Genbank), 16 of *rps16* and 15 of *5 trnK* intron; and (iii) a final combined matrix resulting from

the concatenation of all the DNA regions composed of 18 accessions and 4470 sites (Supplementary Material, Table S2). The matrices were analyzed using Bayesian Inference (BI) with MrBayes software (Ronquist *et al.*, 2012). The best nucleotide substitution model for each DNA region was chosen based on the results obtained in JModelTest v.2.1.10 (Darriba *et al.*, 2012) using AICc (corrected Akaike Information Criterion; Akaike, 1974) (Supplementary Material, Table S2). Region *ITS* was split into three regions, *ITS1*, *5.8 S* and *ITS2*. We performed two independent runs of four Metropolis-coupled Markov chains for five million generations. Sampling was done every 100 generations. The first 20% of the trees generated were discarded as burn-in and the majority rule consensus tree was obtained from the remaining 80% of trees. Finally, the phylogenetic trees were visualized and edited using FigTree v.1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>) and Adobe Illustrator v.14.0 ([www.adobe.com](http://www.adobe.com)).

## Haplotype network

The combined plastid DNA sequences of the four sampled populations of *Carex camposii* and the three problematic individuals from Sierra de Tejada were analyzed with PopART v.1.7 software (Leigh & Bryant, 2015) in order to visualize the nucleotide changes between them.

## Results

The voucher MGC-58571 from Sierra de Tejada was found to be similar to *Carex camposii*, but with some deviant morphological characters (see below). This plant was initially identified as *C. riparia* Curtis, the only record of this plant in eastern Andalusia (Salazar & Quesada, 2011). However, as the specimen does not belong to this species with certainty, its presence in eastern Andalusia should be rejected. In a posterior field trip to Sierra de Tejada (40 km from the nearest population of *C. camposii*), the corresponding population was located and the habitat where we found it (riverside forest in an oceanic-influenced environment) was also characterized as different from that typical of *C. camposii* (Luceño & Escudero, 2008). Only one mature individual (Figure 1) and eleven immature ones were found in the population.

## Morphological study

A total of nine morphological features (Table 1) from the population from Sierra de Tejada did not match the morphology described for *Carex camposii* (Luceño & Escudero, 2008). In contrast to *C. camposii*, the measured plants from Sierra de Tejada present longer ligules, the lowest bract of the inflorescence is longer than the inflorescence length, it generally has more male spikes, the intermediate spikes are usually androgynous, the color of the male and female glumes is yellowish-brown, it has longer utricles that are narrowly ellipsoid and gradually attenuated (rarely abruptly contracted) into the beak, the utricle beak is generally longer and the achenes are longer (Table 1).

Table 1. Main distinctive characters between *Carex* individuals from Sierra de Tejada and *C. camposii*.

Character	<i>Carex</i> from Sierra de Tejada	<i>C. camposii</i> *
Ligule length (mm)	(17)21–41	5–20(30)
Lowest bract of the inflorescence	Longer than the inflorescence length	Shorter than the inflorescence length
Male spikes number	2–3(4)	1–2(3)
Intermediate and lower spikes	Usually androgynous, but the lowest one usually female	Female
Color of the glumes	Yellowish-brown	Dark red-brown to purple-brown
Utricle shape	Narrowly ellipsoid, gradually attenuated, rarely abruptly contracted, into the beak	Widely ellipsoid, abruptly contracted into the beak
Utricle (length x width; mm)	(3.5)4.2–5.3(5.7) x 1.1–1.7	(2)2.5–3.5(4) x 1–2
Achene (length x width; mm)	2.5–3.5 x (0.9)1–1.5	1.4–1.9 x 0.9–1.4
Beak length (mm)	0.8–1.9	0.4–1

\* Measurements of *C. camposii* has been taken from Luceño & Escudero (2008), except the presence of three male spikes, a character not reported to date, that has only been observed in two populations of this species, both in Sierra Nevada, Granada: (UPOS-14127, Balsa del Almiar, Soportújar; GDAC-40821, Barrio river, Lanteira).

### Cytogenetic count

The studied specimen showed a regular pairing with  $36^{\text{II}}$  in MI (Figure 2), the same chromosome number

and the most frequent configuration previously published for *Carex camposii* (Luceño & Castroviejo, 1993).

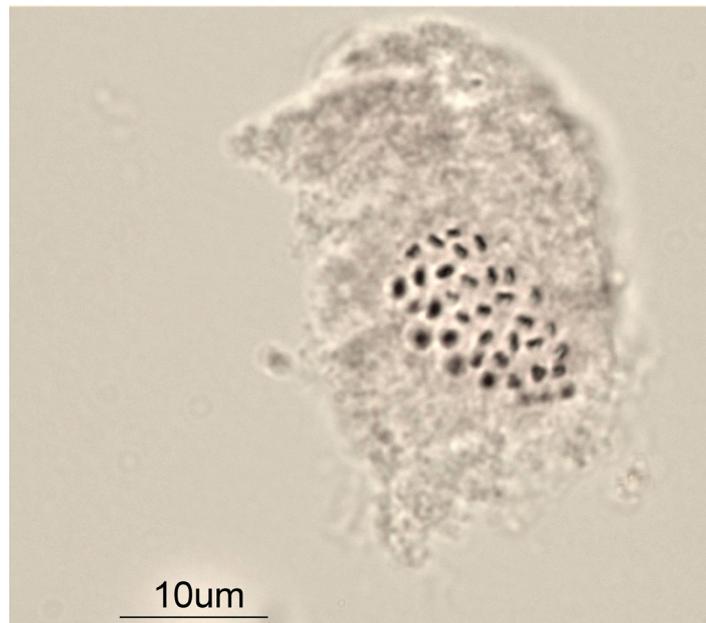


Figure 2. Meiotic configuration of *Carex* from Sierra de Tejada ( $2n = 36^{\text{II}} = 72$ ).

### Phylogenetic study and Haplotype network

Regarding the phylogenetic trees obtained after the analysis of the molecular matrices, it can be observed that the resolution shown by the plastid tree (Figure 3B) is much lower than that of the nuclear (Figure 3A) and combined trees (Supplementary Material, Figure S1) due to the lower number of informative characters in the plastid matrix (Supplementary Material, Table S2).

The species of *Carex* gr. *laevigata* are clustered in a supported clade (0.91–1 *pp*) in all the trees, except in the plastid one, where the clade is not supported. While *C. paulo-vargasii* is monophyletic in all the trees obtained, *C. binervis* and *C. laevigata* remain non-monophyletic. The samples of the problematic

population found in Sierra de Tejada are grouped with those of *C. camposii* in a well-supported clade in all the trees (1 *pp*) except in the plastid one, where the samples appear in polytomy in a non-supported clade. In the nuclear and combined trees, a subclade containing the samples of *C. camposii* from Sierra de los Filabres, Sierra de Baza and one from Sierra Nevada (in addition of one from the population of Sierra de Tejada in the nuclear one) is formed, but none of them are supported. The rest of samples of *C. camposii* and the problematic population from Sierra de Tejada appear in polytomy.

The analysis of the plastid matrix of the samples of *C. camposii* and the problematic individuals from Sierra de Tejada has revealed three different haplotypes (Figure 4). The haplotype 1 is the most represented, as

it includes all the samples from Sierra de Tejada and two samples of *C. camposii* (one from Sierra Nevada and one from Sierra de Baza). Haplotypes 2 and 3 are both found in only one population of *C. camposii*,

one from Sierra de los Filabres and one from Sierra Nevada. While haplotype 2 differs from haplotype 1 by six mutations, haplotype 3 differs from haplotype 1 by two mutations.

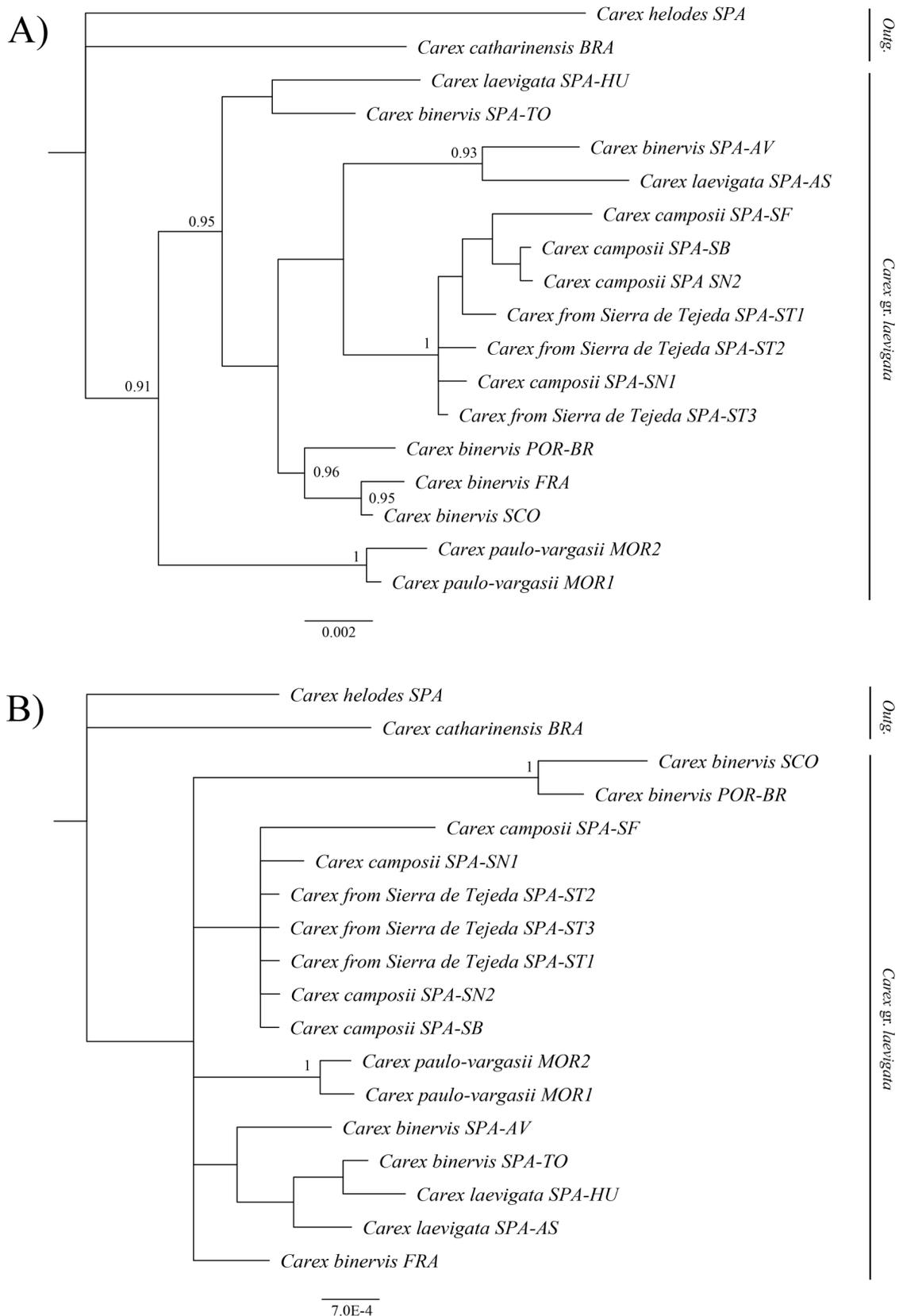


Figure 3. Majority-rule consensus trees obtained from the Bayesian analysis of: A, the combined nuclear (*ITS*, *ETS*, *G3PDH*, *CATP* and *GZF*); B, the combined plastid (*matK*, *rpS16* and *5 trnK* intron) regions. Node labels indicate posterior probability of the clades only when it is higher than 0.9. Tip labels indicate species names and populations (see Supplementary material, Table S1). Scale bar represents substitutions per site.

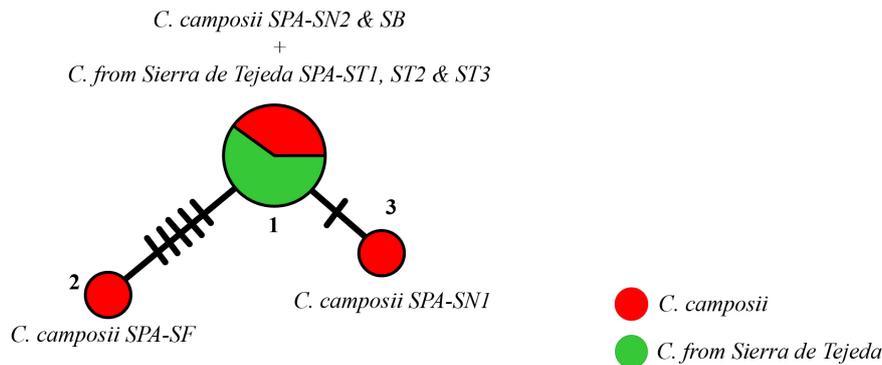


Figure 4. Haplotype network obtained from the analysis of the combined plastid sequences of *Carex camposii* and the individuals from Sierra de Tejada. Short black lines represent inferred extinct or not sampled haplotypes.

## Discussion

Important morphological differences have been found between the individuals studied from Sierra Tejada and those of the remaining populations of *Carex camposii* (Table 1). The description of this species would have to be substantially modified in case if such differences were considered within the range of its morphological variability.

The genetic information provided by the Sanger sequenced DNA regions used in this study shows that the samples included from the problematic population found in Sierra de Tejada are intermingled with those of *Carex camposii* in a well-supported monophyletic group. In addition, in the haplotype network the samples of the plant from Sierra Tejada share the same haplotype with two other samples of *C. camposii*. Although our results do not support that this population is a different phylogenetic species (Mishler & Brandon, 1987) from *C. camposii*, the morphological study shows that *C. camposii* and the population from Sierra de Tejada exhibited important morphological differences. Finally, our cytogenetic results reveal the same chromosome number for the studied population with respect to the known number for *C. camposii*.

As mentioned above, the Sierra de Tejada population shows a different habitat than the known populations of *Carex camposii*. The plants from Sierra de Tejada were found in a more oceanically-influenced habitat than those typical for *C. camposii* (Luceño & Escudero, 2008; Salazar & Quesada, 2011), so that these morphological differences could be due to the phenotypic plasticity of the species. Alternatively, this population could be interpreted as a different taxon. It is important to note that: 1) the greenhouse-grown specimen maintains the characters of the two mature individuals studied from the Sierra de Tejada population; 2) there are already numerous precedents where the new taxa have been described based on only one population, e.g. *Gadoria falukei* Güemes & Mota (Güemes & Mota, 2017), *Rivasmartinezia cazorlana* Blanca, Cueto, Benavente & J. Fuentes (Blanca *et al.*, 2016); and 3) it has been

advocated that some morphologically-differentiated disjunct populations could benefit from the concept of “morphogeographical compartmentalization” proposed by Stuessy (1990) to explain morphological differences between geographically distant populations and which has been used to justify infraespecific taxonomic treatments in the genus *Carex* (Jiménez-Mejías *et al.*, 2017; Míguez *et al.*, 2018). The absence of molecular and cytogenetic differentiation of the newly discovered population could be explained by a recent migration of *C. camposii* to Sierra de Tejada with subsequent interruption of gene flow that has led to a rapid morphological change, perhaps due to the adaptation to a new habitat, without generating significant genetic differentiation between the plant from Sierra de Tejada and the rest of the populations of *C. camposii*, at least for the analyzed markers. A similar process has been reported for *C. borbonica* Lam. and *C. boryana* Schkuhr, two species belonging to subsect. *Elatae* of sect. *Spirostachyae* growing on the Reunion Island (Escudero & Luceño, 2011). These species are morphologically and ecologically well-delimited (Escudero & Luceño, 2011), but genetically and cytogenetically very similar (Escudero *et al.*, 2009; Márquez-Corro *et al.*, 2018). Moreover, it is not uncommon to find groups of plants with such contrasting patterns of morphological-ecological vs genetic differentiation in the Western Mediterranean basin and North Africa. In *Reseda* sect. *Glaucorese* (Martín-Bravo *et al.*, 2010) and *Linaria* sect. *Supinae* (Blanco-Pastor *et al.*, 2012), species are morphologically and ecologically very different, but it is difficult to separate them genetically. For all these reasons, we think that the second hypothesis is the most plausible, and therefore the population from Sierra de Tejada would belong to a new taxon. Considering the morphological and ecological differentiation, as well as the geographic isolation of the population from Sierra de Tejada (Figure 5) and pending a genomic study that sheds more light on the evolutionary history of this lineage, we believe that the most appropriate is to consider the plants of Sierra de Tejada as a different subspecies of *C. camposii*.

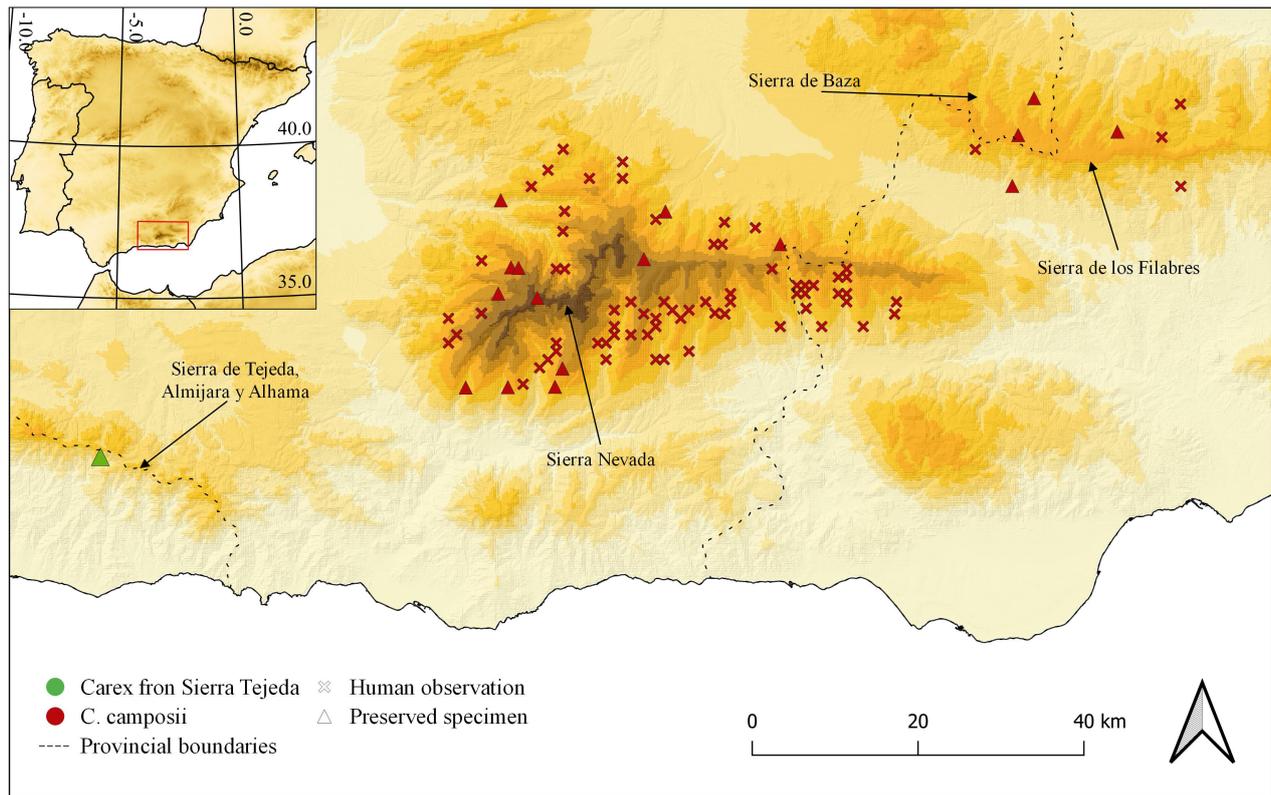


Figure 5. Distribution map of *Carex camposii* subsp. *camposii* and *Carex* from Sierra de Tejada (Supplementary Material, Table S3).

***Carex camposii* subsp. *tejedensis* R. Sánchez-Villegas, M. Escudero & Luceño, subsp. nov.**

**Diagnosis:** Similar to *Carex camposii* subsp. *camposii*, from which it differs by its longer ligules ((17)21–41 mm in subsp. *tejedensis* vs 5–20(30) mm in subsp. *camposii*), its lowest bract of the inflorescence (longer than the inflorescence length in subsp. *tejedensis* vs. shorter than the inflorescence length in subsp. *camposii*), its higher number of male spikes (2–3(4) in subsp. *tejedensis* vs 1–2(3) in subsp. *camposii*), its intermediate spikes (usually androgynous in subsp. *tejedensis* vs female in subsp. *camposii*), the color of its male and female glumes (yellowish-brown in subsp. *tejedensis* vs dark red-brown to purple-brown in subsp. *camposii*), its utricles shape (narrowly ellipsoid and gradually attenuated (rarely abruptly contracted) into the beak in subsp. *tejedensis* vs widely ellipsoid and abruptly contracted in subsp. *camposii*), and length ((3.5)4.2–5.3(5.7) mm in subsp. *tejedensis* vs (2)2.5–3.5(4) mm in subsp. *camposii*), its utricle beak length (0.8–1.9 mm in subsp. *tejedensis* vs 0.4–1 mm in subsp. *camposii*), and the achenes of the subsp. *tejedensis* are longer (2.5–3.5 mm) than in subsp. *camposii* (1.4–1.9 mm).

**Holotype:** Spain, Málaga, P.N. Sierra de Tejada, Almjara and Alhama, Canillas de Albaida, Nevazo stream, 36°52'55.95"N 03°57'2.81"W, 1381 m.a.s.l., riverside forests, 13-V-2021, R. Sánchez-Villegas 65RSV21, B. Quirós, S. Martín-Bravo, J. Algarra, C. Salazar & M. Luceño (UPOS-14126). **Paratype:** Spain, Málaga, Canillas de Albaida, P.N. Sierra de Tejada, Almjara and Alhama, Nevazo stream, VF1582, 1000 m.a.s.l., riverside

forests, 30-V-2003, B. Cabezudo, G. Caballero, A.V. Pérez Latorre & D. Navas R. 1359/03 (I) (MGC-58571).

Rhizome densely caespitose, with short internodes, very stout, dark-brown. Flowering culms 72–84 cm long, obtusely trigonous, smooth, leafy up to the upper third of its length, (2.1)3.1–4.5 cm wide at the middle. Leaves (8)10–21(22) mm wide, shorter or longer than the inflorescence, quite rigid, middle to dark green, plicate in cross section, scabrous along the edges, at least in the upper two-thirds; abaxial surface smooth, but scabrous on the upper third of the midrib; adaxial surface smooth; ligule (17)21–41 mm long, usually obtuse. Basal sheaths entire, lowermost bladeless. Lowest bract of the inflorescence leaf-like, longer than the inflorescence length. Male spikes 2–3(4), 45–65 x (2.5)3–5 mm the upper one and 5–30(45) x 1.7–3.1(4) mm the lower one, fusiform; female spikes 3–4, (35)40–87(91) x 2.9–5.5 mm, cylindrical, the upper one sessile, the lower ones pedunculate and sometimes nodding, the intermediate generally androgynous and the lowest one usually female. Male glumes 4.3–5.1 x 1.1–1.7 mm, oblong to oblong-lanceolate, yellowish-brown with a green central band, shortly mucronate; female glumes 3.1–5 x 1.1–1.6 mm, ovate, yellowish-brown with a green central band, mucronate. Utricles (3.5)4.2–5.3(5.7) x 1.1–1.7 mm, suberect, narrowly ellipsoid-trigonous, smooth to very shortly and dispersely aculeolate at the apex, green to yellowish-brown when mature, with perceptible veins, although the two lateral ones are much more prominent, gradually attenuated (rarely contracted) into a bifid beak up to 0.8–1.9 mm long. Achenes 2.5–3.5 x (0.9)1–1.5 mm, ellipsoid-trigonous.



Figure 6. Analytical drawing of *Carex camposii* subsp. *tejedensis*. A, plant size; B, male glume; C, female glume; D, utricle; E, achene; F, ligule. Drawing by M. Sánchez-Villegas.

**Distribution and habitat:** Endemic to Sierra de Tejada (Figure 5) in Málaga province (Spain), where it has only been found in the Nevazo stream, in riverside forest accompanied by *Castanea sativa* Mill., *Salix atrocinerea* Brot., *Rubus ulmifolius* Schott, *Scrophularia scorodonia* L., *Hypericum tetrapterum* Fries and *Adiantum capillus-veneris* L.

**Etymology:** This taxon is named after the Sierra de Tejada, the only place where this plant has been found to date.

**Iconography:** Figure 1 and 6.

**Conservation:** There is only one known population of *Carex camposii* subsp. *tejedensis*, consisting of one mature individual and eleven immature ones. The population is included in a protected area, Sierra de Tejada, Almijara and Alhama Natural Park. During the years 2021 and 2022, numerous surveys of this taxon have been carried out throughout similar habitats of the Sierra de Tejada, Almijara and Alhama Natural Park, but none has been successful. According to the criterion D of the IUCN Red List (IUCN, 2012), this taxon should be categorized as critically endangered (CR) because the number of mature individuals of the unique population is less than 50. Therefore, we propose here the category CR D for *C. camposii* subsp. *tejedensis*, applicable at both national and global level.

## Conclusions

The population found in Sierra de Tejada is closely related to *Carex camposii*. However, the remarkable morphological and ecological differentiation, as well as the geographical isolation of this population has led us to describe it as a new subspecies, *C. camposii* subsp. *tejedensis*.

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## Conflict of interest

None.

## Authorship contribution

R.S.-V.: Data curation, Formal analysis, Research, Management of the Project, Resources, Software, Visualization, Writing (first draft, review and editing); M.E.: Conceptualization, Methodology, Resources, Supervision, Writing (review and editing); S.M.-B.: Conceptualization, Resources, Supervision, Writing (review and editing); C.S.-M.: Resources, Writing

(review and editing); J.A.A.: Resources, Writing (review and editing); M.L.: Conceptualization, Research, Management of the project, Resources, Supervision, Writing (first draft, review and editing).

## References

- Akaike, H. 1974. A New Look at the Statistical Model Identification. *IEEE T. Automat. Contr.* 19(6): 716–23.
- Benítez-Benítez, C., Míguez, M., Jiménez-Mejías, P. & Martín-Bravo, S. 2017. Molecular and Morphological Data Resurrect the Long Neglected *Carex laxula* (Cyperaceae) and Expand its Range in the Western Mediterranean. *Anales Jard. Bot. Madrid* 74: 1–12. doi:10.3989/ajbm.2438
- Blanca, G., Cueto, M., Benavente, A. & Fuentes, J. 2016. *Rivasmartinezia cazorlana* sp. nov. (Apiaceae) from southern Spain. *Nord. J. Bot.* 34, 517–521. doi:10.1111/njb.01191
- Blanco-Pastor, J.L., Vargas, P. & Pfeil, B.E. 2012. Coalescent Simulations Reveal Hybridization and Incomplete Lineage Sorting in Mediterranean *Linaria*. *PloS one* 7(2): e39089. doi:10.1371/journal.pone.0039089
- Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. 2012. JModelTest 2: More Models, New Heuristics and Parallel Computing. *Nat. Methods* 9(8): 772. doi:10.1038/nmeth.2109
- Doyle, J.J. & Doyle, J.L. 1990. Isolation of Plant DNA from Fresh Tissue. *Focus* 12: 13–15.
- Edgar, R.C. 2004. MUSCLE: Multiple Sequence Alignment with High Accuracy and High Throughput. *Nucleic Acids Res.* 32(5): 1792–1797. doi:10.1093/nar/gkh340
- Escudero, M. & Luceño, M. 2009. Systematics and evolution of *Carex* sects. *Spirostachyae* and *Elateae* (Cyperaceae). *Plant Syst. Evol.* 279(1–4): 163–189. doi:10.1007/s00606-009-0156-x
- Escudero, M. & Luceño, M. 2011. Taxonomic revision of the tropical African group of *Carex* subsect. *Elateae* (sect. *Spirostachyae*, Cyperaceae). *Anal. Jardin Bot. Mad.* 68(2): 225–247. doi:10.3989/ajbm.2256
- Escudero, M., Maguilla, M. & Luceño, M. 2013. Selection by Climatic and Neutral processes in holocentric chromosomes (*Carex* gr. *laevigata*: Cyperaceae): A microevolutionary approach. *Perspect. Plant. Ecol. Evol. Syst.* 15: 118–129. doi:10.1016/j.ppees.2013.01.001
- Escudero, M., Valcárcel, V., Vargas, P. & Luceño, M. 2008. Evolution in *Carex* L. sect. *Spirostachyae* (Cyperaceae): A molecular and cytogenetic approach. *Org. Divers. Evol.* 7(4): 271–291. doi:10.1016/j.ode.2006.08.006
- Escudero, M., Valcárcel, V., Vargas, P. & Luceño, M. 2009. Significance of ecological vicariance and long-distance dispersal in the diversification of *Carex* sect. *Spirostachyae* (Cyperaceae). *Am. J. Bot.* 96(11): 2100–2114. doi:10.3732/ajb.0900134

- Global Carex Group, Waterway, M.J., Ford, K.A., Luceño, M., Martín-Bravo, S., Starr, J.R., Wilson, K.L., Yano, O., Zhang, S.R., Roalson, E.H., Alverson, W.S., Bruederle, L.P., Bruhl, J.J., Chung, K.-S., Cochrane, T.S., Escudero, M., Ford, B.A., Gebauer, S., Gehrke, B., Hahn, M., Hipp, A.L., Hoffmann, M.H., Hoshino, T., Jimenez-Mejias, P., Jin, X.-F., Jung, J., Kim, S., Maguilla, E., Masaki, T., Miguez, M., Molina, A., Naczi, R.F.C., Reznicek, A.A., Rothrock, P.E., Simpson, D.A., Spalink, D., Thomas, W.W. & Villaverde, T. 2015. Making Carex monophyletic: a new broader circumscription. *Bot. J. Linnean Soc.* 179: 1–42. doi:10.1111/boj.12298
- Global Carex Group, Jiménez-Mejías, P., Hahn, M., Lueders, K., Starr, J.R., Brown, B.H., Chouinard, B.N., Chung, K.S., Escudero, M., Ford, B.A., Ford, K.A., Gebauer, S., Gehrke, B., Hoffmann, M.H., Jin, X.F., Jung, J., Kim, S., Lucenõ, M., Maguilla, E., Martín-Bravo, S., Míguez, M., Molina, A., Naczi, R., Pender, J.E., Reznicek, A.A., Villaverde, T., Waterway, M.J., Wilson, K.L., Yang, J.-C., Zhang, S., Hipp, A.L. & Roalson, E.H. 2016. Megaphylogenetic Specimen-Level Approaches to the Carex (Cyperaceae) Phylogeny Using ITS, ETS, and matK Sequences: Implications for Classification. *Syst. Bot.* 41(3): 500–518. doi:10.1600/036364416X692497
- Global Carex Group, Roalson, E.H., Jiménez-Mejías, P., Hipp, A.L., Benítez-Benítez, C., Bruederle, L.P., Chung, K.-S., Escudero, M., Ford, B.A., Ford, K.A., Gebauer, S., Gehrke, B., Hahn, M., Hayat, M.Q., Hoffmann, M.H., Jin, X.-F., Kim, S., Larridon, I., Léveillé-Bourret, É., Lu, Y.-F., Luceño, M., Maguilla, E., Márquez-Corro, J.I., Martín-Bravo, S., Masaki, T., Míguez, M., Naczi, R.F.C., Reznicek, A.A., Spalink, D., Starr, J.R., Uzma, Villaverde, T., Waterway, M.J., Wilson, K.L. & Zhang, S. 2021. A framework Infrageneric classification of Carex (Cyperaceae) and its organizing principles. *J. Syst. Evo.* 59(4): 726–762. doi: 10.1111/jse.12722
- Güemes, J. & Mota, J. F. 2017. *Gadoria* (Antirrhineae, Plantaginaceae): A new genus, endemic from Sierra de Gádor, Almería, Spain. *Phytotaxa*, 298(3): 201–221. doi:10.11646/phytotaxa.298.3.1
- IUCN. 2012. IUCN Red List Categories and Criteria. Version 3.1 Second edition. IUCN Species Survival Commission, Gland and Cambridge.
- Jiménez-Mejías, P., Escudero, M., Guerra-Cardenas, S., Lye, K.A. & Luceño, M. 2011. Taxonomic delimitation and drivers of speciation in the Ibero-North African Carex sect. *Phacocystis* river-shore group (Cyperaceae). *Am. J. Bot.* 11: 1855–1867. doi:10.3732/ajb.1100120
- Jiménez-Mejías, P., Benítez-Benítez, C., Fernández-Mazuecos, M. & Martín-Bravo, S. 2017. Cut from the same cloth: The convergent evolution of dwarf morphotypes of the Carex flava group (Cyperaceae) in Circum-Mediterranean mountains. *PLoS ONE* 12(12): e0189769. doi:10.1371/journal.pone.0189769
- Larridon, I., Zuntini, A.R., Léveillé-Bourret, É., Barrett, R.L., Starr, J.R., Muasya, A.M., Villaverde, T., Bauters, K., Brewer, G.E., Bruhl, J.J., Costa, S.M., Elliott, T.L., Epitawalage, N., Escudero, M., Fairlie, I., Goetghebeur, P., Hipp, A.L., Jiménez-Mejías, P., Kikuchi, I.A.B.S., Luceño, M., Márquez-Corro, J.I., Martín-Bravo, S., Maurin, O., Pokorny, L., Roalson, E.H., Semmouri, I., Simpson, D.A., Spalink, D., Thomas, W.W., Wilson, K.L., Xanthos, M., Forest, F. & Baker, W.J. 2021. A new classification of Cyperaceae (Poales) supported by phylogenomic data. *J. Syst. Evol.* 59(4): 852–895. doi:10.1111/jse.12757
- Leigh, J.W. & Bryant D. 2015. PopART: Full-feature software for haplotype network construction. *Methods Ecol. Evol.* 6(9): 1110–1116. doi:10.1111/2041-210X.12410
- Lorite, J., Valle, F. & Salazar C. 2003. Síntesis de la vegetación edafohigrófila del Parque Natural y Nacional de Sierra Nevada. *Monogr. Fl. Veg. Béticas* 13: 47–110.
- Luceño, M. 1988. Notas caricológicas III. *An. Jard. Bot. Madrid* 45: 189–196.
- Luceño, M. 1992. Distribución de Carex camposii Boiss. & Reuter en la Península Ibérica. *An. Jard. Bot. Madrid* 50(1): 123–125.
- Luceño, M. 2008. Carex L. In: S. Castroviejo et al. (Eds.) *Flora Iberica* 18: 178–191. Real Jardín Botánico, CSIC, Madrid.
- Luceño M. & Castroviejo S. 1993. Cytotaxonomic studies in the sections Spirostachyae (Drejer) Bailey and Ceratocystis Dumort. of the genus Carex L. (Cyperaceae), with special reference to Iberian and North African taxa. *Bot. J. Linn. Soc.* 112: 335–350.
- Luceño, M. & Escudero, M. 2008. Carex sect. Spirostachyae Drejer ex L.H. Bailey. In: S. Castroviejo et al. (Eds.) *Flora Iberica* 18: 178–191. Real Jardín Botánico, CSIC, Madrid.
- Luceño, M. & Marín, J.M. 2002. Carex paulo-vargasii Luceño & J.M. Marín (Cyperaceae), una nueva especie del norte de África. *An. Jard. Bot. Madrid* 59(2): 348–350.
- Maguilla, E. & Escudero, M. 2017. Cryptic Species Due to Hybridization: A combined Approach to Describe a New Species (Carex: Cyperaceae). *Plos One* 12(2): e017209. doi:10.1371/journal.pone.0172079
- Maguilla E., Escudero M., Waterway M.J., Hipp A.L. & Luceño M. 2015. Phylogeny, systematics, and trait evolution of Carex section Glareosae. *Am. J. Bot.* 102: 1128–1144. doi:10.3732/ajb.1500169
- Maguilla E., Escudero, M. & Luceño, M. 2018. Vicariance versus dispersal across Beringian land bridges to explain circumpolar distribution: A case study in plants with high dispersal potential. *J. Biogeogr.* 45: 771–783. doi:10.1111/jbi.13157
- Márquez-Corro, J.I., Luceño, M., Jiménez-Mejías, P., Escudero, M., Martín-Bravo, S., Hipp, A.L., Chung, K., Muasya, A.M., Rothrock, P.E., Weber, J.A. & Naczi, R. 2018. Cyperaceae. In: K. Marhold & J. Kučera (Eds.) *IAPT/IOPB chromosome data* 28. *Taxon* 67(6): 6–8. doi:10.1002/tax.12414
- Martín-Bravo, S., Valcárcel, V., Vargas, P. & Luceño, M. 2010. Geographical Speciation Related to Pleistocene Range Shifts in the Western Mediterranean Mountains (Reseda sect. *Glaucoreseda*, Resedaceae). *Taxon* 59(2): 466–482. doi:10.1002/tax.592012
- Martín-Bravo, S., Jiménez-Mejías, P., Villaverde, T., Escudero, M., Hahn, M., Spalink, D., Roalson, E.H., Hipp, A.L., Benítez-Benítez, C., Bruederle, L.P., Fitzek,

- E., A. Ford, B.A., Ford, K.A., Garner, M., Gebauer, S., Hoffmann, M.H., Jin, X.F., Larridon, I., Lévillé-Bourret, É., Lu, Y.-F., Luceño, M., Maguilla, E., Márquez-Corro, J.I., Míguez, M., Naczi, R., Reznicek, A.A. & Starr, J.R. 2019. A tale of worldwide success: Behind the scenes of *Carex* (Cyperaceae) biogeography and diversification. *J. Syst. Evol.* 57(6): 695–718. doi:10.1111/jse.12549
- Míguez, M., Martín-Bravo, S. & Jiménez-Mejías, P. 2018. Reconciling morphology and phylogeny allows an integrative taxonomic revision of the giant sedges of *Carex* section *Rhynchocystis* (Cyperaceae). *Bot. J. Linn.* 188(1): 34–58. doi:10.1093/botlinnean/boy040
- Mishler, B.D. & Brandon, R.N. 1987. Individuality, Pluralism and the Phylogenetic Species Concept. *Biol. Philos.* 2: 397–414.
- Peñas, J., Pérez-García, F.J. & Mota, J.F. 2005. Patterns of endemic plants and biogeography of the Baetic high mountains (south Spain). *Acta Bot. Gallica* 152(3): 347–360. doi:10.1080/12538078.2005.10515494
- Quèzel, P. 1953. Contribution a l'étude phytosociologique et géobotanique de la Sierra Nevada. *Mem. Soc. Brot.* 9: 5–82.
- Red de Información Ambiental de Andalucía (REDIAM). 2009. Cartografía de vegetación a escala de detalle 1:10.000 de la masa forestal de Andalucía. Consejería de Medio Ambiente. Junta de Andalucía.
- Reznicek, A.A. 1990. Evolution in sedges (*Carex*, Cyperaceae). *Can. J. Bot.* 68(7): 1409–1432. doi:10.1139/b90-180
- Ronquist, F., Teslenko, M., Van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Farget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. 2012. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice across a Large Model Space. *Syst. Biol.* 61(3): 539–542. doi:10.1093/sysbio/sys029
- Salazar, C. 1996. Estudio Fitosociológico de la Vegetación Riparia Andaluza (Provincia Bética): Cuenca del Guadiana Menor. Tesis Doct. Departamento de Biología Animal, Vegetal y Ecología. Universidad Jaén.
- Salazar, C. & Quesada, J. 2011. *Carex* L. In: Blanca, G., Cabezudo, B., Cueto, M., Morales Torres & Salazar, C. (Eds.) *Flora vascular de Andalucía Oriental*, 2ª ed. Pp. 250–263. Universidades de Almería, Granada, Jaén y Málaga. Granada.
- Salazar, C., Lorite, J., García-Fuentes, A., Torres, J.A., Cano E. & Valle, F. 2001. A phytosociological study of the hygrophilous vegetation of Sierra Nevada (southern Spain). *Stud. Geobot.* 20: 17–32.
- Shaw J., Lickey E.B., Beck J.T., Farmer S.B., Liu W., Miller J., Siripun K.C., Winder C.T., Schilling E.E. & Small R.L. 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *Am. J. Bot.* 92: 142–166. doi:10.3732/ajb.92.1.142
- Starr, J. R., Harris, S.A. & Simpson, D.A. 2003. Potential of the 5' and 3' ends of the intergenic spacer (IGS) of rDNA in the Cyperaceae: New sequences for lower-level phylogenies in sedges with an example from *Uncinia* pers. *Int. J. Plant Sci.* 164(2): 213–227. doi:10.1086/346168
- Stuessy, T.F. 1990. *Plant Taxonomy: The Systematic Evolution of Comparative Data*. Columbia University Press, New York.
- Villaverde, T., Jiménez-Mejías, P., Luceño, M., Waterway, M.J., Kim, S., Lee, B., Rincón-Barrado, M., Hahn, M., Maguilla, E., Roalson, E.H., Hipp A.L. & The Global *Carex* Group. 2020. A new classification of *Carex* (Cyperaceae) subgenera supported by a HybSeq backbone phylogenetic tree. *Bot. J. Linn. Soc.* 194(2): 141–163. doi:10.1093/botlinnean/boaa042

## Websites

- Anthos. 2011. Information System of the Plants of Spain. Real Jardín Botánico, CSIC - Fundación Biodiversidad. <http://www.anthos.es> [Accessed on 24 January 2022].
- GBIF 2021. *Carex olbiensis* Jord. In: GBIF Secretariat: GBIF Backbone Taxonomy. Checklist dataset. <https://www.gbif.org/es/species/169565956> [Accessed on 24 January 2022]. doi:10.15468/39omei
- Jiménez-Mejías, P. & Luceño, M. 2011. Cyperaceae. Euro + Med Plantbase - the Information Resource for Euro-Mediterranean Plant Diversity <http://ww2.bgbm.org/EuroPlusMed/> [Accessed on 22 November 2021].
- POWO. 2021. Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. <http://wcp.science.kew.org> [Accessed on 23 July 2021].
- WCSP. 2021. World checklist of selected plant families. Facilitated by the Royal Botanic Gardens, Kew. <http://wcp.science.kew.org> [Accessed on 23 July 2021].

## Supplementary Material

**Figure S1.** Majority-rule consensus tree obtained from the Bayesian analysis of all the combined DNA regions (*ITS*, *ETS*, *G3PDH*, *CATP*, *GZF*, *matK*, *rps16* and *5'trnK* intron). Node labels indicate posterior probability of the clades only when it is higher than 0.9. Tip labels indicate species names and populations (see Supplementary material, Table S1). Scale bar represents substitutions per site.

**Table S1.** Studied material including taxon, population code, locality, voucher and/or herbarium number, and NCBI GenBank accession numbers for each molecular marker (*ITS*, *ETS*, *G3PDH*, *CATP*, *GZF*, *matK*, *5'trnK* intron, *rps16*).

**Table S2.** Characteristics of the nuclear, plastid and combined matrixes.

**Table S3.** Materials used for the elaboration of the distribution map of the two subspecies of *Carex camposii*. The search for the locations of *C. camposii* subsp. *camposii* has been carried out through the GBIF (GBIF, 2021) and Anthos (Anthos, 2011) platforms.

**Table S4.** Morphological characters examined of *Carex camposii* (including subsp. *tejedensis*).

