

DNA barcoding of the rare ant *Oxyopomyrmex krueperi* (Hymenoptera: Formicidae) using *cox1* marker

Vera Antonova¹, Stela Lazarova²

(1) Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Yuriy Gagarin Str, 1113 Sofia, Bulgaria, vera_antonova@yahoo.com

(2) Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Yuriy Gagarin Str, 1113 Sofia, Bulgaria, stela.lazarova@gmail.com

INTRODUCTION & AIM

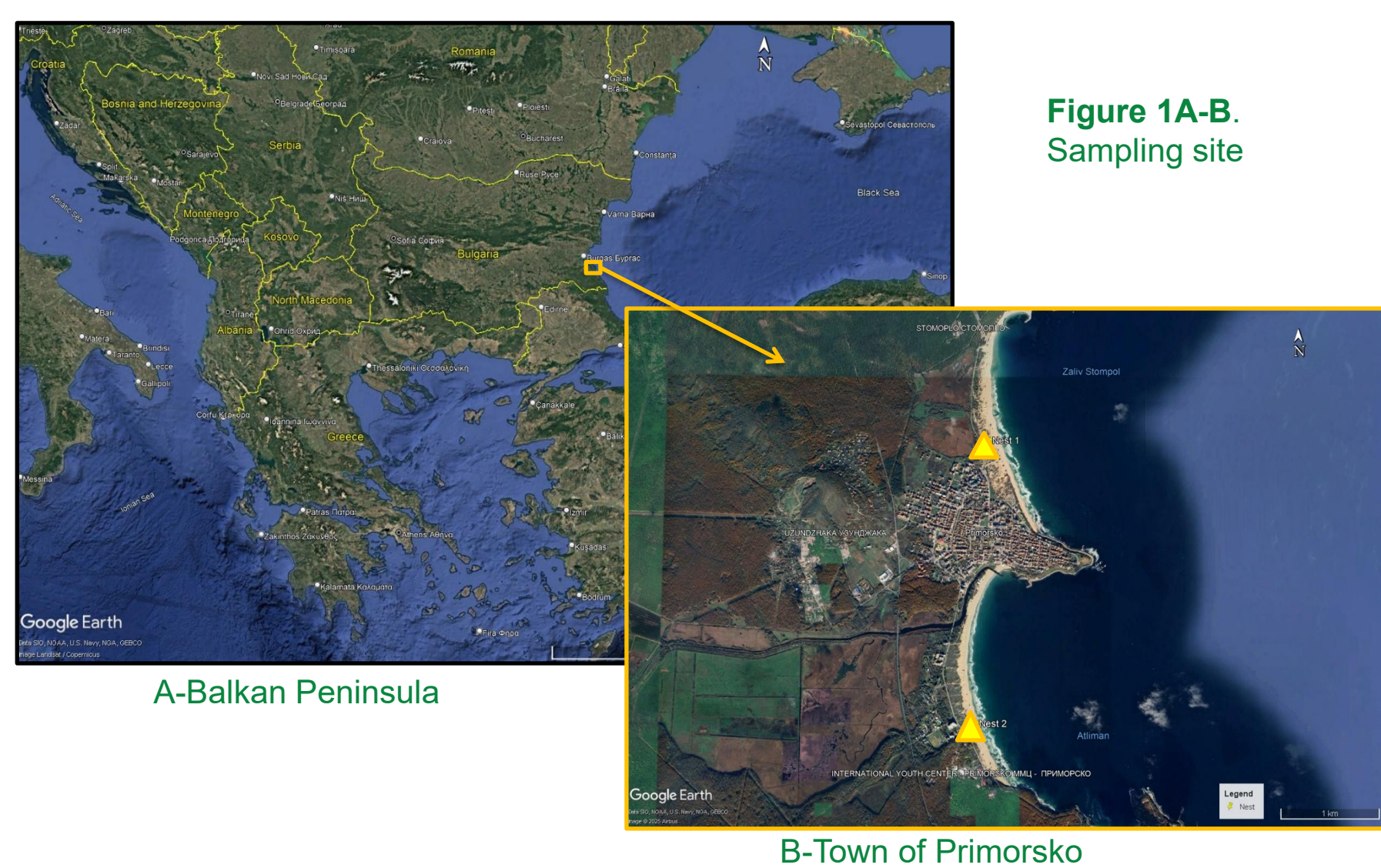
The genus *Oxyopomyrmex* André, 1881 is placed in subfamily Myrmicinae and the tribe Stenammini (Salata, Borowiec, 2015). It includes sixteen valid species of small ants as no new species were discovered in the last ten years (Reyes-López, 2025). The species are found in the arid areas of the Mediterranean region. It is a genus with scarce, fragmentary information as the species are rare with of low density of the populations (Reyes-López, 2025).

Habitats: The genus *Oxyopomyrmex* includes specialized thermophilic species occurred in dry habitats. The ants build their nests under stones from the sea level to 578 m in Greece (Borowiec, Salata 2025). The species belong to the Harvester ants (Espadaler, Gelabert 1981).

Morphology: *Oxyopomyrmex* is easily distinguished from other Mediterranean genera by having 11-segmented antennae and characteristic large eyes, pointed anteroventrally, with anterior margin close to the mandibular insertions. The genus *Goniomma* is very similar by morphological characteristics but differs from *Oxyopomyrmex* with 12-segmented antennae and its areal in the western part of the Mediterranean region. The detailed morphological characteristics of the gynes, males and workers of *Oxyopomyrmex* are given in the taxonomic revision of Salata, Borowiec (2015).

Distribution: One of the species *Oxyopomyrmex krueperi* Forel, 1911 is known in Bulgaria (Lapeva-Gjonova, Kiran 2012). Distribution in EUROPE: Greece: Crete, Dodecanese, Macedonia mainland; North Macedonia; Bulgaria; Turkey (Borowiec, Salata 2025); ASIA: SW Iran (Ghahari et al. 2011). Data on its presence in Bulgaria are scarce, based on one location near the town of Tzarevo. So far, they have not been molecularly supported yet.

METHOD



Sampling site

As part of the INVBG project (“Invertebrate Fauna of Bulgaria and Adjacent Regions – DNA-based Barcoding of Under-represented in Reference Libraries Taxa”), the sampling of rare ant species was carried out along the Southern Black Sea Coast of Bulgaria according to the published data occurrence in Burgas Province from 4th to 7th September 2024. Worker ants were hand collected in different colonies along the protected sandy dunes “Perla” near the North Beach of the town of Primorsko (Figures 1 and 2). The dunes occupy an area of 24 ha and protected by Ramsar Convention. They have the status of a natural monument since 1984 with high biodiversity of psammophyte vegetation. Fixed coastal dunes (habitat No 2130/ N17 by EUNIS) with herbaceous vegetation (grey dunes) are mostly represented by *Aurinio uechtritzi* - *Artemisietum campestris* association (Zingstra et al. 2009; Tsonev et al. 2015). The specimens were preserved in 99% ethanol and kept in refrigerator in the collection of V. Antonova in the Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria. The morphological verification was done by using the identification key of Salata, Borowiec (2015).

DNA Extraction, Amplification, Sequencing and Alignment

Molecular analysis was done by sequencing cytochrome c oxidase subunit 1 (COI-5P) gene. DNA extraction, amplification, and sequencing of the gene fragment of 4 specimens were performed by the Biology department of University of Florence on 20 February 2025. DNA was extracted from the head's tissue of the specimens (workers). DNA was extracted using a non-destructive protocol. Translation Matrix: Invertebrate Mitochondrial (BOLD). BOLD Barcode Index Number (BIN): **BOLD:AGQ0816**

RESULTS & DISCUSSION

The workers were sampled at the protected dunes near the town of Primorsko (Fig. 2, 4). The nests were situated deep in the sands and only a few entrances were visible on the surface (Fig. 3). The small sandy hills around each entrance were about 3–4 cm in diameter.

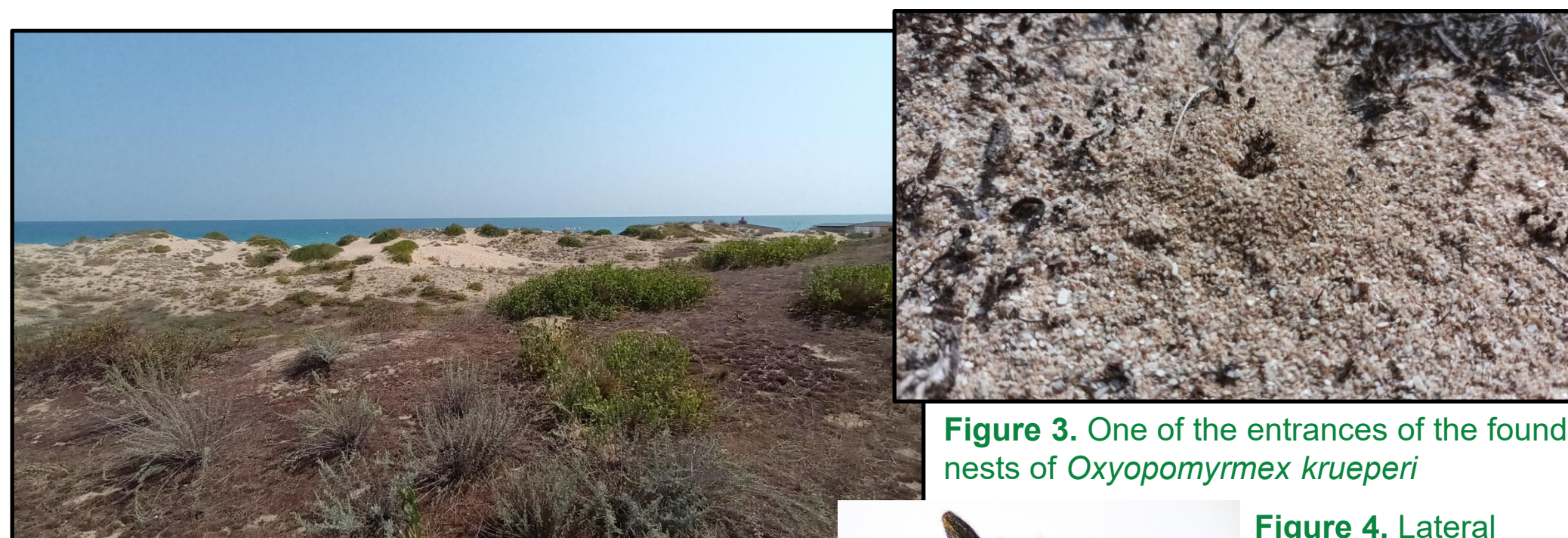
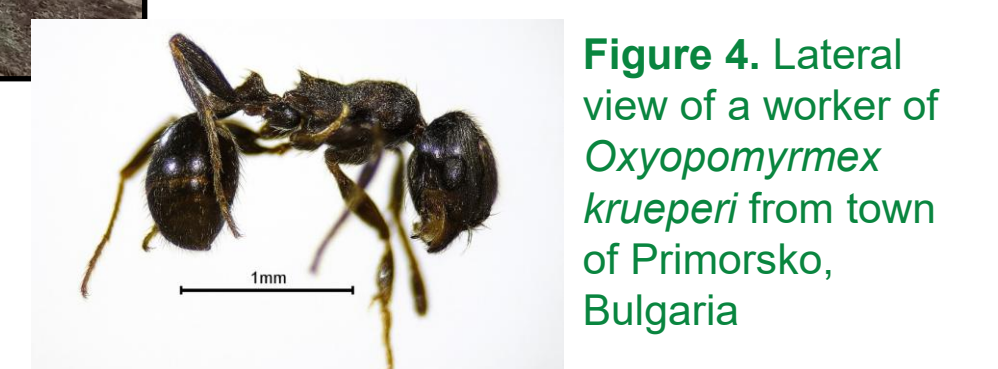


Figure 2. Sampling habitat: protected dunes of Primorsko (Burgas Province, Bulgaria) near the Black Sea coast



Sequence analyses and phylogeny

Our four COI gene sequences were identical, 658 bp in length (BOLD Sequence ID: INVBG654-24.COI-5P). No COI sequences of *O. krueperi* were available in public databases, making these the first molecular records for the species worldwide. Bioinformatic analyses using sequences from both NCBI and BOLD identified three *Oxyopomyrmex* species (*O. magnus*, *O. saulcyi*, and *O. insularis*), showing 87–90% identity, supported the genetic distinctiveness of *O. krueperi*. The phylogenetic relationships of this genus, together with three additional genera within the tribe Stenammini, are shown in Fig. 5.

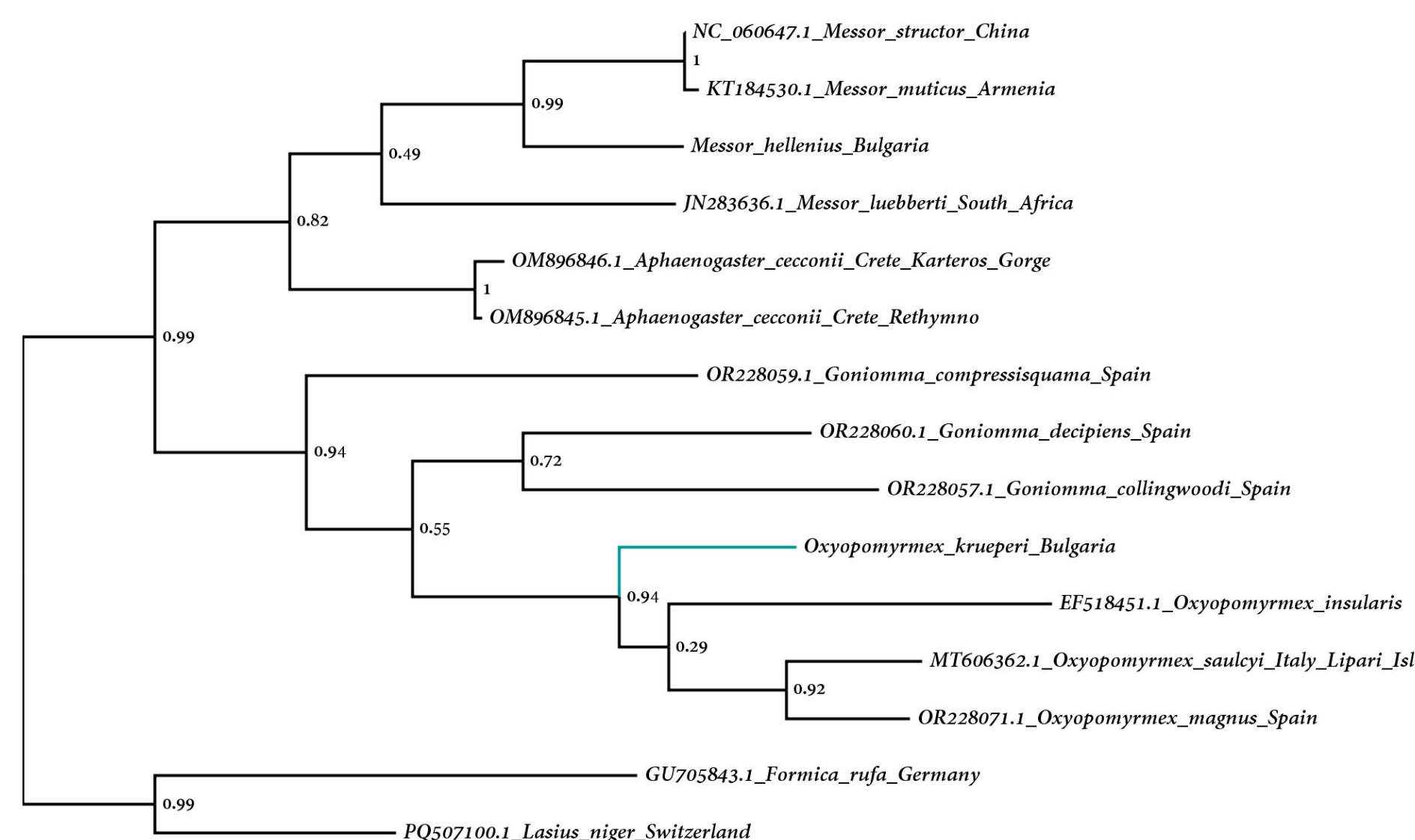


Figure 5. ML tree

CONCLUSION

Although the genus *Oxyopomyrmex* is represented by a few COI sequences in GenBank, *O. krueperi* has remained unsequenced until now. The newly generated data from Bulgarian material confirm its genetic distinctiveness within the genus and support its current morphological delimitation. The sequence similarity (up to 90%) to other species aligns with expected interspecific divergence in Formicidae COI data. These new sequences now enable comparative molecular studies and may assist in resolving phylogenetic placement within Myrmicinae.

ACKNOWLEDGEMENTS

Funding was due to the InBuiLT project “Invertebrate Fauna of Bulgaria and Adjacent Regions – DNA-based Barcoding of Under-represented in Reference Libraries Taxa” (INVBG) led by Institute of Biodiversity and Ecosystem Research (IBER-BAS). It was part of the initiatives from the Biodiversity Genomics Europe (BGE). The work was partly supported by National Science Fund (Ministry of Education and Science), Bulgaria under Grant contract MCCATF number KP-06-N61/6-14.12.2022.