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"Northern tetraploids" clarified: A study of dactylorchids (*Dactylorhiza*, Orchidaceae) from North European Russia

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ABSTRACT

Despite numerous phylogenetic studies in recent decades, dactylorchids still remain taxonomically a poorly resolved group, which is especially a case for the vast territory of Russia. The massive morphological sampling effort was made towards the better understanding of dactylorchids in northern (from 50° latitude) European Russia. Previous study revealed that the majority of plants of *Dactylorhiza maculata* group from North European Russia belong to plants which combine *D. maculata* s.str. and *Dactylorhiza fuchsii* genomes. We applied results of previous molecular studies in the region as "anchors" to establish species identity using advanced multivariate classification techniques, Random forest and recursive partitioning. With Random forest, most samples were successfully discriminated so it became possible to attribute them to the known species. We also found that the majority of our Arctic samples with likely combined genomes comprise the separate group, to which we propose the name *Dactylorhiza psychrophila*. With recursive partitioning, we were able to create the dichotomous key which could be used for discrimination of *Dactylorhiza* species from the area of our study.

1. Introduction

Dactylorchids (*Dactylorhiza* Necker ex Nevski) are well known as taxonomically difficult group (Soó, 1980; Stace, 2010). While at least during the last 15 years this group has been under intensive, both molecular and morphological, investigations in Western Europe (Hedrén et al., 2001, 2007; Bateman and Denholm, 2003; Hedrén, 2002, 2003, 2009; Pillon et al., 2006, 2007; Nordström and Hedrén, 2008; Ståhlberg and Hedrén, 2010; Paun et al., 2011), the amount of studies concerning more eastern parts of its geographical range is scarce. Several papers published in 2000s (Shipunov et al., 2004, 2005; Shipunov and Bateman, 2005) elucidated problems specific to the Russian representatives of the genus. One of them is the presence of plants provisionally called "northern tetraploids" (Shipunov et al., 2004), putative hybrids with haplotype content intermediate between *Dactylorhiza fuchsii* and *Dactylorhiza maculata*. Another problematic group is allotetraploids, which are still in need of both molecular and morphological characterization and taxononomical recircumscription on the territory of European Russia. Up to date, this group has received only limited amount of studies, mainly concerning *Dactylorhiza baltica*, an allopolyploid with *D. incarnata* as a maternal parent (Shipunov and Bateman, 2005; Efimov, 2012). Allotetraploids were mostly out of the scope of the present study, and are represented only by *D. baltica* accessions.

Unfortunately, the level of sampling of dactylorchids from Russia is low. For the studies focused on Russian territory (Shipunov and Bateman, 2005), less than 200 specimens have been investigated with the molecular and classic/geometric morphometric approaches. Better, much larger sampling is necessary to assess the polymorphism of Russian dactylorchids. However, typical capacity of regional molecular studies rarely exceeds first hundreds of samples whereas, the territory of European Russia requires thousands of samples to be studied on the level comparable with the whole Western Europe. To resolve this conflict, researchers may want apply the "anchor" approach described in Shipunov and Bateman (2005). Since molecular features of some samples are well known, and it is also suggested that in dactylorchids, plastid and ITS markers correlate well with morphology (Shipunov et al., 2004), it is possible to use sequenced samples as proxies ("anchors") which help to determine non-sequenced, purely morphological samples. We decided to apply this approach for the massive sampling effort performed in European Russia.

In Northern Russia, genus *Dactylorhiza* is dominated by spotted marsh-orchids group (*D. maculata* s.l.). The contemporary review of spotted marsh-orchids mentions only 4 species: three diploids (*D. fuchsii*, *D. foliosa*, *D. saccifera*) and one tetraploid (*D. maculata*

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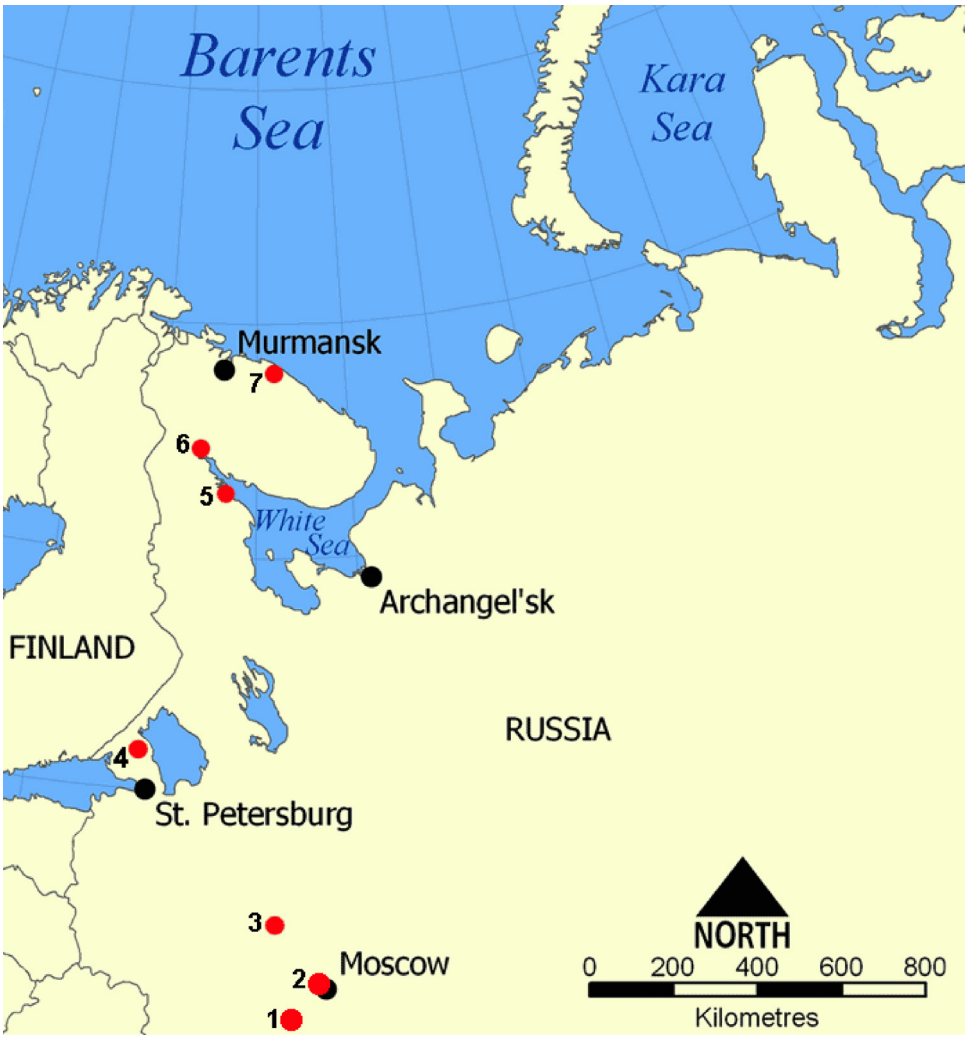

Fig. 1. Regions of sampling: 1–Kaluga region (one locality), 2–Moscow region (3 localities), 3–Tver region (49 localities), 4–St. Petersburg region (7 localities), 5–Karelia (24 localities), 6 and 7–Murmansk region (21 localities).

s.str.). *D. maculata* s.str. is commonly treated as autotetraploid originated from *D. foliosa*-like ancestor (Hedrén et al., 2001), which in its turn may be formally considered as an earlier segregate of polymorphic and widespread *D. fuchsii*. Recent studies focusing on sampling from Western Fennoscandia (mostly outside Russia) have revealed more complex pattern in the group and suggested several autopolyploidization events rather than one (Ståhlberg and Hedrén, 2008). Although sampling from subarctic northwestern part of the area in the latter study was limited, the populations of *D. maculata* from that area were assigned to a special taxonomic entity, namely var. *kolaensis*, whereas, it was "not possible to reliably distinguish any other groups of ssp. *maculata* in Scandinavia" (Ståhlberg and Hedrén, 2008). We find it especially reasonable to compare this var. *kolaensis* with "northern tetraploids" which were reported from neighboring part of the Russian territory.

In all, the main aim of our research was to study morphological disparity of Northern Russian dactylorchids using intensive sampling and comparisons with "anchor" specimens.

2. Material and methods

Most of morphometric data used in this study was collected in seven different regions of northern and central European Russia (Fig. 1), being represented by 642 plants which represent these regions. These samples were collected in 105 localities. Most of samples were characterized with both morphometric and geometric approaches. Morphometric data (see Table 1 for abbreviations

Table 1
Morphological characters used (all measurements are in millimetres).

Abbreviation	Description
BR.L	Length of lowermost bract
INFL.L	Length from the lowest bract to the top of inflorescence
L.WPOS	Position of maximal width (the distance from leaf base to the place of maximal width)
LATER.L	Length of lateral lobe of the lip, from the base of the sinus to the top apex of lobe
LEAF.L	Length of longest leaf
LEAF.SP	Leaf spots (0 none, 1 light, 2 heavy)
LEAF.W	Width of longest leaf
LIP.L	Lip length, from the base to the top of middle lobe
LIP.LM	"Minor length" of lip: from sinus to the end of left lateral lobe
LIP.W	Lip maximum width
MIDD.L	Length of middle lobe of the lip, from the base of the sinus to the top apex of lobe
P.HIGH	Plant height, from the ground to the top of inflorescence
SPOT.D	Spot distribution (0 absent, 1 concentrated at base, 2 equally distributed)
SPUR.L	Spur length, measured from lower side of spur
ST.DIAM	Stem diameter (measured just above the node of longest leaf)