IJM - Italian Journal of Mycology
ISSN 2531-7342 - Vol. 55 (2026): 1 - 7
Journal homepage: https://italianmycology.unibo.it/

Short note

First records of *Phellodon castaneoleucus* in the chestnut forest of Monte Rocca Romana

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ARTICLE INFO
Received 14/05/2025; accepted 09/12/2025
https://doi.org/10.60923/issn.2531-7342/21977

Abstract

We report the finding of the recent species *Phellodon castaneoleucus* growing in late autumn in two sites in the forest of Monte Rocca Romana, inside the Bracciano-Martignano Regional Natural Park in the province of Viterbo, Latium, Italy. Molecular analyses have been performed to obtain reliable taxonomic information. DNA-checked data is necessary to refine the distribution and ecology of the *Phellodon* genus which are usually only known from the few localities where they were originally collected. We have already reported interesting mycological findings (*Hydnum ibericum*, *Hydnum ovoidesporum*, *Entoloma dislocatum*, *Ramariopsis pulchella*) from this area, characterised by a forest composed in prevalence of *Castanea sativa* mixed with some *Fagus sylvatica* trees, indicating a large interesting fungal biodiversity. The annual presence of these stipitate hydnoid fungi is an indicator of the good quality of this forest habitat.

Keywords
Bracciano-Martignano Regional Natural Park, conservation, Castanea sativa, hydnoid fungi

Introduction

The genus *Phellodon* P. Karst. is composed of stipitate hydnoid fungal species recognised by a white spore print, classified in the thelephoroid clade (Binder et al., 2005) affiliated to Bankeraceae (Song et al., 2025). The *Phellodon* genus includes ectomycorrhizal fungi with spiny hymenophore associated with broad-leaved or coniferous trees (Parfitt et al., 2007; Song et al., 2025). Van der Heijden et al. (1998) reported that mycorrhizal symbiosis is relevant for plant biodiversity, ecosystem variability and productivity and the presence of ectomycorrhizal species reflects the conservation state of forest systems. The stipitate hydnoid fungi have been included in several European Red Lists due to the decrease in the presence of basidiomata over the years (Lizon, 1995). Four *Phellodon* species [*P. atratus* K.A. Harrison, *P. excentrimexicanus* R.E. Baird, *P. fuligineoalbus* (J.C. Schmidt) R.E. Baird, *P. secretus* Niemelä & Kinnunen] are assessed as threatened on a global scale in the IUCN

Department of Agricultural and Food Science in partnership with the Italian Mycological Society (UMI).

Red List (IUCN, 2025). *Phellodon castaneoleucus* A.M. Ainsw. together with *P. aquiloniger* A.M. Ainsw & Svantesson, *P. dititomentotus* Svantesson and *P. frondosoniger* A.M. Ainsw & Svantesson belong to four clades not previously assigned to any existing species and interpreted as new by ITS phylogram containing 13 clades of European specimens interpreted as species (Svantesson et al., 2025). *Phellodon castaneoleucus* was recognised as a new species through phylogenetic analyses within the *Phellodon melaleucus* morphological complex. The presence of *P. castaneoleucus* basidiomata was evidenced in Italy and the southern part of the United Kingdom by molecular analyses, in Georgia by sequences obtained from soil samples (Svantesson et al., 2025).

We report the finding of *P. castaneoleucus*, new to Latium, annually present in autumn, in two separate sites in a forest dominated by *Castanea sativa* L. on the internal mountain slope of Monte Rocca Romana, part of the Bracciano-Martignano Regional Natural Park. We have already reported interesting mycological findings from this area characterised by a large fungal biodiversity (Knijn et al., 2021): *Ramariopsis pulchella* (Boud.) Corner, several *Hydnum* species and *Entoloma* species in the *Cyanula* subgenus. One of the latter was identified as *Entoloma dislocatum* Vila, Dima & Noordel. (Noordeloos et al., 2022).

Materials and Methods

Area of study

Monte Rocca Romana was formed by volcanic scoria cones, it is the highest peak (612 m asl) of the Monti Sabatini, a mountainous group of Lazio originating from a quiescent volcanic district. The humid slope facing north is vegetated by a forest prevalently composed of *C. sativa* with some *Fagus sylvatica* L. trees. Besides the chestnut trees, these woods are composed of *Acer campestre* L., *Ostrya carpinifolia* Scop., sporadic *Populus* sp., *Ulmus* sp. and *Corylus avellana* L., the underwood prevalently of *Ilex aquifolium* L. and *Ruscus aculeatus* L. The volcanic soils examined in the territory have a low content of calcium carbonate and a subacid to neutral reaction. We collected the *P. castaneoleucus* specimens in November 2021 (TUF111927) together with *Hydnellum concrescens* (Pers.) Banker (TUF111926) specimens. In the same site, other specimens were found in November 2023 (TUF137800) and in October 2024 (TUF143026), on sloping ground in the moss between the roots of *Castanea sativa* close to *Fagus sylvatica* trees where some specimens also had matured in November 2024. We found *P. castaneoleucus* (TUF143050) on the same day of October 2024 in another site of the forest on the soil along an uphill path under chestnut trees, about 320 meters from the first site. The collected specimens are conserved as a part of the Mycological Collections (acronym TUF) of the Natural History Museum and Botanical Garden of the University of Tartu, Estonia.

Microscopy

Microscopic images were obtained with a digital camera AmScope (Irvine CA, USA) MU 500 mounted on an AmScope B490 microscope, operating at ×800 magnification. Fresh fragments were observed in distilled water. Dimensions of basidiospores are given as “(minimum value)–average value–(maximum value) of length × (minimum value)–average value–(maximum value) of width”. Q_m is the spore quotient (length/width ratio).

Molecular analyses

Ribosomal DNA-based analysis was performed on the specimens in the frame of the UNITE project (Kõljalg et al., 2013). DNA extraction, PCR amplification of SSU partial, ITS1, 5.8S, ITS2, LSU partial regions and sequencing were performed as in Voitk et al. (2018). Initial molecular analyses consisted of BLAST 2.13.0+ (Camacho et al., 2009) alignment against the Full UNITE+INSD v10.0 dataset for Fungi (Abarenkov et al., 2025). Together with the four sequences obtained in this study, other fifty-one were selected for phylogenetic analyses in addition to two sequences from *Amaurodon aquicoeruleus* Agerer and *Amaurodon viridis* (Alb. and Schwein.) J. Schröt. as outgroup. ITS and LSU sequences from the *P. castaneoleucus* holotype K-M00016220, as well as from all other specimens where available, were combined (Supplementary Table S1). The sequences were aligned using the L-INS-I method of the MAFFT v7.520 algorithm (Katoh and Standley, 2013) in Aliview 1.27 (Larsson, 2014) and trimmed to exclude nucleotides from poorly covered regions resulting in an alignment of 1207 nucleotide sites. Bayesian inference was engaged with the model TrN+I+G4 as suggested by ModelTest-NG v0.1.7 (Darriba et al., 2020). In MrBayes 3.2.7a (Ronquist et al., 2012), the Markov Chain Monte Carlo algorithm was run using four chains (three hot, one cold), a temperature of 0.1, a stop value of 0.01 on two trees with a diagnose frequency every 100 generations and 25% burn-in. The phylogenetic tree of the sequences was visualised using FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree) and annotated manually. The sequences of the four specimens are available in the UNITE database at https://unite.ut.ee/ (UDB07672113, UDB07675410, UDB07676782, UDB07676794).

Results and Discussion

Identifying species in the Bankeraceae family based on morphological characteristics only could complicate conservation efforts. Molecular analyses are necessary to obtain reliable information on species' distribution and ecology. In particular, the innovated taxonomy based upon reference DNA sequences allows the detection of specific *Phellodon* species in other localities from which they were originally collected. *P. castaneoleucus* was recently recognised as a new species through DNA sequencing within the *P. melaleucus* morphological complex (Svantesson et al., 2025). This new ectomycorrhizal species forms partnerships with the living roots of sweet chestnut, beech, and oak, and was described from a wooded heathland near Royal Tunbridge Wells in the United Kingdom (Svantesson et al., 2025). We report the presence of *P. castaneoleucus* specimens in two sites of the chestnut forest of the Monte Rocca Romana, inside the Bracciano-Martignano natural regional park in Lazio.

Morphological description

Basidiomata centrally stipitate grow terrestrially in single turbinate elements or small clusters with fused pilei. Pileus up to 4 cm in diameter, during the fungal growth depressed centrally, infundibuliform then flat but slightly convex in the middle, round, with plicate margins. The surface was initially silky or tomentose, whitish or yellowish cream in colour, then brown in the centre, and became rough with concentric zones of colour, brownish, chestnut brown or dark brown with age, the margins always remain whitish yellowish (Fig. 1a-i). Once dry, the surface appears zonate with castaneus brown tones (Fig. 1g). Stipe cylindrical up to 3 cm x 0.3-0.5 cm, with decurrent spines from the hymenium, slightly velvety, cream under the spines to brown along the stem. White spines that became grey with age (Fig 1f). Context brown with a fungal smell and like helichrysum flowers particularly when dry.

Microscopy

The hyphal system is monomitic, hyphae septate without clamp connections (Fig. 1j). Basidiospores globose, echinulate, faintly coloured, (3.01)–4.27–(5.20) × (3.00)–4.13–(5.10) µm, Q_m=1.03, n=19, (measurement including ornaments, to 0.9 µm high). Basidia 4-spored, cylindrical, clavate 19-28×3.7-7.6 µm.

Molecular analyses

The ITS-LSU phylogenetic analysis obtained through Bayesian inference is shown in Fig. 2. Our phylogenetic analysis confirms the *castaneoleucus* clade, perfectly accommodating the four sequences obtained in this study. The interest of highlighting its presence lies in the fact that fungi in the *Phellodon* genus are reported as an indicator of overall good quality forest habitat due to their sensitivity to nitrate pollution. They are of European conservation concern because their basidiomata are disappearing across several European countries, a trend correlated with excess airborne nitrogen (Parfitt et al., 2007; Hasselquist and Högberg, 2014).

Fig. 1 – a-f) *Phellodon castaneoleucus* basidiomata at different stages of growth, specimens from the same site between the roots of *Castanea sativa* close to *Fagus sylvatica* trees: a) 21 November 2021 (TUF111927); b) 6 October 2024 (TUF143026); c) 13 October 2024; d-e) 2 November 2024; f) 16 November 2024; g) Dried specimens; h-i) Specimens from a different site of the forest under *Castanea sativa*, 13 October 2024 (TUF143050). j) Basidiospores and simple septate hyphae, scale bar 10 µm.

The establishment of the Park, twenty-five years ago, aimed at promoting conservation and enhancement of the territory and the natural area of the Sabatini Mountains (Various Authors, 2009). Despite urban expansion, the Park still preserves extensive forests and riparian vegetation zones. The maintenance of these habitats allows the presence of fauna and botanical and mycological flora, some of which are unique or rare, and this should be a reason for continuous surveillance. Therefore, the evidence of the presence of the hydnoid fungal species *P. castaneoleucus* and *H. concrescens*, in the last three years, within the forests of the park is an indicator of the good ecological conservation of the area.

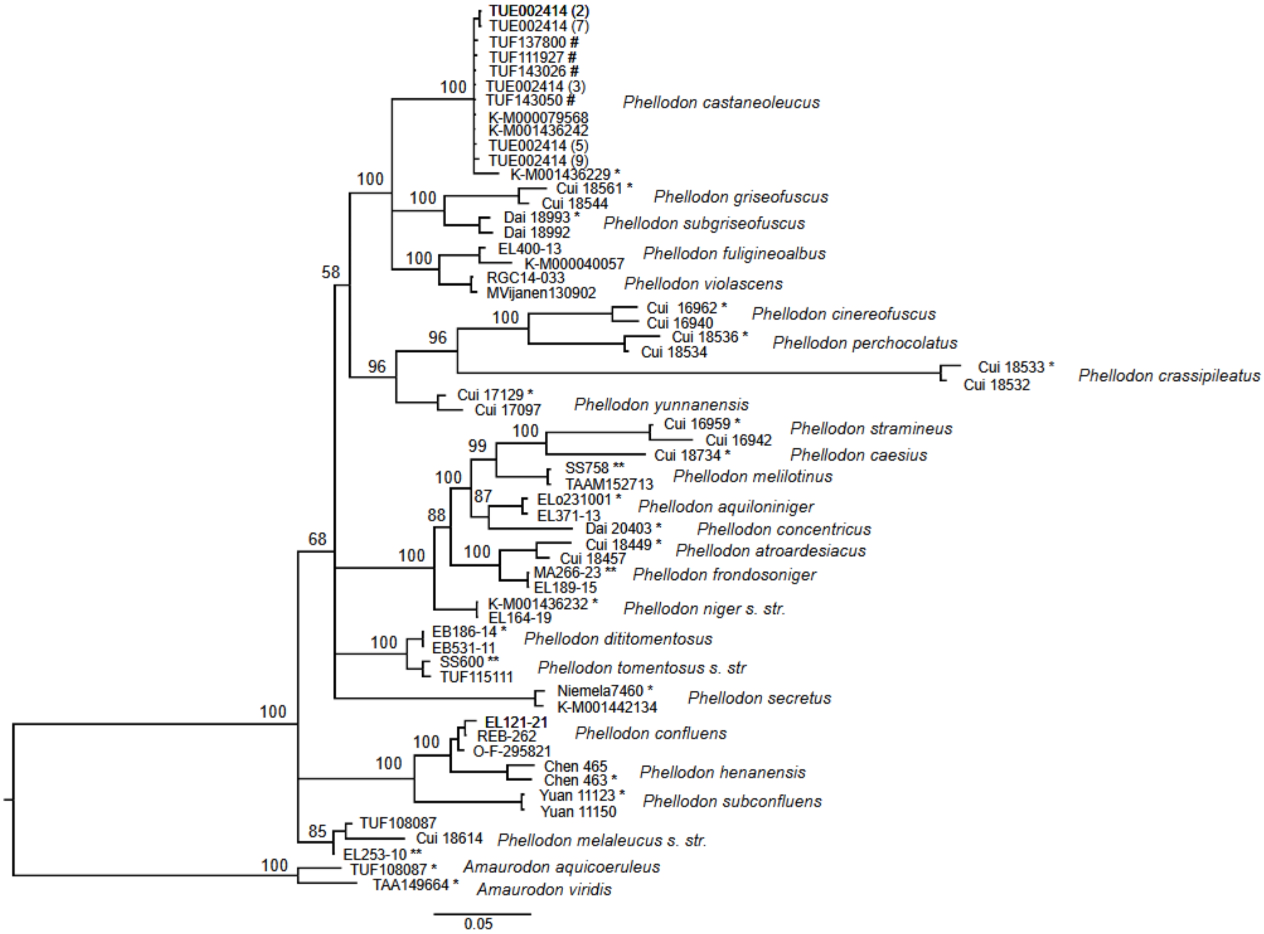

Fig. 2 – Bayesian phylogram of the ITS-LSU dataset for *Phellodon castaneoleucus* with support values reported on the main branches. Newly generated sequences are denoted by hashes, holotypes by asterisks and epitypes by double asterisks. Sequence labels indicate the collection identifier. TUE002414 indicates a soil sample from which five sequences were obtained.

Acknowledgements

IS was supported by the Estonian Research Council grant (PRG1170) and the Estonian Ministry of Education and Research (Centre of Excellence AgroCropFuture "Agroecology and new crops in future climates", TK200).

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