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Carex quixotiana (Cyperaceae), a new Iberian endemic from Don Quixote's land (La Mancha, S Spain)

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Running title: *Carex quixotiana, a new Iberian endemic species*

Abstract

Despite centuries of intense work, the basic taxonomic knowledge of the flora of the Iberian peninsula is still incomplete, especially for highly diverse and/or difficult genera such as *Carex*. In this study, we conducted an integrative systematic study based on molecular, morphological and cytogenetic data to elucidate the taxonomic status of several problematic *Carex* populations from La Mancha region (S Spain) belonging to *Carex* sect. *Phacocystis*. These populations have been traditionally considered of uncertain taxonomic adscription, but close to *C. reuteriana.* A detailed morphological and cytogenetic study was performed with 16 sampled La Mancha problematic populations (Sierra Madrona, Montes de Toledo) in order to compare them with the other Iberian sect. *Phacocystis* species. In addition, a phylogenetic analysis was conducted using two nuclear (ITS, ETS) and two plastid (*rpl*32-*trn*L^{UAG}, *ycf*6-*psb*M) DNA regions, including representatives from the whole sect. *Phacocystis*. We found a significant degree of molecular and morphological differentiation that supports the recognition of these populations as a new Iberian endemic species, *Carex quixotiana.* Our results reveal that, unexpectedly, *C. quixotiana* is more closely related to *C. nigra* than to *C. reuteriana* on the basis of phylogenetic relationships and chromosome number. However, the overall morphological appearance and ecological preferences were misleadingly suggesting affinities with *C. reuteriana*. These contrasting patterns reflect the taxonomic complexity in sect. *Phacocystis* and remark the need of integrative systematic approaches to disentangle fiendish evolutionary scenarios.

Keywords

Cytogenetics, Iberian peninsula, Mediterranean, morphometrics, new species, *Phacocystis*, phylogenetics, *Quercus* forest, taxonomy

Introduction

The Iberian Peninsula is one of the three large peninsulas of Southern Europe projecting into the Mediterranean Sea, and as such, it harbours a high plant species diversity and endemism in relation to the rest of Europe. Phytogeographically, it is part of the Mediterranean Basin biodiversity hotspot (Myers et al. 2000, Mittermeier et al. 2011). *Carex* L. (Cyperaceae) is among the three most speciose angiosperm genera in the Iberian Peninsula (Aedo et al. 2017). Floras, monographs and checklists published from the second half of the 20th century have progressively increased the number of reported *Carex* native taxa in the Iberian Peninsula (Vicioso 1959: 74 taxa; Chater et al. 1980: 95 taxa; Luceño 1994: 98 taxa; Luceño 2008: 101 taxa; Luceño et al. unpublished: 107 taxa). These studies, along with the finding of new Iberian records (*C. cespitosa,* Jiménez-

Mejías et al. 2007), revalorization of neglected taxa (*C. paui,* Benítez-Benítez et al. 2017; Troia et al. 2018) or even description of new ones (*C. lucennoiberica,* Maguilla and Escudero 2016; *C. camposii* subsp. *tejedensis*, Sánchez-Villegas et al. 2022), demonstrate that the taxonomic and chorological knowledge of *Carex* in the territory is still in progress.

Section *Phacocystis* Dumort. (subg. *Carex*) is among the largest sections of *Carex* (ca. 112 spp. in Roalson et al. 2021), although the number of considered taxa may considerably differ among treatments (see Benítez-Benítez et al. 2021). It has a sub-cosmopolitan distribution that somehow mirrors that of the whole genus, with higher species diversity in temperate and cold areas of the Northern Hemisphere, but also with several species present in the Southern Hemisphere (Benítez-Benítez et al. 2021). The typical ecological requirements of the section include habitats with high water availability, such as wetlands, river shores, mountain bogs and wet coastal sands, mostly in freshwater systems, although a few high latitude species also grow in halophytic environments. In the Iberian Peninsula it is represented by six species and eight taxa: *C. acuta* L., *C. cespitosa* L., *C. elata* All. subsp. *elata*, *C. nigra* (L.) Reichard subsp. *nigra*, *C. nigra* subsp. *intricata* (Tineo ex Guss.) Rivas Mart., *C. reuteriana* Boiss. subsp. *reuteriana*, *C. reuteriana* subsp. *mauritanica* (Boiss. & Reut.) Jim.Mejías & Luceño, and *C. trinervis* Degl. Section *Phacocystis* is one of the most controversial *Carex* groups from a taxonomic perspective, as species in the group are affected by the complex interplay of high intraspecific morphological variability, faint species boundaries, and interspecific hybridization (see references in Jiménez-Mejías et al. 2014a; Benítez-Benítez et al. 2021). In addition, the use of certain species as taxonomic hotchpotches (e.g., *C. acuta*, *C. cespitosa*), together with frequent misidentifications during sect. *Phacocystis* taxonomic history, have greatly obscured the knowledge of the group in certain areas (e.g., Jiménez-Mejías and Martinetto 2013; Jiménez-Mejías et al. 2014b). Among the Iberian taxa, that has been the case of the *C. elata*-*C. reuteriana* group, where the different taxa have been miscited or even confounded with other species such as *C. acuta* or *C. nigra* (Luceño and Aedo 1994; Jiménez-Mejías et al. 2011, 2020).

La Mancha (Fig. 1) is a natural and historical region placed in the south-southeastern limit of the Iberian Central Plateau, mostly belonging to the Guadiana river basin. It is often popularly known because it constitutes the main scenario where the globally famous Miguel de Cervantes's (1547-1616) novel *Don Quixote* (de Cervantes, 1605, 1615) takes place. With about 500-600 m of average elevation, La Mancha has a relatively cold semi-arid climate and mostly a low topographic relief. The natural limits of this region are a set of low-elevation ranges: Sierra Morena to the south, Montes de Toledo to the north, and Sierra de Alcaraz to the east, all delimiting the Guadiana basin, plus Las Villuercas to the west, across which the Guadiana river stretches towards the Atlantic Ocean. The slopes of Sierra Morena and Montes de Toledo are considered as integrant parts of La Mancha. While the central part of La Mancha is mostly formed by carbonate sediments, Sierra Morena and Montes de Toledo are composed by metamorphic siliceous materials, including quartzite, schist and slate. The current vegetation in vast parts of La Mancha is mostly an agricultural landscape. The potential vegetation would be primarily evergreen sclerophyllous *Quercus ilex* forest on the lower parts, and marcescent *Q. pyrenaica* or *Q. faginea* woodlands on the more elevated ones (Martín Herrero et al. 2003). According to Ramos-Gutiérrez et al. (2021), there are only two plant species endemic from La Mancha, both belonging to the agamospermic genus *Limonium* (*L. pinillense* Roselló & Peris and *L. squarrosum* Erben) and inhabiting inland saltmarshes. Regarding *Carex* there are about 20 species present in La Mancha (Luceño

et al. unpublished). Most of them are limited to habitats with year-round water availability, such as springs, streams or *bonales* (relictic aquifer-fed peat bogs) (Martín-Blanco and Carrasco de Salazar 2005), although a few species also grow on relatively dry soils as part of the Mediterranean forest understory.

During the preparation of *Flora Iberica* treatment (Luceño and Jiménez-Mejías 2008) a set of problematic populations from Sierra Madrona (a northern subrange of Sierra Morena belonging to La Mancha) were tentatively assigned to *C. reuteriana* (Jiménez-Mejías et al. 2011), but with ambiguous affinities regarding the subspecies (C. *reuteriana* subsp. *reuteriana* and *mauritanica*; treated under *C. elata* in Flora Iberica, Luceño and Jiménez-Mejías, 2008). A preliminar AFLP assessment (Jiménez-Mejías and Martín-Bravo unpublished) associated to a study focusing on *C. reuteriana* (Benítez-Benítez et al. 2018) revealed that, surprisingly, the problematic populations were not as closely related to *C. reuteriana* as expected. Subsequently, we performed fieldwork prospections in La Mancha (Ciudad Real province; Sierra Madrona and southern Montes de Toledo) and found several additional *Carex* sect. *Phacocystis* problematic populations of uncertain taxonomic adscription. The detailed comparison of the materials with other Iberian species of the group revealed superficial morphological affinities with either *C. reuteriana* and *C. nigra*. However, its ecological preferences were somehow confounding, since the problematic populations inhabited generally small streams, rivers and springs and wet meadows in marcescent and sclerophyllous *Quercus* forests, while *C. reuteriana* grows exclusively in permanent streams, and *C. nigra* thrives at much higher altitudes in wet mountain meadows and bogs. Here we conduct a detailed systematic study of these La Mancha problematic populations, including the comparison with the other Iberian sect. *Phacocystis* species, through an integrative approach based on molecular, morphological and cytogenetic data, which have been proven useful to help disentangling the complex taxonomy of this group (e.g., Jiménez-Mejías et al. 2011). Our objective was to elucidate the systematic status of these populations and to warrant their taxonomic recognition if required.

Materials and methods

Sampling

We collected specimens from 10 problematic populations from La Mancha region (Sierra Madrona and Montes de Toledo; Fig. 1) that were deposited at UPOS herbarium, with duplicates at GDA, JAEN, K, MA, NY, P and SALA. A special effort was conducted in Sierra Madrona, resulting in a representative sampling across all the range (Fig. 1). Additional herbarium specimens were found at JAEN, MA, MACB and SALA (Supplementary file 1). Some of them came from very close sites to the 10 mentioned populations (e.g., in the same water course) and/or had imprecise coordinates, so they were considered to belong to the same population, but 6 new populations were identified (Supplementary file 1). Overall, we studied material from 16 problematic populations.

Morphological study

Material from all 16 sampled populations (Supplementary file 1) was carefully compared with all the other sect. *Phacocystis* species present in the Western Palearctic using specialized literature (Schultze-Motel 1968; Nilsson 1985; Kukkonen 1998; Egorova 1999; Jermy et al. 2007; Dean and Ashton 2008; Luceño and Jiménez-Mejías 2008; Jiménez-Mejías et al. 2015), with emphasis in Iberian species and specifically the

morphologically similar *C. nigra* and *C. reuteriana*. We examined the most important morphological characters for the taxonomy of *Carex* sect. *Phacocystis*: general habit, basal sheaths colour and form, stomata distribution in leaf faces, lowermost bract/inflorescence relative length, spike size and sex distribution, and utricle size, colour and indumentum. Measurements above 1 cm were taken with a standard 30 cm rule, and those below 1 cm with an ocular micrometer (only for specimens from the 10 collected populations at UPOS).

Molecular and phylogenetic study

In order to tackle the phylogenetic placement of La Mancha problematic populations, we included these samples in the available molecular phylogeny for sect. *Phacocystis* from Benítez-Benítez et al. (2021). We used the same markers as in that phylogeny: two nuclear ribosomal markers (ITS-ETS) and two plastid markers (*rpl*32-*trn*L^{UAG} and *ycf*6-*psb*M). The new sequenced material (Supplementary file 1) was integrated in the complete singleton's matrix from Benítez-Benítez et al. (2021) (Supplementary file 2), which provided the best phylogenetic resolution in that study. That matrix included the 75% of the species of the section and 80% of the so-called Western Palearctic clade in particular (Benítez-Benítez et al. 2021). Inferred hybrid samples (e.g., *C. acuta, C. buekii, C. randalpina, C. salina*) were excluded of complete singleton's matrix to avoid their deleterious topological effect as explained in Benítez-Benítez et al. (2021). The morphological –and biogeographic– affinities of La Mancha problematic populations clearly points to the Western Palearctic lineage as the best candidate for them to be placed in.

DNA extraction and sequence amplification followed Benítez-Benítez et al. (2021). All PCR products were sequenced by Macrogen (Madrid, Spain). Sequence chromatograms were edited using GENEIOUS v.11.0.2 (Biomatters Ltd., Auckland, New Zealand). The sequences were aligned with Muscle (Edgar 2004). Following Jiménez-Mejías et al. (2016) methodology, we carried out a scaffolding strategy (see details in Benítez-Benítez et al., 2021). First, we compiled those accessions containing both nuclear sequences (ITS and ETS; independently of their having the plastid markers) and built a nrDNA reference tree using maximum likelihood (ML) with RAXML (Stamatakis 2014; 100 bootstrap replicates). The resulting tree was used to build a query tree placing all the remaining accessions through the Evolutionary Placement Algorithm (EPA; Berger et al. 2011), also implemented in RAXML. Branch support for the query tree was calculated using the non-parametric Shimodaira-Hasegawa (SH) from approximate likelihood ratio test (SH-aLRT support; Guindon et al. 2010; Anisimova et al. 2011). All analyses were implemented in CIPRES Science Gateway (Miller et al. 2010).

In order to explore the systematic relationships of La Mancha problematic populations at a finer scale and with respect to the other Iberian representatives of sect. *Phacocystis,* a statistical parsimony analysis of plastid haplotypes was conducted with TCS version 1.2.1 (Clement et al. 2000). We included only those accessions from specimens from the Iberian Peninsula that contained both plastid *rpl*32-*trn*L^{UAG} and *ycf*6-*psb*M regions, except for *C. acuta* and *C. buekii* as explained above. To calculate the most parsimonious haplotype network we set a 95% parsimony connection limit for the minimum number of mutations differentiating the haplotypes. Coding gaps in sequences were treated as a fifth character.

Cytogenetic study

Chromosome counts for five La Mancha problematic populations (Supplementary file 1) were performed. Anthers were fixed during meiosis from young male flowers, following the method by Luceño (1988). The obtained chromosome numbers and meiotic configurations were compared with those of closely related species, since cytogenetics have been demonstrated to have a strong diagnostic potential in *Carex*, including sect. *Phacocystis* (Jiménez-Mejías et al. 2011).

Conservation Assessment

Following the taxonomic recognition of La Mancha problematic populations (see below), we evaluated their conservation status at the global level following criteria, categories, and guidelines from IUCN (2017). Area of occupancy (AOO) and extent of occurrence (EOO) were calculated using the GEOCAT tool (Bachman et al. 2011) based on the 16 studied populations (see morphological study).

Results

Morphological study

The detailed examination of diagnostic morphological characters for La Mancha problematic populations and its comparison with closely related species revealed the constant presence in all the studied populations of both qualitative and quantitative morphological differences regarding the remaining Iberian taxa of sect. *Phacocystis* (Table 1).

Molecular study

The query tree built (see Supplementary file 3) using the singletons matrix from Benítez-Benítez et al. (2021) recovered a topology equivalent to that in the reference paper. The Western Palearctic-clade was well-supported (SH=89) and arranged in two main clades (Fig. 3): one containing *C. reuteriana* and the closely related *C. panormitana* (marginally supported, SH=64), and another with the rest of the species of the clade (SH=90). La Mancha problematic populations were placed unresolved among the species of this latter clade.

The statistical parsimony analysis revealed 14 different haplotypes (Fig. S1). La Mancha problematic populations displayed an exclusive haplotype, not shared with any of the other Iberian species. The most closely related haplotype was that displayed by the Sierra Nevada populations of *C. nigra*, separated by only one mutation.

Cytogenetic study

Studied meiotic plates, representing five different La Mancha problematic populations (Supplementary file 1), mostly displayed 41 chromosomes, with regular pairing (Fig. 4). Interestingly, the population 59SMB17 also showed $40^{II} + 1^{III}$ in Metaphase I, and n=41, 42 in pollen grain mitosis (from 30 counted plates, 16 showed n=41 and 14 n=42). Therefore, the inferred diploid chromosome numbers were 2n=82, 83 (Fig. 4).

Discussion

The present integrative study combining different sources of evidence (morphological, cytogenetic, molecular) supports the systematic distinctiveness of the studied La Mancha populations. The greater morphological affinities of these populations are with *C. reuteriana* and *C. nigra*, however they show clear-cut morphological differences (Table 1). Thus, La Mancha populations differ from these two species by leaf width and basal sheaths colour. In addition, it is different from *C. reuteriana* by leaf stomata distribution, utricle indumentum and chromosome number, and from *C. nigra* by the terminal male spike length.

Remarkably, the DNA markers used in the phylogenetic reconstructions showed that La Mancha problematic populations do not group with *C. reuteriana*, to which they had been traditionally assigned (Jiménez-Mejías and Luceño 2008). The haplotype network pointed to affinities with the populations of *C. nigra* from Sierra Nevada (Fig. S1), which are ca. 150 km south from La Mancha border. However, the appearance and ecology of the Sierra Nevada plants is fairly distinct from that of the La Mancha populations. La Mancha populations are medium-size herbs that grow in low-medium elevation in riparian vegetation within *Quercus* forests, while *C. nigra* in Sierra Nevada is a dwarf sedge that forms dense tufts at habitats mostly above the timberline (from 2000 m and up to 3300 m).

According to the evidence presented, we consider that La Mancha populations deserve taxonomic recognition, so we proceed to describe them as a new species.

Taxonomic treatment

Carex quixotiana Ben.Benítez, Martín-Bravo, Luceño & Jim.Mejías, *sp.nov.*

Diagnosis. Similar in appearance to *C. reuteriana* Boiss. & Reut., from which it differs by the creamy-yellow to reddish-brown basal sheaths (vs. orange to reddish-brown), amphistomatic leaves (vs. hypostomatic), and utricles with high papillae (vs. smooth or rarely with low papillae). It is also similar to *C. nigra* (L.) Reichard, from which it can be distinguished also by the creamy-yellow to reddish-brown basal sheaths (vs. dark brown when present), lower leaf width (1.8)2-3.2(4.7) (vs. 3-6(10)) and higher terminal male spike length (18)20-60(85) (vs. (5)10-30).

Type. SPAIN. Ciudad Real: Sierra Madrona, Fuencaliente, recreational area, Azor stream, stream edges in *Quercus faginea* forests. 733m, 38.44906944 -4.327163889, 10 May 2017, S. Martín-Bravo & C. Benítez-Benítez 41SMB17 (holotype!: UPOS-8925, 41SMB17(5) 2/3; isotypes!: GDA, JAEN, K, MA, NY, P, SALA and UPOS).

Specimens examined (see Supplementary file 1).

Morphological description. Rhizomes from dense and tussock-forming to elongated. **Stems** (48)60–80(95) cm long, (0.8)1–1.2(1.3) mm wide below the inflorescence, sharply trigonous, smooth for most its length, densely scabrid distally on the angles. **Basal sheaths** squamous, sometimes forming a more or less conspicuous lamina, creamy-yellow to reddish-brown, coriaceous. **Leaves** (1.8)2–3.2(4.7) mm wide, pale green to glaucous, amphistomatic, flat to keeled, usually shorter or equalling the stems, introrsely scabrid on the margins, especially distally, and on the mid-vein on the abaxial side to the

apex; **ligule** (2)3–7(10) mm long, usually more than twice longer than wide, apex acute to obtuse, more rarely rounded or truncate, hyaline, the margins brownish to orange-brown. **Inflorescence** (9)12–19(22) cm long, all spikes erect, exceptionally the lowermost slightly nodding. Lowermost bract leaf-like, very rarely setaceous, longer to shorter than the inflorescence, sheathless, with hyaline, pale brownish to dark purplish-brown auricles at its base. Terminal **male spikes** (1)2–3; the uppermost spike (15)20–60(85) × 2-3.5 mm, subsessile to long pedunculate, oblong-cylindrical to narrowly fusiform, densely flowered; subterminal male spikes (0)1–2, (5)10-30(33) × (1)1.5–2.3(2.6) mm, similar in outline to the terminal one, basally overlapping with it or with an internode up to 20 mm. Lateral spikes female or androgynous with the flowers spirally arranged; **female spikes** 0-2(3), (19)25–50(79) × (2)2.8–4(5.2) mm, cylindrical, densely to more or less laxly flowered proximally; **androgynous spikes** (0)1–3(4), (9)20–50(62) × (1)1.7–2.7(5) mm, with the male portion (1)3–15(50) mm long and the female one (6)10–40(50) mm long. **Male glumes** (1.9)2.3–3.9(4.1) × (0.6)0.7–1.2(1.4) mm, oblong to obovate-oblong, apex rounded, usually dark purplish brown, with a green, 1-veined central band, with or without whitish hyaline margins, sometimes also becoming hyaline towards the base. **Female glumes** 1.3–2.3(2.5) × (0.6)0.7–0.9(1) mm, lanceolate to ovate, more rarely elliptical, apex obtuse, acute or mucronate, usually shorter and narrower than the utricles, very rarely shortly surpassing them, dark purplish brown, rarely pale brown, with a green, 1-3 veined central band, with or without whitish hyaline margins, mainly in the distal part. **Utricles** (1.8)2–2.7(3) × (1.1)1.3–1.8(2) mm, plano-convex, widely elliptical to almost suborbicular, green to straw coloured, distally whitish, sometimes purplish-dotted or purplish-tinged towards the apex, with high whitish papillae on the upper half or towards the apex, sometimes also aculeolate at the upper margins, faintly to conspicuously nerved, shortly stipitate, more or less abruptly contracted into a short, cylindrical, truncate, more rarely emarginate beak (0)0.1–0.2(0.3) mm long, whitish, sometimes brown-tinged. **Achenes** 1.5–2.1 × (1.1)1.3–1.5(1.7) mm, widely elliptical to suborbicular, straw-coloured to pale brown, biconvex, more or less stipitate; style base terete to slightly conical, up to 0.3 mm. Stigmas 2.

Distribution. (Fig. 1) Endemic to South-Central Spain (Ciudad Real and marginally Jaén provinces). So far known from 16 populations (Supplementary file 1), mostly located in Sierra Madrona range, but also extending north reaching the southern foothills of Montes de Toledo range. Since it is a small to medium-size sedge rarely collected, there might be additional populations in these areas.

Habitat. Small streams, rivers and springs in riparian forests (*Alnus glutinosa, Fraxinus angustifolia, Salix* sp.), and humid meadows, in marcescent and sclerophyllous *Quercus* forests, on siliceous bedrock substrate. 400-800 m.

Phenology. (April) May-June (July).

Chromosome number. 2n=82, 83.

Iconography. Fig. 5.

Conservation status. So far known from 16 populations (Fig. 1), deduced from the studied material (Supplementary file 1). This implies a relatively restricted distribution range enclosed in an extent of occurrence (EOO) of 4.920 km2. This would point to the application of criteria B1 of the Endangered (EN) category (threshold of 5.000 km^2; IUCN 2017). However, the number of locations (16) prevents the application of EN category since two conditions of criteria B must be fulfilled. In addition, its area of occupancy (AOO) is of 64.000 km^2 and therefore, the species does to fulfil B criterium

to be classified as endangered. The species' overall demographic tendency and number of mature individuals are unknown. Some populations are located in protected land (see below) so they should not be likely submitted to plausible threats in the short term (but see García Río 2007). With the currently available data, we hypothesize that *C. quixotiana* formal IUCN conservation category at the global level would be Least Concern (LC). However, it is an Iberian endemic with a relatively small number of populations and distribution range, which would benefit from legal protection and inclusion in in-situ/ex-situ conservation programmes, at least at the regional level (Castilla-La Mancha, Andalucía).

Etymology. The species epithet, *quixotiana* (pronounced *kee·how·tee·a·na* in English) is derived from Miguel de Cervantes's (1547-1616) masterpiece *Don Quixote* (1605, 1615), considered one of the best works for universal literature, and whose number of editions and translations is only surpassed by the Bible. The setting of *Don Quixote* is La Mancha, the region of Spain where almost all populations of *Carex quixotiana* occur. We want this epithet to serve as a double tribute: (1) First to Cervantes and his novel *Don Quixote*, flagship of Spanish culture. And (2) To Pedro Jiménez-Mejías's father, Pedro Jiménez Gilabert, an enthusiastic reader who always enjoys reading *Don Quixote* above all other books, and who always encouraged Pedro's curiosity and love for nature. Contrasting to the first words in *Don Quixote* ("En un lugar de La Mancha, de cuyo nombre no quiero acordarme (...)" [In a village of La Mancha, the name of which I have no desire to call to mind (…)]), we desire to remember and commemorate the dedication of this epithet we are coining.

Systematic notes. *Carex quixotiana* populations have been consistently assigned to *C. reuteriana*. Local floristic studies have predominantly identified them as *C. reuteriana* subsp. *reuteriana* (e.g., Fernández García-Rojo 2015; Fernández García-Rojo and Salazar-Mendías 2019; García Río 2004, 2007; Martín Blanco and Carrasco 2005), but also as *C. reuteriana* subsp. *mauritanica* (García Río 1999, 2004). Specialized taxonomic treatments have considered the populations as morphologically intermediate between the two *C. reuteriana* subspecies, which has been attributed to hybridization in a putative contact zone between the two *C. reuteriana* subspecies (Luceño and Jiménez-Mejías 2008).

These problematic populations clearly illustrate the taxonomic complexity of sect. *Phacocystis.* Their overall morphological appearance and ecological preferences suggested that the populations were conspecific with *C. reuteriana* (Luceño and Jiménez-Mejías 2008). On the other hand, phylogenetic relationships (Fig. 2) and the relatively high chromosome number (see below) indicate closer genetic affinity with *C. nigra*. These misleading patterns remark the necessity of integrative approaches comprising different data sources to unmask complex systematic scenarios. Further phylogenomic studies based on High-Throughput-Sequencing techniques like GBS provide a greater number of loci and therefore might help to shed more light on the systematic placement of *C. quixotiana* (Benítez-Benítez et al. unpublished).

Chromosome number has been used as a biosystematic tool in sect. *Phacocystis* in the Iberian Peninsula (Luceño and Aedo 1994; Luceño and Jiménez-Mejías 2008). Our chromosome counts indicate that *C. quixotiana* (2n=82-83) have a greater cytogenetic affinity with *C. nigra* (2n=82-86) than with *C. reuteriana* (2n= 73-76).

Biogeographic and conservation issues. Considering the new species described here, the Iberian Peninsula has 12 endemic *Carex* taxa (*C. asturica* Boiss., *C. camposii* Boiss.

& Reut. subsp. *camposii*, *C. camposii* subsp. *tejedensis* R. Sánchez-Villegas, M. Escudero & Luceño, *C. caudata* (Kük.) Pereda & Laínz, *C. durieui* Steud. ex Kunze, *C. furva* Webb, *C. lainzii* Luceño, E.Rico & T.Romero, *C. lucennoiberica* Maguilla & M. Escudero, *C. nevadensis* Boiss. & Reut., *C. quixotiana*, *C. reuteriana* ssp. *reuteriana* and *C. rorulenta* Porta). However, this may appear as a relatively small number of endemics in proportion to the total number of *Carex* Iberian native taxa (107; Luceño et al. unpublished), especially when compared with other species-rich genera (e.g., *Alchemilla*, *Armeria*, *Centaurea*, *Limonium*, *Teucrium*) with much larger numbers of Iberian endemics (>40; Aedo et al. 2017; Buira et al. 2020). This highlights the novelty of the description of an additional Iberian endemic in *Carex*. Indeed, *C. quixotiana*, with a highly restricted distribution in southern-central Spain, would be the first Iberian endemic species belonging to sect. *Phacocystis*, although one subspecies (*C. reuteriana* ssp. *reuteriana*) is also endemic to C-NW parts of the Iberian Peninsula. Other endemics from the Mediterranean basin in sect. *Phacocystis* are *C. reuteriana* ssp. *mauritanica* (S Iberian Peninsula-NW Africa), *C. nigra* ssp. *intricata* (Western Mediterranean mountains), and *C. panormitana* Guss. (Sicily, Sardinia and Tunisia).

As explained above, all known populations of *C. quixotiana* are located in La Mancha limits, in Sierra Madrona and, to a lesser extent, southern Montes de Toledo. Sierra Madrona is one of the secondary mountain ranges within the larger Sierra Morena range, which stretches for about 450 kms from W to E across S Iberian Peninsula, separating the southern half of the Central Plateau from the Guadalquivir Valley. Sierra Madrona runs almost in parallel (NW-SE) along the northern side of the main range for about 80 kms. It includes the highest altitudes of all Sierra Morena (Bañuela peak, 1332 m). As the whole Sierra Morena, it is mainly composed by old Paleozoic siliceous rocks, especially quartzites. On the other hand, Montes de Toledo is a mountain range entirely contained within the southern half of the Central Plateau, separating the Tajo and Guadiana river basins. It stretches for about 350 kms from E to W and is composed by quartzite ridges of relatively uniform elevation, around 1400 m, and a generally eroded relief (Muñoz Jiménez 1976). The immediate foothill landscapes are highly anthropized, with the vast majority of La Mancha dedicated to non-irrigated crops, mostly cereal (wheat, barley and oat).

Sierra Madrona is currently protected as a natural park established in 2011 (Valle de Alcudia and Sierra Madrona). Several restricted plant endemics from Sierra Morena are found in this range and its surroundings (i.e., *Armeria pauana* (Bernis) Nieto Fel., *Coincya longirostra* (Boiss.) Greuter & Burdet; García Río 2004). It also includes many different habitats protected at the European level (Directive "Habitats" 92/43/CEE; see García Río 2004, 2007), including some considered of high conservation priority. *Carex quixotiana* habitats in Sierra Madrona, although included in a protected area, are not free of threats, so their biodiversity has been considered as highly valuable and sensitive (García Río 2007). Thus, they include populations of other endangered and protected plant species at the regional (e.g., *Erica tetralix* L., *Drosera rotundifolia* L.) or even at the national level (e.g., *Myrica gale* L., *Narcissus muñozii-garmendiae* Fern.Casas).

The other known populations of *C. quixotiana* occur mostly in river and creeks in the southern foothills of Montes de Toledo in W Ciudad Real province. These populations are located within or close to remarkable habitats, such as relictual peatbogs (e.g., Bonales de Puebla de Don Rodrigo) and birch forests (e.g., Abedular de Ríofrío), which are on protected land and considered of extraordinary conservation value. In addition, *C. quixotiana* marginally reaches NE Jaén province in Sierra Quintana, a small southern subrange of Sierra Madrona province (Cano-Carmona and Valle-Quintero 1996), which

separates Castilla-La Mancha from Andalucía (Fig. 1). Sierra Madrona and Montes de Toledo are considered biogeographically close (García Río 2007). Interestingly, *C. reuteriana* ssp. *reuteriana* occur in northern parts of Montes de Toledo, whereas *C. reuteriana* ssp. *mauritanica* is widespread in the main Sierra Morena range (Luceño and Jiménez-Mejías 2008; see maps in Benítez-Benítez et al. 2018). It would be interesting to precisely delimit the two species range and to explore potential contact zones in detail in order to investigate the possible co-occurrence and/or hybridization processes.

Key to the Iberian taxa of *Carex* section *Phacocystis*:

1. Leaves strongly plicate to canaliculate, rigid, up to 2,5(3) mm wide; stems obtusely trigonous; plants glaucous ……...***C. trinervis***
- Leaves flat to carinate, soft to medium rigid, (1.8)2-8(10) mm wide; stems sharply trigonous; plants green to glaucous ……2

2. Utricles not veined; basal sheaths dark purple ……***C. cespitosa***
- Utricles faintly to prominently veined; basal sheaths yellowish-brown to reddish-brown ……3

3. Utricles smooth, rarely with some scattered, green, low papillae …….....4
- Utricles densely papillose, at least on the apex, with whitish, more or less high papillae ……... 5

4. Male spike usually solitary, rarely 2; lowest spikes usually female; leaves up to 6 mm wide …… ***C. reuteriana*** subsp. ***reuteriana***
- Male spikes (1)2-4; lowest spikes usually androgynous; leaves (3)4-8(10) mm wide…… ***C. reuteriana*** subsp. ***mauritanica***

5. Leaves epistomatic or amphistomatic ……6
- Leaves hypostomatic …… 8

6. Male spikes (1)2-3, the terminal one (15)20-60(85) mm long; leaves (1,8)2-3,2(4,7) mm wide; plants usually tussock-forming; basal sheaths usually without lamina (squamous), more rarely foliose ……... ***C. quixotiana***
- Male spike solitary, (5)10-30 mm long; leaves 3-6(10) mm wide; plants rarely tussock-forming; basal sheaths frequently foliose ……... 7

7. Leaves densely stomatic in both sides (amphistomatic) ... …… ***C. nigra*** subsp. ***intricata***
- Leaves epistomatic or densely stomatic in the upper side and with a few scattered stomata in the lower one …… ***C. nigra*** subsp. ***nigra***

8. Lowest bract much longer than the inflorescence; utricles strongly biconvex, somewhat inflated; basal sheaths foliose ...…… ***C. acuta***

- Lowest bract always shorter than the inflorescence; utricles plano-convex to slightly biconvex, not inflated; basal sheaths squamous ……***C. elata*** subsp. ***elata***

Conclusion

Integrative approaches based on different sources of evidence are required to unveil complex systematic scenarios. Our study of some problematic Iberian populations belonging to the taxonomically difficult sect. *Phacocystis* revealed that they display a diagnostic and congruent set of morphological, molecular, ecological and cytogenetic characters that allow their distinction with respect to close relatives (e.g. *C. reuteriana*, *C. nigra*). Therefore, we propose a new species (*C. quixotiana)* for these populations, which would constitute a new endemic species from the Iberian Peninsula.

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Legends

Figure 1. Distribution map representing the studied populations of *Carex quixotiana*.

Figure 2. Representative photos of *Carex quixotiana.* A. habitat (riparian forest with *Alnus glutinosa* and *Fraxinus angustifolia*: Ciudad Real, Solana del Pino, Robledillo river; 57SMB17, UPOS-8924; *C. quixotiana* plants are in the lower right corner); B. habitat (stream in *Quercus pyrenaica* open forest: Ciudad Real, between El Viso del Marqués and San Lorenzo de Calatrava; 8CBB18, UPOS16897; *C. quixotiana* are the tussocks along the stream); C. inflorescence; D-E. details of androgynous, female and male spikes; F. details of utricles (Ciudad Real, Fuencaliente, Minas del Horcajo, 54SMB17, UPOS-8922).

Figure 3. Phylogram obtained based on the maximum likelihood phylogenetic reconstruction of the complete singletons tree (ITS, ETS, rpl32-trnL, ycf6-psbM) following scaffolding approach presented by Benítez-Benítez et al. (2021). SH supports>60 are given above branches. Tip codes are included in Supplementary file 2. The samples with asterisk (*) have been newly sequenced for this study.

Figure 4. A. Regular meiotic configuration of *Carex quixotiana* in Metaphase I (2n = 82); **B.** Meiotic configuration showing a trivalent in Metaphase I ($2n = 40^{II} + 1^{III} = 83$); **C.** Pollen grain mitosis of the irregular cytotype in which two cells are visible (2n = 82 and 2n = 83).

Fig. 5. Analytical drawing of *Carex quixotiana* (Spain, Ciudad Real, Sierra Madrona). A. habit; B. plant; C. androgynous spike; D. female glume; E. male glume; F. achene; G. utricle; H. details of papillae in the upper part of the utricle. Drawing by M. Sánchez-Villegas.

Figure S1. Haplotype network obtained from the statistical parsimony analysis of the combined plastid sequences of Iberian *Carex* sect. *Phacocystis* species, including *C. quixotiana*. Small circles represent inferred extinct or not sampled haplotypes and lines mutational connections.

Supplementary file 1. Studied materials of *C. quixotiana*. Those specimens newly sequenced in the present study are also included in the second excel tab, including taxon, sample code, locality, voucher and/or herbarium number, and NCBI accession numbers for each molecular marker (ITS, ETS, rpl32-trnL, ycf6-psbM).

Supplementary file 2. List of studied material, with their accession numbers included, which were previously published by Benítez-Benítez et al. (2021). Tip codes with "spm" denote samples previously published by Global Carex Group works (Jiménez-Mejías et al. 2016a; Martín-Bravo et al. 2019).

Supplementary file 3. Cladogram of sect. *Phacocystis,* including newly sampled sequences, based on the maximum likelihood phylogenetic reconstruction of complete singleton's tree (ITS, ETS, rpl32-trnL, ycf6-psbM) following a scaffolding approach previously implemented by Benítez-Benítez et al. (2021). SH supports > 60 are given above branches.

Table 1. Comparison of main morphological diagnostic characters, chromosome numbers, and habitat (in the Iberian Peninsula) among *C. quixotiana* and all the other Iberian *Carex* sect. *Phacocystis* taxa. Measurements from the other taxa have been taken from Luceño and Jiménez-Mejías (2008).

	C. quixotiana	*C. acuta*	*C. cespitosa*	*C. elata* **subsp.** *elata*	*C. nigra*		*C. reuteriana*		*C. trinervis*
					subsp. *nigra*	**subsp.** *intricata*	**subsp.** *reuteriana*	**subsp.** *mauritanica*	
Habit	From tussock-forming to rhizomes elongated	From more or less caespitose to rhizomes elongated	Tussock-forming	From tussock-forming to laxly caespitose	Rhizomes elongated		Tussock-forming		Rhizomes elongated
Basal sheaths (at the base of fertile stems)	Squamous, creamy-yellow to reddish-brown	Inconspicuous, fertile stems usually foliose (with brown old-leaf remains)	Squamous, dark purple	Squamous to elongate, creamy-yellow	Squamous or inconspicuous, dark brown when present		Squamous, orange to reddish-brown		Inconspicuous, fertile stems usually with straw-colored old-leaf remains
Leaf section	Flat to keeled	Flat	Flat to keeled	Flat to keeled	Flat to keeled		Flat to keeled		Strongly plicate to canaliculate
Leaf width (mm)	(1.8)2-3.2(4.7)	3-5(7)	2-4	3-6(7)	3-6(10)		(3)3.5-5.5(6)	(3)4-8(10)	(0.5)1.5-2.5(3)
Stomata distribution	Amphistomatic	Hypostomatic	Hypostomatic	Hypostomatic	Epistomatic	Amphistomatic	Hypostomatic		Amphistomatic

Relative lowermost bract-inflorescence length	Longer to shorter than the inflorescence	Longer than the inflorescence	Shorter than the inflorescence, rarely equalling it	Shorter than the inflorescence, rarely equalling or exceeding it	Equalling to slightly shorter than the inflorescence, rarely exceeding it	Equalling the inflorescence		Exceeding the inflorescence
Terminal (male) spikes number	(1)2-3	2-4	1	1-2	1(2)	1(2)	(1)2-4	(1)2-3
Terminal (male) spike length	(15)20-60(85)	(15)20-50(60)	(12)15-30	25-80	(5)10-30	(15)20-60(70)	(10)20-70	(15)25-40
Lateral androgynous spikes	(0)1-3(4)	1-2	0	0-3(4)	(0)1	0-3(6)	(0)1-5	2-4
Lateral female spikes	0-2(3)	2-4	1-2(3)	0-2(3)	2-4	0-3(4)	0(1)	2-4
Utricle nerves	Nerved	Nerved	Nerveless	Nerved	Nerved	Nerved		Nerved
Utricle indumentum	With high whitish papillae on the upper half or towards the	Almost entirely covered with high papillae,	With whitish high papillae	With whitish high papillae, at	Covered with high papillae at least the upper half	Smooth, very rarely with some scattered, low papillae towards the apex		Covered with low papillae

	apex, sometimes aculeolate at the apex	somewhat inflated	towards the apex	least towards the apex, not inflated				
Chromosome number (2n)	82,83	84-86	78-80	76	82-86	73-75	72,74,75	82-85
Habitat (Iberian peninsula)	Small streams, rivers and springs in riparian forests (*Alnus glutinosa*, *Fraxinus angustifolia*, *Salix* sp.), humid meadows, in marcescent and sclerophyllous *Quercus* forests, on siliceous bedrock substrate	River shores, usually in deciduous forests, without clear edaphic preferences regarding bedrock substrate	River shores in deciduous forest, on granitic substrates	River shores (or lakes) in diverse types of vegetation, on calcareous bedrock substrates, rarely on siliceous ones, also on coastal swamps	Montane-alpine wet meadows, bogs, and swamps, also in river and lake shores, in diverse types of vegetation and without clear edaphic preferences regarding bedrock substrate	Stream and river shores in diverse types of vegetation, usually on siliceous bedrock substrates, rarely on calcareous ones	Stream and river shores in riparian forests, in marcescent and sclerophyllous *Quercus* forests, without clear edaphic preferences regarding bedrock substrate	Sandy coastal swamps
Altitude (m)	400-800	0-1300	10-40	10-1750	1000-3300	300-1900	20-500	0-30

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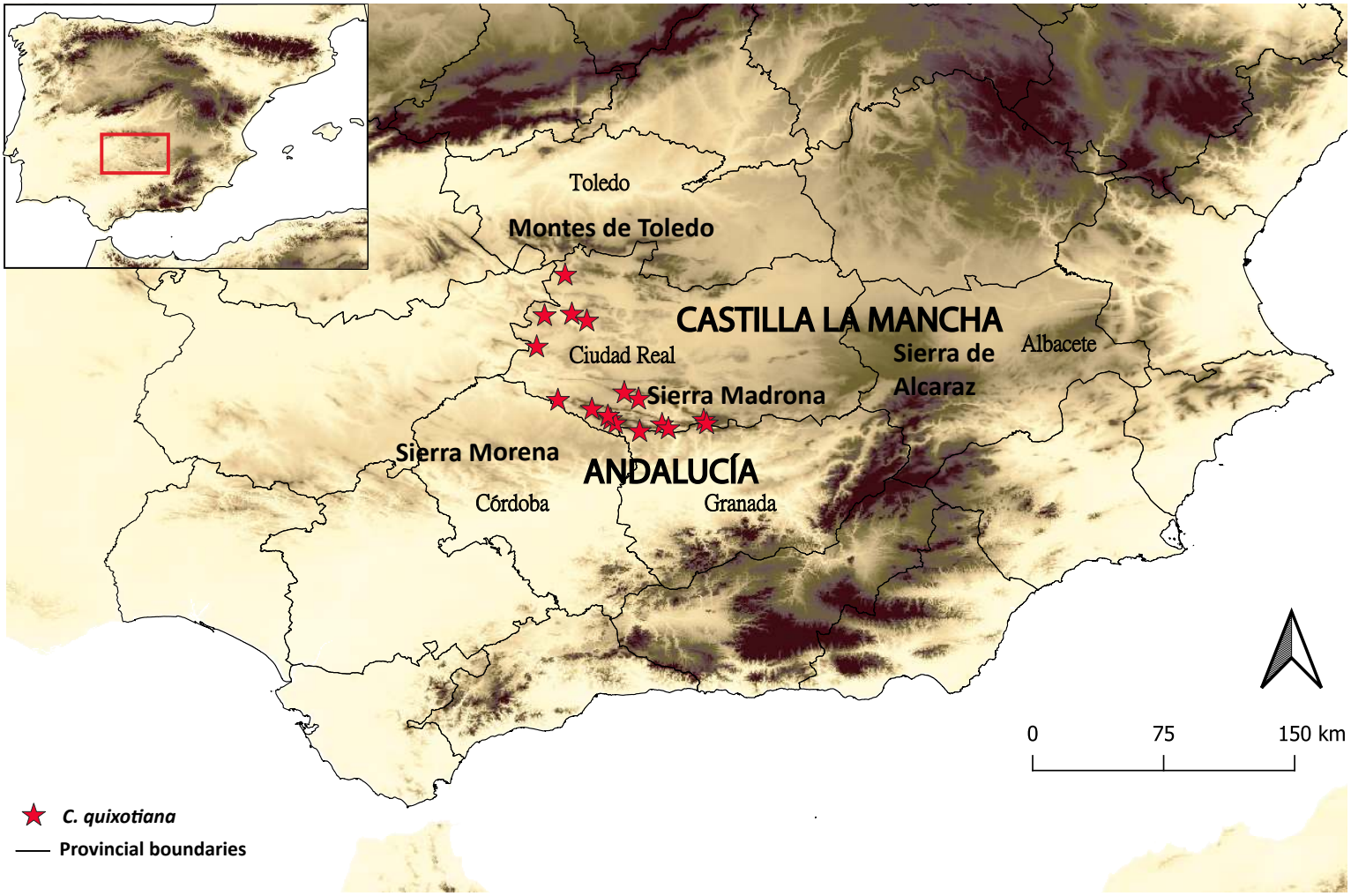

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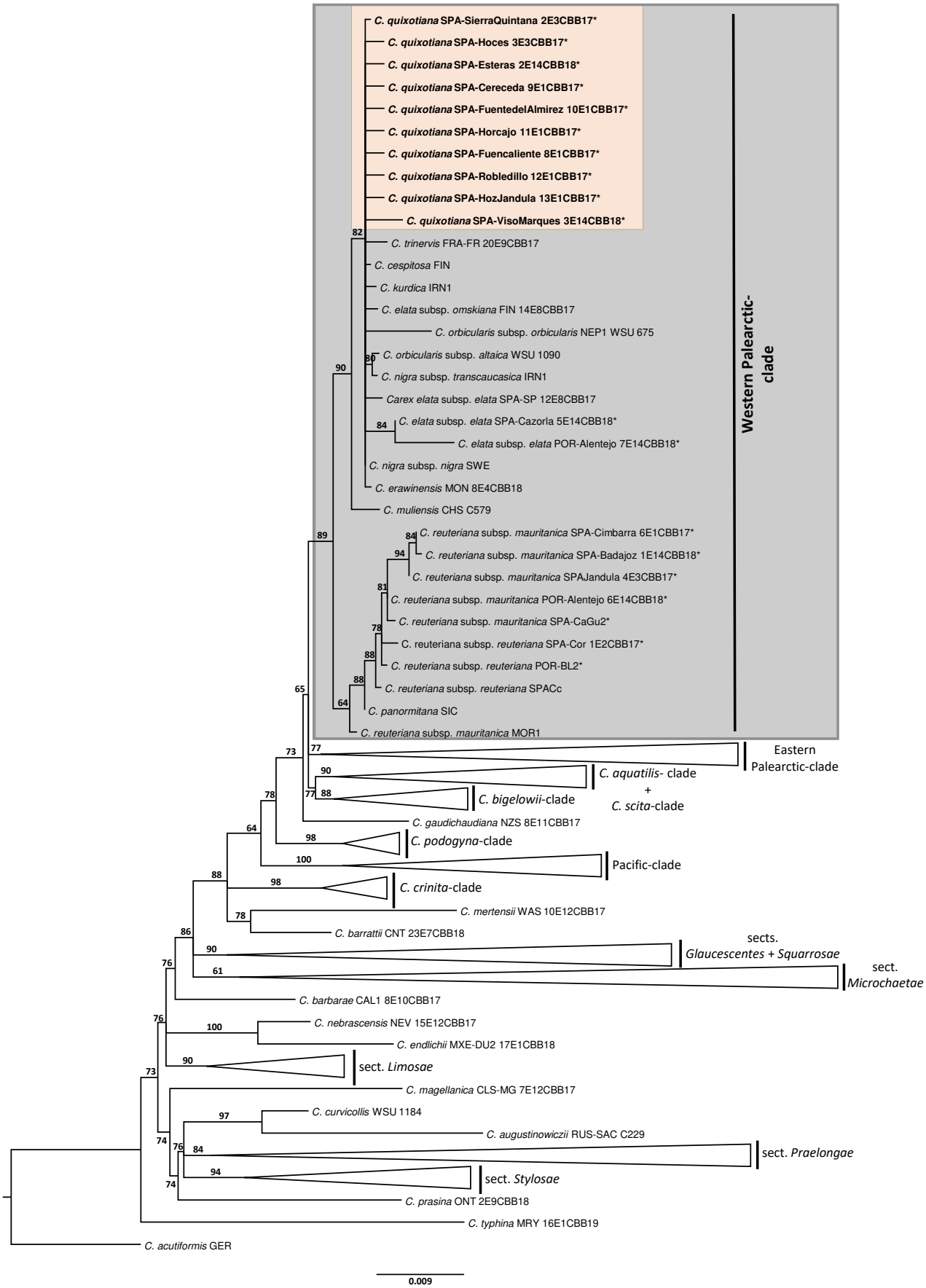
C. quixotiana SPA-SierraQuintana 2E3CBB17*
C. quixotiana SPA-Hoces 3E3CBB17*
C. quixotiana SPA-Esteras 2E14CBB18*
C. quixotiana SPA-Cereceda 9E1CBB17*
C. quixotiana SPA-FuentedelAlmirez 10E1CBB17*
C. quixotiana SPA-Horcajo 11E1CBB17*
C. quixotiana SPA-Fuencaliente 8E1CBB17*
C. quixotiana SPA-Robledillo 12E1CBB17*
C. quixotiana SPA-HozJandula 13E1CBB17*
C. quixotiana SPA-VisoMarques 3E14CBB18*
C. trinervis FRA-FR 20E9CBB17
C. cespitosa FIN
C. kurdica IRN1
C. elata subsp. omskiana FIN 14E8CBB17
C. orbicularis subsp. orbicularis NEP1 WSU 675
C. orbicularis subsp. altaica WSU 1090
C. nigra subsp. transcaucasica IRN1
Carex elata subsp. elata SPA-SP 12E8CBB17
C. elata subsp. elata SPA-Cazorla 5E14CBB18*
C. elata subsp. elata POR-Alentejo 7E14CBB18*
C. nigra subsp. nigra SWE
C. erawinensis MON 8E4CBB18
C. muliensis CHS C579
C. reuteriana subsp. mauritanica SPA-Cimbarra 6E1CBB17*
C. reuteriana subsp. mauritanica SPA-Badajoz 1E14CBB18*
C. reuteriana subsp. mauritanica SPAJandula 4E3CBB17*
C. reuteriana subsp. mauritanica POR-Alentejo 6E14CBB18*
C. reuteriana subsp. mauritanica SPA-CaGu2*
C. reuteriana subsp. reuteriana SPA-Cor 1E2CBB17*
C. reuteriana subsp. reuteriana POR-BL2*
C. reuteriana subsp. reuteriana SPACc
C. panormitana SIC
C. reuteriana subsp. mauritanica MOR1
Western Palearctic-clade
Eastern Palearctic-clade
C. aquatilis- clade + C. scita-clade
C. bigelowii-clade
C. gaudichaudiana NZS 8E11CBB17
C. podogyna-clade
Pacific-clade
C. crinita-clade
C. mertensii WAS 10E12CBB17
C. barrattii CNT 23E7CBB18
sects. Glaucescentes + Squarrosae
sect. Microchaetae
C. barbarae CAL1 8E10CBB17
C. nebrascensis NEV 15E12CBB17
C. endlichii MXE-DU2 17E1CBB18
sect. Limosae
C. magellanica CLS-MG 7E12CBB17
C. curvicollis WSU 1184
C. augustinowiczii RUS-SAC C229
sect. Praelongae
sect. Stylosae
C. prasina ONT 2E9CBB18
C. typhina MRY 16E1CBB19
C. acutiformis GER
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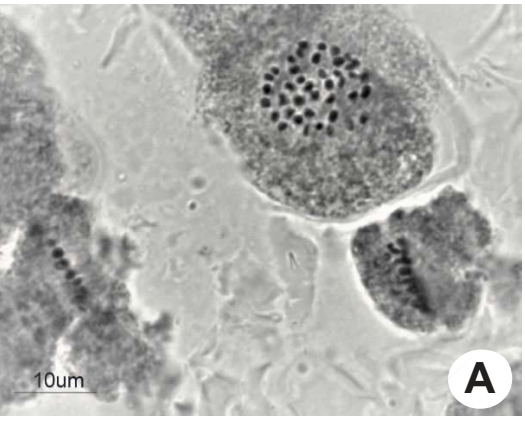

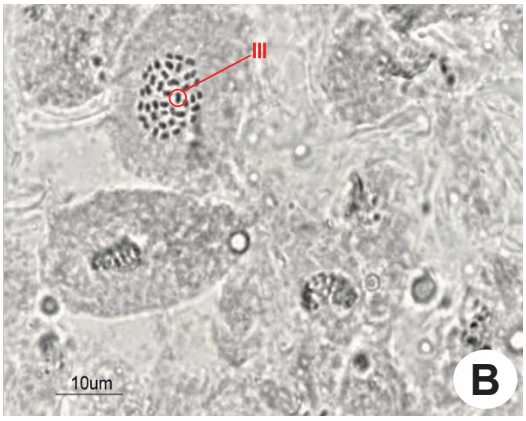

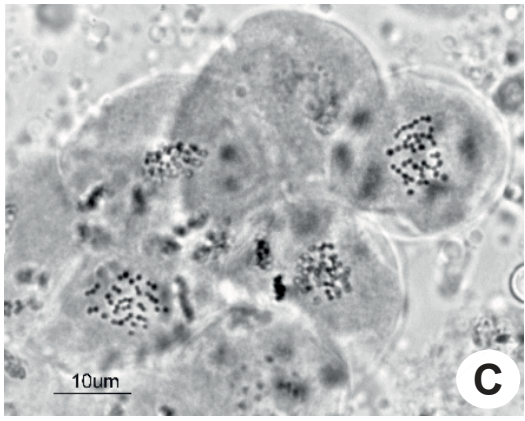
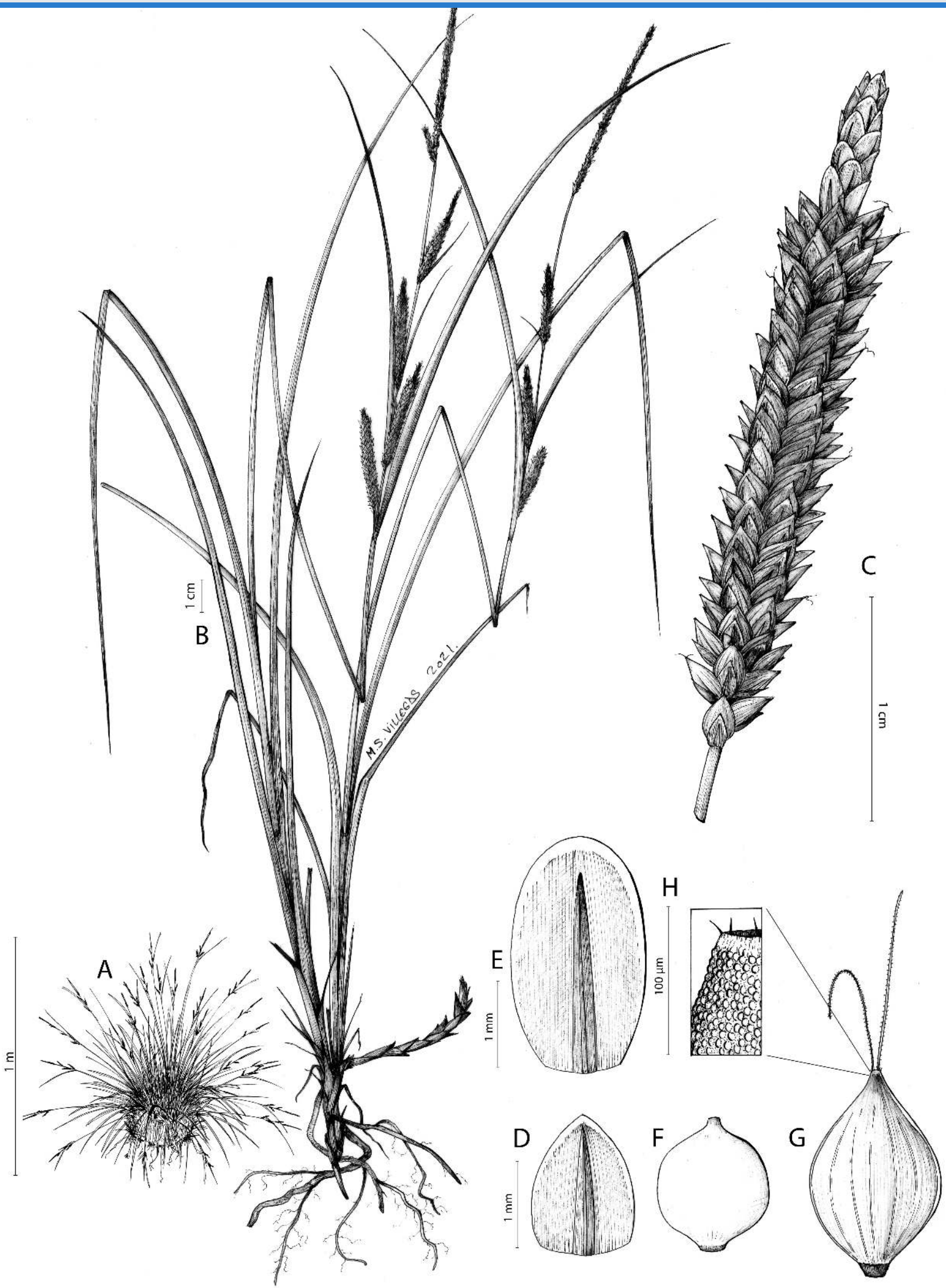
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