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Xerocomus silwoodensis sp. nov., a new species within the European *X. subtomentosus* complex

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ABSTRACT

A recent analysis of the *Xerocomus subtomentosus* complex in Europe using rDNA-ITS sequence data distinguished four taxa in Europe. Two of these corresponded to the established taxa *X. subtomentosus* and *X. ferrugineus*, and a new taxon, *X. chrysonemus*, was described. The fourth taxon was noted but left undescribed owing to lack of material. Here, we describe this taxon as *X. silwoodensis* sp. nov. *X. silwoodensis* is a rare but widespread taxon known from single sites in Italy and Spain, and three in the UK. The features of *X. silwoodensis* basidiomes are very similar to other members of the complex but the pileus colours tend to show richer red–brown tones and the stipe often radicates deeply into the substrate. The taxon also exhibits a strong preference for associating with *Populus* species, whereas the other taxa are associated with either *Quercus* (*X. chrysonemus*) or generalists on broadleaved hosts (*X. subtomentosus*) or conifers and broadleaved trees (*X. ferrugineus*). Microscopically, the spore characteristics of *X. silwoodensis* are similar to the recently described *X. chrysonemus*, but differ significantly from both *X. subtomentosus* and *X. ferrugineus*. *X. silwoodensis* is probably overlooked due to the resemblance to other taxa within the complex. The present study on the identification and description of *X. silwoodensis* should reduce the confusion associated with the identification of taxa within this species complex and lead to a more accurate assessment of the geographic distribution and conservation needs of the taxa.

Introduction

European taxa within the *Xerocomus subtomentosus* (*Basidiomycota*, boletoid clade) species complex have traditionally been distinguished on the basis of cap colour and on the degree of development of a raised network on the upper part of the stipe (Engel *et al.* 1996). However, the usefulness of these characters has been questioned (Redeuilh 1994; Ladurner & Simonini 2003) and recent studies using molecular data have supported these criticisms. Taylor *et al.* (2006) analysed rDNA-ITS

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sequence data from a large number of geographically separated collections attributed to the European *X. subtomentosus* complex and demonstrated that variation in cap colour and network development did not support the recognition of species within the complex. Four taxa were recognized in this study. Two corresponded to the established taxa *X. subtomentosus* and *X. ferrugineus*, and a new taxon, *X. chrysonemus* was described. A fourth taxon was noted but left undescribed owing to lack of material. Here, we describe this taxon as *X. silwoodensis* sp. nov.

Materials and methods

Collection of material and morphological analysis

Material of *Xerocomus silwoodensis* included 16 collections from UK, one from Italy and one from Spain (see type description and Table 1). Material from the other taxa within the *X. subtomentosus* complex, *X. subtomentosus*, *X. ferrugineus* and *X. chrysonemus*, were included for comparative purposes (Table 1). Comparisons of spore measurements among taxa were based on all European material included in Taylor *et al.* (2006). For an analysis of DNA sequence data, a subsample of five collections (Table 1) from each taxon within the complex was chosen to cover the morphological, geographical and molecular variation observed within these taxa (Taylor *et al.* 2006).

Microscopic analysis of spores followed Ladurner (2001) and Peintner *et al.* (2003). Briefly, spores were examined from either deposits at the stipe apex or associated with hymenophoral material. Measurements were made of 30 spores from each collection mounted in 3 % potassium hydroxide aqueous solution. Care was taken to ensure that only mature spores were measured. These usually contain 1–3 guttules and have darker walls than immature spores (Ladurner & Simonini 2003). Spore measurements are given as (min.) mean ± s.d. (max.). The spore quotient (Q) is the ratio of spore length to breadth (Q = l/b). Comparisons of spore characteristics among taxa were carried out using one-way analysis of variance (Minitab, Version 12).

The description of *X. silwoodensis* was prepared by A.H. and is based on fresh and dried material. Colour codes refer to Kornerup & Wanscher (1963).

Molecular analysis

The molecular analyses were based on DNA sequence data from the ITS region of nuRNA genes, including the two spacer regions ITS1 and ITS2 and enclosing the highly conserved 5.8S ribosomal gene. DNA was extracted from dried specimens using PrepMan Ultra (Applied Biosystems, Foster City, CA), 100 µl per sample, and purified with JETquick general DNA cleanup columns (Genomed, Löhne), according to the manufacturers instructions. PCR products were purified with the Viogene PCR clean-up purification kit (Viogene, Sunnyvale, CA), omitting the first washing step described in the Viogene instructions leaflet. Further details of primers, PCR conditions and direct sequencing have been described previously (Taylor *et al.*, 2006). The ITS amplicon obtained from the holotype [K(M)137134] could not be sequenced directly. The PCR product was therefore cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions, but reducing the volumes by half. Two clones were sequenced.

Raw sequence data were edited in Sequencher (version 4.1, Gene Codes Corporation, Ann Arbor, MI). Sequence alignment was initially carried out by Clustal X (version 1.81; Thompson *et al.* 1997) with standard settings and later edited by hand. Due to difficulties in the alignment, no outgroup taxon was included. Gaps were introduced in the alignment where unambiguous alignment of sequences of different taxa could not be accomplished. This was done in order to maintain existing intraspecific variation. Analyses were run on the complete alignment or excluding all gapped positions in order to assess the overall phylogenetic resolution and topology.

ML analysis and NJ with the corresponding BS analyses were carried out in PAUP* (version 4.0b10; Swofford 2002). Modeltest (version 3.7; Posada & Crandall 1998) was used to give an indication of how many and which parameters should be considered in likelihood models. The suggested model was then used in a ML analysis of the complete alignment, calculated in 100 random-addition-of-species replicates of heuristic search with factory settings, complemented by a BS analysis (100 replicates of heuristic search with the same settings as for the original analysis, only reducing the number of random-addition-of-species replicates to one for each BS replicate) under the same model. NJ analyses were performed under the same model, including all positions and excluding gapped positions to see whether the overall topology was affected by the inclusion of the sequence stretches that could not be aligned between species.

Results

Molecular analyses

Sequences obtained from collections of *Xerocomus silwoodensis* (Table 1) comprised 715–745 bp, including the entire ITS1 and ITS2 regions. No differences were observed. As the cloned ITS PCR products from the type were identical in sequence, only one of the sequences was considered in further analyses.

The alignment of sequences from the whole complex, spanning 941 bp, was submitted to TreeBASE (accession no. SN2759). From three *X. subtomentosus* collections, two different ITS sequences were obtained (see Taylor *et al.* 2006): indicated by 'a' and 'b' after the collection code (Fig 1). Hierarchical likelihood ratio tests, implemented in Modeltest, suggested a likelihood model assuming equal base frequencies, and incorporating different substitution rates for transitions and transversions with a transition:transversion ratio of 2.57 and approximating the distribution of variable sites across the alignment by a gamma distribution with $\alpha = 0.14$ (K2P+G model). The ML analysis resulted in a single island of five trees, varying very slightly in the placement of the