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

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ARTICLE INFO

Article history:
Received 26 June 2006
Received in revised form
20 December 2006
Accepted 17 January 2007
Published online 4 February 2007
Corresponding Editor:
Karl-Henrik Larsson

Keywords:
Boletoid clade
Geographic distribution
Host specificity
ITS sequence data
Molecular taxonomy

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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doi:10.1016/j.mycres.2007.01.014

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

Table 1 – Material included in a phylogenetic analysis of the *Xerocomus subtomentosus* complex, GenBank accession numbers of rDNA-ITS data, origin and host tree where known

Taxon	Collection code[a] and herbarium number	GenBank accession no.	Origin	Host tree
Xerocomus chrysonemus	JAM0539	DQ438141	Spain, Cantabria, Las Rozas	*Quercus petraea*
X. chrysonemus	AH1999083	DQ066379	UK, Hampshire, New Forest, Rufus Stone	*Q. robur*
X. chrysonemus	AH2000037	DQ066381	UK, Hampshire, New Forest, Gritnam Wood	*Q. robur*
X. chrysonemus	AH2001095	DQ066385	UK, Kent, North Bishopden Wood, Blean woods	*Quercus* sp.
X. chrysonemus	AH2003040 (K(M) 123243 – holotype)	DQ066376	UK, Hampshire, New Forest, Pig Bush	*Q. robur*
X. ferrugineus	AT1999098	DQ066398	Sweden, Uppsala, City Forest	Mixed woodland
X. ferrugineus	AT2001071	DQ066402	Sweden, Umeå	*Pinus sylvestris*
X. ferrugineus	GS0898	DQ066403	Italy, RE, Villaminozzo	*Fagus sylvatica*
X. ferrugineus var. *citrinovirens*	AH2001110	DQ066393	UK, Perthshire, Kindrogan	*Salix repens*
X. ferrugineus var. *citrinovirens*	GS1920	DQ066405	Italy, TN, Arco, Monte Velo,	*F. sylvatica, Picea abies*
X. subtomentosus	AH2000014	DQ066368	UK, Berkshire, Windsor, Ascot Gate	*Castanea sativa*
X. subtomentosus	AT2004282	DQ066363 DQ066364	Sweden, Lammö	Mixed woodland
X. subtomentosus	GS1284	DQ066367	Italy, RE, Casino, Peconte	Mixed broadleaved forest
X. subtomentosus	GS1135	DQ066359 DQ066360	Italy, RE, Pulpiano, Viano	*Q. cerris*
X. subtomentosus	GS1796	DQ066355 DQ066356	Italy, RE, Reggio Emilia	*Quercus* sp.
X. silwoodensis	AH2004074	DQ066374	UK, Berkshire, nr Ascot, Silwood Park	*Populus* × *canescens*
X. silwoodensis	AH2005031(S F48083)	DQ438145	UK, Berkshire, nr Ascot, Silwood Park	*P.* × *canescens*
X. silwoodensis	AH2005039 (K(M)137134 – holotype)	DQ438143	UK, Berkshire, nr Ascot, Silwood Park	*P.* × *canescens*
X. silwoodensis	AH2005003 (S F48084)	DQ438142	UK, Hampshire, New Forest, Ferny Knap Inclosure	*P. alba*
X. silwoodensis	AH2004223	DQ438146	UK, Hertfordshire, Great Wood Country Park, nr Potters Bar	*Populus* sp.
X. silwoodensis	GS1959	DQ066375	Italy, RE, Carpineti, Marola	*Castanea sativa*
X. silwoodensis	JAM0612	DQ438144	Spain, Araba, Albina	Mixed forest including *P. tremula*

a Collection codes: AH, Alan Hills; AT, Andy Taylor; GS, Giampaolo Simonini; JAM, José A. Muñoz.

terminal branches within the same subterminal groups, which was recovered in 92 of 100 replicates of heuristic search. Fig 1 shows one of the trees, including the results of the BS analyses. NJ analyses on the full alignment and excluding the gapped positions (results not shown) resulted in topologies very similar to each other and to the ML tree in Fig 1. ITS sequence analyses clearly support the existence of four well-separated taxa, one of which corresponds to *X. silwoodensis* sp. nov.

Spore characteristics

The spores of the four taxa within the European *Xerocomus subtomentosus* complex are typically subfusiform to elliptical and vary most noticeably with respect to the length:breadth ratio (Table 2). *X. silwoodensis* shares the spore characteristics of the recently described *X. chrysonemus*, whereas the spores of *X. subtomentosus* are, on average, significantly longer and those of *X. ferrugineus* are significantly narrower (Table 2).

Taxonomy

Xerocomus silwoodensis A. E. Hills, U. Eberhardt & A. F. S. Taylor, **sp. nov.**, (Figs 2–3)

MycoBank number: MB510312

Etym.: silwoodensis, from Silwood Park, Berkshire, UK.

Pileus 20–110 mm, Xerocomi subtomentosi atque X. ferruginei in parte similis sed plus rubrobrunneus. Hymenophorus primo viv-ide pallide flavus tum sordide stramineus, immutabilis. Contextus firmus, albus ad albidum, ad pallide flavum in pileo, nunquam cyanescens. Mycelium albidum ad pallide flavum. Basidiosporae subfusiformes ad late subfusiformes, (9–)12 (−18.5) × (4–)5(−5.5) μm. Consociatus praecipue cum Populo spp. in locis umbrosis.

Typus: **UK**: *Berkshire:* Silwood Park nr Ascot, associated with *Populus* × *canescens,* 30 Aug. 2005, A. E. Hills (K(M)137134 – holotypus).

Pileus 20–130 mm diam, somewhat convex to hemispherical, only when very young with an inrolled margin, becoming plano-convex, initially finely tomentose becoming matt with age, highly variable in colour, reddish-yellow (5B8) to dark brown (9F7), most typically within this range is a rich red–brown sometimes showing lighter shades at the outer edge. With only a slight tendency to crack, if so then only toward the outer edge and never cracking to reveal any colour in the context beneath. *Pileipellis* (Fig 2A) a trichoderm of rounded cells 5.5–18.5 μm diam, 19–110 μm in length, without ornamentation, mostly in short, rarely branched chains, terminal hyphae mostly rounded, bullet-shaped, rarely tapering, easily disarticulating. *Hymenophore* tubulate, adnate to sinuate, decurrent, up to 18 mm in length, bright pale yellow (3A5) when fresh becoming dirty straw yellow (4A6) with age, never showing any colour change on cutting or bruising. *Pores* colours as for hymenophore, ± red spotted with age, almost round when young, becoming angular especially toward the outer edge, not bluing or changing with pressure. *Basidia* narrowly clavate to ± clavate, bearing four sterigmata, seldom three, 29–49 × 6.5–14.5 μm, ± showing granular incrustations. *Cheilocystidia* (Fig 2B) very variable, mostly cylindrical in shape becoming tibiiform or tapering toward the apex, rarely conical to somewhat fusiform, without any ornamentation. 28.5–62 × 6.5–15.5 μm. *Pleurocystidia* similar in shape and size to the cheilocystidia but in general slightly smaller. *Caulocystidia* short, clavate somewhat slightly mucronate 30–38 × 4–6.5 μm. *Clamp connections* none noted. *Stipe* very variable in both size and shape 22–70 (−130) × 7–33 mm, cylindrical to subclavate always tapering at the very base, often deeply rooting (Fig 3), sometimes with up to half of the stipe below soil level. At times, slightly curved but never bent unless below soil level. Stipe apex often with a narrow band of yellow bearing the decurrent tubes that extend into an easily observed crude reticulum. This is over a background colour that is concolorous with the pileus, which becomes straw, buff and dull red–brown at mid-stipe, then toward the base always becoming somewhat buff. The stipitipellis is cartilaginous that often results in the outer stipe splitting and peeling both upwards and downwards as the basidioma matures. *Context* firm, on cutting white to off-white throughout, within the pileus soon changing to light yellow (1A5) to yellow (2A7). Within the stipe varying amounts of colour change can occur, from a mottled pale red (12A3) to greyish rose (12B5), to a uniform

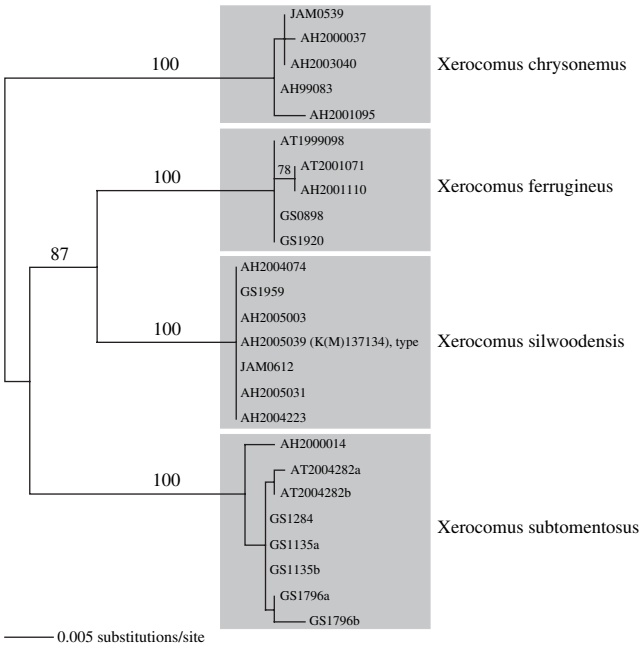

Fig 1 – One of five best trees (unrooted) obtained in a ML analysis of an alignment including ITS sequences from five to seven collections from the four European *Xerocomus* species included in the *X. subtomentosus* complex. Deviating sequences obtained from the same *X. subtomentosus* collections are denoted by 'a' and 'b' following the collection number. BS support values ≥75 % (100 replicates) are indicated.

Table 2 – Spore characteristics (mean ± S.D.) of taxa within the European *Xerocomus subtomentosus* complex

Taxon	Length (μm)	Breadth (μm)	Ratio (L:B)
Xerocomus subtomentosus ($n = 13$[a])	12.3 ± 0.6 c[b]	5.1 ± 0.3 b	2.4 ± 0.2 b
X. chrysonemus ($n = 9$)	11.3 ± 0.5 a	5.2 ± 0.2 b	2.2 ± 0.1 a
X. ferrugineus ($n = 23$)	11.9 ± 0.7 b,c	4.5 ± 0.3 a	2.7 ± 0.2 c
X. silwoodensis ($n = 7$)	11.4 ± 0.6 a,b	4.9 ± 0.3 b	2.3 ± 0.2 a,b
F-value	5.66	20.69	25.11
Significance (P)	0.002	<0.001	<0.001

a *n* Refers to the number of collections included in the analysis. Values within collections are based on 30 spores.
b Values within columns not sharing the same letter differ at $P \leq 0.01$.

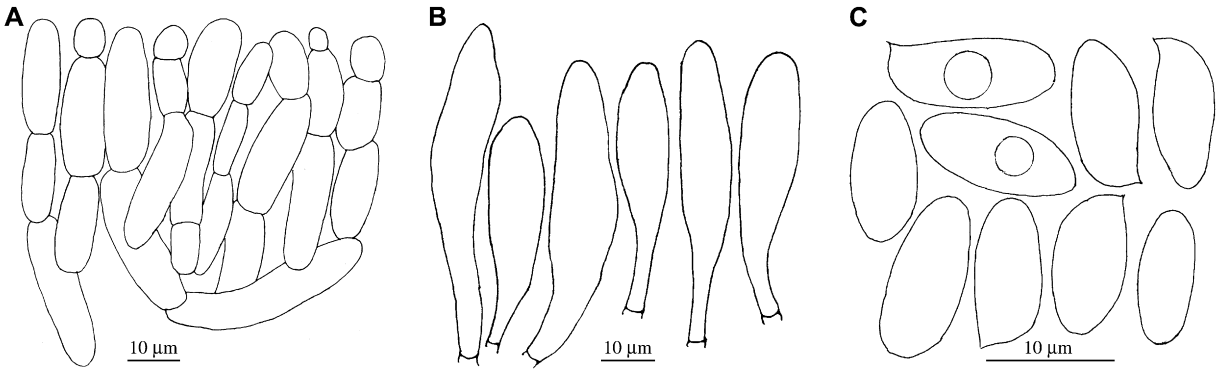

Fig 2 – Microscopical features of *Xerocomus silwoodensis* (K(M)137134 – holotype). (A) Pileipellis end cells. (B) Cheilocystidia. (C) Basidiospores.

development of these colours in the base, rarely there is no colour change. A reddish purple line above the pores and below the pileipellis is present in some collections. Insect larval tunnels yellow in fresh basidiomata, becoming black with age. *Mycelium* off white to pale yellow, never golden yellow, pale yellow in dried specimens. *Spores* (Fig 2C) subfusiform to broadly subfusiform in side view, ellipsoid to broadly ellipsoid in face view, lacking striations, a small percentage with oil drops, size (9–)9.5–14.5(−18.5) × 4–5.5(−7.5) µm, Q = 2.1–2.5, average size 12 × 5 µm, spore deposit olive–brown (4E8).

Chemical reactions: *On pileus*: ammonia—fleeting dark green to dull purple, at other times a faint blue–green ring may be present. Sun-bleached and shaded areas of a pileus may differ in the response to ammonia. *On context*: ammonia—bleaches out any colour; iron sulphate—bleaches out any colour; potassium hydroxide (10 %)—straw; Melzer's reagent—no reaction. On pores: Melzers reagent—blue–green; ammonia—russet.

Odour: Slightly acidic to pleasant.

Fig 3 – A basidiome of *Xerocomus silwoodensis* (AH2005031).

Habitat: Fruiting in shaded places on almost bare soil, pH 6.9, associated with *Populus* species.

Observations: Currently known from three sites in the south of England. *Xerocomus silwoodensis* may have been overlooked until recent times as being a reddish form of *X. ferrugineus*.

Additional specimens examined: **UK:** *Berkshire*: Silwood Park, nr Ascot, *Populus* × *canescens*, 2 Sep. 2000, *S. Kelly* (AH 2000058); *loc. cit., P.* × *canescens*, 27 Aug. 2002, *A. E. Hills* (AH2002073); *loc. cit., P.* × *canescens*, 6 Aug. 2003, *S. Kelly* (K(M) 116907); *loc. cit., P.* × *canescens*, 11 Aug. 2004, *A. E. Hills* (AH2004074); *loc. cit., P.* × *canescens*, 2 Sep. 2004, *A. E. Hills* (AH2004123); *loc. cit., P.* × *canescens*, 16 Aug. 2005, *A. E. Hills* (AH2005014); *loc. cit., P.* × *canescens*, 23 Aug. 2005, *A. E. Hills* (AH2005031 (S F48083)); *loc. cit., P.* × *canescens*, 28 Sep. 2005, *A. E. Hills* (AH2005078); *Hampshire*: New Forest, Ferny Knap, *P. alba*, 1 Oct. 2002, *A. E. Hills* (AH2002150); *loc. cit., P. alba*, 31 Oct. 2002, *A. E. Hills* (AH2002187); *loc. cit., P. alba*, 29 Jul. 2003, *A. E. Hills* (AH2003025); *loc. cit., P. alba*, 14 Jul. 2004, *A. E. Hills* (AH2004024); *loc. cit., P. alba*, 29 Jul. 2004, *A. E. Hills* (AH2005062); *loc. cit., P. alba*, 31 Aug. 2004, *A. E. Hills* (AH2004103); *loc. cit., P. alba*, 15 Jul. 2005, *A. E. Hills* (AH2005006); *loc. cit., P. alba*, 3 Aug. 2005, *A. E. Hills* (AH2005003 (S F48084)); *Hertfordshire*: Great Wood, nr Potters Bar, *Populus* sp., 18 Aug. 2004, *S. Kelly* (AH2004223).

Discussion

Xerocomus silwoodensis is a rare but widespread taxon known from three sites in the UK and single sites in Italy and Spain. The features of *X. silwoodensis* basidiomes are very similar to other members of the complex but the pileus colours tend to richer red–brown tones and the stipe often radicates deeply into the substrate. Despite the close similarity with the other taxa, the uniformity of the ITS sequences of the *X. silwoodensis* collections considered in the molecular analyses strongly suggests: (1) that they represent a single taxon, and (2) that they deserve species rank due to the clear distinction from other species of the *X. subtomentosus* group.

X. silwoodensis also exhibits a strong preference for associating with *Populus* species. Although the Italian collection was recorded as associating with *Castanea sativa*, there were individuals of *Populus tremula* in the vicinity. This contrasts with the other taxa that either associate with *Quercus* (*X. chrysonemus*) or are generalists with a preference for broadleaved hosts (*X. subtomentosus*) or conifers and a range of broadleaved tree genera (*X. ferrugineus*). Microscopically, the taxon shares the

spore characteristics of the recently described *X. chrysonemus*, whereas the spores of *X. silwoodensis* are shorter than in *X. subtomentosus* and broader than *X. ferrugineus*. In the field, *Xerocomus silwoodensis* is probably overlooked due to the resemblance to other taxa within the complex. It should be looked for in damp, partly shaded areas beneath *Populus* spp. where the ground vegetation is sparse or absent. It may be recognised by the crude, but pronounced network and, in particular, by the context within cap changing from white to pale yellow. The latter character separates *X. silwoodensis* from *X. ferrugineus*, which it most closely resembles.

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.

Acknowledgements

We thank Steve Kelly for providing material and monitoring the Hertfordshire site, Alan Lucas for monitoring the Hampshire site, and Mike J. Crawley for all the help and assistance he has given within Silwood Park. We also thank Mieke Verbeken for kindly providing the Latin diagnosis.

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