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Interspecies hybrids in *Carex* sect. *Ceratocystis* (Cyperaceae) of Northwest Russia as revealed by analysis of the internal transcribed spacer (ITS1) and *trn*L sequences

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Summary. *Carex* sect. *Ceratocystis* is one of the most taxonomically complex groups in the genus. Resolving the phylogeny and systematics of this section is challenging due to frequent interspecific hybridisation and introgression. Most species belonging to this group can produce hybrids, suggesting that species boundaries are semipermeable. To trace hybridisation and introgression among the species of the sect. *Ceratocystis* from the territory of Northwestern Russia, we studied the intragenomic polymorphism of the internal transcribed spacer (ITS1) of the 35S rRNA genes in *Carex flava*, *C. serotina, C. scandinavica, C. hostiana*, and their putative hybrids (*C. flava* × *C. scandinavica, C. flava* × *C. serotina, C. hostiana* × ?) and traced maternal lineages using the chloroplast *trn*L marker. In addition, we evaluated comparative morphological data and pollen fertility for these taxa. It was found that pure species have a number of species-specific ribotypes, while putative hybrids have a set of ribotypes from different parents. Pure species always had a high percentage of fertile pollen, as did some hybrids, while other hybrids were completely sterile. PCA analysis based on morphology data showed three clusters: *C. serotina* together with *C. scandinavica*, hybrids, and *C. flava*; the same showed PCoA analysis based on molecular data. Comparison of all obtained data supports a hybrid origin of the specimens having intermediate morphology.

Межвидовые гибриды в секции *Ceratocystis* рода *Carex* (сем. Cyperaceae) на Северо-Западе России, выявленные на основе анализа последовательностей внутреннего транскрибируемого спейсера (ITS1) и *trn*L

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***Ключевые слова*:** гибридизация, Европейская Россия, осоки, *Carex* секция *Ceratocystis*, *Carex flava* agg., ITS1.

Аннотация. Секция *Ceratocystis* рода *Carex* – одна из наиболее таксономически сложных групп в роде. Решение вопросов филогении и систематики этой секции сложны из-за частой межвидовой гибридизации и интрогрессии. Большинство видов, принадлежащих к этой группе, способны образовывать гибриды, что указывает на полупроницаемость видовых барьеров. Для выявления процессов гибридизации и интрогрессии среди видов секции *Ceratocystis* мы изучили внутригеномный полиморфизм внутреннего транскрибируемого спейсера (ITS1) генов 35S рРНК у *Carex flava*, *C. serotina*, *C. scandinavica*, *C. hostiana* и их предполагаемых гибридов (*C. flava* × *C. scandinavica*, *C. flava* × *C. serotina*, *C. hostiana* × ?) и проследили материнскую линию наследования с помощью хлоропластного маркера *trn*L. Дополнительно для этих таксонов были проанализированы сравнительно-морфологические данные и фертильность пыльцы. Было установлено, что чистые виды обладают набором видоспецифичных риботипов, в то время как предполагаемые гибриды несут комбинацию риботипов разных родительских таксонов. Чистые виды всегда характеризовались высоким процентом фертильной пыльцы; аналогичный показатель отмечен у некоторых гибридов, тогда как другие гибридные образцы оказались полностью стерильными. Анализ главных компонент (PCA), основанный на морфологических признаках, выявил три кластера: *C. serotina* совместно с *C. scandinavica*, гибриды и *C. flava*. Сходные результаты показал анализ главных координат (PCoA) на основе молекулярных данных. Сравнение всего массива полученных данных подтверждает гибридное происхождение образцов, обладающих промежуточной морфологией.

Introduction

The genus *Carex* L. – true sedges (Cyperaceae) is one of the largest genera of vascular plants with ca. 2000 herbaceous, mostly perennial, wind-pollinated flowering plants, whose species are spread all over the world (Reznicek, 1990; Egorova, 1999; Jiménez-Mejías et al., 2016; Villaverde et al., 2020). According to current classification, it includes 6 subgenera and 62 formally named Linnean sections, and 49 informal clades (Roalson et al., 2021). Hybridisation was noted only in some sections, one of which is *Ceratocystis* Dumort. (Cayouette, Catling, 1992). Members of the sect. *Ceratocystis* are considered diploids, with chromosome numbers varying from $2n = 56$ to $2n = 72$ (Davies, 1956; Schmid, 1982; Halkka, 1992; Rotreklová et al., 2011; Jiménez-Mejías et al., 2012; Lipnerová et al., 2013). This section includes about 16 species that are distributed mostly in Eurasia and North America, but they can also be found in North and South Africa, South America, Australia, and New Zealand (Roalson et al., 2021). According to Egorova (1999), in the European part of Russia, there are 7 species: *C. hostiana* DC., *C. flava* L., *C. lepidocarpa* Tausch, *C. jemtlandica* (Palmgr.) Palmgr., *C. scandinavica* Dav., *C. serotina* Mérat, and *C. bergrothii* Palmgr., but Tzvelev (2000) also mentioned *C. demissa* Hornem. The most widespread species are *C. flava* and *C. serotina,* while other species are mostly rare as they are confined to specific habitats.

Sect. *Ceratocystis* is one of the most taxonomically complex in the genus *Carex* (Vonk, 1979; Pykälä, Toivonen, 1994; Egorova, 1999; Więcław, 2014). According to Schmid (1982), critical groups of sedges exemplified by section *Ceratocystis* (which includes the *C. flava* agg.) are in a dynamic phase of evolutionary development. Species of this section can hybridise with each other, forming sterile, semi-fertile, and in some cases fertile hybrids (Schmid, 1982; Pykälä, Toivonen, 1994; Hedrén, 2002). Hybridisation events, frequently observed across the *C. flava* agg., are known to yield individuals with phenotypic characteristics that are intermediate between the parent forms (Schmid, 1982; Crins, Ball, 1989; Egorova, 1999; Jiménez-Mejías et al., 2012; Więcław, 2014). Together with the polymorphism and morphological plasticity of the species, this leads to numerous intermediate forms with uncertain taxonomic identity (species, subspecies, varieties) (Palmgren, 1959; Schmid, 1982, 1983; Crins, Ball, 1989; Pykälä, Toivonen, 1994; Egorova, 1999; Hedrén, 2004; Więcław, Koopman, 2013). As a result, there is an ongoing debate regarding the taxonomic status of many species (Crins, Ball, 1988; Pykälä, Toivonen, 1994; Hedrén, 2004; Więcław, 2014; Nygaard et al., 2021). Therefore, targeted research on hybridisation is crucial to resolving the taxonomic diversity of this group.

While there is a wide range of works on the morphology of species and hybrids in the sect.

Ceratocystis (Hedrén, 1998, 2002; Więcław, Koopman, 2013; Jiménez-Mejías et al., 2014; Więcław, 2014; Więcław, Wilhelm, 2014), molecular genetic studies are limited and have been carried out only for the species from Europe and North America. Only a few previous molecular studies within the sect. *Ceratocystis* specifically focused on hybridisation. The first attempts to characterise the genetic diversity of these sedges were made by allozyme analysis (Bruederle, Jensen, 1991; Hedrén, Prentice, 1996; Kuchel, Bruederle, 2000; Hedrén, 2002; Blackstock, Ashton, 2010). Recently, a new cryptic species, *C. viridistellata* Derieg, Reznicek et Bruederle, was found in North America. The taxon was discovered using molecular genetic methods: allozyme analysis and then phylogenetic analysis of nuclear DNA (ITS, ETS), which confirmed the monophyletic nature of the studied specimens (Derieg et al., 2008, 2013). Molecular phylogenetic and cytological studies were conducted for European and North African species of the section, resulting in the identification of three major clades corresponding to perigynium morphology (Jiménez-Mejías et al., 2012). In an attempt to clarify the status of *C. jemtlandica,* RAD sequencing and ecological niche modelling were used on populations of *C. jemtlandica* and *C. lepidocarpa* in Norway. The authors suggested that *C. jemtlandica* is a subspecies of *C. lepidocarpa* (Nygaard et al., 2021). A study of 37 populations of the sedges *C. flava* and *C. viridula* and their hybrid *C.* × *subviridula* Fernald found that these taxa are clearly genetically distinct. Regional genetic differentiation in *C. viridula*, which has relatively small populations in the studied regions (Estonia, as well as high and low Switzerland), is higher than in *C. flava*, despite admixing and hybridisation (Schmidt et al., 2018).

In this study, we performed an analysis of intragenomic polymorphism of ITS variants of rDNA for discrimination between species and to confirm the hybrid origin of plants, which were originally selected in the field based on morphological characters. Plant genomes contain thousands of copies of ribosomal DNA (rDNA) arranged in tandem arrays. Sequence polymorphism of their spacer sequences (ITS) has been highly informative concerning molecular species identification (DNA Barcoding) and phylogenetic inferences. Application of high-throughput sequencing to the ITS amplicons increases its resolution as a taxonomic marker and provides individual-level (intragenomic) information regarding various degrees of heterogeneity of ITS-homeologs among the multiple copies of the region within rDNA (Matyášek et al., 2012). This method significantly increases the resolution of ITS1 rDNA as a taxonomic marker as opposed to Sanger sequencing by providing quantitative information. Analyses of intragenomic polymorphism of ITS variants (ribotypes) have been applied to characterise rDNA diversity in species of various plant groups: grasses (Nosov et al., 2021; Gnutikov et al., 2022; Amosova et al., 2024; Gnutikov et al., 2025), *Sparganium* (Belyakov et al., 2019), *Pulsatilla* (Punina et al., 2024), and *Taraxacum* (Efimov et al., 2024). Some of these studies have connected high rDNA heterogeneity with hybridisation, initially based on the data on morphology. More homogenised rDNA (sometimes represented by a single major ribotype) was characteristic of more stabilised taxonomic units, thus suggesting the discriminatory power of ribotypes in plants.

To date, no studies have investigated chloroplast inheritance in Cyperaceae, but a single cytological observation reported DAPI-positive DNA aggregates in the cytoplasm of mature pollen grains of *Carex baccans*, which raises the possibility of biparental cpDNA transmission (Zhang et al., 2003). However, the mere presence of chloroplasts in male gametes is a necessary but insufficient condition for biparental inheritance (Kuroiwa, 2010). Phylogenetic trees based on biparentally inherited 35S rDNA are generally incongruent with those derived from chloroplast markers in Cyperaceae (Escudero, Luceño, 2009; Gebauer et al., 2015; Muasya, Larridon, 2021). This incongruence is most consistent with uniparental inheritance of cpDNA (Yano et al., 2016; Torimaru et al., 2021; Koopman et al., 2023), whether maternal or paternal, and maternal transmission is by far the more likely scenario, as in the vast majority of vascular plants (Connett, 1986; Birky, 1995; Kuroiwa, 2010).

In our study we sequenced *trn*L to identify maternal lineages of possible hybrids – an application that has not previously been explored for representatives of the *Carex* sect. *Ceratocystis*. This marker (*trn*L) can be amplified with universal primers (Taberlet et al., 1991), making it convenient for a wide range of plants (Taberlet et al., 2007; Matiz-Ceron et al., 2022). Although *trn*L has been used in a number of phylogenetic studies on sedges (Yen, Olmstead, 2000; Waterway, Starr, 2007; Waterway et al., 2009; Jung, Choi, 2013), the NCBI database contains only a few sequences for representatives of the *Carex* sect. *Ceratocystis*. Nevertheless, our sequencing of several specimens revealed nucleotide

differences among *C. flava*, *C. serotina*, *C. hostiana*. In the *trn*L region, our data are congruent with those obtained by Waterway and Starr (2007) for *C. flava* and *C. viridula* (a taxon frequently synonymised with or considered closely related to *C. serotina*).

Although hybrids of the sect. *Ceratocystis* are recorded for the territory of Russia, they are poorly covered in literature and collections. Egorova (1999) didn't provide their description and an identification key. Information on the distribution of hybrids in this section across European Russia is limited (e. g. Tzvelev, 2000; Kravchenko, 2007). This is attributable both to a paucity of collections and the widespread misidentification of hybrid specimens as pure species. The wider distribution of the parent species makes *C. flava* × *C. serotina* (*C.* × *subviridula*) the most frequent hybrid; other combinations are rare.

The present work aims to fill a gap in the study of hybridisation in the *Carex* sect. *Ceratocystis* from European Russia. This was achieved through targeted search of hybrids between species in the field and in herbarium collections, followed by their integrated molecular and morphological characterisation.

Materials and Methods

Nomenclature and section interpretation

In our study, we use the ecological species concept of the section (Hedrén, 2004; Więcław, 2014), according to which *C. scandinavica* is an independent taxon (Davies, 1953; Egorova, 1999). Other taxonomic treatments are given in Table 1. The variation of morphological characters among the studied samples can also be seen in Fig. 1.

Although *C. scandinavica* is considered by some authors as a variety or subspecies of *C. serotina* (Table 1), in the present study, we follow the treatment by Egorova (1999). *Carex scandinavica* occurs mainly on the sandy shores of seas and lakes, on coastal saline low-grass meadows, while *C. serotina* occupies a greater variety of habitats and is distributed much more widely. One of the key morphological differences between these species is the shape of the utricle (perigynium): in *C. scandinavica*, the achene completely fills the utricle, while in *C. serotina* it does not, and the utricles are more or less inflated (Egorova, 1999).

Non-hybrid taxa studied in the present work are the following:

Carex hostiana DC. 1813, Cat. Pl. Horti Monsp.: 88 (Fig. 1I).

Carex flava L. 1753, Sp. Pl.: 975 (Fig. 1A).

Carex serotina Mérat, Nouv. 1821, Fl. Env. Paris, ed. 2. 2: 54 (Fig. 1B).

Carex scandinavica E. W. Davies, 1953, Watsonia 3: 66 (Fig. 1D).

Putative hybrids and plants with intermediate morphological characters

Hybrids of ***C. flava* × *C. scandinavica* (1)** (Fig. 1F) and ***C. flava* × ? (1)** do not have bisexual (androgynous) spikes but form female ones.

One of the notable features of other hybrid specimens is the presence of bisexual (androgynous) spikes instead of female ones (Fig. 1E). Such hybrids in this study are named: ***C. flava* × *C. serotina* (2)**; ***C. flava* × *C. scandinavica* (2)**, and ***C. flava* × ? (2)**.

Ambiguous samples are: ***C. lepidocarpa*** (B1, Fig. 1C), ***C. flava* × *C. serotina* (1)** (B13, Fig. 1G), ***Carex*** sp. **× ?** (B14, Fig. 1H) and ***C. hostiana* × ?** (possibly, ***C.* × *xanthocarpa*** Degl. **(*C. flava* × *C. hostiana*)** (Fig. 1J).

Taxon sampling

The study is mostly based on original material collected in 2023. We collected plants from sites where different combinations of putative parental taxa, as well as their hybrids, were recorded. Members of the *Carex flava* agg. (*C. flava*, *C. serotina*, *C. scandinavica*) grow in loose cespitose clumps, while *C. hostiana* produces culms from short, stout horizontal rhizomes (Crins, Ball, 1989). Our sampling included a "south-north" geographic cline from the Leningrad Region to the Murmansk Region, as well as a variety of habitats within these areas.

One of the most interesting places is our southernmost sampling location in the Leningrad Region, on limestone bedrock in the vicinity of the villages of Dontso and Pyataya Gora, where seven specimens were collected among which four putative parental species were identified: *C. flava* (B11), *C. hostiana* (B3), *C. serotina* (B12) and probably *C. lepidocarpa* (B1) as well as three plants with intermediate trait values or a set of traits that characterise different species (B2, B13, B14). Four specimens were collected on the shore of Lake Krugloe, in Medvezhegorsky District (Republic of Karelia): *C. flava*, *C. scandinavica,* and their hybrids *C. flava* × *C. scandinavica*. Eleven specimens were collected in a spring fen on the White Sea coast near the village of Kovda (Murmansk Region, Fig. 1B): *C. flava* (3), *C. scandinavica* (3) and two types of their hybrids (5).

Table 1. Comparison of some taxonomic treatments of members of the *Carex* sect. *Ceratocystis* analysed in the present study

Author/Taxa	***C. hostiana***	***C. flava***	***C. serotina***	***C. scandinavica***
Kükenthal, 1909*	*C. hornschuchiana*	*C. flava*	*C. oederi* (in part) and *C. urbani*	–
Kreczetowicz, 1935*	*C. hostiana*	*C. flava* and *C. flavella*	*C. viridula, C. oederi, C. philocrena*, and *C. pulchella*	–
Mackenzie, 1935*	*C. fulvescens*	*C. flava* and *C. laxior*	*C. oederi* (in part), *C. viridula*, and *C. chlorophila*	–
Davies, 1953	–	*C. flava*	*C. serotina* and *C. viridula*	*C. scandinavica*
Palmgren, 1959	*C. hostiana*	*C. flava*	*C. oederi* subsp. *euoederi* and *C. oederi* subsp. *fennica*	*C. oederi* subsp. *pulchella*
Chater, 1980*,**	*C. hostiana*	*C. flava*	*C. serotina* subsp. *serotina*	*C. serotina* subsp. *pulchella*
Schmid, 1983	–	*C. flava* var. *flava* or *C. flava* var. *alpina*	*C. viridula* subsp. *viridula* var. *viridula*	*C. viridula* subsp. *viridula* var. *pulchella*
Crins, Ball, 1989	*C. hostiana*	*C. flava*	*C. viridula* subsp. *viridula* var. *viridula*	–
Pykälä, Toivonen, 1994	–	*C. flava*	*C. viridula* var. *viridula*	*C. viridula* var. *pulchella*
Sell, 1996**	–	*C. flava* subsp. *flava*	*C. flava* subsp. *serotina*	*C. flava* subsp. *pulchella*
Egorova, 1999	*C. hostiana*	*C. flava*	*C. serotina*	*C. scandinavica*
Blackstock, Jermy, 2001	–	*C. flava*	*C. viridula* subsp. *viridula* var. *viridula*	*C. viridula* subsp. *viridula* var. *pulchella*
Hedrén, 2002	–	*C. flava*	*C. oederi* var. *oederi*	*C. oederi* var. *pulchella*
Więcław, 2014	–	*C. flava*	*C. viridula*	–
GCG, 2016	*C. hostiana*	*C. flava*	*C. viridula*	–
Koopman et al., 2025	*C. hostiana*	*C. flava*	*C. oederi* var. *oederi*	–
Present study	*C. hostiana*	*C. flava*	*C. serotina*	*C. scandinavica*

Note: * – Cited based on treatment Crins, Ball, 1989; ** – Cited based on treatment Hedrén, 2002.

Voucher specimens are deposited in the Herbarium of the Komarov Botanical Institute, Russian Academy of Sciences, St. Petersburg (LE). Specimen barcodes are given only for samples from the LE herbarium, and scanned voucher herbarium specimens are available via the following link: https://en.herbariumle.ru/.

The remaining specimens were obtained from the herbarium of the Karelian Research Centre, Russian Academy of Sciences, Petrozavodsk (PTZ).

Three specimens originated from the Arkhangelsk Region and five from two locations in the Republic of Karelia. Four herbarium sheets are transferred to the herbarium LE from PTZ (see Table 2) while the other four are stored in PTZ.

The total number of samples is 30 plants, which are listed in Table 2. A map showing the locations where the material was collected is presented in Fig. 2.

Pollen grain fertility assessment test

A carmine test (Marks, 1954) was conducted on 16 samples to check their fertility, because other 14 specimens lacked pollen. For this purpose, some anthers were placed in 200 µl tubes with 10 % acetocarmine solution. After staining, glycerin (~70 %) was added to the solution, and it was analysed under a microscope (Leica DM2500, lens 10× objective). The percentage of stained and unstained pollen grains was counted, with a total of up to 500 grains evaluated. Intensely stained and undeformed pollen grains were considered conditionally fertile (Fig. 4A) while uncoloured pollen grains without contents, or slightly stained and deformed ones were considered abortive (Fig. 4B). Results of the test are shown in Figs. 7–9.

Morphometry

Collected plants of the sect. *Ceratocystis,* as well as herbarium specimens, were identified using the morphological characteristics listed in Table 3, and are shown in Fig. 3, the accuracy of measurements is 0.1 mm. In this analysis, 14 quantitative and 2 qualitative characters were studied using 30 specimens, which were taken for the rDNA analyses.

A medium-height shoot with an entire lowest female spike leaf was selected or a shoot from which DNA was extracted was taken. Utricles and their glumes were measured soaked in cold water under a stereo microscope (Micromed MC-4-ZOOM). The utricle was taken from the middle of the lower female spike. One utricle and its glume were taken from each plant. Some measurements (CH, LFSL, IL) were conducted in ImageJ on herbarium scans. If possible, MPL, DLFS, LFSL, LFSW were checked in ImageJ, too. Measurements are available in Supplementary Materials on the journal's website.

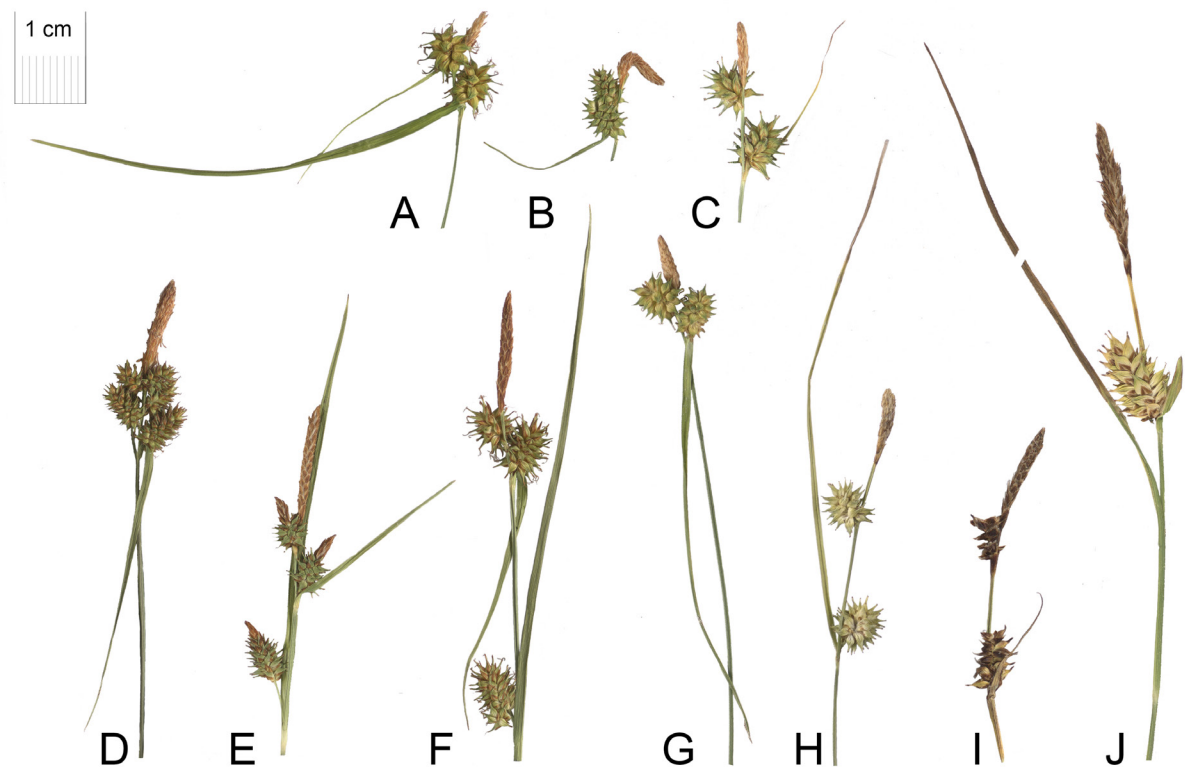

Fig. 1. Appearance of plants used in this study (tops of shoots with spikes), for details see Table 2: A – *Carex flava*; B – *C. serotina* (part of inflorescence with three female spikes); C – *C. lepidocarpa* (B1); D – *C. scandinavica*; E – *C. flava* × *C. scandinavica* (2); F – *C. flava* × *C. scandinavica* (1); G – *C. flava* × *C. serotina* (1) (B13); H – *Carex* sp. × ? (B14); I – *C. hostiana* (B3); J – *C. hostiana* × ? (B2).

Table 2. Samples of *Carex* sect. *Ceratocystis* used in present study

Samples and voucher barcodes*	Place	Habitat	Coordinates	Date collected	Sample ID	Herbarium Barcode
Arkhangelsk Region (A)						
C. flava	Onezhskiy District, Kozhozerskiy Nature Park, NW of the Kozhozero Lake, SW shore of the Vingozero Lake.	lake-side mesoeutrophic mire on sandy shore, sparse.	N63.163858°, E37.935303° (inferred)	27 VIII 2003	B23	PTZ
C. serotina					B22	PTZ
C. flava × *C. serotina* (2)					B29	LE 01271312
Murmansk Region (B)						
C. flava	Kandalaksha District, mouth of the river Kovda.	A small spring fen on the left bank of the river.	N66.695°, E32.854° (measured)	3–4 VII 2023	B7, B18, B19	LE 01271286 LE 01271299 LE 01271300
C. scandinavica					B5, B16, B20	LE 01271289 LE 01271298 LE 01271301
C. flava × *C. scandinavica* (1)					B6, B15	LE 01271290 LE 01271297 (+LE 01230373-375)
C. flava × *C. scandinavica* (2)					B4, B17, B21	LE 01271288 LE 01271335 LE 01271308
Leningrad Region (C)						
C. serotina	Leningrad Region, Volosovsky District, ca. 2 km NW of Dontso.	Edge of the flooded limestone quarry, reedbed (*Phragmites australis*) bordering herb-rich meadow with *Carex hostiana*, *Calamagrostis epigeios*, *Briza media*, *Salix rosmarinifolia*.	N59.43248°, E29.75089° (measured)	09 VII 2023	B12	LE 01271295
C. flava × *C. serotina* (1)					B13	LE 01271294 (+ LE 01310262)

Ending Table 2

Samples and voucher barcodes*		Place	Habitat	Coordinates	Date collected	Sample ID	Herbarium Barcode
C. flava		Leningrad Region, Volosovsky District, ca. 2 km NW of Dontso.	Edge of the flooded limestone quarry, meadow dominated by sedges (mainly *Carex flacca*) with *Calamagrostis epigeios*, *Centaurea jacea*.	N59.43547°, E29.74832° (measured)	09 VII 2023	B11	LE 01271337 (+ LE 01310243)
C. hostiana						B3	LE 01271285
C. lepidocarpa						B1	LE 01271281 (+ LE 01310244)
C. hostiana × ?						B2	LE 01271282
Carex sp. × ?						B14	LE 01271296
Republic of Karelia (D-F)							
D	*C. flava*	Medvezhegorsky District. Krugloe Lake.	Fen bank of the lake Krugloe.	N62.906625°, E34.367639° (measured)	04 VII 2023	B8	LE 01271291 (+LE 01230376)
	C. scandinavica					B9	LE 01271292
	C. flava × *C. scandinavica* (1)					B10, B30	LE 01271293 LE 01310261
E	*C. flava*	Kondopozhskiy District, Kyappeselga village, E part, W of small lake.	Rich fen.	N62.68043°, E34.28495° (measured)	10 VII 2002	B28	LE 01271311
	C. flava × ? (1)					B27	LE 01271310
	C. flava × ? (2)					B26	LE 01271309
F	*C. scandinavica*	province: Kon, Medvezhiegorsky District, Zaonezhie, Tipinitsy village, Varnavolok Peninsula in the Onego Lake.	Boulder shore with sedges of the Onego Lake.	N62.160480°, E35.464841° (inferred)	04 VII 2004	B25	PTZ
	C. flava × *C. scandinavica* (2)					B24	PTZ

The Principal Component Analyses (PCA) on the morphological data were done using IBM SPSS Statistics for Windows, Version 31.0 (IBM Corp., 2025). NMS was excluded from the analysis as the value was constant across all plants.

DNA extraction

DNA was extracted from leaves (herbarium material) using the DNEasy Plant Mini Kit (Qiagen) and innuPREP Plant DNA Kit (Analytik Jena) using protocol with SLS buffer, both protocols were used according to manufacturer's instructions but with some modifications (RNAse was not used).

Illumina library preparation

Illumina sequencing was used to obtain all variants (ribotypes) of the 18S rDNA–ITS1–5.8S rDNA region (~300 b.p.) contained in the genome of a particular plant. PCR was conducted with OneTaq Quick-Load 2X Master Mix with Standard Buffer

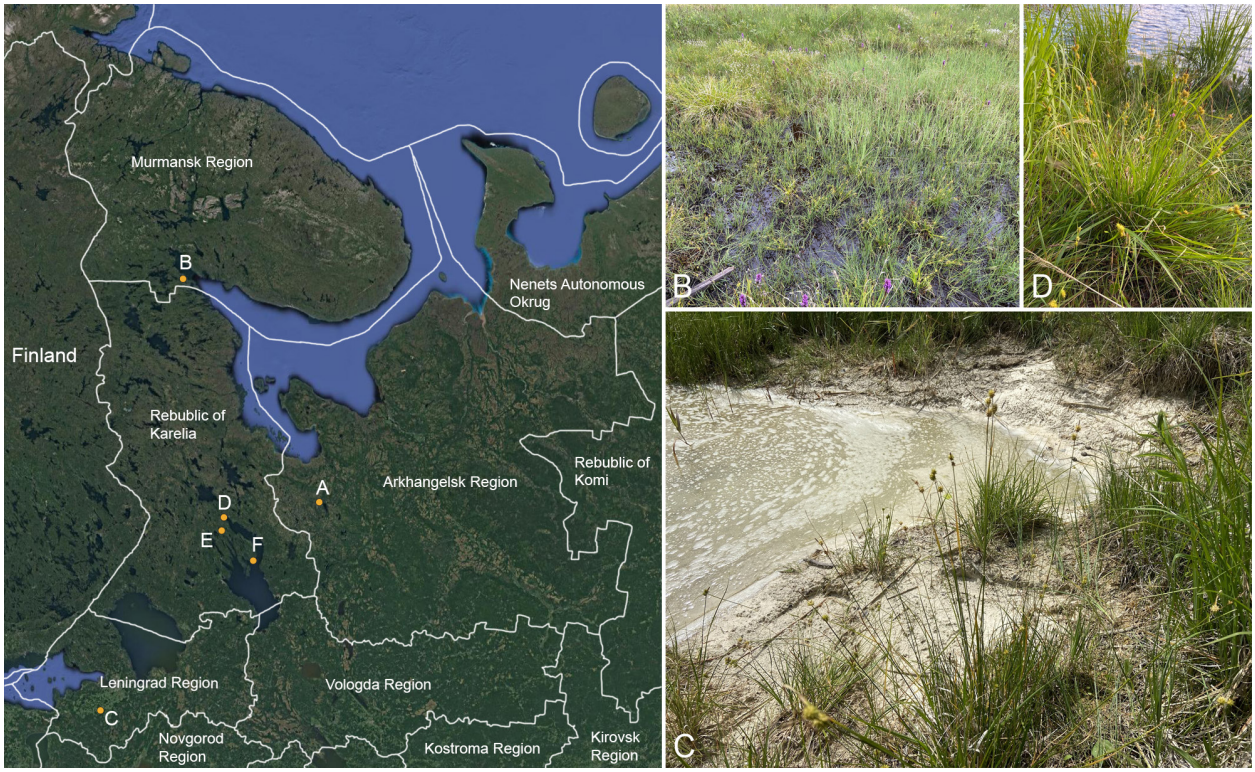

Fig. 2. Map of sampling localities: A – Lake Vingozero, Arkhangelsk Region; B – Kovda, Murmansk Region; C – Dontso, Leningrad Region; D – Lake Krugloe, Medvezhegorsky District, Republic of Karelia; E – Kyappeselga, Republic of Karelia; F – Zaonezhye, Republic of Karelia. Photographs of plant habitats (B, D and C) are given only for the original collections conducted in 2023. The map was created in the QGIS 3.34.1 Prizren while area boundaries are drawn in Photoshop (13.0.1 x 64).

(Biolabmix) and specific primers with adaptors (Beagle or Eurogene): ngsITS1P: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAACCTTATCATTTAGAGGAAGG-3'; ngsITS_5.8_PZ: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTATCGCATTTCGCTACG-3'. PCR was performed according to the following protocol: initial DNA denaturation at 94 °C for 3 min, 40 cycles of denaturation at 94 °C for 30 s, annealing at 45 °C for 15 s, elongation at 68 °C for 30 s; followed by a final elongation at 68 °C for 5 min. PCR products were cleaned with CleanMag DNA PCR (Eurogen). Paired-end sequencing with 300-bp reads was conducted in a single run on an Illumina MiSeq platform (Smorodintsev Research Institute of Influenza).

Bioinformatic analysis

Bioinformatic analysis of raw FASTQ files was conducted using the USEARCH v.11 pipeline (Edgar 2010). We used ZOTU (also called ribotype, an ITS1 variant) in our study. The data analysis was carried out as follows: merged forward and reverse reads of each sample were pooled together, quality filtered, and dereplicated. Then, the detection of chimeras was performed, followed by ZOTU (zero radius OTU) clustering. Resulting ZOTUs were used to construct a table of variants across samples with corresponding read counts. Relative frequencies for each variant were computed.

Variants with frequencies less than 3 % were discarded from further analysis, as possible PCR or sequencing errors. The remaining ribotypes are named with the first letters of the species in which they are more commonly found (host – *C. hostiana*, ser – *C. serotina* (including *C. scandinavica*), fl – *C. flava*). Sequences and a table with the relative frequencies of ribotypes are available in the Supplementary Materials on the journal's website.

Visualisation

Principal Coordinate Analysis (PCoA) was performed on a pairwise distance matrix calculated from ITS ribotype composition. Distances were derived from a custom metric integrating (i) presence/absence of shared ribotypes and (ii) differences in sequence counts for ribotypes occurring in both samples. Graphical presentation

Table 3. Morphological measurements of the studied specimens of *Carex* sect. *Ceratocystis*

N	Abbreviation	Character	Description of measurements
1	CH	Culm height (cm)	Measured from the base of the shoot to the top of the inflorescence.
2	LFSLL	Lowest female spike leaf length (cm)	Length of the leaf blade originating from the lowest female spike sheath.
3	IL	Inflorescence length (cm)	Measured from the base of the lowest female spike sheath to the apex of the male spike.
4	NMS	Number of male spikes (no)	Total count on the measured shoot.
5	MPL	Male spike peduncle length (cm)	Measured from the beginning of the sheath of the last female spike.
6	NFS	Number of female spikes (no)	On the measured shoot.
7	DLFS	Distance between two lower female spikes (cm)	Measured from the top of the sheath (i.e., the place where the leaf comes off or the beginning of the visible part of the spike peduncle).
8	LFSL	Lowest female spike length (cm)	Measured from the attachment point of the lower utricle and its glume to the tip (end of the beak) of the longest utricle.
9	LFSW	Lowest female spike width (cm)	Measured by the middle of the spike, which was set along the middle row of the utricle: from the top of one beak to the other.
10	SSh	Spike shape	Ratio of lowest female spike width to lowest female spike length (LFSW/LFSL).
11	LPL	Lowest female spike peduncle length (cm)	Measured from the lower spike utricle to its exit from the sheath, i.e. the leaf sheath was not taken into account.
12	GL	Glume of female spike length (mm)	Measured on a flattened glume from the base to the apex.
13	UL	Utricle length (mm)	Total length including the beak, measured on a flattened surface.
14	UBL	Utricle beak length (mm)	Measured from the obvious expansion of the utricle.
15	UBL/UL	Ratio of beak length to utricle length (%)	Ratio of beak length to total utricle length (UBL/UL).
16	G/U	Ratio of glume length to utricle length (%)	Ratio of glume length to total utricle length (G/U).

of distances between samples was performed using the Python skbio module (Rideout et al., 2023). The analysis code is available at https://github.com/valentina-domashkina.

Bar charts were visualised using R with used readxl (Wickham, Bryan, 2025) and ggplot2 (Wickham, 2016). The ribotype network (Fig. 10) was constructed in TCS v.2.1 (Clement et al., 2000) and visualised in tcsBU (Santos et al., 2016). In this network, each filled circle represents a unique ZOTU (ribotype), with its total size proportional to the total number of samples in which that ribotype was detected. Each circle is composed of sectors representing individual samples. The size of each sector is proportional to the relative abundance (frequency) of that ribotype within the genome of the respective sample (e. g., a 0.1 sector represents a 1–10 % frequency of the ribotype in that specimen).

The colours of the sectors correspond to the different taxa.

Chloroplast marker analysis

To determine the maternal parent responsible for chloroplast inheritance, *trn*L intron sequences were obtained using Sanger sequencing with C and D primers. PCR was conducted with BioMaster HS-Taq PCR-Color (2×) master mix (Biolabmix) and specific primers: trnL_C: 5'-CGAAATCGGTAGACGCTACG-3'; trnL_D: 5'-GGGGATAGAGGGACTTGAAC-3' (Taberlet et al., 1991). PCR was performed according to the following protocol: initial denaturation at 95 °C for 1 min, 34 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for 15 s, and elongation at 72 °C for 30 sec; followed by a final elongation at 72 °C for 2 min. PCR products were cleaned with CleanMag DNA PCR (Eurogen). Sequences obtained by us via Sanger sequencing for this study were analysed in CodonCode Aligner (v. 10.0.2–12.0.4, Codon Code Corporation, https://www.codoncode.com/aligner). To supplement our dataset, additional sequences of studied *Carex* species were retrieved from the

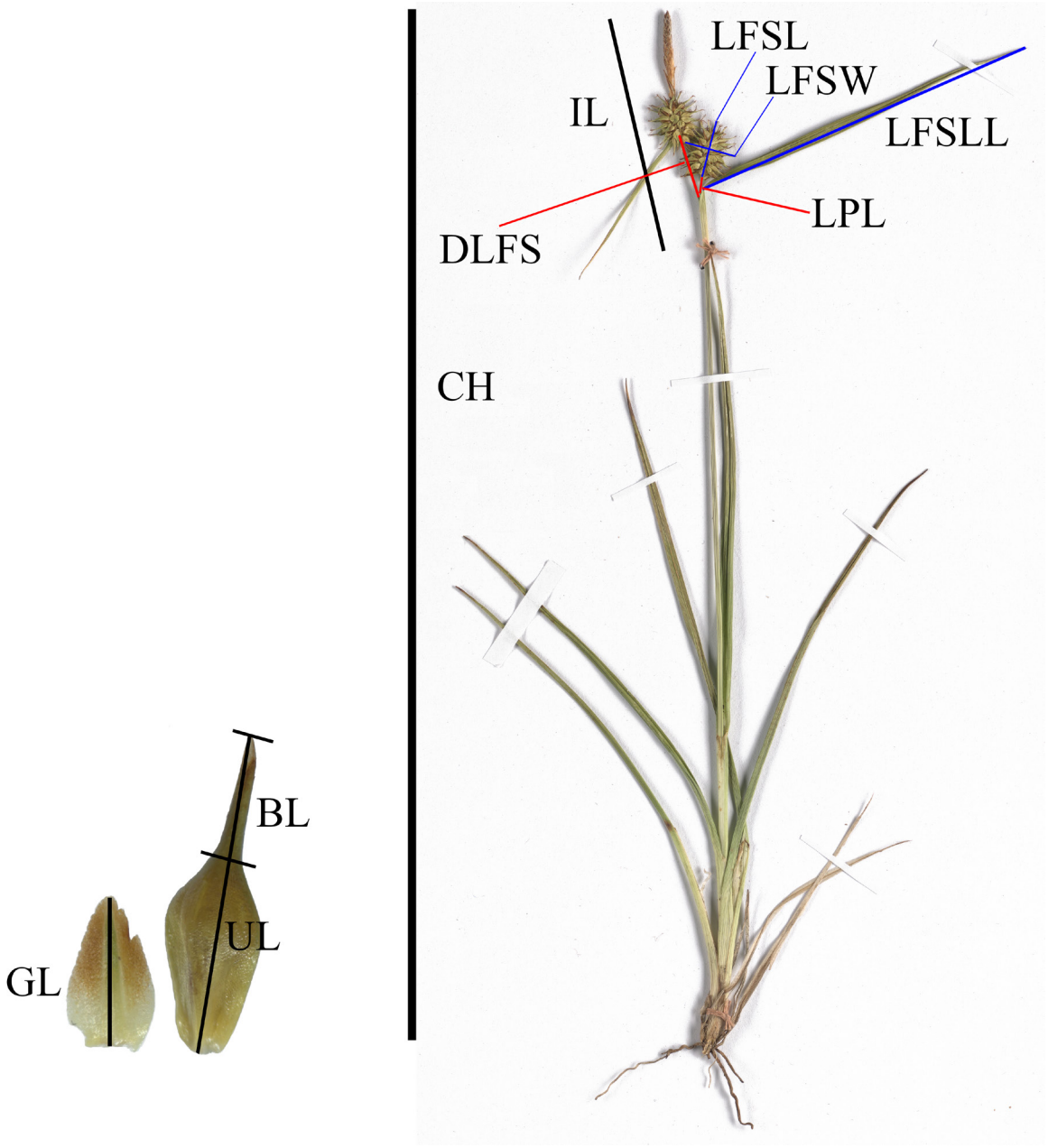

Fig. 3. Illustration of morphological measurements of the studied specimens of *Carex* sect. *Ceratocystis*. Abbreviations are given in Table 3.

NCBI GenBank database (accession numbers: AY757523.1; AY757524.1). The alignment was performed in Aliview with the default alignment program (Larsson, 2014). The resulting tree was obtained using the Maximum Likelihood method in IQ-TREE 3.1.2 (Wong et al., 2026). The best-fit substitution model (F81+F) was automatically selected by ModelFinder (Kalyaanamoorthy et al., 2017) based on the Bayesian Information Criterion (BIC). Branch support was evaluated with 1000 replicates of both the SH-like approximate likelihood ratio test, SH-aLRT (Guindon et al., 2010) and the standard bootstrap (Felsenstein, 1985). It was visualised in iTOL v7 (Letunic, Bork, 2024). Support values below 50 % are not shown. All newly produced sequences were submitted to NCBI (Accession numbers: PZ452550 – PZ452571).

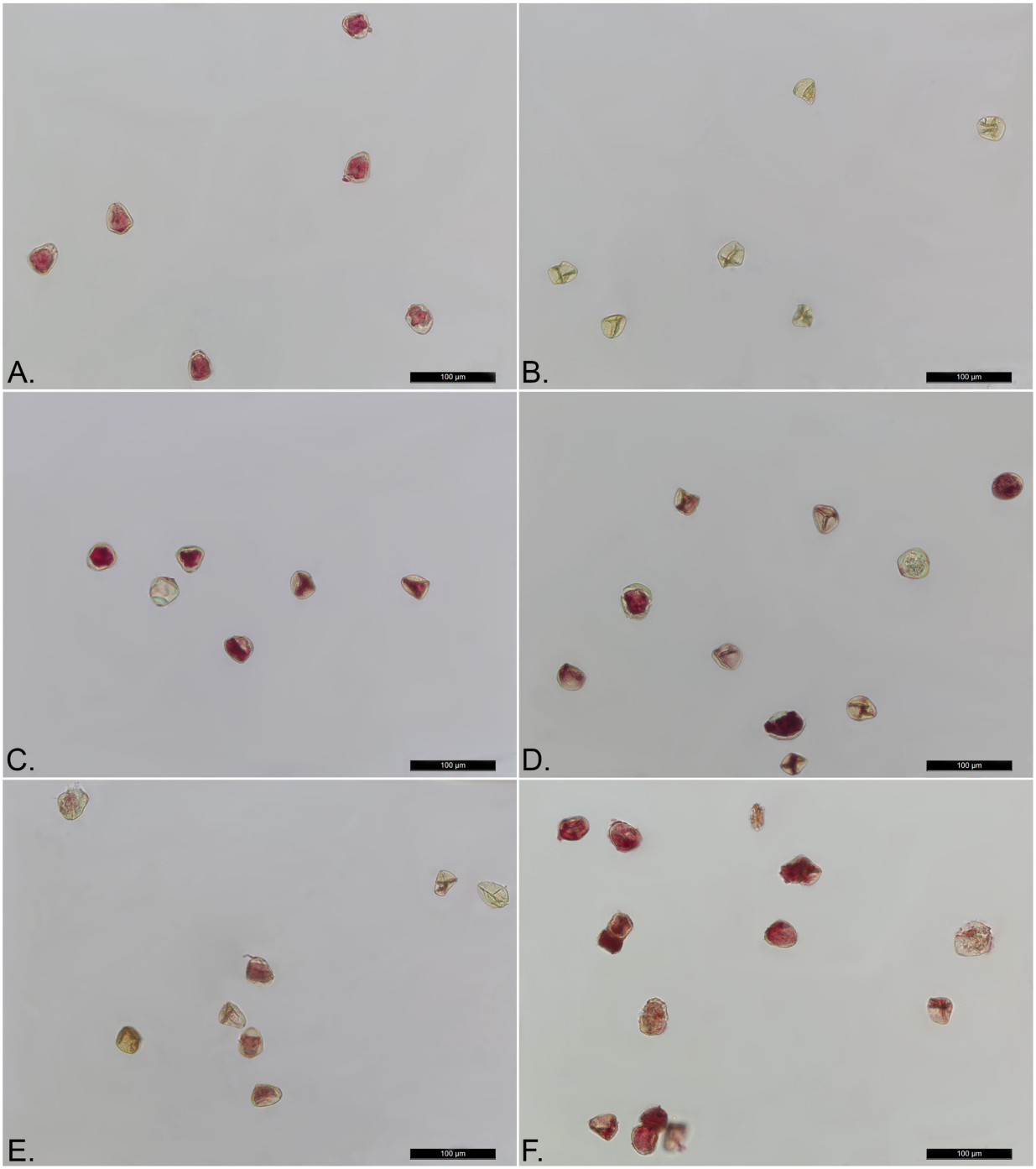

Fig. 4. Photographs of pollen grains, bar = 100 µm: A – *Carex scandinavica* (B20, high percent of fertility); B – *C. flava* × *C. scandinavica* (B24, sterile); C – *Carex* sp. × ? (B14, semi-fertile); D – *C. hostiana* × ? (B2, semi-fertile); E – *C. flava* × *C. serotina* (1) (B13, semi-fertile); F – *C. lepidocarpa* (B1, semi-fertile).

Results

Pollen fertility analysis

Results of pollen grain staining are shown in Figs. 7–9. It was shown that the parental species have fertile pollen grains, *C. flava* (99 %), *C. scandinavica* (90–98 %). Unfortunately, it was not possible to assess the fertility of *C. hostiana* and both *C. serotina* specimens. Hybrids *C. flava* × *C. scandinavica* (1, 2), and *C. flava* × ? (1, 2) were sterile. Other plants with intermediate traits (B1, B2, B13, B14), which are presumed to be hybrids or pure species, are fertile. Nevertheless, B2 and B14 did not form seeds while B1 and B13 did.

Morphometric analysis

According to the PCA results, all samples were classified in three groups: *C. serotina* and *C. scandinavica*; hybrids and *C. hostiana*; and *C. flava*.

The first two principal components (Fig. 5) account for 58.15 % of the total variance, with PC 1 accounting for 40.95 % and PC 2 accounting for 17.20 %. The highest positive values for PC 1 are observed for the spike width (LFSW) and utricle characteristics (GL, UL, UBL), while inflorescence characteristics (IL, LFSLL, DLFS) had the highest component loadings along PC 2. Ordination along PC 1 and PC 2 revealed clusters of *C. scandinavica* together with *C. serotina* and *C. flava* species separated along PC 1. The cluster in the centre of the plot consisted of different putative hybrids.

Illumina sequencing

General notes

ITS1 sequences were obtained for 30 specimens (Figs. 6–10). Of these, seven were identified as *C. flava*, two as *C. serotina*, five as *C. scandinavica*, one as *C. hostiana.* The remainder consisted of hybrids and other ambiguous specimens: four presumable *C. flava* × *C. scandinavica* (1), four *C. flava* × *C. scandinavica* (2), two *C. flava* × *C. serotina* (1, 2), two *C. flava* × ? (1, 2), one *C. hostiana* × ? (B2), one *C. lepidocarpa* (B1), and one *Carex* sp. × ? (B14). The number of reads ranged from 8791 to 120702.

A total of 38 ITS1 variants were identified in the raw data, but as a result of data processing and filtering, the number of variants was reduced to 15 (Fig. 10). In pure species, the diversity of ribotypes varied from 1 to 9, while in hybrids, the set of ribotypes in this study did not exceed six.

Parental species

Three groups of ribotypes were identified (Fig. 10). The first group included all ribotypes found in *C. flava* (N = 11). *Carex flava* samples had the greatest diversity of ribotypes from 1 (B23: fl_1, Arkhangelsk Region) to 9 (B8: fl_1, fl_2, fl_4–fl_7, fl_9–fl_11, Lake Krugloe, and B28: fl_1–fl_4, fl_7–fl_11, Kyappeselga, Republic of Karelia). Putative *C. lepidocarpa* (B1) had a set of ribotypes similar to the plants we identified as hybrids; this sample is addressed in the Discussion section.

The remaining four ribotypes were organised into two distinct groups, each comprising two ribotypes. These four ribotypes were exclusively observed in *C. hostiana* (host_1 and host_2) and *C. serotina* (ser_1 and ser_2), with two ribotypes being specific to each species. Furthermore, *C. serotina* shared a common set of ribotypes with *C. scandinavica*.

However, ribotypes fl_1 and fl_2 were not exclusive to *C. flava* and were also detected in other species. The ribotypes specific to *C. flava* are fl_3 through fl_11. No ribotypes common to *C. serotina* (or *C. scandinavica*) were found in the examined *C. flava* specimens. In contrast, some *C. flava* ribotypes were present in specimens of *C. serotina* s. l. (specifically, fl_2 in *C. scandinavica* samples B5, B16, and B20 from the Murmansk Region, and fl_1 in *C. serotina* sample B12 from the Leningrad Region). *Carex hostiana* shared one ribotype (fl_1) with *C. flava*. However, none of the *C. flava* samples contained ribotypes specific to *C. hostiana*.

Putative hybrids

Plants with intermediate morphological characteristics also possessed the set of parental ribotypes (Figs. 7–10), thus confirming their hybrid nature. All hybrids from the Murmansk Region (Fig. 7) inherited five ribotypes from the parental plants (three from *C. flava*: fl_1–fl_3 and two from *C. scandinavica*: ser_1 and ser_2) and had similar ribotype distribution.

The hybrids *C. flava* × *C. scandinavica* (1) (specimens B10 and B30) shared a similar ribotype profile (Fig. 8). They inherited ribotypes fl_1, fl_2, fl_4, fl_9, and fl_11 from their *C. flava* parent (specimen B8). Notably, these ribotypes were found in higher abundance in this parental specimen (B8) than in other *C. flava* specimens from different sites, with the exception of the sample from Kyappeselga (B28). At the Kyappeselga site, other hybrids exhibited different ribotype sets: *C. flava* × ? (1) (specimen B27: fl_1–fl_3) and *C. flava* × ? (2) (specimen B26: fl_1, fl_9, fl_11).

Plants from Dontso, Leningrad Region (B1, B13, B14, Fig. 9) also possessed a set of ribotypes from the

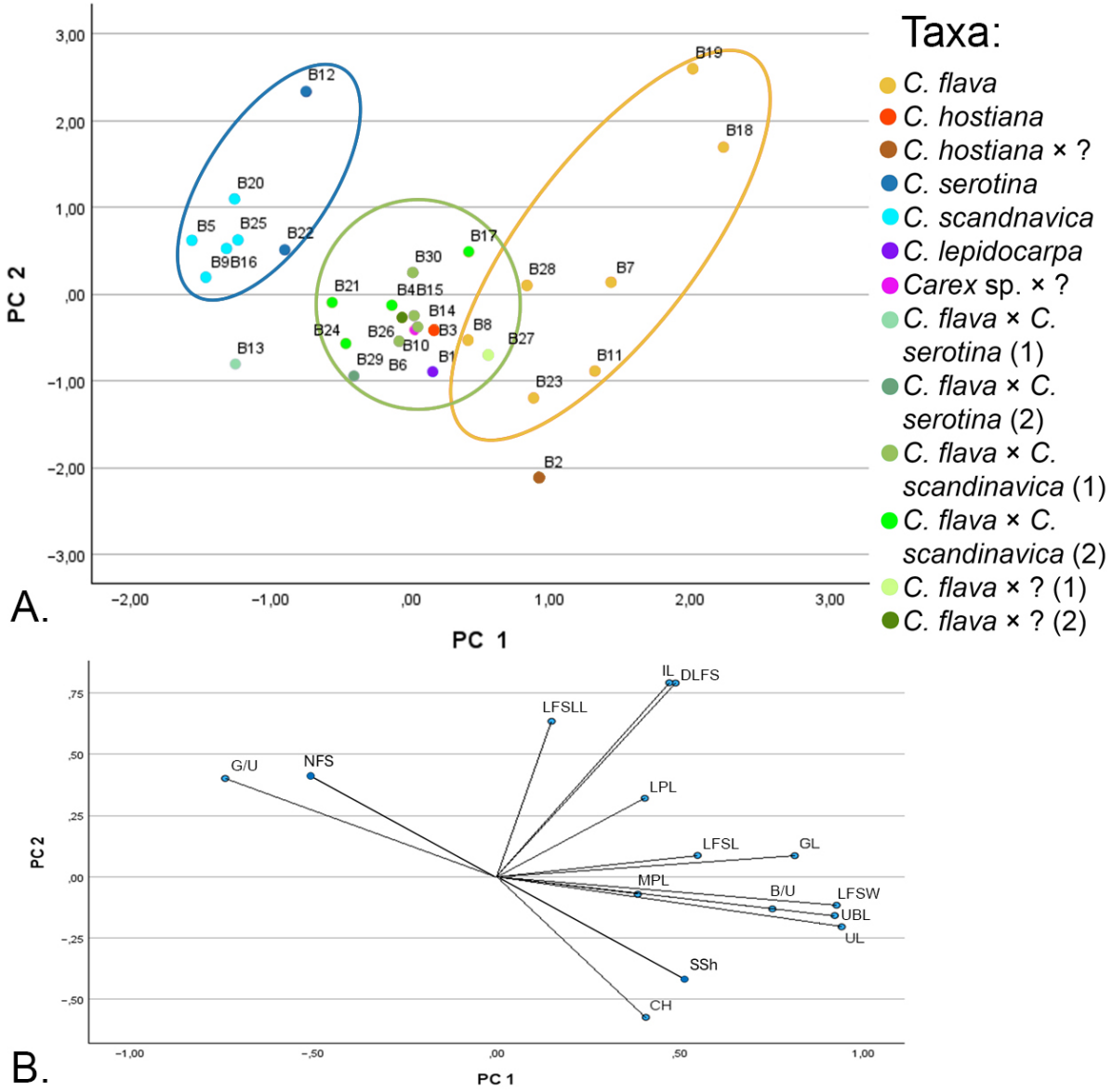

Fig. 5. Results of principal component analysis (PCA) of morphological measurements of studied *Carex* sect. *Ceratocystis* specimens: A – ordination of specimens in the space of PC1 and PC2; B – factor loadings of morphological variables.

parental species, with a quantitative predominance of *C. serotina* ribotypes. B1 and B13 inherited both *C. serotina* ribotypes and fl_1, fl_2, while B14 had only ser_1 and fl_1 ribotypes.

In the *C. hostiana* × ? (B2, Fig. 9) hybrid, in addition to the unique ribotypes that present in *C. hostiana*, the main ribotypes of *C. serotina* (ser_1, ser_2) were also found, as well as those of *C. flava* (fl_1 and fl_2).

PCoA

PCoA identified several groups of parental species and their hybrids that were consistent with the established taxonomy. The presence of groups was probably related to the geography of the sampling sites. *Carex flava* (B7, B18, B19) specimens from the Murmansk Region formed one cluster, while specimens of *C. flava* from the Republic of Karelia, Arkhangelsk and Leningrad Regions formed another; however all these samples could be considered as one large cluster. Other specimens from a single population in the Murmansk Region were grouped in separate clusters (Fig. 6): *C. scandinavica* (B5, B16, B20) and their hybrids *C. flava* × *C. scandinavica* (1) (B6, B15), *C. flava* × *C. scandinavica* (2) (B4, B17, B21). It is worth noting

that *C. scandinavica* specimens from this population possessed the fl_2 ribotype; in a population from the Leningrad Region, we can also observe the presence of *C. flava* ribotypes (fl_1 and fl_2) in *C. flava* × *C. serotina* (1) (B13) and *C. lepidocarpa* (B1) as well as fl_1 in *C. serotina* (B12).

Direction of crosses in hybrids

In this study, 22 sequences of *trn*L fragments were obtained for our samples (Fig. 11, Supplementary Materials are available on the journal's website): one from *C. hostiana*, five from *C. flava*, three from *C. scandinavica*, two from *C. serotina*, and the rest from putative hybrids.

Sequence analysis revealed that the total length in unaligned *C. hostiana* sequence is 636 nucleotides. It possessed a 21-nucleotide insertion and two single-nucleotide substitutions that distinguished it from the other species: a T at position 453 instead of G, and an A at position 495 instead of G (numbers of aligned positions). *Carex flava* differed from *C. serotina* and *C. scandinavica* by a single nucleotide transition (C to T, respectively) at position 319, while *C. serotina* and *C. scandinavica* sequences did not differ. The total length of those unaligned sequences is 615 nucleotides.

It was established that the maternal parent for hybrids with bisexual spikes (*C. flava* × *C. scandinavica* (2), *C. flava* × *C. serotina* (2), and *C. flava* × ? (2)) was *C. flava,* while other examined hybrids and ambiguous plants had the chloroplast DNA variant from *C. serotina*.

Parental formulas for hybrids and specimens with intermediate trait values:

C. flava × *C. scandinavica* (1): *C. scandinavica* (♀) × *C. flava* (♂)

C. flava × *C. scandinavica* (2): *C. flava* (♀) × *C. scandinavica* (♂)

C. flava × *C. serotina* (1): *C. serotina* (♀) × *C. flava* × ?

C. flava × *C. serotina* (2): *C. flava* (♀) × *C. serotina* (♂)

C. flava × ? (1): *C. serotina* s. l. (♀) × *C. flava* (♂)

C. flava × ? (2): *C. flava* (♀) × *C. serotina* s. l. (♂)

C. lepidocarpa (B1): *C. serotina* (♀) × ? × ?

C. hostiana × ? (B2): *C. serotina* (♀) × *C. flava* × *C. hostiana*

Carex sp. × ? (B14): *C. serotina* (♀) × ? × ?

Discussion

Morphological analysis

In the PCA, morphologically distinct *C. flava* and *C. serotina* s. l. form two clear clusters, with hybrids positioned intermediately between them. This pattern confirms earlier recorded results from a detailed study of Polish material (Więcław, 2014).

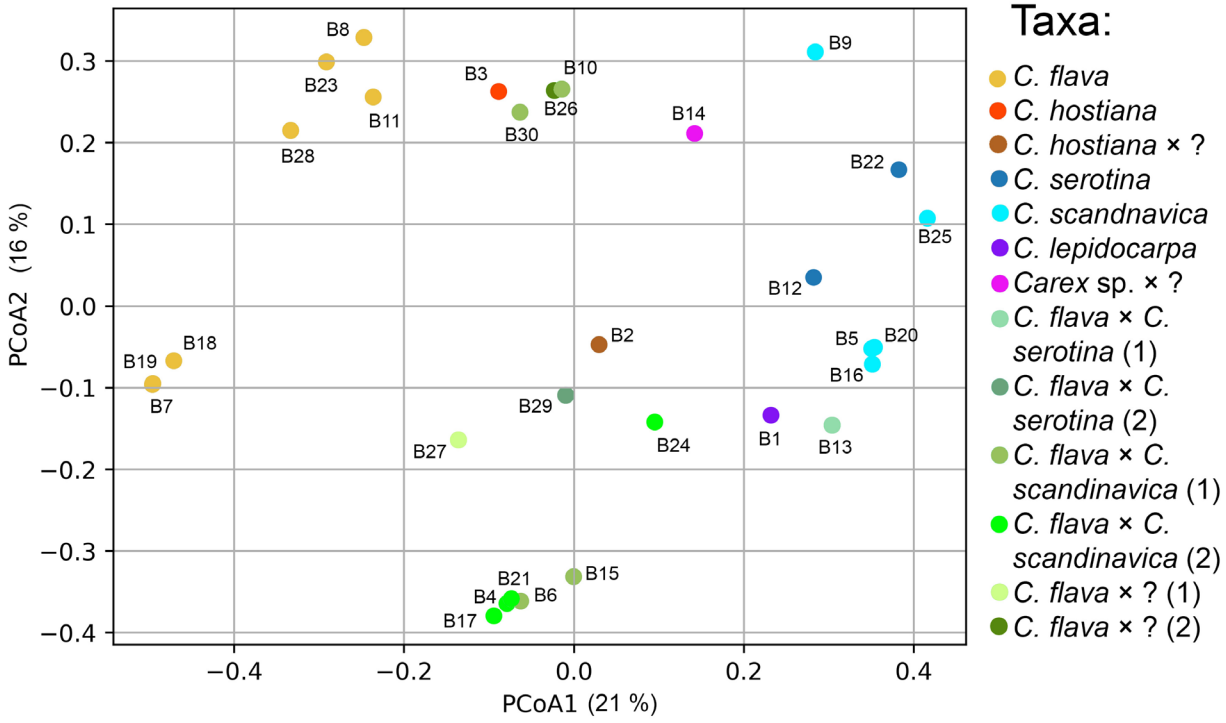

Fig. 6. PCoA of studied *Carex* sect. *Ceratocystis* specimens. Specimen numbering according to Table 2. Consensus of all ribotypes for specimens is shown as one point.

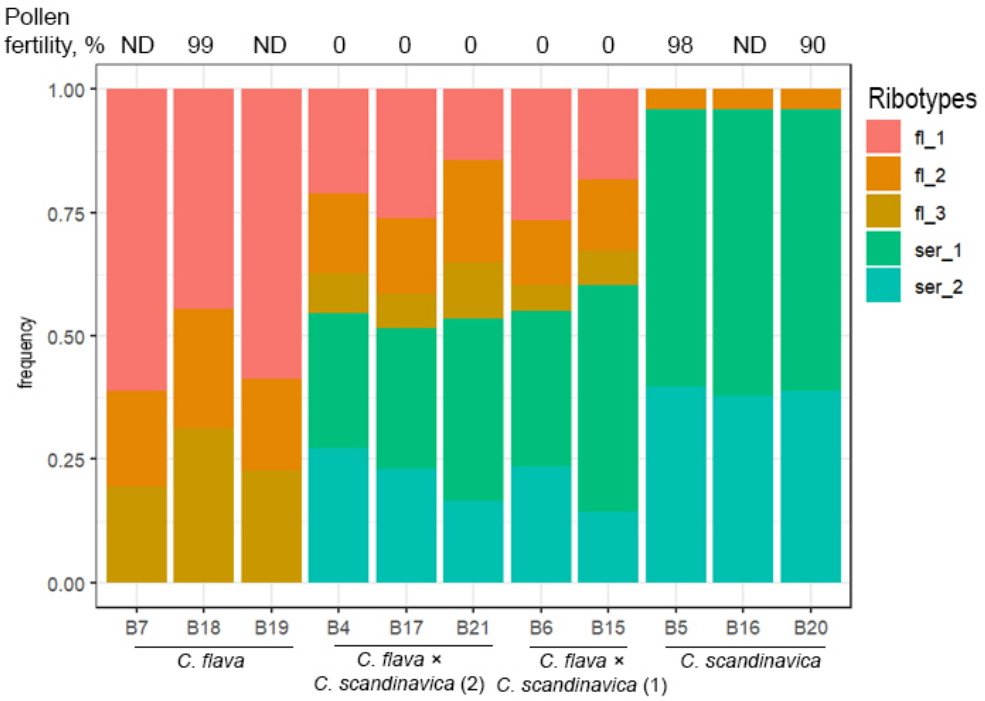

Fig. 7. Distribution of ribotypes in the studied *Carex* sect. *Ceratocystis* specimens from the Murmansk Region. The percentage of fertile pollen is shown above the plot on a separate line; unknown percentages are marked as ND (not determined).

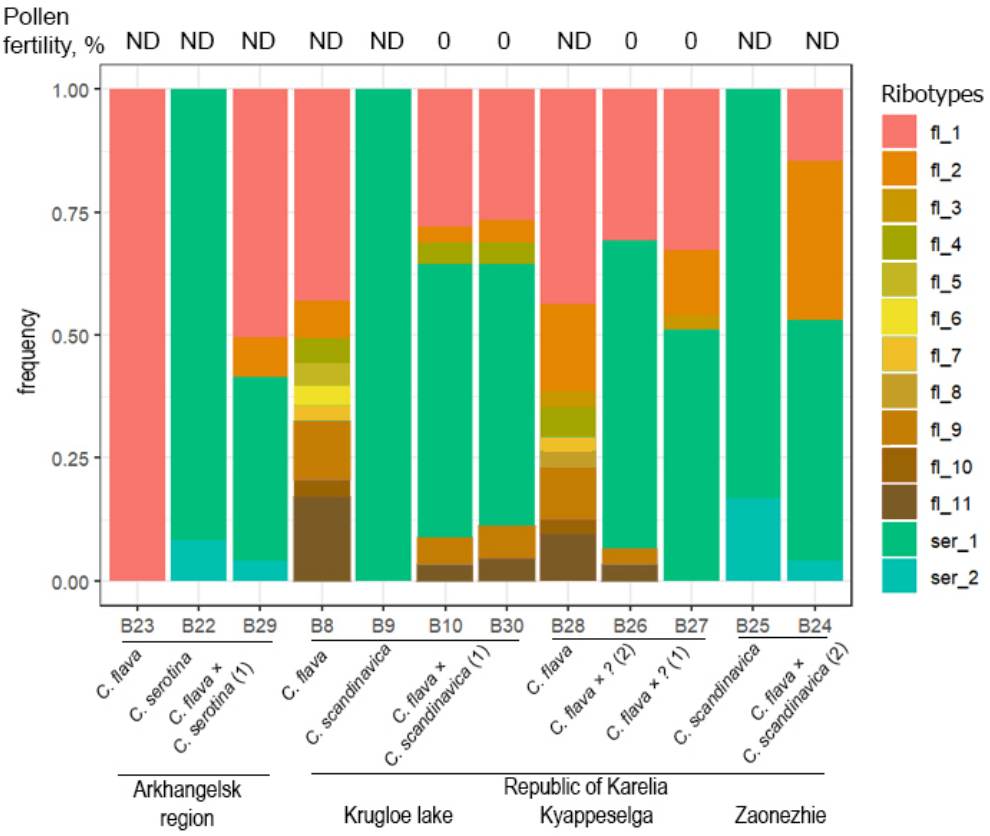

Fig. 8. Distribution of ribotypes in the studied *Carex* sect. *Ceratocystis* specimens from the Arkhangelsk Region and the Republic of Karelia. The percentage of fertile pollen is shown above the plot on a separate line; unknown percentages are marked as ND (not determined).

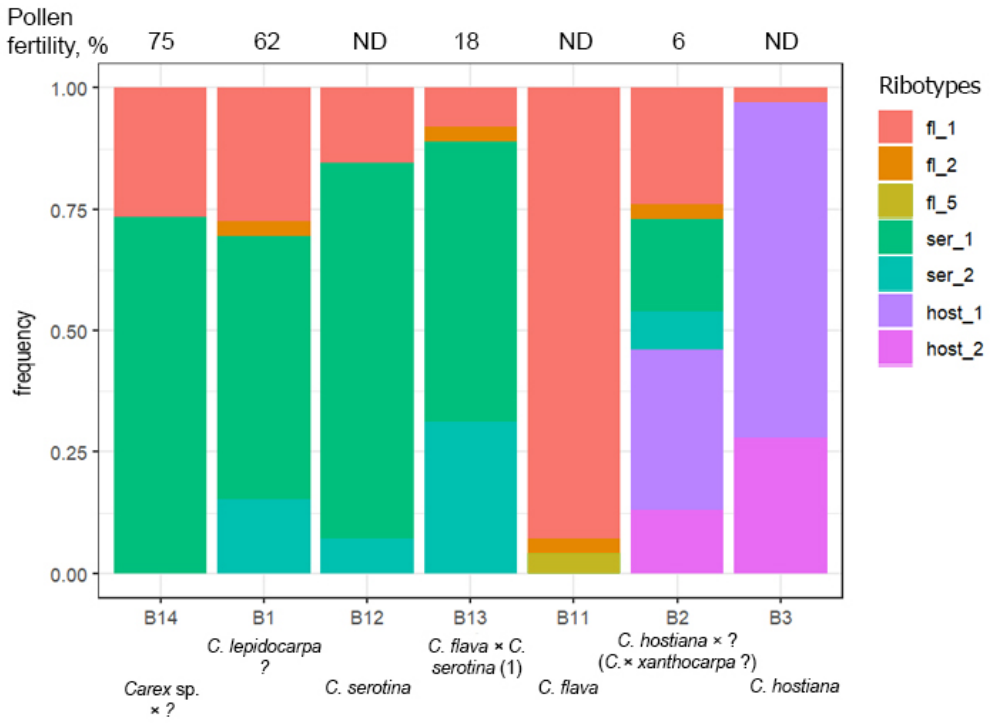

Fig. 9. Distribution of ribotypes in the studied *Carex* sect. *Ceratocystis* specimens from the Leningrad Region. The percentage of fertile pollen is shown above the plot on a separate line; unknown percentages are marked as ND (not determined).

Although that study recognised two varieties of *C. viridula* (*C. viridula* var. *viridula* and *C. viridula* var. *pulchella*) and we treated them as separate species, both approaches resulted in them forming a common cluster.

The variation in morphological characters, such as the average values of utricle length (UL) and beak (B) of *C. flava*, *C. scandinavica*, *C. serotina*, and *C. hostiana* in our study seems largely similar to that found in other regions of Fennoscandia (Hedrén, 2002), the British Isles (Blackstock, Ashton, 2001), as well as Central and Western Europe (Więcław, Koopman, 2013; Więcław, 2014).

Molecular analysis

Illumina sequencing of ITS1 revealed that all species have their own ribotypes, while hybrids and ambiguous specimens with intermediate trait values exhibit a combination of parental ribotypes. Despite the fact that *C. scandinavica* shares the same set of ribotypes as *C. serotina*, the lack of genetic differentiation is not always associated with morphological divergence (Jiménez-Mejías et al., 2012).

The presence of unique ribotypes in each species except *C. scandinavica* coincides with the morphological division of the section into three subsections (Egorova, 1999), as well as with the molecular phylogenetic data (Jiménez-Mejías et al., 2012, 2016), however sometimes *C. hostiana* is treated as a separate monotypic section (Danylyk, Koopman, 2023). This is also consistent with allozyme data showing the presence of unique alleles in the recognised taxa *C. flava*, *C. demissa*, *C. lepidocarpa*, and *C. oederi* s. l. (or *C. viridula* s. l.) (Bruederle, Jensen 1991; Hedrén, 2002). Some specimens in our study (*C. scandinavica* (B5, B16, B20) from Murmansk and *C. serotina* (B12) from the Leningrad Region possess *C. flava* ribotypes, which could indicate introgression. Gene flow may be extensive between some of the taxa, and data obtained from different studies, including morphological (Hedrén, 2002; Więcław, Koopman, 2013), cytogenetic (Schmid, 1982), and molecular (Bruederle, Jensen, 1991; Hedrén, Prentice, 1996; Hedrén, 2002; Jiménez-Mejías et al., 2012), indicated this. However, it is worth noting that *C. flava* differs from *C. serotina* and *C. scandinavica* both morphologically (Więcław, 2014) and genetically, as was shown earlier in the study of mixed populations of these plants, their hybrids, and backcrosses in Estonia, high and low Switzerland (Schmidt et al., 2018).

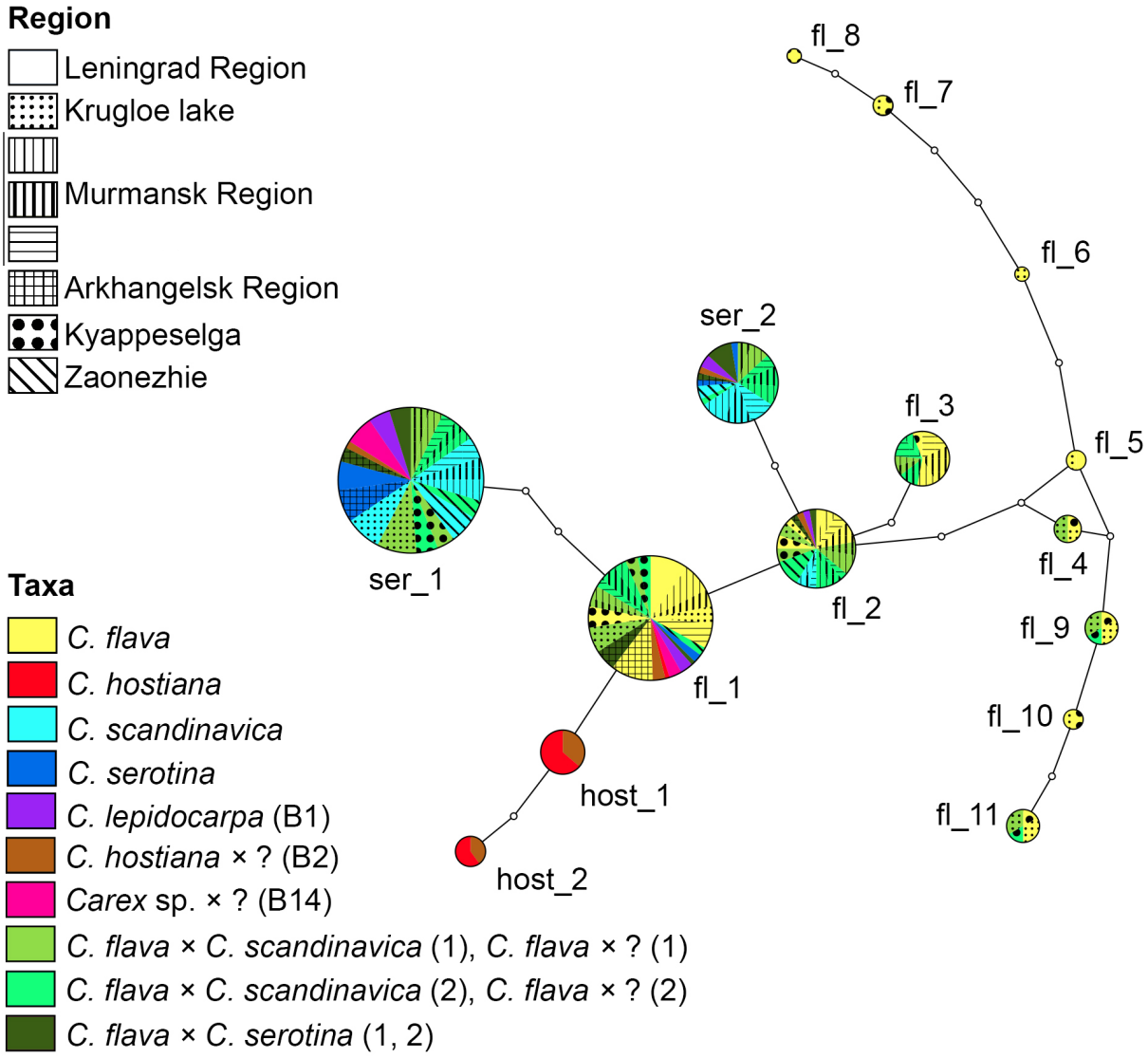

Fig. 10. A network of ribotypes demonstrates the level of intragenome and interspecific variation based on the ITS1 sequences.

Comparison of molecular and morphological data

Ordination analyses (morphological PCA and molecular PCoA) yielded consistent results.

The *C. scandinavica* group clustered closely with *C. serotina* and exhibited low morphological variation. In contrast, *C. flava*, however, was morphologically diverse, though the molecular data confirmed a population-level structure, with individuals from the same site grouping together. Despite being a well-differentiated species, *C. hostiana* was placed with hybrids in PCA and PCoA. This may be due to the set of morphological features used in our analysis, which may not sufficiently distinguish this taxon from others, or to the limited number of samples included in the study. Hybrids have an intermediate position in both ordinations. However, PCoA partially reflects the geographical origin of specimens while PCA on morphological data does not. This is clearly visible in the Murmansk population, which is isolated on the shore of the White Sea, and its representatives have not just a set of identical ribotypes, but an extremely similar distribution of them. It is a valuable site in which only two parents and two of their hybrids grow.

Putative F_1 hybrids characteristics

Apparently, the majority of hybrids *C. flava* × *C. serotina* s. l. (*C. flava* × *C. scandinavica* (1, 2), *C. flava* × *C. serotina* (2), *C. flava* × ? (1, 2)) in our study are F_1 hybrids, as indicated by their complete sterility observed in those specimens for which pollen fertility could be assessed. This is consistent

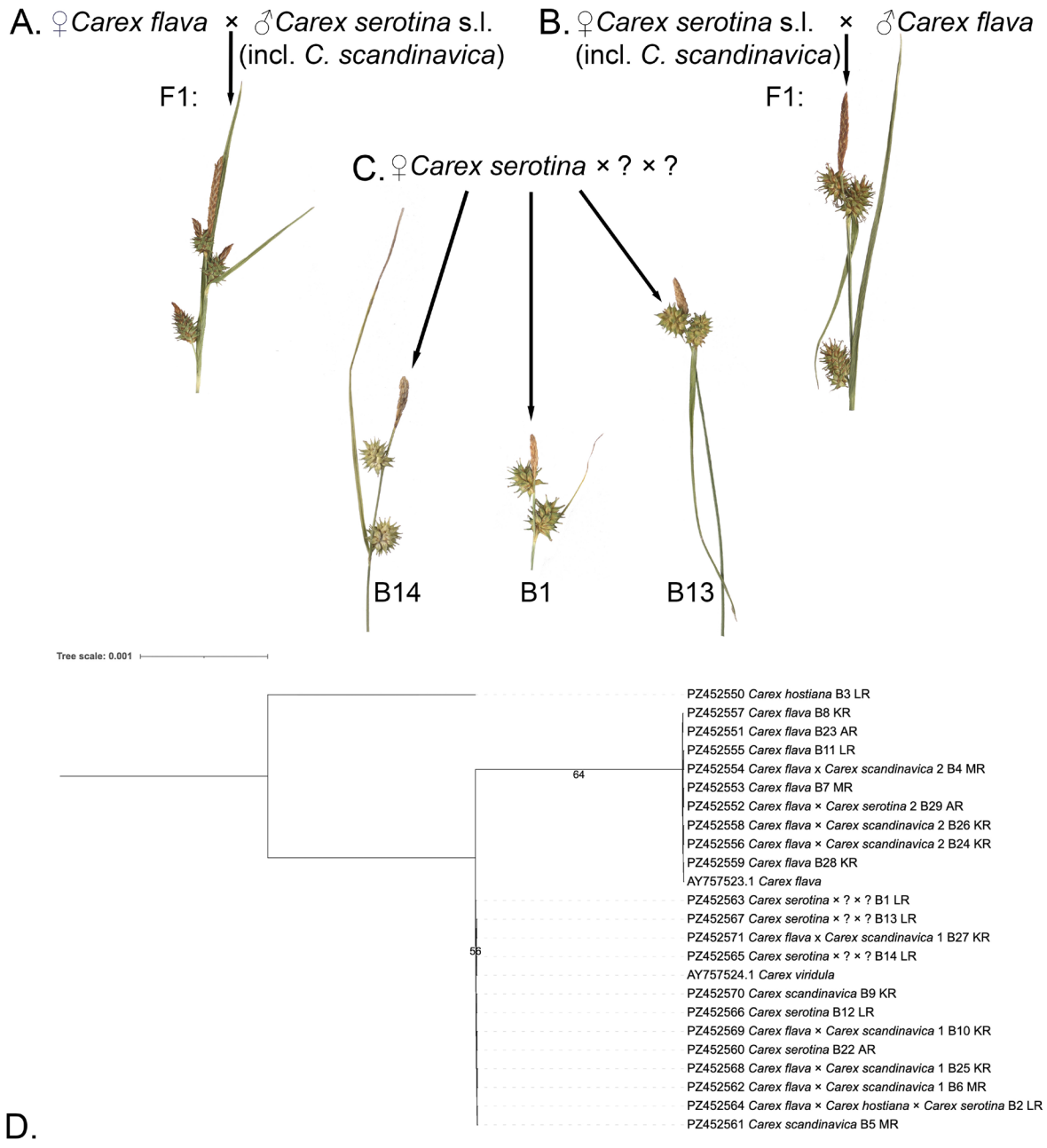

Fig. 11. Breeding schemes of parental *Carex* plants based on *trn*L Sanger sequences and Illumina sequencing of ITS: A, B – F_1 hybrids; C – F_n hybrids or backcrosses; D – phylogeny tree based on *trn*L sequences. Abbreviations: AR – Arkhangelsk Region; KR – Republic of Karelia; LR – Leningrad Region; MR – Murmansk Region.

with previous studies of Swiss populations, where these hybrids showed practically complete sterility (Schmid, 1982). Although our data did not show it, these F_1 hybrids likely retain a marginal level of fertility, as evidenced by the presence of backcrosses in certain populations as also reported by Schmid (1982). It was noted that hybrids exhibited unopened anthers containing sterile pollen; they are a more reliable indicator of hybridity than the absence of seeds (Rich, 1998).

It turned out that hybrids differ in morphology but have a similar set of ribotypes to the parental species and different combinations of parental species. Similar ribotype distribution in Murmansk specimens and in plants from Lake Krugloe can be explained by the rather small area that all these plants occupy, perhaps all these hybrids are descended from one pair of parents, as well as the parental plants.

***C. flava* × *C. serotina* s. l. (1)**

Culms 25.3–50.49 cm high, lowest female spike leaf length is 6.37–12.13 cm which is longer than inflorescence 3.3–4.48 cm long. Male spike single on peduncle 0.09–0.4 cm. Female spikes 3, close together (0.96–1.6 cm), ellipsoid 0.54–1.14 cm long, 0.7–0.77 cm wide, sessile or on short peduncle (0.15–0.4 cm). Utricles 3.4–3.9 mm long with beak 1.3–1.5 mm long (accounting for 37–39 % of total utricle length) and glume 2.2–2.6 mm (accounting for 61–76 % of total utricle length).

***C. flava* × *C. serotina* s. l. (2)**

Culms 25.9–39.73 cm high, lowest female spike leaf length is 6.1–11.43 cm which is longer than inflorescence 1.78–8.2 cm long. Male spike single on peduncle 0.43–1.05 cm. Bisexual spikes 2–3, close together (0.38–2.45 cm), ellipsoid 0.8–1.36 cm long, 0.5–0.59 cm wide, sessile or on short peduncle (0.17–0.8 cm). Utricles 2.7–3.3 mm long with beak 0.9–1.2 mm long (accounting for 33–40 % of total utricle length) and glume 2–2.2 mm (accounting for 65–78 % of total utricle length).

Palmgren (1959) also reported *C. flava* × *C. bergrothii*, *C. flava* × *C. kotilaini, C. flava* × *C. oederi* subsp. *pulchella,* and *C. flava* × *C. oederi* subsp. *fennica* hybrids in Finland, distinguishing two types: one with predominantly *C. flava* traits and another with a stronger imprint of the second parent, including staminate flowers at the apex of the female spikes. This author noted that the first type was clearly predominant, although the second was also quite common, a pattern observed particularly in hybrids with *C. oederi* subsp. *pulchella* and subsp. *fennica*. However, our molecular data from the chloroplast *trn*L sequence reveal that the maternal parent of the first hybrid type is *C. serotina* s. l., while for the second type it is *C. flava*.

Establishing the taxonomic identity of the specimens with intermediate traits

The combined molecular approach utilising Illumina sequencing of ITS1 and Sanger sequencing for a chloroplast marker elucidates the status of morphologically intermediate forms and corrects prior misidentifications of relevant specimens. These morphologically ambiguous plants originated from Dontso, an old limestone bedrock site rich in the *Carex* sect. *Ceratocystis* specimens. This environment, where hybrids and backcrosses are common, suggests that such intermediate morphotypes are likely products within long-established populations.

It was possible to establish that the specimen identified as *C. lepidocarpa* (B1, Figs. 1C, 9) presumably may be a non-first generation hybrid or a backcross with nearly restored fertility, as pollen analysis and the presence of seeds show. This assumption is supported by the fact that the plant has a set of ribotypes from its possible parents, while molecular studies have shown that *C. lepidocarpa* differs genetically from *C. flava* and *C. serotina* (Bruederle, Jensen, 1991; Blackstock, Ashton, 2010; Jiménez-Mejías et al., 2012).

The specimen *C. flava* × *C. serotina* (1) (B13, Fig. 1G) is semi-fertile; perhaps it was formed as a result of backcrossing with parental species or is an F_n hybrid, which explains the partial fertility of pollen and seed formation and morphological similarity of the specimen to parental species (*C. serotina*).

The specimen *Carex* sp. × ? (B14, Fig. 1H) is morphologically different from all the plants studied in this work. It also has fertile pollen but does not form seeds. We suspect it is a hybrid of *C. flava* and *C. serotina* or backcross, which has only 2 dominant ribotypes of these species (fl_1 and ser_1) but no other ribotypes, unlike the hybrids *C. flava* × *C. scandinavica* (1) and *C. flava* × *C. scandinavica* (2) from which it differs morphologically.

However, the *C. hostiana* hybrid used in our study (B2, Fig. 1J), which we initially assigned to *C.* × *xanthocarpa*, likely represents triple (*C. flava* × *C. hostiana* × *C. serotina*) or F_n hybrid. Its *trn*L chloroplast sequence (Fig. 11) matches to *C. serotina*. The hybrid nature of this specimen is confirmed by molecular data, in which the ribotypes host_1, host_2, ser_1, ser_2 are species-specific and fl_1, fl_2 are found not only in *C. flava* but also in other species, possibly as a result of introgression. From a morphological point of view, this hybrid resembles its parent species: while its inflorescence leaf is damaged, it seems to be short like that of *C. hostiana*. However, its utricles are bigger than those in *C. hostiana* and *C. serotina,* and its length does not occupy an intermediate position between them. On the PCA plot, specimen B2 is located quite far from other species and hybrids, while in the PCoA plot, it is in the centre with other hybrids.

Conclusion

In this study, we provided an integrated molecular and morphological characterisation of four *Carex* species and their hybrids from Northwest European Russia by combining Illumina sequencing of ITS1 with chloroplast *trn*L sequencing and pollen fertility analysis.

We showed that analysis of ITS1 polymorphism is an effective tool to study hybridisation and introgression and establish parental taxa or hybrid origin of specimens.

A key finding of this work is the correlation between the maternal lineage and hybrid phenotype. According to our data, hybrids with *C. flava* as the maternal parent (determined by *trn*L sequences) consistently exhibited bisexual (androgynous) spikes. Conversely, hybrids with *C. serotina* s. l. as the maternal parent retained typical female spikes.

Our data confirm that in the *Carex* sect. *Ceratocystis* there are F_1 hybrids, hybrids of other generations (such as the putative triple *C. flava* × *C. hostiana* × *C. serotina* hybrid) and backcrosses are possible.

The diagnostic markers identified in this study (both molecular and morphological) provide a robust framework for improving identification keys and accurately documenting the biodiversity of the section *Ceratocystis* in European Russia.

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