The occurrence of the rare Ciboria aestivalis in Europe

R. Galán¹ and J. T. Palmer²

¹Department of Plant Biology, University of Alcalá, Alcalá de Henares, 28871 Madrid, Spain
²25, Beech Road, Sutton Weaver, via Runcorn, Cheshire WA7 3ER, U. K.


The authors report the finding in Southern Spain of an apparently rare fungus: Ciboria aestivalis (Pollock) Whetzel (Sclerotiniaceae) growing on mummified quinces (Cydonia oblonga Miller). Originally described from apples (Malus sp.) in the United States, the species has also been reported on other pomaceous and stone fruits in Australia. A recent report from France is based on a misidentification, whilst a British reference to “imported fruit” refers to mummified quinces brought from Australia. The inoculation of various fresh fruits produced mummies which developed apothecia, also on blackened pips from a mummified apple, whilst stromata formed in P. D. A. cultures. The apothecia are described in detail and illustrated, including the type. Its generic placement in the Sclerotiniaceae is discussed.

Key words: Ciboria, Sclerotiniaceae, mummification of fruits, Spain


Je referováno o nálezu v jižním Španělsku, zřejmě vzácné houby Ciboria aestivalis (Pollock) Whetzel (Sclerotiniaceae) rostoucí na mumifikovaných malvicích kdoule (Cydonia oblonga Miller). Původně byl druh popsán z plodů jabloně ve Spojených Státech a později byl tento druh hlášen též z jiných jádrovin a peckovin z Austrálie. Inokulace různých čerstvých plodů vytvořila mumifikované plody, které produkovaly apotecia (také na zčernalých jablkách), zatímco stromata se vytvářela v kultuře na bramboro-dextrózovém agaru. Jsou detailně popisována a vyobrazena apotecia včetně typu. Je diskutováno rodové zařazení v rámci čeledi Sclerotiniaceae.

MATERIAL AND METHODS

Measurements of living cells (*) were made in tap water, with dead cells (†) in tap water, Melzer’s reagent (MLZ), KOH 2%, and Aqueous cresyl blue (CRB) c. 0.5. The light microscopes used, both equipped with phase contrast, were a Nikon Labophot-2 (R. G.) and an Olympus BHT, fitted with a drawing attachment (J.T.P.), with magnifications up to 1250× The photomicrographs were made with a Nikon microscope, Labophot-2, equipped with phase contrast and having an incorporated system of automatic photography. Culture methods, using Potato Dextrose Agar (P.D.A.) are in accordance with Galán & al. (1996), whilst apothecial colours follow Kornerup & Wanscher (1967).
Specimens have been deposited in AH (the herbarium of Alcalá University, Spain) and J.T.P. (personal herbarium of the second author) with duplicates in CUP (Cornell University, Ithaca, New York, U. S. A.), and A. M. S. S. (herbarium of the “Asociación Micológica de las Sierras Subbéticas”, Priego de Córdoba, Spain). The holotype is preserved in BPI (Herbarium of the U. S. National Fungus Collections, Beltsville Agricultural Research Center, U. S. A.).

Abbreviations: * = living state, † = dead state

INTRODUCTION

This apparently rare species was collected on old, blackened quinces at Priego de Córdoba, Zagrilla in Córdoba province, Spain, 10. XII. 1995 (A. M. S. S. 956) by J. Gómez and B. Moreno-Arroyo (Gómez & al., 1999) with specimens sent by Mr. Gómez to R. G. for identification (AH 7108 and J.T.P. 4890).

Three mummified fruits from the same locality were later sent to J.T.P., who placed them in damp chambers, i.e. closed plastic boxes. A single apothecium (with one half in JTP 4895 and the other half in AH 7085) developed on one fruit on 19. VIII. 96, and from which cultures were obtained on P.D.A. from a mass-spore discharge, which subsequently formed immersed stromata but no apothecia developed in either this or subcultures. Single apothecia subsequently developed on the same mummy and were harvested on 27-IX. and 11-IX-1997.

Various fresh fruits, apples [Malus domestica Borkh. cv. “Sunset”] and Japonica [Chaenomeles speciosa (Sweet) Nakai], both from J.T.P.’s garden, and quinces [Cydonia oblonga Miller] from Bad Mergentheim, Baden-Württemburg, Germany, were inoculated in October, 1996, with culture material and placed in damp chambers, some of which eventually completely mummified. Apothecia subsequently developed on the mummified pomes of all three fruits and were harvested as they matured.

HISTORY

Found on mummified apples at Ann Arbor and Palmyra, Michigan, U. S. A., from 26th June to 9th August, 1909, and described as Sclerotinia aestivalis by Pollock (1909), the species appears to have been later collected on apples in a neglected greenhouse at College Park, Maryland, U. S. A., in November, 1911, by Demaree (1912), who reported it as a Sclerotinia sp. He thought it could be the perfect state of Monilinia “uredoformis” (sic) = M. urediniformis Ellis et Everhart (1893), when it “would be referred to Sclerotinia as Sclerotinia uredoformis”. The type, therefore, would have been a Monilinia, an anamorph, which is not acceptable for a telemorphic name, but as Demaree very clearly
used the subjunctive, the name was therefore provisional and has no nomenclatural status. A further collection was reported from Maryland in Norton et al. (1923). The fungus was found in 1921 on apples near Sydney, New South Wales, Australia, by Harrison (1922), and later (Harrison 1928 and 1935) also on apricots, quinces and, subsequently, on peaches, pears and plums, from December to March, who, on the advice of H. H. Whetzel, proposed a new combination in *Ciboria*. Seaver (1961) gave a similar description to Pollock’s with the distribution as “Michigan and Maryland?; also in Australia on various fruits”. Cannon & al. (1985) stated “on imported fruit” for the British Isles but enquiries with Dr. Cannon ascertained that it was based on an earlier report of the species under its synonym, recorded in Ramsbottom & Balfour-Browne’s (1951) list of UK discomycetes, which reports “*Sclerotinia aestivalis* Pollack (sic), grown by F. Harrison on quince brought to this country from Australia, May 1931 in Herb. BM”, which herbarium was subsequently transferred to K. Harrison (1935) mentions bringing mummified quinces from Australia, which were moistened to produce apothecia for a British Mycological Association (sic) meeting in November, 1930 etc. Spooner (1987) studied two of Harrison’s collections on quinces and provided a detailed redescriptions. Batra (1991) under “Imperfectly known *Monilinia*, Related Parasites and Excluded taxa” discussed Demaree’s findings and repeated Pollock’s diagnosis, with the comment “After examining the type (BPI), I am unable to ascribe it to the Sclerotiniaceae (sic)” . The collection on a “discarded peach nut” in the Forêt de St. Sever, Normandy, France, on 26th September 1994, mentioned in Shorten (1995) is an error as examination of the alleged “peach nut” by J.T.P. found it to be a typically wrinkled mummified cotyledon of a *Quercus* sp. bearing apothecia of *Ciboria batesiana* (Zopf) Buchw. (CRO 176 and J.T.P. 4980). Enquiries have ascertained that there are no collections of *Ciboria aestivalis* in IMI and only Harrison material from Australia in CUP and K.

**Description**


Apothecia clustered with short stipes in nature but single to sparse, with stipes of varying length, on mummified pome fruits (*Chaenomeles speciosa, Malus domestica* and *Cydonia oblonga*) inoculated with culture media and mummified in closed plastic boxes, also on blackened pips from within a mummified pome of *Malus domestica*. Disc slightly cupulate, becoming plane to shallowly or more deeply cupulate, sometimes convex, with varying reddish shades tending to darken with age, 2–4 mm diam in the Spanish collection (AH 7108), 0.7–4.5 mm diam on.
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Table 1. Disc, flank and stipe colours, all fresh apothecia except for AH 7108 (Spanish collection), which was revived, per Kornerup & Wanscher (1967)

<table>
<thead>
<tr>
<th>Herbarium collection</th>
<th>K. &amp; W. Key colour name – DISC</th>
<th>K. &amp; W. Key colour name – FLANK</th>
<th>K. &amp; W. Key colour name – STIPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH 7108</td>
<td>7A4 Pastel red</td>
<td>7A2 Reddish or pinkish white</td>
<td>7A2 Reddish or pinkish white</td>
</tr>
<tr>
<td>J.T.P. 4890</td>
<td>7A5 Pastel red</td>
<td>8A2 Reddish or pinkish white</td>
<td>8A2 Reddish or pinkish white</td>
</tr>
<tr>
<td>J.T.P. 4890</td>
<td>7B8 Reddish orange</td>
<td>8A2 Reddish or pinkish white</td>
<td>8A2 Reddish or pinkish white</td>
</tr>
<tr>
<td>J.T.P. 4895</td>
<td>8A3 Pale red</td>
<td>5A2 Orange white</td>
<td>5B4 Greyish red</td>
</tr>
<tr>
<td>J.T.P. 4946</td>
<td>6B4 Light orange</td>
<td>8A2 Reddish or pinkish white</td>
<td>8A2 Pale orange</td>
</tr>
<tr>
<td>J.T.P. 4947</td>
<td>7B3 Greyish red</td>
<td>7B3 Greyish red</td>
<td>7B3 Greyish red</td>
</tr>
<tr>
<td>J.T.P. 4950</td>
<td>6A2 Orange white</td>
<td>7B3 Greyish red</td>
<td>7B3 Greyish red</td>
</tr>
<tr>
<td>J.T.P. 4950</td>
<td>6A3 Pale orange</td>
<td>6B4 Light orange</td>
<td>6B4 Light orange</td>
</tr>
<tr>
<td>J.T.P. 4950</td>
<td>6B2 Orange grey</td>
<td>6A2 Orange white</td>
<td>6A2 Orange white</td>
</tr>
</tbody>
</table>

Table 2. Comparison of published measurements for asci and ascospores, showing minimum, average and maximum, with those found by authors in tap water.

<table>
<thead>
<tr>
<th>Publication/ Herbarium collection</th>
<th>ASCI</th>
<th>SPORES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pollock (1909)</td>
<td>51.0-85.0 × 6.0-8.5 μm</td>
<td>6.4-11.9 × 2.0-3.4 μm</td>
</tr>
<tr>
<td>Demaree (1912)</td>
<td>44.0-64.0 × 4.0-6.0 μm</td>
<td>6.0-8.0 × 2.0-3.0 μm</td>
</tr>
<tr>
<td>Harrison (1935)</td>
<td>56.6-81.7 × 4.8-7.7 μm</td>
<td>6.0-8.5-10.0 × 2.0-2.97-3.5 μm</td>
</tr>
<tr>
<td>Spooner (1987)</td>
<td>60.0-82.5 × 6.5-7.5 μm†</td>
<td>7.5-9-10.0 × 2.5-2.7-3.0 μm†</td>
</tr>
<tr>
<td>BPI 573925 Holotype</td>
<td>64.0-70.0 × 4.8-5.6 μm†</td>
<td>4.0-7.0-8.6 × 1.7-2.3-3.2 μm†</td>
</tr>
<tr>
<td>AH 7108 / J.T.P. 4890</td>
<td>75.0-90.0 × 5.0-6.5 μm*</td>
<td>5.6-7.9-11.2 × 2.2-2.6-3.2 μm*</td>
</tr>
<tr>
<td>J.T.P. 4895</td>
<td>68.0-77.0-86.0 × 8.0-8.1-9.0 μm*</td>
<td>8.0-8.9-11.2 × 2.4-2.8-3.6 μm*</td>
</tr>
<tr>
<td>J.T.P. 4945</td>
<td>72.0-82.0-92.0 × 8.0-8.1-9.0 μm*</td>
<td>7.8-9-12.0 × 2.3-2.8-4.0 μm*</td>
</tr>
<tr>
<td>J.T.P. 4946</td>
<td>81.0-92.0 × 5.0-6.8-8.0 μm*</td>
<td>7.7-8-10.4 × 1.8-2.5-3.3 μm*</td>
</tr>
<tr>
<td>J.T.P. 4947</td>
<td>72.0-77.0-86.0 × 8.0-6.1-7.0 μm*</td>
<td>7.8-9-10.4 × 2.0-2.4-2.6 μm*</td>
</tr>
<tr>
<td>J.T.P. 4950</td>
<td>88.0-77.0-90.0 × 5.0-7.0-7.2 μm*</td>
<td>7.4-8-10.4 × 1.9-2.6-3.3 μm*</td>
</tr>
</tbody>
</table>

Inoculated fruit, 0.5–1.3 mm diam in the holotype (after reviving apothecia, see Fig. 1). Colour variation shown in Table 1.

Receptacle tapering downwards into stipe, similar to or concolorous with disc and stipe (see Table 1), minutely downy at surface due to protruding excipular, catenulate cells. Stipe equal width or slightly tapering downwards, sometimes waved, often concolorous with disc or receptacle, sometimes darkening to almost black in Spanish collection, otherwise 1.2–7(-11) × 0.1–0.8 mm. Colour variation show in Table 1. Ectal excipulum very sharply delimited but poorly developed (ca. 3–5 cell layers, 20–40 μm thick) of *textura globulosa- subangularis* comprising hyaline to feebly pigmented cells 7–16 μm † diam, moderately thin-walled,
Fig. 1. *Sclerotinia aestivalis*: Revived apothecia from the type (BPI 573925)

Fig. 2. *Ciboria aestivalis*: Fresh apothecia emerging from an inoculated, mummified apple of *Malus domestica* (AH 7119)
strongly adherent, sometimes protruding as “catenulate hairs” on flank and stipe or as “clavate hairs” at the margin. Medullary excipulum well developed, consisting of hyaline to weakly pigmented loose hyphae (3–6 μm) forming a textura intricata. Subhymenium poorly developed and indistinguishable from the medullar area, apart from the brownish colour and highly intricate texture. Asci narrowly to sometimes broadly cylindrical, tapering at base to form a slender stalk, emerging from croziers, 8-spored, uniseriate to occasionally biseriate towards apex (dead state), which round to truncate, with plug slightly J+ (feebly blue) in Melzer's reagent, 68–92 × 5–9 μm (see Table 2). Spores hyaline, narrowly to broadly elliptical, regularly unicellular, frequently flattened on one side and showing a very characteristic form (i.e. asymmetrical), often with two small terminal apical guttules, less frequently one-guttulate, (5.6–)7.7–11.2–(12) × 1.9–3.3–(4) μm (see Table 2). Paraphyses straight, filiform, obtuse, sparsely septate, simple or branched below, ca. 1.5–2.5 μm diam (†), equalling the asci in length.

Habitat on pomaceous and stone fruits, which it completely mummifies, structure not investigated, with a dark brown stroma developing in P. D. A. cultures, submerging to about 2 mm and comprising irregularly shaped isodiametric (textura angularis), hyaline cells with the upper having a thin, black rind, and the surface whitish pruinose in older cultures. No conidia, sclerotia or similar structures were seen in the cultures.

Material examined


The following fruits all appeared to be in good condition with no sign of any Monilia infection, either prior to inoculation or subsequently. Several pomes of each fruit were inoculated with media from the J.T.P. 4895 culture on P.D.A. and, whilst some fruits deteriorated, others gradually mummified with no sign of conidia, and subsequently developed apothecia, which were harvested by J.T.P. as they matured.
Figs. 3-6. *Ciboria aestivalis*: Vertical sections of apothecia near the margin (Fig. 3) and on flanks (Figs. 4, 5 & 6). Scale bars are valid as follows: Fig. 3 for Fig. 4, Fig. 5 for Fig. 6. (Media: H₂O for Figs. 3 & 4 from AH 7119; Hoyer’s medium in phase contrast for Fig. 5 from AH 7108; KOH for Fig. 6 from AH 7119).
Figs. 7–14. *Ciboria aestivalis*. 7. Details of the ectal excipulum and outer layer of the medullary excipulum in phase contrast (AH 7119); 8. Catenate cells emerging from flanks and stipe after squash (AH 7119); 9. Label on "Sclerotinia aestivalis" type sheet, together with the hand-written annotation of J. B. Pollock; 10–13. Free-lying spores (Figs. 10 & 11 from AH 7119, Figs. 12 & 13 from AH 7108); 14. A group of asci. (AH 7108). Scale bar of Fig. 8 is also valid for the remaining figures. (Media: MLZ for Fig. 7; Lugol for Figs. 8 & 11; KOH for Fig. 10; CR blue for Figs. 12–14).
Fig. 15. *Ciboria aestivalis*: Apothecia which developed on mummified fruits inoculated in England. J. T. P. 4896 on *Cydonia oblonga*: a. Apothecia; b. An ascus; c. A paraphysis; d. Loose spores.

J. T. P. 4950 on *Chaenomeles speciosa*: e. Apothecia; f. Two asci; g. A branched paraphysis; h. Loose spores. J. T. P. 4947 on *Malus domestica*: i. Apothecia and developing stipes on blackened pip; j. Two asci; k. A branched paraphysis; l. Loose spores. Scale bar for Fig. 15e also for Fig. 15a; 15g also for Figs. 15b, 15c, 15f, 15j & 15k; and Fig. 15l also for figs. 15d and 15h. (All apothecia were drawn in fresh condition with microscopical observations made in tap water).

**Remarks**

Described as “light reddish brown” by Pollock (1909), the apothecia were given as “cinnamon-brown to gray” by Demaree (1912). Harrison (1935) reported the typical colour as “flesh pink, darkening appreciably to reddish-brown, and later to brown when drying and turning mealy with age”, with the range “from pale fresh pink to cornelian red, with the majority falling within the colour range between flesh colour and carrot red” in Ridgway (1912), which reasonably agree with our findings (Table 1).

It is noteworthy that cells from the Ectal excipulum show a few refractive walls which, together with their strong compaction, suggest that some could be gelatinized and therefore have a high resistance to separation by strong squashes, a feature rarely reported in this genus. In fact, although individual cells are mainly globose and maintain that form whilst alive and are observed in water mounts, occasionally some appear to be subangular, which could be explained by partial loss of turgescence, although maintaining their high adherence and “adopting” such an “unexpected” form.

Whilst the asci and spores of the Spanish collection and apothecia on inoculated fruits were in general agreement with the published description, Harrison (1935) stated in his discussion of the ascospores “They have no conspicuous oil spots or vacuoles”. Neither Pollock (1909) nor Demaree (1912) mentioned them and Spooner (1987) described them as “eguttulate” in the Harrison collections which he examined. None were seen in the holotype. Concerning the nature of such observed polar guttules, and following Baral (1992), their presence in living water mounts and optically vanishing in dead or lethal mounts (such as KOH 2%) reveals
that they could be "refractive vacuolar bodies" instead of lipid bodies, that are not dissolved in living or dead cells.

**DISCUSSION**

Whilst Harrison (1935) considered *Ciboria aestivalis* to be a possible parasite on fruits mummified by *Monilinia fructicola*, none of the pomes inoculated by J.T.P., and on which apothecia developed after mummification, showed any evidence of a *Monilia* infection.

In view of the statement in Batra (1991) that, after examining the type, he was unable to ascribe it to the Sclerotiniaceae and our findings that the fungus mummified pome fruits, J.T.P. wrote to the late Dr. Batra, who replied "As I recall, there were a few apothecia devoid of any stromatic mass attached to them".

We therefore decided to apply for the type collection in the Pathological and Mycological Collections of the United States Department of Agriculture in BPI. The herbarium sheet, with the typed label "*Sclerotinia aestivalis* Pollock. On *Pyrus malus*" affixed to the base, bore two packets. The first packet, labelled 573925 with "722" in pencil and a "TYPE" label, stated "*Sclerotinia aestivalis* on apple mummies, Ann Arbor, Mich. July 2, 1909 Coll, J. B. Pollock" and contained a hand-written slip "*Sclerotinia aestivalis* on apple mummies, July 2, 1909. Ann Arbor Mich. in 60% alcohol. Prof. J. B. Pollock, Sept. 1931 PC345[?]'(Fig. 9) with the preserved material comprising hard black fragments.

The second packet, labelled 573926, also with "722" in pencil, bore a typed label stating "*Sclerotinia aestivalis* Pollock from apple mummy N. S. Wales, 1921 Coll. T. H. Harrison" and contained a hand written slip "*Sclerotinia aestivalis* from apple mummy, N. S. Wales, T. H. Harrison, Chrome acetic 54°C wax" with the collection comprising 3 stalked apothecia vertically embedded in wax. This was obviously part of an Australian collection received from T. H. Harrison, but, because of the method of preservation, we decided not to examine.

The "hard black fragments" were found on moistening to comprise small, very much wrinkled pieces of mummified fruit, with one ca 1 cm diam fragment bearing a cluster of six apothecia, which were photographed after they had revived (Fig. 1). Two half apothecia were removed and studied with slides bearing microtome sections, dried in aqueous gum arabic or mounted in Hoyer's medium, attached to the type sheet. Although the apothecial structure was identical with our European specimens, the general appearance of the hymenial elements, which had most of their cells more or less collapsed, did not allow their measurement or precise description, apart from some asci and spores.

Our findings of the development of stromata in culture and the mummification of fruits does indeed confirm that *Ciboria aestivalis* belongs in the Sclerotiniaceae, where it should certainly be maintained in *Ciboria* on the basis of the ectal
excipular textura globulosa and the absence of any sclerotial structure. The development of apothecia on apple pips has not previously been reported for this species.

Acknowledgements

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References