



## A reappraisal of the *Carex arenaria* complex in Flanders (Belgium)

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**Illustrations** by the authors (Fig. 1-3, 9: DDB; Fig. 4-8: IVdB) and H. Engledow (BR; Fig. 10).

**ABSTRACT.** – The taxonomy of *Carex* section *Ammoglochin* is complex due to the faint morphological species boundaries and overlapping ecological niches. This study focusses on species boundaries within the *C. arenaria* complex, in particular *C. arenaria*, *C. brizoides* and *C. pseudobrizoides*. *Carex pseudobrizoides* is morphologically very similar to *C. arenaria*, but also shares some features with *C. brizoides*, which has sometimes led to the assumption of a hybridogenic origin. We studied the morphology, ecology and distribution of these species in Flanders, and combined this with DNA sequence data (plastid encoded matK and nuclear rDNA ITS) from a large number of specimens. Our results do not provide evidence for a hybridogenic origin of *C. pseudobrizoides* (although it cannot be rejected either), but instead indicate possible conspecificity or a very recent divergence of *C. pseudobrizoides* and *C. arenaria*, which remains undetected by the two genetic markers that were used. Although there are still outstanding questions, our results further improve our understanding of species boundaries in this species complex in Europe, and highlight the need for further investigations using more variable molecular markers.

**SAMENVATTING.** – Een nieuwe evaluatie van het *Carex arenaria*-complex in Vlaanderen (België). De taxonomie van *Carex* sectie *Ammoglochin* is complex vanwege vage morfologische soortafgrenzingen en overlappende ecologische niches. Deze studie richt zich op de soortafgrenzing binnen het *C. arenaria*-complex, in het bijzonder tussen *C. arenaria*, *C. brizoides* en *C. pseudobrizoides*. *Carex pseudobrizoides* lijkt morfologisch sterk op *C. arenaria*, maar deelt ook enkele kenmerken met *C. brizoides*, waardoor soms een hybridogene oorsprong wordt aangenomen. We bestudeerden de morfologie, ecologie en verspreiding van deze soorten in Vlaanderen, en combineerden dit met DNA-sequentiegegevens van een groot aantal specimens (het chloroplast gen matK en de nucleaire merker rDNA ITS). Onze resultaten leveren geen bewijs voor een hybridogene oorsprong van *C. pseudobrizoides* (hoewel die evenmin kan uitgesloten worden), maar duiden eerder op een mogelijke conspecificiteit of een zeer recente divergentie van *C. pseudobrizoides* en *C. arenaria* (niet detecteerbaar door de twee gebruikte genetische markers). Hoewel een aantal vragen onbeantwoord blijft, werpen onze resultaten nieuw licht op de soortafgrenzing binnen dit soortencomplex in Europa. Verder tonen ze de noodzaak aan van verder onderzoek met behulp van meer variabele moleculaire markers.

**RÉSUMÉ.** – Une réévaluation du complexe de *Carex arenaria* en Flandre (Belgique). La taxonomie de la section *Ammoglochin* du genre *Carex* est complexe en raison des faibles limites morphologiques des espèces et des niches écologiques qui se chevauchent. Cette étude se concentre sur les limites d'espèces au sein du complexe *C. arenaria*, en particulier sur *C. arenaria*, *C. brizoides* et *C. pseudobrizoides*. *Carex pseudobrizoides* est morphologiquement très similaire à *C. arenaria*, mais partage également certaines caractéristiques avec *C. brizoides*, ce qui a parfois conduit à supposer une origine hybridogène. Nous avons étudié la morphologie, l'écologie et la distribution de ces espèces en Flandre et nous les avons combinées avec des données de séquences d'ADN (le gène chloroplastique matK et le marqueur nucléaire ADN ITS) d'un grand nombre de spécimens. Nos résultats ne prouvent pas une origine hybridogène de *C. pseudobrizoides* (bien qu'elle ne puisse pas non plus être rejetée), mais indiquent plutôt une possible conspécificité ou une divergence très récente de *C. pseudobrizoides* et *C. arenaria*, qui reste non détectée par les deux marqueurs génétiques utilisés. Nos résultats améliorent notre compréhension des limites d'espèces dans ce complexe en Europe et soulignent la nécessité de poursuivre les recherches à l'aide de marqueurs moléculaires plus diversifiés.

## Introduction

*Carex* L. section *Ammoglochin* Dum. comprises six species in Europe (Chater 1980): *Carex arenaria* L., *C. ligerica* J. Gay (currently accepted name: *C. colchica* J. Gay), *C. reichenbachii* Bonnet (currently accepted name: *C. pseudobrizoides* Clavaud), *C. repens* Bellardi, *C. praecox* Schreber and *C. brizoides* L. According to Koopman & Więclaw (2016) *C. curvata* Knaf also belongs in this section. Within the *Vignea* subgenus, the section is primarily characterized by the long, crawling rhizomes from which flowering or sterile shoots develop at regular distances. These long rhizomes enable the species to quickly occupy large surfaces. Once established, such a colony can form a dense mat of interconnected rhizomes, which may hinder the growth of other species, including shrubs and small woody plants (Weeda *et al.* 1994).

In Belgium, four species of this section occur: *Carex arenaria*, *C. brizoides*, *C. curvata* [syn.: *C. praecox* subsp. *intermedia* (Čelak.) W. Schultze-Motel] and *C. pseudobrizoides* (Lambinon & Verloove 2012). Their distribution and conservation status are quite different in Flanders and Wallonia (Table 1).

*Carex arenaria* is common on sandy soils throughout Flanders. In the coastal dunes it is almost omnipresent and in the Kempen (provinces of Antwerp and Limburg) it is also very common. Elsewhere it can be found on sandy

**Table 1.** Red list status of the species of the *Carex arenaria* complex occurring in Flanders (Van Landuyt *et al.* 2006b) and Wallonia (Anon. 2020). LC = least concern (currently not threatened); NT = near threatened; EN = endangered; CR = critically endangered; RE = regionally extinct, NE = not evaluated (because presumably non-native).

Name	Red list status	
	Flanders	Wallonia
<i>Carex arenaria</i>	LC	CR
<i>Carex brizoides</i>	NE	EN
<i>Carex curvata</i>	–	CR
<i>Carex pseudobrizoides</i>	NT	RE

river dunes, sand raised sites, etc. It clearly avoids loamy soils; as a consequence it is absent from most of the southern part of Flanders (Van Landuyt *et al.* 2006a). The same goes for Wallonia, where the species is only common west of Mons (e.g. Mer de Sable in Stambruges); elsewhere it is virtually absent [<http://biodiversite.wallonie.be/fr/atlas-en-ligne.html?IDD=6056&IDC=807>]. This species sets quite high demands to be able to germinate, but once established it can withstand long periods of drought (Weeda *et al.* 1994). Usually, it flowers profusely (Fig. 1), but plants growing in shady places can be very reluctant to flower. This can lead to confusion with *C. pseudobrizoides*.



**Figure 1.** Typical habitat of *Carex arenaria* in the edge of a pine forest. (Vorselaar, Sassenhout, 12.07.2017)





**Figure 2.** Typical habitus of *Carex brizoides*. (Brecht, Kooldries, 12.07.2017)

*Carex brizoides* is a species of clearings in deciduous woodland on rather acidic soils with shallow stagnant water (Weeda *et al.* 1994). It is easily distinguished from the other species in this section based on its small inflorescences, the small and indistinctly veined utricles and the colorless glumes of the female florets (Lambinon & Verloove 2012). Its residence status long remained questionable in Flanders. Van Landuyt *et al.* (2006b) did not evaluate its conservation status because there was reasonable doubt as to whether the populations known at that time were truly native. At present, however, there is no longer doubt about the species being indigenous to Flanders. The ecological circumstances observed in a population in Opoeteren – on the banks of a meandering stream in alder woodland – perfectly agree with those found for this species in Central Europe (Weeda *et al.* 1994). In some other Flemish populations, however, an older introduction is more likely, e.g. along a former railway track in Torhout. The leaves of this species were formerly used to fill mattresses and cushions of chairs and seats as a cheap alternative for horsehair or seagrass (*Zostera* div. sp.) (Hohla 2014). *Carex brizoides* is very rare in Flanders. To date, about ten populations have been known. In Wallonia, the species is a little less rare. According to the current distribution map, it has been found in about 20 localities, all south of Sambre-and-Meuse [http://biodiversite.wallonie.be/fr/atlas-en-ligne.html?IDD=6056&IDC=807]. The species is reputed for being very reluctant to flower (Fig. 2), and some populations are known to have seemingly not flowered for several years. The low flowering rate, however, can be partly attributed to the flowering stems that tend to bend back to the ground, together with the leaf, and thus remain hidden from view. A careful inspec-

tion among the leaves lying on the ground, often yields surprisingly many inflorescences. Just like in *C. pseudobrizoides*, large populations often show a kind of 'woven' pattern, with some parts of the plants 'combed' in one direction and others in another (Fig. 2).

Two subspecies of *Carex praecox* are mentioned by Lambinon & Verloove (2012). The nominal subspecies, however, is only known from a few localities in France (Tison & de Foucault 2014) and the Netherlands (Koopman & Więclaw 2016) and has never been reliably recorded in Belgium. A second subspecies, subsp. *intermedia*, is now considered to be a separate species, *C. curvata* (Więclaw *et al.* 2020). It was known since 1961 from only a single locality along the Meuse river in Dinant (De Langhe 1963, Lambinon & Verloove 2012). A targeted search by J. Koopman at the exact locality in 2017 proved the species to have disappeared. Its habitat, the artificial, concrete border of river Meuse, suggested a historical, unintentional introduction (pers. comm. J. Koopman). Since this species has never been recorded in Flanders, it was not considered in this study.

The genuine occurrence of *Carex pseudobrizoides* in Flanders was until recently very uncertain, mostly because most field botanists are not familiar with this species. According to Van Landuyt *et al.* (2006a) it was only known from seven 4 × 4 km squares, only one of them dating from before 1970. Like *C. arenaria*, *C. pseudobrizoides* prefers sandy soils and occurs in similar habitats such as dry forest edges, but also remarkably often in roadsides and field edges (Fig. 3).

Although the taxonomy of this species complex has been well studied based on morphology, the identity and species boundaries of these European species are





**Figure 3.** Copiously flowering population of *Carex pseudobrizoides* along a maize field; population known since at least 1956. (Oud-Turnhout, near bridge over river Aa, 12.07.2017)

not always clear and remain to be tested using molecular data. For *Carex* a number of markers have commonly been used in systematic studies. In particular, the plastid encoded *matK* has been found as a suitable marker for DNA-based identification (DNA barcoding) and species delimitation (Starr *et al.* 2009, Le Clerc-Blain *et al.* 2010). In systematic studies, the use of additional unlinked markers is advisable, and the ribosomal Internal Transcribed Spacer (ITS) has been put forward as a suitable candidate in conjunction with *matK* or other plastid markers (e.g. Roalson & Friar 2004, Villaverde *et al.* 2017). Currently, few molecular data are available for European species in the *C. arenaria* complex. *Carex arenaria* is best represented in public DNA sequence repositories (GenBank, EMBL-ENA) with about 40 available ITS and *matK* sequences, but only a few sequences are available for *C. brizoides*, *C. pseudobrizoides*, and *C. curvata*. In the present paper we discuss the identity and distribution of the Flemish representatives of the *Carex arenaria* complex, with emphasis on the rare species *C. brizoides* and *C. pseudobrizoides*, using a combination of morphological and molecular data. Given the little molecular data available for this species complex, our results will also improve our understanding of species boundaries in the *Carex arenaria* complex in Europe.

## Materials and methods

### • Taxon sampling

For the purpose of our study all localities of *Carex brizoides* and *C. pseudobrizoides* reported on the nature observations platform [waarnemingen.be](http://waarnemingen.be) (<http://waarnemingen.be>) were surveyed in 2017. Herbarium collections

were made and these are preserved in the herbarium of Meise Botanic Garden (BR). An overview of all localities and the corresponding herbarium collections is presented in Table 2.

### • DNA sequencing and phylogenetic analyses

Molecular phylogenetic analyses were based on *matK* and ITS sequences (Table 2). Total genomic DNA was extracted from silica-dried leaf material or herbarium material using a modified CTAB protocol (Doyle & Doyle 1987, Verloove *et al.* 2020). The *matK* gene was amplified using primers *matK* 2.1F and *matK* 5R (Ford *et al.* 2009, Starr *et al.* 2009), and the ITS region was amplified using primers ITS1 and ITS4 (White *et al.* 1990). PCR conditions are described in Verloove *et al.* (2020), and PCR quality control was performed with a BioAnalyzer (Agilent Inc.). After purification using ExoSAP-IT (ThermoFisher Scientific), PCR products were sent for sequencing to Macrogen (Seoul, South Korea). Sequences have been deposited in EMBL/GenBank under study number PRJEB59351 and sequence accession numbers OX420866-OX420898 (*trnK-matK* locus) and OX420899-OX420930 (ITS rDNA).

Two datasets of 46 *matK* (812 positions) and 62 ITS sequences (686 positions) were assembled, including the sequences generated in this study and publicly available sequences of species in the section *Ammoglochin* obtained from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>). We did not include outgroup species from the sister clade section *Remotae* (Jiménez-Mejías *et al.* 2016) because inclusion of these sequences introduced long branches in the phylogenies, possibly creating systematic error. For the same reason, we excluded the highly divergent se-

**Table 2.** Overview of studied populations of *Carex* section *Ammoglochin* in Flanders. Column 1 (DDB): collection number herbarium Dirk De Beer.

DDB	Occurrence	IFBL square	Barcode BR	Corrected identification
1654	Kasterlee, Oosteneind	C5.18.44	BR0000027301555V	<i>C. arenaria</i>
1686	Brecht, Groot Schietveld	B5.32.13	BR0000027301562V	<i>C. arenaria</i>
1688	Meerle, Elsakker	A5.55.21	BR0000027301579V	<i>C. arenaria</i>
1692	Vorselaar, Sassenhout	C5.25.41	BR0000027301586V	<i>C. arenaria</i>
1696	Tessenderlo, Gerhagen	D6.21.23	BR0000027301593V	<i>C. arenaria</i>
1701	Oostmalle, vliegveld (airfield)	B5.54.44	BR0000027301609V	<i>C. arenaria</i>
1702	Geel, Kievermont	C6.31.23	BR0000027301616V	<i>C. arenaria</i>
1703	Kapellen, Fort van Ertbrand	B4.36.44	BR0000027301661V	<i>C. arenaria</i>
1711	Oostmalle, vliegveld (airfield)	B5.54.44	BR0000027301654V	<i>C. arenaria</i>
1712	Oostmalle, vliegveld (airfield)	B5.54.44	BR0000027301647V	<i>C. arenaria</i>
1721	Zandvliet, Ruige Heide	B4.35.32	BR0000027301630V	<i>C. arenaria</i>
1722	Poederlee, Schrieken	C5.25.24	BR0000027301623V	<i>C. arenaria</i>
1723	Poederlee, Boskapel	C5.25.22	BR0000025968965V	<i>C. arenaria</i>
1733	Merksplas, Graafsbos	B5.36.22	BR0000025968972V	<i>C. arenaria</i>
1653	Brecht, Kooldries	B5.42.24	BR0000025968996V BR0000025969009V	<i>C. brizoides</i>
1693	Balen, Kanaal van Beverlo	C6.45.31	BR0000025969016V	<i>C. brizoides</i>
1697	Opoeteren, Volmolen	D7.14.24	BR0000025969023V	<i>C. brizoides</i>
1699	Leopoldsburg, Boskant	C6.56.31	BR0000025969030V	<i>C. brizoides</i>
1704	Grobbendonk, Engels Kamp	C5.34.24	BR0000025969047V	<i>C. brizoides</i>
1707	Aalter; Kraenepoel	D2.16.32	BR0000025969054V	<i>C. brizoides</i>
1724	Torhout, De Groene 62	D1.17.21	BR0000025969061V	<i>C. brizoides</i>
1761	Leopoldsburg, Boskant	C6.55.42	BR0000025968989V	<i>C. brizoides</i>
1655	Oud-Turnhout, Schuurhoven	B5.48.24	BR0000025969207V BR0000025969214V	<i>C. pseudobrizoides</i>
1656	Mol - Wezel, Kasteeldreef	C6.34.21	BR0000025969184V BR0000025969191V	<i>C. pseudobrizoides</i>
1689	Weelde, vliegveld (airfield)	B5.28.41	BR0000025969177V	<i>C. pseudobrizoides</i>
1690	Oud-Turnhout, Schuurhoven	B5.48.22	BR0000025969160V	<i>C. pseudobrizoides</i>
1691	Turnhout, Winkelsbroek	B5.58.33	BR0000025969153V	<i>C. pseudobrizoides</i>
1694	Oud-Turnhout, Lage Mierdse Heide	B6.31.33	BR0000025969146V	<i>C. pseudobrizoides</i>
1700	Arendonk, De Korhaan	B6.41.23	BR0000025969139V	<i>C. pseudobrizoides</i>
1705	Ravels, Gewestbossen	B6.31.13	BR0000025969122V	<i>C. pseudobrizoides</i>
1706	Ravels, Kijkverdriet	B6.31.32	BR0000025969115V	<i>C. pseudobrizoides</i>
1715	Ravels, Kijkverdriet	B6.31.32	BR0000025969108V	<i>C. pseudobrizoides</i>
1752	Kasterlee, Geelsebaan	C5.28.23	BR0000025969085V BR0000025969092V	<i>C. pseudobrizoides</i>
1785	Ravels, Kijkverdriet	B6.31.41	BR0000025969221V	<i>C. pseudobrizoides</i>

quences of *C. siccata* and *C. praecox* (ITS and matK), and *C. repens* (ITS). These species turned out to be not the closest relatives of *C. arenaria*, *C. brizoides* or *C. pseudobrizoides* in preliminary analyses, thus not impacting the conclusions of this study. Sequences of the two markers were aligned using MUSCLE (Edgar 2004) in Mega7 (Kumar *et al.* 2016). Maximum likelihood (ML) and rapid bootstrap analysis were performed using RAXML v. 8 (Stamatakis 2014) under the GTRCAT model.

## Results

### • *Carex pseudobrizoides* and similar species: morphological characteristics

*Carex pseudobrizoides* is a very poorly known species in Flanders (and elsewhere throughout its distribution range) which is frequently confused with *C. arenaria*. This may

partly be explained by its very local occurrence in Flanders, but is also a result of the overall strong resemblance to *C. arenaria*, not only morphologically but also ecologically. In almost all European floras, differences between the two species are not sufficiently weighted against each other and characteristics that are put forward usually are not relevant or too variable (Table 3).

*Carex brizoides*, on the other hand, is easily distinguished from *C. arenaria* and *C. pseudobrizoides* based on its small inflorescence, the small, indistinctly veined utricles and the colorless glumes (Fig. 4).

In practice, the gender distribution within the spikes appears to be the most reliable character to distinguish *Carex arenaria* and *C. pseudobrizoides*. In short, in *C. arenaria* the middle spikes are female at the base (Fig. 5), whereas in *C. pseudobrizoides* (and also in *C. brizoides*) they are male at the base (Fig. 6). This can be seen at a



**Table 3.** Comparison between *Carex arenaria*, *C. brizoides* and *C. pseudobrizoides*, according to [1] = Lambinon & Verloove (2012), [2] = Duistermaat (2020), [3] = Chater (1980), [4] = Tison & de Foucault (2014) and [5] = Koopman & Więclaw (2016). Relevant character states are given in bold preceded by the sign ●.

	<i>Carex arenaria</i>	<i>Carex pseudobrizoides</i>	<i>Carex brizoides</i>
● <b>Spikes: sex distribution</b> [1, 2, 3, 4, 5]	upper entirely ♂; middle ♀ at the base, ♂ higher up; lower ♀	middle ♂ at the base, ♀ higher up	♂ at the base, ♀ higher up
Ratio length/width utricle [1]	2/1	2.5–3/1	3–4/1
Nutlets [1, 2])	2 × 1.5 mm, trapezoidal	2 × 1 mm, narrow oval-egg-shaped	oval-egg-shaped
Rhizome width [1]	3–4 mm	max. 2 mm	max. 2 mm
● <b>Inflorescence length</b> [4]	> 25 mm	> 25 mm	< 25 mm
● <b>Glumes</b> [3, 4, 5]	tan	yellowish brown [3, 4], pale white-greenish [5]	white or straw-colored
Utricles [2, 3, 5]	distinctly veined	distinctly veined	indistinctly veined
Wing of utricle [1, 2, 4]	broadly winged, wing much wider in the middle	more narrowly winged, wing ± equally wide throughout	more narrowly winged, wing ± equally wide throughout

glance, even long after flowering, when only the bracts remain in the male flowers. These bracts then form a cuff around the base of the spikes, which gives them a typical upward curvature. Unfortunately, in most identification keys, this relatively straightforward character is either complicated by the unnecessary use of more extensive



**Figure 4.** Inflorescence of *Carex brizoides*, herbarium DDB 1653. (Brecht, Kooldries, 11.07.2016)



**Figure 5.** Inflorescence of *Carex arenaria*, herbarium DDB 1692. (Vörselaar, Sassenhout, 09.06.2017)

formulations (e.g. Koopman 2015). As a result, most field botanists are confused and rely on other, easier to observe but less reliable characteristics.

The length/width ratio of the utricles and the width of the wings of the utricles are very variable and overlap to such an extent that the three species cannot be reliably told apart based on that character (Fig. 7). In the literature, ‘typical’ utricles are invariably depicted to show these differences, but these are extreme rather than average forms. The same holds true for the size and shape of the nutlets (Fig. 8). Nonetheless, both Delay *et al.* (2016) and Koopman & Więclaw (2016) consider the width of the wing of the utricle as an important feature for distinguishing *C. arenaria* from *C. pseudobrizoides*.

The thickness of the rhizomes is irrelevant because it depends on edaphic factors. Moreover, well-developed rhizomes are often lacking in herbarium material.

It is often claimed that *C. pseudobrizoides* and *C. brizoides* rarely flower or do not flower at all. This is definitely incorrect and entirely depends on the locality. In heavily shaded places, all the species treated here, including *C. arenaria*, rarely flower. However, in full sunlight conditions with sufficient nutrient and water supply, both *C. pseudobrizoides* and *C. brizoides* can flower prolifically.



**Figure 6.** Inflorescence of *Carex pseudobrizoides*, herbarium DDB 1752. (Kasterlee, Geelsebaan, 23.06.2018)



**Figure 7.** Utricles of *Carex arenaria* (a), *C. pseudobrizoides* (b) and *C. brizoides* (c). All the same magnification and taken at random from 8 different specimens.



**Figure 8.** Nutlets of *Carex arenaria* (a), *C. pseudobrizoides* (b) and *C. brizoides* (c). All the same magnification and taken at random from 8 different specimens.

ly (Fig. 9). It has also been pointed out that the flowering stems of both *C. pseudobrizoides* and *C. brizoides* quickly bend towards the ground and are then almost impossible to find between the leaves and the surrounding vegetation.

In the online citizen science platform [waarnemingen.be](https://waarnemingen.be/) (<https://waarnemingen.be/>) several populations of *C. arenaria* were erroneously identified as *C. pseudobrizoides*. In most cases these records refer to plants from vast, rarely flowering populations in forest edges. *Carex brizoides* was always correctly determined and a number of the correctly determined populations of *C. pseudobrizoides* in the Turnhout area are from well-known populations.

A search for other diagnostic characters not yet men-

tioned in the literature, such as the length of the bracts in *C. pseudobrizoides*, and the leaf anatomy, were unfruitful. Nevertheless, Delay *et al.* (2016) were able to distinguish this species based on (microscopic) features in the leaf anatomy.

#### • Distribution and ecology of *Carex pseudobrizoides* in Flanders

Interestingly, all verifiable observations – historical (verified in the BR herbarium) as well as recent (verified in the field) – are confined to the Antwerp part of the Kempen, more specifically from the vicinity of Turnhout (municipalities of Turnhout, Oud-Turnhout, Ravels, Arendonk,





**Figure 9.** Copiously flowering population of *Carex brizoides*. Same population as in Fig. 2. (Brecht, Kooldries, 01.05.2022)

Mol and Kasterlee). All claims from outside this area are erroneous (Fig. 10).

*Carex pseudobrizoides* can sometimes form a very extensive population. A population along the Canal Des-sel-Schoten, near to the ‘Kijkverdriet’ nature reserve in Ravels and known for several decades, forms a virtually uninterrupted vegetation along the northern bank of the canal at least from the N12 to the N118 roads, over a length of five kilometers or even more.

In Wallonia this species has probably disappeared; all herbarium material in BR originates from a single locality in Gerpinnes (province of Hainaut), along the road Charleroi-Florennes, where it has not been observed for a long time (comm. J.M. Lecron 16.02.2023). The online Walloon distribution atlas refers to two recent sites from the Famenne (<http://biodiversite.wallonie.be/fr/atlas-en-ligne.html?IDD=6056&IDC=807>), but these claims could not be verified and require confirmation.

The ecology of *C. pseudobrizoides* in Flanders is

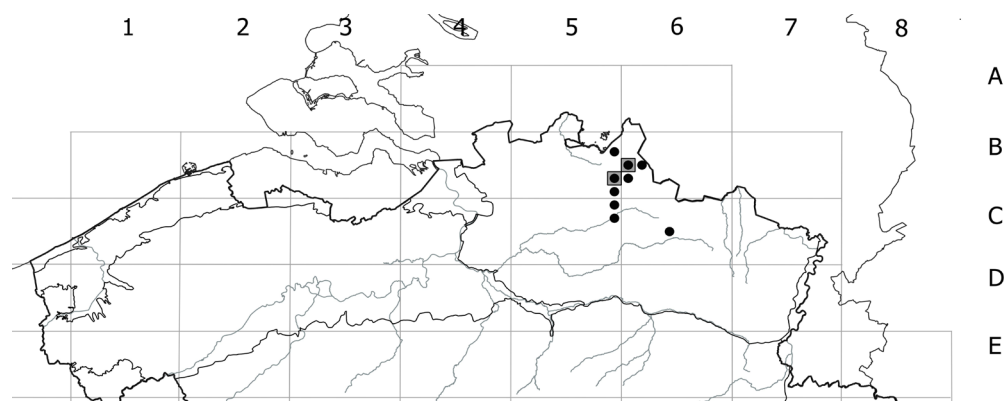
somewhat unclear. It seems to prefer more nitrophilous soils than *C. arenaria* and to be less shade tolerant than *C. brizoides*. However, in some places it occupies the same niche as *C. arenaria* (e.g. on the verge of pine forest) and, although exceptionally so, the same as *C. brizoides* (in swamps, e.g. in Mol-Wezel and in the Winkelsbroek reserve in Turnhout). Most striking are the populations in road verges, perhaps because these usually flower abundantly.

• *Molecular phylogeny of Carex pseudobrizoides and its relatives*

The maximum likelihood phylogenies inferred from the matK and ITS alignments were congruent, showing two main well supported clades (Fig. 11). One clade mainly consisted of specimens identified as *C. arenaria*, *C. pseudobrizoides* and *C. colchica*. Within this clade none of the species was found to form a monophyletic group. Instead, several accessions of *C. arenaria* and *C. pseudobrizoides*

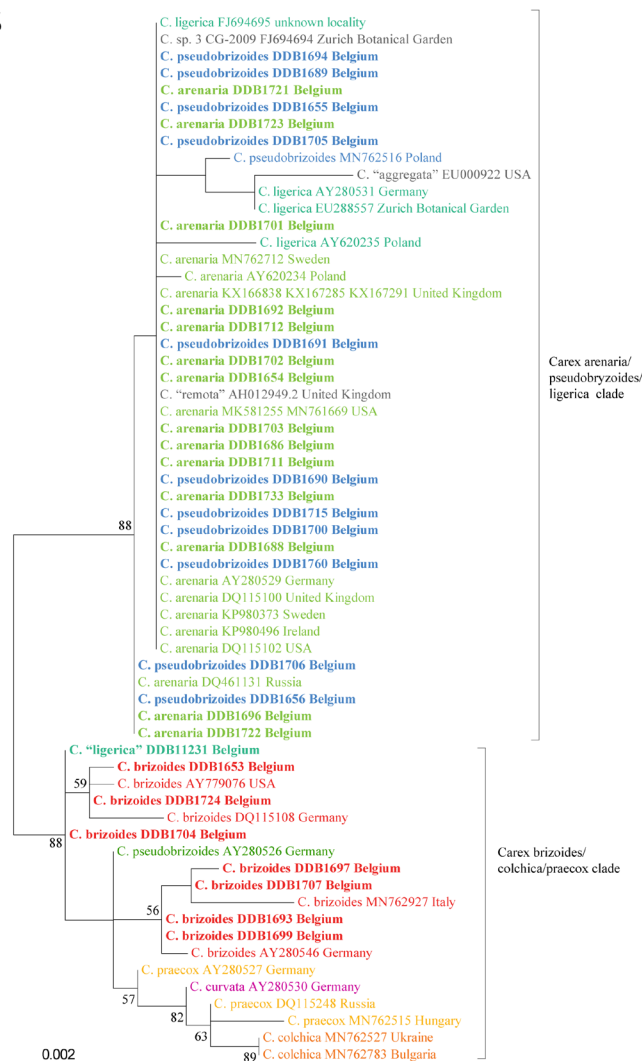
**Figure 10.** Distribution of *Carex pseudobrizoides* in Flanders.

Grey squares: before 2000; black dots (●): after 2000.

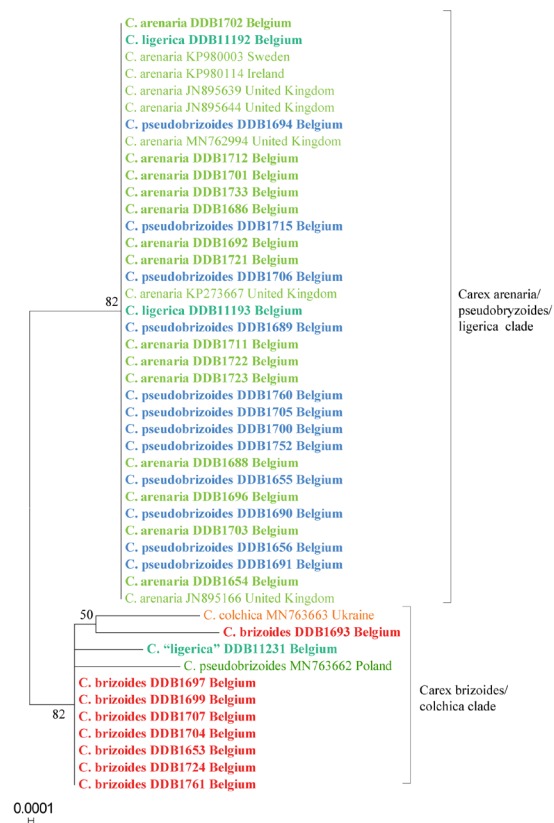




ITS



matK



**Figure 11.** Maximum likelihood (ML) trees of species in the *Carex arenaria* complex inferred from rDNA ITS and matK sequences. Sequences generated in this study are indicated in bold. Colors indicate the different morphospecies. ML bootstrap values (> 50%) are indicated at branches.

from different locations across Europe had identical ITS and matK sequences.

The other clade consisted of *C. brizoides*, *C. praecox*, *C. curvata*, and *C. colchica* (not all species represented by a matK sequence). These species were largely intermixed, thus not forming distinct subclades. This clade also includes one silica gel accession from the Netherlands – identified as *C. colchica* (DDB 11231) but whose identity in fact could not be verified – and specimens identified as *C. pseudobryzoides* from Germany (ITS tree) and Poland (matK tree). We were not able to verify the identity of these German (Hendrichs *et al.* 2004) and Polish (Martín-Bravo *et al.* 2019) accessions.

## Discussion

*Carex pseudobryzoides* is in many ways morphologically intermediate between *C. arenaria* and *C. brizoides* and a potential hybridogenic origin has been put forward by various authors (e.g. Chater 1980, Lambinon & Verloove

2012, Delay *et al.* 2016). In addition, *C. pseudobryzoides* has a much smaller area than the alleged parent species.

In a recent study, Zonneveld (2019) provides the genome sizes of the *Carex* species relevant for our study (Table 4). This information does not provide any argument to confirm the hybridogenic status of *Carex pseudobryzoides*. An F1 hybrid would be expected to have a weight that lies in the middle between the two alleged parent species. A ‘stabilized hybrid’, however, may have crossed further with one of the parents and can there-

**Table 4.** DNA weight of some Dutch *Carex* species (Zonneveld 2019).

	DNA weight (picogram)
<i>Carex arenaria</i>	0.86
<i>Carex brizoides</i>	0.73
<i>Carex pseudobryzoides</i>	0.72

fore shift in weight to that parent. However, according to Zonneveld (l.c.) *C. pseudobrizoides* and *C. brizoides* have more or less equal genome sizes that are different from that of *C. arenaria*.

In our molecular analysis, on the contrary, all our accessions of *C. pseudobrizoides* appear in a clade with accessions of *C. arenaria*. Judging from the origin of the material measured by Zonneveld, it can be assumed that the identification of the material is nevertheless correct. Zonneveld (2019) already stated that in *Carex* “species can unfortunately hardly be distinguished with flow cytometry”.

In section *Ammoglochin* the dominant cytotype is  $2n = 58$  and chromosome numbers are hardly suitable for species identification (Więclaw *et al.* 2020). A clearly different chromosome number only occurs in *C. repens* ( $2n = 70$ ), a species most probably of hybrid origin (Więclaw *et al.* 2020). *Carex pseudobrizoides* has a chromosome number of  $2n = 58$  (Rotreklová *et al.* 2011), the same as its putative parents.

Żukowski and Lembicz (2000) assessed genetic differences between *Carex arenaria*, *C. brizoides* and *C. pseudobrizoides* based on isozymatic phenotypes determined by electrophoresis of enzymatic proteins. Three enzyme markers were identified, distinguishing *C. pseudobrizoides* from its putative ancestors, *C. arenaria* and *C. brizoides*. This did not, however, enable the authors to verify the hybridogenic origin of *C. pseudobrizoides* as proposed by Egorova (1999).

Our molecular phylogenetic data does not provide evidence for a hybridogenic origin of *C. pseudobrizoides* either. A hybridogenic origin would be detectable in our phylogenies as incongruent positions of *C. pseudobrizoides* in the two trees, which are based on unlinked markers. Instead, the observation that *C. pseudobrizoides* and *C. arenaria* cluster in the same clade based on two unlinked markers, and the fact that accessions of the two species collected from different locations in Europe have identical ITS and matK sequences can be interpreted in two ways. Firstly, the two species could be regarded as conspecific. This is supported by the overall strong morphological resemblance between the two species, and similar ecological niche. Secondly, the two markers used may be too conservative to distinguish between closely related species. Although in a large scale regional study to test the performance of DNA barcoding markers, Le Clerc-Blain *et al.* (2010) found that matK resolves the greatest number of species of any single-locus (95%), and when combined in a two-locus barcode, it provides 100% species resolution, it is still possible that both markers were not able to detect very recently diverged species lineages. Whatever the case may be, *C. pseudobrizoides* and *C. arenaria* are certainly very closely related, and additional fine-grained multilocus data will be needed to untangle species boundaries within this complex.

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