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Brief note

The first record of *Densocarpa crocea* in Italy

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Abstract

The first record of *Densocarpa crocea* (Quél.) Healy & M.E. Sm in Italy is reported. The specimen was morphologically described and the ITS sequence was deposited in GenBank.

Keywords: Densocarpa crocea; morphological description; ITS sequence

Riassunto

Per la prima volta *Densocarpa crocea* (Quél.) Healy & M.E. (= *Stephensia crocea* Quél.) è stata trovata in Italia, ai confini della zona A del Parco Regionale dell'Abbazia di Monteveglio (SCI IT4050016). Questa specie europea è stata finora segnalata solo in Francia, Olanda e Spagna. L'ascoma è stato descritto morfologicamente e molecolarmente caratterizzato tramite sequenziamento delle regioni ITS del DNA ribosomiale.

Gli ascomi di *D. crocea* si distinguono morfologicamente da quelli di *Hydnocystis bombycina* (Vittad.) Healy & M.E. Sm. [= *Stephensia bombycina* (Vittad.) Tul. & C. Tul.] solo per le dimensioni inferiori, per il colore rosso aranciato e per le dimensioni sporali (9-15 µm) che sono molto inferiori rispetto a quelle di *H. bombycina.*

Parole chiave: Densocarpa crocea; descrizione morfologica; sequenza ITS

Introduction

The taxonomy of ascomycetes has been revolutionized by the introduction of phylogeny-based approaches in the last 20 years (Lumbsch, 2000). A recent study of Kumar et al. (2017) revised the classification of the Pyronemataceae with smooth, globose, hyaline spores, grouped in the *Tarzetta-Geopyxis* lineage by Tedersoo et al. (2006). This linkage includes the truffle genera *Paurocotylis* and *Stephensia*, and the cup fungus genera *Geopyxis* and *Tarzetta* (Tedersoo et al., 2006). Kumar et al. (2017) study supports the

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abandonment of the genus *Stephensia* (Vittad.) Tul. & Tul. and suggests to transfer *Stephensia bombycina* (Vittad.) Tul. & C. Tul. to *Hydnocystis*, *Stephensia bynumii* Trappe, Bushnell & Castellano to *Paurocotylis*, and *Stephensia shanori* (Gilkey) Gilkey and *Stephensia crocea* Quél. to *Densocarpa*. Of these species, only *H. bombycina* and *D. crocea* are reported in Europe (Montecchi and Sarasini, 2000; Pegler et al., 1993; de Vries, 1985). While *H. bombycina* is quite common (Montecchi and Sarasini, 2000; Gori, 2005, Morara et al., 2009), *D. crocea* was never found in Italy.

D. crocea was firstly found in France by Quélet (1886) who gave a synthetic description of the species. Then, it was reported in several lists of new rare fungi of France (/https://www.biodiversitylibrary.org/name/Stephensia%20crocea) and, later, in Nederland where a detailed description of its ascomata was made by de Vries (1985). Recently, *D. crocea* has been found, for the first time, in Spain (Rubio et al., 2006). In this brief note, we report the first record of *Densocarpa crocea* (Quél.) Healy & M.E. Sm in Italy.

Materials and methods

The ascoma of *D. crocea* was found in the SCI IT4050016 ”Abbazia di Monteveglio” Regional Park (Emilia-Romagna Region, Italy) near the zone A (coordinates 44° 17’ 56.88’’ N, 11° 05’ 9.9599” E) the 28 November 2014, and deposited in the Herbarium CMI-Unibo (herbarium n. 4518). The habitat type is 92A0 (*Salix alba* and *Populus alba* galleries) along the Ramato stream. The ascoma was found near plants of the footpath, in proximity of young plants of *Quercus pubescens* Willd. (Fig. 1).

Fig. 1. Area of the SCI IT4050016 ”Abbazia di Monteveglio” Regional Park (Emilia Romagna Region, Italy) where *D. crocea* was found. On the left side there are young oaks which are not visible in the picture.
Fig. 1. Area del parco Abbazia di Monteveglio (SCI IT4050016) dove è stata trovata *D. crocea.* Sulla sinistra c’è una sponda con giovani roverelle che non sono visibili nella foto.

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Identification of the species was carried out on the basis of spore dimensions and ITS rDNA sequences. Spore dimensions were taken using Nis-Elements AR (v3.10) software (Zeiss) from images captured with a DXM1200F digital camera (Nikon). The primer pair ITS1F–ITS4 (White et al., 1990) was used to amplify ITS regions with the direct PCR approach (Iotti and Zambonelli, 2006). Polymerase chain reaction products were first purified using the NucleoSpin Extract kit (Machery-Nagel) and then sequenced in both directions using forward and reverse primers ITS1F and ITS4. Sequencing reactions were performed on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). The obtained sequence was compared against nucleotide database in the National Centers for Biotechnology Information GenBank (http://www.ncbi.nlm.nih.gov/) by using the search tool Blastn to confirm the species. The sequence of the ascoma described in this study was deposited in GenBank with the accession number MH087211.

Results

The ascoma was 3 mm in diameter, subglobose with a basal cavity, orange encrusted by a brown tomentum (Fig. 2a and c). The microscopic characters correspond to those previously described by de Vries (1985) and Kumar et al. (2017). The peridium was 400-550 µm, with the outermost layer composed by yellowish-brown, of interwoven granule-encrusted hyphae, large 5-10 µm which fuse with a thin layer of small-celled pseudoparenchyma composed by irregular arranged cells; the innermost peridium was plectenchimatous (Fig. 2d, e and f). The gleba was white yellowish, with a unique, large meandered channel (Fig. 2c). Hymenium was composed by paraphyses and asci, filling the fertile channel. Paraphyses were enlonged, hyaline, slightly swollen at the tips, 2.5-5 µm in diameter. Asci were cylindrical to oblong, 120-150 µm x 17.5- 23 µm, with 6-8 uniseriate spores in a single ascus. The spores were hyaline, smooth, globose, thin-walled (1 µm thick), of 9-15 µm (Fig. 2b).
The ITS sequence of the described specimen had a 99.4% of similarity (3 variable positions on 463 bp) with *D. crocea* (KT361829) deposited by Kumar et al. (2017). All the other sequences in the best blast hit list had a sequence similarity < 90% (date of accession 22 March 2018).

Discussion and conclusions

D. crocea is clearly distinguishable by *H. bombycina* for the smaller dimensions (3 mm and 2-3 cm respectively), the orange color and the smaller spores (9-15 µm and 19-22 µm) respectively. The typical morphological characters of this species could exclude that it was previously unnoticeable found in Italy.
This European species was rarely found only in France, Spain and Nederland. In Nederland it is reported in the red list as vulnerable species (https://www.verspreidingsatlas.nl/0695020#; http://minez.nederlandsesoorten.nl/content/oranje-plooitruffel-stephensia-crocea; http://wikipedia.qwika.com/nl2en/Nederlandse_Rode_lijst_(paddenstoelen_K_t/m_O). This species was already included in the red list candidates of European Council for the Conservation of Fungi (ECCF) (https://www.wsl.ch/eccf/activities-en.ehtml). Considering its rarity and threaten habitat, which is commonly visited by truffle hunters in Italy looking for the expensive white truffle (*Tuber magnatum* Picco), we think that *D. crocea* should be considered priority for incorporation into the IUCN red list (http://iucn.ekoo.se/en/iucn/welcom).

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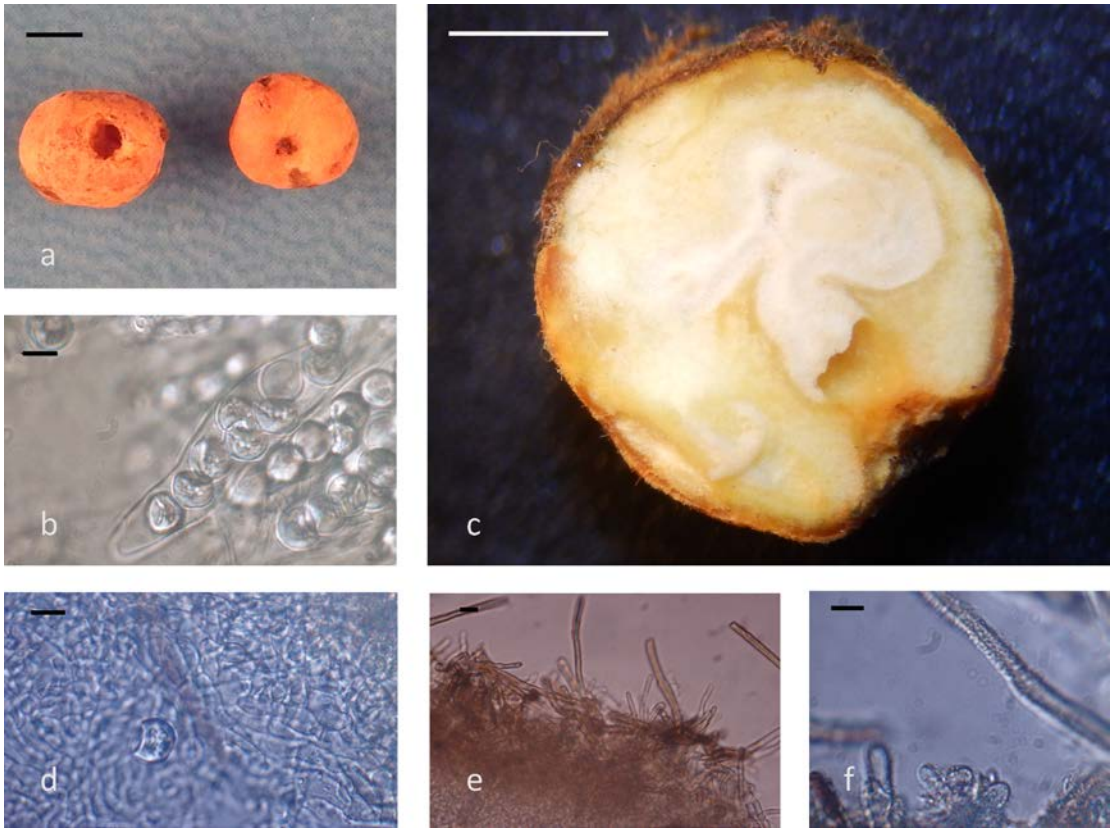

Fig. 2. Ascoma *D. crocea* cut in two parts (a); gleba (c); spores (b); innermost peridium (d); outermost peridium (e); granule-encrusted hyphae (f). a & c bars = 1mm; b, d, e & f bars = 10 µm.
Fig. 2. Ascoma di *D. crocea* tagliato in due parti (a); gleba (c); spore (b); peridio interno (d); peridio esterno (e); ife incrostate (f). a, c barre = 1 mm; b, d, e , f barre = 10 µm.

Acknowledgements

This note is dedicated to my truffle dog Geppa.

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