

# **Species Status**

No. 14

Red List of Fungi for Great Britain: Boletaceae

A pilot conservation assessment based on national database records, fruit body morphology and DNA barcoding

By

A.M. Ainsworth<sup>1</sup>, J.H. Smith<sup>2</sup>, L. Boddy<sup>3</sup>, B.T.M. Dentinger<sup>1</sup>, M. Jordan<sup>4</sup>, D. Parfitt<sup>3</sup>, H.J. **Rogers<sup>3</sup> & S.J. Skeates<sup>5</sup>** 

> Further information on the JNCC Species Status project can be obtained from the Joint Nature Conservation Committee website at http://www.jncc.gov.uk/

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ISSN 1473-0154

<sup>1</sup>Mycology Section, Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey TW9 3AB <sup>2</sup>13 Baden Road, Redfield, Bristol BS5 9QE <sup>3</sup>Cardiff School of Biosciences, Cardiff University, Cardiff CF10 3AX

<sup>4</sup>Association of British Fungus Groups, Harveys, Alston, Axminster, Devon EX13 7LG

<sup>5</sup>British Mycological Society, City View House, 5 Union Street, Ardwick, Manchester, M12 4JD



*Boletus immutatus* at its type locality in Windsor Great Park, Berkshire with stipe base cut to show the bright yellow and characteristically non-blueing flesh within. Photograph and toad management, 04 Sept. 2010, by A.M. Ainsworth.

This publication should be cited as:

A.M. Ainsworth, J.H. Smith, L. Boddy, B.T.M. Dentinger, M. Jordan, D. Parfitt, H.J. Rogers & S.J. Skeates. 2013. Red List of Fungi for Great Britain: *Boletaceae;* A pilot conservation assessment based on national database records, fruit body morphology and DNA barcoding *Species Status* 14. Joint Nature Conservation Committee, Peterborough.

# Contents

1.	Introduction to the series	4
2.	Background and Introduction to this Review	8
3.	Taxonomic scope and nomenclature	10
4.	Data preparation	11
5.	Methods: rationale	11
6.	Methods: application of IUCN Criterion D	16
7.	Methods: Data Deficient (DD) and Not Evaluated (NE)	17
8.	Results: summary of conservation assessments	18
9.	Results: assessments for RDL and NE taxa (omitting LC)	21
10.	Site Protection and Threats	32
11.	Recommendations for future recording of RDL boletes	33
12.	Acknowledgements	33
13.	References and sources of further information	34

# **1.** Introduction to the series

## 1.1 The Species Status Assessment series

This publication is one of a series produced under the auspices of the Species Status Assessment project initiated by JNCC in 1999. The project established the means by which the statutory conservation agencies, in partnership with voluntary conservation organisations and leading specialists, assign conservation statuses to British species. It aims to work towards assessing the status of all native species against standard criteria based on the internationally accepted guidelines developed by the International Union for Conservation of Nature (IUCN) (see IUCN, 2012a,b, 2013).

Comparisons are facilitated by assessing all taxa to the same standards. This is not without difficulty because species have a variety of life and reproductive strategies. Status assessments are prepared on the basis of the best available information for the group concerned, recognising that this will vary according to the intensity of recording and study, the majority of which is carried out by volunteer naturalists.

Assessments are produced as Red Lists or as broader National Reviews of taxonomic groups of species. Both types of publication provide an audit trail of the assessment. To enable assessments to reach as many practitioners as possible, the texts are made freely available via the JNCC website (<u>http://www.jncc.gov.uk/</u>).

## 1.2 The Red List system

The Red List system was initiated by IUCN in 1966 with the publication of the first Mammal Red Data Book. Since then Red Lists, and more detailed Red Data Books, have been published that deal with many plants, fungi and animals at global, regional, country, and even local scales. The aim has been to identify those species at greatest risk from extinction and to identify the critical factors responsible, so that action may be taken to improve the chances of these species surviving in the long term.

In Britain the first published Red Data Book endorsed by a statutory conservation agency was by Perring and Farrell (1977, 2<sup>nd</sup> edition published 1983), dealing with vascular plants. The Red Data Book for insects, edited by Shirt, was published in 1987, with volumes dealing with other animal and plant groups appearing thereafter. The geographic range is normally Great Britain, and hence excludes Northern Ireland as well as the Isle of Man and the Channel Isles. Only one volume has a combined treatment for Britain and Ireland, that by Stewart and Church (1992) for stoneworts, although separate statuses were provided.

The British Red List of vascular plants has had a full update twice (Wigginton, ed. 1999, Cheffings and Farrell, 2005) following the production by the IUCN of a new, quantitative approach to threat assessment (IUCN, 1994, 2001, 2003). The recent Red Lists of British Odonata (Daguet *et al.*, eds., 2008) and butterflies (Fox *et al.*, 2010) and reviews of Diptera (Falk and Crossley, 2005, Falk and Chandler, 2005), water beetles (Foster, 2010) and lichens (Woods and Coppins 2012) have continued to follow the revised IUCN guidelines (<u>http://jncc.defra.gov.uk/page-3352</u>) which are regularly updated (IUCN, 2012a,b 2013).

## **1.3** Status assessments other than Red Lists for species in Britain

Conservation assessments that are broader in scope than the traditional Red Data Books and Red Lists have been produced. These assessments add GB-specific categories based on restricted distribution rather than risk. The term Nationally Scarce, originally coined for plants, is applied to species that are known to occur in 16 to 100 ten-km squares (or hectads). Early assessments of invertebrate taxa used the term Nationally Notable and, for some taxa this category was further split into Notable A (Na) for species occurring in 16 to 30 hectads and Notable B (Nb) for those occurring in 31 to 100 hectads.

A further category that has a very specific application is that of 'Nationally Rare'. This category is only used for plant and lichen species that occur in 15 or fewer hectads in Britain and is used in SSSI designation and Common Standards Monitoring.

The restricted distribution categories have now been standardised to Nationally Rare (used only for plants and lichens) and Nationally Scarce (used for all taxa including plants and lichens), without further subdivision. The GB system of assessing **rarity** based solely on distribution is used alongside the IUCN criteria which, although they also use measures of geographical extent, are concerned with assessing **threat**.

Publications that compile information about Red List species are known as Red Data Books and usually cover broad taxonomic groups (e.g. insects). Publications that include information about both Red Listed and Nationally Scarce species are known as National Reviews. The latter are usually produced for a more restricted taxon group (e.g. dragonflies or water beetles). Both types of publication contain individual species accounts that include information about their biology, distribution and status as well as threats to the species and their conservation needs.

## 1.4 Species Status Assessment and conservation action

Making good decisions to conserve species should primarily be based upon an objective process of determining the degree of threat to the survival of a species, in the present exercise by assigning the species to one of the IUCN threat categories. This assessment of threats to survival should be separate and distinct from the subsequent process of deciding which species require action and what activities and resources should be allocated.

When making decisions as to which species should be treated as priorities for conservation action, factors to be considered other than IUCN threat category include: the likely chances of recovery being achieved; the cost of achieving recovery (and whether sources of funding are available or likely to be available); the benefits to other threatened species of a recovery programme; the fit of a recovery programme with other conservation activities (including conservation actions to be taken for habitats); the likely gains for the profile of conservation; and the relationship and fit between national and international obligations. Under the UK Biodiversity Action Pan (see <u>www.ukbap.org.uk</u>) a list of priority species has been identified as a focus for conservation effort. In addition, certain species are legally protected in Great Britain under legislation such as the Wildlife and Countryside Act 1981, and British wildlife legislation is overlaid by international directives such as the Habitats Directive (Directive 92/42/EEC). For some species groups, threat assessments and rarity assessments also underlie the criteria used for protected site selection, and these species can then constitute protected interest features on the site.

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## 2. Background and Introduction to this Review

There have been two fungal red-listing exercises in Great Britain (GB). The first of these historic Red Data Lists (RDLs) was "A Provisional Red Data List of British Fungi" (Ing 1992) which expressed levels of risk using the IUCN categories available at the time and was based on data from "foray lists, herbarium and literature sources and by discussion with experienced field mycologists". This was superseded by an online list, the current RDL, entitled "Preliminary Assessment: The Red Data List of Threatened British Fungi" (Evans et al. 2006). This covers GB and the Isle of Man, again using IUCN categories, and was compiled on behalf of the British Mycological Society (BMS). The lists were not published by the Joint Nature Conservation Committee (JNCC), a body approved by the International Union for Conservation of Nature (IUCN) for quality assuring regional RDLs, and so they did not achieve official status. The assessments are now in need of revision and, unfortunately, the unofficial status of the current RDL has limited its role in fungal conservation. For example, JNCC stipulated that only official RDLs could be taken into account when the European conservation status of fungi was assessed during the 2005/7 UK Biodiversity Action Plan review. As a result of this constraint, the conservation assessments of Evans et al. (2006) could not be used in this task. Closer to home, the publication of an official fungal RDL is an essential prerequisite for the production of two long-awaited conservation tools. It would enable the "Guidelines for Fungi", a compilation of the criteria upon which selection of the UK's Sites of Special Scientific Interest for their fungal interest depend, to be significantly expanded. This would bring a wider variety of important fungal sites into consideration for designation and protection. Similarly, an official RDL is an essential tool for revising the UK's Important Fungus Areas, a list which was drawn up over a decade ago (Evans et al. 2001).

British fungal records are now held in two national record databases: FRDBI managed by the BMS and CATE2 managed by the Association of British Fungus Groups (ABFG). A pilot RDL project, focussing on the conservation assessment of an entire fungal family, was therefore proposed to investigate how the datasets could be efficiently analysed to secure the first JNCC-approved official RDL for British fungi. The geographic scope was restricted to Great Britain (England, Scotland and Wales) and all database records from the Channel Isles, Ireland and the Isle of Man were excluded from the project. Although JNCC's remit also includes Northern Ireland, it was deemed to be more biologically-relevant to have separate regional assessments for GB and for the island of Ireland. Following a successful outcome, the longer-term goal is to progress towards an official RDL for all accepted GB *Basidiomycota* and regional RDLs for the constituent countries. The *Basidiomycota* has been prioritised for red-listing because most of its members form relatively conspicuous fruit bodies and it is currently the only fungal taxon with a recent British checklist (Legon & Henrici 2005). This list, the Checklist of the British & Irish Basidiomycota, is now maintained and updated online (CBIB).

The bolete family (*Boletaceae*) was selected for a Natural England-funded pilot RDL assessment because its members form large, often colourful, and sometimes commercially important edible fruit bodies (ceps, porcini) and so they are relatively well-known to naturalists and the general public alike. Identification guides to British boletes are very popular with field mycologists and are frequently updated (e.g. Watling 1970, Kibby 2000, Taylor *et al.* 2002, Watling & Hills 2005, Kibby 2006, Hills 2008, Kibby 2011). Several British field mycologists have taken a special interest in the group in recent years, in particular A.E. Hills, whose extensive collections and fruit body abundance data gathered over the last two decades are now deposited at the Royal Botanic Gardens, Kew (RBGK). Boletes are therefore associated with an

extensive recording dataset and ample reference material is available for combined molecular and morphological studies.

Boletes are generally regarded as ectomycorrhizal (ECM) fungi, having an obligate association with roots of living trees and shrubs. Species of *Buchwaldoboletus*, however, are exceptional in feeding on dead wood and/or associating with other wood-inhabiting fungi. In Britain, the ECM boletes associate with oaks, beech, birches or pine and, to a lesser degree, with sweet chestnut, lime, hornbeam, cedars, poplars, willows, rockrose and bearberry. Different species often fruit in close proximity and many of the rarities are found in relatively open and warm wooded sites (thermophilous boletes) such as avenues, rides, parkland, wood pasture or former wood pasture where they can be site-faithful over many decades. Some boletes are relatively common whereas others have only ever been found at a few sites and so the family was therefore thought likely to provide a good representative sample of basidiomycete fungi for a pilot study.

Identification of British boletes, and hence our understanding of their distribution and rarity, has traditionally relied on field and microscopic characters of their fruit bodies. Basidiomycete taxonomy is currently being revolutionised by modern molecular (DNA barcoding) approaches. This has already had profound effects on bolete names. New segregate genera have been erected, although not all have reached universal acceptance, such as *Hemileccinum, Xerocomus* and *Xerocomellus* (Šutara 2008), and several new species have recently been described including two, *Xerocomus chrysonemus* and *X. silwoodensis*, discovered in southern England (Taylor *et al.* 2006, 2007). European members of *Leccinum* peaked at 39 species (Korhonen 1995, Lannoy & Estadès 1995) before falling back to a mere 14 (Kibby 2006, 2011) following the molecular studies of den Bakker & Noordeloos (2005). Nevertheless, some authors continue to uphold the recognition of some currently "molecularly invisible" but morphologically-defined species concepts based on suites of correlated fruit body and ecological characters.

Although *Boletus* section *Boletus*, referring to *B. edulis* and its close allies, has been investigated using molecular techniques (Dentinger *et al.* 2010), other groups of red and yellow-pored *Boletus* have apparently received less attention. A series of combined morphological and molecular studies was therefore carried out, initiated in 2011, to investigate the taxonomy and identification of a prioritised subset of such bolete taxa in Britain. The objective was to provide some DNA sequence-based verification of the fruit body record data upon which all fungal conservation assessments ultimately depend. The questions to be addressed by the sequencing studies ranged from "is taxon X really British?" through "is what we call taxon X in Britain just a colour form of taxon Y?" to "has taxon A ever been collected at site B?"

Analysis and interpretation of recording data and the conservation assessments were carried out by one of us (JHS) with assistance from co-authors following IUCN guidelines, categories and criteria (IUCN 2012a, b, 2013). This publication is based on a project report submitted to Natural England in March 2013 and augmented by the results of DNA sequencing studies carried out at Cardiff University and RBGK during the period 2011–2013.

## 3. Taxonomic scope and nomenclature

The subject of this conservation assessment is the family *Boletaceae* sensu stricto and hence it excludes the following bolete genera which are now accommodated in other families: *Gyrodon, Gyroporus, Paxillus* and *Suillus*. A full list of the genera under consideration can be found by traversing the taxonomic hierarchy of The Checklist of Fungi of the British Isles (<u>GBCHKLST</u>).

A typical bolete fruit body comprises a fleshy cap with an underlying spongy layer in place of gills. Genera with such fruit bodies included in this assessment are: *Aureoboletus, Boletus, Buchwaldoboletus, Chalciporus, Leccinum, Porphyrellus, Pseudoboletus, Rubinoboletus, Strobilomyces, Tylopilus* and *Xerocomus*. The assessment also includes *Phylloporus*, with thick gills below the cap, and the rarely-seen *Octaviania* and *Wakefieldia*, which produce subterranean truffle-like fruit bodies.

Within these genera, the assessed taxa are those that are currently included in <u>CBIB</u> named according to the nomenclature used therein. There are 66 currently accepted species included in <u>CBIB</u> that have been recorded in Great Britain and are within the scope of the current assessment. At varietal level, there are currently two relatively rare boletes accepted as distinct taxa in <u>CBIB</u>, *Boletus luridiformis* var. *discolor* and *B. luridus* var. *rubriceps*, and these were also assessed.

Other names not meeting the above criteria can be found in the national databases but are excluded. These are mostly names which are of uncertain application or synonyms and include: *Boletus auriporus*, *B. badiorufus*, *B. betulicola*, *B. candicans*, *B. carnosus*, *B. citrinus*, *B. crassus*, *B. cruentus*, *B. discoideus*, *B. fagineus*, *B. flavus*, *B. fuligineus*, *B. gregarius*, *B. impuber*, *B. inunctus*, *B. irregularis*, *B. junquilleus*, *B. labyrinthiformis*, *B. laciniatus*, *B. leoninus*, *B. lignatilis*, *B. macweeneyi*, *B. niveus*, *B. obtusus*, *B. paludosus*, *B. pascuus*, *B. persoonii*, *B. polyporus*, *B. procerus*, *B. proliferus*, *B. proteus*, *B. punctatus*, *B. purpureus*, *B. semicircularis*, *B. subfuscus*, *B. subsquamosus*, *B. tenax*, *B. trilobatus*, *B. ungulatus*, *B. vaccinus*, *B. variicolor*, *Buchwaldoboletus hemichrysus*, *Leccinum aerugineum*, *L. ambiguum*, *L. croceostipitatum*, *L. decipiens*, *L. fuscoalbum*, *L. molle*, *L. murinaceum*, *L. nigellum*, *L. oxydabile*, *L. pulchrum*, *L. thallassinum*, *L. umbrinoides*, *L. umbrinum*, *Phylloporus rhodoxanthus*, *Xerocomus erubescens* and *X. quercinum*.

A few database names refer to taxa that are not (yet) accepted in <u>CBIB</u>. For example, the national fungus collection (fungarium) at RBGK currently holds collections made in 1998 and 2011 from Kent filed as *Boletus caucasicus*. At the time of this report, this species had not been considered for inclusion in <u>CBIB</u>, pending molecular analysis, and therefore it was not assessed herein.

# 4. Data preparation

The assessment analysed national fungal database records up to and including December 2011 drawn from FRDBI managed by the British Mycological Society (BMS) and CATE2 managed by the Association of British Fungus Groups (ABFG). It is likely that significant numbers of records made after this date have not yet been incorporated in CATE2 or FRDBI, hence they were excluded. CATE2 records were cleaned by a management team (including standardisation of terminology, removal of replicates and correction of place names and grid references) and, after cleaning and incorporation of additional data from FRDBI, there are currently ca. 40,000 *Boletaceae* records held in CATE2 (Jordan 2013).

These data were incorporated (by JHS) in evidence tables containing recording dates, site details such as locality names and georeferences, and, wherever possible, fruit body abundance data (to assess numbers of fruiting patches). Site georeferences in <u>CATE2</u> and <u>FRDBI</u> are in the form of Ordnance Survey (OS) grid references which are now captured more frequently than hitherto and increasingly derived from GPS data. When database entries lacked recorder-generated georeferencing, grid references were sometimes generated from site names to provide a "best estimate" of site coordinates. This often proved misleading in the current analysis, as large sites could be reduced to six-figure centroid grid references which may or may not place the record in the correct tetrad (OS grid squares of 4 km<sup>2</sup> area represented by two letters and four digits). To try to address this, grid references and site boundaries associated with site names were checked against various online resources such as herbariaunited and <u>MAGIC/Nature on the Map</u>. For records associated with dried fruit body material (voucher specimens), the whereabouts of the specimens were also recorded to assist with sample selection for molecular analysis.

# 5. Methods: rationale

The aim of red-listing is to categorise taxa to show which are at greatest risk of extinction and to provide an assessment of the relative degrees of threat they face. RDLs can then be used to prioritise conservation action to improve the chances of survival for at least some of the most threatened taxa in the long term. Taxa assigned to three of the IUCN Red List categories Critically Endangered (CR), Endangered (EN) and Vulnerable (VU) are regarded as Threatened.

The IUCN criteria represent the accepted method of producing Red Lists, both globally and regionally (IUCN 2012a, b, 2013). Five criteria are used to assign a taxon to the appropriate IUCN Red List category:

Criterion A: Population size reduction

Criterion B: Geographic range in the form of extent of occurrence (EOO) or area of occupancy (AOO) coupled with other factors including fragmentation, decline and extreme fluctuations. Criterion C: Small population size and decline.

Criterion D: Very small or restricted population.

Criterion E: Quantitative analysis, indicating the probability of extinction in the wild.

Boletes, like almost all non-lichenised macrofungi, are organisms that remain hidden for most of their lives and only briefly reveal their whereabouts when they emerge to fruit. Although it is the fruiting stage that forms the basis of all our historic records, each individual fungus generally exists as a network (mycelium) of microscopic tubes (hyphae) both within and sometimes

connecting between its food sources. Mycorrhizal boletes are spatially constrained insofar as their mycelia are tethered to living roots of their plant partners (usually trees) where nutrient exchanges, vital to the health of both symbionts, occur. Bolete mycelium is difficult to culture and manipulate in the laboratory and the underground detection and enumeration of non-fruiting bolete genotypes in natural environments is a relatively new line of research largely driven by innovations in molecular ecology. Consequently, insufficient data have accumulated for use in population modelling methods such as population viability analysis and so there is currently no possibility of using Criterion E for fungi.

The use of Criteria A–C requires an assessment of population decline. Changes in the distribution of fungal fruiting (mapped grid references of records) over time initially seemed to offer the most plausible route for the investigation of possible population decline in fungi. Consequently, a preliminary investigation was carried out to compare the British bolete fruiting populations recorded over two time intervals.

A similar analysis was carried out by Evans *et al.* (2006). For this, the number of pre- and post-1960 occupied hectads (OS grid squares of 100 km<sup>2</sup> area represented by two letters and two digits) was recorded for each taxon assessed. "For a species recorded in **n** hectads since 1960 there is considered to be evidence of decline if it was recorded in at least 2n + 1 hectads prior to 1960" (Evans *et al.* 2006). Of the boletes, only the truffle-like *Octaviania* showed any decline using this method. This result is most likely due to the activities of a single specialist, L.E. Hawker, in the 1950s generating a burst of collections followed by a more recent cessation of organised 'truffle forays' due to environmental concerns about excavation damage.

The selection of 1960 as the cut-off date was linked to the Evans *et al.* (2006) interpretation of Extinction. A British species was assessed as Extinct if there were no records post-1960. Extinction in the current assessment was viewed slightly differently. The open-ended "post-1960" timeframe was replaced by a rolling value of "not found over the last 50 years despite appropriate searching". Based on this interpretation, all mycelia whose fruit bodies have been recorded at any time within the last 50 years should therefore be regarded as extant at the time of assessment (unless their habitat has been destroyed). For each species considered in the preliminary investigation for the current assessment, the chosen proxy measure of its extant GB population was the total number of occupied 1 km OS grid squares (monads) recorded over the last 50 years. This was compared with a corresponding measure of the extant population as it existed 50 years ago and the results are shown in Table 1.

As with the previous hectad-based comparisons (Evans *et al.* 2006), a monad-based comparison of records between two 50-year recording periods (Jan. 1913–Dec. 1962 and Jan. 1963–Dec. 2012) simply highlighted the recent upsurge in recording effort and database usage.

50-year periods (san. 1715–Dec. 1702 and san. 1705–Dec. 2012) in national databases.							
Species	Jan 1913- Dec 1962	Jan 1963- Dec 2012	Species	Jan 1913- Dec 1962	Jan1963 - Dec 2012		
Aureoboletus gentilis	2	95	Boletus ripariellus	0	23		
Boletus aereus	0	95	Boletus rubellus	22	200+		
Boletus appendiculatus	8	200+	Boletus satanas	14	49		
Boletus armeniacus	0	17	Boletus subappendiculatus	0	5		
Boletus badius	75	200+	Boletus subtomentosus	90	200+		
Boletus bubalinus	0	3	Boletus torosus	0	5		
Boletus calopus	15	190	Boletus xanthocyaneus	0	5		
Boletus chrysenteron	120	200+	Buchwaldoboletus lignicola	0	54		
Boletus cisalpinus	0	200+	Buch. sphaerocephalus	2	6		
Boletus declivitatum	0	70	Chalciporus piperatus	50	200+		
Boletus depilatus	0	1	Leccinum albostipitatum	0	6		
Boletus edulis	200+	200+	Leccinum aurantiacum	2	200+		
Boletus fechtneri	0	12	Leccinum crocipodium	3	128		
Boletus ferrugineus	1	140	Leccinum cyaneobasileucum	0	13		
Boletus fragrans	2	11	Leccinum duriusculum	10	100		
Boletus immutatus	0	5	Leccinum holopus	12	200+		
Boletus impolitus	7	145	Leccinum melaneum	0	62		
Boletus legaliae	0	52	Leccinum pseudoscabrum	0	135		
Boletus luridiformis	42	200+	Leccinum scabrum	200+	200+		
Boletus luridus	45	200+	Leccinum schistophilum	0	20		
Boletus moravicus	2	63	Leccinum variicolor	0	200+		
Boletus pinophilus	26	101	Leccinum versipelle	46	200+		
Boletus porosporus	0	200+	Leccinum vulpinum	4	31		
Boletus pruinatus	10	200+	Octaviania asterosperma	7	6		
Boletus pseudoregius	0	14	Phylloporus pelletieri	5	64		
Boletus pseudosulphureus	0	13	Porphyrellus porphyrosporus	13	102		
Boletus pulverulentus	7	200+	Pseudoboletus parasiticus	11	200+		
Boletus queletii	2	200+	Rubinoboletus rubinus	4	45		
Boletus radicans	11	200+	Strobilomyces strobilaceus	22	98		
Boletus regius	0	0	Tylopilus felleus	23	200+		
Boletus reticulatus	19	200+	Wakefieldia macrospora	1	0		
Boletus rhodopurpureus	5	23	Xerocomus chrysonemus	0	20		
Boletus rhodoxanthus	0	0	Xerocomus silwoodensis	0	3		

**Table 1.** Numbers of occupied OS 1 km grid squares for *Boletaceae* species recorded over two 50-year periods (Jan. 1913–Dec. 1962 and Jan. 1963–Dec. 2012) in national databases.

Indeed, a greater number of unique site and date records for many species have appeared in the national databases in the last 15 years than in all the previous 150 years put together. The only *Boletaceae* that were associated with declining records (at a 1 km grid square scale) were the two truffle-like species *Octaviania asterosperma* and *Wakefieldia macrospora* (Table 1, in bold) and this is likely to be due to the factors stated above regarding the earlier hectad assessments of Evans *et al.* (2006).

It is clear that recent increased recording activity has masked any decline in bolete fruiting populations. Indeed it has been speculated that those species showing only modest increases in records during recent years relative to those of an "average bolete" could be experiencing a decline in their actual populations. Comparative methods designed to reveal such species were considered and rejected because the available databases do not hold null records (documentation of site visits resulting in no records of the target species). Regardless of time intervals adopted,

an absence of records in the second recording period means that we simply cannot tell from the available data whether a bolete fruiting population recorded during the first period is now no longer alive or whether it has simply not been adequately surveyed recently. In the absence of any documented losses of bolete sites, the current assessment concluded that existing fruiting record data could not be used to provide evidence of population decline.

Habitat-based assessments of decline can also be used when applying Criteria A and B, but are severely hampered by a lack of spatial and temporal data regarding the microhabitats favoured by bolete species. For example, those species found fruiting under oak might have special requirements regarding site ecology and there might also be a relatively brief "window" of tree ages favouring mycorrhizal establishment and persistence. Therefore, it was not considered appropriate to infer oak-associated bolete decline based on any national decline in the area of oak-dominated habitat. The current project team reached similar conclusions to those reached by the compilers of "The Vascular Plant Red Data List for Great Britain" (Cheffings *et al.* 2005) when considering the conservation assessment of hybrid plants:

"Until more complete data are available, application of IUCN threat criteria is severely limited and criteria A, B & C cannot be used, relying as they do on evidence of decline. For the time being we can only apply criterion D, which assesses population size".

Initial considerations of population size included calculations of extent of occurrence (EOO) and area of occupancy (AOO) as defined in IUCN red-listing literature (IUCN 2012a, b, 2013). To the best of our knowledge, these have not previously been calculated for British fungi. The geospatial conservation assessment tool GeoCAT, developed by Bachman et al. (2011) and endorsed by the IUCN, was used to generate and display the required data. To upload data into GeoCAT, the bolete site OS grid references were converted to latitude and longitude coordinates using OS Grid Inquest. This software calculates AOO in multiples of tetrads (in  $\text{km}^2$ ) thus ensuring the scale is appropriate for use with IUCN criteria. However, it should be noted that this is based on a moveable grid and is therefore unlike that historically used for botanical recording. The latter is sometimes referred to as the "DINTY" system and comprises lettercoded tetrads denoting subdivisions of fixed OS grid hectads. Hence GeoCAT can assign fruit body patches that are, for example, 1.5 km apart but located in neighbouring OS hectads to a single tetrad for AOO purposes. It should be noted in passing that this version of GeoCAT automatically generates a conservation assessment based solely on EOO and AOO values (on the assumption that at least two of the three sub-criteria necessary for using Criterion B are also fulfilled). However such an assessment cannot be valid unless the IUCN sub-criteria (a), (b) and (c) have been evaluated.

Analysis of records revealed that many boletes are so widespread that the area of their EOO polygons was above 20,000 km<sup>2</sup>, thereby exceeding the thresholds of all the IUCN threatened categories. By contrast, some AOOs seemed to offer promise for future fungal Red List assessments using Criterion B, but only if they could be coupled with further data appropriate to the sub-criteria. There were only two species with AOO values < 2,000 km<sup>2</sup> and < 11 locations (*B. fragrans* and *Buchw. sphaerocephalus*) but both had >5 locations which, provided a further sub-criterion was met, would assign them to a less threatened category (VU) than using Criterion D (see below). Sample <u>GeoCAT</u>-derived maps for *Strobilomyces strobilaceus* and *Boletus satanas* are shown in Fig. 1.



**Figure 1.** <u>GeoCAT</u>-derived maps for *Strobilomyces strobilaceus* (upper) and *Boletus satanas* (lower) showing bolete fruit body records (blue dots) derived from national databases (<u>CATE2</u> and <u>FRDBI</u>) used to calculate Extent of Occurrence (EOO), shown as shaded polygons, and Area of Occupancy (AOO). Note that the "traffic light" conservation assessments (LC based on EOO and EN based on AOO for both) are autofilled by <u>GeoCAT</u> applying IUCN Criterion B, but would only be valid if IUCN sub-criteria were also satisfied and so are invalid for these boletes.

Having considered the above approaches and being unable to detect any relevant extreme fluctuations or credible decline in populations, EOO, AOO, habitat-based values, number of locations or subpopulations, the assessment shifted to the application of Criterion D, which is concerned with very small or restricted populations and assessments based on estimates of mature individuals.

# 6. Methods: application of IUCN Criterion D

Although limited above-ground (e.g. Dahlberg 1997) and below-ground (e.g. Zhou *et al.* 2001) mapping of genetically-defined mycelial individuals (genets) has been carried out for several conifer-associated members of the *Suillaceae*, this information is lacking for most boletes. Each genet consists of one or more spatially discrete but clonally related mycelia known as ramets. A similar dearth of information accompanies any estimate of the extent and number of bolete ramets per genet. Ramets are especially important because they equate to the IUCN's preferred "mature individuals" for red-listing purposes. In this context it should be borne in mind that the numbers of ramets per genet are liable to temporal fluctuations. Their number is reduced when genetically identical mycelia meet, fuse and unite in the soil and, conversely, it is increased when mycelia become fragmented.

Dahlberg & Mueller (2011) reviewed the literature regarding genet size in terrestrial macrofungi and concluded that, for those species lacking fairy rings or specialised bundles of exploratory hyphae (rhizomorphs or mycelial cord systems), each genet was generally less than 10 m in diameter. Furthermore, they proposed that each genet comprised "(2–)10 mature individuals (ramets) depending on the distribution of sporocarps [fruit bodies]". By way of explanation, Dahlberg & Mueller (2011) considered that isolated solitary fruit bodies "should be counted as two mature individuals" whereas for those species producing "scattered, sparse to gregarious" fruit bodies, the recommendation was that conspecific fruit bodies separated by up to 10 m (one genet) should be counted as 10 mature individuals (ramets) for red-listing purposes. Dahlberg & Mueller's (2011) pragmatic approach, incorporating what they regarded as conservative assumptions, was incorporated in the IUCN (2013) guidelines for "diffuse organisms": "For diffuse organisms, not wholly visible, in continuous habitats (e.g. subterranean mycelial fungi) assessors may assume that each recorded presence separated by a minimum distance represents an assumed number of individuals. For example, each visible fruiting body may be assumed to represent 10 mature individuals, so long as they are separated by at least 10 metres. This kind of assumption is necessary because the size or area of a fungal mycelium is rarely known".

Boletes produce "scattered, sparse to gregarious" fruit bodies. Therefore, following the IUCN (2013) guidelines would mean that, for example, if one bolete fruit body was found under an oak with a group of four conspecific fruit bodies occurring 12 m away and a further pair growing 10 m further from those, the population would be conservatively assumed to consist of 30 mature individuals (ramets) for red-listing purposes regardless of the number of other conspecific fruiting patches occurring directly in between.

Even in the 21<sup>st</sup> century, fungal records are almost always casually generated and are usually made in an attempt to boost the numbers of taxa recorded within a particular geographical boundary such as a site or county. The resulting records often omit any documentation of fruiting abundance, which hampers the assessment of numbers and spacing of discrete fruiting patches. Where bolete fruit body information was available in the national databases, it usually

specified a patch or cluster of 1–5 fruit bodies associated with a single record (possibly the reproductive output of an individual ramet). For these cases and when no abundance details were available, following the above IUCN guidelines, it was assumed that each record constituted 10 mature individuals for red-listing purposes. Fruiting patches larger than this are much more likely to be commented upon by recorders and in such cases their data were used to calculate the number of estimated ramets associated with the record.

Criterion D applies to extant populations and therefore, following the rationale outlined in Section 4, estimates of extant mature individuals (ramets) for the 2013 assessment were obtained from compilations of the last 50 years' recording data for each bolete taxon considered. Applying Criterion D, the following thresholds were used to assign taxa to the three threatened categories:

CR D: <5 discrete fruiting patches representing <50 mature individuals EN D: 5–24 discrete fruiting patches representing 50–240 (<250) mature individuals VU D1: 25–99 discrete fruiting patches representing 250–990 (<1,000) mature individuals

IUCN defines a taxon as Near Threatened (NT) when it does not qualify for threatened status, but is close to qualifying or is likely to qualify in the near future. Therefore we assumed: NT: 100–110 discrete fruiting patches representing 1,000–1,100 mature individuals and hence the category of Least Concern (LC) is defined thus:

LC: >110 discrete fruiting patches representing >1,100 mature individuals (Criterion D2 not met).

# 7. Methods: Data Deficient (DD) and Not Evaluated (NE)

Several bolete taxa, mainly in *Boletus* and *Leccinum*, were assigned to the Data Deficient (DD) category. These include recently described species and those that have only been included in the checklist (<u>CBIB</u>) since 1995 but have less than 110 discrete fruit patches recorded (<1,100 mature individuals) and so do not currently qualify for LC.

*B. armeniacus* was assessed as DD because the species concept was only recently clarified with regard to British collections. Hills & Kibby (2005) noted that it has been the subject of 121 years of confusion. It remains a relatively poorly understood taxon and is likely to have been historically overlooked and misidentified in Britain, hence the records are expected to be particularly unreliable. It was therefore assessed as DD due to taxonomic uncertainty regarding British records and insufficient information being available to place it in another category.

In accordance with IUCN (2013) guidelines, DD has also been used for taxa where there are few known sites and the taxonomic concept, at least as understood in Britain, probably represents aberrant forms, mutations or a rare colour morph of another species. This has been employed for example with *B. immutatus* which, when originally described from Windsor Great Park, Berkshire, was merely recognised as a variety of *B. luridiformis*. *B. luridiformis* var. *immutatus* was distinguished from *B. luridiformis* var. *luridiformis* by having yellow flesh that failed to turn blue when cut or bruised (Pegler & Hills 1996). However, subsequent collections from the original site have occasionally shown localised blueing of the cut flesh and further DNA sequencing work is required to resolve its correct taxonomic placement.

Similarly, it was long-suspected that British *B. xanthocyaneus* might represent a yellow colour morph of *B. rhodopurpureus*. Four British collections named as *B. xanthocyaneus* were selected for molecular analysis and yielded DNA barcode sequences which unequivocally clustered with those of *B. rhodopurpureus*. There is, therefore, no molecular support for recognising a distinct yellow-fruited *B. xanthocyaneus* in Britain and all records of *B. xanthocyaneus* were considered to be of *B. rhodopurpureus* for assessment purposes. The taxon *B. xanthocyaneus* was, therefore, not evaluated (NE) and the next checklist (CBIB) update will include it as a synonym of *B. rhodopurpureus* rather than as a species in its own right. DNA sequences of continental European specimens named as *B. xanthocyaneus* should be compared with those obtained from British material to investigate whether a distinct taxon exists elsewhere.

Whilst there is a degree of uncertainty as to whether a distinct *B. xanthocyaneus* exists somewhere in Europe, this is not the case for *B. regius*. In Britain this is a legally protected species (Schedule 8, Wildlife and Countryside Act) for which good European DNA sequence data exist. However, DNA sequences obtained from English material named as this failed to match those of authentic *B. regius* (see species accounts below) and so this taxon was assigned to the NE category. The remaining bolete in the NE category is *B. rhodoxanthus*, currently included in <u>CBIB</u> on the strength of a single verified collection from Northern Ireland and so it is beyond the scope of this assessment.

## 8. Results: summary of conservation assessments

The results of the 2013 assessments are shown in Table 2 alongside the two previous RDL assessments (Ing 1992, Evans *et al.* 2006). Table 2 categorises all taxa not listed in the first RDL as NE, but this could be an overestimate because Ing (1992) did not document any taxa that were assessed as LC or DD. Almost the same caveat applies to the NE category with respect to the second RDL. For this, however, the names of those taxa regarded as LC by Evans *et al.* (2006) were recovered from unpublished RDL documents and these assessments are shown in Table 2.

Of the 68 accepted GB *Boletaceae* taxa (66 species and two varieties) assessed, 37 (54%) are now considered to be on the Red List (CR, EN, VU, NT, DD) with the following breakdown by category:

EX	00	00%	NT	06	09%
CR	00	00%	LC	28	41%
EN	05	07%	DD	18	27%
VU	08	12%	NE	03	04%
			Total	68	100%

Red-listed bolete taxa by category:

EN: Boletus fechtneri, Boletus fragrans, Boletus pseudoregius, Boletus pseudosulphureus, Buchwaldoboletus sphaerocephalus.

VU: Boletus legaliae, Boletus moravicus, Boletus rhodopurpureus, Boletus satanas, Buchwaldoboletus lignicola, Leccinum vulpinum, Phylloporus pelletieri, Rubinoboletus rubinus.

NT: Aureoboletus gentilis, Boletus aereus, Boletus pinophilus, Leccinum duriusculum, Porphyrellus porphyrosporus, Strobilomyces strobilaceus.

DD: Boletus armeniacus, Boletus bubalinus, Boletus declivitatum, Boletus depilatus, Boletus immutatus, Boletus luridiformis var. discolor, Boletus luridus var. rubriceps, Boletus ripariellus, Boletus subappendiculatus, Boletus torosus, Leccinum albostipitatum, Leccinum cyaneobasileucum, Leccinum melaneum, Leccinum schistophilum, Octaviania asterosperma, Wakefieldia macrospora, Xerocomus chrysonemus, Xerocomus silwoodensis.

Taxon	Ing (1992) RDL	Evans <i>et al.</i> (2006) RDL	2013 assessment	Estimated post-1962 mature individuals	Notes on 2013 assessment
Aureoboletus gentilis	NE	NE	NT	1020	
Boletus aereus	NE	NE	NT	1080	
Boletus appendiculatus	NE	NE	LC	2000+	From Table 1
Boletus armeniacus	NE	NE	DD		See Section 6
Boletus badius	NE	NE	LC	2000+	From Table 1
Boletus bubalinus	NE	NE	DD		
Boletus calopus	NE	NE	LC	1900	From Table 1
Boletus chrysenteron	NE	NE	LC	2000+	From Table 1
Boletus cisalpinus	NE	NE	LC	2000+	From Table 1
Boletus declivitatum	NE	NE	DD		
Boletus depilatus	NE	NE	DD		
Boletus edulis	NE	NE	LC	2000+	From Table 1
Boletus fechtneri	NE	NT	EN D	120	
Boletus ferrugineus	NE	LC	LC	1400	From Table 1
Boletus fragrans	NE	NT	EN D	110	
Boletus immutatus	NE	VU D2	DD		See Section 6
Boletus impolitus	NE	NE	LC	1450	From Table 1
Boletus legaliae	NE	LC	VU D1	590	
Boletus luridiformis	NE	NE	LC	2000+	From Table 1
Boletus luridiformis var. discolor	NE <sup>1</sup>	NT	DD		
Boletus luridus	NE	NE	LC	2000+	From Table 1
Boletus luridus var. rubriceps	NE	NE	DD		
Boletus moravicus	VU	LC	VU D1	630	
Boletus pinophilus	NE	NE	NT	1080	
Boletus porosporus	NE	NE	LC	2000+	From Table 1
Boletus pruinatus	NE	NE	LC	2000+	From Table 1
Boletus pseudoregius	$NE^2$	NT	EN D	140	
Boletus pseudosulphureus	NE <sup>1</sup>	NT	EN D	130	
Boletus pulverulentus	NE	NE	LC	2000+	From Table 1
Boletus queletii	NE	NE	LC	2000+	From Table 1
Boletus radicans	NE	NE	LC	2000+	From Table 1
Boletus regius	$E^2$	CR B	NE	0	Not in GB
Boletus reticulatus	NE	NE	LC	2000+	From Table 1
Boletus rhodopurpureus	VU <sup>3</sup>	EN B	VU D1	400	Incl. B. xanthocyaneus
Boletus rhodoxanthus	EX	NE	NE	0	In UK but not GB
Boletus ripariellus	NE	NE	DD		

**Table 2.** Conservation assessments of 68 British *Boletaceae* (all 2013 assessments based on IUCN Criterion D)

Boletus rubellus	NE	NE	LC	2000+	From Table 1
Boletus satanas	$R^4$	LC	VU D1	540	
Boletus subappendiculatus	NE	NE	DD		
Boletus subtomentosus	NE	NE	LC	2000+	From Table 1
Boletus torosus	NE	VU D2	DD		
Boletus xanthocyaneus	NE	DD	NE	0	Not distinct in GB
Buchwaldoboletus lignicola	VU	LC	VU D1	540	
Buchwaldoboletus	E <sup>5</sup>	VU D2	EN D	60	
sphaerocephalus					
Chalciporus piperatus	NE	NE	LC	2000+	From Table 1
Leccinum albostipitatum	NE	NE	DD		
Leccinum aurantiacum	NE	NE	LC	2000+	From Table 1
Leccinum crocipodium	NE	NE	LC	1280	From Table 1
Leccinum cyaneobasileucum	NE	LC	DD		
Leccinum duriusculum	NE	NE	NT	1100	
Leccinum holopus	NE	NE	LC	2000+	From Table 1
Leccinum melaneum	NE	NE	DD		
Leccinum pseudoscabrum	NE	NE	LC	1350	From Table 1
Leccinum scabrum	NE	NE	LC	2000+	From Table 1
Leccinum schistophilum	NE	NE	DD		
Leccinum variicolor	NE	NE	LC	2000+	From Table 1
Leccinum versipelle	NE	NE	LC	2000+	From Table 1
Leccinum vulpinum	VU	DD	VU D1	300+	
Octaviania asterosperma	NE	DD	DD		
Phylloporus pelletieri	NE	LC	VU D1	640	
Porphyrellus porphyrosporus	NE	NE	NT	1020	
Pseudoboletus parasiticus	NE	NE	LC	2000+	From Table 1
Rubinoboletus rubinus	VU <sup>6</sup>	LC	VU D1	450	
Strobilomyces strobilaceus	VU	LC	NT	1020	
Tylopilus felleus	NE	NE	LC	2000+	From Table 1
Wakefieldia macrospora	NE	DD	DD		
Xerocomus chrysonemus	NE	NE	DD		
Xerocomus silwoodensis	NE	NE	DD		

<sup>1</sup> *B. junquilleus* was assessed as VU in 1992 but the underlying records are difficult to interpret and probably comprise *B. pseudosulphureus* and *B. luridiformis* var. *discolor*.

 $^{2}$  *B. regius* was assessed as E (Endangered), an IUCN Criterion in 1992, but it is likely that the underlying records comprise misdetermined *B. pseudoregius* and yellow-pored *B. legaliae*.

 ${}^{3}B.$  purpureus was assessed as VU in 1992. Historic records named thus are difficult to interpret but are likely to be mainly *B. rhodopurpureus*.

<sup>4</sup> B. satanas was assessed as R (Rare), an IUCN Criterion in 1992.

<sup>5</sup> Buchwaldoboletus sphaerocephalus was assessed as E (Endangered), an IUCN Criterion in 1992, as Buchwaldoboletus hemichrysus.

<sup>6</sup> *Rubinoboletus rubinus* was assessed in 1992 as *Chalciporus rubinus*.

# 9. Results: assessments for RDL and NE taxa (omitting LC)

## Aureoboletus gentilis (Quél.) Pouzar

Previous assessment: NE

2013 assessment: NT

Mature individuals: 1020

Estimated population: 1–10 fruit bodies recorded at each of 94 unique georeferenced sites (940 mature individuals) plus 30+ fruit bodies recorded at one site (assumed to span 30 m and therefore to represent 30 mature individuals), 10+ fruit bodies at another site (20 mature individuals) and 3 "sites" noted at one further grid ref (30 mature individuals). A very small population (Criterion D) assessed as NT.

A thermophilous bolete mainly fruiting in older woodlands of central and southern England with *Quercus* and to a lesser extent, *Fagus, Castanea, Corylus, Pinus* and *Picea*. One of a group of species found in bolete 'hotspots' with a stronghold in the New Forest, Hampshire.

## Boletus aereus Bull.

Previous assessment: NE

2013 assessment: NT

Mature individuals: 1080

Estimated population: 1–10 fruit bodies recorded at each of 92 unique georeferenced sites (920 mature individuals) plus 10–15 fruit bodies (or in "quantity") recorded at eight further sites (assumed to represent 160 mature individuals). A very small population (Criterion D) assessed as NT.

A thermophilous bolete mainly fruiting in older woodlands of central and southern England with *Quercus*, and to a lesser degree *Fagus*, *Tilia* and *Castanea*. One of a group of species found in bolete 'hotspots' with a stronghold in the New Forest, Hampshire, and on the Crown Estate, Windsor, Berkshire.

## Boletus armeniacus Quél.

Previous assessment: NE 2013 assessment: DD Mature individuals: 170+ (Table 1)

Population cannot be estimated due to historic confusion (mainly with *B. declivitatum* and *B. rubellus*), relatively recent clarification of the species concept with regard to British collections (Hills & Kibby 2005, Hills 2008) and lack of confirmed records (see Section 6). This species is usually associated with *Quercus* and *Fagus* in parkland and woodland and is also known as *Xerocomus armeniacus* or *Xerocomellus armeniacus*.

## Boletus bubalinus Oolbekk. & Duin

Previous assessment: NE

2013 assessment: DD

Mature individuals: 30+ (Table 1)

Population cannot be estimated because the species was originally described in 1991 and only recently recognised in GB following a collection made in Berkshire in 2007 (Hills 2008), hence there has been insufficient recording time. Predicted to be quite common and widespread and likely to have been misidentified as *B. chrysenteron* or *B. declivitatum* in the past (Hills 2008, Burnham & Kibby 2011, Kibby 2011).

A species apparently associated with open parkland, garden or street-side trees including *Populus, Tilia* or *Carpinus*.

#### Boletus declivitatum (C. Martin) Watling

Previous assessment: NE

2013 assessment: DD

Mature individuals: 700+ (Table 1)

Population cannot be estimated because the species was only recently recognised in GB (1995), hence there has been insufficient recording time. Predicted to be quite common and widespread and likely to have been misidentified as *B. chrysenteron*, *B. rubellus* or *B. armeniacus* in the past.

A relatively early-fruiting bolete associated with *Quercus* (and to a lesser extent, *Tilia, Fagus* and *Castanea*) in a variety of open woodland and parkland sites. It is also known as *Xerocomus* communis or *Xerocomellus engelii* (Hills 2008, Kibby 2008, 2011).

#### Boletus depilatus Redeuilh

Previous assessment: NE 2013 assessment: DD Mature individuals: 10+ (Table 1) Population cannot be estimated because the species was only recently recognised in GB (2008), associated with *Carpinus* in Kent (Kibby & Burnham 2008), hence there has been insufficient recording time.

### Boletus fechtneri Velen.

Previous assessment: NT (2006)

2013 assessment: EN D

Mature individuals: 120

Estimated population: fewer than 5 fruit bodies recorded at each of 12 unique georeferenced sites (120 mature individuals). A very small population (Criterion D) assessed as EN D. A thermophilous bolete, mainly found in central and southern England associated with *Fagus* and *Quercus*, apparently with single trees on each site. Four out of five British specimens that were sequenced from the collections preserved at Kew (and currently entered as *B. fechtneri* on national databases) were found to represent other species (*B. calopus*, *B. pseudoregius* and *B. radicans*). Therefore, although now confirmed as occurring in GB (Wye Valley), it seems likely that this is one of the most poorly known of all the British boletes and all future sightings should be supported by voucher material suitable for sequence-based confirmation. If all the remaining voucher collections in Kew were sequenced and found to be misdetermined, it is possible that the estimated population could fall below the threshold for EN D and satisfy CR D, but such an assessment is not justified on the evidence currently available.

## Boletus fragrans Vittad.

Previous assessment: NT (2006) 2013 assessment: EN D Mature individuals: 110 Estimated population: fewer than 5 fruit bodies recorded at each of 11 unique georeferenced sites (110 mature individuals). A very small population (Criterion D) assessed as EN D. A thermophilous bolete, mainly found in central and southern England associated with *Quercus*, apparently with single trees on each site.

### Boletus immutatus (Pegler & A.E. Hills) A.E. Hills & Watling

Previous assessment: VU D2 (2006)

2013 assessment: DD

Mature individuals: 50+ (Table 1)

Estimated population: three unique georeferenced sites (30 mature individuals) plus around 30 fruit bodies a year in two distinct patches (separated by 10 m) at the original site (20 mature individuals). Although a very small population of 50 mature individuals is estimated, the application of Criterion D does not seem justified in view of the ongoing taxonomic uncertainty (see below).

Originally described from Windsor Great Park, Berkshire, its most reliable fruiting site, this bolete has a very long fruiting period with several flushes at the same site occurring from June to November. It is associated with Fagus and Quercus and with just a few trees at each site. Its taxonomic status has been repeatedly questioned since its description as a variety of the common B. luridiformis (Pegler & Hills 1996) from which it is distinguished by having fruit body flesh that fails to turn blue when cut or bruised. Any specimens of B. luridiformis with weak bruising reactions could be assigned to this taxon, indeed it has been suggested that the concept consists entirely of such forms. Nevertheless, B. immutatus was elevated to specific rank eight years later (Watling 2004). However, more recent collections from Windsor have on occasion shown some patchy blueing response, and Kibby (2011, 2013) regarded it as a colour form of B. luridiformis (=B. erythropus) citing unpublished DNA analysis. While our own limited molecular studies do not currently support recognition of non-blueing B. luridiformis as a distinct taxon, further DNA sequencing work is required to resolve the detailed structure of the B. luridiformis complex. Since there is a strong likelihood that this taxon does represent a series of aberrant forms or mutations of another species, it has been assessed as DD in accordance with IUCN (2013) guidelines. Interestingly, a similarly non-blueing variant of B. pulverulentus has recently been found in Herefordshire (Kibby 2013) whose DNA barcode sequence matched that of more typical (strongly blueing) B. pulverulentus.

#### Boletus legaliae Pilát

Previous assessment: LC (2006) 2013 assessment: VU D1 Mature individuals: 590

Estimated population: usually 1-10 fruit bodies recorded at each of 47 unique georeferenced sites (470 mature individuals) plus two further sites with two and three distinct fruiting areas noted (assumed to represent 50 mature individuals), one with 12–20 fruit bodies (20 mature individuals), one with 24 fruit bodies (20 mature individuals) and one with 30 fruit bodies (30 mature individuals). A very small population (Criterion D) assessed as VU D1. A thermophilous bolete, mainly found in central and southern England, associated with Quercus and, to a lesser extent, with Fagus, Castanea and Carpinus in open woodland, wood pasture or roadside situations. It is often found in bolete 'hot spots' such as parts of the New Forest and the Crown Estate, Windsor, Berkshire. DNA sequencing revealed that a confusing form with almost entirely yellow pores occurs in two areas in Windsor Great Park, sometimes fruiting close to normal orange- or red-pored specimens. The collector's notes revealed that such aberrant specimens had pores with faint orange tints, the intensity of which increased in the zone immediately surrounding the stipe apex. Nevertheless, the predominantly yellow pore colour resulted in specimens being misdetermined as B. pseudoregius for several years. Six such collections, from both Windsor sites, were sequenced, including the material photographed in Marren (2000), and all sequences were found to cluster with B. legaliae. These specimens have now been redetermined as *B. legaliae* and, in view of the DNA analysis, all records of *B*.

*pseudoregius* on the Crown Estate at Windsor must now be regarded as doubtful and were disregarded in this assessment.

## Boletus luridiformis var. discolor (Quél.) Krieglst.

Previous assessment: NT (2006) 2013 assessment: DD

Mature individuals: 350+

Estimated population: the population cannot be estimated with any confidence due to confusion surrounding the taxonomic concept. This is a member of the *B. luridiformis* complex with remarkably varying degrees of yellow, rather than brown, on the cap and elsewhere on the fruit bodies. Specimens with only faint red pigments on pores and stipe can resemble the all-yellow *B. pseudosulphureus*, indeed these taxa might represent points on a continuum, and historically the name *B. junquilleus* has probably been applied to both. Preliminary DNA analysis suggests that a distinct yellowish taxon does exist but that there are also yellow forms of *B. luridiformis* with which it has undoubtedly been confused. The available data are, therefore, insufficient for a precise categorisation and so the assessment is currently DD.

## Boletus luridus var. rubriceps (Maire) Dermek

Previous assessment: NE 2013 assessment: DD Mature individuals: 20+

Estimated population: the population cannot be estimated because the species was only recently recognised in GB (1998), associated with *Fagus* in Berkshire and with *Quercus* in North-east Yorkshire, hence there has been insufficient recording time. DNA sequencing is required to investigate whether this is a colour morph of *B. luridus* or perhaps a distinct taxon worthy of recognition at specific rank.

## Boletus moravicus Vacek

Previous assessment: VU (1992 as *Xerocomus leonis*), LC (2006) 2013 assessment: VU D1 Mature individuals: 630 Estimated population: usually 1–10 fruit bodies recorded at each of 63 unique georeferenced sites (630 mature individuals). A very small population (Criterion D) assessed as VU D1. A thermophilous bolete, mainly found in central and southern England, associated with *Quercus* and, to a lesser extent, with *Fagus, Castanea* and mixtures of *Betula* and *Pinus*. It is often found in open conditions and in bolete 'hot spots' such as parts of the New Forest and the Crown Estate, Windsor, Berkshire.

## Boletus pinophilus Pilát & Dermek

Previous assessment: NE

2013 assessment: NT

Mature individuals: 1080

Estimated population: usually 1–5 fruit bodies recorded at each of 105 unique georeferenced sites (1050 mature individuals) plus one further site with three distinct fruiting areas noted (assumed to represent 30 mature individuals). A very small population (Criterion D) assessed as NT.

Favours *Pinus* in Scottish native and planted woodlands, but also occasionally found in English and Welsh pine plantations. Collections from English sweet chestnut coppices, apparently in the absence of nearby pines, require molecular work to check their taxonomic affinities.

### Boletus pseudoregius (Hubert) Estadès

Previous assessment: NT (2006)

2013 assessment: EN D

Mature individuals: 140

Estimated population: usually 1-10 fruit bodies recorded at each of 14 unique georeferenced sites (140 mature individuals). A very small population (Criterion D) assessed as EN D1. A thermophilous bolete apparently restricted to woodlands of central and southern England favouring calcareous sites with Quercus. It is found under single trees on most sites in open mature woodland or wood pasture, often in bolete 'hot spots' such as parts of the New Forest and Wye Valley. All the available British collections named as B. regius and their accompanying notes and photographs were examined by A.E. Hills and all except one were redetermined as B. pseudoregius (Kibby 1998). This was largely based on comparisons with authentic Italian material and a search for evidence of any blueing reaction seen when the flesh, especially the tube layer, was cut. The flesh of true *B*. regius is virtually unchanging whereas that of *B. pseudoregius*, particularly the tube layer and cap, gives an unequivocal blue reaction. DNA sequencing revealed that the fruit body morphology of *B. pseudoregius* can overlap with that of other species and so verification of future records is recommended. Sequencing of collections preserved at RBGK revealed that one from the New Forest named as B. fechtneri was misdetermined *B. pseudoregius*, whereas six collections from two areas of Windsor Great Park named as B. pseudoregius were yellow-pored variants of B. legaliae (see above). Based on this evidence, we do not accept that B. pseudoregius is extant on the Windsor Estate and Windsor records were excluded from the assessment of mature individuals for this species. There is a proposal that the earlier name *B. fuscoroseus* should be used for this taxon based on a 1912 description that includes similar, but not identical, spore dimensions (Assyov 2012, Mikšik 2012). However, it might be prudent to await further sequencing results before following this lead. Our DNA analysis confirmed that the American species B. speciosus is a related, but distinct, taxon.

#### Boletus pseudosulphureus Kallenb.

Previous assessment: VU (1992 as *Boletus junquilleus* but possibly including specimens of *B. luridiformis* var. *discolor*), NT (2006)

2013 assessment: EN D

Mature individuals: 130

Estimated population: usually 1–10 fruit bodies recorded at each of 13 unique georeferenced sites (130 mature individuals). A very small population (Criterion D) assessed as EN D1. A widespread bolete (from Cornwall almost to the Cairngorms) associated with various trees such as *Quercus*, *Fagus*, *Pinus* and *Picea* in open, mature woodland, parkland or wood pasture. It has been suggested that the entirely yellow fruit bodies of this species merely represent one end of a range of colour forms within the of *B. luridiformis* complex. Further DNA sequencing work is required to resolve the taxonomy of this critical group.

#### Boletus regius Krombh.

Previous assessment: E (1992), CR B (2006)

2013 assessment: NE

Mature individuals: 0

This species is one of the four non-lichenised fungi included on Schedule 8 of the Wildlife and Countryside Act and so is legally protected. A.E. Hills' study of British collections, records and associated data filed under *B. regius* resulted in all except one being reassigned to *B.* pseudoregius (Kibby 1998 and see above). Modern identification guides refer to the sole survivor as the "only one certain record" (Watling & Hills 2005) and the "only one authentic British record" (Buczacki et al. 2012). An outdoor photograph of the single British B. regius specimen, and likely to have been taken at or near its collection site at Ashurst, New Forest, Hampshire, by its finder M. Kratochvila in 1987, is included in Marren (1998). Kratochvila's notes state that specimen was found under oak (although there is evidence of pine and birch in the photo), the cap colour was "rose pink" and the "pores and flesh [were] unchanging when cut" (Marren 1998). A small fragment of the Ashurst collection is in the Kew fungarium and it has now been sequenced. This sequence failed to match those of several authentic continental European specimens of *B. regius* and of British and continental collections of *B. pseudoregius*. The true affinities of the New Forest specimen are currently being investigated, meanwhile B. regius is being considered for exclusion from the British list before being proposed for removal from Schedule 8 of the Wildlife and Countryside Act.

Given the history of this taxon, it is important that some measure of DNA-based quality assurance is carried out should it be proposed for inclusion on the British list in the future. An unconfirmed record of *B. regius* from Worcestershire in October 1996 has appeared relatively recently in the national databases but lacking any details of voucher material. Until an authentic British *B. regius* DNA barcode sequence can be generated, this species will remain excluded from the British list. On the currently available evidence, *B. regius* is not thought to occur in GB and so is Not Evaluated.

#### Boletus rhodopurpureus Smotl.

Previous assessment: VU (1992 as *Boletus purpureus*), EN B (2006) 2013 assessment: VU D1

Mature individuals: 400 (incl. those determined as *B. xanthocyaneus*, see below) Estimated population (named as *B. rhodopurpureus*): usually 1–10 fruit bodies recorded at each of 19 unique georeferenced sites (190 mature individuals) plus one further site with two distinct fruiting areas noted (assumed to represent 20 mature individuals) and three other sites with large numbers of fruit bodies (30, 60+, ca. 100) recorded in some years but little guidance is available regarding discrete fruiting patches (assumed to represent 130 mature individuals in total). This gives a total of 340 mature individuals. *B. xanthocyaneus* is almost always found fruiting in company with *B. rhodopurpureus*. The hypothesis that British *B. xanthocyaneus* merely represents a yellow colour form of *B. rhodopurpureus* was supported by DNA sequencing data (see below). Hence the records were amalgamated and all treated as *B. rhodopurpureus*. The additional mature individuals were recorded from 4 unique georeferenced sites (40 mature individuals) plus one site with two distinct fruiting areas noted (20 mature individuals). The grand total for *B. rhodopurpureus* is 400 mature individuals. A very small population (Criterion D) assessed as VU D1.

A thermophilous bolete, mainly found in southern England and, although of restricted distribution, it can sometimes produce up to 100 fruit bodies at one site. It is associated with *Quercus* or *Fagus* in open mature woodland or wood pasture, often in bolete 'hot spots' such as parts of the New Forest or Windsor Great Park. There is a possibility that the few British records assigned to *B. torosus* (see below) also represent yellow colour forms of *B. rhodopurpureus* and

molecular work on *B. torosus* collections is ongoing. However, if the *B. torosus* records from sites lacking any records of *B. xanthocyaneus* are also included, this would only add 10 extra mature individuals to the *B. rhodopurpureus* total and so the overall assessment would remain unchanged.

#### Boletus rhodoxanthus (Krombh.) Kallenb.

Previous assessment: EX (1992)

2013 assessment: NE

Mature individuals: 0

This was recently discovered in Northern Ireland in 2009 (Wright 2011) and now confirmed by our DNA analyses. There are two historic records from England: an unconfirmed report from Worcestershire by Rea in 1895 (CBIB) and a specimen held at the Royal Botanic Garden, Edinburgh, collected from Buckinghamshire by Corner in 1933 (RBGE online catalogue). However, in view of the historic confusion surrounding the red-pored boletes, and in the absence of any supporting sequence data, we concluded that there are no authentic collections known from Great Britain and so *B. rhodoxanthus* was Not Evaluated in this assessment.

## Boletus ripariellus (Redeuilh) Watling & A.E. Hills

Previous assessment: NE 2013 assessment: DD Mature individuals: 230+ (Table 1) although 250 fruit bodies have been recorded at a single New Forest site. Population cannot be estimated because the species was originally described in 1997 and only

Population cannot be estimated because the species was originally described in 1997 and only recently recognised in GB, hence there has been insufficient recording time. It is associated with wetter soils and broadleaved trees such as *Salix* near watercourses, with good populations in the New Forest. Undoubtedly it has been confused with *B. chrysenteron* and *B. rubellus* in the past.

## Boletus satanas Lenz

Previous assessment: Rare (1992), LC (2006) 2013 assessment: VU D1 Mature individuals: 540 Estimated population: usually 1–10 fruit bodies recorded at each of 49 unique georeferenced sites (490 mature individuals) plus one site with up to 240 fruit bodies recorded (assumed to represent 50 mature individuals). A very small population (Criterion D) assessed as VU D1.

A thermophilous bolete mainly found in central and southern England associated with *Fagus*, *Quercus* and *Helianthemum* on calcareous sites. British records of this species were compiled in Marren (1997), it was illustrated on a British postage stamp (possibly misdetermined *B. luridiformis*) and it was a Priority Biodiversity Action Plan species until 2007.

## Boletus subappendiculatus Dermek, Lazebn. & J. Veselský

Previous assessment: NE

2013 assessment: DD

Mature individuals: 50+ (Table 1).

Population cannot be estimated because the species has only been recognised as being British since 2003, hence there has been insufficient recording time. Most British records thus far have been associated with conifers in Scotland.

#### Boletus torosus Fr. & Hök

Previous assessment: VU D2 (2006)

2013 assessment: DD

Mature individuals: 50+ (Table 1)

Population cannot be estimated due to uncertainty whether British specimens named as this are distinguishable from those named as *B. xanthocyaneus* and therefore merely yellow colour forms of *B. rhodopurpureus* (see above). Further molecular studies of British collections are required to resolve their taxonomic placement and so they have been assessed as DD for now. If British material is confirmed as belonging to a distinct taxon then, on currently available record data, it is likely that it would be assessed as EN D.

### Boletus xanthocyaneus (Ramain) Romagn.

Previous assessment: DD (2006) 2013 assessment: NE

Mature individuals: 0

DNA sequencing studies supported the hypothesis that British collections named as this are merely yellow colour forms of the normally pinkish-capped *B. rhodopurpureus* (see above), with which it is often seen fruiting, and not worthy of recognition at specific rank. All records have therefore been included in the assessment for *B. rhodopurpureus*.

### Buchwaldoboletus lignicola (Kallenb.) Pilát

Previous assessment: VU (1992), LC (2006)

2013 assessment: VU D1

Mature individuals: 540

Estimated population: 1–10 fruit bodies, usually at the lower end of this range, recorded at each of 54 unique georeferenced sites (540 mature individuals). A very small population (Criterion D) assessed as VU D1.

Associated with dead roots of various conifers (saprotrophic?), rarely with broadleaved trees and fruit bodies are often found near to those of the economically important, wood-inhabiting bracket fungus *Phaeolus schweinitzii* (mycoparasitic?).

#### Buchwaldoboletus sphaerocephalus (Barla) Watling & T.H. Li

Previous assessment: E (1992 as *Buchwaldoboletus hemichrysus*), VU D2 (2006) 2013 assessment: EN D Mature individuals: 60 Estimated population: 1–21 fruit bodies recorded at each of 6 unique georeferenced sites (60 mature individuals). A very small population (Criterion D) assessed as EN D. Associated with decayed trunk wood of various conifers or sawdust piles and generally regarded as saprotrophic.

## Leccinum albostipitatum den Bakker & Noordel.

Previous assessment: NE 2013 assessment: DD

Mature individuals: 60+ (Table 1)

Population cannot be estimated because the species was only recently described (den Bakker & Noordeloos 2005), hence there has been insufficient recording time. This species has a relatively pale-stiped fruit body and is ectomycorrhizal with *Populus*. Den Bakker & Noordeloos (2005) distinguished this taxon from *L. aurantiacum*, a species with darker stipe squamules found fruiting near a variety of broadleaved trees including *Populus*. Confusingly for British conservation assessments, *L. aurantiacum* was the name historically used for the pale-stiped

species, found mainly with Scottish aspens, which is now distinguished as *L. albostipitatum*. Therefore the British concept of *L. aurantiacum* has recently changed (Burnham & Kibby 2011) so that it now includes *L. populinum* and *L. quercinum* and great care should be taken when attempting to interpret the historic records.

#### Leccinum cyaneobasileucum Lannoy & Estadès

Previous assessment: LC (2006)

2013 assessment: DD

Mature individuals:140 + (Table 1)

Generally regarded as one of the more common components of what was historically recorded as *L. scabrum* (brown birch bolete) in Britain (Kibby 2006, 2011), *L. cyaneobasileucum* was only described in 1991. It was distinguished from *L. brunneogriseolum*, described by the same authors in the same year, until the molecular studies of den Bakker & Noordeloos (2005) indicated that they were colour forms of a single species henceforth to be known as *L. cyaneobasileucum*. The relatively few confirmed British records of *L. cyaneobasileucum* (2009 onwards) and ongoing confusion regarding the correct naming of historic finds of brown birchassociated boletes indicates that the population cannot be satisfactorily estimated and DD is the appropriate assessment category. It is likely to be assessed as LC once recorders become more familiar with its distinguishing characteristics.

## Leccinum duriusculum (Schulzer ex Kalchbr.) Singer

Previous assessment: NE

2013 assessment: NT

Mature individuals: 1100

Estimated population: 1–20 fruit bodies recorded at each of 97 unique georeferenced sites (970 mature individuals) plus 30 fruit bodies recorded at one site (assumed to span 30 m and therefore to represent 30 mature individuals) and two sites noted with over 100 fruitbodies on single visits (2 x 50 mature individuals). There is no indication of the area of ground that supported the largest numbers of fruit bodies and if the population was even slightly underestimated it would be large enough to breach the upper limit for NT (1100 mature individuals). However, there is some uncertainty regarding these estimates and so, as a precaution, *L. duriusculum* was considered to be a very small population (Criterion D) assessed as NT.

A species associated with *Populus* (white, grey and aspen group) that can be found fruiting in open woodlands, parks and gardens.

## Leccinum melaneum (Smotl.) Pilát & Dermek

Previous assessment: NE

2013 assessment: DD

Mature individuals: 620 + (Table 1)

Undoubtedly formerly confused with the common *L. scabrum* (brown birch bolete) in Britain (Kibby 2006, 2011), *L. melaneum* was confirmed as a distinct darker-capped birch associate during the molecular studies of den Bakker & Noordeloos (2005) although the name may be invalidly published. Unfortunately, these two species are often difficult to distinguish without recourse to DNA sequencing. Further confusion is likely to arise between these taxa and another dark-capped species, *L. roseofractum*, which, although previously distinguished in Britain, was recently synonymised with *L. scabrum* (den Bakker & Noordeloos 2005). The confusion surrounding the identification of brown birch boletes indicates that the population of *L. melaneum* cannot be satisfactorily estimated and DD is the appropriate assessment category.

#### Leccinum schistophilum Bon

Previous assessment: NE 2013 assessment: DD

Mature individuals: 200 + (Table 1)

Another brown-capped, birch-associated species and likely to have been historically confused with *L. scabrum*, *L. holopus* and *L. variicolor* in Britain (Kibby 2006, 2011), although usually found in wetter habitats than the first species. It was only recently recognised in GB and as the database records currently commence in 2000, there has been insufficient recording time.

#### Leccinum vulpinum Watling

Previous assessment: VU (1992), DD (2006) 2013 assessment: VU D1

Mature individuals: 300+

Estimated population: 1 fruit body (if any number specified) recorded at each of 28 unique georeferenced sites (280 mature individuals) plus one site with "widely scattered" and one with "several groups of" fruit bodies. There are therefore estimated to be at least 300 mature individuals. A very small population (Criterion D) assessed as VU D1.

This is predominantly a Scottish species strictly associated with *Pinus* woodland or with *Arctostaphylos* (bearberry) at sites where *Pinus* once grew (Watling & Hills 2005). It is characteristic of Caledonian pine forests and there is a single record with planted pine in Devon.

#### Octaviania asterosperma Vittad.

Previous assessment: DD (2006) 2013 assessment: DD

Mature individuals: 60 + (Table 1)

Associated with *Fagus*, this truffle-like ectomycorrhizal species is extant at six widely distributed sites in England, Scotland and Wales according to database records. However very few searches have been carried out to assess the true distribution of such subterranean-fruiting fungi and the records of this species and *Wakefieldia* constitute an unsatisfactory evidence base for any meaningful assessment of extant populations.

#### Phylloporus pelletieri (Lév.) Quél.

Previous assessment: LC (2006) 2013 assessment: VU D1 Mature individuals: 640

Estimated population: usually 1–2 fruit bodies recorded at each of 64 unique georeferenced sites (640 mature individuals). A very small population (Criterion D) assessed as VU D1.

Associated with *Fagus* and *Quercus*, often on mossy wood-banks in older woodlands, also with *Corylus, Castanea* and other broadleaved trees. This species often produces just one or two fruit bodies per site in each season. Although the fruit bodies have highly distinctive thick yellow gills, molecular studies indicate a close relationship with poroid *Xerocomus* species; indeed it is sometimes known as *Xerocomus pelletieri* (Hills 2008, Kibby 2011).

### Porphyrellus porphyrosporus (Fr. & Hök) E.-J. Gilbert

Previous assessment: NE 2013 assessment: NT Mature individuals: 1020 Estimated population: usually 1–10 fruit bodies recorded at each of 102 unique georeferenced sites (1020 mature individuals). A very small population (Criterion D) assessed as NT. Chiefly associated with *Quercus* and *Fagus* in older woodlands of England, Scotland and Wales.

#### Rubinoboletus rubinus (W.G. Sm.) Pilát & Dermek

Previous assessment: VU (1992), LC (2006)

2013 assessment: VU D1

Mature individuals: 450

Estimated population: usually 1–10 fruit bodies recorded at each of 45 unique georeferenced sites (450 mature individuals) although up to 24 fruit bodies have been recorded along one woodland ride on the Crown Estate, Windsor. A very small population (Criterion D) assessed as VU D1.

A thermophilous bolete associated with *Quercus* and *Fagus* mainly fruiting in older woodlands and parks from northern England (Rae 2006) southwards with one collection known from Wales. It is also known as *Chalciporus rubinus* (Ing 1992, Kibby 2011).

#### Strobilomyces strobilaceus (Scop.) Berk.

Previous assessment: VU (1992), LC (2006)

2013 assessment: NT

Mature individuals: 1020

Estimated population: usually 1–10 fruit bodies recorded at each of 97 unique georeferenced sites (970 mature individuals) plus five "sites" noted at one further grid ref (50 mature individuals). A very small population (Criterion D) assessed as VU D1.

A potential ancient woodland indicator species found in Scotland and England during the 50 y recording period but possibly extinct in Wales. It is associated with various trees, mainly *Fagus*, and can be found fruiting in leaf litter in shade.

#### Wakefieldia macrospora (Hawker) Hawker

Previous assessment: DD (2006) 2013 assessment: DD

Mature individuals: 0

Associated with *Fagus* on calcareous soils, this truffle-like ectomycorrhizal species has no known GB records since 1954 (nr Wotton-under-Edge) and so is potentially extinct. However, very few searches have been carried out to assess the true distribution of such subterranean-fruiting fungi and the records of this species and *Octaviania* constitute an unsatisfactory evidence base for any meaningful assessment of extant populations. This species was collected in the same area from 1949 to 1954 but it is not known whether there have been any attempts to re-find it, although this is thought to be unlikely. A targeted survey effort is now required to ascertain whether this species can be rediscovered near the Cotswold escarpment.

#### Xerocomus chrysonemus A.E. Hills & A.F.S Taylor

Previous assessment: NE 2013 assessment: DD Mature individuals: 200+ (Table 1) Population cannot be estimated because the species was originally described in 2006 (Taylor *et al.* 2006), hence there has been insufficient recording time. Currently only known with *Quercus* and *Fagus* in southern England, with a stronghold in the New Forest, it is likely to have been misidentified as *B. subtomentosus*, *B. ferrugineus* or *B. moravicus* if recorded in the past (Hills 2008, Kibby 2011).

### Xerocomus silwoodensis A.E. Hills, U. Eberhardt & A.F.S. Taylor

Previous assessment: NE

2013 assessment: DD

Mature individuals: 30+ (Table 1)

Population cannot be estimated because the species was originally described in 2007 (Taylor *et al.* 2007), hence there has been insufficient recording time. Currently only known with *Populus* (white and grey group) from three sites in southern England, it is likely to have been misidentified as *B. ferrugineus* if recorded in the past (Hills 2008, Burnham & Kibby 2011, Kibby 2011).

# **10. Site Protection and Threats**

Over 60% of sites where red-listed *Boletaceae* are recorded are designated Sites of Special Scientific Interest (SSSIs), National Nature Reserves (NNRs), Local Nature Reserves (LNRs) or Country Parks (CPs). Even so, fungi are very rarely named among the special interest features and therefore usually only receive indirect protection.

Threats to bolete sites include:

- Habitat loss through development, tree felling (e.g. sweet chestnut).
- Appropriate management at inappropriate times such as carrying out mowing, leafblowing and mechanical bracken control during the fruiting season; use of tractors in wet conditions leading to localised ploughing and severing of mycorrhizal roots.
- Lack of management such as allowing coarser vegetation to encroach, e.g. bramble, bracken, grasses which inhibits fruiting and may be detrimental to bolete mycelium over a long period.
- Picking of edible (and non-edible) fruit bodies.
- Trampling and compaction of mycelium and/or fruit bodies resulting from poorly-sited public activities such as mountain biking, car parking and organised events with checkpoints/refreshment/toilet areas sited on bolete-rich areas of parkland.

## 11. Recommendations for future recording of RDL boletes

- 1. Estimated or actual fruit body numbers to be recorded as well as the number of discrete patches of fruit bodies under each individual host tree or at least 10 metres apart.
- 2. 8-digit (GPS) grid references to be taken, where possible, for each discrete fruiting patch.
- 3. Targeted surveys to be undertaken of all red-listed boletes concentrating initially on those sites with the longest gaps since the last records were made.
- 4. Voucher specimens (even a small section of a single fruit body) to be deposited in national fungaria (RBG, Kew and Edinburgh), which can then be used for DNA-barcode verification of records or for taxonomic studies. In this regard it should be noted that scientific collecting of fungal fruit bodies of species protected under Schedule 8 of the Wildlife and Countryside Act is currently not authorised unless the collector holds a permit for such work.
- 5. Literature used for identification to be cited when a record/voucher is documented to try to future-proof the record against taxonomic change.
- 6. Information on exact locations of red-listed species to be sent to owners/managers of all designated and otherwise protected sites.

# 12. Acknowledgements

Special thanks go to the Association of British Fungus Groups (Michael Jordan) and to the British Mycological Society (Paul Kirk) for provision of full database records held respectively in <u>CATE2</u> and <u>FRDBI</u>. Thanks are also due to all fungal fieldworkers who support the two national databases and provide the foundation for all RDL assessments and, for bolete recording, particular thanks to Alan Hills who lodged a copy of his records database at Kew.

We acknowledge the financial support of the following organisations: Natural England for the pilot assessment project; Cardiff University, NE and RBG Kew for DNA sequencing work; and NE for the bolete sampling project carried out by Alan Lucas, to whom our thanks are also due.

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