The British species of *Otidea* (2). *O. alutacea* and related taxa

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Ascomycete.org, 7 (6) : 295-302.
Novembre 2015
Mise en ligne le 30/11/2015

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Summary: The typification of *Peziza alutacea* Pers., the basionym of *Otidea alutacea*, is discussed, together with an analysis of British collections of *O. alutacea* and related taxa. Amongst British collections of *O. alutacea sensu lato* at least four closely related phylogenetic taxa are present. One of the latter is morphologically indistinguishable from that referred to *O. alutacea sensu stricto* by Olariaga *et al.* (2015), two others have smaller spores than those of the epitype designated by Carbone (2011), but are indistinguishable from each other except for subtle difference in hymenium colour. One taxon is morphologically distinct from the epitype in spore size range and in hymenium colour. An ITS sequence from a British collection is identical to those of clade 1 of Olariaga *et al.* (*loc. cit.*). This clade is strongly supported within the *O. alutacea* complex and warrants recognition at varietal level. It is described here as *O. alutacea* var. *parvispora*. In addition, the interpretations of *O. felina* and of *O. alutacea* var. *microspora* are considered, and an aberrant specimen with ovoid spores belonging to a fourth clade is reported. A lectotype is selected for *O. alutacea* var. *microspora*.
Keywords: Ascomycota, *Pyronemataceae*, Otidea alutacea, morphology, phylogeny, typification.

Introduction

Based on studies of multilocus phylogenies of *Otidea* (Pers.) Bonord., Hansen & Olariaga (2015) estimated the number of species involved in the genus to be 47, although a monograph of the genus simultaneously published by Olariaga *et al.* (2015) recognised 33 species. Both studies concluded that *O. alutacea* (Pers.) Massee is a complex of taxa, and the latter demonstrated the presence of five phylogenetic groups or clades within this complex. As their study excluded many British collections, an analysis is presented here of the UK *O. alutacea* collections and related taxa, based on morphology and examination of a small number of molecular data obtained at Royal Botanic Gardens, Kew (K), in comparison with other molecular data available in GenBank.

Material and methods

During the current *Otidea* study, 548 British collections held at K were examined, as well as 74 from other European countries and 6 from North America. Also examined were Persoon's type collections held at L and other type collections held at MICH, PRM and HMAS. Thin hand-sections, to examine excipulum and hymenial elements, were mounted initially in water to observe surface encrustation, and later stained with Melzer's reagent (the slides later prepared as semi-permanent by addition of lactic acid). Drawings using a drawing tube were prepared of spores, paraphyses, and ectal excipular elements under oil immersion at ×1600. UV light reflection of dried and, when available, fresh specimens was studied under a 6W UV lamp in a dark box, switchable between two wavelengths of 254 and 365 nm, viewed through a UV filter. The reflection was compared with the colour chart by Ridgway (1912). Duration of exposure of each specimen to one UV light source was limited to 5 seconds at most. As well as DNA samples extracted by Brock *et al.* (2009), five sets of internal transcribed spacer (ITS) sequences obtained by Martin Bidartondo of Royal Botanic Gardens, Kew and Imperial College, London, were studied. The sequences were first compared with each other and with selected ITS sequences from other European *Otidea alutacea* collections (Hansen & Olariaga, 2015; Olariaga *et al.*, 2015) downloaded from the GenBank website, by nucleotide BLAST analysis. Those downloaded from GenBank were aligned together with five British sequences (Fig. 4) under the maximum likelihood criterion, with RAxML programme version 8 (Stamatakis, 2014) along with raxmlGUI v1.3.1 (Silvestro & Michalak, 2012). Sequences of *Otidea papillata*, also downloaded from GenBank, were used as the outgroup. The GTRGAMMA substitution model of DNA evolution was employed and branch support assessed, using 10,000 non-parametric, bootstrap replicates.

Specimen citation for the United Kingdom is in chronological order. Publication dates of individual plates are based on Stafleu & Mennega (1993, 1995) and subsequent online supplements. All scale bars on illustrations correspond to 10 µm. Kew accession numbers are in the form K(M)12345.

Typification

The original description of *Peziza alutacea* (Persoon, 1799: 78) was discussed by Carbone (2011) who demonstrated that an illustrated plate by Bulliard (1783-84: t. 154B) represented the sole element cited in the protologue, and hence served as the holotype of *Peziza alutacea*. This plate includes six illustrations, marked A, B, C, D, E as well as one unmarked figure which, judging from the accompanying text, should have been marked F. As noted by Carbone (*loc. cit.*) and also by Parslow & Spooner (2013), the illustration B shows a pale otideoid fungus with three clustered fruitbodies, while C and D are of a segment of the same or a similar fungus, the rest referring to a different, dark-coloured otideoid species, or multiple species. Subsequently, Persoon (1822: 221) chose the illustration B for *P. alutacea*. To reflect this choice, Carbone (*loc. cit.*) designated 154B as lectotype of *P. alutacea*. Carbone (*loc. cit.*) also studied Persoon's two original collections of *P. alutacea* held at L, and considered that both collections are of *P. alutacea*. He selected one of them, L910.261-13, to serve as epitype of *P. alutacea*, and hence of *Otidea alutacea*.

Having also studied Persoon's original collections at L, the current authors (Parslow & Spooner, 2013) found that the epitype specimen matched Persoon's description of *P. alutacea*, whereas L910.261-12 proved referable to another species, *O. bufonia*, as also concluded by Harmaja (2009).

Given that *O. alutacea* was shown by Olariaga *et al.* (*loc. cit.*) to be a species complex, it is unfortunate that the epitype cannot provide molecular data which would be required for a precise application of the name. This problem will be rectified at a later date.

Results

1. Description and morphological characters of *O. alutacea sensu stricto s.* Olariaga *et al.*

Otidea alutacea (Pers.) Massee, *Brit. fung.-fl.*, 4: 446 (1895).
Basionym: *Peziza alutacea* Pers., *Obs. mycol.*, 2: 78 (1799).
Scodellina alutacea (Pers.) Gray, *A natural arrangement of British plants*, 1: 668 (1821).
Peziza alutacea Pers. subgen. *Otidea*, *Mycol. eur.*, 1: 221 (1822).

The description below is amended slightly from that given by Olariaga *et al.* (2015) and serves to contrast with the new taxon described below. It is based on the epitype, L910.261-13 (Fig. 1), examined by the current authors at L, supplemented with characters of K(M)159266 (Fig. 2).

The ITS sequence from this specimen (GenBank accession, KT818925) is almost identical (Ident. 99%) to others in GenBank (accession numbers KM010075, KM010074, KM010073, KM010072, and KM010071; BLAST query coverage 100%), to which Olariaga *et al.* (*loc. cit.*) applied the concept of *O. alutacea sensu stricto*. This concept was based solely on spore size which they perceived to differ from that of the other clades, although stating it to be 'slightly overlapping' with that of another clade, numbered 3b. However, the spore size for these two clades is actually identical based on their measurements and that from British material K(M)142010 (Fig. 3; GenBank accession KT818924) so that there is no basis for application of '*sensu stricto*' to either of these clades.

Apothecia solitary or clustered, ear-shaped to almost cup-shaped when young, later mostly broad truncate, split on one side, sessile or short-stipitate. Outer surface Fawn Color (XL13‴) when fresh, drying Pale Ochraceous-Buff (XV15'f) or partly Warm Buff (XV17'd), hymenium Russet (XV13'k) when fresh [K(M)159266], drying Ochraceous-Tawny (XV15'd). **Ectal excipulum** a *textura globulosa* to *subangularis*, cells thin-walled, up to 20 µm in diam., with irregularly outgrowing chains of cells, hyaline, unencrusted. **Medullary excipulum** a *textura intricata*, yellow or pale yellow, cell contents pale yellow, cell walls hyaline or pale yellow, with no encrusted hyphae. **Subhymenium** of interwoven, thin-walled hyphae, yellowish due to pigmentation of the walls, showing as a dark date-brown line when dry. **Ascospores** smooth, bi-guttulate, ellipso-cylindric, almost parallel-sided in face view, ends broad, bluntly rounded, (14.5–)15.0–17.5(–18.5) × (6.5–)7.0–8.0(–8.5) µm, Q=1.98–2.45, average 2.16 (n=54). **Asci** operculate, cylindric, 200–210 × 11–11.5 µm, arising from croziers [K(M)159266]. **Paraphyses** with hyaline to very pale yellow content, inclusions appearing granulate or with minute bubbles, slender, 1.6–2.5 µm at middle and below, apex curved or hooked, some with a distinct or inconspicuous notch. **UV reaction** on excipulum, 254nm, Light Ochraceous-Buff (XV15'd); 365nm, Pale Ochraceous-Buff (XV15'f). On hymenium, 254nm, Pale Ochraceous-Buff (XV15'f); 365nm, Light Ochraceous-Buff (XV15'd), partly white.

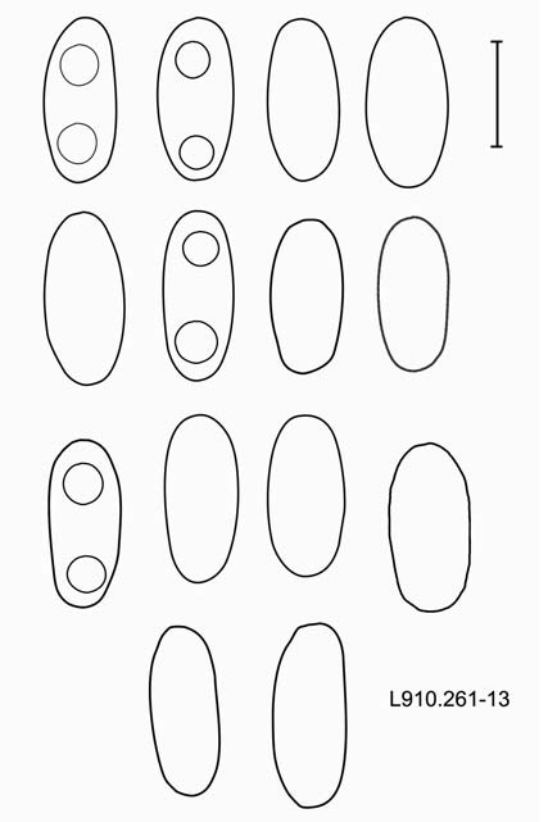

Fig. 1 – *Peziza alutacea*, epitype, *sine loc.*, Persoon, L910.261-13. Scale bar = 10 µm.

Selected collection based on morphology and molecular data: UK, England, South Essex, Epping, under *Fagus sylvatica*, 25 Aug. 2008, Kibby, K(M)159266.

Selected illustrations based on morphology: Cooke (1876), *Mycogr.*, pl. 54, fig. 211 *sub Peziza leporina*, drawn by W. Phillips from his collection, Elv. Brit. 11, K(M)155639; Bresadola (1898), *Fung. Trident.*, 2: 69, pl. 181; Boudier (1908), *Icones mycol.*, pl. 327.

Habitat: *Otidea alutacea*, in common with other members of the genus, is considered ectomycorrhizal (Rinaldi *et al.*, 2008; Hansen & Olariaga, 2015), although there are no available nucleotide data which have been directly obtained from an ectomycorrhiza and are explicitly identical to *O. alutacea sensu stricto* (*s.* Olariaga *et al.*, 2015; GenBank data). Most of the British collections under this name have been made in woodland with *Fagus sylvatica* and/or *Quercus* sp., from August to early November. However, one collection was obtained from under *Tilia* sp. and another from amidst *Salix repens*, but which infraspecific taxon each of them represents is still to be investigated.

Distribution: Judging from the *Otidea* collections at K, *O. alutacea sensu lato* is one of the most commonly recorded in Britain, surpassed only by *O. onotica* (Pers.) Fuckel and *O. bufonia* (Pers.) Boud., except in Scotland where it is apparently rare, perhaps due to lack of appropriate host trees. In the current study, of 548 British fungarium collections of *Otidea* examined, 144 prove referable to *O. alutacea* or to a closely related taxon. 93 of these are morphologically referable to *O. alutacea sensu stricto* (*s.* Olariaga *et al.*, 2015), and were recorded from 27 vice counties (VCs) in England, 5 VCs in Wales and 2 VCs in Scotland. However, distribution of the phylogenetic taxon strictly identical to K(M)159266 (Genbank EU818925) is not yet clear.

2. Interpretation of the epithet by British authors

Although this epithet is well known to British mycologists, its earliest interpretation, both within Britain and elsewhere, was inconsistent, as demonstrated by Carbone (2011). When Massee (1895: 446) made the combination in *Otidea*, he wrote "spores elliptic oblong, ends obtuse, continuous, 2-guttulate, smooth at first, then minutely verruculose and with a faint tinge of brown, 14–16 × 6–7 µ, 1-seriate; paraphyses slender, apex clavate, brownish", referring to Fuckel (1864), Fung. Rhen. 1229. Two examples of this exsiccatum examined at K [K(M)158712 and K(M)158713, Germany] both proved referable to *Peziza petersii* Berk., thus Massee's concept was clearly not of an *Otidea*. It is probable that Massee followed Cooke (1876) and Phillips (1887), both of whom wrongly applied this name to Fung. Rhen. 1229.

Being aware of this confusion, we re-examined British collections of *Otidea* at K, comparing them to available type material from Britain and elsewhere. As noted, there are 144 British collections at K which belong to the *O. alutacea* species group, of which 93 are morphologically consistent with the epitype. Many had previously been misnamed as: *Peziza* or *Otidea leporina* (named by M.J. Berkeley, M.C. Cooke, C.E. Broome, W. Phillips, W.D. Graddon); *cochleata* (by Berkeley, Broome, Phillips, C. Crossland, A. Bloxam, J.D. Hooker); *bufonia* and its synonym *umbrina* (Dennis). Based on morphology only, the oldest British specimen of *O. alutacea* at K originated from herb. J.D. Hooker [*sub Peziza cochleata* Huds., Scotland?, 1830, K(M)154583]. The oldest specimen which correctly carried the epithet *alutacea* originated from herb. M.J. Berkeley [*sub Peziza alutacea*, Wales, Denbighshire, Abergele, Coed Coch, 1877, Berkeley, K(M)155742]. At the same time, the epithet *alutacea* had erroneously been applied not

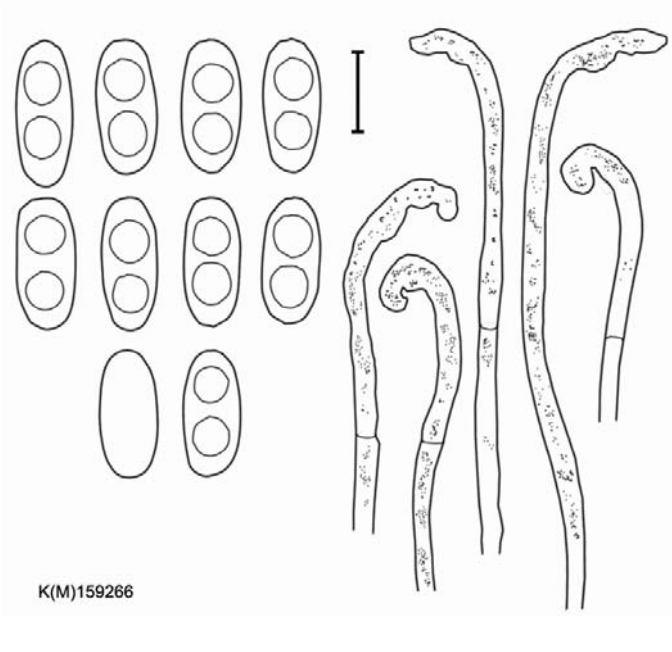

Fig. 2 – *Otidea alutacea sensu stricto s.* Olariaga *et al.*, UK, England, South Essex, Epping, under *Fagus sylvatica*, 25 Aug. 2008, Kibby, K(M)159266. Scale bar = 10 µm.

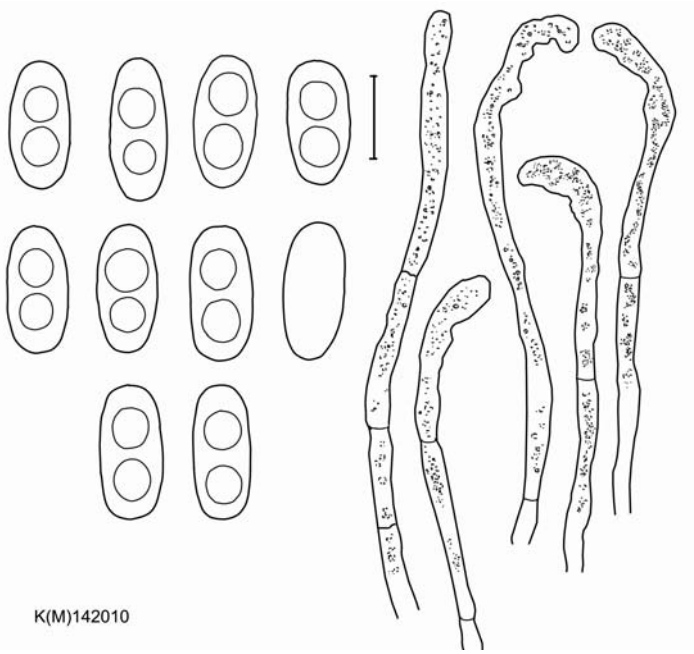

Fig. 3 – *Otidea alutacea*, UK, England, West Gloucestershire, Tetbury, Westonbirt Arboretum, under *Fagus sylvatica*, 15 Oct. 2006, Alder, K(M)142010. Scale bar = 10 µm.

only to related taxa, but also to other species of *Otidea*, including *O. bufonia*, *O. onotica*, and two other currently unnamed taxa with encrusted excipulum.

During the present study, we became aware that there were at least two taxa involved amongst British collections referred to *O. alutacea*, differing most obviously in spore size. It became apparent that Dennis (1960, 1968) based his concept of *O. alutacea* on determinations by Le Gal [as in K(M)157726, Northern Ireland, Co. Down, Clandeboye, Sep. 1948] and by Nannfeldt [as in Fung. Suec. Exic. 3276, K(M)158714, and 3277, K(M)158715], i.e. a taxon with smaller spores than those of the epitype. Nannfeldt noted on the labels of these exsiccata, "the fungi distributed here as nos. 3276-3277 fall both within what is conventionally named *O. alutacea*, but their name remains doubtful until the genus is critically revised."

It is notable that, among the older specimens of *O. alutacea* held at K, the majority were large spored, morphologically agreeing with *O. alutacea sensu stricto s.* Olariaga *et al*. The proportion of the total number of these against the total number of the *O. alutacea* group was highest in the 19th century, but has declined since then, as further discussed below.

3. Interpretation of molecular data

Hansen & Olariaga (2015) provided a strong hypothesis for species delimitation within the genus, by recognising phylogenetic species by genealogical concordance, and using multilocus phylogenies to study the interspecific relationships. Based on this study, Olariaga *et al.* (2015) concluded that *O. alutacea* was part of a complex of taxa. Their large subunit rRNA phylogeny resolved several closely related clades, which are also supported in their multigene phylogeny (Hansen & Olariaga, *loc. cit.*). They demonstrated that ITS sequences were too variable to align throughout the genus, but were effective to distinguish between closely related taxa. The concept of *O. alutacea sensu stricto* they assigned to what they considered to be the only clade in which the spore size range matched that of the epitype.

The British collections of *O. alutacea* involved at least two taxa, both with relatively narrow spore ranges, hardly overlapping with each other. Provisionally, they had been accessioned at K as *O. alutacea* and *O. alutacea* (aff.). However, analysis of ITS sequences of British collections using GenBank data deposited by Olariaga *et al*., shows that both of them consist of two or more taxa. Included in the former are *O. alutacea sensu stricto s.* Olariaga *et al.*, represented by K(M)159266 (GenBank KT818925), and clade 3b represented by K(M)142010 (GenBank KT818924). Included in the latter are clade 1, represented by K(M)70199 (GenBank EU784380), and a new clade, represented by K(M)81596 (GenBank EU784381) and K(M)145938 (GenBank KT818926). The single best tree from RAxML Maximum Likelihood analysis is shown in Fig. 4.

K(M)159266 and K(M)142010 are morphologically identical, except for a slightly denser subhymenial pigmentation in the latter (appearing as a marginally thicker line of date brown colour than the former when dry). The spore range of the latter is 15.5–17.0 (–18.0) × 7.0–8.0 µm, Q=2.00–2.21, average 2.14 (n=36), almost exactly coinciding with that of the former. Due to the lack of sequence data from the epitype, the concept *sensu stricto* cannot be unequivocally referred to one or other of the two morphologically identical clades as noted above.

The ITS sequence of K(M)70199 (EU784380) is phylogenetically identical (Coverage 100%, Ident. 99%) to clade 1 of Olariaga *et al.* (*loc. cit.*). There is no overlap in the spore size between *O. alutacea sensu stricto s.* Olariaga *et al.* and K(M)70199, as also stated by Olariaga *et al.* (*loc. cit.*: 183) for clade 1. This taxon warrants recognition as a variety of *O. alutacea* as proposed below.

Otidea alutacea* var. *parvispora, var. nov. — IF 551565

Diagnosis: pale fawn coloured, macroscopically as in the typical variety of *Otidea alutacea* (Pers.) Massee, differing only in the pale hymenium which dries buff, and microscopically in less yellow pigmentation on cell walls in the medullary excipulum and subhymenium, in smaller ascospores and shorter asci than those of the typical variety.

Holotype: UK, England, Surrey, Kew, near Quercus ilex, Legon, 19 Oct. 2000, K(M)70199 (Fig. 5a, 5b).

GenBank accession number (ITS) of holotype: EU784380.

Etymology: referring to the spore size, which is markedly smaller than that of the typical variety.

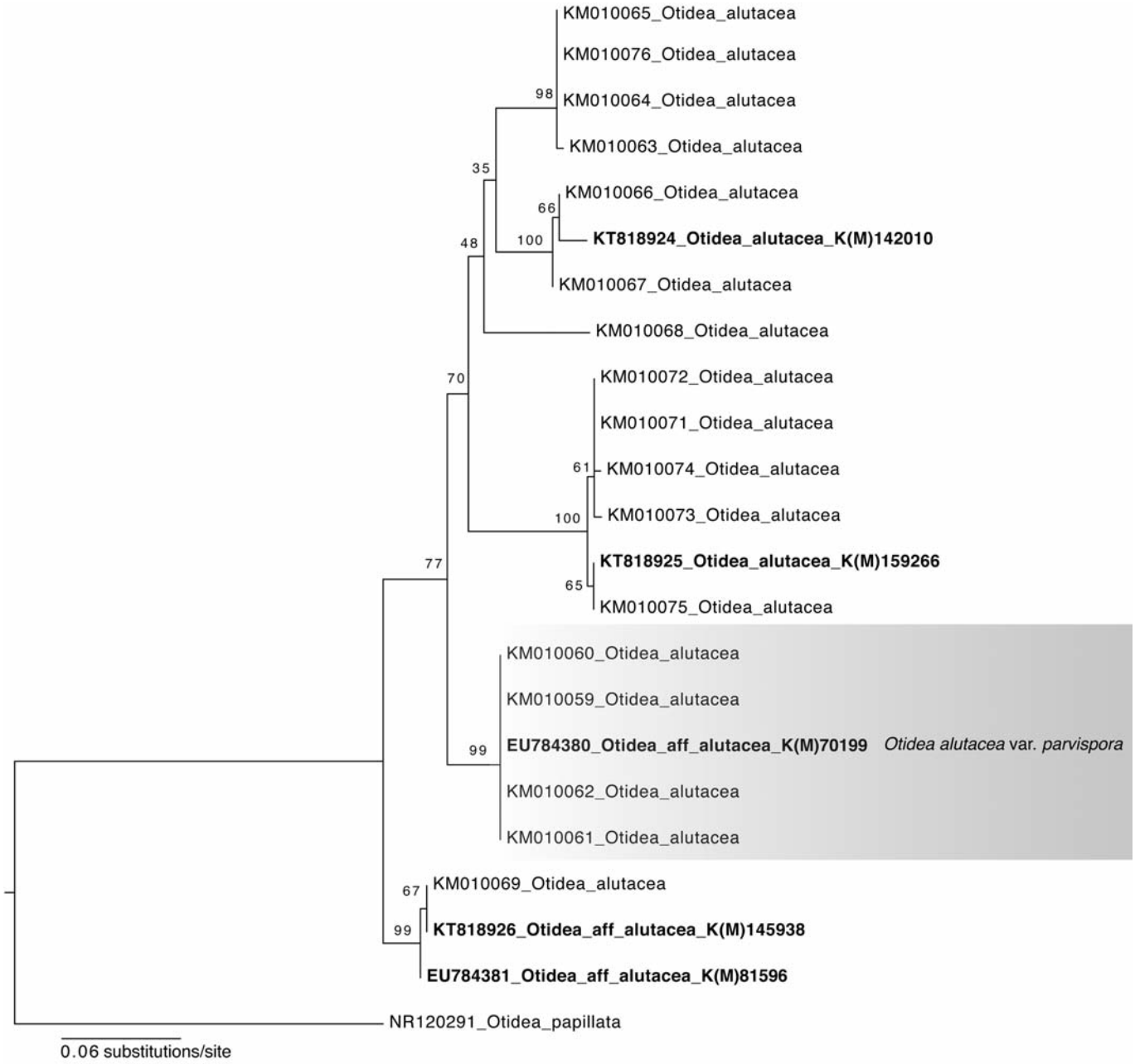

Fig. 4 – Maximum likelihood tree using ITS sequences from specimens belonging to the European *Otidea alutacea* complex including five British collections, with *Otidea papillata* as the outgroup. Numbers above branches are non-parametric bootstrap values.

Apothecia clustered, truncate or ear-shaped when young, outer surface pale Fawn Color (XL13‴) when fresh, drying Warm Buff (XV17′d), hymenium Light Ochraceous-Buff (XV15′d) when fresh, drying Warm Buff (XV17′d). **Ectal excipulum** a *textura globulosa* to *angularis*, cells mostly 20–27 (–35) µm diam., often smaller at the surface, thin-walled, with short outgrowing chains of cells, hyaline or pale yellow, unencrusted. **Medullary excipulum** a *textura intricata*, pale yellow. **Subhymenium** pale yellow when fresh, showing as a thin, pale brown zone when dry; hyphal walls apparently lacking pigmentation. **Ascospores** smooth, biguttulate, ellipsoid, almost parallel-sided in face view but less so than in the typical variety due to shorter spore lengths, ends broad, bluntly rounded, (11.0–) 11.5–13.0 × 5.0–6.5 µm, Q=1.73–2.28, average 2.04 (n=41). **Asci** 165–185 × 9.0–10.0 µm, 8-spored, arising from croziers. **Paraphyses** (2.5–) 3–4 µm wide at middle, apex curved to hooked, slightly enlarged to 4-5 µm wide, with pale yellowish granular inclusion, deeper staining in Melzer's reagent.

Habitat and distribution: the precise distribution of this taxon which phylogenetically corresponds to K(M)70199 is still to be investigated.

This taxon may correspond to Bresadola's (1900) description of *O. felina* (Pers.) Bres., but not to the holotype of *Peziza felina*, L910.256-815. Olariaga *et al.* (2015: 184) suggest that the relevant clade (their clade 1) should be compared to *O. kunmingensis* W.Y. Zhuang which they refer to *O. alutacea* complex despite the fact that they did not examine the type. The spore range of this species, 10.3–11.7 × 5–6 µm (in the protologue, Zhuang & Yang, 2008: 238), hardly overlaps with that of K(M)70199. Furthermore, the apothecia are described as having olivaceous yellow tones, and it is unlikely that this species belongs to the same taxon as K(M)70199. Molecular data for the type or an authentic *O. kunmingensis* are not available.

This taxon, or morphologically indistinguishable taxa, represented in K by 51 collections which currently lack sequence data, are widespread in Southern England, East Anglia and The West Mid-

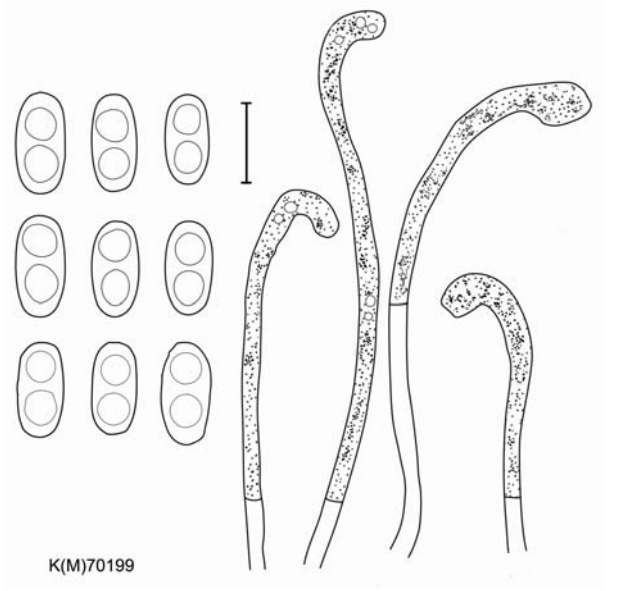

Fig. 5a – *O. alutacea* var. *parvispora*, holotype, England, Surrey, Kew, near *Quercus ilex*, 19 Oct. 2000, Legon, K(M)70199. Scale bar = 10 µm.

lands, being recorded from 24 English vice counties. Three originate from Northern Ireland and two from Scotland; none is yet known from Wales. They are most frequently recorded from near *Quercus* spp. or *Fagus sylvatica*, between August and early December. Whether or not those collected under *Pinus* sp. and other trees belong to the same phylogenetic taxon is still to be investigated.

Selected illustrations based on morphology: Dennis (1968), VIIIB [drawn from his collection, Northern Ireland, Clandeboye, Dennis, Sep. 1948, K(M)157726].

Two other British collections, K(M)81596 (England, Oxford, Harberton Mead, near *Quercus* sp., 12 Oct. 2000, Webb) and K(M)145938 (see below), have ITS regions which are identical to *O. alutacea* no. 17 (S-F267085, i.e. MC201005) of Olariaga *et al.* (*loc. cit.*) and form another clade. K(M)145938 is aberrant in having uniformly ovoid spores, and was initially considered to be a new species. It is reported below. K(M)81596 is morphologically similar to the type of *O. alutacea* var. *parvispora* except for a slight difference in the colour of the hymenium, and the amount of yellow pigmentation in subhymenium, which in the latter are lighter.

Interpretation of *Otidea felina*

Otidea felina (Pers.) Bres., *Fung. Trident.*, 2: 103, f. 215 (1900).
Basionym: *Peziza felina* Pers., *Mycol. eur.*, 1: 223 (1822).

Having examined the holotype (L910.256-815) of *Peziza felina*, Harmaja (2009), Van Vooren & Carbone (2012) and the current authors (Parslow & Spooner, 2013) all considered *O. felina* a taxonomic synonym of *O. alutacea*. However, the measurements of ascospores and asci of the holotype published by Van Vooren & Carbone (*loc. cit.*; asci average 150 µm in length, spores 14–16 × 6–6.5 µm) were slightly smaller than those made by the current authors, i.e. asci 160–170 × 10–11 µm, spores 15–17.5 × 6.5–7.5 µm, average 16.1 µm (n=22). This latter spore range agrees well with that of *O. alutacea sensu stricto s.* Olariaga *et al.*, and also with that of clade 3b as represented by a British specimen, K(M)142010, with spores 15.5–17 (–18) × 7–8 µm. *Peziza felina* may belong to one of these two clades, but was

Fig. 5b – *O. alutacea* var. *parvispora*, holotype, K(M)70199, dried specimen, demonstrating the buff hymenium lacking brown tone, photographed under daylight bulb, 6000K, with white balance adjusted. Photo: RBG, Kew.

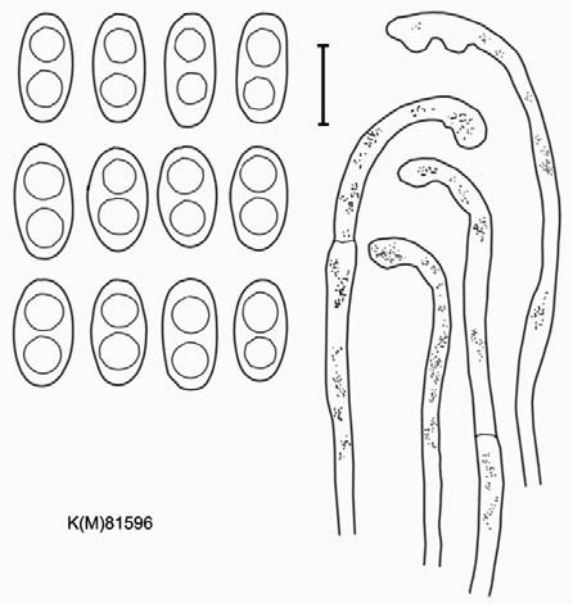

Fig. 6 – *O. alutacea sensu lato*, England, Oxford, Harberton Mead, near *Quercus* sp., 12 Oct. 2000, Webb, K(M)81596. Scale bar = 10 µm.

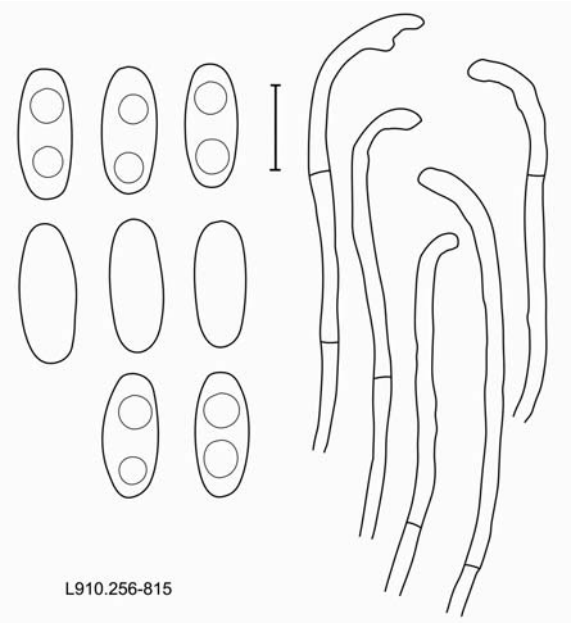

Fig. 7 – *Peziza felina*, holotype, France, Meudon, near Paris, Persoon, L910.256-815. Scale bar = 10 µm.

described '*ex vivo denuo describenda*' as grey, hence unlike other known collections from these clades. However, Van Vooren & Carbone (2012) stated that the holotype collection was heavily parasitized and, during the present study, the outer surface of this collection was found to bear numerous brown-pigmented, globose spores. The grey colour of the fresh apothecia may have been due to this fungus, either an infection or contaminant. *Peziza felina* clearly requires an epitype, as also noted by Olariaga *et al.* (*loc. cit.*), and this will be resolved at a later date.

Otidea felina has apparently not been formally reported from Britain, although there are two collections at K which were previously held under this name, K(M)155397 (on *Picea*, medulla non yellow in KOH), and K(M)155461 (on *Picea*, medulla yellow in KOH but lacking encrusted hyphae). These both have dark reddish ectal encrustations and represent two distinct taxa, but are currently unidentified.

Interpretation of *Otidea alutacea* var. *microspora*

Otidea alutacea* var. *microspora Kanouse, *Mycologia*, 41(6): 668 (1950).

Homotypic synonym: *O. microspora* (Kanouse) Harmaja, *Karstenia*, 15: 32 (1976).

As noted by Olariaga *et al.* (2015), the Latin diagnosis by Kanouse (1950: 668-669) designated A.H. Smith no. 9351 as type, whereas under "Material Examined" following her English text (*loc. cit.*: 669), another specimen, A.H. Smith 17699, is given as type. Smith 9351 is also cited here but not marked as type, together with another specimen, Smith 30502. As two different collections, Smith 9351 and 17699, are indicated as type, they should both be regarded as syntypes; 30502 is a paratype for this species.

Having studied these three collections preserved at MICH, the current authors support the conclusion by Olariaga *et al.* (*loc. cit.*) that Smith 9351 (MICH barcode 14407) belongs to the *O. alutacea* species group, and has larger spores than given in the protologue. Both Smith 17699 (MICH barcode 14406) and Smith 30502 (MICH barcode 02016) have pale yellow apothecia, with yellowish brown, encrusted excipular cells and spores 10–11.5 × 5.5–6.5 µm. They are conspecific and correspond to the diagnosis of *O. alutacea* var. *microspora*. As the two syntypes represent different species, a lectotype is required for *O. alutacea* var. *microspora* (McNeill *et al.*, 2012, Melbourne Code, Art. 9.12), as also suggested by Olariaga *et al.* (*loc. cit.*). Only Smith 17699, MICH barcode 14406, matches the diagnosis and this is here designated as the lectotype of *Otidea alutacea* var. *microspora* Kanouse.

Olariaga *et al.* (*loc. cit.*) demonstrated that an ITS sequence from the paratype of *O. alutacea* var. *microspora* (Smith 30502, GenBank AF072094) is almost identical to the sequence they obtained from the holotype of *O. rainierensis* Kanouse (Smith 30553, MICH barcode 14410, GenBank KF717583). Further, they found that this latter sequence and that of the holotype of *O. kauffmanii* (MICH barcode 14409, GenBank KF717579) are also almost identical. They hence concluded that *O. rainierensis* and *O. kauffmanii* are synonymous, and chose *O. rainierensis* as the name for the species. It should be noted that on the GenBank website, the holotype of *O. kauffmanii* appears under *O. rainierensis*, with no reference to its original name. It should also be noted that 30502 involves two elements, i.e. one caespitose group of 4 yellow-brown, stipitate apothecia and one solitary, pale-coloured, sessile apothecium. It is not known from which of these apothecia the ITS sequence was derived.

Otidea kauffmanii has previously been reported from the British Isles based on two English collections [K(M)143475, South Devon, Torquay, 18 Dec. 2006, Roberts; K(M)137477, North-east Yorkshire, Thornton Dale, 10 Sep. 1960, Sledge], both now revised as *O. phlebophora* (Berk. & Broome) Sacc.

Discussion

1. UV reaction

Lack of strongly pigmented encrustation on the ectal excipulum distinguish *O. alutacea* and related taxa from most other *Otidea* species, except *O. apophysata* (Cooke & W. Phillips) Sacc. and *O. platyspora* Nannf., in which encrustation is absent or scarce respectively. This lack of encrustation is reflected in the UV reaction of the outer surface which in these taxa is most strikingly pale ochraceous buff under 264 nm or white 365 nm. The hymenium of the *O. alutacea* complex is also white under UV light, whereas in other unencrusted taxa it is darker (Hare Brown XLVI 17""i, or Light Drab XLVI 17""b). A white UV reaction from the hymenium is also characteristic of *O. onotica*, but the outer surface of that species is UV dark.

2. Suspected dynamism amongst related taxa in the *Otidea alutacea* complex

Amongst British collections of *O. alutacea s. lato*, the relative proportion of the four clades is still to be investigated when molecular data are available. However, the current study revealed that, in the 19th Century, 91.2% (n=34) of collections of all *alutacea s. lato* had spores 15.0–17.5 (–18.5) × (6.5–) 7.0–8.0 (–8.5) µm, and hence are representative of one the two larger-spored clades. Curiously, this proportion decreased to 58.6% (n=70) in the 20th Century and to 52.5 %, (n=40) since 2000. The reason of this change is unknown, but such a dynamism has also been reported in Britain for the two species of *Sarcoscypha*, *S. coccinea* (Gray) Boud. and *S. austriaca* (Beck ex Sacc.) Boud. (Butterfill & Spooner, 1995).

3. A morphological anomaly

An apparently teratological collection [K(M)145938] of an *Otidea* which macroscopically resembled *O. alutacea* but exhibited unusual, uniformly ovoid, small ascospores was made in 2006, in England. The ITS4 sequence of this specimen, banked at K, is identical to that of an Italian collection of the *O. alutacea* complex (no. 17, S-F257085, GenBank KM010069 (Ident. 100%), in Olariaga *et al.*, 2015), as well as to K(M)81596, GenBank EU784381 (Ident. 99%), which form a well-supported clade. It is worth noting that a similar teratological collection, referred to *O. bufonia* based on molecular data, was previously reported by Olariaga *et al.* (*loc. cit.*: 208). The causes of these striking abnormalities are unknown. A description of K(M)145938 is given below.

Apothecia macroscopically as in the nominate variety, clustered, truncate, outer surface pale Fawn Color (XL13‴) when fresh (from photograph), drying Warm Buff (XV17'd), hymenium Warm Buff to Tawny (XV13'i) when fresh (from photograph), drying Warm Buff (XV17'd). **Ectal excipulum** a *textura subangularis*, cells up to 20 µm diam., thin-walled, with short outgrowing chains of cells, pale yellow, unencrusted. **Medullary excipulum** a *textura intricata*, pale yellow when fresh. **Subhymenium** pale yellow when fresh, pale date-brown when dry, hyphae appearing hyaline sub-microscope. **Ascospores** smooth, biguttulate, uniseriate but irregularly arranged, ovoid, one end broad and bluntly rounded in outline, other end markedly narrowed, mostly with one large guttule or very rarely with two guttules, 12.0–13.5 × 7.5–8.5 µm (n=20). **Asci** 170 × 10.0 µm, 8-spored, arising from croziers. **Paraphyses** 2–3 µm wide at middle, 3–4 µm wide at apices, with pale yellowish inclusion. Fig. 8a, b.

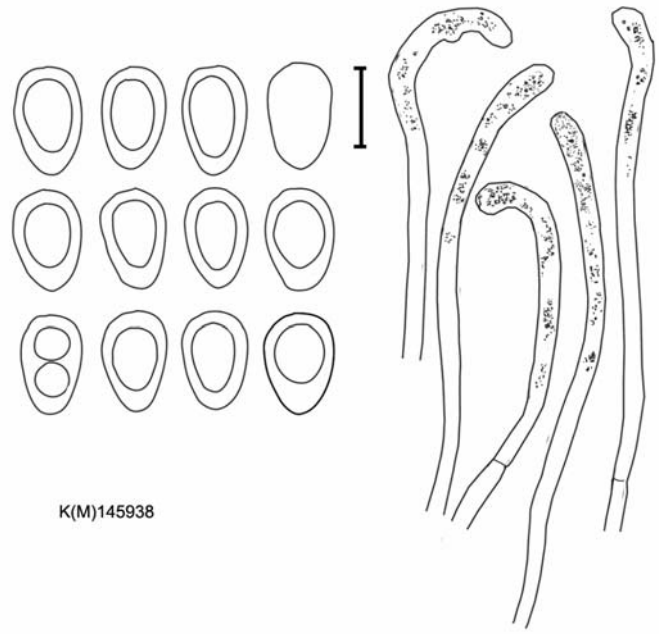

Fig. 8a – *O. alutacea sensu lato*, with unusual ovoid spores, UK, England, Leicester, 28 Nov. 2006, Rixon, K(M)145938. Scale bar = 10 µm.

Specimens examined: UK, England, Leicestershire, Leicester, 28 Nov. 2006, Rixon, K(M)145938.

Conclusion

The *O. alutacea* species complex is represented in Britain by taxa referable to at least four clades. One of the four clades (i.e. clade 1 of Olariaga *et al.*, 2015) is well-delimited both morphologically and phylogenetically and is described here as a new variety, *O. alutacea* var. *parvispora*. The taxonomy of the remaining three clades (i.e. larger-spored clades 3b, and *s. stricto* of Olariaga *et al.*, *loc. cit.*, and another unnumbered smaller-spored clade newly recognised here

Fig. 8b – *Otidea alutacea sensu lato*, with unusual ovoid spores, K(M)145938. Photo: T.F. Hering.

Fig. 8c – *O. alutacea sensu lato*, with unusual ovoid spores, K(M)145938. Fragments from dried specimen, demonstrating the ochraceous hymenium with brown tone, photographed under daylight bulb, 6000K, with white balance adjusted. Photo: RBG, Kew.

based on British collections) remains unclear and is in need of further study. All four clades appear to be associated in Britain with broad-leaf trees, especially *Fagaceae*, as also evident from specimens cited by Olariaga *et al.* (*loc. cit.*), but, at least among the two larger-spored clades, *Fagus sylvatica* rather than *Quercus*, is the most commonly recorded associate.

Acknowledgements

Our thanks are due to the curator of Nationaal Herbarium Nederland (Leiden branch) for allowing examination of Persoon's type collections; to the curators of Mycology Department of Herbarium, National Museum in Prague (PRM), Herbarium of University of Michigan (MICH) and the Mycology Herbarium, Institute of Microbiology, Academia Sinica (HMAS), for allowing loan of collections; to Martin Bidartondo (Imperial College, London, and Royal Botanic Gardens, Kew) for supplying additional molecular data: to Laura Martinez-Suz (Royal Botanic Gardens, Kew) for help in interpreting data from GenBank and for creating a phylogram; to Paul Kirk for his advice in nomenclature and his help in usage of Index Fungorum website. Thanks are also due to Ken Hudson (CABI) and Wen-ying Zhuang (CAS) for literature, and to Tom Hering for permission to publish the image of the aberrant specimen.

References

Boudier J.L.É. 1908. — *Icones Mycologicæ, ou iconographie des champignons de France principalement Discomycètes, avec texte descriptif*. Sér. 4, livr. 17. Paris, Paul Klincksieck, 19 plates.

Bresadola G. 1898. — *Fungi tridentini novi, vel nondum delineati, descripti, et iconibus illustrati*. Ser. 2, fasc. 11-13. Trento, J. Zippel, 47-81, pl. 151-195.

Bresadola G. 1900. — *Fungi tridentini novi, vel nondum delineati, descripti, et iconibus illustrati*. Ser. 2, fasc. 14. Trento, J. Zippel, 83-106, pl. 196-217.

Brock P.M., Döring H. & Bidartondo M. 2009. — How to know unknown fungi: the role of a herbarium. *New Phytologist*, 181 (3): 719-724.

Bulliard J.B.F. 1783-1784. — *Herbier de la France*. Tome 4, fasc. 39. Paris, Didot jeune, Belin, pl. 153-156.

Butterfill G.B. & Spooner B.M. 1995. — *Sarcoscypha* (*Pezizales*) in Britain. *Mycologist*, 9 (1): 20-26.

Carbone M. 2011 [2010]. — Il genere *Otidea*. III. Identità e tipificazione di *Peziza alutacea*. *Bollettino dell'Associazione Micologica ed Ecologica Romana*, 80-81: 22-38.

Cooke M.C. 1876. — *Mycographia, seu Icones fungorum*. Vol. 1, Discomycetes. Part 3. London and Edinburgh, Williams and Norgate; Leipzig, A. Durr; New York, Westerman & Co., 87-136, pl. 41-60.

Dennis R.W.G. 1960. — *British Cup Fungi and their allies*. London, Ray Society, 280 p.

Dennis R.W.G. 1968. — *British Ascomycetes*. Lehre, J. Cramer, 455 p., pl. 1-40, fig. 1-31.

Fuckel L. 1864. — *Fungi Rhenani exsiccati a Leopoldo Fuckel collecti. Fasc. 13. Hostorichiae ad Rhenum Nassoviorum*. Exsic. 1201-1300.

Hansen K. & Olariaga I. 2015. — Species limits and relationships within *Otidea* inferred from multiple gene phylogenies. *Persoonia*, 35: 148-165.

Harmaja H. 2009. — Studies in *Otidea* (*Pezizales*). *Karstenia*, 48: 33-48.

Kanouse B.B. 1950 [1949]. — Studies in the genus *Otidea*. *Mycologia*, 41 (6): 660-677.

Massee G. 1895. — *British Fungus-Flora*. Vol. 4. London and New York, George Bells & sons, 522 p.

McNeill J., Barrie F.R., Buck W.R., Demoulin V., Greuter W., Hawksworth D.L., Herendeen P.S., Knapp S., Marhold K., Prado J., Prud'Homme van Reine W.F., Smith G.F. & Wiersema J.H. (eds.) 2012. — *International Code of Nomenclature for algae, fungi, and plants (Melbourne Code)*. Regnum Vegetabile 154. Königstein, Koeltz Scientific Books, 208 p.

Olariaga I., Van Vooren N., Carbone M. & Hansen K. 2015. — A monograph of *Otidea* (*Pyronemataceae*, *Pezizomycetes*). *Persoonia*, 35: 166-229.

Parslow M & Spooner B.M. 2013. — The British species of *Otidea*: overview and the large-spored species. *Mycosystema*, 32 (3): 347-365.

Persoon C.H. 1799. — *Observationes Mycologicae seu descriptiones tam novorum quam notabilium fungorum exhibitae a D.C.H. Persoon*. Pars. 2. Leipzig and Lucerne, Gessner, Uster et Wolf, Lucerne, 106 p.

Persoon C.H. 1822. — *Mycologia Europaea seu completa omnium fungorum in variis Europaeae regionibus detectorum enumeratio, methodo naturali disposita; descriptione succincta, synonymia selecta et observationibus criticis additis*. Sectio 1. Erlangen, Johann Jakob Palm, 356 p.

Phillips W. 1887. — *A Manual of the British Discomycetes with Descriptions of All the Species of Fungi Hitherto Found in Britain, etc*. London, Kegan Paul, Trench & Co., 462 p.

Ridgway R. 1912. — *Color Standards and Color Nomenclature*. Washington, Robert Ridgway, 44 p., pl. 1-53.

Rinaldi A.C., Comandini O. & Kuyper T.W. 2008. — Ectomycorrhizal fungal diversity: separating the wheat from the chaff. *Fungal Diversity*, 33: 1-45.

Silvestro D. & Michalak I. 2012. — RaxmlGUI: a graphical front-end for RAxML. *Organisms Diversity and Evolution*, 12 (4): 335–337.

Stafleu F.A. & Mennega E.A. 1993. — *Taxonomic literature*. A selective guide to botanical publications and collections with dates, commentaries and types. 2nd edition. Suppl. 2. Be-Bo. Königstein, Koeltz Scientific Books, 464 p.

Stafleu F.A. & Mennega E.A. 1995. — *Taxonomic literature*. A selective guide to botanical publications and collections with dates, commentaries and types. 2nd edition. Suppl. 3. Br-Ca. Königstein, Koeltz Scientific Books, 550 p.

Stamatakis A. 2014. — RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics*, 30 (9): 1312–1313.

Van Vooren N. & Carbone M. 2012. — The genus *Otidea*. VI. *Otidea felina* and its interpretations. *Ascomycete.org*, 4 (4): 29-34.

Zhuang W.Y. & Yang Z.L. 2008 [2007]. — Some Pezizalean Fungi from Alpine Areas of Southwestern China. *Mycologia Montenegrina*, 10: 235-249.

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