CRYPTIC INVASION OR NATIVE SPECIATION? THE CASE OF PHRAGMITES IN BELARUS

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Introduction. Biological invasions are currently one of the most pressing problems facing humanity. The ecological consequences of the introduction of alien species can be dramatic, but the regulation and prevention of negative impacts is often hampered by the presence of cryptic species – species that are practically indistinguishable in morphological characteristics, but have significant differences at the genetic level (Morais & Reichard, 2018; Jarić et al., 2019). The problem of cryptic species and cryptic invasions has become especially urgent recently in connection with the development of molecular genetic methods and the possibility of reliable identification of such taxa. Cryptic invasions require a lot of attention, since, like any other invasions, they can cause changes in the functioning and productivity of ecosystems, but at the same time they go unnoticed, as a result, their monitoring becomes much more difficult.

The genus *Phragmites* Adans. is practically cosmopolitan in distribution, its species are distributed from the tropics to the cold temperate regions of the northern and southern hemispheres of the Earth, which makes it a good subject for research. Most of the species of this genus demonstrate high phenotypic, cytological and genetic variability and, as recent studies show, are complexes of cryptic species. The number of species of the genus *Phragmites* has not yet been unambiguously established. According to various researchers, the genus contains from 4–7 to 11–12 species. According to N.N. Tsvelev, the real number of reed species can reach 20 species (Tsvelov, 2011).

Study of the genetic diversity of *P. australis* s.l. showed a very wide range of general genotypic variability and allelic polymorphisms. As a result of these studies, the monophilia and independence of *P. japonicus* and *P. americanus* were confirmed. In addition, the AFLP analysis showed a significant difference at the genetic level between the Eurasian tetraploid plants and the Australo-East Asian octoploids (Lambertini et al., 2006). Therefore, instead of *P. australis* s.str. (the species described from Australia) we use the name *P. communis* Trin for the widespread Eurasian tetraploids.

Species of the genus *Phragmites* are one of the best studied examples of cryptic invasions among higher plants. In North America, a detailed study of the displacement of the native *P. americnus* by the invasive European *P. communis* is being carried out (Saltonstall, Peterson & Soreng, 2004). It has been convincingly shown that the cryptic invasion of *P. communis* in North America is comparable in scale (if not more) to other easily detectable invasions (*Lythrum salicaria*, *Tamarix* sp., etc.) (Saltonstall, 2002). Unfortunately, in Europe this issue has hardly been studied.

For the territory of Belarus, most sources provide only one reed species – *P. australis* (Cav.) Trin. ex Steud. (syn. *P. communis* Trin.). In the second volume of “Flora of Belarus” DI Tretyakov mentions two more invasive species – *P. altissimus* (Benth.) Mabille and *P. chrysanthus* Mabille (Tret'yakov, 2013). In addition, we found *P. americanus* (Saltonstall, P.M. Peterson, & Soreng) A. Haines (*P. australis* ssp. *americanus* Saltonstall, P.M. Peterson, & Soreng) in Belarus. For the territory of Belarus and Europe as a whole, this species is presented for the first time (Nobis et al., 2019).

In addition to the mentioned species, we found populations of reeds that are very close phenotypically to the invasive *P. altissimus*, but differ from it in the pubescence of leaves and flower scales, as well as in the seasonal rhythm of development, and so morphologically correspond to *P. communis* var. *pseudodonax* Rabenh. Nosov et al. suggested that this taxon was formed as a result of the ancient hybridization of *P. communis* and *P. altissimus* (Nosov et al., 2020). In order to confirm this assumption, we assessed the genetic polymorphism of the *Phragmites* species using ISSR analysis.

Materials and methods. Populations of *Phragmites* from various habitats in Belarus (Brest, Minsk, Gomel, Vitebsk and Grodno regions) were taken as objects for assessing intra- and interspecific polymorphism.

Eight di-, tri-, and tetranucleotide microsatellite sequences were used for the analysis (ISSR-04, ISSR-09, ISSR-09a, ISSR-10, ISSR-17, ISSR-22, ISSR-23, ISSR-24), which showed high efficiency according to the results of preliminary studies.

Results. The dendrogram was constructed using the neighbor-joining method (Saitou & Nei, 1987), the probability of the dendrogram topology is supported by the bootstrap values in the cluster nodes. The calculation of distances and the construction of a dendrogram of phylogenetic relationships between the samples were carried out using the TREECON for Windows v.1.3b program (Van de Peer & De Wachter, 1994).

The average level of ISSR markers polymorphism for the *Phragmites* populations was 90.4 %. Polymorphic ISSR markers were used to construct a phylogenetic dendrogram of the *Phragmites* species, shown in the figure.

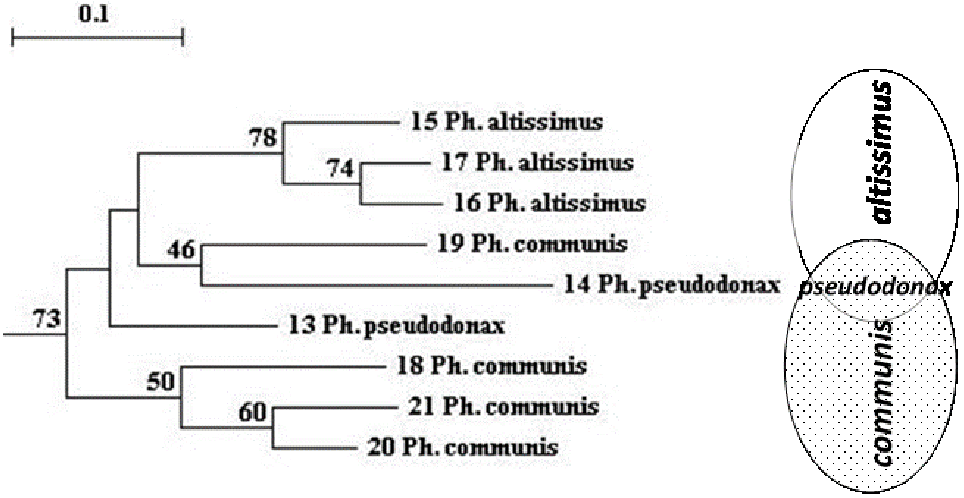

Fig. Dendrogram of genetic similarity of the populations *P. communis*, *P. altissimus* and *P. communis* var. *pseudodonax*, constructed by the NJ method (Saitou & Nei, 1987) based on ISSR markers polymorphism.
Top scale – genetic distances by Nei (bootstrap values are indicated in cluster nodes)

Analysis of ISSR polymorphism of plants from different *Phragmites* populations showed that they are grouped into several clusters, which correspond to the phenotypic clusters of *P. communis*, *P. communis* var. *pseudodonax* and *P. altissimus*. Moreover, *P. communis* var. *pseudodonax* forms a subcluster included in the cluster of *P. altissimus*. This character of the genetic variability is a feature of the hybrid nature of the taxa. Thus, we confirm the hypothesis of the origin of *P. communis* var. *pseudodonax* as a result of hybridization of northern (*P. communis*) and southern (*P. altissimus*) races (Nosov et al., 2020).

Conclusion. The data about the hybrid origin of *P. communis* var. *pseudodonax* can be interpreted as a modern hybridization of invasive *P. altissimus* and native *P. communis*, or as an ancient (most likely postglacial or even earlier) hybridization of parental species and further independent evolution of the formed hybrid taxon. Considering that the invasion of *P. altissimus* in Eastern Europe (including Belarus) has been observed only since the 1990s–2000s (Tret'yakov, 2013; Papchenkov, 2008), and herbarium collections of *P. communis* var. *pseudodonax* from the territory of the republic have been known since the middle of the 19th century; the fact that in the weather conditions of Belarus *P. communis* var. *pseudodonax* blooms very early and bears fruit well, unlike *P. altissimus*, which blooms very late and does not have time to form fruits, as well as the confinement of this taxon to the Baltic Sea basin (Tsvelov, 2011), it can be assumed with a high degree of confidence that that *P. communis* var. *pseudodonax* should be considered as a result of the ancient hybridization of *P. communis* and *P. altissimus*. Currently, this species is spreading independently of the parental species and is native to Belarus.

References

Morais P. & Reichard M. 2018. Cryptic invasions: A review. *Sci. Total Environ.*, **613-614:** 1438–1448.

Jarić I., Heger T., Castro Monzon F., Jeschke J.M., Kowarik I., McConkey K.R., Pyšek P., Sagouis A., & Essl F. 2019. *Crypticity in Biological Invasions. Trends in ecology & evolution*, **34** (4): 291–302.

Tsvelov N.N. 2011. O rodakh trostnik (*Phragmites* Adans.) i zmeyevka (*Cleistogenes* Keng) semeystva zlakov (Poaceae) v Rossii [On the genera *Phragmites* Adans. and *Cleistogenes* Keng (Poaceae) in Russia]. *Novosti sistematiki vysshikh rasteniy*, **43**: 30–44. (In Russian).

Lambertini C. et al. 2006. A phylogeographic study of the cosmopolitan genus *Phragmites* (*Poaceae*) based on AFLPs. *Plant Systematics and Evolution*, **258** (3–4): 161–182.

Saltonstall K., Peterson P.M. & Soreng R.J. 2004. Recognition of *Phragmites austraus* subsp. *americanus* (*Poaceae*: *Arundinoideae*) in North America: Evidence from morphological and genetic analyses. *SIDA, Contributions to Botany*, 683–692.

Saltonstall K. 2002. Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. *PNAS*, **99** (4): 2445–2449.

Tret'yakov D.I. 2013. Rod 64 – *Phragmites* Adans. – Trostnik – Trysneg. *In:* Flora Belarusi. Sosudistyye rasteniya. **2**. Minsk: Belaruskaya navuka. 348–351. (In Russian).

Nobis M. et al. 2019. Contribution to the flora of Asian and European countries: new national and regional vascular plant records. *Botany Letters*, **166** (2): 163–188.

Nosov N.N., Gnutikov A.A., Punina Ye.O. et al. 2020. O razlichii vidov trostnika (*Phragmites, Poaceae*) po molekulyarno-filogeneticheskim dannym [On distinction of the reed species (Phragmites, Poaceae) according to the molecular phylogenetic data]. *Probl. botan. Yuzhnoy Sibiri i Mongolii*, **19** (1): 8–13.

Saitou N. & Nei M. 1987. The Neighbor-joining Method: A New Method for Reconstructing Phylogenetic Trees. *Mol. Biol. Evol.*, **4** (4): 406–425.

Van de Peer Y. & De Wachter R. 1994. TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Comput. Applic. Biosci*, **10**: 569–570.

Papchenkov V.G. 2008. O rasprostranenii *Phragmites altissimus* (Benth.) Nabille (Poaceae) [About distribution of *Phragmites altissimus* (Benth.) Nabille (Poaceae)]. *Rossiyskiy zhurnal biologicheskikh invaziy*, **1**: 36–41.