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The red-listed *Cetrelia cetrarioides* (Parmeliaceae) is confirmed by molecular data in Belarus

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The new locality of the Red-listed lichen *Cetrelia cetrarioides* was discovered in Belovezhskaya Puscha National Park. The occurrence of this species was confirmed by the sequence of the ITS region.

Keywords: biodiversity, distribution, Red Data Book, lichens, ITS.

Новое место произрастания краснокнижного лишайника *Cetrelia cetrarioides* выявлено в национальном парке «Беловежская пушта». Определение вида подтверждено секвенированием фрагмента ITS оперона 5.8S гена ядерной рДНК.

Ключевые слова: биоразнообразие, распространение, Красная книга, лишайники, ITS.

The genus *Cetrelia* W.L. Culb. & C.F. Culb. is currently represented by 18 species worldwide [1], of which only four species occur in Europe [2], [3]. In Belarus, three species were confirmed by means of thin-layer chromatography (TLC) during the recent revision of 203 collections from the herbaria of GRSU, GSU, MSK, MSKH and MSKU, namely *Cetrelia cetrarioides* (Duby) W.L. Culb. & C.F. Culb., *C. monachorum* (Zahlbr.) W.L. Culb. & C.F. Culb. and *C. olivetorum* (Nyl.) W.L. Culb. & C.F. Culb. [4]. Of these, *C. cetrarioides* appeared to be the least frequent one being known from 13 records only.

A fieldtrip to Belovezhskaya Puscha National Park was made by the first and the second authors in August 2018. The purposes of the trip were taxonomical investigations of the isidiate *Parmelia* species [5] and populational studies to re-assess the species boundaries between *Usnea subfloridana* and *Usnea florida* [6]. The detection of new localities of rare and Red-listed species was also among the main goals of the excursions.

During the fieldtrip, seven new localities of well-developed populations of the *Cetrelia* species were found. Of these, two appeared to be *Cetrelia monachorum* and five were identified as belonging to *Cetrelia olivetorum* by TLC. No new localities of the rarest *Cetrelia cetrarioides* were believed to have been found [7]. However, the investigation of one isidiate *Parmelia* collection revealed few young small *Cetrelia* thalli. Molecular studies suggested these as belonging to *Cetrelia cetrarioides*.

ITS-sequences were amplified with the primers ITS5 and ITS4 [8], and extraction of total DNA and amplification of the ITS1-5.8S-ITS2 ribosomal RNA region followed the standard methods [9]. BLAST searches were performed for the sequences [10] which were aligned by MUSCLE algorithm in MEGA software package and compared with sequences from GenBank (<http://www.ncbi.nlm.nih.gov>).

The evolutionary history was inferred using the Maximum Parsimony method for five sequences, of which four were downloaded from GenBank (Table 1). The phylogenetic tree shows a well delimited *Cetrelia cetrarioides* clade which includes the Belarusian specimen (Figure 1).

There are two specimens of *Cetrelia cetrarioides* known from the two last decades, both from the Northern Belarus [4]. The studied specimen confirms the presence of this species in Belovezhskaya Puscha National Park nowadays.

Table 1 – Location, collector and GenBank accession numbers of sequences used in the analyses

Species	Location, collector, voucher	GenBank Accession Number
<i>Cetrelia cetrarioides</i>	Norway, Rui and Timdal O-L-200022	MK812062
<i>Cetrelia cetrarioides</i>	Spain, Ruibal MAF:Lich 15552	JN943844
<i>Cetrelia cetrarioides</i>	Belarus, Tsurykau s.n.	MZ028437
<i>Cetrelia olivetorum</i>	Estonia, Randlane and Saag CKM59	KX685871
<i>Cetrelia monachorum</i>	Russia, Notov CKM52	KX685865

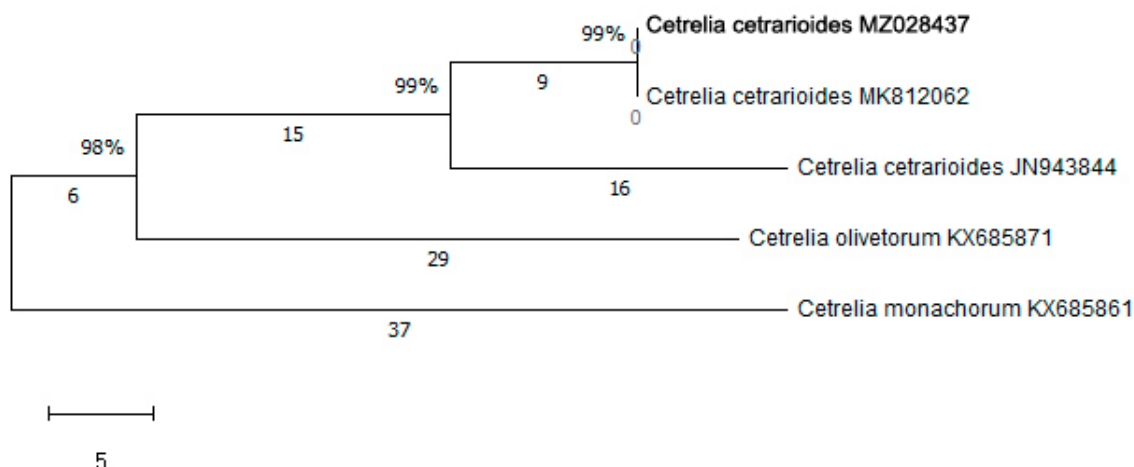


Figure 1 – A Bootstrap consensus tree based on an analysis of 5 ITS *Cetrelia* sequences using Maximum Parsimony method. Belarusian specimen is marked in bold

Specimen examined. Belarus, Brest region, Kamenets district, Belovezhskaja Puscha National Park, Khvoinik forest, 349 q., 5 km N of Khvonik village, 52°44'N, 23°58'E, oak forest, on fallen moss-covered branch of *Quercus robur*, leg. A. Tsurykau, 09.08.2018.

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