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Towards an accurate taxonomic interpretation of *Carex* fossil fruits (Cyperaceae): a case study in section *Phacocystis* in the Western Palearctic.

Pedro Jiménez-Mejías[2,3] and Edoardo Martinetto[4]
[2]Botany Area, Department of Molecular Biology and Biochemical Engineering, Pablo de Olavide University, Seville.
[3]Department of Biodiversity and Conservarion, Real Jardín Botánico de Madrid.
[4]Department of Earth Sciences, Turin University.
[5]Corresponding author: pjimmej@upo.es

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Abstract
Premise of the study: Despite the growing interest in the systematics and evolution of the hyperdiverse genus *Carex*, few papers study its evolution using absolute time framework. It is partly due to the limited knowledge of the fossil record. However, *Carex* fruits are not rare in certain sediments. In this study, we analyse carpological features of modern materials from *Carex* sect. *Phacocystis* to characterize taxonomically the fossil record.
Methods: We studied 374 achenes from modern materials (18 extant species), as well as representatives from related groups, to establish the main traits within and among species. 99 achenes from sediments of living populations were also studied to assess their modification process after decay. Additionally, 145 fossil achenes from ten different locations (4-0.02 Myr) were characterized, and their taxonomic adscription discussed.
Key results: Five main characters were identified for establishing morphological groups of species (epidermis morphology, achene-utricle attachment, achene base, style robustness, and pericarp section). Eleven additional characters allowed the discrimination at species level of most of the taxa. Fossil samples were assigned to two extant species, and one unknown, possible extinct species.
Conclusions: The analysis of fruit characters allows the distinction of groups, even up to species level. Here carpology is revealed as an accurate tool in *Carex* palaeotaxonomy, which could allow the characterization of *Carex* fossil fruits and assign them to subgeneric or sectional categories, or to certain species. Our conclusions could be crucial in order to include a temporal framework in the study of evolution of *Carex*.

Key words: approximately unbiased test; carpology; fossil record; Gower's coefficient; systematics; taphonomical alteration; UPGMA.

Accurate identification of fossil fruits of different ages is a key issue in order to incorporate an absolute time framework into phylogenetic, and biogeographic studies (Denk and Grimm, 2009; Meseguer and Sanmartín, 2012). The genus *Carex* L., with approximately 2000 species and a cosmopolitan distribution, is one of the most widespread and ecologically important angiosperm genera (Reznicek, 1990). Understanding its diversification patterns has challenged botanists for over a century. Despite the great effort that is being done to improve the current systematic knowledge of the entire tribe, few papers evaluate the evolutionary trends of Cariceae (and of Cyperaceae in general) in a temporal context. Published studies are based on mutation rates (Hipp et al., 2010; Escudero et al., 2010), estimations from other studies at higher evolutionary scales (Escudero et al., 2009) or geological events (Dragon and Barrington, 2009). Escudero et al. (2012) calibrated the stem node of *Carex* using the age of a fossil (*C. tsagajanica* Krassil., from early Paleocene; Krassilov, 1976), considered to have ancestral traits (Egorova, 1999). This species has not usually been considered by other authors (cf. Mai and Walter, 1988; Mai, 2000; Smith et al., 2010), illustrating the scarcely developed taxonomy of the *Carex* fossil record.

It seems contradictory that there is scarce taxonomic knowledge of the *Carex* fossil record when *Carex* fossilized fruits are found rather frequently in particular types of sedimentary deposits. Certain kinds of fossils are characterized by excellent preservation of organic matter due to fossilization in wet anoxic conditions (Scott and Collinson, 1983). They are called "mummified" when they are very similar to modern counterparts, and "coalified" when more compressed and altered. Nevertheless, there is a complete and gradual transition between the two states, so that intermediate forms are difficult to classify without ambiguity (Taylor and Taylor, 1993). The taxonomy of such fossil remains is hindered by the alteration of the silica bodies of the epidermis achene, a feature widely used in micromorphological studies in *Carex* (e.g. Menapace and Wujek, 1987; Standley, 1987; Olgun and Beyazoğlu, 1997; Waterway, 1990; Starr and Ford, 2001). Observation of the relatively few scanning electron microscopy (SEM) images of mummified achenes from Cenozoic sediments thus far published (e.g., Martinetto, 1994a; Mai, 2000; Ravazzi et al., 2005; Ghiotto, 2010) suggests that the silica body is generally absent, although there are apparent traces of its presence, indicating that the microstructure of these fossils has been radically changed by silica dissolution. On the contrary, a much rarer type of fruit preservation, so-called permineralisation, produces excellent undeformed fossils, with surprising micromorphological details (Thomasson, 1983).

One of the main problems for assessing taxonomy of *Carex* using fruits is that carpological characters are often neglected in favour of more evident features. Extant *Carex* species are distinguished using a wide range of characters, both vegetative (rhizomes, basal sheaths, stem section and scabrousness, or leaf surface) and reproductive (bracts, inflorescence configuration, sex distribution, spikes morphology and utricles). Among reproductive characters, utricles or perigynia (bottle-shape structures that envelop the fruit) are by far the most important (cf. Schultze-Motel, 1968-1969; Chater, 1980; Egorova, 1999; Luceño, 2008; among others). However, regarding the fruits, few characters have been habitually recorded in the literature (with the exception of trigonous section in species with 3-stigmas, and the biconvex section in those with 2-stigmas). The achene has a rather limited taxonomic value in descriptions and keys, used only for very concrete cases, and always together with other supporting characters. For example, the achene outline (obovate vs. elliptical) is one of the characters that allows the distinction between the closely related sections *Ceratocystis* and *Spirostachyae* (Luceño, 2008), or the presence of thickened style-base is characteristic in certain species (*C. depressa*, *C. oedipostyla*; Luceño, 2008) or groups (section *Mitratae* p.p. (Egorova, 1999), section *Graciles* (Dai and Koyama, 2010)). However, the analysis of the fruit morphology from modern material showed that there are meaningful taxonomic characters, as illustrated by Nilsson and Hjelmquist (1967) in the southern Scandinavian species, or Ercole et al. (2012) for NW Italy.

Fruits of Cyperaceae are rather resistant to decay and are frequently found in Cenozoic fossil assemblages (Smith et al., 2009). However, considering the mentioned complexity, the taxonomic

adscription of fossil achenes is frequently problematic, and only done superficially (Mai and Walther, 1988; Velichkevich and Zastawniak, 2006; Smith et al., 2010). Mai (1999) reported that the geologically oldest *Carex* utricle was *C. colwellensis* Chandler, found in the British Isle of Wight, and dated to the late Eocene according to Smith et al. (2010). Alernatively, Egorova (1999) considered that the most ancient known *Carex* material dates back to the late Paleocene (ca. 59-56 Myr) from eastern Siberia (*C. tsagajanica*; Krassilov, 1976), described from a specimen currently unlocated (M. Tekleva pers. comm.). However, the assignment of such fossils to *Carex* is not fully supported. More numerous fossil fruits that are described accurately enough as to be reliably assigned to *Carex* date back to the Early Oligocene (Reid and Chandler, 1926). Mummified fossils of *Carex* achenes, and sometimes utricles, became common only in the Neogene fossil assemblages (25-2.5 Myr). In Siberia, Dorofeev (1963) reported *Carex* fruits only in the Miocene, and not in the Oligocene, even when rich fruit assemblages are available. In Europe a considerable diversity has been attained for the Early Miocene (Mai and Walther, 1991; Czaja, 2003), and several species of the Middle and Late Miocene have been documented with an excellent iconography by Mai (2000). In the Pliocene (5.3-1.8 Myr), many species have been reported (e. g. Mai and Walther, 1988; Reid and Reid, 1915; Martinetto, 1994b; Matthews et al., 1990; Van der Burgh and Zetter, 1998). The geographic coverage of Neogene fossils is rather broad, ranging from Western Siberia (Dorofeev, 1963; Nikitin, 2006) throught Eastern Europe (Palamarev et al., 2005; Velichkevich and Zastawniak, 2003), central and Western Europe (Reid and Reid, 1915; Van der Burgh and Zetter, 1998) to Italy (Martinetto, 1994b; Cavallo and Martinetto, 2001). Whereas specimens from Miocene are mostly assigned to "fossil" species (e.g. morphospecies in the sense of ICBN; McNeill et al., 2006), specimens from Pliocene are assigned to both "fossil" and extant species (Mai and Walther, 1988). Many studies report *Carex* fruit records from the Quaternary, however just a few overviews exist (Dickson (1970) for Britain, Jankovská and Rybníček (1988) for the Czech Republic, and Velichkevich and Zastawniak (2006) for Poland and Eastern Europe). These Quaternary fossils are assigned to extant species, with a few exceptions such as *Carex paucifloroides* Wieliczk. (Velichkevich, 1982).

Carex sect. *Phacocystis* Dumort belongs to subgenus *Carex*. With ca. 70 taxa it is one of the largest sections of *Carex*, and has a center of diversification in Asia and North America. In Europe, section *Phacocystis* is easily distinguished from the other sections of subgenus *Carex* by having two stigmas, biconvex to plano-convex utricles, beakless or with a truncate short beak, rarely with a well-developed beak, and lenticular achenes (Chater, 1980). In its current delimitation, section *Phacocystis* includes the hybrid complex of seashore taxa previously considered as belonging to section *Temnemis* (Rafin) Krec. (= *Cryptocarpae* Tuck.; cf. Dragon and Barrington (2008) and Volkova et al. (2008)), and excludes section *Praelongae* (Kük.) Nelmes (M. Waterway pers. comm.), traditionally included by American taxonomists (cf. Standley et al., 2002). Section *Phacocystis* was considered by Egorova (1999) to presumably be a close relative to sections *Forficulae* (Kük.) Raymond, *Praelongae*, *Tuminenses* Y. L. Chang, *Graciles* (Kük.) Ohwi and *Abditispicae* Wheeler, although the latter two have already been refuted as phylogenetically close (cf. Roalson et al., 2001; Starr et al., 2004). Phylogenetic analyses by Dragon and Barrintong (2008, 2009) revealed that section *Phacocystis* proper can be arranged, at least, into six main clades: clade I- Eurasian clade; clade II- *C. aquatilis* clade (including seashore species); clade III- *C. lenticularis* clade (mainly American); clade IV- the *C. bigelowii-C. stricta* clade; clade V- the Australasian clade; and clade VI- the Pacific *C. alligata-C. obnupta* clade. To these clades, a seventh clade should be added (clade VII) formed by *C. reuteriana* and *C. panormitana*, a Mediterranean group not included within the representatives of clade I (Jiménez-Mejías, 2011). Section *Phacocystis* is a relatively recent group, that has been dated back to Mio-Pliocene boundary (5.32 Myr; Escudero et al., 2012) using four molecular markers (ITS, ETS, *trn*L intron, and *trn*L-F) and mixed calibration taking into account both fossil (*C. tsagajanica*) and an indirect calibration (Anderson and Janssen, 2009). Dragon and Barrington (2009) also inferred rather recent divergence times (3.01 to 1.20

Myr) for some lineages of this section. In addition, results inferred from AFLPs suggest that the Pleistocene glaciations would have played a key role in the phylogeographic structure of some taxa, contributing to genetic differentiation processes and subsequent speciation (Jiménez-Mejías et al., 2011, 2012). Therefore the accurate systematic interpretation of fossils of the last 4 Myr would be especially useful to estimate fine scale divergence times in section *Phacocystis*.

Fossils from section *Phacocystis* are frequently found in aquatic palaeoenvironments (e.g. Jankovská and Rybníček, 1988; Birks et al., 1993; Brooker et al., 2001; Szczepanek, 2001; Nita and Szymczyk, 2010; among others). As some of their representatives are dominant or co-dominant in some plant communities (e.g. *Caricion fuscae, Magnocaricion elatae*), presence of remains is not unexpected in such palaeohabitats. The earliest reports from this section date back to Miocene (van der Burgh, 1987: achenes referred to "*Carex acuta*") and Pliocene (achenes referred to "*Carex nigra*" by Bůžek et al. (1985), and to "*Carex cespitosa*" by Van der Burgh and Zetter (1998)). References of other extant species date back to Early Pleistocene (2.6-0.8 Myr) (*C. nigra*; Ghiotto, 2010; Ravazzi et al., 2005), late Middle Pleistocene (ca. 0.4-0.1 Myr) (*C. elata;* Mai, 2010), Late Pleistocene (*C. bigelowii* s.l., sub *C. lugens*; Kienast et al., 2011) or Late Holocene (*C. aquatilis, C. subspathacea,* Kienast et al., 2001; *C. paleacea*, Arlen-Pouliot and Bhiry, 2005). We are not aware of any fossil record of the remaining section *Phacocystis* members from the western Palearctic.

In this study we focus on the representatives of section *Phacocystis* from the Western Palearctic to 1- assess the guidelines for the detection and recognition of diagnostic characters in the achenes; 2- taxonomically characterize the fruits of extant species; 3- in light of our results, to interpret the fossil record of the group, mainly from North Italy.

MATERIAL AND METHODS

Sampling—

Delimitation of the study group—

The European representatives of section *Phacocystis* and their closest allies from North Africa and Western Asia are relatively few species (20), and they belong to at least five of the main identified clades (Table 1). Nevertheless, the majority of the European species expected belong to clades I, II and VII. Misidentification or confusion of materials belonging to section *Phacocystis* with other *Carex* groups in the study area is quite limited because fruits are distinct. The examination of extensive iconography (Berggren, 1969; Hurd et al., 1998; Ercole et al., 2012) strongly suggests that the representatives of subgenus *Vignea*, which mostly have biconvex or plano-convex achenes with two stigmas, can be easily discriminated by the style jointed to the base (cf. Hurd et al., 1998). Mainly the risk of potential misidentification would be given by other distigmatic groups from the subgenus *Carex*, as five additional sections are characterized by systematically having two-stigmas and lenticular achenes: *Abditispicae*, *Forficulae*, *Graciles*, *Praelongae* and *Tuminenses* (Egorova, 1999). Nevertheless, none of them have European representatives. In our study, two sections can be *a priori* excluded: section *Abditispicae*, which is strictly endemic from Andean America, and section *Graciles*, since their achenes can be easily distinguished by the thickened style-base (Dai and Koyama, 2010). In order to ensure the identification of European fossil samples as belonging to section *Phacocystis*, representatives from the sections *Forficulae* (*C. heterolepis* Bunge, *C. sadoensis* Franch), *Praelongae* (*C. dimorpholepis* Steud., *C. gynandra* Schwein, *C. phacota* Spreng.), and *Tuminenses* (*C. coriacea* Hamlim, *C. darwinii* Boott.) were also included (Table 2). In addition, transitions to lenticular achenes with two stigmas sometimes are found in groups mainly with trigonous fruits and three stigmas. Among European representatives of the subgenus *Carex* and the so-called Caricoid-clade, only achenes from *C. bicolor* All., *C. saxatilis* L. (both from subgenus *Carex*), and *C. capitata* L. (from subgenus *Psyllophora*) are similar in their outline to those displayed by the *Carex* sect. *Phacocystis* species, and therefore are also included for comparison (Table 2).

Fresh modern materials—

A total of 275 modern achenes of all the extant terrestrial and freshwater section *Phacocystis* taxa of Europe, North Africa and Western Asia (21 taxa; (1)5-10(20) achenes, depending on the sample) from herbarium materials and field collections (Table 2) were included in this study. Only three representatives of the hybrid complex of seashore species were included. We did not perform an intensive sampling of this set of taxa, as they form a monophyletic and recently diversified group (Dragon and Barrington, 2009). In addition, high quality illustrations of the achenes of most of the species available in Berggren (1969; see below), were used to complement our observations. As explained above, we also included representatives from sections *Forficulae, Praelongae*, and *Tuminenses*, as well as samples of *C. bicolor, C. capitata*, and *C. saxatilis* (Table 2). These samples were incorporated into the Modern Carpological Collection of Turin University (MCC, see Ercole et al., 2012).

Sediment-derived modern materials—

Ninety nine achenes from seven populations belonging to five taxa (*C. acuta*, *C.* cf. *buekii*, *C. elata*, *C. nigra*, and *C. reuteriana* ssp. *reuteriana*) were sampled from the sediment beneath living plants, in order to achieve a morphological characterization of the modern achenes after decay (Table 2). These materials have also been incorporated into the MCC. Observations on sediment-derived achenes illustrate that they are already altered by the taphonomic process (Smith et al., 2009), even if they were shed relatively recently (e.g. less than 1 year; Vassio and Martinetto, 2012). The changes involved in the decay of the soft parts frequently imply a loss of characters, but also reveal a few important features that are hidden in fresh material (e.g. style junction to the base, lignification and type of fracture; see Results).

Fossil materials—

A total of 145 achenes of the CENOFITA collection of the Earth Sciences Department of Turin University (CCN), collected from eight different locations in Italy (Martinetto and Vassio, 2010), and a few fossil specimens from two additional localities, stored in other collections (CNR-IDPA, Institute for the Dynamics of Environmental Processes, Bergamo; Cenophytic collection of the Museum für Naturkunde of Berlin), previously studied in detail by one of the authors (EM)), were identified from section *Phacocystis* (Table 2). We considered as fossil specimens from section *Phacocystis* those achenes displaying the following morphological characters (see Results): lenticular shape, persistent not-thickened/not-jointed style and cells below the epidermis clearly longer than 1/5 of the achene. The frequent presence of utricle remains sticking to the base of the achene was also considered as a good indicator of section *Phacocystis* fruits (see Results). Additionally,

Previous observations of a few achenes using SEM revealed that the silica body structure was greatly altered in mummified-coalified achenes (i.e., Martinetto, 1994a; Mai, 2000; Ravazzi et al., 2005; Ghiotto, 2010). Thus, stereomicroscope-based observations were used instead of SEM.

Bibliographic sources—

Previous works with information about carpology of *Carex* sect. *Phacocystis* and related taxa are critical for a good understanding of the morphological variation. The detailed works of Nilsson and Hjelmquist (1967) and Berggen (1969), as well as the SEM study performed by Nakamatte (2009), constitute an exhaustive compilation of data for section *Phacocystis*. Photographs and comments gathered in these works have been integrated as an important source of data for our morphological characterization of the samples.

Morphological characterization protocol—

Modern (fresh and sediment-buried) materials were characterized using the following procedure: 1–photographic documentation of 4-5 selected utricles that were opened later for achene extraction (at least 5 utricles were conserved for further documentation); 2– achene extraction by using needles from wet utricles and exhaustive morphological characterization of the extracted fruits, giving particular attention to variation within characters; 3– photographic documentation of the achenes; 4– cross-section of an achene with a razor blade; 5– immersion of another achene in a drop of water and manipulation of the outer cell wall with a needle.

Characterization of fossil achenes followed a protocol similar to that used for the modern ones: 1– preliminary detection of achenes with identical or similar diagnostic morphological traits (i.e. interpreted as belonging to the same taxon, in order to reduce the risk of mixing fruits from different taxa together); 2– characterization of utricles with achenes inside; 3– morphological characterization of isolated achenes, with particular attention to variation of characters observed in modern materials; and 4– selection of utricles and achenes (4-5) for photographic documentation.

The characters were chosen from our own observations, and described using the nomenclature of Berggren (1969) and Hurd et al. (1998) (Table 3, Figs. 1-2). Characters were observed using a stereomicroscope (Wild M3B). Stereomicroscope analysis was chosen in order to accurately compare the numerous achene samples. A few quantitative characters that were difficult to measure directly (cell dimensions, relief of anticlinal walls and callus dimensions) were evaluated on a relative scale by direct comparison to 6 standard-achenes which exhibited extreme values for such characters (RA1-6, Figs. 3-13). We strongly recommend this approach to be used in future studies, because it permits collecting data, in a successful and time-saving way, of several hundred specimens of a single species as well as of many different species. For the observation of inner pericarp cell-layers, whole achenes were submerged in water for 30 seconds. In some cases the achenes that were enclosed into the utricles, were left into a 5% solution of sodium hypochlorite (bleach) for 2 or 24 hours (Fig. 5); the aim of this treatment is to produce a sort of pseudo-taphonomic effect, which permits the evaluation of some structures that are resistant (callus, style, epidermal cell walls). Additionally, it is effective in facilitating the photographic documentation of the cell structure (Fig. 6).

The chosen characters (Table 3) were coded for each studied sample as unordered multistate characters (Appendix S1). By application of such an approach we were able to assess that each character was: 1) consistent within all the samples of one species (and therefore potentially a good diagnostic for such species), when the scores for all the samples of such species gave the same result; 2) variable within different samples of one species, when the scores for different samples of such species gave discordant results; 3) exclusive for one species, when a state was scored only for the samples of such species; 4) shared among few species; 5) useless for characterization of each species, when it was scored for several samples of different species. In a preliminary analysis of our material we tried to highlight as many characters as possible, and after the above-mentioned comparative observations we excluded those characters which responded simultaneously to the conditions 2 and 5.

Clustering analysis—

A clustering analysis was performed in order to evaluate the groups considered by our observations, and the taxonomical affinities of the fossil materials. The 25 selected characters (Table 3; see also Results) were coded in a multistate unordered matrix (Appendix S1). Ambiguous, extremely variable within a sample, or not observable (in fossil specimens) characters were coded as missing data. A hierarchical clustering with all the specimens (extant species and fossil materials) was performed to obtain clusters. Pairwise dissimilarities between samples was calculated using Gower's coefficient. This coefficient allows for the calculation of similarity between sampling units using qualitative or quantitative data (Gower, 1971) and it has been successfully used to study phenetic relationships in taxonomically problematic groups belonging to

genus *Carex* (Molina et al., 2008a,b; Smith and Waterway, 2008a,b). Unweighted pair group method with arithmetical averaging (UPGMA) was carried out to obtain a dendrogram from Gower's distances. Approximately unbiased (AU) test (Shimodaira, 2002) and bootstrap probabilities (BP) were calculated (1000 replicates) to assess the support of the branches using pvclust R package (Suzuki and Shimodaira, 2011). All statistical analysis were implemented in R (R Development Core Team, 2005)

RESULTS

Modern materials—

Images representative of the different studied taxa and main characters and variations are depicted in Figs. 15-91.

Taxa that could potentially be confusing with Phacocystis *representatives—*

The comparative study of the selected species that could potentially misled the identification of samples as a member of section *Phacocystis*, allowed us to pinpoint several characters that are not present in achenes of section *Phacocystis* (Table 4). Only for the members of section *Tuminenses* no differences were found, with a pericarp thickness, cell pattern and style robustness matching those of *C. lyngbyei* (Table 5). A key to distinguish section *Phacocystis* from the potentially confusing sections and species is provided in Appendix I.

Main characters in section Phacocystis *fruit taxonomy—*

Our study reveals that the characterization of each species using achene characters is a not easy task. The achene outline and dimensions at a first glance seem to be useful characters to discriminate among species, but the variation at the individual and/or population scale can be very broad. It is especially pronounced in species that are widely distributed, such as *C. acuta*, *C. elata* or *C. nigra*, in which a broad morphological variation has been recognized (cf. Jiménez-Mejías et al., 2011, 2012, and manuscript in press). The widest population-scale variation was observed in 23 achenes of *C. elata* (Ela_1; Fig. 14) all obtained from a single soil sample. The fruit sampling from individual fresh spikes of this population (Ela_2) showed that individual plants certainly produce less variable achenes. Thus the achene diversity level observed in the soil sample can be attributed to variation among individuals within this population. In contrast, large samples of *C. nigra* (especially the samples Nig_5-8 from NW Italy) have a very consistent morphology.

Careful observation at the stereomicroscope led us to identify 25 characters, making a total of 72 different states, as those most significant for achene taxonomy (Tables 3, 5; Appendix S1). Five groups of characters were found to be especially relevant in making morphological groups.

Epidermis morphology—

Nilsson and Hjelmquist (1967) noted the importance of the achene surface for distinction of members of section *Phacocystis*. We found that the characters that are the most consistent within each species are related to the cell pattern: cell dimensions, silica bodies (visibility at 40×), anticlinal walls protrusion, and texture of the outer cell wall (established by manipulation with a needle in a water-borne achene). We observed five main states of the outer cell wall: 1) mucilaginous = easily removed in masses similar to jelly; 2) ephemeral-membranaceous = spontaneously falling apart in small and very thin cuticle-like fragments; 3) chartaceous, similar to a very thin sheet of paper, usually whitish and somewhat resistant; 4) vitreous = shining and transparent, rather resistant; 5) coriaceous = opaque, thick, and very resistant.

Utricle remains—

Berggren (1969, p. 19) already pointed out the importance of the the "wall remains of perigynium" on basal part of the achene as a differentiating character for "section *Acutae*" (= section *Phacocystis*). In this study the achene-utricle attachment is defined as strong when the gentle manipulation of the utricle's base permits a complete removal of utricle remains with 5-30 needle-hits (Fig. 31). The attachment of the achene to the utricle was defined as weak when the

action of the needle permitted a complete (Fig. 44) or almost complete removal (Fig. 9) of utricle remains with 1 to 4 hits. When present, the utricle remains on the achene base are so strongly attached, that these remain even after rubbing by hands free achenes.

Achene base—

In some taxa, the base of achenes is bordered by a sort of ridge (CT in Fig. 8), whose conspicuousness varies between species up to being an apparent ring. This structure, termed *callus* by Berggren (1969), is apparent as a basal ridge even when the achene is still inside the utricle. In general, a stronger achene-utricle attachment is linked to a thicker callus. As it is difficult to measure precisely the dimensions of the callus, either for the presence of utricle remains or for its lateral variability, we distinguish two main types of callus: thick, as in RA3 (Fig. 7), and thin, as in RA5 (Fig. 11) or thinner. In several samples both presence and thickness of the callus were not constant, so that variation was considered to score this character (see Table 3). In some cases a large callus can show, as an additional feature, small irregular granules ("callus granulose": Fig. 8); otherwise it appeared completely smooth (Fig. 88). The relative size of the base was also scored as a character (Table 3), as we noticed that it is either around ¼ or ½ of the achene's width; only the sample Rec_1 fell in between (ca. 1/3), so that we did not score it in any of the two states.

Style robustness and response to fracture—

The style jointed to the base (Fig. 92) is the best character to differentiate the otherwise very similar achenes of the species from subgenus *Vignea* (cf. Berggren, 1969) or *C. bicolor* and *C. capitata*. In members of section *Forficulae* (Table 4) we observed a distinct type of non-lignified style. In all the examined species of section *Phacocystis* the style is persistent, never jointed to the base, and varying in length, shape and degree of lignification (e.g.: Figs. 7, 12, 17, 24, 25, 27, 29, 31, 38, 50, 81). We observed in the achenes separated from the utricles by vigorous friction, and in the soil samples, that the style tends to break at 1/10-1/5 of the achene's length (Fig. 9) or at the base (Fig. 3) depending on the pattern of lignification. The style morphology is rather constant in different samples of the same species, even when the general achene shape varies considerably. Some authors have referred to the resistent part of the style as the "beak" (cf. Nilsson and Hjelmquist, 1967), but we did not use this term to avoid confusion with the utricle's beak..

Pericarp thickness—

The pericarp thickness has not been previously used in *Carex* taxonomy despite it being known to hold a critical taxonomic value in other Cyperaceae genera. Pericarp thickness is used for the taxonomy of the extant genus *Bolboschoenus* (Browning and Gordon-Gray, 2000; Hroudová et al., 2007), and also for distinction of fossil taxa (Smith et al., 2009). In our samples, pericarp thickness was directly measured in cross-sections of completely ripe achenes filled by the seed (immature achenes are thinner). This divided them into two classes, thin (<0.03 mm, flexible, easily deformed using forceps) and thick (≥0.03 mm, rigid), being the maximum observed thickness 0.05 mm.

Additional characters of limited taxonomic value—

Eleven additional characters allow the distinction of single samples but not groups of them. They are useful in order to discriminate between morphologically close species found within the main types of achene.

In several cases, the length-width ratio (L:W) is so variable within single samples that we consider its uses for classification as limited. Only those samples that had a L:W roughly close to 1 were scored, as this was only the case in two species of Clade I, and *C. orbicularis* ssp. *kotschyana* (see Appendix S1).

The mean length of the achene displays the same situation, being variable within single samples. This character was only considered for those samples which were clearly shorter than RA4

(1.9 mm, excluding stile base; Fig. 9) because it was useful for differentiating otherwise similar species (e.g., *C. elata* and *C. nigra*).

Despite the achene outline being broadly used to characterize different species (Nilsson and Hjelmquist, 1967; Berggren, 1969), we find that it shows a broad intraspecific variability too often. Thus, it should be taken into account only in combination with more precise diagnostic characters. The studied achenes included all types of elliptic and obovate outlines (see for example Fig. 14). We considered that it was useful to score only six main states (circular, elliptic, narrowly obovate, obovate, broadly obovate, and variable from elliptic to obovate, according to the definitions of Berggren, 1969; Fig. 2, Table 3), because of their prevalent occurrence in some species, but not in others. We also noticed that, on each side of the lateral outline of the achene (Fig. 1), one or two slight changes of curvature (flexa: see AF and BF in Fig. 1) can be distinguished, allowing species discrimination.

The presence of a substipitate base (Hurd et al., 1998; Fig. 1) gives a distinct appearance to the achenes, but too often a combination of specimens within the same sample is observed, where some have a substipitate base, while others do not (e.g. Fig. 14). However, in most achenes, the lack of this character in a single sample seems to be more consistent within taxa in terms of frequency. This substipitate base of the achene has also been called "stipe" or "stalk" (e.g. Olgun and Beyazoğlu, 1997), but this term is used in an inconstant and confusing way in most literature. We would suggest to use the term stipe only for a structure which is distinctly separated from the achene base, providing a connection to the utricle base (Fig. 16).

We also introduced, as shown in Fig. 1, the measurement of an apical and basal angle of the achenes and we found it was useful for distinction between individual taxa. Despite the basal angle (Fig. 1) being a very variable character with values generally ranging from 70º to 100°, the occurrence of very narrow angles (40-60°) is clearly more constant in several samples. The apical angle is more consistent in several species, with a prevalence of values in the range of 140-180°.

Certain samples displayed a striation pattern of inner pericarp cell layers. This was observed after the immersion of achenes in water, and under strong oblique light (Fig. 87). In this way the epidermal cells became invisible and the ornamentation of the internal layers of the achene was apparent. The achenes of the majority of species showed an apparent longitudinal striation in such condition. Conversely, *C. panormitana* exhibited a strong transverse striation. An analogous, though for the most part less apparent, transverse striation was shown by *C. aquatilis* and *C. reuteriana*. Treatment with bleach of *C. acuta* achenes (Fig. 90) enhanced the visibility of such transverse striation, suggesting that striation may be more apparent in fossil achenes (where taphonomical alterations may be similar to the effect of bleaching) than in the corresponding fresh achenes. In *C. elata* and *C. nigra* we noticed that the transverse striation is apparent in immature achenes, and not in the completely ripe ones. Due to its connection to the degree of ripening and fossilization, as well as with the wall thickness, this character was not scored, although it was considered as characteristic for the *C. reuteriana*-type (Table 5).

Four of the considered characters were exclusively present in one species (real well-defined stipe, independent from the achene in *C. bigelowii* (Fig. 16); inflated achenes in *C. cespitosa* (Fig. 27); median furrow in *C. rufina*(Fig. 91); and achene base slightly winged in *C. dacica* (Fig. 62); see Tables 3, 5, Appendix S1). The presence of invagination was restricted to a few members of the seashore taxa (Appendix S1), although also observed in *C. gynandra* of section *Praelongae*.

Individualized types of achenes in section Phacocystis—

Sixteen different achene types could be discriminated under stereomicroscope observation. These types of achenes, and their main morphological features are summarized in Table 5, and figured in Figs. 15-91. Most achene types have been observed in only one species: *C. bigelowii*-type, *C. palaeacea*-type, *C. elata*-type, *C. cespitosa*-type, *C. dacica*-type, *C. orbicularis*-type, *C. nigra*-type, *C. lyngbyei*-type, *C. trinervis*-type, *C. kurdica*-type, *C. rufina*-type. Additionally, four

types occur in more than one species: *C. acuta*-type (*C. acuta* and a sample of *C.* cf. *randalpina*), *C. aquatilis*-type (*C. aquatilis*, *C. recta*, and *C. subspathacea*), *C. buekii*-type (*C. buekii*, *C. randalpina*), and *C. reuteriana*-type (*C. reuteriana* and *C. panormitana*). The inclusion of *C. recta* and *C. subspathacea* within the *C. aquatilis*-type is not definitive, since the achenes of the first two species are often easily distinguishable from *C. aquatilis* for the presence of invagination and/or small folds (Table 5). In general, the achene types proposed here on the basis of one or a few achene samples should be verified by means of the occurrence of their diagnostic characters in more numerous and specimen-rich samples.

Persistence of characters in decayed achenes—

The observation of achenes treated with bleach showed that a few characters have a poor possibility to be recorded in the fossil state, for example outer periclinal walls and silica bodies. Thus, those characters that can be singled out as more useful for the characterization of fossils are the achene outline and wall thickness, the cell size and the relief of anticlinal walls, and the base and style features. Achenes treated with bleach for 24 hours (e.g. *C. acuta*; Figs. 5, 6), showed a complete degradation of the external periclinal walls, and, in some patches, even of the entire epidermal layer (Fig. 6). In these cases, a consistent deterioration of the apparently robust basal callus and style was also observed. It contrasts with the perfect preservation of the same structures in achenes extracted from soil samples, which have already undergone a marked decay (Figs. 11, 88). Considering this, the utility of some epidermal characters in certain well-preserved fossil achenes (especially those found inside the utricle) should not be ruled out.

Fossil materials—

The results of our overview of modern fruits indicate that fossil achenes (Figs. 93-104) can be assigned to section *Phacocystis* when they display the following character combination: biconvex section, non-jointed style, and inner longitudinal cells longer that 1/5 achene length. Additionally, the presence of utricle remains sticking to an achene's base is a diagnostic character, but possibly only present in those achenes that were fossilized inside the utricles. We detected the presence of an apparent callus only in the clades I, II and III of section *Phacocystis*, and a special abundance of utricle remains in members of clade I, as well as in *C. rufina*. This may be a good character for the intra-sectional assignment of fossils. However, the callus could be present, although in rare cases, in other *Carex* groups, and a continuing effort would be necessary in order to exhaustively characterize *Carex* fruits to ensure a more accurate determination. Another problem is the secondary loss of the callus due to taphonomical alteration. This was observed in a few fossil samples, particularly in TB_3 (Table 2; Figs. 99, 103), where a few achenes showed similar morphological damage as those of *C. elata* and *C. acuta* (Fig. 87), when treated for 24 hours with bleach. Actually, we must bear in mind that extreme conditions of decay or chemical alteration during fossilisation may produce a loss of diagnostic characters. However, most of our fossil samples appeared well preserved, and most of the *Carex* fruits preserved the original diagnostic features (e.g. the callus in Fig. 104a). Thus, on the basis of the combination of characters reported above, fossil materials belonging to section *Phacocystis* were recognized among other materials.

The few SEM analyses we did showed that the cell lumina were filled with compressed remains of the outer periclinal walls, and the silica bodies altered and scarcely apparent (Fig. 104).

With the new insight of the variation seen in modern monospecific populations (see above), the morphological analysis of the populations from each fossil locality suggest that, probably, a single taxon was represented in each sampled site. The studied fossil specimens could be assigned to three taxa.

Taxon A—

Records found from 4 to 2.5 Myr (Figs 93-98). Achene compatible with the *C. reuteriana*-type, but also somewhat related to *C. paleacea*-type (in the rare cases lacking invagination): cell

size as in RA6, very few utricle remains at the base of achene; no callus (Fig. 98); apical angle 140-180°; style poorly lignified, and therefore for the most part not preserved; thin and flexible pericarp with apparent internal transverse striation; two flexa in the lateral outline; base not winged (Table 2; Figs. 93-98). Additionally, a peculiar micro-granulation of the surface was observed with oblique light (Fig. 97), which, among modern materials, was only observed in specimens of *C. reuteriana* ssp. *reuteriana* (Fig. 55) that have decayed (soil sample).

Taxon B—

Records found from 2.0 to 0.02 Myr (Figs. 99-103). Achenes of the *C. elata*-type: cell size as in RA4 (Fig. 102), few utricle remains; callus thin (Fig. 100), sometimes absent (Fig. 103); apical angle mostly 140-180°; style much lignified in the basal half, which is the single part of the style preserved in most fossils (Fig. 101); apical part of the style soft, and only preserved inside the utricle (Fig. 99); thick and rigid pericarp; L:W mainly 1.2-1.3; mostly two flexa in the lateral outline (Fig. 103, see Fig. 1 for explanation), rarely only one (Fig. 100); achene not inflated; base not winged (Table 2; Figs. 99-103).

Taxon C—

Records found from ca 1.8-1.0 Myr (Fig. 104). Achenes related to *C. nigra*-type: cell size as in RA4, abundant utricle remains at the base of achene; thick callus (Fig. 104); apical angle 90-120° (to 160°); style lignified only in a basal acute part; achene length less than RA4; two flexa in the lateral outline; base not winged (Table 2; Fig. 104). This type of achenes was already detected as *C. nigra* by Ravazzi et al. (2005) and Ghiotto (2010, pl. 10, fig. 13) for the Pleistocene, and also is reported by Bůžek et al. (1985) for the Pliocene.

Clustering analysis—

The results obtained in the clustering analysis (Fig. 105) confirm the distinctiveness and cohesiveness of most of the groups considered (see above), as well as the taxonomical affinities inferred for fossil materials. Samples of recent materials showing achenes of the *C. acuta*, *C. aquatilis*, *C. buekii*, and *C. elata* types were grouped in independent and well-supported clades. On the other hand, samples belonging to *C. cespitosa* and *C. nigra* types were grouped each in a different, marginally-supported clade. The *C. reuteriana*-type samples clustered together, but without significant support, although the clade containing *C. reuteriana* ssp. *reuteriana* samples was well-supported. Three morphological types were placed within more broadly defined types: *C. lyngbyei* (within *C. aquatilis*), *C. paleacea*-type (within *C. reuteriana*-type), and *C. rufina*-type (within *C. nigra*-type). Probably, the inclusion of more samples of these types would contribute to enhance the resolution of the clustering analysis. Regarding fossil materials, Taxon A was strongly supported together with *C. panormitana*, revealing affinities with the *C. reuteriana*-type, and Taxon B was strongly supported and showed affinities with *C. elata*-type. Taxon C was marginally supported within *C. nigra* clade.

DISCUSSION

Carpology as a tool for *Carex* sect. *Phacocystis* taxonomy—

Species identification using achene characters is a difficult task. Nevertheless, it does not imply that achene characters are useless (Nilsson and Hjelmquist, 1967). Our results show promise for the further interpretation of *Carex* fossil achenes. Despite the fact that authors have commonly neglected the carpological characters of *Carex*, and even stated the impossibility of discrimination of some taxa from others (e.g. *C. bigelowii* s.l. from *C. aquatilis*; Schönswetter et al., 2008), the distinction at quite fine taxonomical scale seems to be possible at least in European members of section *Phacocystis*. In this sense, our analysis found remarkable differences between the achenes of *C. bigelowii* s.s. and *C. dacica* (previously considered a *C. bigelowii* subspecies). While most

authors consider them to be closely related plants (e.g. Chater, 1980; Egorova, 1999), recent data suggested that they could be different, poorly differentiated, species (Nakamatte and Lye, 2007).

We used our detailed morphological analysis of achene samples for the segregation and description of precise morphological types (as used, e.g. for *Atriplex*, by Berggren, 1981), defined by unequivocal characters, easily detectable using a stereomicroscope. Although several characters are missing in fossils, the combined analysis of wall thickness, cell size, outline, style and base characteristics are revealed to be sufficient to assign fossils to a given morphological type. The assessment of precise achene types in an unidentified population, either modern or fossil, is a necessary prerequisite for a correct description and for the final taxonomic interpretation of an achene sample of *Carex* sect. *Phacocystis*, and of *Carex* in general by extension. The assignment of an unidentified achene population to a definite species can be attempted in a secondary refinement, by detection of one of these conditions: 1) the same achene type occurs in a single modern species; 2) the sample shows an association of characters, within an achene type, which is only present in a single modern species (e.g. achenes of the *C. buekii*-type with callus "absent" and style "shortly conical" are only present in *C. buekii*, and not in *C. randalpina*); 3) one or more specimens of the sample exhibit a character or a combination of characters (possibly not very apparent) which is highly diagnostic for a single modern species sharing the same achene type (e.g. achenes of the *C. acuta*-type with style "longely cylindrical" are only present in *C. acuta*, and not in *C.* cf. *randalpina*); 4) achene characters, when combined to utricle characters, are diagnostic for a single modern species (e.g. achenes of the *C. buekii*-type associated to utricles with very apparent ribs are only present in *C. randalpina*).

The analysis of *Carex* fossils should be extended to the abundant materials preserved in a few rich palaeobotanical collections (Berlin, Krakow, St. Petersbourgh, Utrecht, among others). New analyses are also needed for the Neogene records of achenes referred to as extant species, such as "*C. acuta*" (Van der Burgh, 1987), "*C. nigra*" (Bůžek et al., 1985), and "*C. cespitosa*" (Van der Burgh and Zetter, 1998), because the specimens illustrated by the authors do not show such fundamental characters as cell size and style characteristics, thus precluding an assignment to the fruit types described in this paper.

Problems on the taxonomic interpretation of *Carex* achenes—

Given the lack of connectivity between the palaeocarpological studies and the majority of modern works in botany systematics, as well as the knowledge gap in *Carex* fossil record, the assignment of fossil achenes to a morphological type, instead of to a taxon, could be a useful nomenclatural artefact. For example, the assignment of fossils of considerable age (> 3 Myr) to a modern species give rise to the potential error of tracing the origin of modern species too far back in time. In this sense, referring only to achenes, the ca. 20-25 Myr-old *C. klettvicensis* Mai (Mai, 2000) matches very well with our *C. acuta*-type. However, this does not imply that it actually represents a record of *C. acuta* (also denied by utricle characters), which would be in total discordance with the recent divergence hypothesized for section *Phacocystis* (Dragon and Barrington, 2009; Escudero et al., 2012). The generic naming as an achene type applied to a particular set of fossils, could avoid such interpretation problems.

The occurrence of the same type of achene in different species should be carefully evaluated. For example, the *C. acuta*-type occurs basically in *C. acuta*, but also in a sample tentatively assigned to *C. randalpina* (cfRan_1, in need of revision; Fig. 105). Processes of convergence, hybridization (widely reported in section *Phacocystis*; Chater, 1980; Egorova, 1999, among others), or the simple lack of differentiation in closely related species, could be involved in the unexpected presence of an additional achene type within a rather homogeneous taxon. We cannot rule out that our limited taxonomic sampling could be behind some cases of apparent heterogeneity, such as the inclusion of *C. paleacea* between the samples of the *C. reuteriana*-type (Fig. 105), and the lack of support of the cluster grouping these samples. In any case, further

sampling would be required for a better understanding of the carpological features of the different section *Phacocystis* taxa.

Carex sect. _Phacocystis_ in the fossil record of Europe—

We verified the presence of the characters diagnostic for section *Phacocystis* in all our studied fossil samples. The ages detected for those samples are according the ranges given for section *Phacocystis* in previous studies (Dragon and Barrington, 2009; Escudero et al., 2012).

The following considerations point to a more precise assessment of the taxonomic affinities:

Taxon A has possible affinities with living species which produce the *C. reuteriana*-type of achenes (probably *C. panormitana*; see Fig. 105). Affinities to *C. paleacea*-type can be discarded because the typical invagination is not observed. Therefore the fossils may be interpreted as the record of a member of clade VII, perhaps an extinct taxon.

Taxon B, with achenes of the *C. elata*-type, is assigned to *C. elata* (or, as for the oldest records, to a closely related ancestor) because the observed achene characters (Fig. 105; Appendix S1) are shared with the modern representatives of this species, and it shows the same variability as in the modern achene assemblages of *C. elata* from waterlogged sediments (e.g. Ela_5). The poorly lignified upper half of the style, the two flexa in the lateral outline, the apical angle 140-180° and, additionally, the utricle remains displaying nerves, all allow to support Taxon B as closely related to *C. elata* (cf. Chater, 1980; Egorova, 1999; Luceño and Jiménez-Mejías, 2008). Conversely, a few individual achenes of Taxon B (TB_3) overlap in variation with a few specimens of modern *C. nigra* (Nig_2) that are large and narrow, but in general the population as a whole can be well differentiated.

Taxon C, with thick callus and short-acute style-remain attached to a robust, broadly conical, achene apex (Fig. 104), could be defined as similar to the *C. nigra*-type. Although the clustering analysis was unable to distinguish between *C. nigra* and *C. rufina* (Fig. 105), this latter can be discounted because of the absence of style remain (Fig. 3) and epidermal cells distinctly visible at 6.4×. The affinities between Taxon C and *C. nigra* were already pointed out by Ravazzi et al. (2005) and Ghiotto (2010), who classified these samples as *C. nigra*.

Conclusions—

The genus *Carex* L. is one of the largest genera of flowering plants, occurring in nearly all habitat types, but particularly common in temperate wetlands of the world. These habitats, and the relatively robust structures of the achenes, facilitate *Carex* fruits to be preserved in the fossil record. Given that the utricle is rarely preserved, achene characters are fundamental for the taxonomic interpretation of fruit fossils. The analysis of fossil fruit characters is a powerful tool for tracing historical distribution of *Carex* groups, and to accurately estimate divergence time of clades. Our analyses of species of *Carex* sect. *Phacocystis* concludes that achene morphology allows for the establishment of affinities at quite fine taxonomical scale. Despite this, we found that the different characters, and particularly the shape, may be very variable (either in a single individual, monospecific populations, or in geographically close conspecific samples). Nevertheless, each modern species, and even local varieties, may be characterized by the "mean" morphology of that population of achenes.

Here we present the guidelines for an effective morphological analysis of fruit characters of a *Carex* group (section *Phacocystis*) and its taxonomical application to fossil specimens. In light of our results, the record of fossil achenes assigned to "*Carex* gr. *cespitosa*" from Europe should be revised more accurately on the basis of "macromorphological" (non-SEM) characters, in order to effectively verify the occurrence of characters typical for the types accepted here.

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Table 1. European, N African and W Asian representatives of *Carex* sect. *Phacocystis* (modified from Chater (1980), Egorova (1999), and Jiménez-Mejías and Luceño (2011)). Clade arrangement is given according to Dragon and Barrington (2008, 2009) with the indicated modifications; for clade numbering see Introduction.

	Clade / *Taxon*	Current distribution
	Clade I – Eurasian	
	C. acuta L.	Eurasia
	C. buekii Wimm.[1]	C and E Europe, Anatolia and Caucasus
	C. cespitosa L.	Eurasia
	C. elata All.	
	ssp. *elata*	Europe, N Africa and SW Asia
	ssp. *omskiana* (Meinsh) Jalas	NE Europe and NW Asia
	C. kurdica Hand.-Mazz.[1]	Greece, S Anatolia and Middle East
	C. nigra (L.) Reichard s.l.	Europe, N Africa, W Asia and E North America
	C. randalpina B.Walln.[1]	C Europe and N Italy
	C. trinervis Degland	Atlantic coasts of Europe, N to Denmark
	Clade II – *C. aquatilis* clade	
	C. aquatilis Wahl. s.l.	Circumboreal
	C. lyngbyei Hornem.	Circumboreal, absent from most N Europe
Seashore taxa	*C. paleacea* Wahl.	Amphi-Atlantic
Seashore taxa	*C. recta* Boot s.l.	Amphi-Atlantic, also in N Russia
Seashore taxa	*C. salina* Wahl.	Amphi-Atlantic, also in N Russia
Seashore taxa	*C. subspathacea* Hornem.	Circumboreal
	Clade III – *C. lenticularis* clade	
	C. rufina Drejer	Amphi-Atlantic, N of 50ºN.
	Clade IV – *C. bigelowii* clade	
	C. bigelowii Schwein. s.s.	Amphi-Atlantic
	Clade VII – Mediterranean	
	C. panormitana Guss.	Tirrenian
	C. reuteriana Boiss.	
	ssp. *mauritanica* (Boiss.) Jim.-Mejías & Luceño	S Iberian Peninsula and NW Africa
	ssp. *reuteriana*	C and NW Iberian Peninsula
	Incertae sedis taxa	
	C. dacica (Heuff.) Egorova [2,3]	C and N European mountains, Iceland
	(=*C. bigelowii* ssp. *rigida* (Gooden.) W. Schultze-Motel)	
	C. orbicularis ssp. *kotschyana* (Boiss & Hohen) Kukkonen [3]	SW Asia

[1]Found to be included within clade I (cf. Jiménez-Mejías, 2011); [2]Recent data suggest the distinctiveness of *C. dacica* from *C. bigelowii* s.s. (Nakamatte and Lye, 2007, Schönswetter et al.,

2008; Nakamatte, 2009); [3]Jiménez-Mejías (2011) found phylogenetic affinities between these taxa and the Australasian clade V.

Table 2. Taxon, sample labelling, geographic location, estimated age (in fossil materials), voucher or fruit sample including where the collection is deposited (in brackets), number of studied fruits (*N*), and achene type of the studied materials. For modern fruit samples, the herbarium acronym where the voucher is deposited, and/or the code for Turin University collections (MCC = Modern Carpological Collection; CCN = CENOFITA Collection Number, for fossils; see Material and methods) is provided. Modern samples from soil are indicated with an asterisk in the label. For section *Phacocystis* species, the clade adscription is given as heading underlined line before species groups (see Introduction and Table 1).

***Taxon* / clade / sample label**	**Geographic location / age**	**Collection reference**	***N***	**Achene type**
		MODERN MATERIALS		
Section *Phacocystis*				
Clade I				
C .acuta				
Acu_1	Finland, Varnisais-Suomi, Nummi-Pusula, Salo	I. Kukkonen 12845 (SEV)	2	*acuta*
Acu_2	Poland, Pommern, Breslau	A.R. Paul s.n. (MA)	7	*acuta*
Acu_3	Serbia, Vlasina lake	P. Jiménez-Mejías 67PJM10 (UPOS)	5	*acuta*
Acu_4*	Italy, Roppolo, Mulecchia hamlet	E. Martinetto s.n. (MCC1960)	7	*acuta*
Acu_5	Italy, Roppolo, Mulecchia hamlet	E. Martinetto 023EM12 (TO)	20	*acuta*
Acu_6	Italy, Moncalieri, Mulino di Carpice	Ferrari (TO)	9	*acuta*
Acu_7	Italy, Foglizzo	E. Martinetto (MCC1966)	5	*acuta*
C. buekii				
Bue_1	Bulgaria, Sofia	P. Jiménez-Mejías 175PJM10 (UPOS)	5	*buekii*
Bue_2	Czech Republic, Bzenec, Bzinek wood	R. Řepka nr.5-1988 (MCC1952)	2	*buekii*
Bue_3	Czech Republic, Jánské koupele, Moravice river	R. Řepka nr.8-1984 (MCC1953)	1	*buekii*
Bue_4	Slovakia, Velká Fatra Mts., Rojkov, Váh river	R. Řepka nr.9-1983 (MCC1954)	5	*buekii*
C. cf. *buekii*				
cfBue_1*	Italy, Piedmont, Albano Vercellese	E. Martinetto s.n. (MCC1962)	18	*buekii*

cfBue_2	Italy, Piedmont, Albano Vercellese	E. Martinetto 024EM12 (TO, MCC1955)	6	*buekii*
cfBue_3	Italy, Piedmont, Albano Vercellese	E. Martinetto 025EM12 (TO, MCC1965)	8	*buekii*
C. cespitosa				
Ces_1	Spain, Navarra, Lesaka	P. Jiménez-Mejías et al. 99PJM06 (UPOS)	1	*cespitosa*
Ces_2	Sweden, Uppland, Uppsala, lake Ramsen	K.A. Lye 33392 (UPOS)	4	*cespitosa*
Ces_3	Sweden, Södermanland, Nyköping kom	K.A. Lye 33391 (UPOS)	3	*cespitosa*
Ces_4	Sweden, Södermanland, Vingåker	Negri's Herbarium s.n. (TO)	8	*cespitosa*
C. elata ssp. *elata*				
Ela_1*	Italy, Piedmont, San Carlo Canavese, Baima hamlet	E. Martinetto s.n. (MCC1959)	23	*elata*
Ela_2	Italy, Piedmont, San Carlo Canavese, Baima hamlet	E. Martinetto 016EM12 (TO, MCC1963)	20	*elata*
Ela_3	Italy, Trento province, Maso della Busa	F. Prosser s.n. (ROV, MCC1956)	13	*elata*
Ela_4	Spain, Burgos, Miranda del Ebro	C. Pau s.n. (MA)	3	*elata*
Ela_5*	France, Jura, Lac de Sainte Point	E. Martinetto s.n. (MCC1958)	9	*elata*
Ela_6	Spain, Cuenca, Uña	S. Cirujano s.n. (MA)	2	*elata*
Ela_7*	Spain, Guadalajara, Póveda	P. Jiménez-Mejías 62PJM11	12	*elata*
Ela_8	Portugal, Algarve, Tavira	P. Rodríguez-González & A. Albuquerque s.n. (UPOS)	5	*elata*
Ela_9*	Italy, Piedmont, San Benigno Canavese, Orco River	E. Martinetto s.n. (MCC0900)	6	*elata*
C. elata ssp. *omskiana*				
Oms_1	Finland, North Häme, Virrat, Hauhuu	I. Kytövuori 339I (MA)	4	*elata*
C. kurdica				
Kur_1	Iran, Kurdestan, 45-50 kms from Sanandaj to Tangi-Sar village	Amini-Rad s.n. (UPOS)	5	*kurdica*
C. nigra				
Nig_1	Spain, Sierra Nevada, Pto de la Ragua	M. Luceño s.n. (MA)	3	*nigra*
Nig_2	Spain, Ávila, Gredos, Garganta de los Conventos	J.M. Marín 5104JMM (UPOS)	3	*nigra*
Nig_3	Spain, Ávila, Gredos, Hoyocasero	J.M. Marín & M. Luceño 3404JMM (UPOS)	4	*nigra*
Nig_4	Spain, Huesca, Pyrenees, Panticosa	P. Jiménez-Mejías & M. Escudero 85PJM06 (UPOS)	5	*nigra*

Nig_5	Italy, Usseglio, Lac Falin	E. Martinetto s.n. (MCC1899)	5	*nigra*
Nig_6*	Italy, Usseglio, Lac Falin	E. Martinetto s.n. (MCC1964)	20	*nigra*
Nig_7	Italy, Balme, Pian della Mussa	E. Martinetto s.n. (MCC0252)	10	*nigra*
Nig_8	Italy, Cavalese, Maso Baldessalon	F. Prosser s.n. (ROV, MCC1951)	17	*nigra*
Nig_9	Poland, Zakopane, Rekowianska	E. Martinetto 001EM98 (TO, MCC0276)	12	*nigra*
Nig_10	Norway, Troms, Skjervoy	M. Luceño & M. Guzmán 3805ML (UPOS)	5	*nigra*
C. randalpina				
Ran_1	Germany, Bayern, Ehring	K.P. Buttler 31830 (M)	1	*buekii*
Ran_2	Italy, Lipoi	E. Martinetto 031EM12 (TO)	3	*buekii*
C. cf. *randalpina*				
cfRan_1	Italy, Paluck di Cesiomaggiore	C. Lasen s.n. (ROV, MCC1950)	3	*acuta*
C. trinervis				
Tri_1	Portugal, Figueira-da-Foz, Lagoa das Braças	J. Fernández Casas s.n. (MA)	4	*trinervis*
Clade II				
C. aquatilis				
Aqu_1	Sweden, Piteå	E. Lundberg s.n. (UPOS)	2	*aquatilis*
Aqu_2	Norway, Troms, Tromsø	P. Jiménez-Mejías 186PJM09 (UPOS)	5	*aquatilis*
C. lyngbyei				
Lyn_1	Iceland, between Djúpivogur and Hofn	M. Luceño 7406ML (UPOS)	4	*lyngbyei*
C. paleacea				
Pal_1	Norway, Nordland, Narvih	H. Rickman s.n. (UPOS)	2	*paleacea*
C. recta				
Rec_1	Norway, Finnmark, Talvik	M. Luceño & M. Guzmán 6305ML (UPOS)	2	*aquatilis*
C. subspathacea				
Sub_1	Norway, Finnmark, Talvik	M. Luceño & M. Guzmán 6405ML (UPOS)	3	*aquatilis*
Clade III				

C. rufina				
Ruf_1	Norway, Troms, Tromsø, Rødryggen	P. Jiménez-Mejías 184PJM09 (UPOS)	5	*rufina*
Clade IV				
C. bigelowii s.s				
Big_1	Greenland, between Qassiarsuk and Tassiussaq	M. Luceño 2807ML (UPOS)	4	*bigelowii*
Clade VII				
C. panormitana				
Pan_1	Italy, Palermo Botanic Garden	G. Domina, cultivated material (MCC1957)	8	*reuteriana*
C. reuteriana ssp. *mauritanica*				
Mau_1	Morocco, Chef-Chaouenne, Sifladu river	A.J. Chaparro et al. 08AJC05 (UPOS)	5	*reuteriana*
C. reuteriana ssp. *reuteriana*				
Reu_1	Spain, Ávila, Barco de Ávila	J.M. Marín & M. Luceño 2604JMM (UPOS)	5	*reuteriana*
Reu_2*	Spain, Madrid, La Pedriza	P. Jiménez-Mejías 61PJM11 (MCC sn)	4	*reuteriana*
Incertae sedis taxa				
C. dacica				
Dac_1	Norway, Hedmark, Lake Muvatn	J. Prudhomme s.n. (STU)	5	*dacica*
C. orbicularis ssp. *kotschyana*				
Orb_1	Iran, Teheran	M. Amini-Rad s.n. (UPOS)	4	*orbicularis*
Section *Forficulae*				
C. heterolepis	Japan, Honshu, Okayama	T. Hoshino et al. s.n. (UPOS)	2	-
C. sadoensis	Japan, Honshu, Iwate	T. Hoshino et al. s.n. (UPOS)	2	-
Section *Praelongae*				
C. dimorpholepis	Japan, Honshu, Okayama	H. Hatooka s.n. (UPOS)	5	-
C. gynandra	Canada, Quebec, Pointe-du-Lac	F. Louis-Arsène s.n. (MA)	3	-
C. phacota	Japan, Honshu, Okayama	T. Hoshino et al. s.n. (UPOS)	3	-
Section *Tuminenses*				

C. coriacea	New Zealand, Christchurch, Riccarton	A.J. Healy 66/17 (CHR)	2	-
C. darwinii	Chile, Region XII, Última Esperanza, Lago Sofía	E. Pisano & J. Henríquez 6793 (MA)	2	-
Other distigmatic European *Carex* species				
C. bicolor	Italy, Balme, Pian della Mussa	E. Martinetto (MCC0217)	5	-
C. capitata	Iceland, Djúpivogur, Berufjördur	M. Luceño 6906ML (UPOS)	2	-
C. saxatilis	Finland, Kalastajasaurento	A. Segura Zubizarreta s.n. (MA)	2	-
		FOSSIL MATERIALS		
Taxon A				
TA_1	Italy, Piedmont, Villafranca d'Asti; Pliocene (Ca. 3Myr)	CCN0645	40	*reuteriana*
TA_2	Italy, Piedmont, Nole Canavese; Pliocene (Ca. 3 Myr)	CCN0607	8	*reuteriana*
TA_3	Italy, Piedmont, Levone; Pliocene (Ca. 4 Myr)	CCN1817	10	*reuteriana*
Taxon B				
TB_1	Italy, Carmagnola; Middle or Late Pleistocene (Ca. 0.8-0.02 Myr)	CCN1350	10	*elata*
TB_2	Italy, Zubiena; Middle Pleistocene (Ca. 0.8-0.15 Myr)	CCN1017	13	*elata*
TB_3	Italy, Buronzo; Early Pleistocene (Ca. 2-1.5 Myr)	CCN0695	40	*elata*
TB_4	Italy, Casnigo; Early Pleistocene (Ca. 2 Myr)	CCN1352	18	*elata*
TB_5	Germany, Neumark-North; Middle Pleistocene (Ca. 0.5-0.15 Myr)	Cenophytic collection, Museum für Naturkunde, Berlin	2	*elata*
Taxon C				
TC_1	Italy, Ranica; Early Pleistocene (Ca. 1.1-1.0 Myr)	CNR-IDPA Coll. Bergamo	1	*nigra*
TC_2	Italy, Steggio; Early Pleistocene (Ca. 1.8-1.0 Myr)	CCN1483	3	*nigra*

Table 3. Characters and states observed for the morphological characterization of the achenes and scoring for morphological analysis. For a further explanation of the characters see the heading "Modern materials" in Results and Figures 1-2. Reference achenes RA1-6 are shown in Figures 3-13.

Character	**States**
Epidermal cells size[1]	Distinctly visible at 6.4×, as in RA1 (0); poorly visible at 16×, as in RA2 (1); distinctly visible at 40×, as in RA4 (2); poorly visible at 40×, as in RA6 (3)
Outer layer consistency (outer anticlinal walls)	Mucilaginous (0); ephemeral thin-membranaceous (1); chartaceous (2); vitreous (3); coriaceous (4)
Anticlinal walls protrusion	Scarce or null protrusion as in RA6 (0); medium protrusion as in RA2 (1); or clearly protruding as in RA1 (2)
Silica bodies size	Visible at 20× in all samples (0); visible at 40× in all samples (1); visible at 40× after removal of the outer cell wall (2); not visible at 40× even after removal of the outer cell wall (3)
Pericarp thickness	Thin (<0.03mm) and flexible as in RA6 (0); thick (≥0.03mm) and rigid as in RA2 (1)
Utricle remains at the base of manipulated achenes	None (0); a few (1); abundant (2)
Callus	Absent (0); thin as in RA5 or less (1); absent or thin but never thick (2); thick as in RA3 or more (3); thick or thin but never absent (4)
Callus granulose	Granulose (1); or not (0)
Style fracture	Tending to break at the base (0); tending to break at 1/10-1/5 achene length (1)
Style lignification	Only lignified at the base (less than 1/4 achene length) (0); much lignified in the basal half and poorly lignified in the distal one (1); much lignified for most its length (2)
Style shape	Inconspicuous to shortly cylindrical and truncate (0); shortly cylindrical, acute (1); shortly conical (2); cylindrical in the basal half, soft and wrinkled in the apical half (3); longely subcylindrical, attenuated towards the apex (4); longely cylindrical (5)
Length	Mean length less than RA4, 1.9 mm (0); or greater (1)
Length-width ratio (L:W)	Mostly equal to 1.0 (1); or not (0)
Outline (see Fig 2)	Elliptic (0); mostly narrow obovate (1); mostly obovate (2); mostly wide obovate (3); mostly circular (4); from elliptic to obovate (5)
Flexa in outline	Only one flexum (0); two flexa (1)
Apical angle	Mostly 90-120º (0); mostly 120-160º (1); mostly 140-180º (2)

Basal angle	Equal to 40-60º (1); greater than 60º (0)
Base relative size	1/4 achene width approx. (0); 1/2 achene width approx. (1)
Base slightly winged	Slightly winged (1); or not (0)
Sub-stipitate base	Null in most specimens (1); present in some specimens (0)
Real short stipe at the base	Present (1); or not (0)
Achene inflated	Inflated (1); or not (0)
Median depression	Present in some achenes (1); or not (0)
Invaginations	Present (1); or not (0)
Folds and pits	Present (1); or not (0)

[1]For specimens with ambiguous assignation, the largest cell size was scored.

Table 4. Main carpological differences between *Carex* sect. *Phacocystis* taxa and other potentially confusing taxa

Taxon	Style junction	Outer epidermal cell wall	Inner epidermal cell wall	Cells below the epidermal cell layer	Achene/utricle attachment
Section *Phacocystis* (incl. section *Tuminenses*)	Not-jointed, mostly much lignified in the basal part, but often poorly lignified in the apical part.	Variable.	Cells mostly sub-isodiametric, but in clade VII, clearly longer than wide. In Clade IV and VII cell size as in RA6, in clades I and II mostly larger cell sizes (as in RA1, RA2, RA4).	Very long, not measurable at the stereomicroscope.	Easily detached from utricle and without any remain, or more or less strongly attached, thus with scarce to abundant utricle remains.
C. bicolor	Sub-jointed: the style base is made of a strong lignified basal portion (1/8 of achene length), and a long apical portion which becomes soft when stored in water for 1 minute.	Mucilaginous.	Cells slightly longer than wide, mean size larger than in any member of section *Phacocystis*.	Very long, not measurable at the stereomicroscope.	Easily detached from utricle without any remain.
C. capitata	Jointed to the base.	Thin-membranaceous, ephemeral.	Cells sub-isodiametric, size as in RA6.	Very long, not measurable at the stereomicroscope.	Easily detached from utricle without any remain.
C. saxatilis	Not-jointed.	Thin-membranaceous, ephemeral.	Cells sub-isodiametric, poorly visible, size as in RA4.	No longer than 1/5 achene length.	Easily detached from utricle without any remain.
Section *Forficulae*	Style not-lignified, leaving a small apical notch on the apex of the achene.	Thin-membranaceous, ephemeral.	Cells clearly longer than wide, size as in RA6,	Very long, not measurable at the stereomicroscope.	Easily detached from utricle without any remain.
Section *Praelongae*	Not-jointed. much lignified in the basal part and poorly lignified in the apical part.	Mucilaginous in *C. dimorpholepis* and *C. phacota*; in *C. gynandra*	Cells sub-isodiametric, smaller (in *C. gynandra*) or larger (in *C. dimorpholepis*) than in any section	Very long, not measurable at the stereomicroscope.	Easily detached from utricle without any remain in *C. gynandra,* or more strongly

	thin-membranaceous, ephemeral.	*Phacocystis* sample; thick marginal sclerenchyma, which in *C. phacota* is raised resulting in a surrounding ridge.
		attached, with scarce utricle remains, in *C. dimorpholepis* and *C. phacota.*

Table 5. Summary of the achene types detected and its main morphological features. Samples nomenclature is according to Table 2. ECS = epidermis cell size; ECW = external cell wall according to a reference achene; SBS = silica bodies size (observable at magnification rate); AWP = anticlinal walls protrusion according to a reference achene; PT = pericarp thickness; UR = utricle remains at the base. In CS and AWP, data within brackets represent alternative, much rarer states. For a further explanation of the characters, see the heading "Modern materials" in Results.

Type	**Samples** (Figure numbers)								
	ECS	**ECW**	**SBS**	**AWP**	**PT**	**UR**	**Callus**	**Style**	**Additional characters**
***C. bigelowii* type**	**Big_1** (Figs. 15-16, 52)								
	<RA6	Mucilaginous	>40×	RA6	Thick	No	Absent	Soft, non-preservable, only a very short remains	Achene with a very short, real stipe, separated from the achene
***C. reuteriana* type**[1]	**Pan_1** (Figs. 17, 58-59), **Reu_1-2** (Figs. 18-19, 55-56), **Mau_1** (Figs. 20, 57)								
	RA6	Membranaceous ephemeral	>40×	RA6	Thin	Few	Absent	Lignified only at the base or in the basal half, shortly cylindrical	Transverse striation in inner pericarp a submerged in water
***C. paleacea* type**	**Pal_1** (Figs. 21-22, 53-54)								
	RA6	Membranaceous ephemeral[2]	>40×	RA6	Varia ble	Few	Absent	Much lignified for most its length, cylindrical	Lateral invaginations
***C. elata* type**	**Ela_1-9** (Figs. 23-24, 64, 66), **Oms_1** (Fig. 25, 65)								
	RA4	Chartaceous	40×	RA2	Thick	Few	Thin	Much lignified in the basal half, rarely only at the base (Oms_1), soft and wrinkled toward the apex	Outline wide obovate[3], L:W mainly 1. 1.3; mostly 2 flexa
***C. cespitosa* type**	**Ces_1-4** (Figs. 26-27, 60-61)								
	RA4	Chartaceous	40×	RA2	Thick	Few	Thin	Much lignified for most its length, subcylindrical	Achene inflated; outline circular[3], L:W mainly 1.0-1.2; only 1 flexum
***C. dacica* type**	**Dac_1** (Fig. 28, 62-63)								

	RA4	Chartaceous	40×	RA2	Thick	Few	Thin	Lignified only at the base, cylindrical	Outline from elliptic to narrow obovate[3], L:W variable; 2 flexa; base slightly winged
C. orbicularis	**Orb_1** (Figs. 47, 78-79)								
type	RA4	Chartaceous	40×	RA2	Thick	Few	Thin	Lignified only at the base, cylindrical	Outline circular[3]; L:W mainly 1.0-1.1; 1-2 flexa
***C. nigra* type**	**Nig_1-10** (Figs. 29-30, 67-69)								
	RA2-RA4	Chartaceous	40×	RA2	Thick	Abundant	Thin to thick	Only lignified at the base, with a short-acute remain after hand rubbing or dacay; rarely more lignified and subcylindrical	Length mostly < RA4
***C. aquatilis* type**	**Aqu_1-2** (Figs. 39, 71), **Sub_1** (Figs. 40, 80, 83), **Rec_1** (Fig. 41, 75-76)								
	RA4	Vitreous	40×	RA6	Thick	Few	Absent	Much lignified in the basal half, cylindrical	Silica bodies only visible after remova outer cell walls;in *C. subspathacea* an *recta*, lateral invaginations and/or sma folds and furrows
***C. lyngbyei* type**	**Lyn_1** (Figs. 42, 81-82, 84)								
	RA4	Ephemeral membranaceous	40×	RA6	Thick	Few	Absent	Much lignified for most its length, cylindrical	Lateral invaginations and/or small fold and furrows
***C. trinervis* type**	**Tri_1** (Figs. 35, 86)								
	RA4	Coriaceous	>40×	RA2	Thick	Few	Thin	Mostly only lignified at the base, conical	Epidermis cell pattern close to RA1
***C. buekii* type**	**cfBue_1-3** (Fig. 36), **Bue_1-4** (Figs. 44,46, 72-73), **Ran_1-2** (Figs. 43, 85)								
	RA2 (RA4)	Chartaceous	40x	RA2	Thick	Few	Thin to absent	Only lignified at the base, shortly cylindrical to conical	Narrow obovate to obovate[3]; 1 or 2 obscure flexa.
***C. kurdica* type**	**Kur_1** (Figs. 37-38, 74, 77)								

	RA2	Chartaceous	40x	RA2	Thick	Few	Thin	Lignified in the basal half; subcylindrical	Narrow obovate[3]; 1 flexum
***C. acuta* type**	**Acu_1-7** (Figs. 48-51, 87-90), **cfRan_1** (Fig. 70)								
	RA2	Chartaceous	40×	RA2 (RA6)	Thick	Abundant	Thin to thick	Lignified in its entire length, cylindrical, rarely attenuated	L:W ≠ 1.0-1.1; mean length similar to RA4
***C. rufina* type**	**Ruf_1** (Figs. 45, 91)								
	RA2	Chartaceous	>40×	RA1	Thick	Abundant	Thick	Only lignified at the base, shortly cylindrical	Inconstant presence of a median furrow

[1] The different taxa that display this type seem to be distinguished for minor achene characters, as reported in Appendix S1.

[2] Despite our sample having an ephemeral-membranaceous external cell wall, this does not seem to be representative of the entire variation in the species, since Nilsson and Hjelmquist (1967), Berggren (1969) and Haines (2000) describe the achenes of this species as lustrous, which is equivalent to a vitreous external cell wall. We suggest assigning achenes with either ephemeral-membranaceous or vitreous external cell wall to the *C. paleacea*-type, given that all the other characters agree with the above description.

[3] See Fig. 1 for outline definition.

Fig. 1. Parts and main outline traits in biconvex *Carex* sect. *Phacocystis* achenes. Nomenclature according to Berggren (1969) unless explicitly stated. 1, achene body; 2, achene base; 3, style base; AA, apical angle; AF, apical flexum; AI, apical introflession; BA, basal angle; BF, basal flexum; BI, basal introflession; BS, basal scar; CA, callus; SB, sub-stipitate base (Hurd et al., 1998); ST, style. Reference subdivisions of the achene length (1/8, ¼, etc.) are shown to the right.

Fig. 2. The most common simple symmetrical shapes detected within the achene populations studied, with reference numbers selected from Berggren (1969): 4, elliptic; 6, circular; 48, narrowly obovate; 49, obovate; 50, broadly obovate.

Figs. 3-13. Stereomicroscope pictures of the six reference-achenes (RA1-6) used for estimation of mean cell size, anticlinal walls protrusion, and callus dimensions of all the other samples. Figs. 3, 4. RA1 = *C. rufina* (Ruf_1), anticlinal walls protruding more than in all other RAs, epidermal cells quite apparent. Figs. 5, 6. RA2 = *C. acuta* (Acu_1) treated with bleach for 24 hours: notice the absence of basal callus (arrow in Fig. 5) and short remain of style, as well as the complete disappearance of the epidermal layer in two patches; anticlinal walls protruding less than in RA1, epidermal cell less apparent than in RA1. Figs. 7, 8. RA3 = *C. nigra* from a soil sample (Nig_6), notice the thick basal callus (arrow): CR = callus relief, CT = callus thickness. Figs. 9, 10. RA4 = *C. elata* ssp. *elata* (Ela_8), epidermal cells less apparent than in RA2, anticlinal walls protruding more or less as in RA2. Fig. 11. RA5 = *C. elata* ssp. *elata* from a soil sample (Ela_9), notice the still thin basal callus (arrow), even if this is one of the thickest observed in this species. Figs. 12, 13. RA6 = *C. reuteriana* ssp. *reuteriana* (Reu_1), epidermal cell size very small, slightly smaller than in RA4, anticlinal walls protruding less than in RA2, essentially barely visible. Continuous scale bar = 1 mm, dashed bar = 0.5 mm (only Figs. 4, 6, 8, 10, 13).

Fig. 14. Achenes picked out from a soil sample collected under an isolated population of *C. elata* ssp. *elata* at San Carlo Canavese, NW Italy (Ela_1). Notice the extreme variability of the outline and dimensions. The fruit sampling from living specimens collected in May 2012 showed that individual plants produce clearly less variable achenes, this means that the consistent achene diversity observed in this soil sample can be attributed to a large variation among the different individuals (ca. 200 in total) forming the same population.

Figs. 15-33. Stereomicroscope pictures of modern achenes of *Carex* sect. *Phacocystis*: *C. bigelowii* s.s., Figs. 15-16 (Big_1); *C. panormitana*, Fig. 17 (Pan_1); *C. reuteriana* ssp. *reuteriana,* Fig. 18 (Reu_1), Fig. 19 (Reu_2, soil sample); *C. reuteriana* ssp. *mauritanica*, Fig. 20 (Mau_1); *C. paleacea*, Figs. 21-22 (Pal_1); *C. elata* ssp. *elata*, Fig. 23 (Ela_8), Fig. 24. (Ela_1, soil sample; notice the strongly lignified, cylindrical base of the style (arrow), with a soft and wrinkled portion at the top); *C. elata* ssp. *omskiana*, Fig. 25 (Oms_1); *C. cespitosa*, Figs. 26-27 (Ces_2) *C. dacica*, Fig. 28 (Dac_1); *C. nigra*, Figs. 29-30 (Nig_3), Fig. 31 (Nig_7), Figs. 32-33 (Nig_4), notice the abundant utricle remains at the base (arrow below) and the short-acute remain of the style (arrow above). Scale bar = 1 mm**.**

Figs. 34-51. Stereomicroscope pictures of modern achenes of *Carex* sect. *Phacocystis*: *C.* cf. *randalpina*, Fig. 34 (cfRan_1); *C. trinervis*, Fig. 35 (Tri_1)*; C.* cf. *buekii*, Fig. 36 (cfBue_1). *C. kurdica*, Figs. 37-38 (Kur_1); *C. aquatilis*, Fig. 39 (Aqu_1); *C. subspathacea*, Fig. 40 (Sub_1); *C. recta*, Fig. 41 (Rec_1); *C. lyngbyei*, Fig. 42 (Lyn_1); *C. randalpina*, Fig. 43 (Ran_1); *C. buekii*, Figs. 44, 46 (Bue_1); *C. rufina*, Fig. 45 (Ruf_1); *C. orbicularis* ssp. *kotschyana*, Fig. 47 (Orb_1); *C. acuta*, Fig. 48 (Acu_4), Fig. 49 (Acu_3), Fig. 50 (Acu_6), Fig. 51 (Acu_2). Scale bar = 1 mm**.**

Figs. 52-67. Detailed stereomicroscope pictures of modern achenes of *Carex* sect. *Phacocystis*: *C. bigelowii* s.s. Fig. 52 (Big_1); *C. paleacea*, Fig. 53, achene base and attachment to utricle (Pal_1), Fig. 54, cell pattern and style (Pal_1); *C. reuteriana* ssp. *reuteriana*, Fig. 55 (Reu_2, soil sample), Fig. 56 (Reu_1); *C. reuteriana* ssp. *mauritanica.* Fig. 57 (Mau_1); *C. panormitana*, Fig. 58, cell pattern (Pan_1), Fig. 59, style (Pan_1); *C. cespitosa*, Fig. 60, cell pattern (Ces_2), Fig. 61, achene base (arrow) and attachment to utricle (Ces_2); *C. dacica*, Fig. 62, cell pattern and detail of the slightly winged achene base (arrow) (Dac_1), Fig. 63, style (Dac_1); *C. elata* ssp. *elata*, Figs. 64, 66 (Ela_9); *C. elata* ssp. *omskiana*, Fig. 65 (Oms_1); *C. nigra*, Fig. 67 (Nig_4). Scale bar = 1 mm**.**

Figs. 68-84. Detailed stereomicroscope pictures of modern achenes of *Carex* sect. *Phacocystis*: *C. nigra*, Figs. 68-69 (Nig_4);. *C.* cf. *randalpina*, Fig. 70 (cfRan_1); *C. aquatilis*, Fig. 71 (Aqu_1); *C.* cf. *buekii,* Figs. 72-73 (cfBue_1); *C. kurdica*, Fig. 74, 77 (Kur_1); *C. recta*, Figs. 75-76 (Rec_1); *C. orbicularis* ssp. *kotschyana*, Figs. 78-79 (Orb_1); *C. subspathacea*, Fig. 80, 83 (Sub_1); *C. lyngbyei*, Figs. 81, 82, 84 (Lyn_1). Continuous scale bar = 1 mm, dashed bar (only Figs. 73, 81) = 0.5 mm.

Figs. 85-92. Detailed stereomicroscope pictures of modern achenes of *Carex* sect. *Phacocystis* and *C. echinata* Murray (subgenus *Vignea*): *C. randalpina*, Fig. 85 (Ran_1); *C. trinervis*, Fig. 86 (Tri_1); *C. acuta,* Fig. 87 (Acu_1), achene in water, observation under strong oblique light after 30 seconds: notice the transverse striation; Fig. 88 (Acu_4), RA2-sized cells with walls protruding as in RA2 and thin basal callus; Fig. 89 (Acu_6), RA2-sized cells with walls protruding as in RA6 and thicker basal callus; Fig. 90 (Acu_1), RA2-sized cells with walls protruding as in RA2, *C. rufina*, Fig. 91 (Ruf_1); *C. echinata*, Fig. 92, notice the jointed style (arrow).

Figs. 93-102. Stereomicroscope pictures of fossil achenes assigned to *Carex* sect. *Phacocystis*. Taxon A, Figs. 93 (TA_2), 94-95 (TA_1), 96 (TA_2), 97 (TA_2), 98 (achene base without callus (arrow); TA_2). Taxon B, Figs. 99 (TB_3), 100-101 (TB_5), 102-103 (TB_3). Continuous scale bar = 1 mm, dashed bar (only Figs. 97, 98, 101, 102) = 0.5 mm.

Fig. 104. SEM pictures of a fossil achene (TC_1) assigned to *C. nigra* (a) entire achene; b) detailed cell pattern with apparent anticlinal walls.

Fig. 105. Hierarchical clustering from UPGMA using Gower's coefficient calculated using 25 variables and 67 different locations of the *Carex* sect. *Phacocystis* samples analysed in this study. AU and BP supports are given above and below the branches respectively (or indicated with an arrow if no room is available). Fossil samples are indicated in bold. Achene types found in more than two samples are depicted using brackets. Gaps in the brackets indicate the presence of an additional achene-type included within the other more broadly defined types.

Appendix I. Key to distinguish the section *Phacosystis* from the other potentially confusing di-stigmatic groups and taxa.

1. Style not lignified, leaving a small apical notch on the apex of the achene

Section *Forficulae*

1. Style at least partly lignified, not leaving a notch on the achene when removed 2

2. Style jointed to the base *C. capitata*, and subgenus *Vignea*

2. Style not jointed to the base 3

3. Style distinctly thickened towards the base Section *Graciles*

3. Style not conspicuously thickened at the base 4

4. Inner epidermal cell wall isodiametric to sub-isodiametric 5

4. Inner epidermal cell wall longer than wide 7

5. Cells below the epidermal cell layer no longer than 1/5 achene length *C. saxatilis*

5. Cells below the epidermal cell layer much longer 6

6. Outer epidermal cell-wall mucilaginous to thin-membranaceous; achene easily detached from utricle, without or with scarce utricle remains; epidermal cells size larger than RA1 or smaller than RA6 (in the studied taxa) Section *Praelongae*

6. Outer epidermal cell-wall from mucilaginous to vitreous; achene attachment to utricle variable, from weak and thus without utricle remains, to strong and with abundant utricle remains; epidermal cells size between RA1 and RA6 Section *Phacocystis*, and section *Tuminenses*

7. Outer epidermal cell-wall mucilaginous; inner epidermal cell wall size larger than RA1

C. bicolor

7. Outer epidermal cell-wall membranaceous-ephemeral; inner epidermal cell wall size as in RA6 Section *Phacocystis* (*C. reuteriana*-type)

Appendix S1. Table with the morphological data as analysed for the clustering analysis. Samples are presented in rows. Characters are presented in columns. An additional row including the number of achenes studied in each sample is also included. Codification of the morphological characters follows Table 3.