

Georgi Bonchev^a, Miroslava Zhiponova^b, Anita Tosheva^c, Valya Vassileva^a



^a Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
^b Department of Plant Physiology, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria
^c Department of Botany, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria



E-mails for correspondence: georgi.bonchev71@gmail.com; zhiponova@biofac.uni-sofia.bg

INTRODUCTION

The mint family Lamiaceae has been well known for its importance as a source of aromatic oil, wood, ornamentals, culinary and medicinal herbs, which determines the strong interest towards detailed studies of related members. Lamiaceae has been annotated as the sixth largest angiosperm family including 12 subfamilies. The subfamily Nepetoideae is monophyletic and comprises nearly all aromatic species within Lamiaceae and the presence of rosmarinic acid is one of their characteristics. Nepetoideae is the largest subfamily (approximately 3400 species) distributed throughout all continents except Antarctica, which is divided into three tribes, Elsholtzieae, Mentheae, and Ocimeae. The tribe Mentheae comprises the largest number of genera and species of any tribe within Nepetoideae and Lamiaceae. Many plants in this group are of great economic and ecological importance, which attracts the attention of scientists.

Nepeta nuda L. (Lamiaceae) is a medicinal plant with a wide distribution in Europa and Asia. In Bulgaria, *N. nuda* is also known as "naked (or hairless) catmint", which likely refers to the naked or sparse short hairy stem and leaves (Fig. 1).



Fig. 1. *N. nuda*. (A) Distribution in Bulgaria from 0 to 1900 m a.s.l. (B) Habitat and a plant specimen from natural population in Pirin. (C) *In vitro* plants at 5 weeks (Scale bar = 1 cm).

OBJECTIVE OF THE STUDY

The aim of this study is to generate DNA barcodes for a specimen of a native for Bulgaria species *N. nuda* and to precisely determine its phylogenetic position in reference to other *Nepeta* species. To achieve this goal, we applied the DNA barcoding technique based on conserved nuclear (internal transcribed spacer ITS) and chloroplast (*rbcL*, *matK*, *trnH-psbA*) DNA regions.

RESULTS

The generated DNA barcode sequences were submitted to the Barcode of Life Data System (BOLD) database (<https://www.boldsystems.org>; accession number BUL002-22). After a BLAST search in NCBI and BOLD databases, we retrieved *Nepeta* accessions which resulted in a set of 56 sequences for ITS, 44 for *rbcL*, 44 for *matK* and 5 for *trnH-psbA*. For the construction of phylogenetic trees and the taxonomic assignment of the studied *N. nuda* we used a reduced dataset of database sequences for each gene (one to two accessions per *Nepeta* species while short sequences were discarded).

The data based on the ITS region show that *N. nuda* is clustered in a subclade together with closely related species *N. sheilae*, *N. deflersiana*, *N. isaurica*, *N. congesta*, *N. heliotropifolia*, *N. schirziana* and *N. cataria* and with the closest phylogenetic distance to the recently assigned *N. sheilae*. (Fig. 2).

Chloroplast markers allowed us to relate *N. nuda* to *N. italica*, *N. cataria* and *N. grandiflora* (Fig. 3). *N. nuda* displays the highest identity (the mean value of three markers was 99.3%) to *N. italica*. The consensus tree of all three chloroplast markers supported this taxonomic affiliation of analyzed *N. nuda*.

CONCLUSIONS

1. We provided the first DNA barcode record for *N. nuda* in the workbench and repository BOLD system.
2. Chloroplast markers show comparable results and can be considered as more reliable for accurate taxonomic assignment of representatives of the genus *Nepeta*, as compared to ribosomal markers.
3. The assignment of the Bulgarian accession of *N. nuda* to *N. italica* (Greek origin) strongly supports the presence of a distinct genetic pool of *Nepeta* species on the Balkan Peninsula
4. Our results imply the evolutionary divergence of the Balkan *Nepeta* species with the presence of species and/or ecotype variability influenced by ecogeographical and demographic factors.

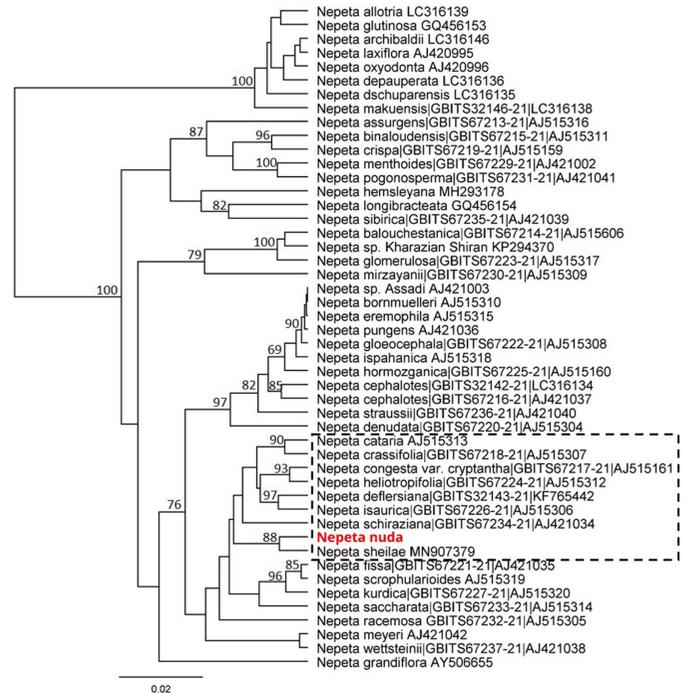


Fig. 2. Taxonomic assignment of *N. nuda* against accessions from BOLD and NCBI GenBank based on DNA barcoding marker ITS. The tree was constructed in Geneious software using the UPGMA method and Tamura-Nei model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The analysed *N. nuda* sequence is depicted in red bold. Accessions from databases are shown with their respective numbers. Punctuated rectangular points to species in closest proximity to *N. nuda*.

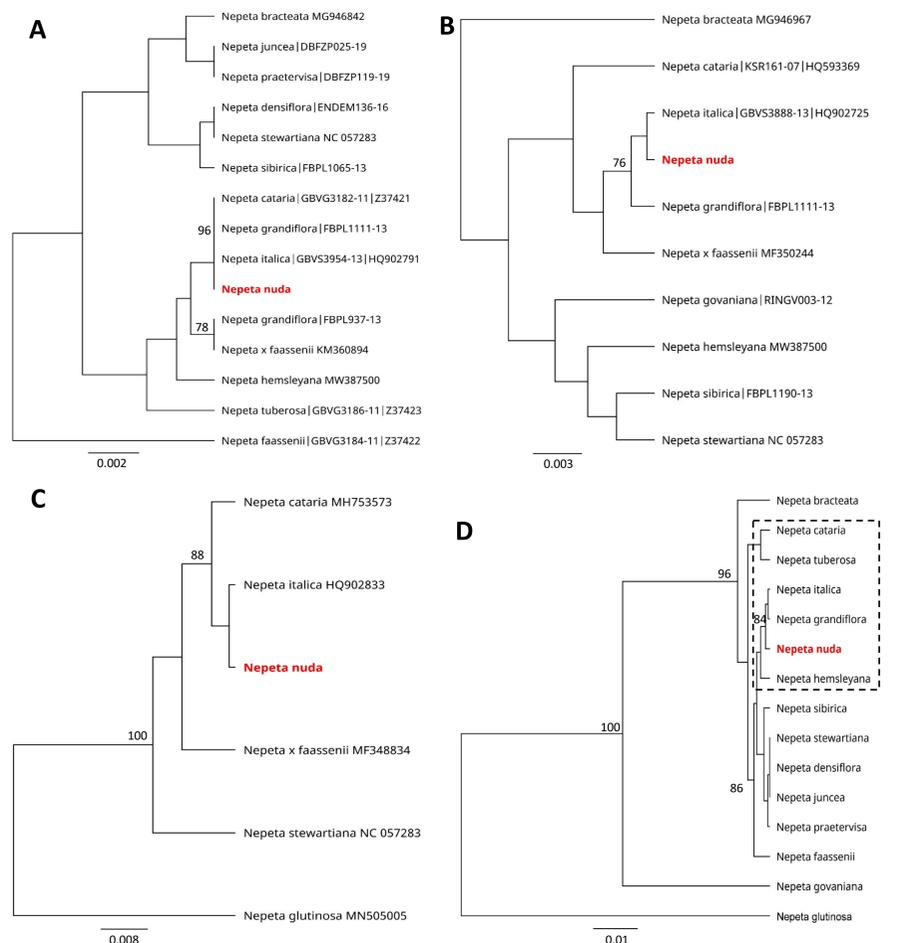


Fig. 3. Taxonomic assignment of *N. nuda* against accessions from BOLD and NCBI GenBank based on the DNA barcoding markers *rbcL* (A), *matK* (B) and *trnH* (C), and a consensus tree of all three markers (D). The trees were constructed in Geneious software using the UPGMA method and Tamura-Nei model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The analysed *N. nuda* sequence is depicted in red bold. Accessions from databases are shown with their respective numbers.

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