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In search of hybridity: the case of Karelian spruces

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Highlights

- Karelian spruces have morphology that is typical for *P. obovata* and characterized with genetic variation, described previously for *P. abies*.
- Karelian spruces evolved due to introgression between *P. abies* and *P. obovata*. However, it is unclear whether Karelian spruces could be treated as *P. fennica*, because unequivocal morphological and genetic characters of the latter are still absent.

Abstract

Distribution ranges of spruces, European *Picea abies* and Siberian *P. obovata* intersect in the Eastern Europe and Urals, forming wide zone of introgressive hybridization where species discrimination is difficult. We applied both molecular (mtDNA and cpDNA sequences) and morphological approaches with goals of elucidating the origin of spruces in undisturbed forests of Russian Karelia (considered as part of the abovementioned introgression zone). Karelian spruces have morphology that is typical for *P. obovata* and characterized with genetic variation, described previously for *P. abies*. This contradiction between morphology and organellar DNA could be itself an evidence of hybrid origin because morphological data should have a genetic basis. If the genes responsible for the observed morphological differences are nuclear, that explains why we did not see any deviation of Karelian spruces towards *P. obovata* in organellar markers. In this situation nuclear gene flow from *P. obovata* should be performed via pollen. Thus, we should expect Karelian spruces to have cpDNA haplotypes (inherited paternally in conifers) typical for *P. obovata*. However, it is not the case for the majority of plants sampled and requires additional explanation like chloroplast capture in the introgression zone. In all, we think that Karelian spruces evolved due to introgression between *P. abies* and *P. obovata*. However, it is unclear whether Karelian spruces could be treated as *P. fennica*, because unequivocal morphological and genetic characters of this hybridogenous species are still absent.

Keywords *Picea*; plant mtDNA; cpDNA; introgression; northern taiga

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Abbreviations

ANOSIM	Analysis of similarity
CP	Coefficient of projection
CQN	Coefficient of narrowing
DC	Distance from the base of cone to the position of maximal width
DS	Distance from the base of scale to the position of maximal width
DSL	Scale width in 0.1 of scale maximal width distance from the tip
HC	Cone maximal width
HS	Scale maximal width
LC	Cone length
LS	Scale length
PCA	Principal component analysis
RAPD	Random amplified polymorphic DNA

1 Introduction

High importance of forest ecosystems is beyond doubt. Knowledge of species composition and genetic variability for dominant trees is essential for rational use of natural resources (Brautingam et al. 2013). However, this is sometimes difficult to achieve. Well-known examples are spruces, *Picea* spp., an important genus of dominant forest trees. In Eurasia, one of the largest continuous distribution areas for forest trees is formed by two spruce species: *P. abies* (L.) Karst. and *P. obovata* Ledeb. The first has European range; the second grows in Siberia and Eastern Asia. Distribution ranges of these species intersect in the Eastern Europe and Urals, forming wide zone of introgressive hybridization which appeared due to secondary contact between *P. abies* and *P. obovata* after the last glaciation (Krutovskii and Bergmann 1995; Popov 2005; Popov and Melnik 2006; Tollefsrud et al. 2008b). In this introgression zone the discrimination between species is difficult; spruces with intermediate morphology are believed to be hybrids and often considered as a hybrid species *P. × fennica* (Regel) Kom., distributed across almost all Russian plain and Scandinavia (Ramenskaja and Andreeva 1982; Potokina et al. 2012). Difficulties with morphological delimitation of *P. abies* and *P. obovata* impelled some authors (e.g. Schmidt-Vogt 1974) to treat them as geographical varieties of one species, which had a support from allozyme data (e.g. Krutovskii and Bergmann 1995).

Characters of cones and seed scales are widely accepted as the most useful for the discrimination of these spruces (Ramenskaja and Andreeva 1982; Popov 2003; 2005; 2010). *Picea abies* is characterized by longer (10–16 cm vs. 4–8 cm) cones, and seed scales of different shape (rhomboid with toothed tip vs. fan-shaped with rounded tip). To add objectivity into seed scale shape determination, several ratios (coefficients) were suggested for species determination, e.g., coefficient of narrowing and coefficient of projection and their difference (Popov 2003; 2005; 2010; Popov and Melnik 2006). A geometric morphometric approach (Zelditch et al. 2004) which could take into account the essential shape of scales instead of linear measures and their ratios, could be useful in this case but as far as we know, this method has not been applied here. There are some other morphological characters of vegetative shoots and needles which are sometimes used for species discrimination (Orlova and Egorov 2011; Potokina et al. 2012), but their applicability is doubtful. However, morphological characters alone could hardly be used for delimitation of the three spruce species in case of their sympatry as there exist a smooth morphological gradient between typical *P. abies* and *P. obovata* (Popov 2005) and high morphological variability of putative *P. fennica* was revealed (Popov 2007; Potokina et al. 2012).

Molecular data could provide a more definitive answer to the question about the nature of spruce populations attributed to *P. fennica*. Both genetics of *Picea* as a whole (Nystedt et al. 2013) and population structure of *P. abies* in particular are well-examined (Lagercrantz and Ryman 1990; Sperisen et al. 2001; Tollefsrud et al. 2008a; 2008b; 2009). In our case (delimitation between *P. abies* and *P. obovata*), mitochondrial *nad1* and *trnL-trnF* chloroplast markers have been proved to be useful (Tollefsrud et al. 2008a; 2008b). In spruces, mitochondrial genome inheritance is maternal whereas the chloroplast genome is coming from wind-distributed pollen (Sutton et al. 1991). This fact provides opportunity for studying gene flow in the introgression zone from each of the two “pure” species separately (Hipkins et al. 1994). We should bear in mind, however, that species delimitation detected by molecular markers is complicated by interactions between introgression, incomplete lineage sorting (Zhou et al. 2010) which are especially typical for conifers (Du et al. 2009), and also historic and contemporary gene flow and environmental selection (Hamilton and Aitken 2013). Various combinations of these evolutionary forces seem to result in different genetic. Greater diagnostic value for delimitation between *P. abies* and *P. obovata* was attributed to mtDNA (Tollefsrud et al. 2008a; 2008b), whereas in numerous conifer species complexes cpDNA was more species specific and mtDNA variation matched with geography rather than with taxonomy (Du et al. 2009; Zhou et al. 2010).

Nowadays the only considerable areas of relatively undisturbed forests in Europe are located in Russian North Karelia (Lampainen et al. 2004). In the same time taxonomical status of spruces frequently dominating in northern taiga forest remains uncertain. According to morphological data, all the three species (namely, *P. abies*, *P. obovata* and *P. fennica*) occur there (Ramenskaja and Andreeva 1982; Orlova and Egorov 2011). However, neither RAPD (random amplified polymorphic DNA, Kopylova et al. 2011) nor nuclear microsatellite data (Potokina et al. 2012) were able to verify this hypothesis. Unfortunately, North Karelia was not included in the only large-scaled study of Eurasian spruces, based on mitochondrial and chloroplast markers (Tollefsrud et al. 2008b). In our work, we tried to use both molecular and morphological approaches with goals of elucidating the origin of spruces in North Karelia and finding the characters that discriminate them best.

2 Materials and methods

2.1 Morphology

Most material was collected in 2010–2012 from 104 trees in 9 locations of North Karelia (environs of Chupa, not far from the Arctic Circle and the coast of White Sea, Table 1). For comparison, we also collected plant material in Germany (48 trees and 4 locations, Table 1) and in Siberia (25 trees, 3 locations from different climatic zones in Krasnoyarsk region and in Buryatia Republic, Table 1) where each of the parental species occurs solely (*P. abies* and *P. obovata* correspondingly: Komarov 1968). We have chosen *P. abies* from southern genetic domain for the comparison to analyze “pure” species, because gene flow from Siberian spruces was reported for northern domain of *P. abies* (Tollefsrud et al. 2008a, 2008b). Unfortunately, as far as we know, cone morphology of southern domain of *P. abies* was not studied, but published data on northern domain suggest that morphological variation in cones is more longitudinal than latitudinal (Popov 2003) making thus German populations comparable with *P. abies* from northern domain. Typically, we picked ripe cones from trees stayed at least 10 m apart, one cone per tree. Seed scale contours were projected on paper and then scanned.

In 2001, we also collected cone and scale material in the same region as a part of preliminary study. This time, we did not distinguish between trees, but pooled all cones collected on the

Table 1. Geographic origin, mtDNA and cpDNA haplotypes and number of plants subjected to morphological analysis in the investigated locations of *Picea* spp.

Population	Geographic coordinates		Haplotype														N plants for morphometry	
	Long., N	Lat., E	Chloroplast haplotypes (cf. Fig. 5) ^(a)										Mitochondrial haplotypes (Tollefsrud et al. 2008b) ^(a)					
			GCCA	_CCA	_CCC	GCAC	_CAC	GCCC	_TCC	712s	721bp	755bp	857bp	891bp	925bp	959bp		
North Karelia (Russia), environs of town Chupa: unknown species identity ("Karelian spruces")																		
a	66.3124	33.3346	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	321
b	66.3378	33.6499	2	0	1	0	0	5	1	0	—	—	—	—	—	—	—	20
c	66.3408	33.6751	2	0	1	0	0	14	0	1	0	3	0	0	0	0	0	20
d	66.3182	33.4714	1	1	2	1	1	4	0	0	—	—	—	—	—	—	—	20
e	66.3495	33.6232	3	1	0	0	0	6	0	0	—	—	—	—	—	—	—	20
l	66.3401	33.6687	2	0	1	0	0	5	0	0	0	2	2	0	0	4	2	10
m	66.4279	33.7777	3	0	0	0	0	2	0	0	0	0	0	0	1	0	0	5
o	66.2652	32.9747	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	9
Central Siberia (Russia): "pure" <i>Picea obovata</i>																		
g	55.9466	92.7433	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	11
h	61.6008	90.0100	1	0	0	0	0	0	0	0	—	—	—	—	—	—	—	10
n	50.8507	107.3085	4	0	0	0	0	0	0	0	4	0	0	0	0	0	0	4
Southern Germany: "pure" <i>Picea abies</i>																		
i	49.5838	9.0216	0	0	1	0	0	0	0	0	—	—	—	—	—	—	—	18
j	49.5080	8.0744	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8
k	47.9947	7.8497	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
p	49.5838	9.0216	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	20

a) According to Tollefsrud et al. (2008b), haplotypes that are given in bold font have Siberian origin and are typical for *P. obovata*; haplotypes given in regular font have European origin and are more frequent in populations of *P. abies*.

transect in every 10 m together. Data of these 321 cones (location “a”) were used only once, in the “extended” dataset (see below).

Cones and scales were measured to obtain the most frequently used morphological characters, namely, (1) LC, cone length; (2) HC, cone maximal width; (3) DC, distance from the base of cone to the position of maximal width; (4) LS, scale length; (5) HS, scale maximal width; (6) DS, distance from the base of scale to the position of maximal width and (7) DSL, scale width in 0.1 of HS distance from the tip. Two more coefficients: coefficient of narrowing (CQN, equal to $DSL/HS*100$) and coefficient of projection (CP, equal to $(LS-DS)/HS*100$) and their difference (Popov 2005) were also added to the character set.

The shape of seed scales was analyzed using the thin-plate spline approach (Zelditch et al. 2004). Instead of true landmarks, we used semi-landmarks which were distributed evenly along the contour of every seed scale (counter-clockwise, starting from the base). Screen digitizer software tpsDig2 (Rohlf 2010a) was used to catch coordinates of every landmark. Weight (principal warps) matrices were obtained from tpsRelw (Rohlf 2010b), and super-imposed images of scales – from tpsSuper software (Rohlf 2004). Classic and geometric morphometry data was visualized with multivariate methods like PCA (principal component analysis). The significance of PCA grouping was tested with ANOSIM (analysis of similarity, Warton et al. 2012) based on 99 or more permutations. For the comparison between populations, non-parametric tests were used, with Bonferroni correction for multiple comparisons, if applicable. R statistic environment (R Core Team 2013) was used for most calculations and plots.

2.2 Molecular data

In 2010–2012, needle material was collected from the majority of trees accessed for morphological data (Table 1). These needles were used for DNA extraction. DNA was extracted using a MO BIO PowerPlant DNA Isolation Kit (MO BIO Laboratories, Carlsbad, California, USA). Dry needle material (~ 0.1 g) was powdered using a sterile mortar and pestle and then processed in accordance with the supplied protocol.

Two fragments were sequenced: chloroplast *trnL-trnF* region using primers trnt_trnf_c_new (5'-GGAGGATAATAACATTGCAT-3', Tollefsrud 2008b) and trnt_trnf_d (5'-GGGGATAGAGG-GACTTGAAC-3', Taberlet et al. 1991), and mitochondrial nad1 region, using primers nad1for (5'-CTCTCCCTCACCCATATGATG-3', Sperisen et al. 2001) and nad1rev (5'-AGATCCCCATA-TATTCCCGG-3', Sperisen et al. 2001; with correction from Dr. Mari Mette Tollefsrud, Norwegian Forest and Landscape Institute, pers. comm. in 2013). PCR was carried out as follows: the reaction mixture in a total volume of 20 µL contained 5.2 µL of *Taq* PCR Master Mix (QIAGEN Corporation, Germantown, Maryland, USA), 1 µL of 10 µM solutions of forward and reverse primers, 1 µL of DNA solution from the extraction above and 11.8 µL water. Samples were incubated in a thermal cycler: 35 cycles of 94° for 1 min; 47 or 53° (for cpDNA and mtDNA, respectively) for 1 min, 72° for 2 min, and finally 72° for 10 min. Single band PCR products were supplied for purification and sequencing to Functional Biosciences, Inc. (Madison, Wyoming, USA) and sequenced there in accordance with standard protocol with both primers (for mitochondrial fragment) or just with one primer (chloroplast fragment, most of cases): Table 1. Sequences were then assembled and edited using Sequencher™ 4.5 (Genes Codes Corporation, Ann Arbor, Michigan, USA). Chloroplast sequences were aligned with ClustalX (Thompson et al. 1997) using gap opening cost 9, gap extension cost 0.05 and IUB weight matrix, followed by manual adjustments; mitochondrial DNA sequences were aligned manually. All haplotype sequences were submitted to GenBank (Table 2). For the alignment, we also used haplotype sequences provided by Dr. Mari Mette Tollefsrud (Norwegian Forest and Landscape Institute, pers. comm. in 2013).

Table 2. GenBank accession numbers for chloroplast and mitochondrial haplotypes discovered.

Haplotype	Haplotype full name	GenBank accession number
_CCC	_CCC_abies_i2	KF896137
_CAC	_CAC_abies_m3	KF896138
_CCA	_CCA_obovata_d11	KF896139
_TCC	_TCC_abies_c9	KF896140
GCAC	GCAC_abies_d4	KF896141
GCCA	GCCA_obovata_g3	KF896142
GCCC	GCCC_abies_b8	KF896143
721bp	721bp_obovata_c5	KF896145
755bp	755bp_na_l3	KF896146
857bp	857bp_obovata_m1	KF896147
891bp	891bp_abies_m5	KF896148
925bp	925bp_obovata_l1	KF896149
959bp	959bp_obovata_l2	KF896150

3 Results

3.1 Morphology

Most morphological characters were able to distinguish *Picea abies* from *P. obovata* and from Karelian spruces (Fig. 1), which was supported with the non-parametric pairwise Wilcoxon test ($p < 0.001$ after Bonferroni correction). However, in most cases Karelian spruces were not separated from “pure” *P. obovata* (pairwise Wilcoxon test $p > 0.05$). Only width of scale tip (DSL) allowed for some differentiation between these two groups (pairwise Wilcoxon test: $p = 0.006$). Nevertheless, DSL values were largely intersecting (interquartile range: 6–8 mm vs. 7–9 mm for Karelian and Siberian trees respectively), making this character less useful for discrimination.

Ratios (Fig. 2) had not more discrimination power than raw morphological characters with an exception of coefficient of narrowing (CQN) that was able to separate (Wilcoxon pairwise test: $p < 0.01$) “pure” *P. abies* (interquartile range: 28–42), Karelian spruces (50–64) and “pure” *P. obovata* (62–75). However, within-group variation of this character was still quite high (Fig. 2). The mean difference between coefficients of narrowing and projection for Karelian spruces (the most important diagnostic character, according to Popov 2005) were equal to 6.6, which was typical for *P. obovata* (i.e. more than 5.0: Popov 2005), the difference between these two groups was not significant (Wilcoxon pairwise test: $p = 0.06$). Interquartile range of differences between coefficients of narrowing and projection for “pure” *P. abies* was from –54 to –12 which is typical for this species (less than –5.0: Popov 2005).

We obtained the general “picture” of spruce diversity using PCA on scaled raw characters (i.e., without ratios). Two first components acquired 80.5% of variation (64.8% and 15.7% for PC1 and PC2, respectively) and therefore were used in subsequent analysis (Fig. 3). The most influential morphological characters (characters with biggest loadings on the particular component) were LS and LC for PC1 and DS for PC2. Similar to individual characters, multivariate analysis was able to distinguish *P. abies* from pooled Siberian and Karelian samples (ANOSIM $R = 0.86$, significance = 0.01), but was not able to separate Karelian samples from “pure” *P. obovata* (ANOSIM $R = -0.05$, significance = 0.85).

To understand if any heterogeneity existed in our Karelian populations, we added here location “a” of our preliminary sampling and obtained the extended dataset of 510 samples. However,

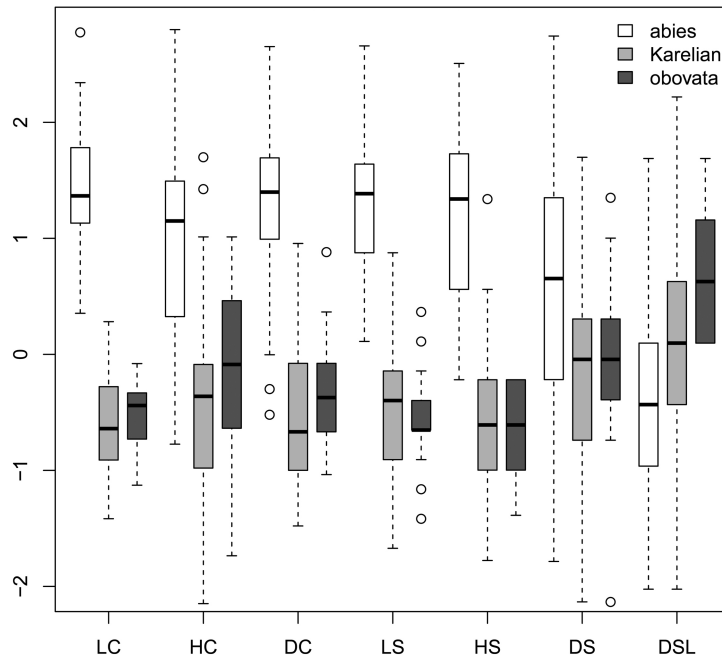


Fig. 1. Boxplots showing the variation of most frequently used scaled morphological characters. Characters are represented in values of standart deviations to facilitate comparison of variability in different characters. Absolute and quartile range along with median and outliers are given. Abbreviations are: LC, cone length; HC, cone maximal width; DC, distance from the base of cone to the position of maximal width; LS, scale length; HS, scale maximal width; DS, distance from the base of scale to the position of maximal width; DSL, scale width in 0.1 of HS distance from the tip.

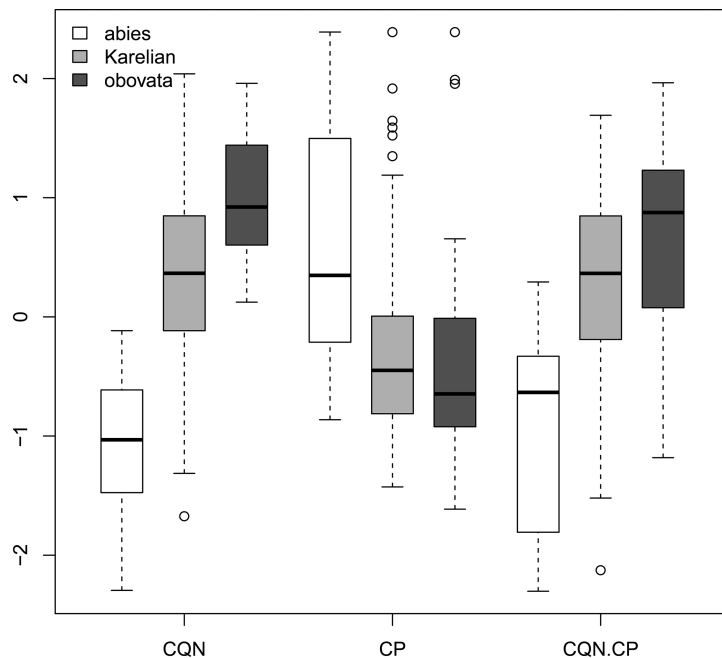


Fig. 2. Boxplots showing the variations of scaled morphological coefficients (ratios). Abbreviations are: CQN, equal to (scale width in 0.1 of scale maximal width distance from the tip)/(scale maximal width)*100; CP, equal to [(scale length)–(distance from the base of scale to the position of maximal width)]/(scale maximal width)*100; CQN.CP, equal to CQN–CP.

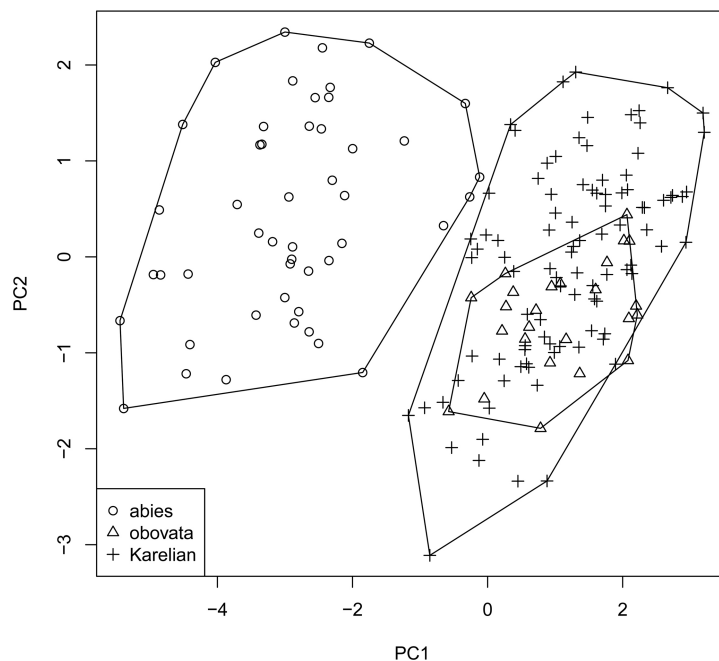


Fig. 3. Principal component ordination plot representing morphological diversity of studied spruces.



Fig. 4. Superimposed images of seed scales of studied spruces. From left to right: *Picea abies*, “Karelian spruces”, *Picea obovata*.

neither PCA nor the advanced classification methods (like recursive partitioning) could split this dataset in well-supported subgroups (not shown).

Geometric morphometric analysis allowed us to construct super-imposed (“averaged”) images of seed scales (Fig. 4). It also resulted in matrix of principal warps (“weight matrix”) which was scaled and used in PCA together with morphological characters. The resulting graph was similar with “classical morphology” PCA (not shown). There was again no significant difference between Karelian samples and “pure” *P. obovata* (ANOSIM $R = -0.06$, significance = 0.8).

3.2 Molecular analysis

We obtained chloroplast sequences for 5–18 trees from each locality in Northern Karelia with except for location “o” from where one tree was analyzed (60 trees in total: Table 1). In all, we discovered 7 chloroplast haplotypes in our Karelian samples. These haplotypes were different in two substitutions and one G-insert (Fig. 5). Majority (60%) of the trees were characterized by

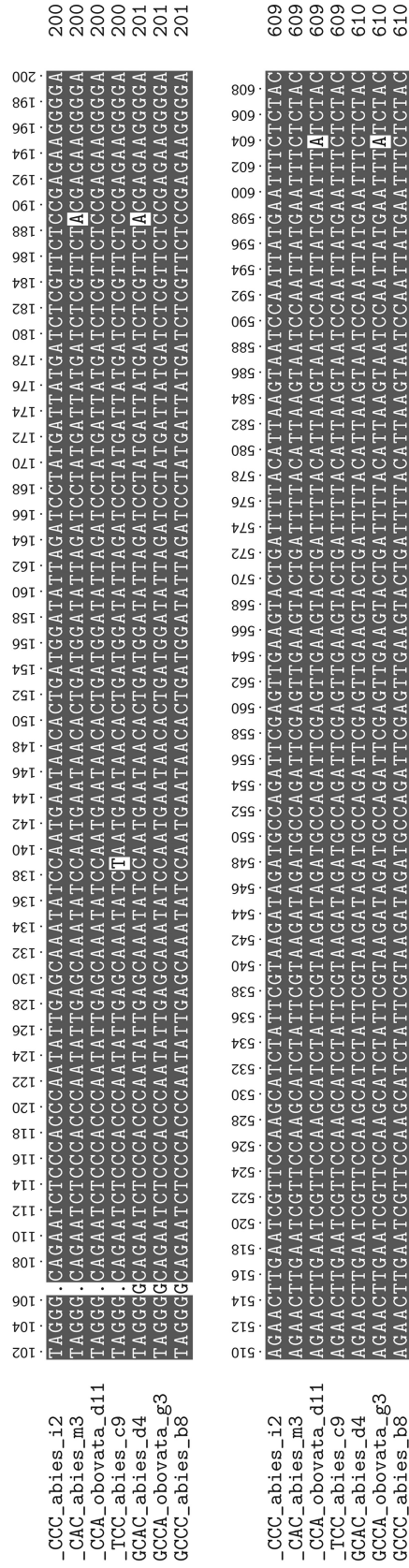


Fig. 5. Two fragments of the alignment of chloroplast haplotypes found, species identity and sample code are shown immediately after haplotype names.

haplotype _CAC, haplotypes _CCC and GCCA were also quite abundant (10% and 21% correspondingly), single trees also had haplotypes _CCA, GCAC, GCCC and _TCC (Table 1). Different haplotypes always co-existed in same locality (Table 1). Morphological analysis of samples to which haplotypes data was available, returned no significant difference in any morphological character between trees with cpDNA haplotypes of Siberian origin (with CA end: Tollefsrud et al. 2008b, see Discussion) and with other haplotypes of European origin (for every raw morphological character, Wilcoxon test p-values > 0.29).

In general, amplification of mitochondrial nad1 marker was less successful (1–10 trees from four localities in Northern Karelia, 19 trees in total: Table 1) but we were able to find 6 mitochondrial haplotypes (721bp, 755bp, 857bp, 891bp, 925bp and 959bp, names from Tollefsrud et al. 2008a) in Karelian samples.

We also sequenced cpDNA and mtDNA of several trees from different locations of “pure” *P. obovata* (7 and 5 trees respectively). All the individuals were characterized by chlorotype GCCA and mitotype 712s (Table 1), typical for this species (Tollefsrud et al. 2008b).

4 Discussion

Morphological heterogeneity within Karelian samples was not enough to split them in any reliable way, even using an extended sampling. In accordance with morphological data and in line with previously published results (Popov 2003; 2007), Karelian spruces have morphology that is typical for *P. obovata*. Although seed scales of Karelian samples have slightly more acute tip than “pure” *P. obovata* (as indicated by values of narrowing coefficient), these differences are too small and were not revealed by shape analysis (geometric morphometry: Fig. 4).

However, molecular analysis resulted otherwise. All the revealed in Karelian spruces mtDNA haplotypes were typical for *P. abies* (Tollefsrud et al. 2008b). According to Tollefsrud et al. (2008b), our cpDNA haplotypes with CA end have Siberian origin and are much more abundant in populations of *P. obovata*, whereas other haplotypes widely distributed in Europe and are more frequent in *P. abies* populations. Haplotypes with G-insert have been also once considered as typical for *P. obovata* (Ran et al. 2006) but since there was only one sample of *P. obovata* in the cited research, we decided not to use this insert as diagnostic. Taking all these considerations into account, 75% of Karelian spruces had chloroplast haplotypes of European origin. Thus, genetic variation in all the analyzed locations of Karelian spruce was typical for *P. abies* (some admixture of *obovata*-specific chloroplast haplotypes is typical for *P. abies* populations and explained by extensive pollen flow: Tollefsrud et al. 2008b).

This contradiction between morphology and organellar DNA could be itself an evidence of hybrid origin because morphological data should have a genetic basis. It was shown for different conifers that cone morphology is under not only environmental, but also genetic control (e.g. Kumar et al. 2007; Hamilton and Aitken 2013). Specifically, main diagnostic character, i.e. shape of seed scales, of *P. abies* and *P. obovata* mainly depends on the genetic characteristics of the plant (Popov 2003). Nuclear microsatellite data delimited Karelian *P. fennica* (pooled together with *P. obovata*) from *P. abies* (species were initially determined on morphological basis: Potokina et al. 2012). However, this delimitation was not perfect and the authors failed to differentiate *P. fennica* from *P. obovata*. Extensive pollen flow could notably reduce differentiation at nuclear markers (Tollefsrud et al. 2008b and references therein) but what is more important, Potokina et al. (2012) used samples only from previously glaciated North-Western Russia where introgression between spruce species is pronounced (Tollefsrud et al. 2008b). Thus, plants referred to *P. abies* and *P. obovata* in the cited work could already represent genetic mixture of “pure” parent species.

There are several possible explanations for the discrepancy between morphological and organellar DNA data. First, natural selection and/or epigenetic variation (Flatscher et al. 2012; Brautingam et al. 2013) of morphological characters could not be excluded. The climate of Northern Karelia is much colder and more continental than even in north of Western Europe (Kuznetsov 1960), and selective pressure towards the Siberian phenotype may take place. For example, rounded cone scales of Siberian phenotype were shown to reflect adaptation to cold climate (Schmidt-Vogt 1974). Clear influence of environmental gradient from maritime to continental climate on clinal variation in both morphological and nuclear genetic characters was recently revealed in *P. sitchensis* × *P. glauca* hybrid zone (Hamilton and Aitken 2013). Interestingly, more rounded cone scales in *P. sitchensis* × *P. glauca* hybrid zone were typical for more continental climate (Hamilton and Aitken 2013), mirroring morphological differences between more maritime *P. abies* and more continental *P. obovata* and variation in contact zone between these species (Ramenskaja and Andreeva 1982; Popov 2003; 2005; 2010).

If the genes responsible for the observed morphological differences are nuclear (the indirect evidence provided by Potokina et al. 2012), that explains why we did not see any deviation of Karelian spruces towards *P. obovata* in neutral organellar markers. Differentiation identified with organellar and nuclear markers has often been found to be independent in conifers (as was shown also for *P. abies*), and populations fixed for a single organelle haplotype do not necessarily show low diversity at nuclear markers (Tollefsrud et al. 2009 and references therein). In this situation nuclear gene flow from *P. obovata* should be performed via pollen as was shown earlier (Tollefsrud et al. 2008b). Abundance of chlorotypes typical for *P. abies* in Karelian spruces could have the following explanations. One possibility is the common phenomenon of closely related species sharing cpDNA haplotypes in the introgression zone, probably by chloroplast capture (i.e. the introgression of a chloroplast from one species into another, resulting in the exchange of chloroplast genomes: Rieseberg and Soltis 1991), which is typical for conifers (Hipkins et al. 1994). Fixation of *P. abies* mitotype in all Karelian locations possibly reflects postglacial recolonization history of Northern Karelia. *Picea abies* migrated to Fennoscandia shortly after retreat of the last maximum glaciation and even sustained there in small ice-free refugia during most of the last ice age (Parducci et al. 2012). Thus, established *P. abies* trees were the likely seed parents of early generations of Karelian spruces, receptive to some pollen flow from *P. obovata*. These processes appear to have contributed to patterns of “mitochondrial capture” of the maternally inherited *P. abies* mitotype in Karelian hybrid zone, as was shown in *P. sitchensis* × *P. glauca* hybrid zone (Hamilton and Aitken 2013). One more reason of the contradiction between morphology and DNA could be the relative scarcity of Siberian spruce data: those haplotypes which are now considering as typical for *P. abies* could be later discovered in *P. obovata*.

Thus, Karelian spruces evolved due to introgression between *P. abies* and *P. obovata*. However, it is unclear whether Karelian spruces could be treated as *P. fennica*, because unequivocal morphological and genetic characters of the latter are still absent (Ramenskaja and Andreeva 1982; Popov 2003; Kopylova et al. 2011; Potokina et al. 2012). Moreover, obtained data suggest treating *P. abies* and *P. obovata* as geographical varieties (or subspecies) of one biological species as was suggested earlier (e.g. Schmidt-Vogt 1974; Krutovskii and Bergmann 1995) and also adopted in some recent treatments (e.g. Annotated Checklist of the Panarctic Flora 2014). This treatment is supported by clinal morphological variation (Popov 2003; 2005; 2010), which is likely adaptive to increasing of climate continentality from west to east (Schmidt-Vogt 1974; Hamilton and Aitken 2013) and also by molecular delimitation between European and Siberian spruces mainly on mtDNA and not cpDNA markers (Tollefsrud et al. 2008b) reflecting geographical and not taxonomical structure (Du et al. 2009; Zhou et al. 2010). These considerations should be taken into account in guiding management of genetic resources, biotechnology and ecosystem conservation.

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