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DESCRIPTION OF THE FIRST FINDING IN ITALY OF *XEROCOMUS CHRYSONEMUS*

Abstract

The first finding in Italy of Xerocomus chrysonemus is described and illustrated with photos and line drawings. A phylogenetic tree of the genus Xerocomus s. str. including an ITS sequence of the studied sample is also presented. The Italian version of the article is available on the AMER website www.ameronlus.it.

Riassunto

La prima raccolta in Italia di Xerocomus chrysonemus viene descritta e illustrata con foto e disegni. Viene inoltre presentato un albero filogenetico del genere Xerocomus s. str. che include la sequenza del campione studiato. La versione in Italiano dell'articolo è disponibile sul sito AMER www.ameronlus.it.

Keywords Basidiomycota, Boletales, Boletaceae, *Xerocomus*, *chrysonemus*, Molecular phylogeny, Umbria, Italy.

Introduction

Every year the CEMM (European Confederation of Mediterranean Mycology) organises a mycological meeting with the aim of investigating the knowledge of fungal coenosis in the Mediterranean environments of Italy, France, Portugal and Spain. In 2012, the AME (Gubbio Mycological Association) in cooperation with local Umbrian mycological associations hosted in Italy the *XX Giornate Micologiche della CEMM* in study areas of particular environmental value. The foray was held in Gubbio (PG) from 13 to 19 October 2012. Field research was mainly carried out in specific sites of the vast territory surrounding Gubbio, including some areas of the municipality of Gualdo Tadino (PG), and a limited area close to the neighbouring Marche region, specifically the municipality of Cagli (PU), near Pianello. The surveyed areas are characterized by widespread, dense forests and are located in different environments. They were also selected according to the different dominant vegetation in order to focus the attention on the associated fungal communities. From the foothills to the uppermost limit of the beech forest, the territory is largely covered by periodically coppiced woods, with forest formations consisting of the European hop-hornbeam (*Ostrya carpinifolia* Scop.), the South European flowering ash (*Fraxinus ornus* L.), the Sycamore (*Acer pseudoplatanus* L.), and to a lesser extent on the sunnier slopes of Downy oak (*Quercus pubescens* Willd.). In the valleys there are woods dominated by Turkey oak (*Quercus cerris* L.). Sub-mountain and mountain plains of the Apennines mainly consist of beech forests (*Fagus sylvatica* L.), which find favourable pedo-climatic conditions at these altitudes. These beech woods extend at an altitudinal gradient comprised between 600 and 1 000 m a.s.l. up to the timberline. Grasslands and pastures are also present in the area and can be divided into two categories: those of the foothills and those of the mountain plain above the woodland line. The former are essentially the result of deforestation of large areas to create pastures and rural land. The latter represent the most typical aspect of the Apennine mountain landscape. These places are dominated by Graminaceae, in particular by the Apennine Sesleria (*Sesleria tenuifolia* Schrad.) and also harbour rare floristic species. Research in the aforementioned areas proved to be fruitful as 216 fungi species were surveyed during the forays, including some that were very interesting from a taxonomic and ecological point of view.

The findings herein documented refer to *Xerocomus chrysonemus* A.E. Hills & A.F.S. Taylor 2006, a species collected in the municipality of Cagli (PU), near Pianello, on 15 October 2012, at 500 m a.s.l., in mixed forest of *Quercus cerris* and *Quercus pubescens*, with the presence of *Fagus sylvatica*, *Ostrya carpinifolia*, *Carpinus betulus*, *Prunus spinosa* L., *Crataegus monogyna* Jacq., *Rubus ulmifolius* Schott. and *Clematis vitalba* L.

Antonio Gennari, the Scientific Director and official supervisor of the event, promptly distinguished certain characters of the species already in the field, such as the yellow mycelium, which led him to study the collection with Tomaso Lezzi and Mario Iannotti, the other two authors of the present work. As a result of their preliminary studies, some distinctive characters appeared evident: the bright yellow context at the base of the stipe, the absence of any discoloration of the tissues on bruising, the presence of a yellow to bright yellow basal mycelium, the association with oaks, as well as characteristic microscopic details such as the size of the spores and their Q ratio.

Since the species has been only relatively recently established (Taylor *et al.* 2006), and is so far known only from sporadic findings in the United Kingdom (Taylor *et al.* 2006; Taylor *et al.* 2007; Hills 2008; Kibby 2011; Ainsworth *et al.* 2013), Spain (Taylor *et al.* 2007; Muñoz 2008), France (Deconchat 2010; Chalange 2013), Hungary (Siller *et al.* 2013), Sweden (Taylor & Eberhardt 2006; Knudsen & Taylor 2008 as "*X. chrysonema*") and the Czech Republic (Janda *et al.* 2013, 2014; Mikšík 2017), and was not yet reported from Italy, we considered it appropriate to also verify its identity by performing an ITS molecular analysis, which confirmed the correctness of the determination previously hypothesised on the basis of macroscopic and microscopic characters.

Materials and methods

Morphology

The study was carried out on fresh and dried material. The images were taken in habitat with Nikon D80 and D300S cameras, with Nikkor Micro 60 mm lenses. For the microscopic study, a Nikon Eclipse E400 with Moticam 580 and an Optika B-500Ti with Optikam Pro 5 camera, equipped with 10 ×, 20 ×, 40 ×, 60 × and 100 × oil, plan-achromatic objectives were used. The macroscopic description was based on fresh material, while the micro-morphological characters were observed from dried material. H_2O, KOH and 6% ammonia were used to rehydrate the herbarium material, while Congo red was used to stain the cells. Spore dimensions were detected with the Piximètre program (Henriot & Cheype 2020) through 80 measurements.

Spore dimensions are reported as follows: (d0) d1-d9 (d10), where the values in parentheses are the exceptional measured values, respectively the decile d0 and the decile d10; while the deciles d1 and d9 are the limits of the range, which correspond to 80% of the measurements made, excluding the values from decile d0 to d1 and from decile d9 to d10. Q is the ratio between length and width, N is the number of measurements taken, Average represents the average values of length × width, and Qa is the average ratio between length and width.

DNA extraction, amplification and sequencing

DNA extraction, amplification and sequencing were performed by the Alvalab molecular biology laboratory in Oviedo (Spain). Total DNA was extracted from dried specimens using a modified protocol based on Murray & Thompson (1980). PCR reactions (Mullis & Faloona 1987) included 35 cycles with an annealing temperature of 54 °C. Amplification of the ITS region of the rDNA was performed with the ITS1F and ITS4 primers (White *et al.* 1990; Gardes & Bruns 1993). The PCR products were checked on 1% agarose gel and the positive PCR amplifications were sequenced.

Phylogenetic analysis

The dataset was constructed using 23 sequences downloaded from GenBank, in addition to the sequence generated for the present work (TL121015-01). *Hemileccinum impolitum* (Fr.) Šutara was chosen as outgroup. The sequences were selected on the basis of the results presented in Taylor *et al.* (2006); Gelardi *et al.* (2013) and Gelardi *et al.* (2014). The sequences were visualized with the software MEGA X (Kumar *et al.* 2018), aligned using the "Muscle" algorithm implemented in the program, leaving the basic parameters unchanged, and edited to remove the extremes of the sequences and to check for possible reading errors of the chromatograms. RAxML GUI 8.2.12 software (Stamatakis 2014) was used to construct a phylogenetic tree based on Maximum Likelihood with the standard search algorithm and 1 000 bootstrap replicates (Felsenstein 1985) using the GTR + GAMMA model. The software MrBayes 3.2.7 (Ronquist *et al.*, 2012) was used for phylogenetic analysis based on Bayesian Inference with the evolutionary model GTR + G, with two separate analyses of four chains for 10 000 000 generations and a sampling every 1 000 generations until the convergence parameters were reached, discarding 25% of the sampled trees as burn-in. The resulting filogram (**Figure 7**) shows the **MLB** (Maximum Likelihood Bootstrap) values with a threshold ≥ 75 in bold, and the BPP (Bayesian Posterior Probability) values with a threshold ≥ 0.75 in plain.

TAXONOMY

Xerocomus chrysonemus A.E. Hills & A.F.S. Taylor, *Myc. Res.* 110 (3): 283, 2006 (Basionym)

Etymology

The epithet *Xerocomus* derives from the Greek *ξηρός* (*xerós*) = dry, and *κόμη* (*kómh*) = hair, due to the dry pileus surface. The epithet *chrysonemus* derives from the Greek *χρυσός* (*krysós*) = gold, and *νῆμα* (*nema*) = filament, due to the yellow colour of its mycelium.

Original diagnosis

Xerocomus chrysonemus A.E. Hills & A.F.S. Taylor sp. nov.
Pileus 25–70 mm, flavus sinapis vel olivaceo-flavus, mutabilis. Poris e aureo-luteo mox leviter viridiflavis, postremo viridi-flavis, haud cyanescentibus. Contextus albidus vel pallido-citrinus, stipes basi aureus, immutabilis ubi scissus vel contusus. Mycelium profunde aureum vel flavum sinapis, color exsiccatorum similis. Basidiosporae (9-) 11.5 (-14.5) (4.5-) 5 (-7.0) μm, laeves, ellipsoideae vel latosubfusoideae, crassotunicatae.
Typus: UK: Hampshire: New Forest, Pig Bush, 13 Aug. 2003, S. Kelly (K(M) 123243 – holotypus).

Macroscopic characters (Figures 1-4)

Pileus 50-70 mm, convex, scarcely fleshy, chamois, reddish-brown especially towards the edge.

Pores large, angular, golden-yellow, unchangeable on bruising (**Figures 2, 4**).

Stipe reddish-brown, with a stretched, elongated network, bright yellow at the base, due to the presence of yellow mycelium (**Figure 3**).

Context unchanging when cut, yellow, especially at the base of the stipe where it exhibits a mustard yellow to bright yellow colour (**Figure 1**).

Smell acidic-fruity, very similar to that of *Xerocomus subtomentosus*.

Microscopic characters (Figures 5-6)

Spores (8.4) 9.7-11.3 (11.5) × (3.9) 4.1-5.0 (5.1) μm; Q = (1.9) 2.1-2.5 (2.6); N = 80; Average = 10.3 × 4.5 μm; Qa = 2.3 (**Figures 5; 6B**).

Basidia clavate, predominantly 4-spored, with fairly elongated sterigmata (**Figure 6C**).

Cheilocistidia fusiform, sometimes rostrate.

Pleurocistidia similar to cheilocystidia.

Pileipellis formed by a trichoderma of cylindrical, subparallel hyphae, with rounded terminal cells and devoid of encrustations (**Figure 6A**).

Clamp connections not observed.

Habitat occurring on soil, in woods dominated by *Quercus cerris* and *Quercus pubescens*, with the presence of *Fagus sylvatica*, *Ostrya carpinifolia*, *Carpinus betulus*, *Prunus spinosa*, *Crataegus monogyna*, *Rubus ulmifolius* and *Clematis vitalba*.

Coenosis other fungal species found in the same habitat: *Amanita franchetii* (Boud.) Fayod, *Russula sororia* (Fr.) Romell and *Hygrophorus penarioides* Jacobsson & E. Larss.

Examined material Italy. Umbria, municipality of Cagli (PU), near Pianello, 15 Oct 2012, TL121015-01, preserved in the authors' private *herbarium*.

Notes

The genus *Xerocomus* Quél. has in recent years undergone a noticeable upset based upon morphological investigation and molecular studies carried out by Binder & Hibbert (2006); Eberhardt & Taylor (2005); Bakker & Noordeloos (2005) and Šutara (2008). In particular, several new genera have been segregated from *Xerocomus* as traditionally circumscribed (namely *Hemileccinum*, *Hortiboletus*, *Imleria*, *Pseudoboletus*, *Rheubarbariboletus*, *Xerocomus* s. str. and *Xerocomellus* s. str.), or some species have been transferred to pre-existing genera (*Phylloporus* and *Aureoboletus*), see Taylor *et al.* (2006); Šutara (2008); Binder & Hibbett (2006); Klofac (2010).

Phylloporus includes the only European species *P. pelletieri* (Lév.) Quél., which is characterized by a strongly anastomosing hymenophore that is generally considered an intermediate morphology between a lamellate hymenophore and a poroid hymenophore; *P. pelletieri* also displays a blue reaction of the pileus surface with ammonia and a mediostratum of the hymenophoral trama not clearly differentiated. The hyphae of the lateral strata are only slightly divergent, rather dense and not gelatinized (Ladurner & Simonini 2003).

Fig. 1. *Xerocomus chrysonemus* (TL121015-01). Photo by Tomaso Lezzi

Fig. 2. *X. chrysonemus* (TL121015-01). Pores and stretched, elongated ribs on the stipe. Photo by Tomaso Lezzi

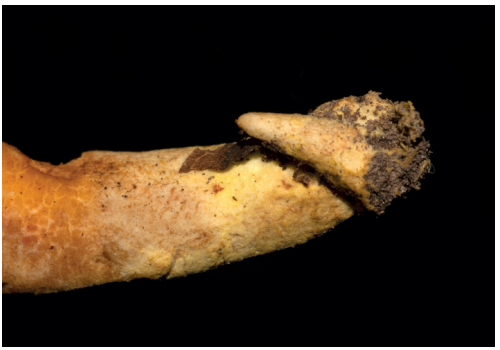

Fig. 3. *X. chrysonemus* (TL121015-01). The yellow base of the stipe. Photo by Tomaso Lezzi

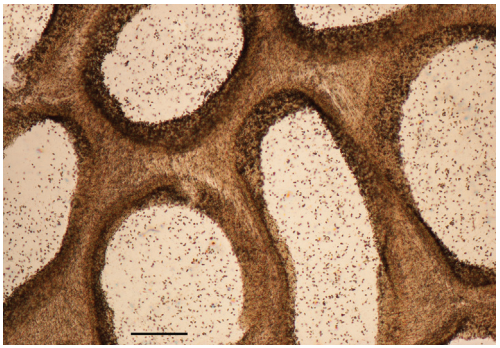

Fig. 4. *X. chrysonemus* (TL121015-01). Pore section in L4, 40×. Scale bar 500 μm. Photo by Tomaso Lezzi and Mario Iannotti

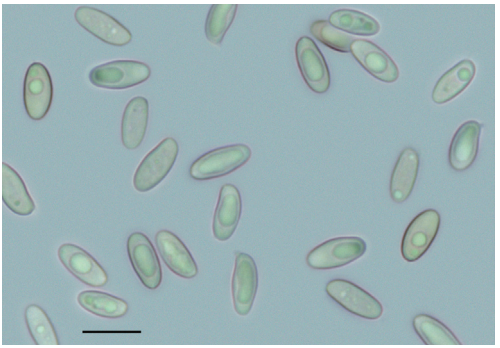

Fig. 5. *X. chrysonemus* (TL121015-01). Spores in L4, 1 000×. Scale bar 10 μm. Photo by Tomaso Lezzi and Mario Iannotti

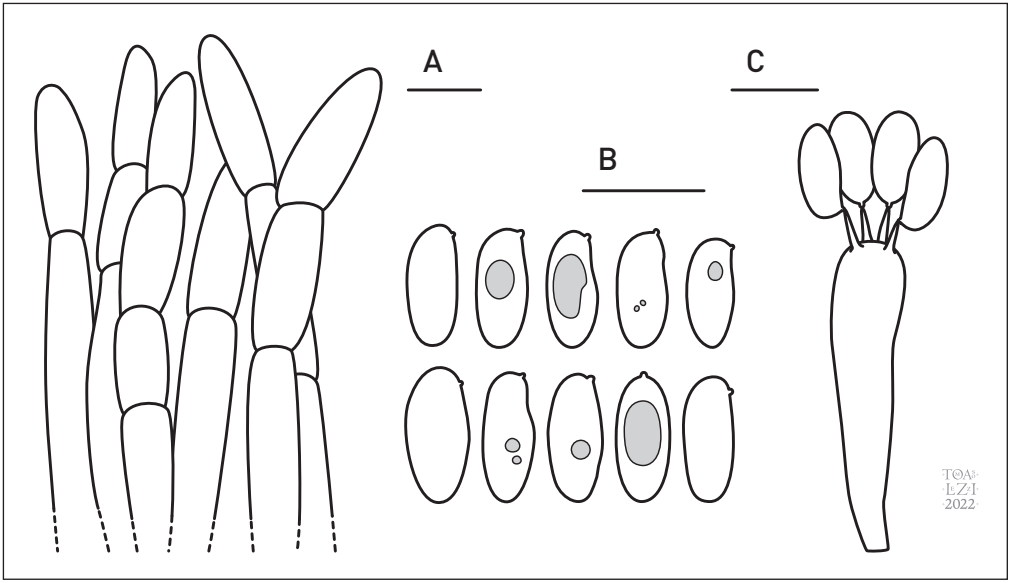

Fig. 6. *X. chrysonemus* (TL121015-01). **A**. Pileipellis; **B**. Spores; **C**. Basidia. Scale bars 10 μm. Drawing by Tomaso Lezzi

Pseudoboletus includes the only European species *P. parasiticus* (Bull.) Šutara growing as a parasite on *Scleroderma citrinum* Pers. : Pers.

Imleria includes the only European species *I. badia* (Fr.) Vizzini, characterized by a slimy pileus in wet weather.

Aureoboletus is represented in Europe by two endemic species: *A. gentilis* (Quél.) Pouzar, and *A. moravicus* (Vaček) Klofac, other than the exotic *A. projectellus* (Murrill) Halling (not reported from Italy to date). The species recorded in Italy are characterized by the terminal hyphae of the pileipellis without encrustations.

Hemileccinum includes two European species: *H. depilatum* (Redeuilh) Šutara and *H. impolitum* (Fr.) Šutara. This genus is characterized by the smell of iodine at the base of the stipe and spores with a smooth surface.

Xerocomellus encompasses nine European species: *X. chrysenteron* (Bull.) Šutara, *X. cisalpinus* (Simonini, H. Ladurner & Peintner) Klofac, *X. poederi* G. Moreno, Heykoop, Esteve-Rav., P. Alvarado & Traba, *X. porosporus* (Imler ex Watling) Šutara, *X. pruinatus* (Fr. & Hök) Šutara, *X. redeuilhii* A.F.S. Taylor, U. Eberh., Simonini, Gelardi & Vizzini, *X. ripariellus* (Redeuilh) Šutara, *X. sarnarii* Simonini, Vizzini & U. Eberh., and *X. fennicus* (Harmaja) Šutara, this latter not yet found in Italy. This genus is characterized by the pileus surface that does not stain blue-green with ammonia, the presence of a white mycelial tomentum enveloping the base of the stipe, terminal hyphae of the pileipellis with encrustations, and spores with a faintly longitudinally striate or smooth surface, sometimes truncated at the apex.

Rheubarbariboletus is represented by two European species: *R. armeniacus* (Quél.) Vizzini, Simonini & Gelardi, and *Rheubarbariboletus persicolor* (H. Engel, Klofac, H. Grünert & R. Grünert) Vizzini, Simonini & Gelardi. This genus is characterized by the pileal surface and the context at the base of the stipe which become blue, dark green to blackish with $FeSO_4$, the terminal hyphae of the pileipellis with congophilous plaques, and the ellipsoidal-fusiform, smooth-walled spores with Qa > 2.5.

Hortiboletus includes three European species: *H. bubalinus* (Oolbekk. & Duin) L. Albert & Dima, *H. engelii* (Hlaváček) Biketova & Wasser and *H. rubellus* (Krombh.) Simonini, Vizzini & Gelardi. This genus is characterized by a pileus surface that does not stain blue-green with ammonia, the presence of tiny orange dots at the base of the stipe, the encrusted terminal hyphae of the pileipellis, the smooth-walled, ellipsoidal-fusiform spores, with Qa < 2.5.

Finally, the genus ***Xerocomus*** **s. str.** comprises four European species: *X. chrysonemus* A.E. Hills & A.F.S. Taylor, *X. ferrugineus* (Schaeff.) Alessio, *X. subtomentosus* (L.) Quél. and *X. silwoodensis* A.E. Hills, U. Eberh. & A.F.S. Taylor. The genus is characterized by a trichodermal pileipellis, with terminal cells devoid of encrustations and bacillate spores under SEM, a bilateral divergent hymenophoral trama of the "*Phylloporus-type*" (Redeuilh 1994; Taylor *et al.* 2001, 2002; Ladurner & Simonini 2003; Gelardi 2011; Simonini *et al.* 2016). The main distinguishing characters of these four species of *Xerocomus* s. str. are summarized in **Table 1**.

Tab. 1. Comparative chart of the European *Xerocomus* s. str.

SPECIES	COLOUR OF THE CONTEXT	CONTEXT DISCOLORATION	BASAL MYCELIUM
X. chrysonemus	Yellow.	Unchangeable to occasionally turning light blue.	Yellow / Golden yellow.
X. subtomentosus	Yellowish but pinkish in the lower third of the stipe.	Light blue above the tubes.	White / Light yellow.
X. ferrugineus	White/Whitish.	Unchangeable to slowly and faintly reddening throughout.	Yellow.
X. silwoodensis	White/Whitish in the stipe, pale cream in the pileus.	More vividly yellowing in the pileus, unchangeable to slightly reddening in the stipe.	White to light yellow.

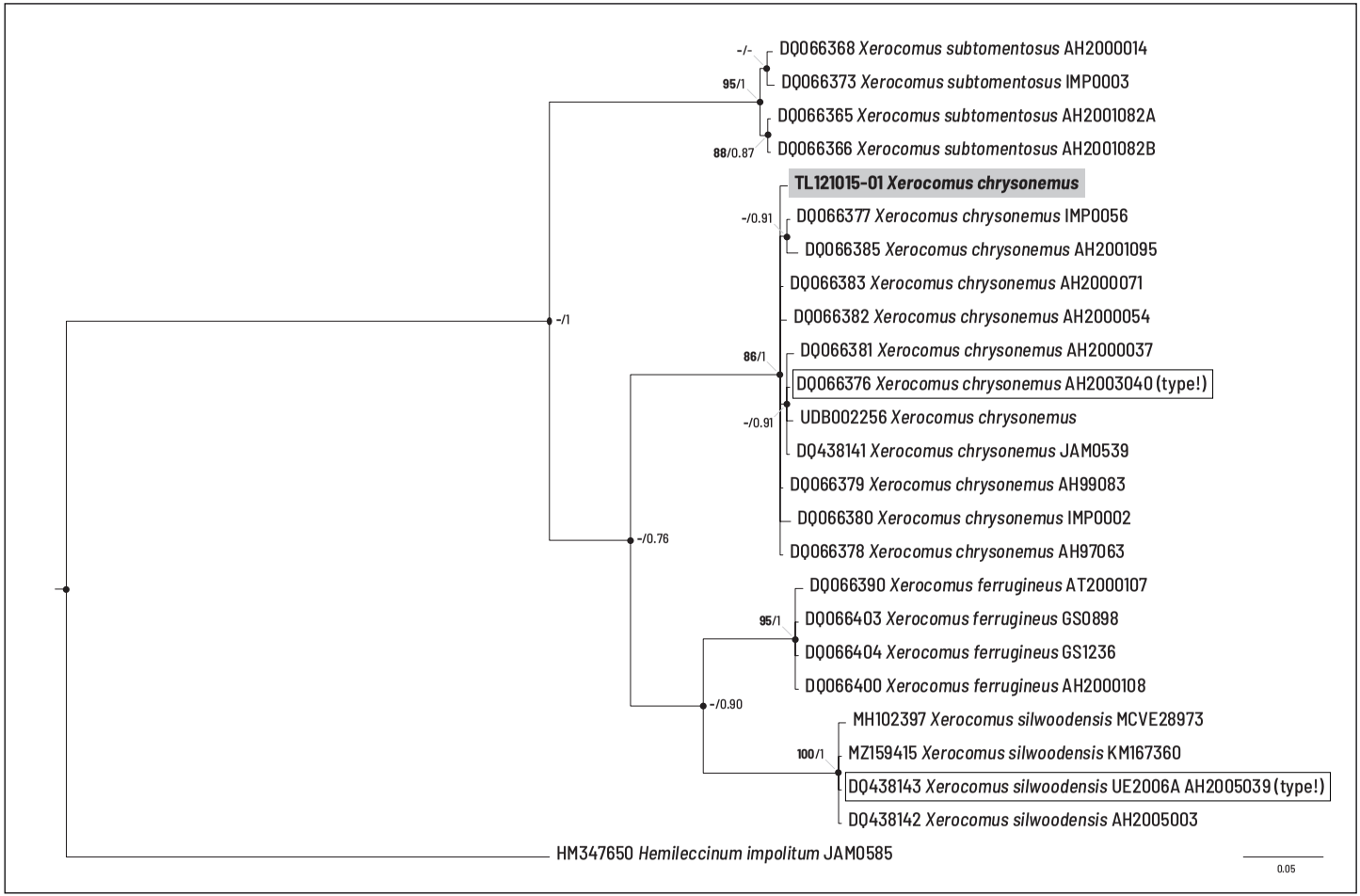

Fig. 7. Filogram obtained from ITS sequences. *Hemileccinum impolitum* was used as an outgroup. The **MLB** (Maximum Likelihood Bootstrap) values ≥ 75 are shown in bold, and BPP (Bayesian Posterior Probability) values ≥ 0.75 in plain. The sequence generated in the present work (TL121015-01) is highlighted in grey.

In the four species belonging to *Xerocomus* s. str. the Q value of the spores can be useful to recognise *X. chrysonemus*, which has shorter and wider spores than those in the other three taxa. This is reflected in a lower Q = 2.1-2.3 (Taylor *et al.* 2006).

The two phylograms obtained using Maximum Likelihood and the Bayesian posterior probability methods produced similar topologies, therefore in **Figure 7** only the Bayesian Inference is shown. **MLB** (Maximum Likelihood Bootstrap) values are in bold, whereas BPP (Bayesian Posterior Probability) values are in plain. The obtained results show that the sequence TL121015-01 is nested in a clade together with other sequences determined as *Xerocomus chrysonemus*. This genus currently includes only three other European species: *X. subtomentosus*, *X. ferrugineus*, and *X. silwoodensis* (Taylor *et al.* 2007; Gelardi 2011).

As far as we know, this is the first official documented and molecularly confirmed record of *Xerocomus chrysonemus* from Italy. This species is probably more frequent than generally assumed but it has most likely been overlooked or misidentified in the past. We hope this contribution will turn out to be helpful for a proper identification of future reports of *X. chrysonemus*.

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