

Molecular and morphological identification of *Chroogomphus mediterraneus* new to the Hungarian funga

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Chroogomphus (Singer) O.K. Mill is a mycorrhizal fungal genus associated with *Pinaceae*, containing more than 30 species worldwide. It is characterized by small to large basidiomata with pale orange to ochraceous lamellae when young, an ochraceous pileal trama, a moist to viscid pileipellis, an ephemeral fibrous veil, and amyloid hyphae at the base of the stipe. The type species of the genus is *Chroogomphus rutilus* (Schaeff.) O.K. Mill., which has economical importance due to its edibility and pharmaceutical properties.

INTRODUCTION

In Hungary, *Chroogomphus* had previously been examined solely based on morphology, and accordingly, published data were only available for the species *C. rutilus* (Schaeff.) O.K. Mill. and *C. helveticus* (Singer) M.M. Moser. However, based on literature data, *C. mediterraneus* (Finschow) Vila, Pérez-De-Greg. & G. Mir with a similar appearance to *C. rutilus* s. str. was presumed to occur in Hungary as well. Though its morphology, host range and ecology were well described, its more distinguishing features and regional ecological preferences are still unrevealed.

MATERIALS & METHODS

The studied *Chroogomphus* samples were collected in the past few years in Hungary. Primer pairs ITS1F/ITS4B was used for PCR amplification and Sanger-sequencing of the nrDNA ITS region. Chromatograms were analyzed using BioEdit. Our dataset was complemented with sequences downloaded from GenBank. Alignments were made and adjusted with MAFFT v. 7.0 and SeaView4. Phylogenetic reconstruction was inferred using RAxML in raxmlGUI2.0 with 1000 rapid bootstraps and GTRGAMMA substitution model. Phylogenetic tree (**Figure 3.**) was visualized in MEGA 7.

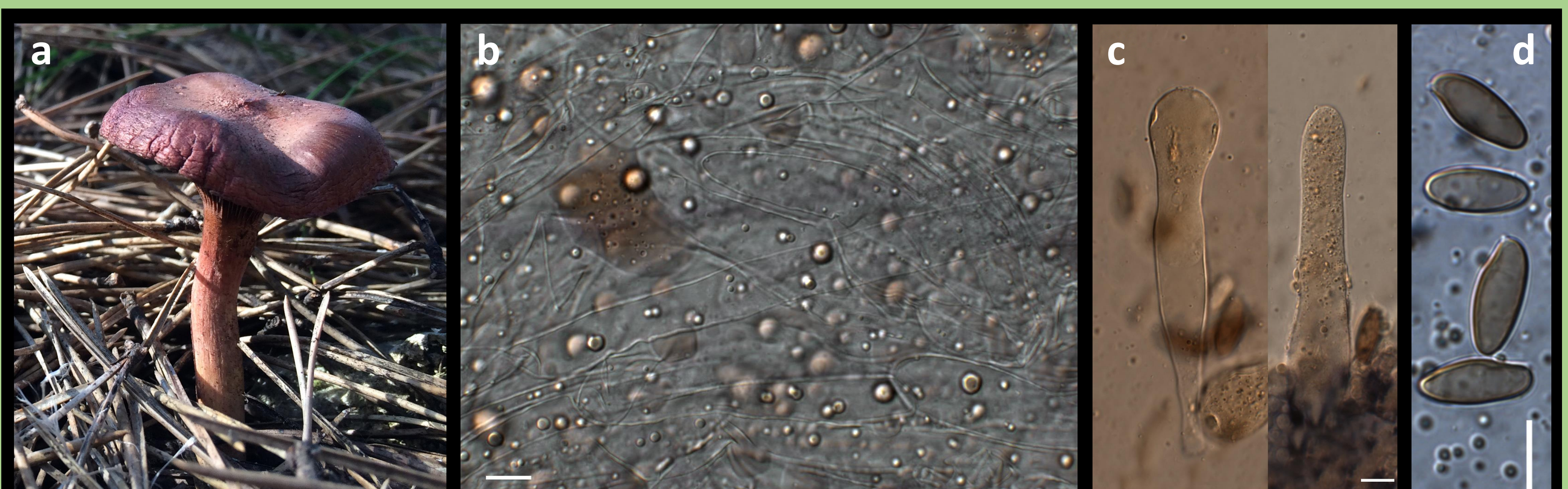


Figure 1. Morphology of *Chroogomphus mediterraneus*. a) basidiocarp, b) pileipellis elements, c) cheilocystidia, d) spores. Photos: B. Palla.

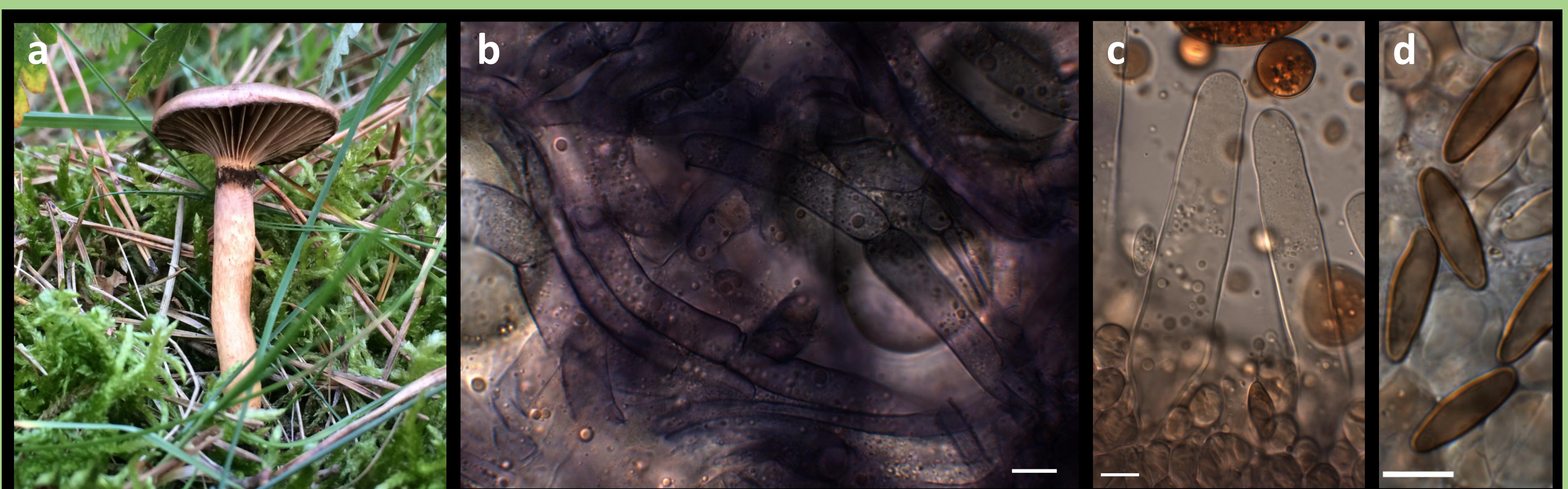


Figure 2. Morphology of *Chroogomphus rutilus*. a) basidiocarp, b) pileipellis elements, c) cheilocystidia, d) spores. Photos: B. Palla.

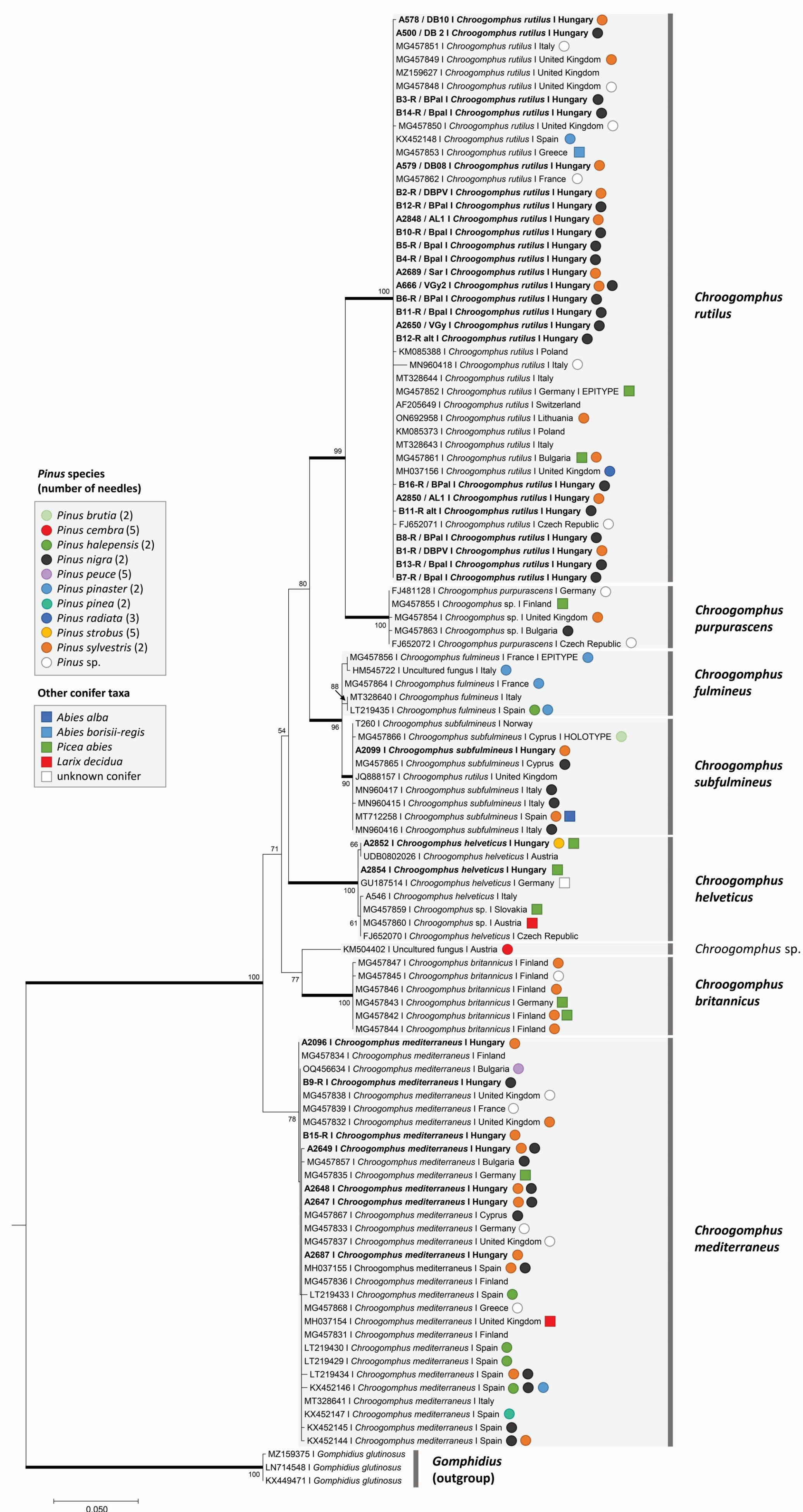


Figure 3. Maximum Likelihood (ML) phylogenetic tree derived from ITS-sequences of *Chroogomphus mediterraneus* and its relatives. ML bootstrap values > 50% are shown above or below branches. The bar indicates 0.05 expected change per site per branch. New sequences are marked with bold. Legend indicate host species.

RESULTS & DISCUSSION

The phylogenetic tree derived from the nrDNA ITS region of the Hungarian *Chroogomphus* collections revealed the presence of *Chroogomphus mediterraneus* from Hungary (Figure 3.). Morphological evidence reinforced the findings, as cheilocystidia wall thickness and spore width showed differences between *C. mediterraneus* (Figure 1. c, d) and *C. rutilus* (Figure 2. c, d). Additionally, the pileipellis elements of *C. mediterraneus* proved to be inamyloid (Figure 1. b) , whereas for *C. rutilus* amyloid (Figure 2. b). Analysis of bioclimatic data indicate the high level of edaphic similarities between the collection sites of the two species (data not shown). As *C. rutilus* is much more abundant than *C. mediterraneus*, the former species could be an earlier colonist of its hosts, pre-empting the shared resource before the later arriving *C. mediterraneus*.



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