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Reconciling morphology and phylogeny allows an integrative taxonomic revision of the giant sedges of *Carex* section *Rhynchocystis* (Cyperaceae)

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Species delimitation in *Carex* section *Rhynchocystis* has remained relatively constant through its taxonomic history. The section is currently composed of five species distributed in the Western Palaearctic (*C. microcarpa* and *C. pendula*) and subSaharan Africa (*C. bequaertii*, *C. mossii* and *C. penduliformis*). Recent phylogenetic studies revealed that the monophyly of *C. bequaertii* and *C. mossii* was not well supported and that *C. pendula* comprises two divergent sister lineages. To evaluate the taxonomic significance of these unexpected results, we performed a rigorous statistical procedure based on morphometrics. We found morphological support for our molecular background, uncovering (1) characters that reflect the evolution of the group and were overlooked by traditional taxonomy and (2) the overlapping of some previously considered diagnostic characters. Our results suggest five species, but only *C. microcarpa* and *C. penduliformis* were supported in their traditional concepts. The two lineages of *C. pendula* corresponded to two morphologically distinct, biogeographically congruent groups: *C. pendula s.s.* in the western part of the range and *C. agastachys* in the eastern part. In congruence with the molecular results, *C. bequaertii* and *C. mossii* were weakly morphologically differentiated and thus better treated as subspecies of a single species. We propose a revised taxonomic treatment for the group.

ADDITIONAL KEYWORDS: Afrotropics – cryptic species – morphometrics – molecular and morphological congruence – multivariate analyses – Western Palaearctic.

INTRODUCTION

The combined use of different sources of data and analytical tools has been shown to contribute to a better performance and rigour in taxonomy (Schlick-Steiner *et al.*, 2010). Such good practice, known as integrative taxonomy (Dayrat, 2005), is necessary in groups in which species delimitation is unclear, especially those with reduced morphological diagnostic features. The evaluation of molecular and morphological data has uncovered errors in traditional taxonomy, and it has reconciled conflicts between traditional, morphology-based taxonomy and molecular phylogenies (e.g. Martín-Bravo & Jiménez-Mejías, 2009; Jiménez-Mejías, Martín-Bravo & Luceño, 2012; Vigalondo *et al.*, 2016).

Traditional classifications of *Carex* L. (Cyperaceae), as for most plants, have relied largely on morphological characters, especially those related to reproductive structures (Kükenthal, 1909; Chater, 1980; Egorova, 1999). Misconceptions about which characters are diagnostic and which are not is often the main cause of incongruence between morphology-based classifications and the phylogenetic relationships revealed by DNA sequences in *Carex* (Global *Carex* Group, 2015, 2016). The identification of synapomorphic combinations of diagnostic characters in sedges is frequently problematic, mainly because reproductive structures in *Carex* are morphologically reduced and, as a consequence, species may look superficially similar (González-Elizondo & Peterson, 1997; Starr, Harris & Simpson, 2004). In addition, prominent homoplastic or plesiomorphic features may obscure taxonomy in plant groups with such reduced morphologies (e.g. Valcárcel & Vargas, 2010; Jiménez-Mejías *et al.*, 2013; Otero *et al.*, 2014; Gebauer, Roeser & Hoffmann, 2015; Więcław *et al.*, 2016), resulting in

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the over-valuation of these characters and the neglect of less evident synapomorphic ones. This is, in many cases, responsible for the disagreements between morphology-based taxonomic classification and DNA-based phylogenetic trees (Naczi, 2009; Jiménez-Mejías & Vargas, 2015; Global *Carex* Group, 2016).

Taxonomic treatments of *Carex* section *Rhynchocystis* Dumort. have remained fairly stable throughout recent history. They differ only in the number of species included, but with little controversy in their delimitation (five to ten species; Kükenthal, 1909; Chater, 1980; Egorova, 1999). The section was recently narrowed to include only the five Western Palaearctic and Afrotropical species (Global *Carex* Group, 2016; Table 1; Fig. 1). As newly delimited, the section is remarkably morphologically well-defined throughout its entire range from Northern Europe to the Cape Region (Nelmes, 1940; Gehrke, 2011). Despite being an old group, the origin of which has been traced to the Miocene (Míguez *et al.*, 2017), its species display a relatively homogeneous morphology, probably as a consequence of morphological stasis (i.e. retaining many plesiomorphic traits). Accordingly, the species of the section are distinguished by: being large, densely caespitose perennial sedges, with stems and leaves usually around 1 m or even more; having racemose inflorescences, with proximal long-sheathing leaf-like bracts, the blade equalling or surpassing the inflorescence; and having elongated cylindrical, densely flowered and usually flexuous spikes, the lowermost ones often pendent (Kükenthal, 1909; Egorova, 1999). Recent molecular phylogenetic analyses (Míguez *et al.*, 2017) showed that relationships among the lineages of section *Rhynchocystis* were not entirely congruent with taxonomic treatments based on morphological characters. Molecular evidence suggests that the section constitutes a well-supported monophyletic group arranged in two major lineages (Fig. 1). The first lineage includes the Mediterranean *C. microcarpa* Bertol. ex Moris as sister to the African species *C. bequaertii* De Wild., *C. mossii* Nelmes and *C. penduliformis* Cherm. In this group, *C. microcarpa* was found to be monophyletic, whereas *C. bequaertii* and *C. mossii* were sisters, but neither received strong support for monophyly (Fig. 1). Only one sample of *C. penduliformis* was included, and this was sister to *C. bequaertii* and *C. mossii*. The second main lineage comprised *C. pendula* Huds. (as traditionally conceived, e.g. Kükenthal, 1909; Schultze-Motel, 1968; Chater, 1980; Egorova, 1999; Reznicek, 2002), which is widespread in the Western Palaearctic. However, despite being monophyletic, *C. pendula* was found to include two highly differentiated genetic lineages, with an ancient divergence dating back to the Miocene (Míguez *et al.*, 2017). These two lineages were parapatric, with an eastern lineage distributed from Central Europe to the Caucasus and western Iran and a western lineage inhabiting Western Europe east to Germany, the Mediterranean Basin and Macaronesia. The lack of support for the reciprocal monophyly of *C. bequaertii* and *C. mossii* and the deep split in *C. pendula* posed the main incongruences to the previous morphology-based classifications (Table 1).

Multivariate statistical methods based on large sets of morphological data have long been used as morphology-based predictors for species delimitation. These methods help in detecting significant morphological discontinuities between taxa and in identifying diagnostic characters. In contrast to treatments based on often subjective 'taxonomic expertise', these techniques standardize the measure of morphological variability and objectively quantify the differences among groups (Valcárcel & Vargas, 2010). In addition, in sedges, the inclusion of micromorphological and anatomical characters has been demonstrated to support taxonomic units based on macromorphological characters (Naczi, 2009). Scanning electron microscopy (SEM) has been used to observe microstructures of the achene epidermis in *Carex*, which revealed significant interspecific variation in certain groups of species (Starr & Ford, 2001; Zhang, 2006; Naczi, 2009; da Silva *et al.*, 2011).

In this study, we performed a morphometric study as part of our comprehensive systematic revision of *Carex* section *Rhynchocystis*. Our main aim is to propose a new taxonomic treatment of the group using our previously published DNA-based phylogenetic results in combination with the morphological variation studied here. The particular objectives are: (1) to re-evaluate the morphological affinities among the five traditionally considered species; (2) to assess if the genetic differentiation between the two lineages in *C. pendula s.l.* is correlated with morphological differences; and (3) to evaluate the taxonomic status of the phylogenetically poorly differentiated *C. bequaertii* and *C. mossii*.

MATERIAL AND METHODS

Study group and sampling

We follow the most recent taxonomic treatment of the group (Míguez *et al.*, 2017) that includes *C. bequaertii*, *C. microcarpa*, *C. mossii*, *C. pendula* and *C. penduliformis*, plus *C. agastachys* L.f. (Table 1), which refers to the eastern lineage of *C. pendula s.l.* (Míguez *et al.*, 2017; Jiménez-Mejías *et al.*, 2017; Fig. 1), whereas *C. pendula* refers only to its western lineage hereafter.

We studied 310 herbarium specimens (Supporting Information, Appendix S1) from the following 30 herbaria: B, BM, BR, E, FI, GOET, HUB, K, J. Koopman's personal herbarium, KRA, LE, LINN, LISU, M, MA, MADJ, MICH, MO, MTMG, MHA,

Table 1. Comparison of the main treatments of *Carex* section *Rhynchocystis* Dumort. and the treatment derived from our own results

This study	Global *Carex* Group (2016) Section *Rhynchocystis* Dumort.	Egorova (1999) Section *Rhynchocystis* Dumort.	Kükenthal (1909) Section *Maximae* Asch.	Native distribution
C. agastachys L.f	(included in *C. pendula*)	(included in *C. pendula*)	(included in *C. pendula*)	Central Europe to the Caucasus and northern Iran
C. bequaertii De Wild. subsp. *bequaertii*	*C. bequaertii* De Wild.	*C. bequaertii* De Wild. *C. petitiana* A. Rich*	*C. petitiana* A. Rich*	Eastern Tropical Africa, north to Ethiopia and south to Tanzania
C. bequaertii subsp. *mossii* (Nelmes) Míguez, Martín-Bravo & Jim.-Mejías.	*C. mossii* Nelmes	*C. mossii* Nelmes	*C. mossii* Nelmes	Eastern parts of southern Africa
C. microcarpa Moris	*C. microcarpa* Moris	*C. microcarpa* Moris	*C. microcarpa* Moris	Corsica, Sardinia and the Tuscan Archipelago; reported from mainland Italy
C. pendula Huds.	*C. pendula* Huds.	*C. pendula* Huds.†	*C. pendula* Huds.	Central and western Europe, Mediterranean Basin and Macaronesia
C. penduliformis Cherm.	*C. penduliformis* Cherm.	*C. penduliformis* Cherm.	[undescribed at that time]	Madagascar
			C. joorii L.H. Bailey‡	North America, from south-eastern USA to Texas
			C. shortiana Dewey‡	North America, from southern Ontario to north-central and eastern USA
			C. jaluensis Komarov§	North-eastern Asia, from Russian Far East to Korea
			C. maculata Boott§	Tropical and subtropical Asia to south-western Pacific
			C. vicinalis Boott§	Southern India

**Carex petitiana* was erroneously used to refer to *C. bequaertii* as Gehrke (2011) already noted.
†According to our results, *C. pendula* is absent from the area covered by Egorova's (1999) treatment, and thus all the natural populations in this work should correspond to *C. agastachys*.
‡The North American *C. joorii* and *C. shortiana* were transferred to sections *Glaucescentes* and *Shortianae*, respectively, which is supported in recent phylogenetic analyses (Global *Carex* Group, 2016).
§The Asian *C. jaluensis* and *C. maculata* have been transferred to section *Anomalae* (Dai *et al.*, 2010); accordingly, the characters displayed by *C. vicinalis*, endemic from southern India (e.g. bracts sheathless), also place this species as a member of section *Anomalae*, as already recognized by the Global *Carex* Group (2016).

NY, P, PRE, SS, SZUB, TUM, UNEX, UPOS, UPS and Z (abbreviations according to Thiers, 2016). We measured morphological characters of 108 specimens: 24 specimens of *C. agastachys*, 21 of *C. bequaertii*, nine of *C. microcarpa*, 17 of *C. mossii*, 34 of *C. pendula* and three of *C. penduliformis*. This number was lower than the number of studied specimens because many specimens did not bear all the considered characters, and thus we could not include them in the morphometric analyses. Particular effort was made to cover the distribution range and morphological variability of each taxon. The sampling of *C. penduliformis* and *C. microcarpa* was limited due to their scarce representation in herbaria. The distribution of taxa was revised using TDWG geographical codes at level 3 ('Botanical countries') for specifying the ranges (Brummitt, 2001).

Morphological characters and measurements

We selected 25 quantitative continuous, four quantitative discrete and four qualitative, potentially diagnostic

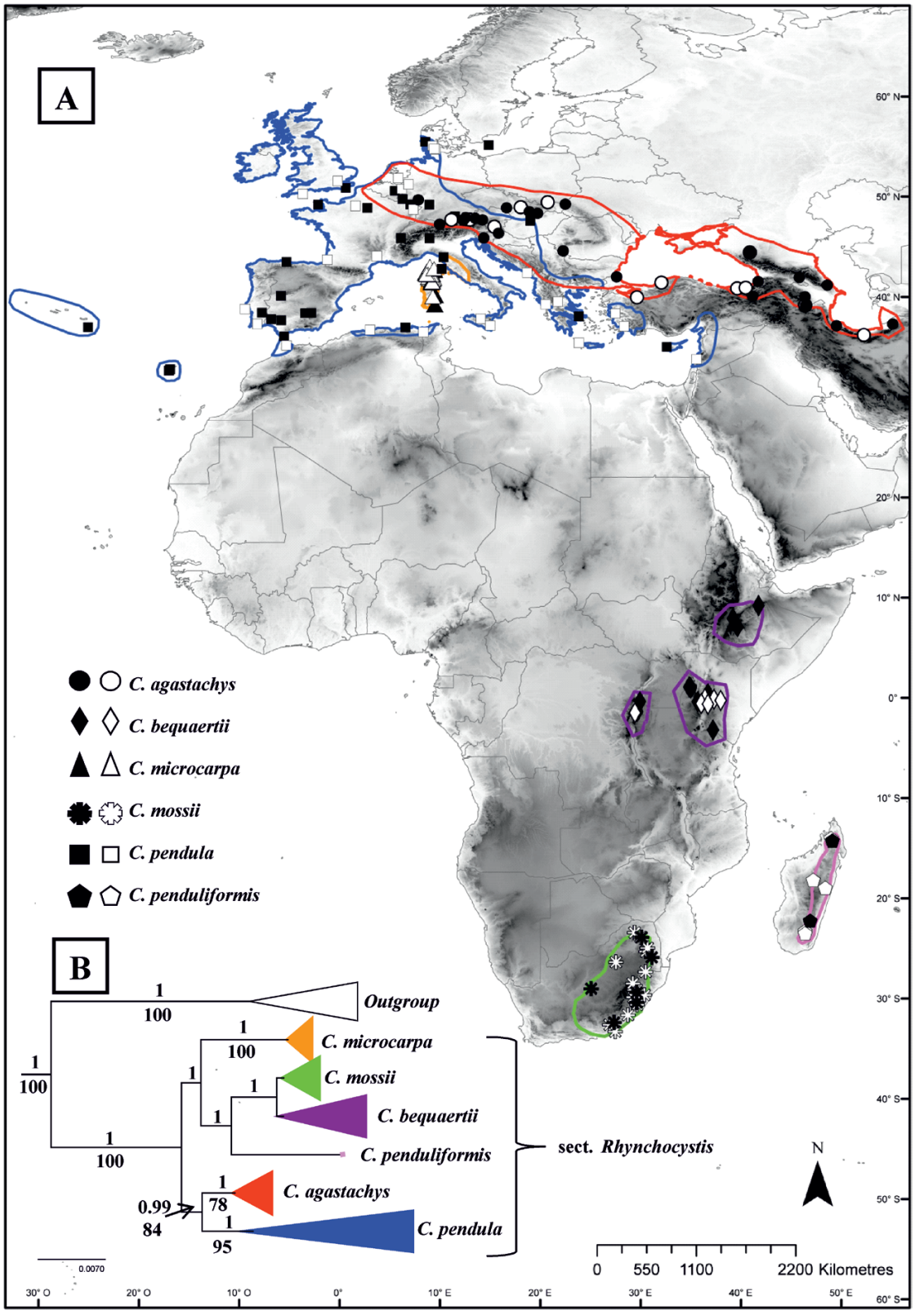

Figure 1. A, distribution of *Carex* section *Rhynchocystis* taxa. Symbols representing the different taxa are shown in the key. Black symbols represent specimens included in the morphometric study; white symbols represent specimens studied

but not included in the morphometric study. The drawn areas are approximate and must be considered indicative, especially the overlapping area between *C. agastachys* and *C. pendula*. B, majority-rule consensus tree of *Carex* section *Rhynchocystis* inferred under Bayesian inference using a combined nuclear ribosomal DNA–plastid DNA matrix (ETS, ITS, *matK* and *rpl32-trnL*UAG regions; modified from Míguez *et al.*, 2017); scale bar indicates substitutions per site. Numbers above and below branches indicate clade support values: maximum parsimony bootstrap and Bayesian posterior probability, respectively. Clades were collapsed to summarize the main lineages.

characters (Table S1) based on previous taxonomic accounts of the group (Kükenthal, 1909; Nelmes, 1940; Chater, 1980; Haines & Lye, 1983; Gordon-Gray, 1995; Egorova, 1999; Gehrke, 2011) and our own observations. Measurements were taken using an ocular micrometer, with the exception of the largest characters (> 10 mm), which were measured using a standard 30-cm rule. The number of prickles on the female spike peduncle was counted on the 0.5-cm distal portion of the peduncles of the proximal and distal female spike. Two or three mature stems were measured per specimen and their averages were included in the analyses. Qualitative characters were codified according to states reported in the above-mentioned treatments of section *Rhynchocystis* and from our own observations (Table S1).

Statistical analyses

Three different types of multivariate statistical analyses were carried out: principal components analysis (PCA); discriminant function analysis (DFA); and the Mann–Whitney *U*-test (MWUt). Our analytical approach is inspired by the hierarchical procedure presented in Valcárcel & Vargas (2010) for *Hedera* L. (Araliaceae), applied in *Carex* by Jiménez-Mejías, Luceño & Martín-Bravo (2014).

Principal component analysis

Consecutive PCAs were used to identify morphogroups (Fig. 2). We started with a PCA including all the samples. To achieve the best split among morphogroups we performed a first exploratory PCA with the 32 variables, retaining later only those with the highest principal component (PC) loadings and with the highest correlation coefficients, whenever the correlation between characters was found not to be redundant (character purge). When the analysis identified morphogroups, i.e. separate clusters containing more than one species, the samples on these morphogroups were split as a new subset and subsequently re-analysed separately, including again all the characters and performing a new character purge. Due to the lack of male spikes in *C. mossii* and *C. bequaertti*, the variables INFLM and INFWM were discarded for those analyses in which these species were included (Table S1). Kaiser's measure of sampling adequacy and Barlett's test of sphericity were computed to evaluate the suitability of the data (Valcárcel & Vargas, 2010; Jiménez-Mejías *et al.*, 2014). Only principal components with eigenvalues > 1 were retained. Analyses were performed using the software IBM SPSS statistics v.22 (Chicago, IL, USA). For a better understanding of the consecutive PCAs explained below, the reader may refer to Figure 2 and Table S1.

A first PCA (PCA-I, complete data exploration, Fig. 3A) was performed using 13 variables and 108 specimens

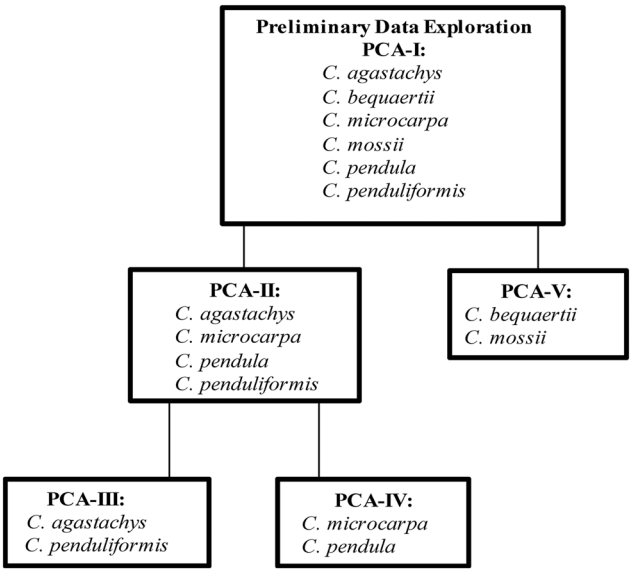

Figure 2. Schematic representation of the datasets and analyses performed in our hierarchical approach to study morphological variation in *Carex* section *Rhynchocystis*. The complete data exploration (PCA-I) was performed using 13 variables and the 108 specimens to explore the complete dataset (*C. agastachys*, *C. bequaertii*, *C. microcarpa*, *C. mossii*, *C. pendula and C. pendulifomis*). PCA-II was perfomed using six variables and 70 specimens to explore the dataset containing *C. agastachys*, *C. microcarpa*, *C. pendula* and *C. penduliformis*. PCA-III was performed using 13 variables and 27 specimens to explore the dataset containing *C. agastachys* and *C. penduliformis*. PCA-IV was performed using 13 variables and 43 specimens to explore the dataset containing *C. microcarpa* and *C. pendula*. PCA-V was performed using 13 variables and 38 specimens to explore the dataset containing *C. bequaertii* and *C. mossii* subset 2.

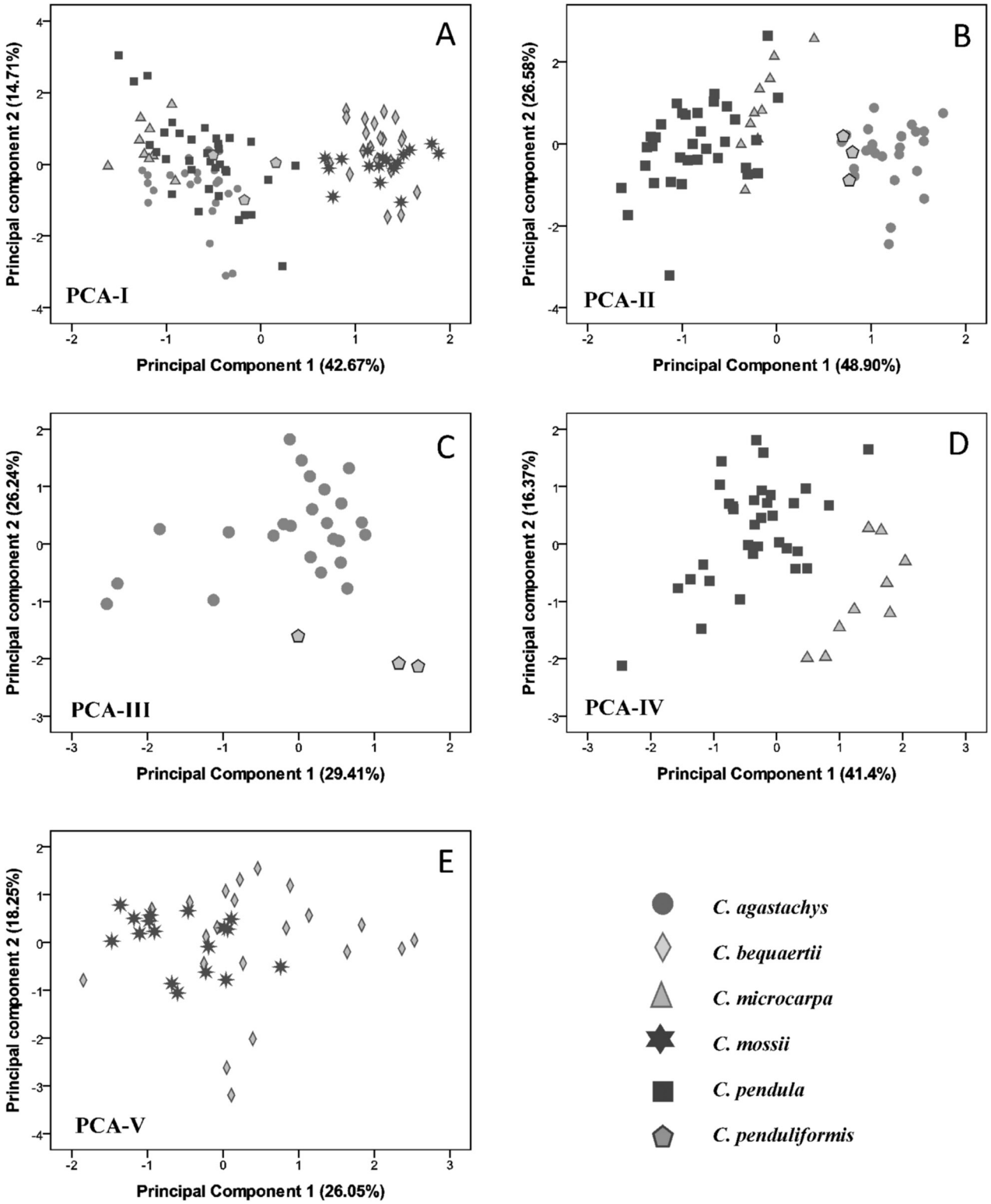

Figure 3. Scatter plot of the first two principal components extracted from the PCA as described in Figure 2. A, PCA-I, including all the species of section *Rhychocystis*; B, PCA-II, including *C. agastachys*, *C. microcarpa*, *C. pendula* and *C. penduliformis*; C, PCA-III, including *C. agastachys* and *C. penduliformis*; D, PCA-IV, including *C. microcarpa* and *C. pendula*; E, PCA-V, including *C. bequaertii* and *C. mossii*. Symbols depicting the different taxa are shown in the key.

to explore the complete dataset (*C. agastachys*, *C. bequaertii*, *C. microcarpa*, *C. mossii*, *C. pendula* and *C. pendulifomis*). This PCA split the dataset into two main clusters. New PCAs were performed for each of these subsets (PCA-II and PCA-V, respectively). PCA-II was performed on subset 1, containing *C. agastachys*, *C. microcarpa*, *C. pendula* and *C. penduliformis*, analysing six variables and 70 specimens. PCA-V was performed on subset 2, containing *C. bequaertii* and *C. mossii*, analysing 13 variables and 38 specimens.

Two new subsets were obtained from the PCA-II of subset 1, split and re-analysed: PCA-III was conducted on subset 1.A, containing *C. agastachys* and *C. penduliformis*, and analysing 13 variables and 27 specimens, and PCA-IV was performed on subset 1.B, containing *C. microcarpa* and *C. pendula*, and including 13 variables and 43 specimens.

The final morphogroups detected at the end of each chain of consecutive PCAs were considered indicative of morphological distinctiveness within the previously known phylogenetic framework of the section. Accordingly, we refer to these groups as homogeneous morphogroups.

Discriminant function analysis

After the identification of homogeneous morphogroups, DFA was used to test the taxonomic significance of the identified morphogroups and our a priori delimited five-species taxonomic treatment, as described in Valcárcel & Vargas (2010). We randomly selected 70% of all samples to perform the DFA using a cross-validation of the model over these samples. The remaining 30% of the samples were then randomly excluded from the analyses and used as a confirmatory blind control. We considered as significant those groups for which > 80% of the excluded cases were correctly classified. All the analyses were performed using IBM SPSS statistics v.22. DFA-I was performed on the entire dataset using all the 28 quantitative variables and the five morphogroups corresponding to *C. agastachys*, *C. microcarpa*, *C. pendula* and *C. pendulifomis* and the homogeneous morphogroup formed by subset 2 (*C. bequaertii* and *C. mossii*). Due to the absence of a clear split between *C. bequaertii* and *C. mossii* in PCA-V (see Fig. 3E, and Results), both taxa were treated in this analysis as a single homogeneous morphogroup. The variables INFLM and INFWM were discarded because *C. mossii* and *C. bequaertii* do not have male spikes.

DFA-II was performed to find taxonomic significance between the homogeneous morphogroup of subset 2 (*C. bequaertii* and *C. mossii*). We used the same 13 variables as in the PCA-V (Table S1).

Mann-Whitney U-test

The MWUt was used to statistically validate the most discriminant characters differentiating species pairs. We selected this test because most of our data did not meet normality. We performed MWUt in R (http://www.r-project.org/), setting a significance level of 0.01.

Micromorphological study

A micromorphological study was performed to complement the descriptions in our taxonomic treatment and to search for additional diagnostic characters. Micromorphology of achenes was examined using SEM (GeminiSEM 300, Zeiss, Oberkochen, Germany). One representative achene per taxon was examined. Achenes were pre-treated using a procedure modified from Salo, Pykälä & Toivonen (1994). Achenes were digested in a solution of acetic anhydride and sulphuric acid (9:1) for 24 h at room temperature, washed with distilled water and then placed in an ultrasonic bath in a Ultrasonic cleaner (Branson 2510E-MT; Danbury, CT, USA) for 10 min. Finally, achenes were air-dried at room temperature on Petri dishes. This treatment allowed removal of the anticlinal and outer periclinal walls of the epidermis cells to expose the silica bodies, which are placed on the inner anticlinal walls. When the anticlinal and outer periclinal walls were not totally removed by this treatment, we repeated the treatment with new achenes but increasing the time in the ultrasonic bath to 20 min. Prior to SEM observation, the achenes were gold-coated. Two pictures at different magnifications were taken from each sample: one of the entire achene to visualize its shape and one focusing on the micromorphological features of the epidermis.

RESULTS

Statistical analyses

In all datasets Kaiser's measure of sampling adequacy was > 0.5, and Barlett's test of sphericity was significant. This implies that the sampling sizes were suitable to be explored using PCA (cf. SPSS, 2009; Valcárcel & Vargas, 2010; Jiménez-Mejías *et al.*, 2014). Principal components (PCs) extracted in each PCA are numbered using roman numerals.

Principal component analysis

Figure 2 provides the consecutive partitions we performed under our analytical procedure. Scatter plots of the two first PCs are shown in Figure 3. Scatter plots of additional PCs are shown in Figure S1. The contribution of the different characters to the PCs is shown in Table S1.

In PCA-I the first three PCs accounted for 66.4% of the total variance (42.67, 14.71 and 9.02% respectively). The scatter-plot PC-1 vs. PC-2 revealed two major

morphogroups (Fig. 3A), which were even better separated in the scatter-plot PC-1 vs. PC-3 (Fig. S1). One morphogroup included *C. pendula*, *C. microcarpa*, *C. penduliformis* and *C. agastachys*. The second morphogroup included the African species *C. mossii* and *C. bequaertii*. The characters that contributed the most to the first two components were SUS, PSCLL, SPKMN and DSFS.

For PCA-II first two PCs explained 75.5 % of the total variance (48.90 and 26.58%, respectively; Fig. 3B). PCA-II revealed two morphogroups, one corresponding to *C. agastachys* and *C. penduliformis* and another to *C. pendula* and *C. microcarpa*. The characters that contributed the most to the first two components were PLC, ACHL, UL and DLC.

In PCA-III the four first PCs explained 73.7% of the total variance (29.41, 26.24, 10.14 and 7.94%, respectively; Fig. 3C). The scatter-plot PC-1 vs. PC-2 revealed two morphogroups clearly separating *C. agastachys* and *C. penduliformis* (Fig. 3C). This separation was not found in the scatter-plots PC-1 vs. PC-3 or PC-1 vs. PC-4. The characters that contributed the most to the first two components were UBL, SSCLW and INFWF.

In PCA-IV the three first PCs explained 67% of the total variance (41.41, 16.37 and 9.12 %, respectively). The scatter-plot PC-1 vs. PC-2 revealed two morphogroups separating *C. microcarpa* and *C. pendula* (Fig. 3D). The scatter-plot PC-1 vs. PC-3 also showed a slight overlap between the two species (Fig. S1). The characters that contributed the most to the first two components were LUMWD, DLC and LTPSM.

For PCA-V the first four PCs explained 68.48% of the total variance (26.05, 18.25, 14.05 and 10.13%, respectively). In all scatterplots, PC-1 vs. PC-2, PC-1 vs. PC-3 or PC-1 vs. PC-4, *C. mossii* and *C. bequaertii* remained intermingled, and no clear separation of the samples of each species as homogeneous morphogroups was obtained (Figs 3E, S1).

Discriminant function analysis

Results of DFA are showed in detail in Appendix S2. In DFA-I, the variables with the highest discriminant scores for the five considered homogeneous morphogroups were SSCLL, ACHL, SPKMN, UL, ACL, USL, INFLF and LUMWD. Validation using excluded cases significantly supported a clear distinction among *C. agastachys*, *C. microcarpa*, *C. pendula*, *C. pendulifomis* and the homogeneous morphogroup formed by *C. bequaertii* and *C. mossii*. Thus, a high correspondence between the identified homogeneous morphogroups and classifications resulting from DFA (Wilks' lambda = 0–0.3; $P = 0$) was retrieved, correctly classifying 97.2% of unselected original grouped cases. All morphogroups were classified correctly at 100% for unselected cases, except for *C. pendula* which was correctly classified at 93.3%.

For DFA-II, the highest scores discriminating between *C. bequaertii* and *C. mossii* were obtained by the characters ACHW, LTPSD and DLC. However, validation using excluded cases was not significant for the discrimination of *C. bequaertii* from *C. mossii*, as only 69.2% of all the excluded cases were correctly classified (Wilks' lambda = 0.237; $P = 0.033$). In particular, 71.4% of all *C. bequaertii* and 66.7% of *C. mossii* unselected cases were correctly classified. These values did not meet the levels of significance considered in this study (80%), further supporting the consideration of these species as a single homogeneous morphogroup.

Mann–Whitney U-test

Despite some overlap in the range of many characters between morphogroups, MWUt retrieved significant differences ($P < 0.01$) between morphogroups for a number of variables (Table S2). Between two and 20 characters were found to be significantly different ($P < 0.01$) among all pairwise comparisons at homogeneous morphogroup level. Boxplots and histograms of the most discriminant characters retrieved by DFA, with an indication of significant differences as found by MWUt or < 25% overlap are shown in Table S2 and Figures S2 and S3.

Micromorphological study

The micromorphological study supported the differences in achene shape among species revealed by the macromorphological study (Fig. 4A, C, E, G, I, K). The achenes of all species in section *Rhynchocystis* were trigonous in cross section, in most cases constricted proximally into a substipitate base, and epapillose. *Carex agastachys* and *C. microcarpa* had obovate achenes, with the widest point near the top, whereas *C. pendula* had elliptical achenes, with the maximum width at the middle or slightly above it. The samples of *C. bequaertii* and *C. mossi* had achenes ranging from obovate to elliptical. *Carex penduliformis* achenes were obovoid to ± ellipsoid. The micromorphology of the epidermic cells was similar in all the studied samples and no differences for any of the species were evident. The epidermis cells were polygonal and the anticlinal wall straight in all species (Fig. 4B, D, F, H, J, L). The silica platforms on the inner periclinal wall were flat or slightly concave for all species. For most samples only one large central silica body was observed within each cell. Conversely, a few sparse cells of *C. bequaertii* and *C. mossi* displayed two smaller bodies (Fig. 4H). No smaller satellite silica bodies or pits were found.

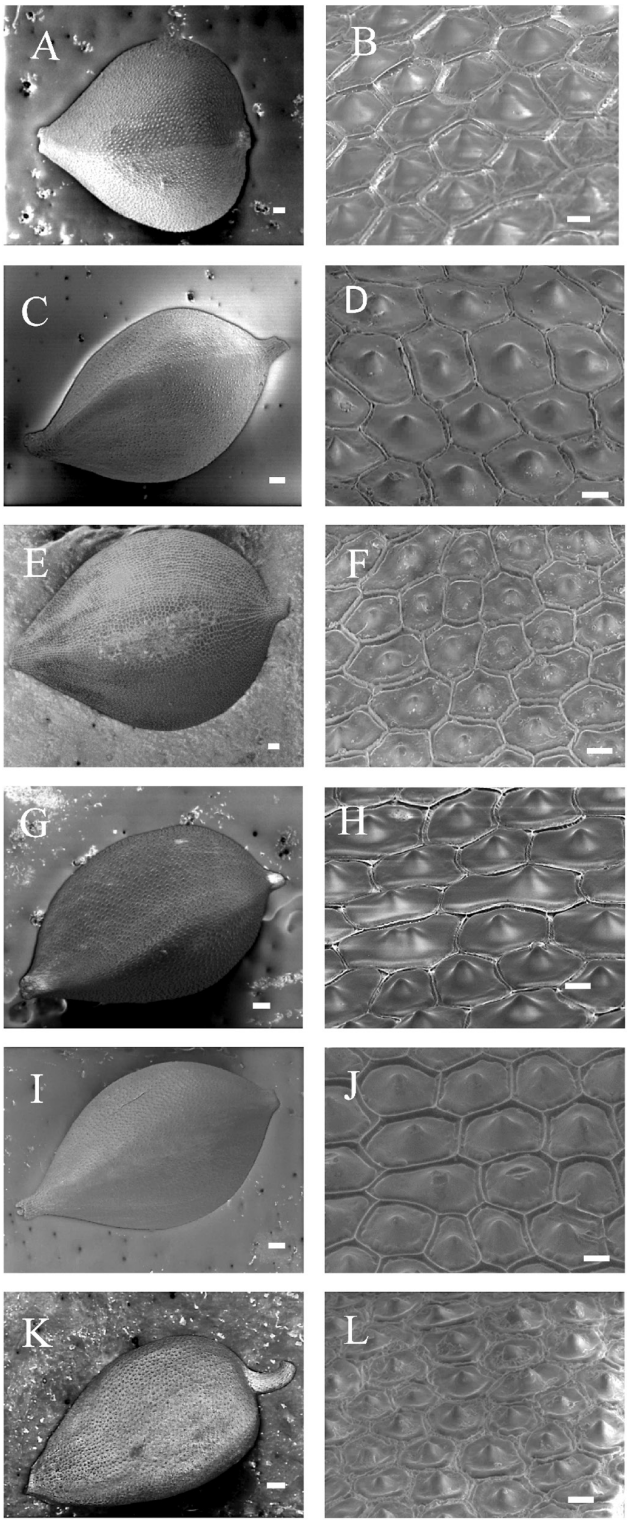

Figure 4. Scanning electron micrographs of the entire achene (A, C, E, G, I, K; scale bar = 100 μm) and detail of the achene surface (B, D, F, H, J, L; scale bar = 10 μm) in *Carex* section *Rhynchocystis*. *Carex agastachys* (A, B); *C. bequaertii* (C, D); *C. microcarpa* (E, F); *C. mossii* (G, H); *C. pendula* (I, J); *C. penduliformis* (K, L).

DISCUSSION

Reconciling phylogeny and taxonomy using standardized multivariate analyses

Phylogenetic studies of *Carex* have revealed multiple incongruences between previous morphology-based classifications and the DNA-based phylogenetic trees (Roalson, Columbus & Friar, 2001; Escudero & Luceño, 2009; Dragon & Barrington, 2009; Gebauer *et al.*, 2015; Global *Carex* Group, 2015, 2016). The main reason for these incongruences is that the more obvious morphological characters, traditionally used to diagnose groups and species in *Carex*, are frequently homoplastic (Hipp *et al.*, 2006; Molina, Chung & Hipp, 2015; Global *Carex* Group, 2016; Jiménez-Mejías & Noltie, 2017). Examination of morphological variation in *Carex* section *Rhynchocystis* using our multivariate analyses revealed that some characters traditionally reported as diagnostic in the species of the section (e.g. utricle size or pistillate glume length: Nelmes, 1940; Gehrke, 2011) showed wide overlap (Figs S2, S3). Conversely, other characters including achene shape or ligule colour are useful diagnostic characters that have not previously been considered. Our study demonstrates how traditional treatments might give excess weight to certain characters, whereas diagnostic characters that reflect evolution remain unnoticed. It highlights the importance of integrated molecular/morphological approaches and of methodological standardization in taxonomy, especially when complex evolutionary processes, such as hybridization, recent divergence, morphological stasis, reduced morphology or a combination of these, is involved in the diversification of the group.

Taxonomic consequences: overlooked species and overvalued characters

Five morphologically well-defined species should be recognized in *Carex* section *Rhynchocystis*: *C. agastachys*, *C. bequaertii* (including *C. mossii*), *C. microcarpa*, *C. pendula* and *C. penduliformis*. Most of these were shown to be monophyletic in a previous molecular phylogenetic reconstruction (with the exception of *C. penduliformis*, from which only one sample could be included; Míguez *et al.*, 2017). Our multivariate analyses reveal the morphological background behind the phylogenetic units. Only two species (*C. microcarpa* and *C. penduliformis*) were supported in their traditional circumscriptions.

The high degree of morphological differentiation between the eastern and western lineages of what was traditionally considered *C. pendula* and the ancient split between them (*c.* 10 Mya, Miocene; Míguez *et al.*, 2017) led us to reinstate the name *C. agastachys* for the eastern lineage. The type of this name (Jiménez-Mejías

et al., 2017) corresponds well to the studied individuals belonging to this lineage. *Carex agastachys* and *C. pendula* (Fig. 3B; Figs S2, S3, Table S2) differ in the shape of the achenes, the prickles on the spike peduncles and the colour of the ligules, but have similar overall morphology; thus, the relatively small differences remained unnoticed. In addition, their parapatric distributions that overlap in a still unclear area (Fig. 1) probably prevented their recognition in treatments, which are mostly national or regional. We did not identify any hybrids in our study, but their overlapping distribution might allow occasional hybridization (but see the taxonomic treatment), which should be more carefully explored in the future.

In contrast, a distinct allopatric distribution led to the past treatment of *C. bequaertii* and *C. mossii* as different species. No proper comparison between the two taxa has ever been performed beyond the key in Gehrke's (2011) monographic work on subSaharan Africa. Nelmes (1940) described the species *C. mossii* as 'scarcely specifically distinct from *C. bequaertii*' and emphasized their distinctiveness mainly relying on their different distributions, *C. bequaertii* in East Tropical Africa and *C. mossii* in South Africa (Gordon-Gray, 1995; Gehrke, 2011). The two taxa are indeed distributed in two disjunct patches unconnected due to the absence of suitable montane habitats in between. This taxonomic decision might look reasonable because such disjunction might limit gene flow via geographical isolation, frequently causing allopatric speciation (Gavrilets, 2003). However, *C. bequaertii* and *C. mossii* were not clearly differentiated in our phylogenetic study (Míguez *et al.*, 2017) and only subtly in our morphological evaluation (Fig. 3D; Figs S1, S2, Table S2). It appears that the divergence between the two sets of populations is relatively recent and thus morphological differentiation is still incipient. However, the slight differences between the two taxa are somewhat emphasized by their geographical separation (morphogeographical compartmentalization; Stuessy, 1990). According to all this evidence, we propose to consider the two taxa as subspecies *C. bequaertii* subsp. *bequaertii* and *C. bequaertii* subsp. *mossii* comb. nov.

As noted above, we found congruence between the phylogenetic and morphological patterns for *C. microcarpa* and *C. penduliformis*. Gehrke (2011) indicated morphological resemblance between *C. penduliformis*, *C. bequaertii* and *C. mossii*, but she referred mostly to the morphological homogeneity in *Carex* section *Rhynchocystis* rather than to a true lack of differentiation. Despite being able to study only a small number of vouchers of *C. penduliformis*, two facts support it as an independent species. First, the few samples of *C. penduliformis* were found to be reasonably well differentiated morphologically from the similar *C. agastachys* (and the rest of the species) (Figs 3C, S1, 2). Up to ten characters were identified to show little or no overlap between *C. agastachys* and *C. penduliformis* (Table S2). Other *C. penduliformis* specimens examined but not included in the morphometric study fitted the reported diagnostic characters well. Second, our previous molecular phylogenetic study (Míguez *et al.*, 2017) revealed that *C. penduliformis* is genetically more closely related to the other African species than to *C. agastachys*.

In summary, our study demonstrates that taxonomy is an essential discipline for the study of biodiversity. Even in relatively well-studied areas like Europe or within groups traditionally free from taxonomic controversy, such as *Carex* section *Rhynchocystis*, revisionary work is still required. Indeed, this work can be added to a growing list of recent taxonomic rearrangements in the genus in Europe (*Carex* section *Phaestoglochin* Dumort.: Molina, Acedo & Llamas, 2008a, b; *Carex* section *Ceratocystis* Dumort.: Jiménez-Mejías *et al.*, 2014; *C. furva* Webb *s.l.*: Maguilla & Escudero, 2016; *C. sylvatica* Huds. *s.l.*: Benítez-Benítez *et al.*, 2017). The changes in species delimitations derived from studies like ours might have important consequences in other disciplines, such as nature conservation and studies of invasive species.

Taxonomic treatment

The taxonomic treatment is based on our data and previous treatments, including data relating to distribution, ecology and phenology (Kükenthal, 1909; Chermezon, 1923; De Wildeman, 1927; Nelmes, 1940; Maire, 1976; Haines & Lye, 1983; Hooper, 1985; Nilsson, 1985; Gordon-Gray, 1995; Kukkonen, 1998; Egorova, 1999; Reznicek, 2002; Jermy *et al.*, 2007; Luceño, Escudero & Jiménez-Mejías, 2008; Gehrke, 2011), which were critically re-evaluated. According to the terminology proposed by Jiménez-Mejías *et al.* (2016a) we refer to the prophyll enclosing the flower as an utricle and to the prophyll embracing the base of the spike as a cladoprophyll. Distributions by botanical countries are summarized according to TDWG codes (Brummitt, 2001). Measurements are provided as the ranges of the minimum and maximum values found in the specimens, with outliers given in parentheses (below and above the 5th and 95th percentiles, respectively).

Carex* section *Rhynchocystis Dumort., Fl. Belg. 147. 1827.

Type: *Carex maxima* Scop. (=*C. pendula* Huds.), the section being described as monotypic.

Description: Perennial, densely caespitose, with short strong rhizomes, usually forming large ± conspicuous tussocks. Stems 40–230(300) cm long and 2–6 mm in diameter, sharply trigonous, smooth or scabrid

distally, sometimes reddish-purple at base. Leaf blades shorter than stem, (8)10–20 mm wide, dark green or yellowish green above, somewhat glaucous beneath; linear, smooth or scabrid on margins or towards the tip, flat to M-shaped in cross section, usually with two adaxial lateral veins more prominent that midvein, abaxially only the midvein prominent; ligule present, membranous, reddish or hyaline, becoming darker when dry, apex acute, subacute or emarginate; sheath fronts reddish, greenish or hyaline, veined; basal sheaths weak, entire and scale-like but promptly decomposing into fibres. Inflorescences racemose, with five to eight spikes, only one per node, the uppermost one (two) erect or slightly arching, entirely male or also with female flowers at top, base or at the middle, the two to eight lateral ones usually long, from arching to pendulous, entirely female or shortly androgynous; proximal bracts leaf-like, long-sheathing. Males spikes, when present, terminal, sessile, erect, long and narrowly cylindrical, reaching 35–180 × 4–7 mm. Lateral spikes 60–160(260) mm, terete or narrowly fusiform, often flexuous, densely flowered, usually with up to 100 utricles or more, the utricles spreading or slightly ascending, proximal-most spikes usually long-pedunculate, distal-most ones with the peduncle progressively shorter, all with a tubular cladoprophyll at the base. Staminate glumes 3.0–7.0 × 0.2–1.5 mm, linear lanceolate or spatulate, brown with green midrib, with or without hyaline margins, apex mucronate or aristate, sometimes sparsely and irregularly erosed. Pistillate glumes 1.9–5.6 × 0.5–2.3 mm, oblong, narrowly obovate or narrowly ovate, mucronate or awned, reddish-brown, sometimes straw-coloured when dry, with a greenish or hialine midrib. Stigmas three. Utricles 2.0–4.0 × 0.8–1.7 mm, elliptical or ovate, obtusely and usually asymmetrically trigonous, smooth, glabrous, with two strongly marked marginal veins, veinless or weakly veined on faces, cuneate and sessile at base, the apex ± abruptly contracted into a beak up to 0.5 mm, ± cylindrical, smooth, truncate or shortly bidentate, the teeth < 0.1 mm. Achenes elliptical, obovate or ovate, trigonous, much smaller than the utricles, ± cuneate at base and often contracted into a substipitate base, rounded at apex, with the style base persistent, shortly cylindrical.

Etymology: From the Greek *rhynchos*, beak, and *kystis*, bladder, probably in reference to the short-beaked utricles.

Distribution: South-western Palaearctic including north-western Africa and Macaronesia (Azores and Madeira), mountains of East Tropical Africa and eastern South Africa, Madagascar (Fig. 1).

Fossil record: The section is one of the oldest *Carex* sections traceable in the fossil record. Fossil samples ascribed to section *Rhynchocystis* have been identified as two extinct taxa, *C. limosioides* Negru and *C. plicata* Lańc.-Środ. These remains are known from Central and Eastern Europe, and span from the early Miocene to the late Pliocene, with doubtful presence in the late Oligocene (Jiménez-Mejías *et al.*, 2016b). These fossils support the long-lasting presence of the section in Europe since the early Neogene. The scarce differentiation between the achenes assigned to each of the two fossil names needs further research and may point to one or several chronospecies in a sequential development pattern.

Observations: The fact that *C. agastachys* and *C. pendula* have been treated as conspecific to date poses a problem with certain data attributed to *C. pendula*. *Carex agastachys* and *C. pendula* seem to co-occur through a yet to be defined overlapping area through north-western, central and south-eastern Europe (Fig. 1), east to Hungary (A. Mesterházy, pers. comm.), and south to the Balkan Peninsula. *Carex agastachys* is distributed to the east of this contact area, whereas *C. pendula* is found mostly to the west and south of it. Certain specimens in the putative contact area seem to be intermediate in the characters (S. Gebauer, pers. comm.), which might point to a certain degree of introgression between the two taxa. In Turkey, the two species seem to be allopatric, and we confirmed that the populations of the northern half are *C. agastachys*, and those in Mediterranean Turkey are *C. pendula*. According to this parapatric distribution, we consider that reports from localities west and south or north and east of the overlapping area belong respectively to *C. pendula* and to *C. agastachys*, although further confirmation would be desirable. The chromosome report of $2n = 62$ for *C. pendula* (Druškovič, 1982) could not be assigned to *C. agastachys* or *C. pendula* because it is from the former Yugoslavia, where both species co-occur, and no herbarium voucher is known.

In the same sense, the reports of *C. pendula* as an introduced species must be taken with caution. *Carex pendula* in its broad sense has been reportedly introduced into North America [CAL ORE VRG WAS] and New Zealand [NZS] (Reznicek, 2002; Govaerts *et al.*, 2017). Plants introduced to New Zealand are *C. pendula* (K. Ford, pers. comm). Our observations on plants from North America revealed that both *C. pendula* and *C. agastachys* are present in the USA. Critical examination would be needed to identify the introduced plants reported as *C. pendula* in other American localities. In addition, some of the studied cultivated materials display a somehow unstable combination of the characters of the two species, which might point to a hybrid origin of the plants sold in American nurseries, but additional studies are needed to confirm this.

KEY TO THE SPECIES OF *CAREX* SECTION *RHYNCHOCYSTIS*

1. Uppermost spike with male and female flowers, rarely entirely male; body of the pistillate glumes (excluding the mucro) conspicuously longer than the utricles ..... **2. *C. bequaertii***
   1.a. Pistillate glumes brown, usually with a wide middle central nerve lighter than the scale sides ... . **2a. *C. bequaertii* subsp. *bequaertii***
   1.b. Pistillate glumes pale brown, with a ± narrow middle nerve usually darker than the scale sides ..... **2b. *C. bequaertii* subsp. *mossii***

1′. Uppermost spike entirely male, rarely bearing female flowers intermingled with the male ones; body of the pistillate glumes (excluding the mucro) equal to or shorter than the utricles, rarely longer 2

2. All spikes erect or slightly spreading, subsessile, rarely with a peduncle up to 50 mm; leaves strongly coriaceous, 4–9 mm wide; stems 40–100 cm long; peduncle of the proximal female spike smooth .... **3. *C. microcarpa***

2′. At least the lowermost spike conspicuously pendulous when mature, with a peduncle (0)25–100(160) mm; leaves herbaceous, not coriaceous, (6)8–19 mm wide; stems usually more than (50)100 cm long; peduncle of the proximal female spike smooth to scabrid 3

3. Uppermost two to five lateral spikes sessile or subsessile, closely arranged, separated by short internodes 5–7 mm long; mature utricles and achenes dark-brown to blackish .....**5. *C. penduliformis***

3′. Uppermost two lateral spikes usually pedunculate, rarely sessile or subsessile, separated by conspicuous internodes (2)20–100 mm long, more proximal ones with internodes even longer; mature utricles and achenes greenish, yellowish or light brown 4

4. Achenes obovate, with the widest point near the top; ligule of the lower and middle leaves conspicuously reddish-purple; peduncle of the lowermost spike and internode between the two uppermost female spikes conspicuously scabrid, rarely sparsely scabrid ..... **1. *C. agastachys***

4′. Achenes elliptical, with the widest point at the middle or slightly above it; all ligules whitish, becoming brownish when dry, rarely reddish tinged in the lower leaves; peduncle of the lowermost spike and internode between the two uppermost female spikes smooth to sparsely scabrid, rarely conspicuously scabrid.....**4. *C. pendula***

1. *Carex agastachys* L.f., Suppl. Pl. 414 (1782). [Figs 4A, B, 5]

Ind. Loc.: 'Habitat in Germania' [Germany].

Type: 19 Agastachys. *Carex agastachys* L., Hannoverae, *F. Ehrhart* [Phytophylacium ehrhartianum]; *lecto-:* LINN-HS1441-174-1! (designated by Jiménez-Mejías *et al.*, 2017); *iso-:* GOET-002819!, LE-00010157!, M!.

=*Carex mutabilis* Willd., Fl. Berol. Prodr. 37 (1787).

Ind. Loc.: 'Spandau hinter den Schülerbergen' [Germany].

Type: lecto-: Willdenow, 1787, Fl. Berol. Prodr., Tab. II, Fig. 6 (designated by Jiménez-Mejías *et al.*, 2017); *epi-:* B-W-17248! (designated by Jiménez-Mejías *et al.*, 2017).

Description: Stems 50–90 cm × 2–7 mm, densely scabrid distally. Leaf blades 9–16 mm wide; ligule 10–27(28) mm, acute, conspicuously reddish-purple at least in the middle and lower leaves, often also in the upper leaves and bracts, rarely whitish in the latter; basal sheaths faint and absent, the stem bases covered by old-leaf remnants, dark ferrugineous-red. Inflorescence with 1(2) male spikes at the apex, and (three) four to six lateral female spikes, exceptionally shortly androgynous, the two uppermost lateral spikes separated by an internode (2)20–100 mm, lower ones often more distant; lowermost bract leaf-like, usually larger than the inflorescence. Male spikes 23–110(140) × 3–8 mm, fusiform or cylindrical, erect, spreading or pendulous, sessile or with a peduncle up to 55 mm. Lateral spikes 62–170 × 3–6 mm, long cylindrical, flexuose, spreading or at least the lowermost one pendulous, usually with peduncles 15–85(100) mm, rarely sessile or subsessile; peduncle of the lowermost spike and internode between the two uppermost female spikes conspicuously scabrid, rarely sparsely scabrid. Staminate glumes (3.7)4.3–6.5 × 0.4–1.1 mm, linear-spatulate, apex ciliate, dark reddish brown. Pistillate glumes (1.9)2.0–2.6 (2.7) × (0.5)0.6–1.2(1.3) mm, oblong or narrowly elliptical, mucronate, usually shorter than utricle, reddish brown with a light green to white midrib. Utricles 2.0–3.0 × 0.5–1.3 mm, narrowly ellipsoid, yellowish green to brownish green when mature, sometimes with sparse small elongated purplish spots, beak conspicuously bidentate or

Figure 5. Analytical illustration of *Carex agastachys* L.f. (Serbia: S Carpathians, Djerdap National Park, 21 June 2010. *P. Jiménez-Mejías 86PJM10*. UPOS-4208). A, culm base; B, leaf apex; C, ligule; D, inflorescence; E, proximal spike peduncle; F, male spike; G, staminate glume; H, female spike; I, pistillate glume; J, utricle; K, achene. Drawing by F. Míguez.

truncate. Achenes 0.7–1.5 × 0.3–1.2 mm, markedly obovate, brownish.

Distribution: Central and Eastern Europe, west to Belgium, the Netherlands, Germany and Austria and possibly NE France, south to the Balkan Peninsula and northern Greece, in south-western Asia in northern Anatolia, Caucasus and the Alborz range in northern Iran; there are additional problematic records from Afghanistan. Introduced in North America at least in Oregon, Virginia and Washington State (Fig. 1) [AFG? AUT BGM BUL CZE_CZ CZE_SL FRA? GEO GER GRC HUN IRN KRY NCS NET ore POL ROM TCS_AR TCS_AZ TUR UKR vir was YUG_SE YUG_SL]; see also comments under the section *Rhynchocystis* heading at the beginning of the Taxonomic Treatment.

Habitat: Moist woods and along streams; 60–1000 m altitude.

Phenology: March–August.

Etymology: From the Greek *aga*, large, and *stachys*, spike.

Chromosome number: $2n = 58$ (Hindakova, 1978).

Observations: The World Checklist of Cyperaceae (Govaerts *et al.*, 2017) points to the presence of '*C. pendula*' in Afghanistan. To be geographically consistent, these materials might be *C. agastachys* rather than *C. pendula*, However, we have not been able to trace any material or published record that support that statement. The easternmost populations that we have studied are from mountains on the eastern shores of the Caspian Sea in Iran, which are *c.* 630 km from the Afghanistan border. This citation might also represent a point introduction (perhaps of *C. pendula*) or be the result of a misidentification. Therefore, the presence of species of section *Rhynchocystis* in Afghanistan should be considered doubtful and in need of revision.

A specimen from Turkey (E-00305402!) is recorded in Nilsson (1985) as problematic: 'female spikes with several small branches at the base, arising through sterile utricles'. After study of this material, we confirm that the ligule and female spike peduncle characters of these plants match *C. agastachys*, but the undeveloped achenes seem to be elliptical, as in *C. pendula*. We consider these plants probably to be malformed specimens of *C. agastachys*.

We have confirmed that the reports of introduced *C. pendula* in Washington State (USA) are actually *C. agastachys.* The apparently weedy populations reported as *C. pendula* in Belgium and the Netherlands seems to belong to *C. agastachys*, and the presence of the species at least in the north-eastern border of France could be possible (F. Verloove, pers. comm.).

2. *Carex bequaertii* De Wild., Pl. Bequaert. 4: 246 (1927). [Figs 4C, D, G, H, 6]

Ind. Loc.: 'Ruwenzori mountains, vallée du Lanuri' [Democratic Republic of Congo]

Type: DR Congo, Ruwenzori Mts, Lanuri Valley, 3000 m, *J.C.C. Bequaert* 4677; *lecto-*: BR-863827! (here designated); *iso-*: BR-863828!, BR-863829!, K-000363485!.

Description: Stems 60–200 cm × 2–4 mm, smooth. Leaf blades 7–21 mm wide; ligule 5–25 mm, hyaline, subacute or emarginate; basal sheaths subcoriaceous, keeled, dark brown. Inflorescence with six to nine pendulous spikes, the uppermost spike mostly with female flowers, with male flowers scattered and diversely mixed between the female ones, rarely entirely male, the rest of the spikes entirely female or rarely androgynous; lowermost bract leaf-like, equal to or slightly shorter than the inflorescence. Spikes 60–220 × 5–10 mm, cylindrical, pendulous when mature, with peduncles up to 16 cm long, smooth. Staminate glumes (3.0)4.0–6.0 × 0.5–1.2(1.8) mm, lanceolate, apex acute, frequently, shortly awned, brown with a pale midrib. Pistillate glumes 2.6–5.4 × 0.9–1.5 mm, deltoid-lanceolate, oblong-lanceolate or lanceolate-elliptical, acute, subulate, mucronate or awned, longer and narrower than the utricles, brownish with a distinct midrib. Utricles 2.2–3.4 × 0.9–1.5 mm, ovate or elliptical, green yellowish green or blackish, with a short bidentate beak. Achenes (1.0)1.4–1.9(2.1) × (0.3)0.7–1.1 mm, from obovate to elliptical, yellowish brown to brown, sometimes with dark spots.

Distribution: Mountains of eastern tropical and south-eastern Africa (Fig. 1).

Habitat: Swamps and wet and moist soils, often along streams and lake shores, also in shady forests; 500–4000 m.

Phenology: January–December.

Etymology: Dedicated to J.C.C. Bequaert (1886–1982), an American naturalist of Belgian origin, who collected the type material.

Observations: The name *C. petitiana* A.Rich has been sometimes used to refer to *C. bequaertii* (e.g. Kükenthal, 1909), but it is now applied to a different species in a different section (Escudero & Luceño, 2011). The confusion was already noted by Gehrke (2011). Although Lye (1997) and Gehrke (2011) mentioned the location of type material of *C. bequaertii* at BR, they did not select a lectotype from the duplicates of the original material; thus, a formal lectotypification was still needed.

2a. *Carex bequaertii* subsp. *bequaertii* [Figs 4C, D, 6A–F, H, I]

=*Carex bequaertii* var. *maxima* Lye in Nordic J. Bot. 3 (2): 244 (1983).

Ind. Loc.: 'Uganda, Karamoja district, Mt. Morungole' [Uganda]

Figure 6. Analytical illustration of *Carex bequaertii* De Wild. subsp. *bequaertii* [(A–F, H–I) Kenya: Aberdares National Park. 26 July 2007. *M.L. Buide et al. 82UPO-K*, UPOS-3288] and *C. bequaertii* subsp. *mossii* (Nelmes) Míguez, Martín-Bravo

Type: Uganda, Karamoja district, Mt Morungole, 2440 m, iv.1960, *J. Wilson* 1012; *holo-:* EA; *iso-*: K-000363589!.

Carex robusta Hochst. ex Boeckeler in Linnaea 40: 412 (1876), *pro syn*.

Carex robusta Hochst. in Pl. Schimp Abyss. exs., Sect. 1, n. 100 (1850), *nom. nud.*

Description: Stems 2.5–3.0 mm wide. Ligule subacute, sometimes emarginate. Pistillate glumes deltoid-lanceolate, acute, the proximal-most ones subulate, brown, with a distinct paler midrib. Achenes 1.0–2.1 × (0.3)0.7–1.0 mm.

Distribution: Mountains of eastern tropical Africa, north to Simien mountains, west to the Rwenzori mountains and south to Kilimanjaro (Fig. 1) [CON ETH KEN TAN RWA TAN UGA].

Habitat: Swamps and wet and moist soils, along streams and lake shores; 2200–3700 m altitude.

Phenology: March–November.

Chromosome number: 2*n* = 58 (Hedberg & Hedberg, 1977).

Observations: The name *C. robusta* Hochst. was never validly published. The name was first recorded on a sample collected by Schimper and later reproduced as a synonym in the above-mentioned publication of Boeckeler. A voucher of the original Schimper collection has been located in P herbarium: 'Flora Abyssinica 100 *Carex robusta* Hoschst., in montibus Debra Eski, 11000, *Schimper*, 27 Octobre 1850' (P-01825501!).

2b. *Carex bequaertii* subsp. *mossii* (Nelmes) Míguez, Martín-Bravo & Jim.-Mejías, comb. nov. [Figs 4G, H, 6G]

≡*Carex mossii* Nelmes in Bull. Misc. Inform. Kew 1940: 137 (1940) [basionym]

Ind. Loc.: South Africa, Eastern Cape Province, Hogsback [Republic of South Africa]

Type: South Africa, Eastern Cape Province, Hogsback, 01.i.1927, *C.E. Moss* 999; *holo-:* K-000363605!; *iso-*: BR; *para-*: Hogsback, C.G. Hope (E), 01.i.1927, *E.M. Young* 15336, K-000363649!, BM-000624846!.

Description: Stems 2.5–4.0 mm wide. Ligule emarginate. Pistillate glumes oblong-lanceolate or lanceolate-elliptical, pale brown, margin narrowly hyaline, with the midrib usually darker than the sides, apex acute mucronate. Achenes 1.4–1.8 × (0.5)0.8–1.1 mm.

Distribution: Drakensberg mountains in south-eastern Africa (Fig. 1) [CPP NAT TVL].

Habitat: Wet and moist soils, along streams or in shady forests; 500–2000 m altitude.

Phenology: October–December(February)

Etymology: Dedicated to C.E. Moss (1870–1930), British botanist who collected the type material.

Observations: The type material of *C. mossii* is problematic. Nelmes (1940) designated a holotype ['Hogsback, 1 January 1927, *C. E. Moss* 999 (Kew, type)'] and a paratype ('*Miss E. M. Young* 15, 336'). The herbarium sheet on which the holotype of *C. mossii* is mounted bears two different barcodes, one referring to the specimen *Moss* 999 and the other apparently to Young's collection. The specimen consists of two flowering stems, without a clear correspondence of either to the barcodes. The specimen bears a single label, that indicates 'Leg. *C. E. Moss*, No 999 […] Date 1-1-27. Habitat and locality Hogsback, E. Province'. However, there is an envelope containing mature utricles with the following text: 'C. E. Moss 15336 Hogsback, 1 Jan 1927. Leg Miss E. M. Young', which is a partial match of the paratype. In our opinion, the two flowering stems most probably belong to the holotype, whereas the material in the envelope, as indicated, contain utricles of another specimen. Such practice seems usual among other British cyperologists, and we have also observed it in other specimens studied by F. M. B. Boott, C. B. Clarke or Nelmes himself. The partial match between the paratype citation and the text on the envelope is probably just the result of a mistranscription. We think that Moss is the actual collector of the specimen, and Young perhaps the donor of the specimen.

3. *Carex microcarpa* Bertol. ex Moris, Stirp. Sard. Elench. 1: 48 (1827). [Figs 4E, F, 7]

Ind. Loc.:'in montibus juxta rivulos' [Sardinia, Italy, not explicit].

Type: Juxta rivulos in montibus Sardinia, Aprili, Majo; *lecto-*: FI-002960! (designated by Arrigoni, 1984; second-step lectotypification performed here: specimen on the right).

=*Carex corsica* Degl. ex Loisel. in J.L.A. Loiseleur-Deslongchamps, Fl. Gall., ed. 2, 2: 307 (1828).

Ind. Loc.: 'In Corsica; juxta Bonifacio' [Corsica, France]

& Jim.-Mejías [(G) South Africa: KwaZulu-Natal, Ntabamhlophe. 10 November 2012, *E. Maguilla et al. 50EMS12*(BIS5), UPOS-5195]. A, culm base; B, ligule; C, inflorescence; D, lateral spike; E, staminate glume; F, pistillate glume of subsp. *bequaertii*; G, pistillate glume of subsp. *mossii*; H, utricle; I, achene. Drawing by F. Míguez.

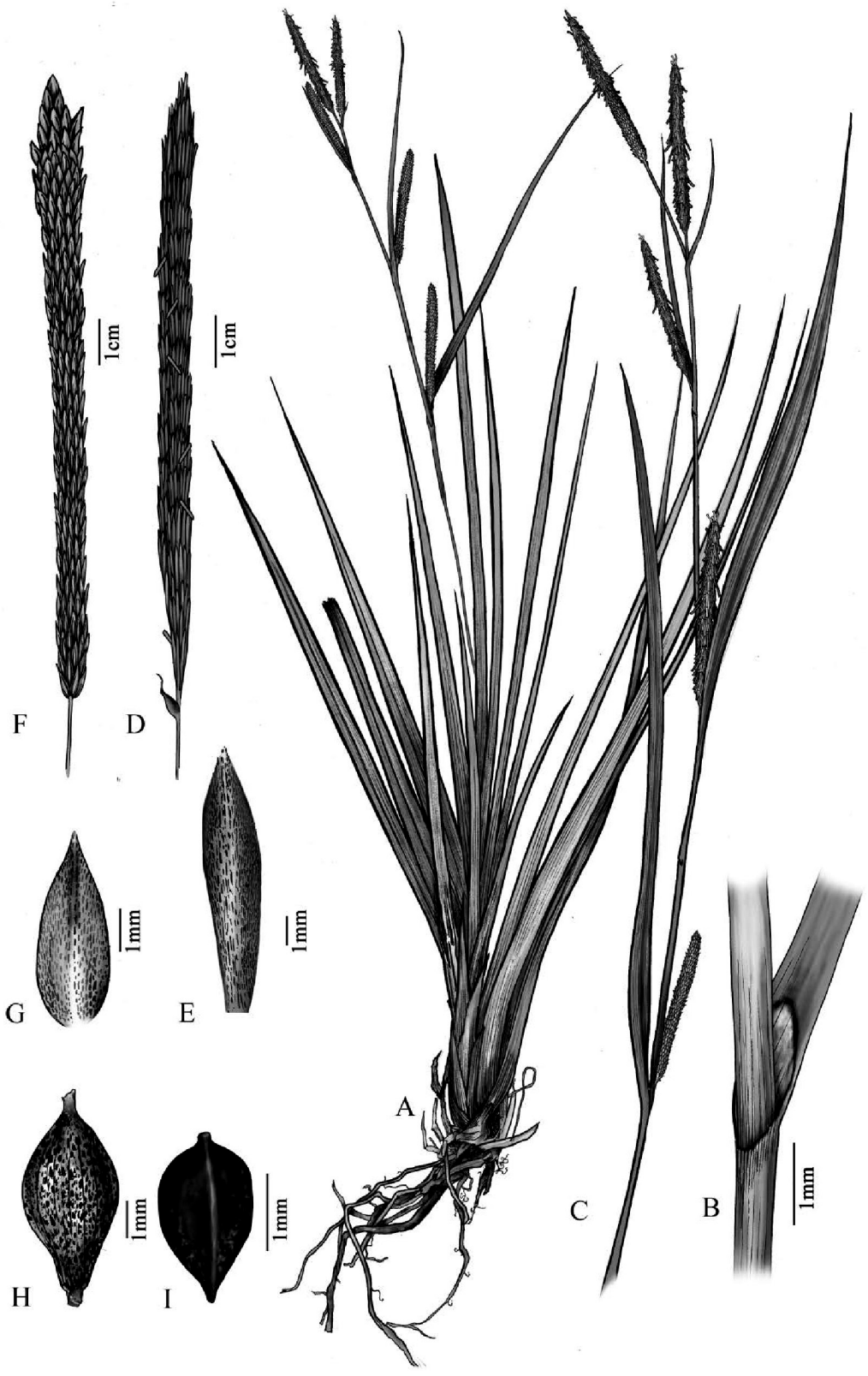

Figure 7. Analytical illustration of *Carex microcarpa* Moris [(A, B, D–I) France: Corsica, Ghiosimi. 4 May 2007, *M. Escudero 88ME07*, UPOS-4720; (C) France: Corsica, Ghiosimi. 5 May 2007. *M. Escudero 104ME07*, UPOS 4723]. A, habit; B, ligule; C, inflorescence; D, male spike; E, staminate glume; F, female spike; G, pistillate glume; H, utricle; I, achene. Drawing by F. Míguez.

Type: Not found, probably at AV (cf. Stafleu & Cowan, 1976–1997).

Description: Stem 40–100 cm × 2–3 mm, smooth or scabrid. Leaf blades 4–9 mm wide, strongly coriaceous; ligule 4–30 mm, hyaline, from acute to obtuse; basal sheaths inconspicuous, the stem bases covered by old-leaf remnants. Inflorescence with one (three) male spike(s) at the apex, and four (five) lateral female spikes, sometimes one or two of which are shortly androgynous, the two uppermost lateral spikes separated by an internode 17–60 mm; lowermost bract leaf-like, shorter or equalin length to the inflorescence. Male spikes 58–105 × 3–5 mm, fusiform, erect, with a peduncle 10–30 mm. Lateral spikes 75–110 × (3) 4–7 mm, female or shortly androgynous, cylindrical, erect or the lowermost one slightly spreading, sessile or subsessile, rarely the lowermost one with a peduncle up to 50 mm. Staminate glumes 4.8–6.9 × 0.7–1.2 mm, lanceolate, acute or acuminate, brown with a hyaline midrib. Pistillate glumes 2.7–3.7 × 0.9–2.0 mm, ovate-lanceolate, acute, equal to or slightly longer than the utricles, reddish-brown, with a greenish midrib. Utricles 2.0–3.3 × 0.8–1.4 mm, elliptical, pale green and purplish-brown puncticulate, beak bifid. Achenes (1.4)1.5–1.7(1.9) × (0.7)0.9–1.1(1.3) mm, obovate, dark-brown.

Distribution: Corsica, Sardinia, Tuscan Archipelago, and reported from the central Italian Peninsula, but needed from confirmation (Fig. 1) [COR ITA SAR].

Habitat: Marshes and wet meadows; 0–1800 m altitude.

Phenology: March–April (August).

Etymology: From the Greek *mikros*, small, and *karpos*, fruit.

Chromosome number: $2n = 60$ (Contandriopoulos, 1962).

Observations: The lectotype designated by Arrigoni (1984) bears two stems. The left one is *C. pendula*. The one at the right is *C. microcarpa*, which we here designate as the lectotype in a second-step lectotypification.

4. *Carex pendula* Huds., Fl. Angl.: 352 (1762). [Figs 4I-J, 8]

Ind. Loc.:'Habitat in sylvis et sepibus humidis; in sepibus inter Hampstead et Highgate copiose' [England, UK].

Type: neo-: Morison, 1699, Pl. Hist. Univ. Oxon. 3m sect. 8, tab. 12, fig. 4 (designated by Egorova, 1999); *epi-*: London, Hampstead Heath, between Hampstead and Highgate, Ken Wood lake –vc 21, Middlesex, *M.A. Spencer* MAS-2012-040, BM-001074530! (designated by Jiménez-Mejías *et al.*, 2017); *iso-*: UPOS-5004!.

= *Carex maxima* Scop., Fl. Carniol., ed. 2, 2: 229 (1772)

Ind. Loc.: Not explicit [Carniola, Slovenia].

Type: G. A. Scopoli, s.n.; *lecto-:* LINN-HL110-94! (designated by Jiménez-Mejías *et al.*, 2017).

= *Carex myosuroides* Lowe, Trans. Cambridge Philos. Soc. 4(1): 10 (1833), nom. illeg., non *Carex myosuroides* Vill., Prosp. Hist. Pl. Dauphiné: 17 (1779).

Ind. Loc.: 'Hab. In Maderae ora septentrionali' [Madeira, Portugal].

Type: '653. *Carex myosuroides*, Madeira, from Rev. M. Lowe. 1837'. *Neo-*: K-000363419! (here designated).

= *Carex pendula var. myosuroides* Boott, Ill. Gen. Carex 4: 197 (1867)

Ind. Loc.: 'from Madeira' [Madeira, Portugal].

Type: '653. *Carex myosuroides*, Madeira, from Rev. M. Lowe. 1837'. *lecto-*: K-000363419! (here designated).

Description: Stems 50–180(240) cm × 2–6 mm, smooth or slightly scabrid distally. Leaf blades (6)8–19 mm; ligule (12)20–37(65) mm long, whitish, becoming brownish when dry, rarely slightly reddish-tinged in the lowermost leaves, acute to subacute; basal sheaths faint, scale-like, dark brown or reddish, often the stem bases covered by old-leaf remnants, pale brown. Inflorescence with one (two) male spike(s) at the apex, and (four) six to eight lateral female spikes, exceptionally shortly androgynous, the two uppermost lateral spikes separate by an internode 14–63(80) mm; lowermost bract leaf-like, equaling or slightly shorter than the inflorescence. Male spikes 60–160(180) × (2.5)4.0–9.0 mm, fusiform or cylindrical, erect, spreading, or pendulous, sessile or subsessile, sometimes with a peduncle up to 2.5 cm. Lateral spikes 85–190(260) × (3.0)6.5–8.0 mm, long-cylindrical, flexuose, spreading or pendulous, subsessile or with peduncles 20–100 mm; peduncle of the lowermost spike and internode between the two uppermost female spikes smooth to sparsely scabrid, rarely conspicuously scabrid. Staminate glumes 3.6–6.9(8.7) × 0.2–1.9 mm, linear, oblong or narrowly obovate, acute, reddish-brown with a hyaline midrib. Pistillate glumes (2.2)2.4–3.7(3.9) × 0.6–1.2 mm, narrowly ovate to narrowly obovate, mucronate, the body generally shorter than the utricles or shortly surpassing them, reddish-brown with a greenish midrib. Utricles (1.4)1.9–3.6 × 0.5–1.5 mm, ovoid or ellipsoid, greenish or yellowish green, beak 0.2–0.5 mm, truncate. Achenes (1.0)1.1–1.8(2.1) × (0.4)1.4–1.5 mm, elliptical, with maximum width at the middle or slightly above it, brownish to yellowish.

Distribution: Europe and the Mediterranean, including north-western Africa and the Mediterranean shores

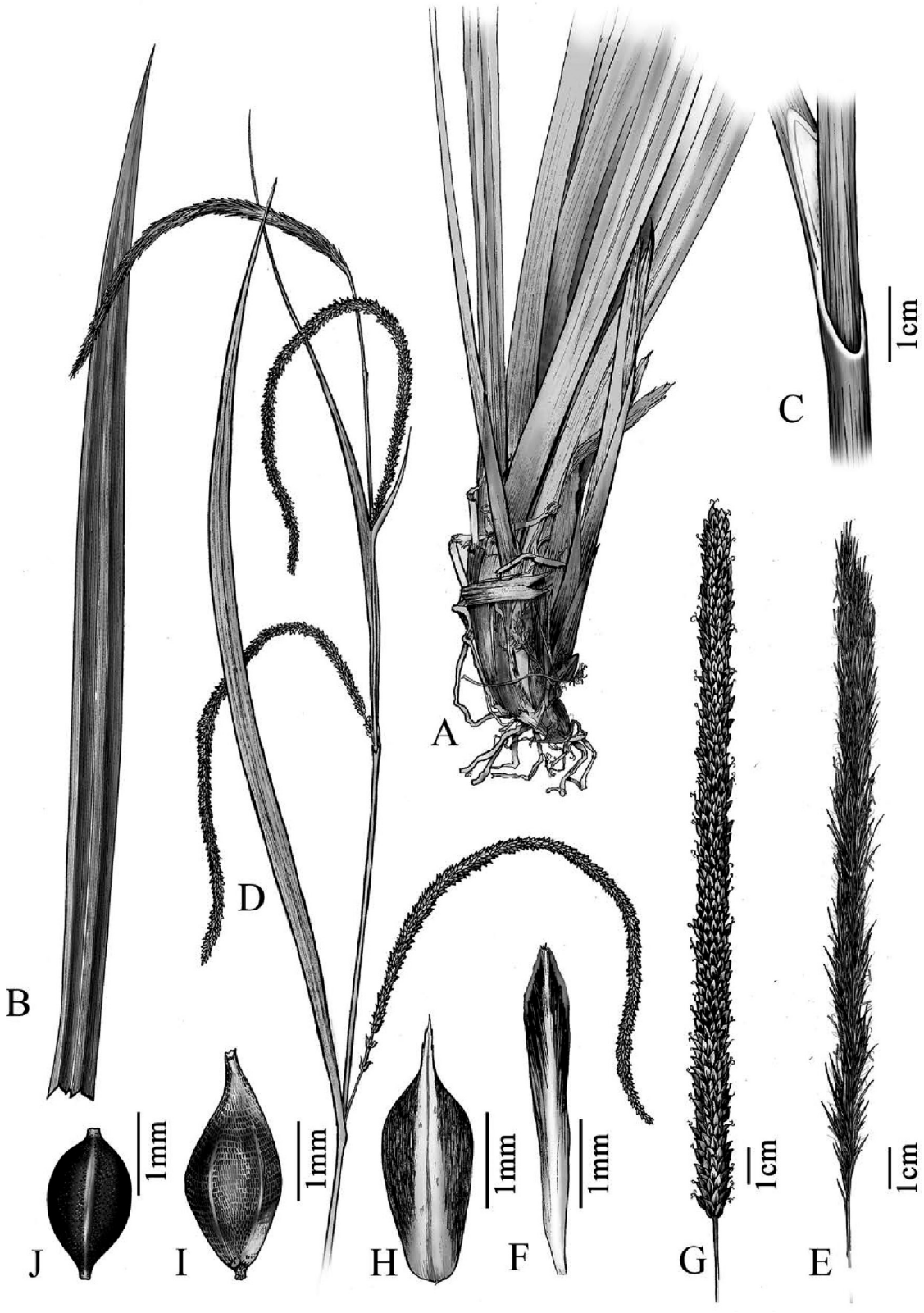

Figure 8. Analytical illustration of *Carex pendula* Huds [(A, B, D, I, J) Italy: Tuscany. 13 June 2010. *P. Jiménez-Mejías 24PJM10*, UPOS-4136; (F, H) France: Cévennes National Park. 25 June 2009. *P. Jiménez-Mejías 104PJM09*, UPOS-5878;

of south-western Asia, in Europe north to Denmark and east to central Germany and western Hungary, also in the Atlantic archipelagos of Azores and Madeira (Fig. 1); apparently introduced in southern Scandinavia, and confirmed also as introduced in the southern island of New Zealand and North America at least in California and Oregon (see comments under the section heading); a problematic record from Iraq [ALG AZO BGM cal COR CYP DEN EAI FRA GER GRB GRC BEL DEN HUN IRE IRQ? ITA KRI LBS MDR MOR NET? ore nzs PAL? POR SAR SIC SPA SWE SWI TUN TUR YUG_CR YUG_MN YUG_SL].

Habitat: Riparian forests, beside streams, usually on damp clayish soils; 25–1370 m altitude.

Phenology: (March) April–August (December).

Etymology: From the Latin *pendulus*, hanging, in reference to the pendulous lateral spikes.

Chromosome number: $2n = 58$ (Kjellqvist & Löve, 1963; Löve & Kjellqvist, 1973; Strid & Franzen, 1981), $2n = 60$ (Ottonello, Romano & Attiata, 1985).

Observations: We have not seen materials from the eastern Aegean Islands, Ireland and Palestine, but because of geographical consistency we consider that these materials very probably belong to *C. pendula* and not to *C. agastachys* (see comments at the beginning of the treatment).

We have examined the voucher cited in *Flora of Iraq* (Hooper, 1985) (see Appendix S1) and its identity points to *C. pendula* (ligule whitish, female spike peduncles smooth). However, our identification must not be taken as entirely conclusive, because the plant is an immature specimen. The Iraqi locality of *C. pendula* seems to be highly isolated, as the closest *Carex* section *Rhynchocystis* populations appear to be *c.* 350 km to the north-east in the Iranian Alborz Mountains near the Caspian Sea (*C. agastachys*), *c.* 500 km to the north-west in the Turkish Pontic Mountains between the Black Sea and the Caucasus (*C. agastachys*), and *c.* 700 km to the west in the Levant coast (*C. pendula*). We cannot rule out a one-off introduction of *C. pendula* in the area, although it would certainly be unexpected.

We have not studied specimens of *C. pendula* from the Netherlands. According to F. Verloove (pers. comm.), the species might have (or had) natural occurrences in the southernmost part of the country. Checking of additional materials would be desirable to confirm the current or former presence of the species in the Netherlands.

For comments on *C. pendula* introductions outside its native range, the reader is referred to the notes under the section heading.

The selected neotype for the illegitimate name *C. myosuroides* Lowe matches the author's taxonomic concept of this name, as the specimen seems to have been sent by Lowe to Boott in 1837 as '653. *C. myosuroides*'. Accordingly, this same material was seen by Boott prior to the coining of the name *C. pendula* var. *myosuroides*. When Boott (1867) created the name *C. pendula* var. *myosuroides*, he considered his variety to be a match of Lowe's *C. myosuroides*. Thus, we also consider the neotype of *C. myosuroides* Lowe to be also original material of *C. pendula* var. *myosuroides*, and accordingly we propose the lectotypification of this name on it.

5. *Carex penduliformis* Cherm., Bull. Soc. Bot. France 70: 414 (1923). [Figs 4K, L, 9]

Ind. Loc.: 'Forêt d'Andasibé […], Massif de l'Andringitra' [Madagascar]

Type: Madagascar Centre, forêt d'Andasibé (basin de l'Onive), novembre 1911, *H.P. de la Bâthie* 2535; *lecto-*, P! (designated by Gehrke, 2011; second-step lectotypification here performed: specimen barcode P-00346091!); *iso-:* P-00346092!, MO-6300685!; *syn-:* Madagascar, Centre, Andringitra Mts, 1922, *H.P. de la Bâthie* 14554, P-00346089!, P-00346090!.

Description: Stem 60–100 cm × 2–3 mm, smooth to scabrid. Leaf blades 9–12 mm wide; ligule 17–30 mm, reddish, subacute; basal sheaths scale-like, dark brown or ferruginous-red. Inflorescence 1(2) terminal spike(s) entirely male, rarely bearing male and female flowers intermingled, and six or seven lateral female or shortly androgynous spikes, the upper ones aggregated at the apex and separated by short internodes 5–7 mm; lowermost bract leaf-like, usually exceeding the length of the inflorescence. Male spikes 120–138 × 3.3–4.5 mm, fusiform, erect, spreading or pendulous, sessile or subsessile. Female and androgynous spikes 160–230 × 4.5–5.0 mm, long cylindrical, flexuose, spreading or at least the lowermost ones pendulous and with a peduncle 2–4 cm, scabrid, Staminate glumes 5.1–6.4 × 1.1–1.5 mm, ovate to lanceolate, aristate or mucronate, brown with a lighter midrib. Pistillate glumes 1.9–3.6 × 1.0–1.3 mm long, ovate, mucronate or aristate, the body shorter than the utricles, dark brown, with a lighter midrib and no hyaline margin. Utricles 2.0–2.4 × 0.8–0.9 mm, elliptical, dark-brown, sometimes red-punctate, beak truncate

(C, E, G) Italy: Piedmont, 8 June 2012. *P. Jiménez-Mejías & E. Martinetto 64PJM12*, UPOS-5348]. A, culm base; B, leaf apex; C, ligule; D, inflorescence; E, male spike; F, staminate glume; G, female spike; H, pistillate glume; I, utricle; J, achene. Drawing by F. Míguez.

Figure 9. Analytical illustration of *Carex penduliformis* Cherm. (Madagascar: Mahajanga, Bealanana, Mangindrano, Ambohimirahavavy, Bemafo, Campement 02 Bemafo. *S. Wohlhauser et al. 795*, P-01874870). A, Culm base; B, ligule; C, inflorescence; D, male spike; E, staminate glume; F, female spike; G, pistillate glume; H, utricle; I, achene. Drawing by F. Míguez.

or shallowly bidentate. Achenes 1.0–1.5 × 0.8–0.9 mm, obovoid to elliptical, dark brown.

Distribution: So far only known from eastern Madagascar (Fig. 1) [MDG].
Habitat: Stream edges on mafic rocks, such as gneisses and basalts; 1350–2700 m altitude.
Phenology: Plants in flower and fruit are only known from November.

Etymology: Carex pendula-like.

Observations: The doubtful reports of *Carex boryana* Schkuhr from Madagascar (see Escudero & Luceño, 2011) seem to correspond to *C. penduliformis*, as some of the studied specimens bear labels with the name '*Carex boryana*' as originally identified by de la Bâthie.

Gehrke (2011) designated a voucher with the collection number *H.P. de la Bâthie* 2535, deposited at P, as lectotype. However, two specimens in P bear this number and thus we perform a second-step lectotypification and here designate the voucher with the barcode P-0034091, which is a complete ripe specimen of *C. penduliformis*, as lectotype.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Appendix S1. Representative specimens of *Carex* section *Rhynchocystis* examined. The 108 numbered sheets are those used in the morphometric study. The rest of the specimens (202 different mounted herbarium sheets belonging to 166 different collections) indicated with an asterisk were examined carefully, although they were not included in the multivariate analyses.

Appendix S2. Main results of the discriminant function analysis (DFA) resulting from study of the entire *Carex* section *Rhynchocystis* dataset (DFA-I) and from the *C. bequaertii–C. mossii* dataset (DFA-II).

Table S1. Variables included in the analyses performed and scores obtained in the first plotted principal components of the PCA analyses of the different subsets of *Carex* section *Rhynchocystis*. Species (and number of samples included) abbreviated as follows: aga (*C. agastachys*; $N = 24$), bea (*C. bequaertii*; $N = 21$), mic (*C. microcarpa*; $N = 9$), mos (*C. mossii*; $N = 17$), pen (*C. pendula*; $N = 35$), pfo (*C. penduliformis*; $N = 3$).

Table S2. Characters that showed < 25% overlap in pairwise comparisons at species level marked by an asterisk (abbreviations specified in Table 1). Characters found to be significantly different by the Mann–Whitney *U* test are marked with a hash. Qualitative characters are marked with a plus sign.

Figure S1. Scatter plots considering the additional principal components extracted from the PCAs as described in Figure 1. All the species of *Carex* section *Rhynchocystis* (PCA-I): PC1 vs. PC3 (A); *C. agastachys* and *C. penduliformis* (PCA-III): PC-1 vs. PC-3 (B) and PC-1 vs. PC-4 (C); *C. microcarpa* and *C. pendula* (PCA-IV): PC-1 vs. PC-3 (D); *C. bequaertii* and *C. mossii* (PCA-V): PC-1 vs. PC-3 (E) and PC-1 vs. PC-4 (F). Symbols depict the different taxa considered: *C. agastachys* = circles, *C. bequaertii* = diamonds [*C. bequaertii* subsp. *bequaertii* according to our revised treatment], *C. microcarpa* = black squares, *C. mossii* = stars [*C. bequaertii* subsp. *mossii* according to our revised treatment], *C. pendula* = squares and *C. penduliformis* = pentagons.

Figure S2. Boxplots of the most discriminant characters retrieved by DFA or with < 25% overlap. The *x*-axis represents the considered species labelled as follows: AGA (*C. agastachys*), BEA (*C. bequaertii*), MIC (*C. microcarpa*), MOS (*C. mossii*), PEN (*C. pendula*), PFO (*C. penduliformis*). The boxes cover 50% of the data values ranging between the 25th and 75th percentiles, and the lines show 90% of the values between the 5th and 95th percentiles. The line within the box represents the median. Outlying values are indicated by small circles and extreme values are indicated by asterisks (*).

Figure S3. Histograms of the qualitative variables: distal ligule colour (0 whitish, 1 pale brownish, 2 reddish-purple); proximal ligule colour (0 whitish, 1 pale brownish, 2 reddish-purple); relative position on the lowermost spike (0 if the spike is pendulous, 1 if it is erect); sex of upper spike (0 if an upper completely male spike is absent, 1 if it is present) as scored for the morphometric study of the six considered species. Species are abbreviated as follows: AGA (*C. agastachys*), BEA (*C. bequaertii*), MIC (*C. microcarpa*), MOS (*C. mossii*), PEN (*C. pendula*), PFO (*C. penduliformis*). The *x*-axis represents the measurement and the *y*-axis the frequency.