

Phaeocalicium populneum

new to South-East Britain

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Introduction

The calicioid – or pin – fungi encompass tiny, mostly dark-coloured members of the *Ascomycota* with a simple or rarely branched stipe and a capitate, spore-bearing apex. Most are saprotrophs or weak parasites, found on twigs or bark of various trees and are usually less than 1 mm high. These fungi, though all similar in appearance and once referred mostly to the order *Caliciales*, have proved to be not all closely related and are placed now in at least three separate orders, *Caliciales*, *Coniocybales* and *Mycocaliciales* (Prieto *et al.* 2012). They differ in various ways, discussed further below, but in general the first two of these orders comprise lichenised species, associated with algae forming a thin though often rather scant basal thallus, whereas the *Mycocaliciales* are non-lichenised. These fungi also differ in other characters, notably their method of spore liberation. In the *Mycocaliciales* the asci are persistent, cylindrical, bearing usually eight dark-coloured, sometimes simple but often 1- to 3-septate ascospores; they hence exhibit active spore discharge. In the other orders the asci are diffluent, leaving the dark-coloured ascospores in an exposed mass termed a mazaedium, to be dispersed by wind or abrasion.

Having been previously referred to *Caliciales*, these fungi tend all to be dealt with in the lichen literature despite the lack of lichenisation in many of them. Around 60 species of calicioid fungi are known in Britain (Smith *et al.*, 2009), placed in nine genera distinguished morphologically by spore and ascus characters (see keys in Smith *et al.*, 2009). Amongst them are two species referred to the genus *Phaeocalicium* (*Mycocaliciales*): *P. praecedens* and *P. populneum*, both on *Populus*, and both scarce and mainly Scottish in distribution. Of these *P. populneum*, is reported here for the first time from S.E. England.

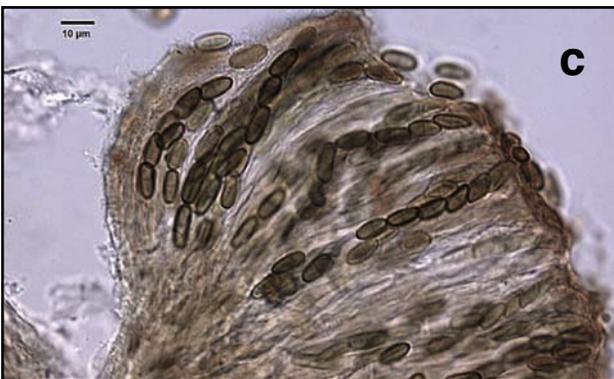
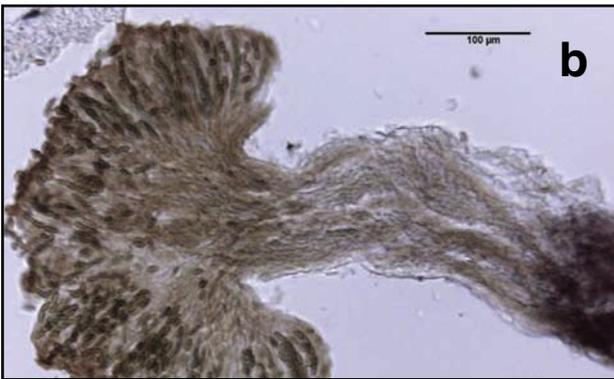
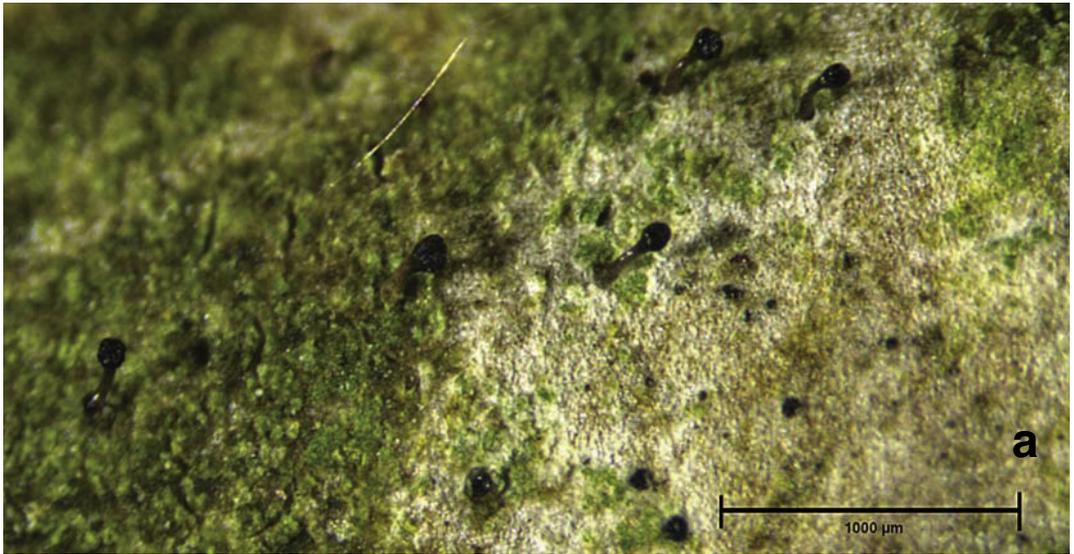
Position and nature of *Phaeocalicium*

The genus *Phaeocalicium* was described by Schmidt (1970) to accommodate three European species with stalked, blackish-brown, bell-shaped ascomata, growing as saprotrophs or weak parasites on twigs of alders and poplars: *P. compressulum*, *P. praecedens* (the type), and *P. populneum*. These three species had been previously treated in *Caliciaceae* in the genera *Calicium* and *Mycocalicium*, but all of them present active spore discharge, long asci (usually more than 80 µm), and large, smooth, pigmented ascospores. In the same work, Schmidt also created the family *Mycocaliciaceae* to accommodate *Phaeocalicium* and the genera *Chaenothecopsis* Vain., *Mycocalicium* Vain., *Stenocybe* Nyl. ex Körb., and *Strongyleuma* Vain. The new family was separated from the *Caliciaceae* especially by the presence of ‘narrow cylindrical asci with thickened walls at the apex and a persisting hyphal layer covering the hymenium’.

Schmidt’s five non-lichenised *Mycocaliciaceae* genera were still treated in the order *Caliciales* by Tibell (1984), though the genus *Strongyleuma* was there synonymised under *Chaenothecopsis*. This classification was based on morphological and chemical features. However, a cladistic analysis of the data had already shown the assemblage to be heterogeneous, and Tibell suggested further developmental studies were needed to clarify the relationships in the group. Later, Wedin & Tibell (1997) and Tibell & Wedin (2000) used sequence data from nuclear SSU rDNA to evaluate the monophyly of the group and found the *Mycocaliciaceae* only distantly related to the *Caliciaceae*. This prompted Tibell & Wedin (2000) to describe a new order, the *Mycocaliciales*.

This new order was also supported by Tibell & Vinuesa (2005) using the genetic markers LSU rDNA and ITS rDNA. In addition to molecular

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Figures of *Phaeocalicium populneum*

1a = habit, showing scattered apothecia

1b = verical section through apothecium

1c = close-up of v.s. of capitulum showing palisade of asci with ascospores, and excipular tissues

1d = 1-septate ascospores

data, the species in this new order were morphologically differentiated from the members of the *Caliciaceae* by the way in which ascospore ornamentation develops, as observed under Transmission Electron Microscopy. They showed that ornamentation is 'formed beneath the plasmalemma (cell wall), and not by the rupture of the perispore' as in the *Caliciaceae*. They also showed that the current morphological delimitation of the genera *Chaenothecopsis* and *Phaeocalicium* based on spore size and lifestyle is not well supported by DNA, and that *Phaeocalicium populneum* clusters with *Stenocybe pullatula*. The latter is a species also found in the British Isles, but on decaying branches of *Alnus glutinosa*, morphologically distinguished from *P. populneum* by branching ascomata, and 1-3 septate ascospores.

All 22 species currently accepted in the genus *Phaeocalicium* (Index Fungorum: <http://www.indexfungorum.org/Names/Names.asp>; accessed March 2019) are weak parasites or saprobes on bark of vascular plants and known mainly from temperate and cool areas in the Northern Hemisphere. In the British Isles, as noted, only two species have been recorded: *P. populneum* and *P. praecedens*, both found on living or decaying twigs and branches of poplars (*Populus* spp) (Giavarini & Purvis 2009). These two species can be unambiguously separated only by ascospore septation. The colour of the stalk after application of 10% potassium hydroxide (K) has been recorded in *P. populneum* as reddish brown by Tibell (1996). Yet Giavarini & Purvis (2009) mention in their identification key for British material the presence of an additional blue-green pigment which helps to differentiate this species from *P. praecedens* (which remains reddish brown in K). We have encountered this emerald green pigment in the following vouchers: E00817771, E00817779, K(M) 205103 and K(M) 235981. However, in the other material examined and listed below, colour changes after the application of K were seen only in the capitulum; we were unable to observe any colour change in the stalk.

Older British collections such as vouchers: E00817778, E00817779, E00817780 formerly identified as *Stenocybe tremulicola* (syn. *Phaeocalicium tremulicola* (Norrl. ex Nyl.) Tibell) are now accepted misidentifications pertaining to *P. populneum* (Giavarini & Purvis 2009). We can

confirm the ascospores in the material examined are always 1-septate rather than 3-septate as in *P. tremulicola*, and the shape of the capitulum is lenticular rather than campanulate.

British distribution

In the British Isles, both *Phaeocalicium* species are largely confined to Scotland, occurring especially in native aspen stands (Ellis, 2008). They have been recorded mostly from Central and East Highlands, with a few records from the Northern Highlands (BLS database; Janet Simkin pers. comm.), and a single early record of *P. populneum* from Eire (Turner & Borrer 1839). Woods & Coppins (2012) evaluated their conservation status and both were given the categories of 'least concern' (LC), but 'nationally rare' (NR), and were regarded as priority taxa for biodiversity in Scotland.

Phaeocalicium praecedens is known outside Scotland from a single collection from Cumberland (England). A collection of *P. populneum* from S. Devon (VC 3), Morwhellan Quay SSSI, made in Oct. 2006, was the first and hitherto only collection from England, and another from Carmarthenshire (VC44), Caerfyrddyn, 30 Mar. 2014, S. Bosanquet, is the only Welsh collection to date (BLS database; J. Simkin pers. comm.). We have not seen vouchers for either of these collections.

The collections reported here from Surrey (VC 17) are thus from the second English site and the first in the South-east. A collection from Esher Commons SSSI, West End Common, was made in May 2016 [K(M) 205103], and further material was collected from this site a year later [K(M) 235981], both near tips of slender, still attached, living but fading twigs of the introduced host *Populus x canadensis*. The host, a mature but young tree c. 40–50 years old, grows near a wet area at the edge of a scrub/grassland area developed over an old land-fill site. It is perhaps worth noting that fallen branches were almost devoid of apothecia. Unfortunately, since then, this tree has partly collapsed (2018) but the *Phaeocalicium* is still present.

In this contribution, we provide a detailed description of the species based on the Surrey collections and others examined. These include one of the earliest Scottish collections, from Argyll, Aug 1876, ex herb. J.M. Crombie, which is now housed within the reference collections of the

Natural History Museum, London (BM). An earlier voucher from Killarney (Eire) collected by Sir T. Gage and described as a new variety by Turner & Borrer (1839): *Calicium curtum* var. *populinum* (fide Hawksworth & Seaward, 1978), now regarded as a synonym of *P. populneum*, is also stored in BM; no historical vouchers of this taxon were found in the separate K-Borrer collection.

Phaeocalicium populneum (Brond. ex Duby) A.F.W. Schmidt Mitt. Staatsinst. Allg. Bot. Hamburg 13: 132 (1970). **Figs 1a–d.**

Calicium populneum Brond. ex Duby, *Bot. Gall.*, Edn 2 (Paris) 2: 638 (1830)

Embolidium populneum (Brond. ex Duby) Vain., *Acta Soc. pro Fauna et Flora Fennica* 57: 59 (1927)

Syn. *Calicium curtum* var. *populinum* Turner & Borrer, *Spec. Lich. Brit.*: 149 (1839) [BM000975694, lectotype!]

Thallus: absent; though in some vouchers small groups of chlorococcoid algae are seen surrounding the base of the stalks; less than 65 µm in height over the host.

Anamorph: not observed; Tibell (1997) successfully grew in axenic culture both *P. populneum* and *P. praecedens*, but neither produced anamorphs. Three different types of media were used in his investigations: MEA (malt extract peptone), MYE (malt yeast extract) and PDA (potato dextrose agar), but it is not clear from the publication whether these *Phaeocalicium* species grew on all three media.

Teleomorph: Ascomata epruinose apothecia, stalked, reddish to dark brown, appearing blackish in the field, 0.45 – 0.7 mm high, shiny. Capitulum almost globose when young, becoming lenticular with age, consisting of a thin layer of reddish brown hyphae, which in microscope mounts resemble irregular shells, forming a textura epidermoidea. Stalk paler than the capitulum, sometimes almost grey and glistening, but under the microscope reddish to pale brown throughout, consisting of periclinally arranged, slightly intertwined dark hyphae up to 4 µm diam.; middle part of stalk swelling after application of 10% KOH, the loose hyphae becoming paler and, in a few vouchers, the stalk, usually K–, becoming K+ emerald green at first, and later darker brown, at the base in a few vouchers. Capitulum 0.2 – 0.3 mm wide, darker brown

than the stalk. Exciple up to 15 µm wide, formed by few (4–5) hyphal layers, in fascicular or anticlinal arrangement, consisting of thick-walled, elongated cells 13–15 x 3–3.5 µm. Hymenium up to 110 µm, pale brown, hypothecium of same coloration, and paraphyses simple and slightly capitate, all tissues mostly K–, but in a few vouchers intensifying reddish-brown, in 50% Nitric Acid HNO₃ (N+) the colour intensifying reddish to almost claret (fleeting). Asci narrowly cylindrical, 75–85 x 4–5.5 µm, with uniformly thickened apex penetrated by a short and blunt canal, 8-spored. Ascospores uniseriately arranged in the ascus, (0-) 1-septate, brown, narrowly ellipsoidal, 11–15.5 x 4.5–5.5 µm, with 0.4 µm thick, smooth to finely ornamented outer walls, and a lightly pigmented septum.

Chemistry: The presence of a green pigment in the stalk after the application of 10% KOH is only recorded in a few British vouchers; for comparison see the monographic works on the genus from northern Europe (Tibell 1996), Central Europe (Zimmermann 2012), or the Iberian Peninsula (Muñiz & Hladun 2011) in which the colour change reported was reddish. We cannot correlate the presence or absence of the pigment to the age of collection or to their hosts, though we can confirm that it is only found at the base of the stalk in all the collections examined. In some vouchers the reddish colour is only observed in the capitulum; e.g. in collection E00817780.

Key to species in Britain

- Ascospores 1-septate; apothecial stalk K–,
occasionally K+ emerald green at the base...
.....*P. populneum*
- Ascospores simple; apothecial stalk often K+
emerald green, especially at the base of the
capitulum spreading to the top of the stalk
.....*P. praecedens*

Material examined

[Note that the records listed below are not included in the following British Lichen Society online links:

http://www.britishlichensociety.org.uk/resources/species-accounts/Phaeocalicium_praecedens and http://www.britishlichensociety.org.uk/resources/species-accounts/Phaeocalicium_populneum]

Eire, Killarney, on smooth bark, T. Gage s.n. (BM s.n. as *Calicium populneum* [*curtum* erased];

possibly part of BM000975694 – lectotype of *Calicium curtum* var. *populinum*). – England, Surrey, Esher (near), West End Common, on attached twigs of *Populus x canadensis*, 22 May 2016, B.M. Spooner (K(M) 205103); loc. cit., 13 Feb 2017, B.M. Spooner (K(M) 235981). – Scotland, Argyll, Appin, Airds, on branches of poplars, Aug 1876, J.M. Crombie] (ex herb. J.M. Crombie; BM s.n. as *Calicium populneum*). Easternness (Nairn), Cawdor Wood, on twigs of *Populus tremula*, 9 Sep 2009, B.J. Coppins 23571 (E00660945 as *Phaeocalicium populneum*). East-Perthshire, Blairgowrie (near), Enochdhu, Kindrogan Field Centre (by drive), 850 feet, on twig of *Populus* sp. (2 year old), 27 Nov-1971, U.K. Duncan s.n. (E00817780 as *Stenocybe tremulicola*); loc. cit., on twigs of *Populus* sp., 30 Mar 1977, B.J. Coppins 2788 (E00817778 as *Stenocybe tremulicola*).

Acknowledgements

We wish to thank Janet Simkin for records of *P. populneum* from the BLS database, Dr Holger Thüs for access to collections in BM, and the fungal curators of E for loan of material. Finally, we would like to acknowledge Prof. Mats Wedin (S) and Alick Henrici for review comments and improvements to the text, and Geoffrey Kibby for assistance in preparing the accompanying images.

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