

# Molecular and morphological data resurrect the long neglected *Carex laxula* (Cyperaceae) and expand its range in the western Mediterranean

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## Abstract

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*Carex sylvatica* subsp. *pau* is a poorly studied taxon considered endemic from a few places in the western Mediterranean. It has been frequently misidentified as *C. sylvatica* subsp. *sylvatica*. To date, it has been reported only from the NE Iberian Peninsula and the NW Africa. We use molecular —nuclear ribosomal and plastid sequences— and morphological data to shed light on the taxonomic circumscription and distribution of this taxon, especially regarding its distinction from the type subspecies. The genetic data support the recognition of *C. sylvatica* subsp. *pau* as an independent taxon, and confirm new records from the Balearic and Tuscan archipelagos. It implies a considerable increase in its range and a new taxon for the Italian flora. Strikingly, the morphometric analyses revealed that the Sicilian type specimen of *C. laxula* identifies this species with *C. sylvatica* subsp. *pau*. We consider that the taxon should be ranked at the species level. Based on the priority of the name *C. laxula* over *C. pau*, we subsume *C. sylvatica* subsp. *pau* in *C. laxula*. We also provide notes on the ecology of the species.

**Keywords:** Balearic archipelago, *Carex* sect. *Sylvaticae*, ITS, Mediterranean flora, taxonomy, Tyrrhenian, Tuscan archipelago, *5'trnK*.

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## INTRODUCTION

The genus *Carex* L. —with more than 2,000 species— comprises about 40% of the total number of taxa of the family Cyperaceae (Reznicek, 1990; Global *Carex* Group, 2015). It has a cosmopolitan distribution, with most species diversity distributed in temperate regions of the Northern Hemisphere. *Carex* sect. *Sylvaticae* Rouy is a morphologically well-defined small section that currently comprises 6 species (Table 1) distributed in temperate Europe, western Asia, northern and southern Africa. It is placed in *C.* subg. *Carex*, and is nested in a well-supported clade together with *C.* sect. *Rhynchocystis* Dumort., *C.* sect. *Ceratocystis* Dumort., *C.* sect. *Spirostachyae* Drejer

## Resumen

Benítez-Benítez, C., Míguez, M., Jiménez-Mejías, P. & Martín-Bravo, S. 2017. Datos moleculares y morfológicos resucitan la olvidada *Carex laxula* (Cyperaceae) y aumentan su área de distribución en la cuenca mediterránea occidental. *Anales Jard. Bot. Madrid* 74(1): e057.

*Carex sylvatica* subsp. *pau* es un taxon poco estudiado considerado endémico de unos pocos lugares del oeste del Mediterráneo. Ha sido frecuentemente confundida con *C. sylvatica* subsp. *sylvatica*. Hasta la fecha, se ha citado solo del noreste de España y el noroeste de África. Utilizamos datos moleculares —secuencias nucleares y plastiales— y morfológicos para estudiar la delimitación taxonómica y distribución de este taxon, especialmente en relación con la subespecie tipo. Los datos genéticos apoyan el reconocimiento de *C. sylvatica* subsp. *pau* como un taxon independiente y confirman su presencia en los archipiélagos balearico y toscano. Esto implica un considerable aumento de su área de distribución y un nuevo taxon para la flora italiana. Sorprendentemente, los análisis morfométricos han mostrado que el espécimen siciliano y tipo de *C. laxula* es *C. sylvatica* subsp. *pau*. Consideramos que este taxon debería ser reconocido al nivel de especie. Dada la prioridad del nombre *C. laxula* sobre *C. pau*, sinonimizamos *C. sylvatica* subsp. *pau* a *C. laxula*. Además, proporcionamos información sobre la ecología de esta especie.

**Palabras clave:** Archipiélago balear, archipiélago toscano, *Carex* sect. *Sylvaticae*, flora mediterránea, ITS, taxonomía, tirreno, *5'trnK*.

ex Bailey, and *C.* sect. *Rostrales* Meinsh. (Global *Carex* Group, 2016). *Carex sylvatica* Huds. is native to the Old World and it is the most widespread species of *C.* sect. *Sylvaticae*, being distributed in Europe, western Asia, and northern Africa; it has also been reported as introduced in North America and New Zealand (Govaerts & al., 2016). Three subspecies are currently recognized within *C. sylvatica*: *C. sylvatica* subsp. *sylvatica*, *C. sylvatica* subsp. *latifrons* (V.I. Krecz.) Ö. Nilsson, and *C. sylvatica* subsp. *pau* (Sennen) A. Bolòs & O. Bolòs (Egorova, 1999; Jiménez-Mejías & Luceño, 2011). *Carex sylvatica* subsp. *sylvatica* is widely distributed across most Atlantic and Eurosiberian Europe and western Asia (Egorova, 1999;

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**Table 1.** Taxonomic treatment of *C. sect. Sylvaticae* according to Egorova (1999), Jiménez-Mejías & Luceño (2011), and Martín-Bravo & al. (2013), modified after the results of this study and Global *Carex* Group (2016). Synonyms at species level follow Govaerts & al. (2016).

Accepted taxa	Synonyms	Global distribution
<i>C. cretica</i> Gradst & J. Kern		Crete
<i>C. hypaneura</i> V.I. Krecz.		Southern Caucasus
<i>C. laxula</i> Tineo ex Boott	<i>C. algeriensis</i> Nelmes; <i>C. pauti</i> Sennen	Western Mediterranean: northeastern Spain, Balearic Islands, Tuscan archipelago, northeastern Algeria, northern Tunisia, and Sicily
<i>C. rainbowii</i> Luceño & al.		South Africa
<i>C. sylvatica</i> Huds. subsp. <i>sylvatica</i>		Europe to western Asia
<i>C. sylvatica</i> subsp. <i>latifrons</i> (V.I. Krecz) Ö. Nilsson.	<i>C. latifolia</i> Boiss. & Balansa	Northeastern Anatolia, Georgia, and adjacent Caucasus (Nilsson, 1985)
<i>C. vulcani</i> Hochst. ex Seub.		Azores archipelago

Jiménez-Mejías & Luceño, 2011). *Carex sylvatica* subsp. *latifrons* is distributed in southwestern Asia, from Turkey to the western Caucasus, and inhabits forests and wet meadows (Nilsson, 1985). Eventually, *C. sylvatica* subsp. *pauti* has been considered a Mediterranean element with a hitherto known distribution restricted to the north-eastern Iberian Peninsula —Catalonia and Navarra— (Bolòs & Vigo, 2001; Luceño & al., 2008; Aizpuru & al., 1999, Jiménez-Mejías & Luceño, 2011), and northwestern Africa —Algeria and Tunisia— (Jiménez-Mejías & Luceño, 2011; Martín-Bravo & al., 2013).

Despite being an especially well-known area from the floristic point of view, the Mediterranean region is still revealing some taxonomic and biogeographic novelties. Such new findings have been especially remarkable in family Cyperaceae due to its complicate taxonomy. Recent studies have revealed the presence of previously unknown species in different areas —v.gr., *Schoenoplectus corymbosus* (Roth ex Roem. & Schult) J. Raynal (Jiménez-Mejías & al., 2007) and *Cyperus glaber* L. (Verloove & Mesterházy, 2013) in Spain, *Cyperus erythrorrhizos* Muhl. (Verloove & Saiani, 2015) alien in Italy, and *C. castroviejoi* Luceño & Jim. Mejías (Jiménez-Mejías & Luceño, 2009) in Greece.

The finding of *C. sect. Sylvaticae* materials resembling *C. sylvatica* subsp. *pauti* from the Balearic and Tuscan Archipelagos and Sicily led us to conduct a revision of the taxonomy of *C. sylvatica*-like plants in the western Mediterranean. Interestingly, the voucher from Sicily is the type material of *C. laxula* Tineo ex Boott, which has been to date considered a synonym of *C. sylvatica* subsp. *sylvatica* (Govaerts & al., 2016).

In this paper we use sequences from two genomes —nrDNA ITS and ptDNA 5'trnK— and morphological data from herbarium specimens to clarify the identity of these taxonomically problematic populations. These molecular regions have been widely and successfully used for systematic purposes in *Carex*, including *C. sect. Sylvaticae* (Martín-Bravo & al., 2013) and other closely related groups (v.gr., Escudero & Luceño, 2009; Jiménez-Mejías & al., 2012). Interestingly, to the best of our knowledge, *C. sylvatica* subsp. *pauti* has never been included in a molecular phylogenetic study. We aim to gain insights on the taxonomic delimitation and geographic distribution of *C. sylvatica* subsp. *pauti*, especially in regards to its distinction from its close relative *C. sylvatica* subsp. *sylvatica*.

## MATERIAL AND METHODS

### Molecular study

We performed a phylogenetic reconstruction to infer the phylogenetic placement of the Balearic and Tuscan individuals and to assess the degree of genetic differentiation between *C. sylvatica* subsp. *sylvatica* and *C. sylvatica* subsp. *pauti*. Our sampling (Appendix 1) included: 18 samples of *C. sylvatica* subsp. *sylvatica*, selected to representatively cover its distribution area; 5 samples previously classified as *C. sylvatica* subsp. *pauti* from northwestern Africa and northern Spain; 3 samples of the *C. sylvatica* subsp. *pauti*-like plants from the Balearic and Tuscan Archipelagos; 4 samples of two other species of *C. sect. Sylvaticae* —*C. rainbowii* and *C. cretica*—; and 6 samples representing two species of each of the three sections phylogenetically closely related to *C. sect. Sylvaticae* (Waterway & Starr, 2007; Martín-Bravo & al., 2013): *C. demissa* Hornem. and *C. flava* L. —*C. sect. Ceratocystis*—; *C. distans* L. and *C. punctata* Gaudin —*C. sect. Spirostachyae*—; *C. pendula* Huds. and *C. bequaertii* De Wild. —*C. sect. Rhynhocystis*—. DNA was extracted from the specimens using a DNeasy Plant Mini Kit —Qiagen—. Materials were PCR-amplified following PCR conditions from Escudero & Luceño (2009). Sequence chromatograms were visualized and edited using the program Geneious v. 6.1.7 —Biomatters—. Two matrices were built, one containing the ITS sequences —nrDNA matrix—, and the second containing the 5'trnK sequences —ptDNA matrix—. Informative indels were coded as binary characters. We performed Maximum Likelihood —ML— and Bayesian Inference —BI— phylogenetic analyses on each matrix as explained in Escudero & al. (2008), Martín-Bravo & al. (2013), and Villaverde & al. (2015) for the ITS and 5'trnK datasets individually. ML analyses were run with RAxML v. 7.2.6 (Stamatakis, 2010), using a GTR+GAMMA model of sequence evolution, as implemented in a Phylocluster —California Academy of Sciences—. Bootstrap support for branches was calculated through 1,000 replicates. BI analyses were run with MrBayes v. 3.2.5 (Ronquist & Huelsenbeck, 2003). Four simultaneous Markov Chain Monte Carlo —MCMC— chains were run for 5 million generations, sampling trees every 100 generations. The simplest models of nucleotide evolution that best fit the data for each studied DNA region were HKY for 5'trnK, HKY+I for ITS1 and ITS2, and JC for 5.8S region. Characters corresponding to coded indels were analysed

with a F81 model. Congruence between the two resulting topologies was checked by eye and using Hompart test as implemented in PAUP\* v. 4.0b10 (Swofford, 2002) in the same Phylocluster —California Academy of Sciences—. As no significant incongruences were found, the two data sets were combined into a total evidence matrix —combined matrix—, which was analysed again with ML and BI using the same parameters stated above. In order to assess and compare the degree of genetic differentiation between the studied taxa of *C. sect. Sylvaticae*, we calculated pairwise Kimura-2-parameter genetic distances between pairs of samples with MEGA v. 5.2 (Tamura & al., 2011), using the ITS, 5′trnK, and concatenated sequences.

### Morphological study

22 herbarium specimens of typical *C. sylvatica* subsp. *sylvatica* (Appendix 1) and 11 specimens previously classified as *C. sylvatica* subsp. *pau* (Appendix 1; including the 3 vouchers from the Balearic and Tuscan Archipelagos, and the type of *C. laxula* from Sicily) were included in our study. For the morphological characterization of the materials we measured the diagnostic characters reported in previous taxonomic studies of *C. sylvatica* and allied taxa (Chater, 1980; Nilsson, 1985; Luceño & al., 2008; Egorova, 1999; Martín-Bravo & al., 2013), as well as additional characters derived from our observations, making a total of 32 quantitative and one qualitative traits (Table 2). Measurements were taken using a binocular micrometer —Nikon SMZ645—, with the exception of the largest macromorphological characters, which were measured using a standard 30-cm rule.

All statistical analyses of morphometric data were performed using the software SPSS Statistics 20 —IBM Corp., New York, Armonk—. First, we chose those variables with a higher correlation level  $\rightarrow 0.8$ —. Secondly, we removed those variables that contributed less to the first significant principal components. When those characters were removed, a clear morphological discontinuity was found between the 2 subspecies as analyzed through Principal Component Analysis —PCA—. A total of 7 variables were kept for the final analysis. We performed boxplot analysis for these variables in order to show the degree of overlapping between the two studied taxa. In the boxplot analysis the Navarran samples previously classified as *C. sylvatica* subsp. *pau* were treated as *C. sylvatica* subsp. *sylvatica* —see Results.

## RESULTS

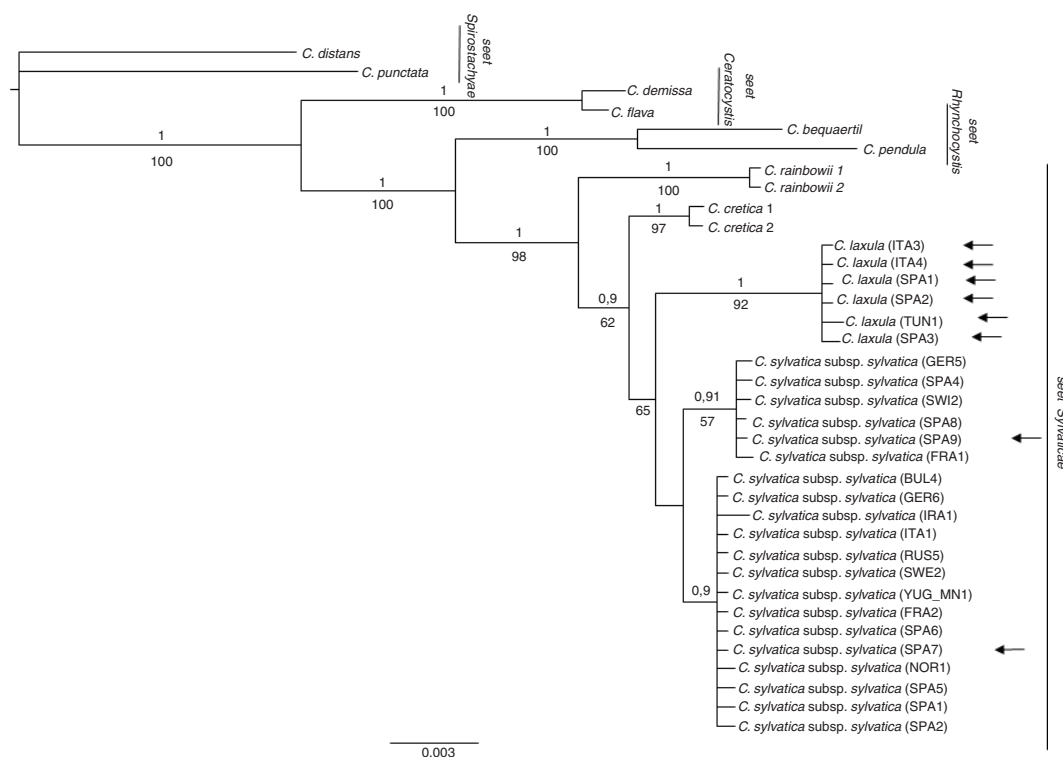
### Molecular study

ITS and 5′trnK sequences from the 3 problematic *C. sylvatica* subsp. *pau*-like specimens from the Balearic and Tuscan archipelagos clustered with those from typical specimens from northwestern Africa and northeastern Spain —Catalonia—, both in separate —nuclear vs. plastid data sets— and combined analyses (Figs. 1, 6, 7). The northern Spanish Navarran samples, previously classified as *C. sylvatica* subsp. *pau*, however grouped with *C. sylvatica* subsp. *sylvatica*. The phylogenetic relationships revealed by the separate nrDNA and

**Table 2.** Variables included in the morphometric analysis reported as potential diagnostic characters [<sup>1</sup>The longest flowering stem is measured up to the beginning of the upper male spike; <sup>2</sup>three measures were taken in each character —base, center, and beak of the glume—; <sup>3</sup>measures taken from three different utricles].

Continuous quantitative variable	Description (measure)
SW	Stem width (mm)
SL <sup>1</sup>	Stem length (cm)
LeafW	Leaf width (mm)
LeafL	Leaf length (cm)
LL	Ligule length (mm)
INFL <sup>1</sup>	Inflorescence length (cm)
BRINFLW	Inflorescence bract width (mm)
BRINFL	Inflorescence bract length (cm)
MSW	Male spike width (mm)
MSL	Male spike length (mm)
FSW	Female spike width (mm)
FSL	Female spike length (mm)
MGW <sup>2</sup>	Male glume width (mm)
MGL <sup>2</sup>	Male glume length (mm)
MGBL <sup>2</sup>	Male glume beak length (mm)
FGW <sup>2</sup>	Female glume width (mm)
FGL <sup>2</sup>	Female glume length (mm)
FGHMW <sup>2</sup>	Female glume hyaline margin width (mm)
UW <sup>3</sup>	Utricle width (mm)
UL <sup>3</sup>	Utricle length (mm)
BULMW <sup>3</sup>	Base utricule length to maximum width (mm)
SUL <sup>3</sup>	Stigma utricule length (mm)
UBL <sup>3</sup>	Utricle beak length (mm)
ACHW <sup>3</sup>	Achene width (mm)
ACHL <sup>3</sup>	Achene length (mm)
SAL <sup>3</sup>	Stipe achene length (mm)
<b>Discrete quantitative variable</b>	
SPKMN	Male spikes number
SPKFN	Female spikes number
SPKAN	Androgynous spikes number
BUN	Beaks utricule number
NUN	Nerves utricule number
PBUN	Prickles beak utricule number
<b>Qualitative variable</b>	
PAP	Presence/absence papillae on the upper leaf

ptDNA trees and the combined tree were slightly different (Figs. 1, 6, 7), though they were not significantly incongruent after the Hompart test — $p > 0.05$ —. The topology of the combined tree mostly agreed with the one yielded by the nrDNA data due to the higher number of informative characters from this data set in comparison with those from the ptDNA data set (Table 3). *Carex sect. Rhynchocystis* was strongly supported as sister group to *C. sect. Sylvaticae* in the combined and nrDNA tree —1.0 PP, 100% BS; 1.0 PP, 98% BS; Fig. 1 and 7, respectively—, whereas in the ptDNA tree *C. sect. Rhynchocystis* and *C. sect. Ceratocystis* were resolved as sister groups and in turn sister to *C. sect. Sylvaticae*,



**Fig. 1.** Bayesian phylogenetic tree obtained from the concatenation of nrDNA ITS and ptDNA 5'trnK sequences for *C.* sect. *Sylvaticae* —incl. *C. cretica*, *C. laxula*, *C. rainbowii*, and *C. sylvatica* subsp. *sylvatica*— and closely related sections —*C.* sect. *Ceratocystis*, *C.* sect. *Spirostachyae*, and *C.* sect. *Rhynchozystis*—. [36 samples were included in this analysis; numbers above or below branches correspond to the posterior probability —PP > 0.9, above branches— and Bootstrap support —BS > 50%, below branches— values; arrows depict the samples previously classified as *C. sylvatica* subsp. *pau*.]

**Table 3.** Summary statistics of the analysed molecular markers. Informative sites have been considered only for ingroup sequences.

Molecular markers	5'trnK	ITS	ITS 1	5.8 S	ITS 2
Lenght aligned matrix (pb)	651	614	225	159	230
Number of sequences	32	29	29	29	29
Indels (pb)	5 (1-8)	1 (1)	1	0	0
Conserved sites	607	525	175	157	193
Variable sites	46	89	50	2	37
Informative sites	27	63	38	2	23

but without high support (Fig. 7). ITS and combined analyses yielded a strongly supported monophyletic *C.* sect. *Sylvaticae* —1.0 PP, >90% BS; Figs. 1, 6—, whereas ptDNA analysis showed a moderate support for the monophyly of *C.* sect. *Sylvaticae* —0.91 PP, < 50% BS (Fig. 7)—. The South African *C. rainbowii* was resolved as sister to the remaining lineages of the section —*C. sylvatica* and *C. cretica*— in the combined and nrDNA trees (Figs. 1, 6), with *C. cretica* being resolved as sister to *C. sylvatica* only in the combined analysis (Fig. 1). The 3 species collapsed in a basal polytomy in the ptDNA tree (Fig. 7). *Carex sylvatica* subsp. *sylvatica* and *C. sylvatica* subsp. *pau* were monophyletic but showed low support in the combined analysis —PP < 0.9, BS 65%

(Fig. 1)—, while their sequences collapsed in a polytomy in the nrDNA and ptDNA phylogenies (Figs. 6, 7). Interestingly, while *C. sylvatica* subsp. *sylvatica* was only recovered as monophyletic in the ptDNA tree —0.99 PP, 81% BS (Fig. 7)—, *C. sylvatica* subsp. *pau* was strongly supported as a monophyletic group by all markers and analyses —1.0 PP, > 90% BS (Figs. 1, 6, 7).

The calculated genetic distances (Table 4) revealed that *C. sylvatica* subsp. *sylvatica* —including the Navarran samples mentioned above— was more distantly related to *C. sylvatica* subsp. *pau* —ITS 0.016; 5'trnK 0.007; concatenated sequences 0.009— than to *C. cretica* —ITS 0.004; 5'trnK 0.005; concatenated sequences 0.006— and to *C. rainbowii* for 5'trnK sequences —0.003.

### Morphological study

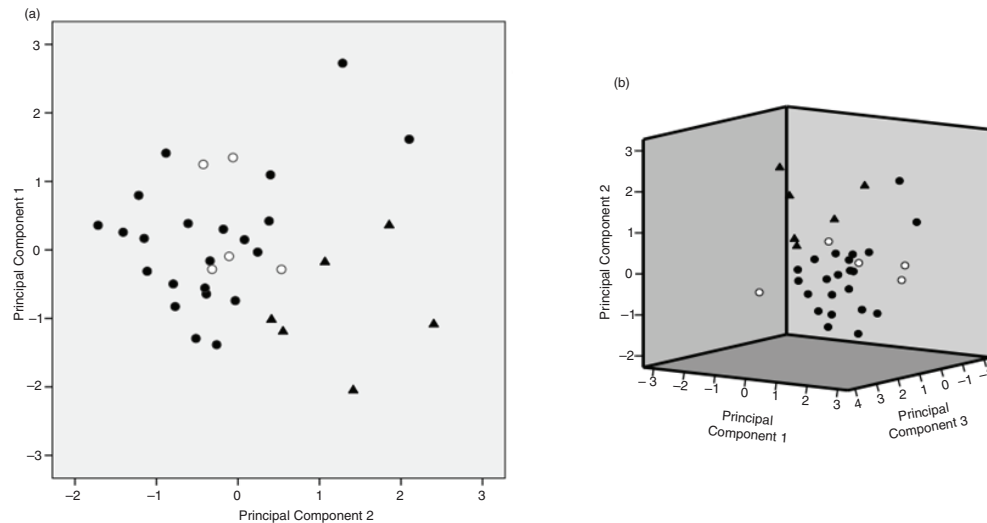
For the sake of simplicity, and according to our molecular results, we considered the Navarran samples previously classified as *C. sylvatica* subsp. *pau* to belong to *C. sylvatica* subsp. *sylvatica*.

The PCA including only seven variables —SL, INFL, UL, UBL, SPKMN, SPKAN, and PAP (Table 2)— revealed a clear separation between *C. sylvatica* subsp. *sylvatica* and *C. sylvatica* subsp. *pau* (Fig. 2). The first three principal components —PCs— accounted for 73.51% of



**Table 4.** Pairwise genetic distances calculated for species pairs in *C. sect. Sylvaticae*.

Species compared		Genetic distance		
		ITS	5'trnK	Combined
<i>C. cretica</i>	<i>C. rainbowii</i>	0.022	0.002	0.018
<i>C. cretica</i>	<i>C. laxula</i>	0.020	0.005	0.014
<i>C. cretica</i>	<i>C. sylvatica</i> subsp. <i>sylvatica</i>	0.004	0.005	0.006
<i>C. rainbowii</i>	<i>C. laxula</i>	0.039	0.003	0.014
<i>C. rainbowii</i>	<i>C. sylvatica</i> subsp. <i>sylvatica</i>	0.022	0.003	0.012
<i>C. sylvatica</i> subsp. <i>sylvatica</i>	<i>C. laxula</i>	0.016	0.007	0.009

**Fig. 2.** Morphometric study: **a**, scatter plot of first and second principal components; **b**, three first principal components extracted in the PCA analysis of the morphometric data. [*C. sylvatica* s.str. is represented by circles and *C. laxula* by triangles; empty circles depict these individuals of *C. sylvatica* s.str. previously classified as *C. sylvatica* subsp. *pauii*.]

the total variance —31.74%, 23.88% and 17.89% respectively—. The characters that contributed the most to the first 3 components were related to plant and utricle sizes, as well as the androgynous spike number (Table 6).

Boxplots showed that at least UL, UBL, SPKAN, and PAP displayed less than 25% overlap between the two studied taxa (Fig. 3).

Table 5 summarizes those diagnostic characters that distinguish between both taxa according to our own results and previous studies (Luceño & al., 2008; Martín-Bravo & al., 2013).

## DISCUSSION

*Carex sylvatica* subsp. *pauii* should be considered a distinct species: *C. laxula*

Our molecular phylogenies revealed that the samples identified as *C. sylvatica* subsp. *pauii* formed a well-supported clade, sister to the *C. sylvatica* subsp. *sylvatica* clade in the combined tree (Fig. 1). Moreover, the genetic distance between these 2 taxa was larger than that found between *C. sylvatica* subsp. *sylvatica* and *C. cretica* (Table 4), being this latter taxon usually considered a separate species, morphologically distinct from any other member of *C. sect. Sylvaticae* (Escudero & Luceño, 2009; Martín-Bravo & al., 2013). In addition,

the results retrieved by the morphometric study revealed a good degree of differentiation and a number of diagnostic characters separating the 2 taxa, despite the small number of specimens examined—including the type of *C. laxula* (Fig. 2)—. At least 4 characters displayed no or few —< 25%— overlap (Fig 3, Table 2). The number of male spikes has been considered the most important and clear diagnostic character in previous studies (Table 5). Our study has revealed the existence of other important distinctive characters between both taxa: the number of androgynous spikes, the length of the stem, the inflorescence, the utricle, and the beak utricle, all of which are larger in *C. sylvatica* subsp. *pauii* than in *C. sylvatica* subsp. *sylvatica* (Fig. 4). Also, the adaxial leaf surface is strongly rough in *C. sylvatica* subsp. *pauii*, but smooth or slightly rough in *C. sylvatica* subsp. *sylvatica* (Fig. 4, Table 5). Our data indicate that the taxon should be recognized at the species level. Based on the nomenclatural priority of the *C. laxula* name over *C. pauii* Sennen, *C. sylvatica* subsp. *pauii* is subsumed under *C. laxula*.

*Carex laxula* new for the Balearic and Tuscan archipelagos and excluded from northern Spain

Our study clearly shows that the studied specimens from the northeastern Spain —Catalonia— and the Balearic and Tuscan archipelagos, as well as the type specimen of

**Table 5.** Main morphological characters differentiating *C. sylvatica* subsp. *sylvatica* from *C. laxula*, according to Martín-Bravo & al. (2013) and the current study.

	<i>C. sylvatica</i> subsp. <i>sylvatica</i>	<i>C. laxula</i>
Longest flowering stem (cm)	100	200
Leaf upper side	Smooth to slightly rough	Strongly rough
Leaf width (mm)	(2)4-7(8)	(6)8-14
Male spikes number	1(2)	(1)2-4(7)
Female spike length (mm)	(23)25-55, not branched	(22)30-53, sometimes branched at the base
Utricle length (mm)	(3.8)4.0-5.0	(4.0)4.5-5.3
Utricle beak	Smooth, rarely with a few prickles towards the apex	Aculeolate

*C. laxula* formed indeed a well-characterized morphological taxon (Fig. 2). It is also in agreement with the phylogenetic nesting of the samples included in the molecular study (Figs. 1, 6, 7). By contrast, the Navarran samples previously classified as *C. sylvatica* subsp. *pau* fall within the variation of *C. sylvatica* subsp. *sylvatica* in both molecular and morphometric studies (Figs. 1, 2) and should therefore be classified within the latter taxon.

*Carex sylvatica* subsp. *pau* was first described at the specific rank —*C. pau* Sennen (Sennen, 1925)—, but the taxon was later recognized at the subspecific rank —*C. sylvatica* subsp. *pau* (Sennen) Bolòs & Bolòs— by Bolòs & Bolòs (1950) and later authors (Luceño & al., 2008; Jiménez-Mejías & Luceño, 2011; Govaerts & al., 2016). Early works indicated that the taxon was only known from Spain (Luceño, 1994; Luceño & al., 2008). Later, the populations of *C. algeriensis* Nelmes from Algeria and Tunisia (Maire, 1957) were synonymized to *C. sylvatica* subsp. *pau* based on morphological data (Jiménez-Mejías & Luceño, 2011; Martín-Bravo & al., 2013). This treatment has been supported by our phylogenetic study —v.gr., the nesting of the Tunisian sample in the phylogenetic trees (Figs. 1, 7)—. The finding of *C. sylvatica* subsp. *pau* to be conspecific to *C. laxula*, from Sicily, and its new records from the islands of Mallorca —Balearic Islands— and Elba —Tuscan archipelago—, greatly expands the presence of this taxon in the western Mediterranean (Fig. 5). There were no previous reports of this species for the Balearic and Elba Islands (Pignatti, 1982; Innamorati, 1991; Bolòs & Vigo, 2001; Conti & al., 2005). It implies an important range expansion of *C. laxula* —*C. sylvatica* subsp. *pau*— in Spain and a new taxon for the Italian flora.

Misidentifications of *C. laxula* and *C. sylvatica* have been common as a consequence of the very subtle morphological differences between both taxa (Table 5). Thus, the finding of more populations of *C. laxula* in other adjacent areas of the western Mediterranean could be expected. The misidentification of the Navarran populations of *C. sylvatica* subsp. *sylvatica* as *C. laxula* —*C. sylvatica* subsp. *pau* (Luceño, 1994; Luceño & al., 2008)— depicts a classical taxonomic problem. The Navarran individuals of *C. sylvatica* subsp. *sylvatica* display larger morphological variation than expected for the taxon (Global *Carex* Group, 2016). The detailed examination of these samples reveals that they show characters, such as a smooth upper side and margins of leaves, and the presence of only a few sparse prickles at the utricle

beak, that match those detected in other studied samples of *C. sylvatica* subsp. *sylvatica* (Fig. 3). Nevertheless, these specimens also have a higher number of male and androgynous spikes and longer inflorescences than those usually found in *C. sylvatica* subsp. *sylvatica*, explaining previous misidentification of these plants.

### Ecology

The ecology of *C. laxula* contrasts with that of *C. sylvatica* subsp. *sylvatica*. The latter taxon inhabits Eurosiberian and Atlantic forests, being part of the understorey in broad-leaf deciduous woods of beech —*Fagus sylvatica* L.—, oaks —*Quercus* spp.—, and riparian forests, mostly on moist to wet soils on sandy or stony-clay substrates (Hegi, 1969; Luceño, 2008; Nilsson, 1985; Pignatti, 1982). In contrast, *C. laxula* mostly grows in shady humid Mediterranean forests dominated by evergreen oak —*Quercus ilex* L.— and *Corylus avellana* L. woods, as well as in riparian forests with *Alnus glutinosa* (L.) Gaertn. and *Populus* spp., mainly on siliceous substrates such as sandstones, at relatively low altitudes —150-300 m s.n.m.— (Maire, 1957; Luceño, 1994; Luceño & al., 2008). The previous reports of *C. laxula* in beech forests —v.gr., *C. sylvatica* subsp. *pau* (Luceño, 1994; Luceño & al., 2008)— correspond to the Navarran populations here identified as *C. sylvatica* subsp. *sylvatica*, in agreement with the newly circumscribed niches of both taxa.

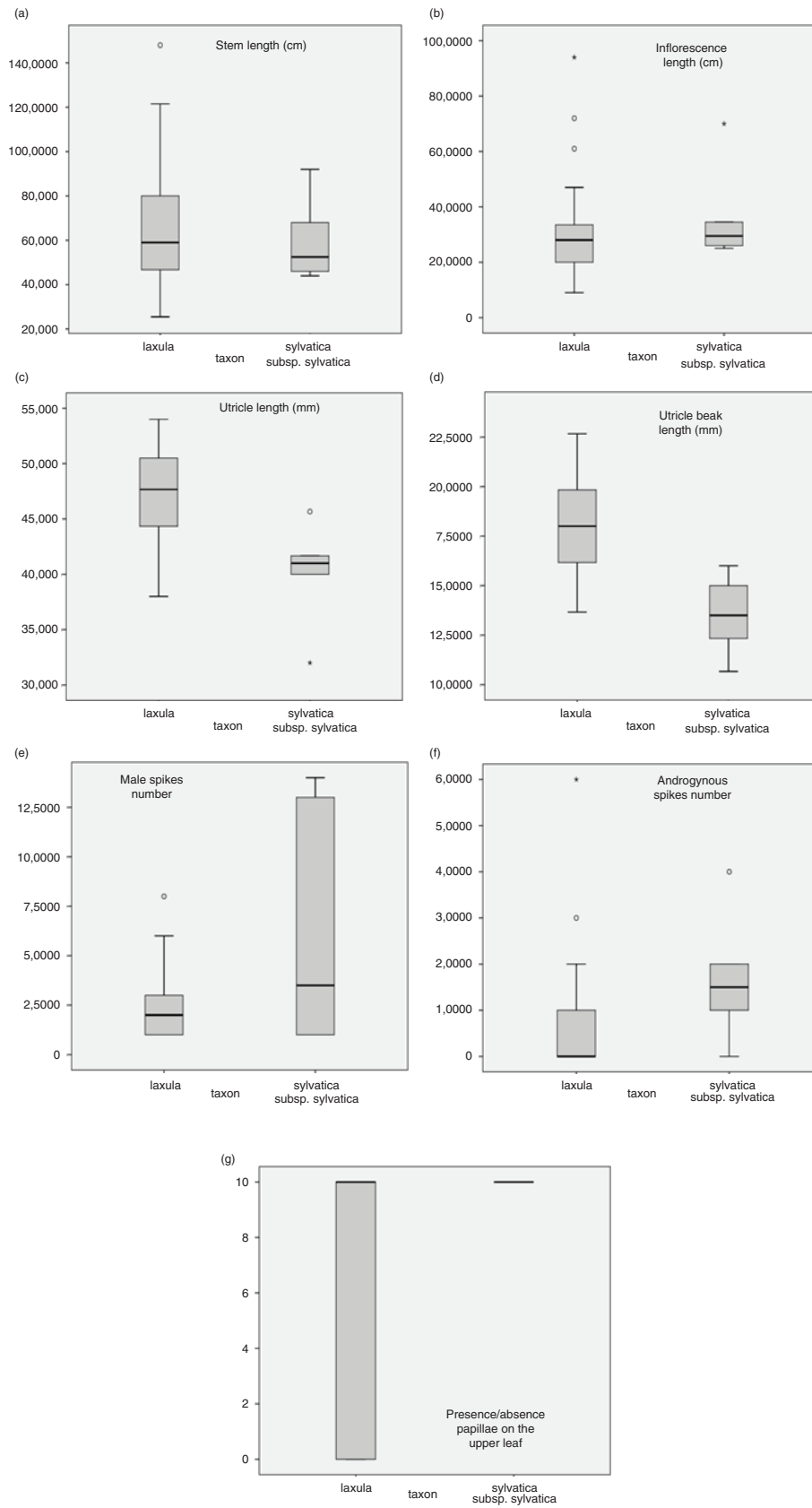
### Description

We provide an updated description for *C. laxula* and an identification key to separate it from *C. sylvatica* subsp. *sylvatica*:

***Carex laxula*** Tineo ex Boott, *Carex* 4: 202 (1867). TYPE: [Italia:] Sicily, Palermo, 1867, *V. Tineo s.n.* [lectotype, here designated: BM 001067082!]. Iconography: Luceño & al. (2008: 166).

*C. algeriensis* Nelmes, Bull. Misc. Inform. Kew 1939: 99 (1939). *C. sylvatica* subsp. *algeriensis* (Nelmes) Maire & Weiller, Fl. Afrique N. 4: 154 (1957). [Algeria:] Yacouïren, between Bougie and Tizi Ouzou, ravine in deciduous oak forest, 27 April 1937, *Alston & Simpson 37614* [lectotype, designated here: K 000363433 photo!; isotypes: BM 000922723 photo!, BM 000922724 photo!].

*C. pau* Sennen, Exsicc. Pl. Espagne 1925: n° 5435 (1925). *C. sylvatica* subsp. *pau* (Sennen) A. Bolòs & O. Bolòs in Bolòs, Veg. Com.



**Fig. 3.** Statistic boxplots of main morphological characters retrieved by PCA analyzed in the studied *C. laxula* and *C. sylvatica* subsp. *sylvatica* samples.

Barcelon.: 246 (1950). [Spain:] Barcelone, massif du Tibidabo, dans les barrancos, 6 June 1925, *Fr. Sennen n.º 5435* [lectotype, designated by Luceño & al. (2008): MA 18049!; isotype: MA 417028!].

Caespitose plant. Flowering stems up to 200 cm long, sharply trigonous, smooth. Inflorescences length 25-35(70) cm. Leaves 10-14 mm wide, shorter than stems,

**Table 6.** Variables included in the PCA (abbreviations specified in Table 2).

Variable	Component		
	1	2	3
SL	0.719	0.546	-0.309
INFL	0.736	0.560	-0.225
UL	0.706	-0.563	0.263
UBL	0.793	-0.446	0.333
SPKMN	-0.023	0.458	0.084
SPKAN	0.068	0.413	0.729
PAP	-0.176	0.404	0.623

± carinate, ± rigid, with the adaxial surface scabrid; ligule 1.5-3(5) mm long, longer than wide, apex obtuse; basal sheaths pale brown, entire, rarely fibrous. Lowermost bract shorter than the inflorescence. Male spikes (1)2-4(7), 14-45 mm long, fusiform, sometimes with a few utricles at the base, very rarely with utricles also at the top. Female spikes 3-4, (22)30-53 mm long, occasionally shortly branched at the base, at least the lower ones separated from the upper ones, with long filiform and pendant peduncles, the upper ones with shorter peduncles, sometimes arising very close to each other. Androgynous spikes (0)1-2(4). Male glumes oblong to obovate, light brown, acute, subacute or obtuse, rarely mucronate; female glumes elliptic, shorter than the utricles, hyaline or, exceptionally, pale brown with a wide scarious margin. Utricles 4-4.5 × 1-1.2(1.5) mm, suberect, ovoid, trigonous, greenish or brownish, with only 2 prominent veins, more or less abruptly contracted into a narrow, slender, bifid beak, 1.2-2(2.3) mm long, conspicuously scabrid, with prickles towards the top. Achenes (2)2.2-2.5 × 0.9-1.4 mm, ovoid, trigonous, greenish to pale brown.



**Fig. 4.** Detailed photographs of diagnostic characters separating *C. laxula*—left column— and *C. sylvatica* subsp. *sylvatica*—right column—: **a, b**, male spike(s) of the inflorescence; **c, d**, utricule beak and complete utricule (inset); **e, f**, leaf upper side. [Scale bars: a, b = 0.5 mm; c, d = 0.2 mm (0.1 mm in inset); e = 0.2 mm; f = 0.1 mm. Specimens: a, *J. Höller s.n.* (M 0223070); c, e, *P. Jiménez-Mejías & al. 76PJM13* (UPOS 6141); b, d, f, *M. Luceño & al. 1608ML* (UPOS 3427).]





Fig. 5. Known distribution of *C. laxula*. [Studied populations are represented by black triangles and literature records by white circles.]

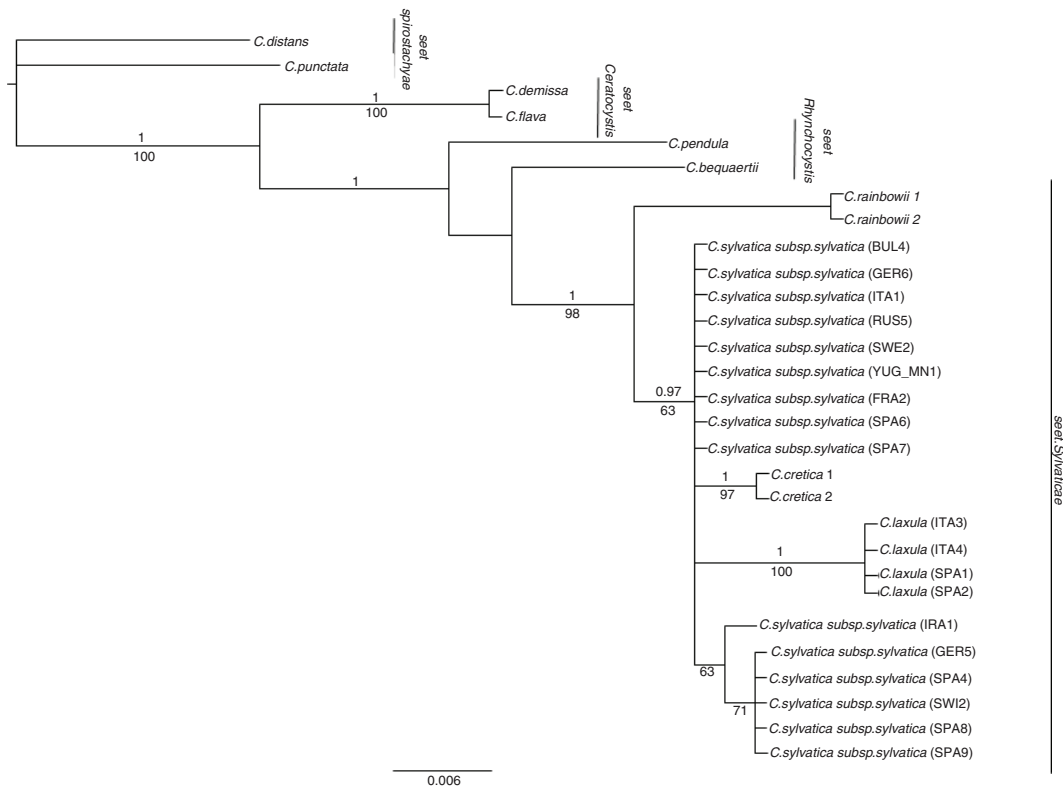
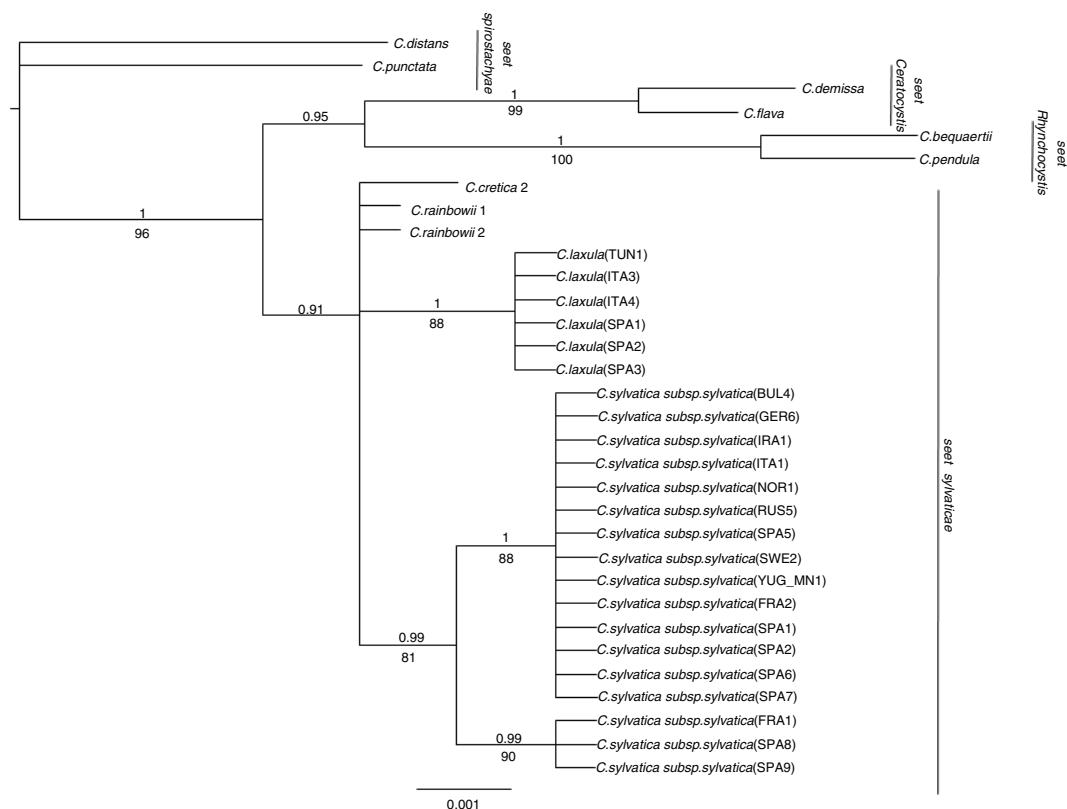


Fig. 6. Bayesian phylogenetic tree obtained from the analysis of the nrDNA ITS sequences for *C. sect. Sylvaticae* —incl. *C. cretica*, *C. laxula*, *C. rainbowii*, and *C. sylvatica* subsp. *sylvatica*— and closely related sections —*C. sect. Ceratocystis*, *C. sect. Spirostachyae*, and *C. sect. Rhynchocystis*—. [29 samples were included in this analysis; numbers above or below branches correspond to the posterior probability —PP > 0.9, above branches— and Bootstrap support —BS > 50%, below branches— values.]



**Fig. 7.** Bayesian phylogenetic tree obtained from the analysis of the ptDNA 5' *trnK* sequences for *C.* sect. *Sylvaticae*—incl. *C. cretica*, *C. laxula*, *C. rainbowii*, and *C. sylvatica* subsp. *sylvatica*—and closely related sections—*C.* sect. *Ceratocystis*, *C.* sect. *Spirostachyae*, and *C.* sect. *Rhynchosystis*— [32 samples were included in this analysis; numbers above or below branches correspond to the posterior probability—PP > 0.9, above branches— and Bootstrap support—BS > 50%, below branches—values.]

### Distribution and notes

It is a western Mediterranean endemic: northeastern Iberian Peninsula, Balearic Islands, Elba Island, Sicily, northern Algeria, and northern Tunisia (Fig. 5).

One of the isolectotype specimens we listed for *C. algeriensis* housed at BM—BM 000922724—displays a label that says it was collected in May—5—instead of April, as indicated in the protologue and other specimens. However, the collector number—37614—, and also the day of the month—27— and year—1937—, are the same. We consider that the difference in the label is just a typo when transcribing the new label, thus the material should be considered an isolectotype.

### Identification key

1. Male spikes 1(2); androgynous spikes absent or very rarely 1-2(3); utricle beaks smooth, very rarely with a few sparse prickles at the tip; leaves soft, smooth to slightly scabrid on the upper side and the margins ..... *C. sylvatica* subsp. *sylvatica*
2. Male spikes (1)2-4(7); androgynous spikes 1-4, very rarely absent; utricle beaks conspicuously scabrid, with prickles towards the top; leaves  $\pm$  rigid, conspicuously scabrid on most of the upper side ..... *C. laxula*

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APPENDIX 1. Herbarium materials of *C. sylvatica* subsp. *sylvatica* and *C. laxula* included in the morphological and molecular studies. Data between square brackets indicate the specimens also included in the molecular study—including sample labeling in the phylogeny, and ITS and 5'*trnK* Genbank accession numbers for the new sequences generated in this study; if a marker is missing, it is replaced by a dash—. Asterisks depict those samples included in the molecular study but not in the morphometric study. Herbaria acronyms follow *Index Herbariorum* (Thiers, 2015).

**Carex sylvatica** subsp. *sylvatica*. ARMENIA. Lori: Pushkin pass, G. Fayvush & al. 03-0537 (MSB 123515).

BOSNIA-HERZEGOVINA. Magli: P. Orendi s.n. (M 0183079).

BULGARIA. Central Balkans: P. Jiménez-Mejías & F. Madroñal 113PJM10 (UPOS 4054); *ibidem*, P. Jiménez-Mejías & F. Madroñal 109PJM10 (UPOS 4049) [BUL4; KU242691, KU242704]\*.

CROATIA. Umag S.: J. Höller s.n. (M 0183078).

DENMARK. Odense: H.F. Poulsen s.n. (V 572147).

FRANCE. Atlantic Pyrenees: Pau Urdo, Espelunguère, Les Forges d'Abel, P. Montserrat s.n. (JACA 227895) [FRA2; KX426304, KX426309]; Haute-Normandie: Eure, P. Jiménez-Mejías 16PJM10 (UPOS 4112) [FRA1; -, KC122386]\*.

HUNGARY. Budapest: Ungvár, P. Erzberger 3-1615 (B 100343844).

GERMANY. Bavaria: München, P. Jiménez-Mejías & G. E. Rodríguez-Palacios 171PJM13 (UPOS 5559) [GER6; KU242692, KU242705]\*.

IRAN. Tanagerah: Golestan National Park, H. Akhani 10385 (M 0183092) [IRA1; KU242693, KU242706].

ITALY. Abruzzo: Central Apennines, Monti Della Laga National Park, P. Jiménez-Mejías & al. 246PJM10 (UPOS 4133) [ITA1; KU242694, KU242707]; Piedmont: colina di Turín, P. Jiménez-Mejías & E. Martinetto 113PJM12-2 (UPOS 5350); *ibidem*, ponte dei Preti, P. Jiménez-Mejías & E. Martinetto 63PJM12-2 (UPOS 5347).

MONTENEGRO. Durmitor National Park: Zabljak, P. Jiménez-Mejías 228PJM10 (UPOS 4026) [YUG-MN1; KU242702, KU242716].

NORWAY. Asker: Konglungen, P. Jiménez-Mejías & K. Lye 188PJM09 (UPOS 4547) [NOR1; -, KC122387].

POLAND. Upper Silesia: Rybnik, Krystof 10362 (B 100118074).

ROMANIA. Cotofanesti: D. Mititelu & al. s.n. (M 0183082).

RUSSIA. Kazan: Semenenko & Nekrasova s.n. (B 100448059). Moscow: Bei Dorf Weschke, A.K. Skvortsov s.n. (M 0183087) [RUS5; (KU242700, KU242712)\*].

SERBIA. Carpatians: Djerdap, P. Jiménez-Mejías 83PJM10 (UPOS 4204).

SOUTH AFRICA. Western Cape: Knysna Diov, G. Lindeberg s.n. (V 571678).

SPAIN. Jaén: Siles, Las Acebas, S. Martín-Bravo & al. 121SMB15 (UPOS 6320) [SPA8; KX426307, KX426314]. Gerona: Olot, La Moixina, P. Jiménez-Mejías & al. 106PJM13 1/2 (UPOS 5270) [SPA2; -, KX426311]\*. Huesca: Ansó, P. Montserrat s.n. (JACA 80782) [SPA6; KX426305, KX426312]; National Park Ordesa, M.L. Buide & J.M. Marin s.n. (UPOS 161) [SPA5; -, KU242714]\*. Lérida: Les Bordes, Artiga de Lin, E. Maguilla & M. Luceño 39EMSI2 (10) 1/2 (UPOS 5048) [SPA1; -, KX426310]. Palencia: Piedrasluengas, J.M. Marin & al. 14004JMM (UPOS 163) [SPA4; KU242699, -]\*.

SWEDEN. Öland: Högrum, C.M. Norrman s.n. (V 572178). Västergötland: Västra Tunhem, J. Sjögren s.n. (V 572152) [SWE2; KU242701, KU242715]\*.

**Carex sylvatica** subsp. *sylvatica*—materials erroneously classified as *C. sylvatica* subsp. *pauii*—. SPAIN. Navarra: Artikutza, I. Aizpuru & P. Catalán s.n. (ARAN 22918); Foz de Arbayun, J. & G. Montserrat 87-JACA-0911-08102 (JACA 810287) [SPA9; KX426308, KX426315]; Garralda, G. & J. Montserrat s.n. (JACA 797187); Isaba, L. Villar s.n. (JACA 10045273) [SPA7; KX426306, KX426313]; Ochagavía, L. Villar & G. Montserrat s.n. (JACA 118787).

**Carex laxula**—including materials previously classified as *C. sylvatica* subsp. *pauii*—. ITALY. Sicily: 1877, V. Tineo s.n. (BM 001067082; *C. laxula* lectotype). Tuscany: Elba, J. Höller s.n. (M 0223069) [ITA3; KU242695, KU242708]; Elba, Mt. Perone, J. Höller s.n. (M 0223070) [ITA4; KU242696, KU242709].

SPAIN. Catalonia: Barcelona, Massif du Tibidabo, 1925, Fr. Sennen n.º 5435 (*C. pauii* lectotype, MA 18049, isolectotype MA 417028); Montnegre, P. Jiménez-Mejías & al. 85PJM13 1/13 (UPOS 6142) [SPA1; KU242697, KU242710]; Sant Carles, P. Jiménez-Mejías & al. 76PJM13 1/4 (UPOS 6141) [SPA2; KU242698, KU242711]. Balearic Islands: Mallorca, Lluch, H. Merxmüller & W. Wiedmann 7709 (M 0223072) [SPA3; -, KU242713].

TUNISIA. Medjerda: Bei Les Chènes, H. Hertel 8305 (M 0183088) [TUN1; -, KU242703]\*.

APPENDIX 2. Accession numbers for ITS and 5'*trnK* sequences downloaded from Genbank and included in the molecular study.

**Carex bequaertii**: EU288572, KC122385; **C. cretica** 1: DQ384117; **C. cretica** 2: DQ384118, EU812677; **C. demissa**: AY278307, JN627690; **C. distans**: EU483663, JN627754; **C. flava**: AF285007, JN627705; **C. pendula**: AY757600, KC122384; **C. punctata**: DQ384180, EU812618; **C. rainbowii** 1: KC122380, KC122382; **C. rainbowii** 2: KC122381, KC122383; **C. sylvatica** subsp. *sylvatica* GER5: AY278306; **C. sylvatica** subsp. *sylvatica* SWI2: AY757599.