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The enigmatic woolly aphid associated fungus '*Cucurbidothis pithyophila*', now threatening Scots pine in the UK, is revealed as two distinct but co-occurring species

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Abstract: In October 2022, disease symptoms in the form of multiple blackened cankers and dieback of shoots and branches were noted in Scots pine (*Pinus sylvestris*) in Scotland. Trees were affected across a range of site types, including natural Caledonian pine forests, planted native woodland and commercial plantations. We investigated the geographical extent of symptoms in the UK, which occurred across most regions of Scotland, and identified one of the primary causal agents to be a previously obscure stroma-forming fungal taxon '*Curreya pithyophila'*. The fungus harbours immature colonies of the native Scots pine woolly adelgid, *Pineus pini*, which live beneath the stroma and feed on the tree, initiating wounds. These wounds can then be invaded by secondary agents, such as a fungal pathogen of pine, *Crumenulopsis sororia*, which is thought to cause the blackened cankers. Historical reports suggest that previous outbreaks of '*Curreya pithyophila*' occurred on plantation Scots pine in Perthshire in the 1900s and in north-east Scotland in the 1960s. A literature review of the taxonomy and ecology of '*Curreya pithyophila*' is presented. The perplexing ability of this fungus to produce two ascospore forms (phragmospores and dictyospores) from apparently morphologically identical stromata, at the same site and often on the same tree or branch, was investigated. After morphological, culture and genetic analyses of single spore isolates, we conclude that the current population of '*C. pithyophila*' in the UK comprises two distinct but co-occurring species. Multigene analyses show that the closely related but separate species occur in the family *Leptosphaeriaceae* in a clade with *Alloleptosphaeria* and sister to *Leptosphaeria*. Morphological comparisons with historical syntypes confirmed that the dictyospore-producing species corresponds to *Sphaeria pithyophila* (as '*pityophila*'), while the phragmospore-producing species matches a syntype of *Sphaeria parmeliarum*. We retain *Cucurbidothis* as the correct generic name, designate a lectotype for the type species *Cucurbidothis pithyophila*, and recombine the second species as *Cucurbidothis parmeliarum comb. nov.*, also designating a lectotype. These species differ not only in spore morphology but also in culture appearance, with *C. pithyophila* producing grey and *C. parmeliarum* producing apricot coloured cultures. Both species can also produce a coelomycetous asexual morph comprising brown, ellipsoidal conidia, with pycnidial conidiomata often occurring adjacent to the ascomata in the same stroma. Histological studies showed that both taxa only penetrate between the periderm layers with no evidence of fungal growth in the living phloem and cambium below, supporting the hypothesis that these species are dependent on the adelgids for nutrition. A third species, *Cucurbidothis shangrilana comb. nov.*, is also discussed.

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INTRODUCTION

Caledonian pine is the name given to the relict populations of endemic Scots pine (*Pinus sylvestris*) which colonised Britain from refugia in Europe following the last ice age (Kinloch *et al.* 1986). These populations are found in the Scottish Highlands and, despite their fragmented nature, have huge ecological, conservation and cultural value due to the diversity of native flora and fauna which they support. For this reason, Caledonian pine is regarded as Scotland's most iconic tree

species. Recently, however, its vulnerability to increasing biotic health impacts has been highlighted, in particular the needle cast pathogen *Dothistroma septosporum* (Piotrowska *et al.* 2018).

In late 2022, blackened cankers and dieback symptoms were reported on shoots, branches and stems of Caledonian pine of all ages in the Cairngorm region of Scotland (Supplementary Material Fig. S1). In addition, on many symptomatic Scots pine trees an unusual black stroma-forming fungus was observed which encircled shoots and branches (Green *et al.* 2024). Beneath each stroma were dense colonies of the nymph stage of a normally free-living species of adelgid. These are host-specific sap-sucking insects (Family Adelgidae: Hemiptera) also known as woolly aphids due to the white wax 'wool' they produce (Havill & Foottit 2007). Preliminary investigations suggested that the stroma-forming fungus, in its association with the adelgids, may be a primary agent of the canker disease (Green *et al.* 2024). The feeding activity of the adelgids beneath the stromata cause wounds on Scots pine which can become infected by the fungal wound parasite *Crumenulopsis sororia*, resulting in the blackened cankers (Green *et al.* 2024).

The stromatal fungus was provisionally identified as two distinct forms, based on ascospore morphology, of a species currently known as *Curreya pithyophila*, a sporadically occurring species normally considered rare (Green *et al.* 2024). As the disease symptoms were concerning and widespread on natural as well as planted Scots pine, this study was undertaken to investigate further the potential role and identity of the black stromatal fungus, and the relationship between the two morphological forms. A comprehensive literature review was undertaken on this previously obscure fungus and is detailed below in chronological order.

Taxonomic literature review

What was thought to be a single variable species, or at best varieties of that species, was known until now as '*Curreya pithyophila*' (or one of the synonyms, www.speciesfungorum.org). For this section, reference will be made to a single organism in line with its treatment in the literature. Images of protologues and sanctioning works, and other relevant literature are given in Supplementary Material Fig. S2.

The taxonomy of this fungus has been problematic due to the presence of two ascospore 'forms' (phragmospore and dictyospore). It was first described in 1817 (by Schmidt & Kunze 1817) and in 1823 the name *Sphaeria pithyophila* [as '*pityophila*'] was sanctioned by Fries (1823). It was recorded on the living trunks and branches of *Pinus sylvestris* during spring in Germany and was distributed in the exsiccatum Deutschl. Schwämme (no. 133). Only the superficial stroma morphology was described, with no discussion of the adelgids or ascospore descriptions. However, inspection of syntype material showed that the ascospores are dictyospores and several publications have noted this also (for example see Saccardo 1883: 311, Holm 1967).

In 1863 there were two treatments of this fungus: *Cucurbitaria pithyophila* (Cesati & De Notaris 1863), with phragmospores illustrated, according to Holm (1967) based on material of Erbar. Crittog. Ital. no. 989 (De Notaris 1863: 60); and *Diplodia pityophila*. Holm (1967) commented that the placement in *Cucurbitaria* was inappropriate as species in this genus have dictyospores [as in the modern circumscription by Jaklitsch *et al.* (2018)]. A fungarium label for *Diplodia pityophila* (Fuckel, Fungi Rhen. Exs. fasc. 6: no. 538) notes that the fungus was very rare on dry branches of pine in the autumn with ascospores described as 'septate' (see notes in Supplementary Material Fig. S2). Neither treatment mentioned adelgids, which are dry and unrecognisable in fungarium specimens.

In 1873 *Cucurbitaria pithyophila* var. *cembrae* was described (Rehm 1881; Rehm, Ascomyc. no. 147) representing the phragmospore variety to distinguish it from the dictyospore form.

The first published record in the UK was in Wales in 1876 when the fungus *Sphaeria parmeliarum* was described as a 'parasite' of the lichen *Parmelia saxatilis*, on a living 'spruce fir' (possibly Nordmann fir, *Abies nordmanniana*) (Phillips & Plowright 1876), distributed in exsiccatum Sphaeriacei Brit. [Cent. 3] no. 52. In 1883 the species was renamed *Leptosphaeria parmeliarum* (Saccardo 1883: 83). The type specimen upon which these names were based has

Table 1. Fungal PCR primers and conditions used in the present study.

Gene region	Primer pairs	PCR mix	PCR programme	Reference for primer sequences
ITS	ITS1F / ITS4	40 µL reaction volume: 20 µL Quick-Load Taq 2× Master Mix (New England BioLabs); 2 µL of each primer (10 µM); 2 µL template DNA	95 °C 2 min, (95 °C 35 s, 55 °C 55 s, 72 °C 45 s) 30 cycles, 72 °C 10 min	White *et al. (*1990), Gardes & Bruns (1993)
tef1-α	EF1-728F / EF1-986R	As for ITS	95 °C 30 s, (95 °C 30 s, 53 °C–58 °C [across 6 zones] 30 s, 68 °C 1 min) 35 cycles, 68 °C 5 min	Carbone & Kohn (1999)
β-tubulin (*tub2*)	TUB2Fd / TUB4Rd	As for ITS	As for *tef1-α*	Aveskamp *et al.* (2009)
γ-actin	ACT-512F / ACT-783R	As for ITS	As for *tef1-α*	Carbone & Kohn (1999)
nuLSU	LR0R / LR5	25 µL reaction volume: 12.5 µL Quick-Load Taq 2× Master Mix (New England BioLabs); 1 µL of each primer (10 µM); 1 µL template DNA	95 °C 5 min, (95 °C 30 s, 55 °C 50 s, 72 °C 90 s) 35 cycles, 72 °C 10 min	Rehner & Uecker (1994), Wanasinghe *et al.* (2020)

phragmospores [see illustration by Berlese (1900) p. 61 and tab XLVII, fig. 4]. No mention was made of adelgids in the descriptions above or by Holm (1967) who studied the type material. Holm (1967) mentions three homotypic synonyms: *Melanomma parmeliarum* (in Cooke 1887); *Psilosphaeria parmeliarum* (in Cooke & Plowright 1879); *Heptameria parmeliarum* (in Cooke 1889); and also cites *Phaeospora parmeliarum* a name given by Vouaux (1913).

In North America in 1876 (in the same issue of Grevillea as *Sphaeria parmeliarum*), a fungus was described with dictyospores ('vertical septa') on *Pinus strobus* in Massachusetts under the name *Melogramma spraguei* (Berkeley 1876) and synonymised in 1883 as *Thyridium spraguei* (Saccardo 1883). Holm (1967) saw material and synonymised these names with *Cucurbidothis pithyophila* var. *pithyophila*.

In Europe by 1883, *Cucurbitaria pithyophila* had been described from the bark of spruce and pine in Germany, Sweden, France, Italy and Belgium (Saccardo 1883). Cavara (1897) recorded *Cucurbitaria pithyophila* var. *cembrae* on *Abies* in Italy, attributing the callousing (hypertrophy) to the fungus but not mentioning the insects. The subsequent formation of cankers infested by wound pathogens was noted. Cavara (1897) indicated that the disease occurred on young and old firs both at high and lower altitudes in the Apennines.

The first record of this fungus in Scotland dates back to 1907 when McIntosh (1915) found the disease in Scots pine plantations (in Perth and Kinross), suspected to be grown from 'foreign seed' due to their uncommon growth form. There was no mention of adelgids here and no micromorphological details.

Holm (1967) suggested that von Höhnel (1918) was the first to notice the asexual morph naming it *Microsporella pityophila* [which was later (Petrak & Sydow 1927) synonymised under *Coniothyrium pityophilum*]. No mention of the sexual morph (and the ascospore type) was made. A previous record of *Phragmotrichum* by Fuckel (1870) reported to be the asexual stage of *Cucurbitaria pithyophila* was considered to be incorrect (Holm 1967, Casagrande 1969).

In 1921, the name *Cucurbidothis pithyophila* was given to this fungus by Petrak (1921) when the new genus *Cucurbidothis* was introduced for this species. Petrak (1921) noted that the development and structure of the ascomata differed from *Cucurbitaria* and observed dictyospores but no adelgids.

In 1926, Welch (1926: 81) first reported the association with 'scale insects', in a monograph of *Cucurbitaria* where *Cucurbitaria pithyophila* is excluded. Adelgids ('chermes') were also noted on collections made in North America in the 1920's and 30's (see notes on specimens on GBIF using the search term *Cucurbitaria pithyophila*). Boyce (1952) examined specimens from pine of *Cucurbitaria pithyophila* (with no mention made of the spore form) and noted adelgids, describing the fungus as 'entomogenous'. Boyce (1952) also discussed that, in the USA, the adelgid association was known about since the 1920's with speculation that the insects had been introduced from Europe.

von Arx (1954) transferred *Cucurbidothis pithyophila* to *Gibberidea* (*G. pithyophila*) considering the earlier name *Gibberidea* to be congeneric, and therefore *Cucurbidothis* could not be maintained. A justification for the synonymy was that apparently only phragmospores were observed, characteristic of *Gibberidea*.

Adelgids (*Adelges piceae*) were first reported in Europe by Franz (1955) with *Cucurbitaria pithyophila* (phragmospore form), on *Abies alba*, in what was the first of three early studies on the biology of the fungus (see 'Discussion').

Petrak (1963) provides more detail about the morphology of *Cucurbidothis pithyophila,* based on a specimen with phragmospores but he also includes dictyospores in the updated generic description given, stating that the difference in ascospore morphology is of no importance. There is no mention of the synonymy by von Arx (1954) with *Gibberidea*.

Holm (1967) agreed with Petrak (1921, 1963) that the correct genus was *Cucurbidothis* and that the fungus was not related to *Cucurbitaria* based on the anatomy of the stroma (including around the ascomata), or *Gibberidea* (von Arx 1954); noting that it was reminiscent of 'true *Leptosphaeriae*' both in peridium anatomy (scleroplectenchyma) and the phragmospores. There is no mention of adelgids, despite three published records then available (Welch 1926, Boyce 1952, Franz 1955). Holm (1967) notes the taxonomic treatments of this fungus in Europe and North America, and based on differences in ascospore morphology and size, outlines how the two forms should be recognised as two varieties of *Cucurbidothis* with the dictyosporous *Cucurbidothis pithyophila* var. *pithyophila* and the phragmosporous *Cucurbidothis pithyophila* var. *cembrae*. Holm (1967) concludes this after studying many specimens from different *Pinaceae* hosts in Europe and North America, and being able to divide specimens into dictyospore or phragmospore forms, finding no 'truly transitional material'.

In the second study of the biology of the fungus, Murray & Parry (1969) recorded adelgids (*Pineus pini*) and found the dictyospore form on Scots pine in north east Scotland, referring to the fungus as *Cucurbitaria pithyophila.* Simultaneously, Casagrande (1969) published the most detailed study to date on the biology of the fungus, in addition to a survey of its host and geographical distribution. Casagrande (1969) considered that there was only one species and stated that the description needed to be updated to include dictyospore and phragmospore forms. It was noted that there were many specimens available at the ETH Zurich fungarium (ZT), plus cultures kept there, although the cultures are no longer available (R. Berndt, pers. comm.). A summary of the systematics of the sexual and asexual morphs was given and it was concluded that *Cucurbidothis* was the correct name for the sexual morph and *Coniothyrium pityophilum* for the asexual morph.

Petrak (1969) compared *Cucurbidothis* with the genus *Gemmamyces* (Casagrande 1969), another stromatic ascomycete occurring on *Picea*, and concluded that there was no difference between the two, synonymising *Gemmamyces* (now reinstated in the *Melanommataceae* (Jaklitsch & Voglmayr 2017)).

Takahashi & Saho (1972) report the dictyosporous form of *Cucurbitaria pithyophila* on various species of plantation and native pines and fir in Japan, describing the disease symptoms on fir as common on young trees, especially in cold regions. No mention of adelgids is made.

von Arx & Müller (1975) synonymised *Cucurbidothis* under *Curreya* in the family *Pleosporaceae* (*Curreya pithyophila*)

and stated that the asexual morph was a '*Coniothyrium*', whereas Barr (1981) did not accept the synonymy and after examining the type specimens of both genera concluded that *Cucurbidothis* was 'certainly distant' from the type of *Curreya* (*C. conorum*) and that *Cucurbidothis* 'must be retained in a separate genus' but did not discuss it any further. von Arx & van der Aa (1983) acknowledged that the placement of this species was debatable but retained it in *Curreya* providing *Curreya pithyophila* var. *cembrae* for the phragmospore form. Barr (1990) reinstated *Cucurbidothis* (monotypic with the two varieties of *Cucurbidothis pithyophila*) and placed it in the family *Cucurbitariaceae* (*Pleosporales*) with *Cucurbitaria*. However, Species Fungorum recognised the treatment by von Arx & Müller (1975) and so retained *Curryea pithyophila* (but in the *Cucurbitariaceae*) as the current name. Curiously in the notes on *Cucurbidothis pithyophila*, Barr (1990) states that the phragmospore form is 'known from Europe', while fungarium specimens originating from North America have, to date, only exhibited the dictyospore form (J.E. Taylor, pers. obs.). Genetic studies are being undertaken on the origin of the two forms in the UK (unpublished data).

Ariyawansa *et al.* (2014) retain *Cucurbidothis pithyophila* (*Cucurbitariaceae*) and omit in their description that it is associated with adelgids, and also mistakenly state that the specimen examined (UBC-F3787, recorded in 1950 in BC, Canada and referred to as a 'paratype') was on 'dead wood' of *Pinus monticola*, a detail of the host substrate that was not mentioned in the label. No mention is made of the stroma but it is described as having 'superficial ascomata growth on conifer wood'. Jaklitsch *et al.* (2018) treat *Cucurbitariaceae* but state that, due to its coniothyrium-like asexual morph, *Cucurbidothis* '*pityophila*' does not belong in the *Cucurbitariaceae,* but go no further to suggest where it might belong. According to Valenzuela-Lopez *et al.* (2018), *Curreya pithyophila* is shown as a member of the *Didymosphaeriaceae*, but see 'Discussion' for an explanation of this finding.

Modern treatments lack molecular data (Ariyawansa *et al.* 2014, Jaklitsch *et al.* 2018) and, as will be discussed later, all the strains available for '*C. pithyophila*' housed at CBS are misidentified, and thus the sequences available on GenBank and cited in past literature are also incorrect. Information compiled in the course of the present study based on taxonomy, culture studies and multigene molecular data of numerous specimens mainly from Scotland, reveal that there are two species (as has been alluded to previously, see Holm 1967) which share the same unique ecological niche, and are indistinguishable macroscopically but differ in microscopic anatomical features such as spore morphology (of both ascospores and conidia), culture morphology and molecular data (Green *et al.* 2024).

MATERIALS AND METHODS

Survey of Scots pine, sample collection and processing

Samples were collected of living material from Scots pine bearing stromata, between November 2022 and November 2023 with general site information, grid reference and collection date recorded for each sample (details of all 76 specimens examined are given in Supplementary Material Table S1). Stromata were examined under a dissecting microscope (Leica S9D or Leica Wild M10) and micromorphology of the sporulating structures was assessed with a light microscope (Leica DM 2500 LED or Leica DN 750). Macro- and micromorphology were recorded (images captured with a Leica K3C or Leica DFC425C digital camera) and measurements from specimens mounted in water were made of asci, ascospores and conidia (minimum of 10 values), as well as other structures, and presented as (min–) $\bar{x}$ ± SD(–max), *n* = the number of measurements. Herbarium specimens were air dried and stored in paper envelopes at room temperature and are deposited at the herbarium of the Royal Botanic Garden Edinburgh (E).

For single spore isolations, individual fruiting bodies were removed from stromata, squashed in 10 % glycerol solution on a sterile microscope slide with a cover slip, and spores were verified under a light microscope and photographed for record keeping. Spore suspensions in glycerol were then diluted, if necessary, and spread onto 2 % malt extract agar (Oxoid, Bacteriological Agar or Thermo Scientific, 1.5 %) supplemented with 0.25 g/L streptomycin sulphate (Thermo Scientific), and incubated at room temperature for a few days. Individual germinating spores, as visualised under a dissecting microscope, were removed from the spread plates using a fine needle, transferred to fresh MEA and incubated

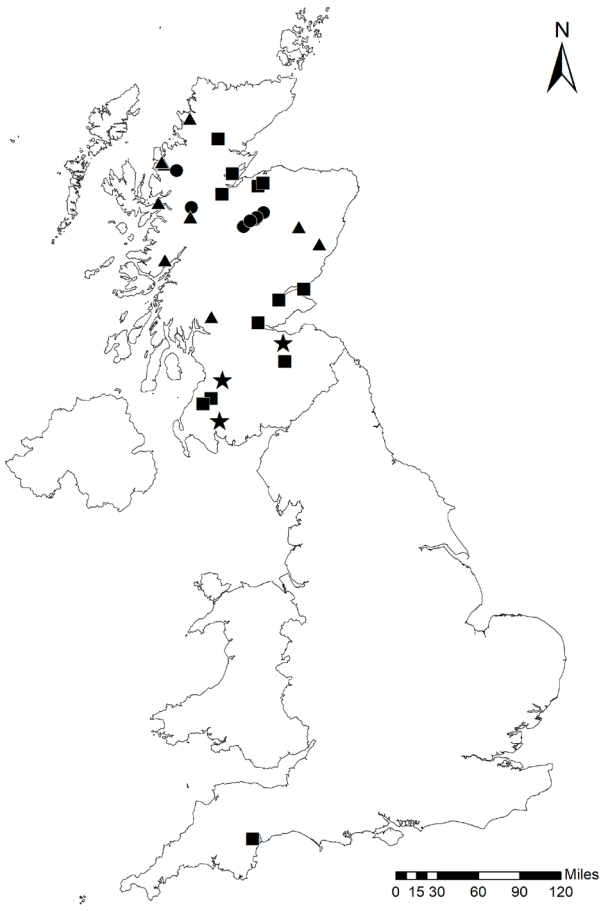

Fig. 1. Location of 31 sites in the UK (mainly Scotland with one site in Devon in the southwest of England) from which samples were collected of Scots pine with stroma present. Symbols represent Caledonian pinewood (circle), native woodland planting (triangle), commercial plantation (square) and amenity/shelterbelt planting (star).

at 15 °C to obtain single spore isolates. Isolates are stored at Forest Research, Northern Research Station, UK.

Genetic analyses

DNA extraction, PCR and sequencing

Genomic DNA was extracted from the mycelium of single spore isolates of 37 specimens (Supplementary Material Table S1). A fragment of mycelium was collected from each culture with a sterile pipette tip, placing it in 20 µL of 25 mM sodium hydroxide, incubating in a thermal cycler (Biometra TGradient) at 99.9 °C for 15 min, followed by 4 °C for 5 min, then adding 20 µL 40 mM Tris-HCL pH 5 (Forest Research SOP rapid DNA extraction protocol modified from Collado-Romero *et al.* 2006). The DNA used for the nuLSU PCR was extracted from freeze-dried mycelium with a Fungal/Bacterial Quick-DNA extraction kit (Zymo Research) following the manufacturer's protocol. Isolates (1–3 per fungal specimen) were subject to PCR amplification of the internal transcribed spacer region of the ribosomal DNA (ITS) (Schoch *et al.* 2012) using the universal ITS1/ITS4 primers (White *et al.* 1990). Up to three of the same isolates were additionally tested using three further DNA barcode regions commonly used for genetic comparisons among fungal species (Tekpinar & Kalmer 2019): i) the partial translational elongation factor 1-alpha (*tef1-α*) gene using the EF1-728F/ EF1-986R primers (Carbone & Kohn 1999)); ii) the partial β-tubulin (*tub*2) gene using the TUB2Fd/TUB4Rd primers (Aveskamp *et al.* 2009); iii) the partial γ-actin gene using the ACT-512F/ACT-783R primers (Carbone & Kohn 1999); iv) the partial large subunit nuclear ribosomal DNA (nuLSU) using the LR0R/LR5 primers (Rehner & Uecker 1994, Wanasinghe *et al.* 2020) (Table 1). was Amplification reactions were carried out on a Biometra TGradient or Applied Biosystems VeritiPro thermal cycler and PCR conditions are given in Table 1. The PCR products were verified by visualisation on a 1 % agarose gel and remaining primers removed with a DNA Clean & Concentrator kit (Zymo Research) before Sanger sequencing at the James Hutton Institute. All sequences generated in the present study are outlined in Supplementary Material Table S1.

Phylogenetic analysis

Consensus sequences were checked and edited using Sequencher v. 5.4.6 and searched against published sequences in the GenBank NCBI nucleotide database using BLASTN+ (Altschul *et al.* 1990). Closely related taxa with verifiable sequences derived from voucher specimens or published taxonomic papers were selected (including Ariyawansa *et al.* 2015, Aiello *et al.* 2020, Phukhamsakda *et al.* 2020, Thiyagaraja *et al.* 2021, Xu *et al.* 2022, Gao *et al.*

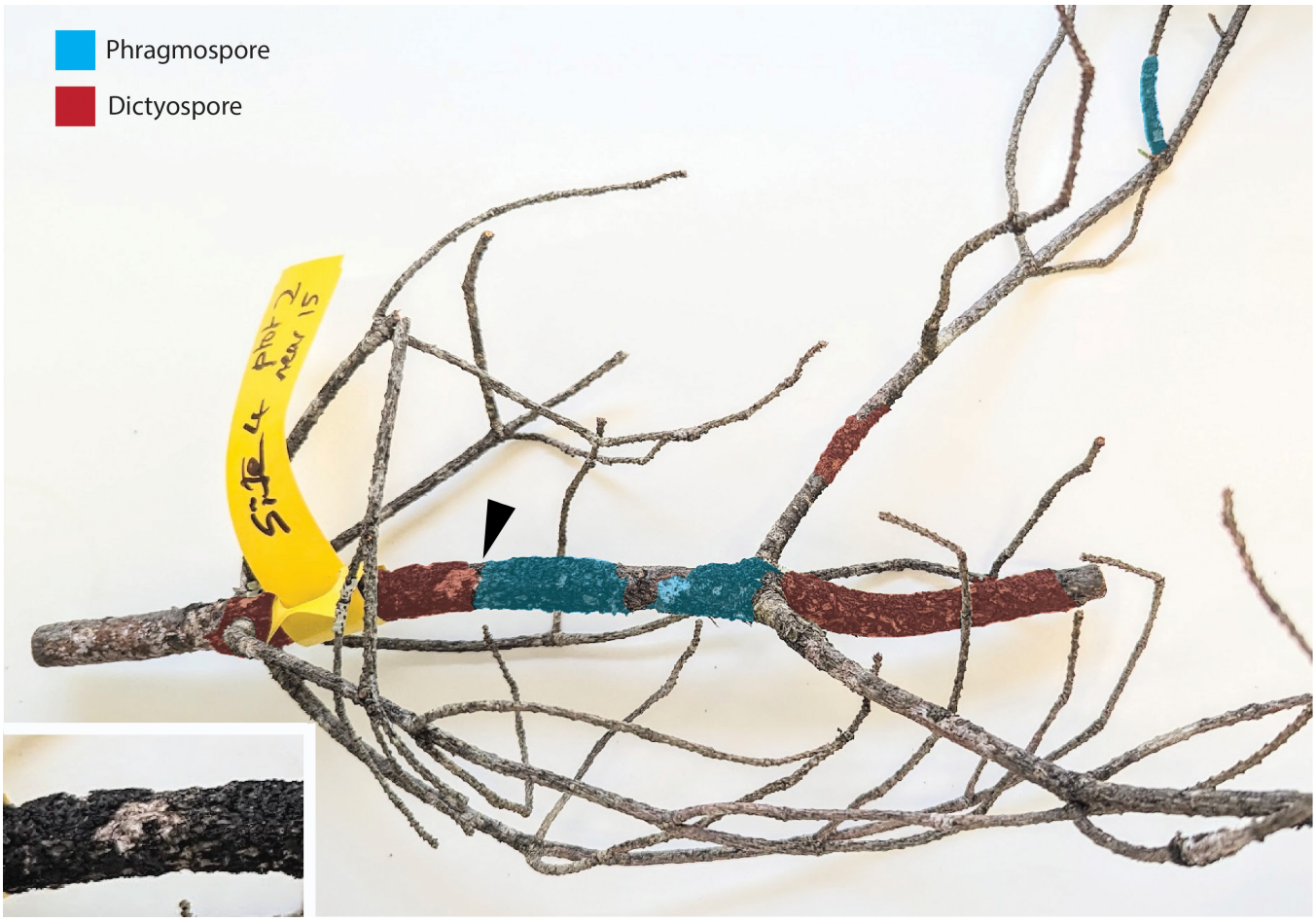

Fig. 2. A single small branch of Scots pine from an immature tree collected at Little Assynt (SP23-67) in Scotland. The multiple stromata on the branch were of both *Cucurbidothis parmeliarum* and *C. pithyophila* (indicated by presence of phragmospores or dictyospores), and in one instance stromata of both species were adjacent and touching (arrowed, shown in inset), giving the impression of a single stroma producing both spore types.

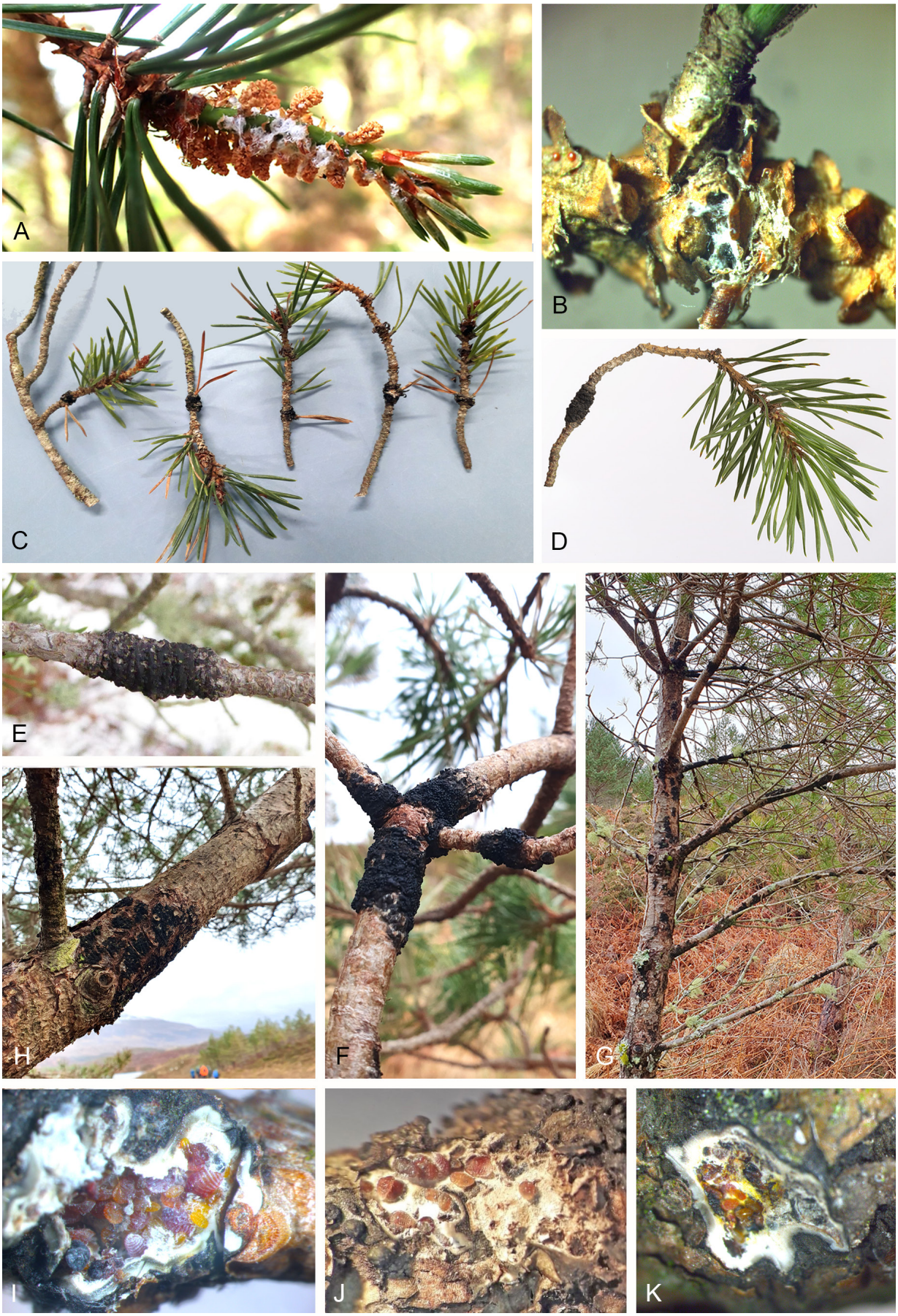

Fig. 3. Stromata and adelgids. **A.** Free-living *Pineus pini* on Scots pine indicated by white 'woolly' wax deposits often visible at bases of buds/flowers and needles. **B.** A woolly deposit and the beginning of a stroma forming at the base of a needle. **C.** Small branches (*c.* 3 mm diam.) with numerous small stromata, often forming at needle bases. **D, E.** Stromata expanding longitudinally and tangentially, forming a cylindrical stroma that girdles the shoots and branches. **F–H.** Stromata encircling larger branches (**F**), forming patches over an immature tree and causing dieback (**G**), and developing as a patch on a large diameter branch (**H**). **I–K.** Exposed *Pineus pini* colonies (showing adults, larvae and eggs) visible after removal of the overlying stroma, and showing signs of being flattened (**J**) and crushed (**K**) within the growing stroma. Image credits: D = Stuart Greig, SASA © Crown Copyright.; E = Stewart Taylor.

2023, Xu & Li 2025) (see Supplementary Material Table S2).

Two sequence alignments were analysed (Supplementary Material Files S1, S2). In the first, the two '*Curreya pithyophila'* ascospore forms were compared to 34 reference taxa for which ITS and partial nuLSU and β-tubulin sequences were available for each taxon (Supplementary Material Table S2). In the second, the two '*Curreya pithyophila'* ascospore forms were compared to 21 reference taxa for which ITS and partial nuLSU sequences were available; by not requiring β-tubulin, the second analysis was able to include, and focused on, a larger number of more closely related taxa (Supplementary Material Table S2). Sequences were aligned using CLUSTALW (Thompson *et al.* 1994), all sites with a gap in any sequence were excluded, and the alignments were then concatenated for the phylogenetic analysis.

Maximum likelihood trees were estimated from the nucleotide sequence alignments using PhyML v. 3.0 (Guindon *et al.* 2010) with the general time reversible (GTR) model of substitution and gamma-distributed rate variation across sites (with 4 categories), using subtree pruning and regrafting (SPR), and 1000 nonparametric bootstrap analyses were performed. Phylogenies were displayed using FigTree v. 1.4.2 (http://tree.bio.ed.ac.uk/software).

Adelgid identification

Adelgid nymphs were removed from beneath fungal stromata of three Scots pine samples from geographically distinct locations in Scotland (Highland, Midlothian and East Ayrshire). The DNA extraction and sequencing were carried out according to the EPPO protocol PM 7/129 (EPPO 2016). In brief, samples were homogenised by placing several individuals into a 2 ml microfuge tube with two 3-mm-diam. steel ball bearings, freezing in liquid nitrogen, and then shaking at 25 beats s^{-1} for 30 s using a Retsch 300 mixer mill (Retsch GmbH, Germany). Total genomic DNA was extracted from the adelgid nymphs using the Qiagen DNeasy Blood & Tissue Kit (Cat. No./ID: 69506, Qiagen). The 709 bp region of the mitochondrial cytochrome c oxidase subunit I (*COI*) gene, as described by Folmer *et al.* (1994), was amplified using the primers LCO1490 and HCO2198.

Temperature growth-rate study of fungal isolates

For the growth study, isolates from eight specimens were cultured (Table 2), comprising three single spore isolates per specimen with three replicates per isolate. Mycelial plugs (5 mm diam.) were cut from the margin of actively growing colonies and plated onto MEA at the centre of cross hairs marked on the bottom of each plate. Plates were incubated at 5, 10, 15, 20 and 25 °C for 8 wk and colony diameter was measured weekly. At the end of the experiment, growth rate was calculated for each isolate as the average weekly increase in colony diameter over the 8-wk period, based on measurements from each replicate.

RESULTS

Survey of Scots pine

Samples of stromata on Scots pine were collected from 31 sites across Scotland, plus one site in Devon, England (Fig. 1). Sampled sites comprised native Caledonian pine forests, new native woodland plantings, commercial plantations as well as amenity and shelterbelt trees. From the 31 sampled sites, a total of 76 herbarium specimens were obtained (Supplementary Material Table S1). In all specimens, the fungi were found to be growing over adelgid nymph colonies. The fungi were never observed in the absence of the adelgids. Of the 76 herbarium specimens collected in this study from the 31 sampled sites, 28 were the dictyospore form and 48 were the phragmospore form. Both dictyospore and phragmospore specimens were often found co-occurring at the same sites (SP23-18 and SP23-19), on the same trees (SP23-45D and SP23-45P) (see Supplementary Material Table S1) and even on the same branch where they could occur directly adjacent and without close examination could be interpreted as a single stroma (Fig. 2). Specimens with only anamorph forms were also recorded but could only be confidently identified with culture or DNA data, or if they occurred alongside the sexual stage on the same stroma, so not all specimens are included in the dataset.

Description of symptoms from observations in the UK on Scots pine

The stromata initially develop at the base of needles (dwarf shoots) and at branching junctions of long shoots of Scots pine. They are possibly initially associated with scale leaves and bracts. The stromata expand longitudinally and tangentially, forming a cylinder that girdles the shoots and

Table 2. Specimens and collection locations of the different strains used in the growth study.

Specimen	Spore type	Location
SP23-18	Phragmospore	Scotland, Highland, Little Assynt tree 7
SP23-19	Dictyospore	Scotland, Highland, Little Assynt tree 8
SP23-25	Dictyospore	Scotland, Highland, Rosehall
SP23-25A	Asexual morph dictyospore	Scotland, Highland, Rosehall
SP23-29	Phragmospore	England, Devon
SP23-32A	Asexual morph phragmospore	Scotland, Highland, Loch Maree
SP23-45D	Dictyospore	Scotland, Aberdeenshire, Muir of Dinnet
SP23-45P	Phragmospore	Scotland, Aberdeenshire, Muir of Dinnet

SP23-45 specimens were from different stroma on the same tree at that site, whereas SP23-18 and SP23-19 were from different trees at the same site. SP23-25 were different spore types on the same stroma.

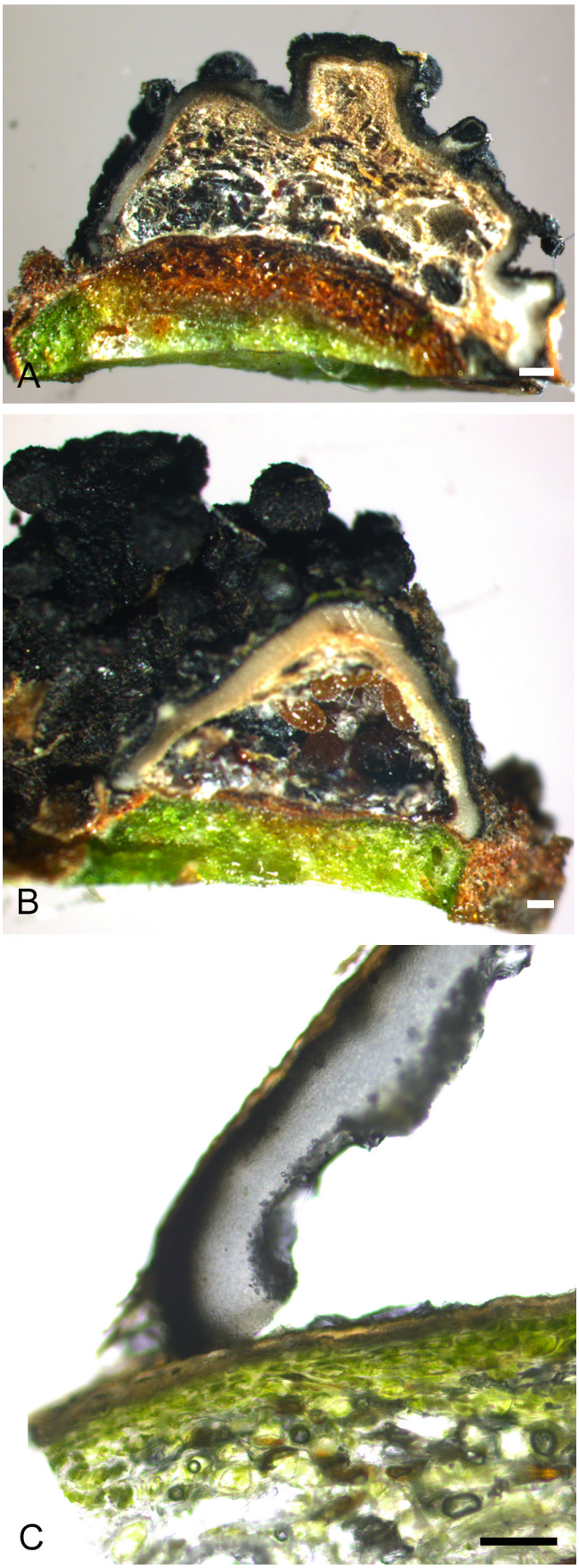

Fig. 4. Interface between stromata and host tissues. **A.** Section of stroma in the area where the host tissue is necrotic and the adelgids are dead. **B.** Section through expanding edge of the stroma with live adelgids and little evidence host tissue death. **C.** Section through the expanding edge of the stroma showing no evidence of penetration of the host tissues by the fungus. A, B. SP23-52D (dictyospore). C. SP23-52P (phragmospore). Scale bars: A = 200 µm; B, C = 100 µm.

branches (Fig. 3), often resulting in dieback of the distal portion of the tree. When girdling happens on the main stems of young trees the dieback can affect all tree parts above the stroma. On larger diameter stems the stromata will often only develop as patches on one side.

The stromata are black, coriaceous and have a 'caviar' appearance caused by the spherical fruiting structures of the fungi embedded in the stroma (Fig. 3). These include both sexual (ascomata) and asexual (conidiomata) structures which appear shiny to dull depending on the condition and age of the stroma. The stromata develop between the inner and outer bark layers as well as between the outer bark layers but otherwise do not invade the host tissue, and often can be easily peeled off. Stromata do not adhere to the bark but instead rise to form cavities inhabited by the adelgids. By pushing up the outer bark the fungus allows the adelgid direct feeding access to the phloem. Consistently observed beneath the fungal stromata, at their expanding margins, are dense clusters of live adelgid nymphs located in the cavity between the fungal stroma and the inner bark of the tree. The adelgids are parthenogenic and propagate beneath the expanding stroma. Away from the expanding stroma margins, but still encapsulated within it, the nymphs are dead and appear shrivelled and dark. Of note is the fact that the fungi are never observed without the adelgid, but free-living adelgids are frequently observed without the fungi in tufts of white woolly wax at needle bases (Fig. 3).

Host tissue at the margins of the stroma appears healthy whereas towards the centre of the stroma the host tissue is necrotic (Fig. 4), often to the depth of the cambium. Attempts to isolate fungi from necrotic phloem, cortical and cambial tissues were unsuccessful suggesting that the death of the host tissues was caused by the adelgid feeding and not by the fungi, the stromata of which grow only between the bark layers (Fig. 4). The stromata are often partially covered in disrupted layers of thin bark. When the adelgid population below a stroma dies or escapes, the stroma also dies and drops off. Several previous studies provide detailed descriptions of stromatal development, adelgid behaviour and their impact on host tissues (Casagrande 1969, Murray & Parry 1969).

Sequencing of fungal isolates and phylogenetic analyses

The ITS sequences from the phragmospore (specimen SP23-18) and dictyospore (specimen SP23-19) forms (GenBank PV990051 and PV990052, respectively) were 603 base pairs (bp) in length and differed at six positions (Supplementary Material Fig. S3A). The ca 1 % ITS divergence (6/603 bp) between the phragmospore and dictyospore forms is within the range used to separate species in *Leptosphaeriaceae* (usually ca 1–2 % Xu *et al.* 2022). The closest match in the GenBank NCBI nucleotide database was to type material of a species recently described from China, *Alloleptosphaeria shangrilana* (Thiyagaraja *et al.* 2021), which had 94 % (534/567 bp) and 95 % sequence identity (534/565) to our phragmospore and dictyospore sequences, respectively. When ITS sequences were compared for 22 UK phragmospore specimens, SP23-48 differed at one position (445) in the 603 bp alignment. For ITS sequences of 14 dictyospore specimens, one isolate each of SP23-53D and

SP23-53A differed at the same two positions (82 and 191) in the 603 bp alignment and all isolates of SP23-25 differed at one position (462).

For *tef1-α* sequences, the two forms differed at 11 positions across 242 bp (Supplementary Material Fig. S3B). For the phragmospore form, two of five sequences (specimens SP23-45P and SP23-48) differed by the same single position (81) in the 242 bp alignment, whereas there was no variation observed in dictyospore sequences. The β-tubulin sequences of the two forms differed at 21 positions across the 348 bp alignment (Supplementary Material Fig. S3C), with no sequence variation within the seven specimens sequenced for each spore form. An alignment of the γ-actin sequences of the two forms showed that they differed at 6 positions across 270 bp (Supplementary Material Fig. S3D) for each of two phragmospore specimens and three dictyospore specimens, with sequences within each species being identical. The nuLSU sequences of each form differed at 4 positions across the 706 bp alignment (Supplementary Material Fig. S3E).

A maximum likelihood (ML) phylogeny was inferred from analysis of a concatenated multigene matrix of 1333 sites of ITS, and partial nuLSU and β-tubulin sequences of the two UK *Cucurbidothis* strains with 32 reference taxa from the *Leptosphaeriaceae* (Fig. 5). The tree is rooted with *Didymella exigua* and *D. maydis* (*Didymellaceae*) as the outgroup. In addition, an ML phylogeny was inferred from a combined matrix of 1114 sites of ITS and partial nuLSU sequences of 23 taxa in *Cucurbidothis*, *Alloleptosphaeria* and *Leptosphaeria*, with *Leptosphaeria* species as the outgroup (Fig. 6). The phylogenetic analyses (Figs 5, 6) clearly show that the two ascospore forms are distinct from each other (ML bootstrap 99 % in the ITS+LSU+ β-tubulin phylogeny; Fig. 5). Both ascospore forms occur in the *Leptosphaeriaceae* in a clade with *Alloleptosphaeria* and sister to *Leptosphaeria,* but with low support. In the ITS+LSU phylogeny (Fig. 6) the two ascospore forms are most closely related to *Alloleptosphaeria shangrilana* (ML bootstrap 97 %) and these three species are distinct from other species in *Alloleptosphaeria*, and are considered to be members of *Cucurbidothis* (see 'Taxonomy' below).

Adelgid identification

The *COI* sequencing of both single and multiple adelgid specimens yielded 620 bp of clean sequence. There were two single base differences in sequences across the three sampling sites. Samples from Midlothian and East Ayrshire had a T at position 327 on the sequence, whereas the sample from Highland had a C, and at position 459 on the sequence the samples from Midlothian and Highland had a C whereas the sample from East Ayrshire had a T.

The *COI* sequences had 100 % similarity to *Pineus pini* (pine woolly aphid) voucher specimen (GenBank MH721206.1) and *Pineus orientalis* (the spruce-pine adelgid) voucher specimen (GenBank FJ502615.1).

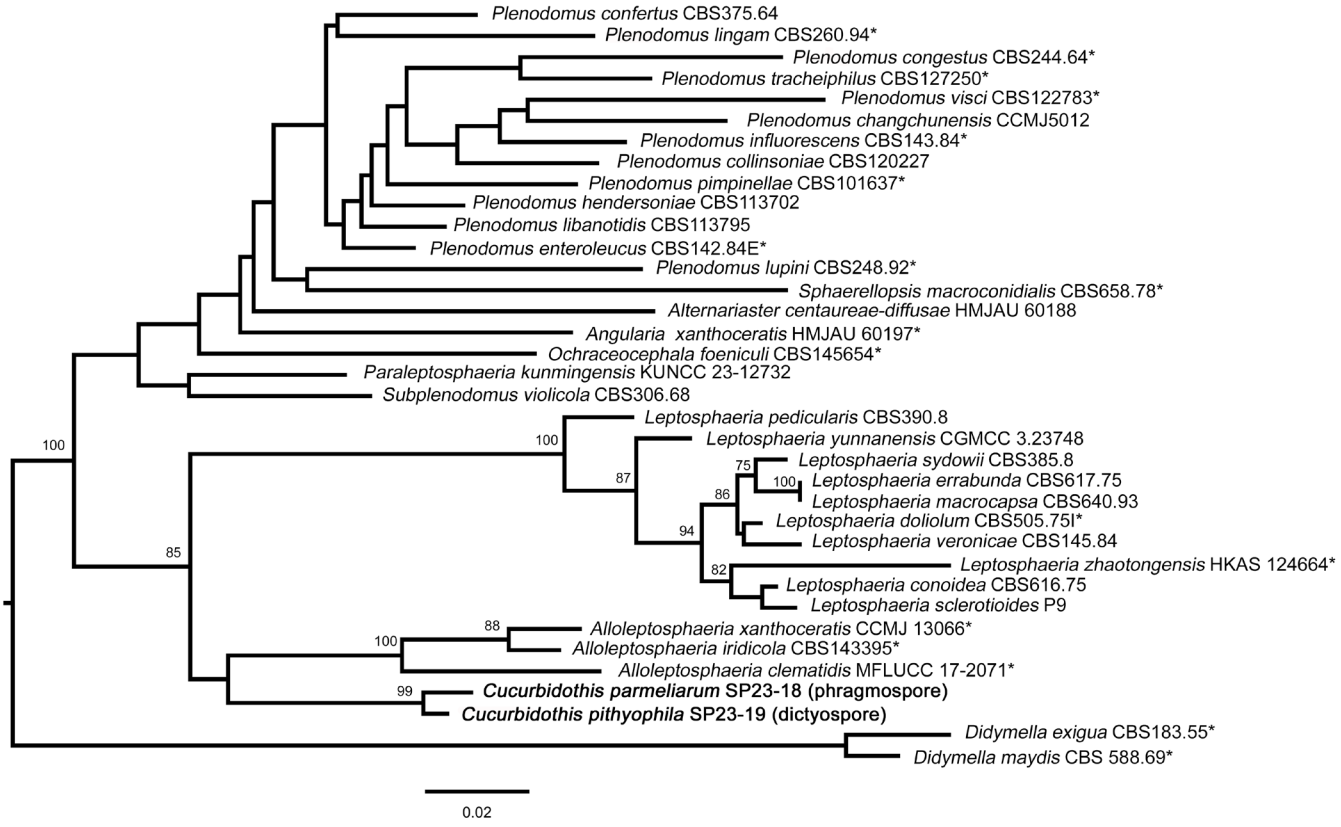

Fig. 5. Maximum likelihood (ML) phylogeny of a multigene matrix of ITS and partial nuLSU and β-tubulin sequences of the two UK *Cucurbidothis* strains (bold) with 32 reference taxa from the *Leptosphaeriaceae*. The ML bootstrap values > 75 % are indicated. Taxon names with '*' are sequences from ex-type strains. The tree is rooted with *Didymella exigua* and *D. maydis* (*Didymellaceae*) as the outgroup. The number of sites included in the analysis is 1333; scale bar indicates 0.02 expected substitutions per site.

Temperature growth-rate studies

All isolates grew fastest at 15 °C (Fig. 7). Growth was minimal at 5 °C and inhibited at 25 °C (measurements were discontinued after 4 wk at 25 °C). The isolates at 5 °C resumed growth after returning them to 15 °C whereas those that were incubated at 25 °C were dead and did not grow. The dictyospore form showed the widest range of growth rates at 15 °C and less so at 10 °C, whereas at 20 °C phragmospore isolates were more variable (Fig. 7). The growth rates of cultures derived from conidia, 32A(P) and 25A(D), were consistent with isolates derived from ascospores with the exception of 32A(P) at 20 °C (Fig. 7).

Taxonomy

The taxonomic emphasis placed on the spore septation has caused the placement of '*Curreya pithyophila*' to be under different genus names, and in different fungal families. Additionally, at no point has '*Curreya pithyophila*' been described as two separate species although Holm (1967) did acknowledge that the two spore types might represent distinct species but only went as far as confirming them as varieties and provided updated synonymies at that taxonomic level. Neither spore form shows obvious or consistent differences in the morphology of the stroma, ascomata or conidiomata; both produce asexual morphs and they share the same ecological niche. However, there are consistent micromorphology differences in some structures, such as the ascospore and conidial morphology, and ascus, ascospore and conidial dimensions. Differences in the micromorphology correspond with variations in culture characteristics (see below), as well as genetic differentiation (Figs 5, 6), which confirms that these are two distinct species.

Morphological comparison with available syntypes of *Sphaeria pithyophila* [as '*pityophila*'] and a syntype of *Sphaeria parmeliarum* shows the Scottish dictyospore and phragmospore specimens are conspecific, respectively, with these types. On the basis of the above findings and on comparison with all species of *Alloleptosphaeria* (Table 3), the genus *Cucurbidothis* is retained (see Ariyawansa *et al.* 2014) but within the family *Leptosphaeriaceae*, and comprises three species: the generic type *Cucurbidothis pithyophila* (dictyospores), *Cucurbidothis parmeliarum comb. nov.* (phragmospores) and *Cucurbidothis shangrilana comb. nov.*

As the two type specimens of *Cucurbidothis pithyophila* and *Cucurbidothis parmeliarum* have been recently examined they are described and illustrated below (and lectotypes designated), along with descriptions of fresh material representing the specimens collected in Scotland which provide extra details (see Supplementary Material Table S1 for a full list of specimens examined).

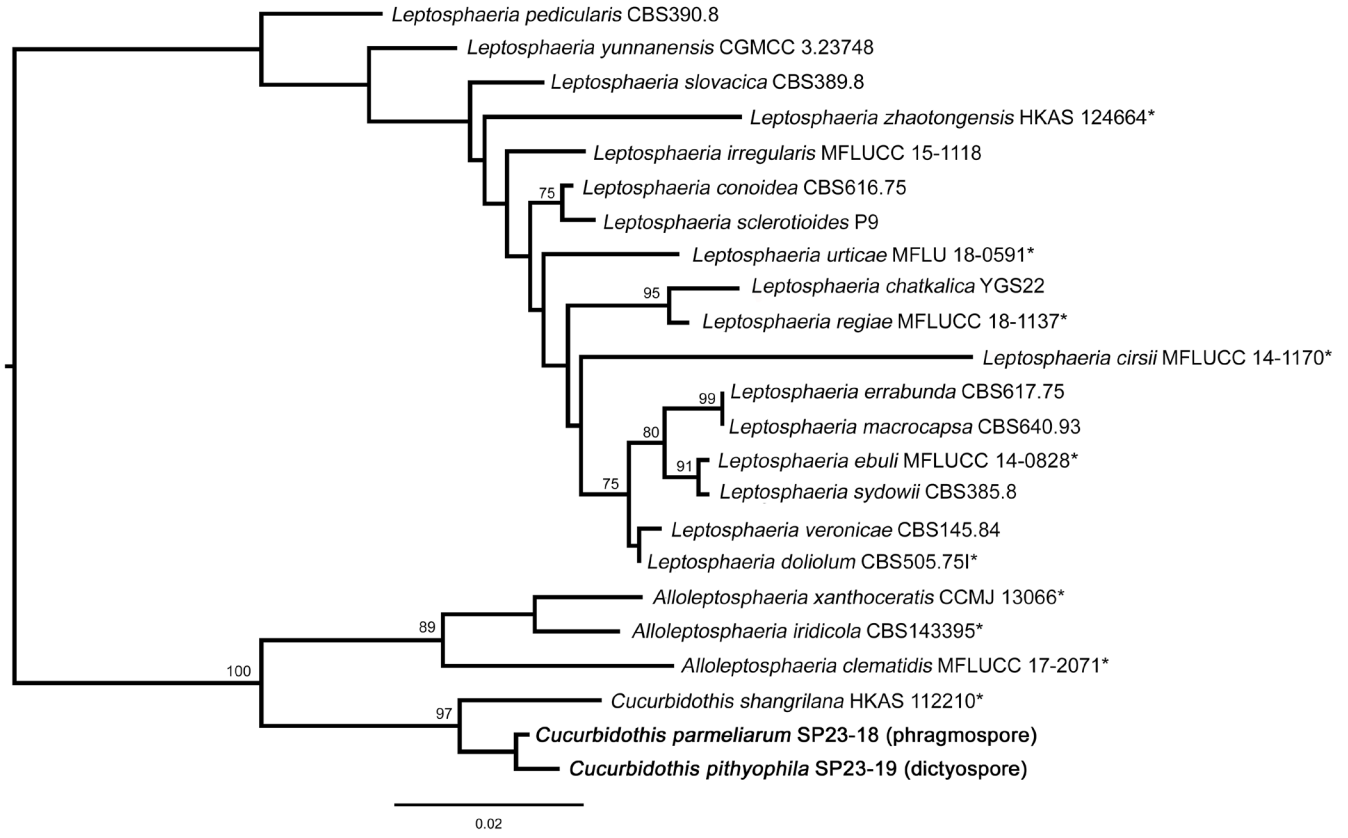

Fig. 6. Maximum likelihood (ML) phylogeny of a combined matrix of ITS and partial nuLSU sequences of 23 taxa in *Cucurbidothis* (UK strains in bold), *Alloleptosphaeria* and *Leptosphaeria*. Note that *Alloleptosphaeria shangrilana* has been renamed *Cucurbidothis shangrilana* (see 'Taxonomy' section). The ML bootstrap values > 75 % are indicated. Taxon names with '*' are sequences from ex-type strains. The tree is rooted with *Leptosphaeria* species as the outgroup. The number of sites included in the analysis is 1114; scale bar indicates 0.02 expected substitutions per site.

Cucurbidothis pithyophila (J.C. Schmidt & Kunze) Petr., *Ann. Mycol.* **19**(3-4): 201. 1921. Fig. 8.
Basionym: *Sphaeria pithyophila* J.C. Schmidt & Kunze [as '*pityophila*'], *Deutschl. Schwämme, Sechste Lieferung*: 3. 1817, nom. sanct. [Fr., *Syst. Mycol.* **2**(2): 425. 1823].
Synonyms: *Cucurbitaria pithyophila* (J.C. Schmidt & Kunze) De Not., *Comment. Soc. Crittog. Ital.* **1**(fasc. 4): 214. 1863.
Diplodia pithyophila (J.C. Schmidt & Kunze) Fuckel, *Fungi Rhen. Exs., Fasc. 6*: no. 538. 1863.
Gibberidea pithyophila (J.C. Schmidt & Kunze) Arx, *Acta Bot. Neerl.* **3**(1): 90. 1954.
Curreya pithyophila (J.C. Schmidt & Kunze) Arx & E. Müll., *Stud. Mycol.* **9**: 80. 1975.
Melogramma spraguei Berk. & M.A. Curtis, *Grevillea* **4**(no. 31): 99. 1876.
Thyridium spraguei (Berk. & M.A. Curtis) Sacc., *Syll. Fung. (Abellini)* **2**: 325. 1883.
Microsporella pityophila Höhn., *Hedwigia* **60**: 146. 1918.
Coniothyrium pityophilum (Höhn.) Petr. & Syd., *Repert. Spec. Nov. Regni Veg. Beih.* **42**: 391. 1927.

Type specimen: **Lectotype** (*lectotypus hic designatus*, MBT 10030111): **Germany**, *Ad corticem truncorum et ramorum adhuc vigentium Pini sylvestris in Lusatia. Vernalis.* Schmidt & Kunze, Deutschl. Schwämme no. 133, JE07002971; **isolectotypes** JE07002912 and HAL 3073 F.

Stroma developing on bark from a thick branch or trunk, covering almost the whole specimen (45 × 25 mm); black, overlayed by patches of thin, flaky brown bark layers and lichen thalli, folded and raised in places to form cavities under which dried adelgids can be observed amongst white filamentous material. In section, comprising an outer stratum of occluded melanised cells in a *textura angularis*, becoming mid brown inwardly with pigmentation restricted to thick cell walls; cells walls remain thick in innermost stratum but become pale brown to hyaline. *Ascomata* numerous, covering the stroma, clustered in tight groups, sometimes interspersed with small patches of bare stroma, pseudothecial, black, smooth to granular, somewhat shiny, subglobose to globose, sometimes misshapen, (280–)320–*360*–400(–430) µm diam., *n* = 20; stromatic, sometimes largely superficial and almost entirely surrounded by stroma and dislodging easily, but often partially embedded in the pale brown to hyaline stratum at the base or up to a third of its height, with all strata covering the remaining upper parts; ostiolate with a pore or small papilla and hyaline periphyses. *Peridium* formed inwardly of stroma, thin, comprising compressed layers of thin walled cells, pale brown becoming hyaline inwardly with the hymenium lining the base and sides of the ascoma. Wall of ascoma (stroma + peridium) 50–120 µm thick. *Cellular pseudoparaphyses* narrow, septate, branched, numerous, forming a dense layer above the asci, anastomosing between and above asci, 1–2.5

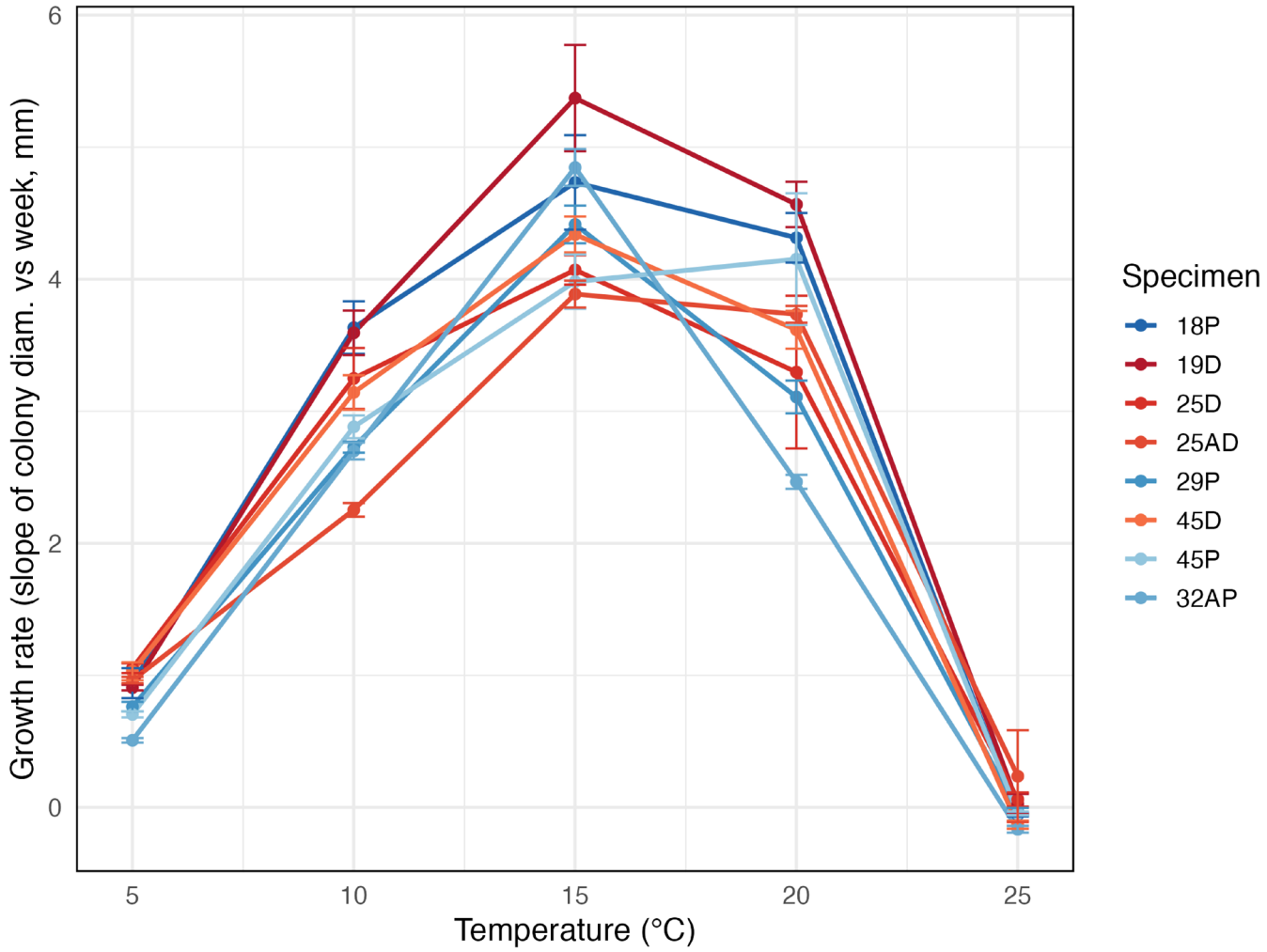

Fig. 7. Growth curves of dictyospore (D = red shades) and phragmospore (P = blue shades) isolates and their asexual morphs (A), at different temperatures; based on the average weekly increase in colony diameter over an 8 wk period, from measurements of each replicate.

µm. *Asci* cylindrical, 8-spored, numerous, bitunicate with a bluntly rounded apex and no apical structures visible, short stipitate, narrowing to a distinct pedicel, ≥ 112 × 9–*10.5*–12 µm. *Ascospores* mid yellowish brown, fusiform, dictyosporus, euseptate, (3–)4–5(–6) transverse septa with vertical septa mainly in the middle cells, but sometimes additionally in other cells and sometimes oblique, slightly constricted at septa particularly at the median septum, smooth, partially overlapping uniseriate (16.5–)18.0–*19.5*–21.0(–23.5) × (6.5–)7.0–*7.5*–8.0(–9.0) µm, *n* = 30; ascospore length/width = 2.6. *Conidiomata* not observed.

Notes: Confirmed specimens of the dictyospore form, *Cucurbidothis pithyophila*, have been recorded from *Abies*, *Picea* and *Pinus* in Asia, Europe (including the UK) and North America (e.g. Holm 1967, Casagrande 1969, Murray & Parry 1969, Takahashi & Saho 1972, Barr 1990, Ariyawansa *et al.* 2014). It remains to be seen if this represents a single species or a complex of closely related species, especially given the range of *Pinaceae* host species, the widespread distribution throughout the Northern Hemisphere (further discussed below) and the variety of host associated adelgid species.

Although Casagranda (1969) provides a very detailed description of '*Cucurbidothis pithyophila*', it is considered therein to be a single species with variable spore types (phragmospores and dictyospores). Therefore, to supplement the above type description of this species, further morphological details are provided below of fresh vouchered material collected in the UK and for which culture characteristics and molecular analyses are also presented. In addition, the specimens investigated (JE07002971, JE07002912 and HAL 3073 F) lack the asexual morph, so a description is given of the conidial morph that has been grown in culture and the identity confirmed.

Further material examined of sexual morph: **Great Britain**, Scotland, Highland, nr Lochinver, Little Assynt Estate, tree no. 8, 58.178003 -5.1391833, on small living branch of Scots pine (*Pinus sylvestris*) and associated with *Pineus pini*, 12 Jan. 2023, *E. Purser*, SP23-19, E01515837. GenBank: ITS, PV990052; *tef1-α*, PX680593; *tub2*, PX625931; nuLSU, PX668390). Fig. 9.

Stroma black, coriaceous, situated across a 5 mm diam. branch junction, 55 × 9 mm, encircling branch, developing

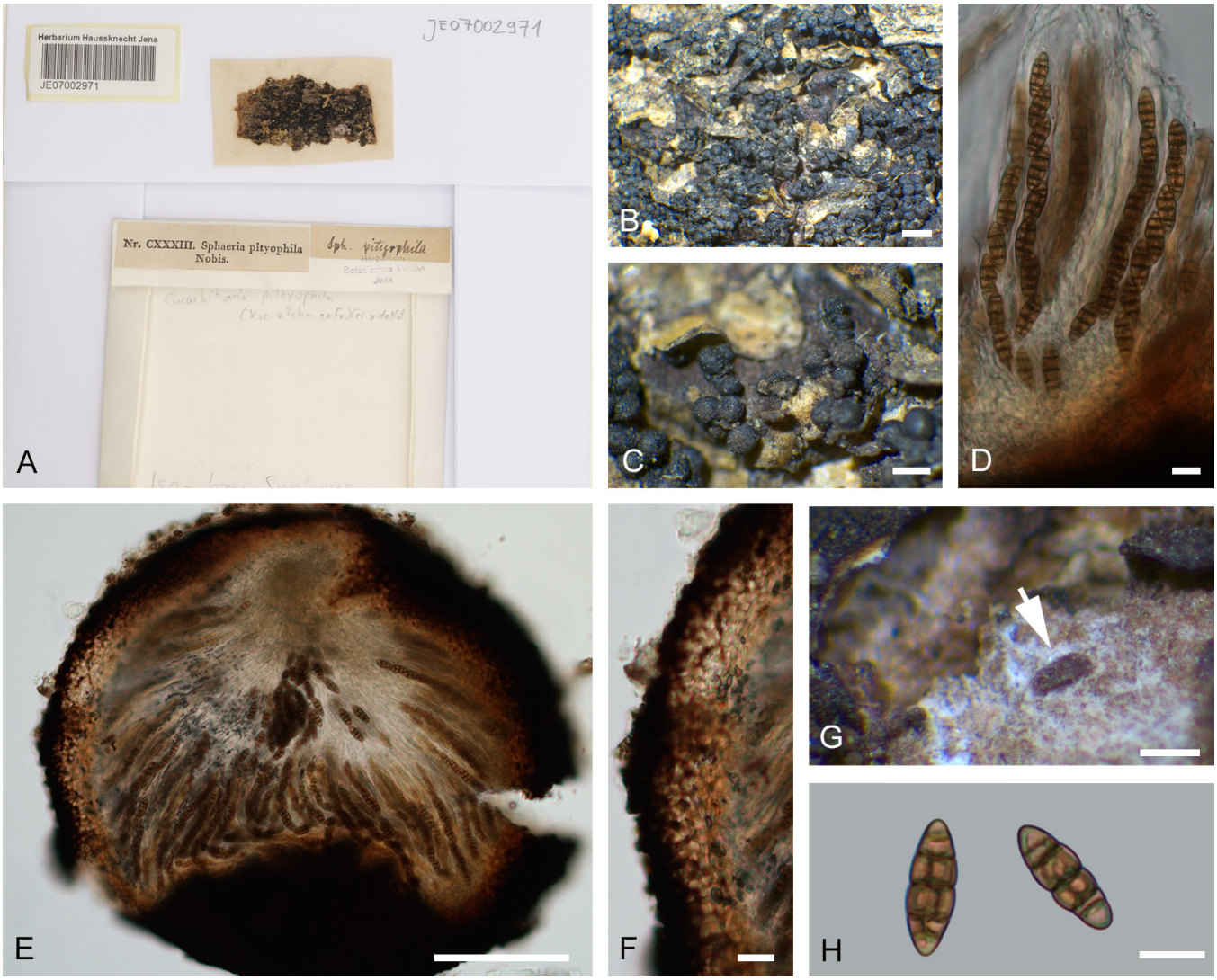

Fig. 8. *Cucurbidothis pithyophila* (JE07002971 – lectotype). **A.** Type specimen and fungarium packet. **B.** Detail of stroma and ascomata. **C.** Ascomata. **D.** Asci and pseudoparaphyses. **E.** Section of ascoma. **F.** Section of ascoma wall showing stroma and peridium. **G.** Dried adelgid (woolly aphid; arrowed). **H.** Ascospores (dictyosporous). Scale bars: B, G = 1 mm; C = 500 µm; D, H = 10 µm; E = 100 µm; F = 20 µm.

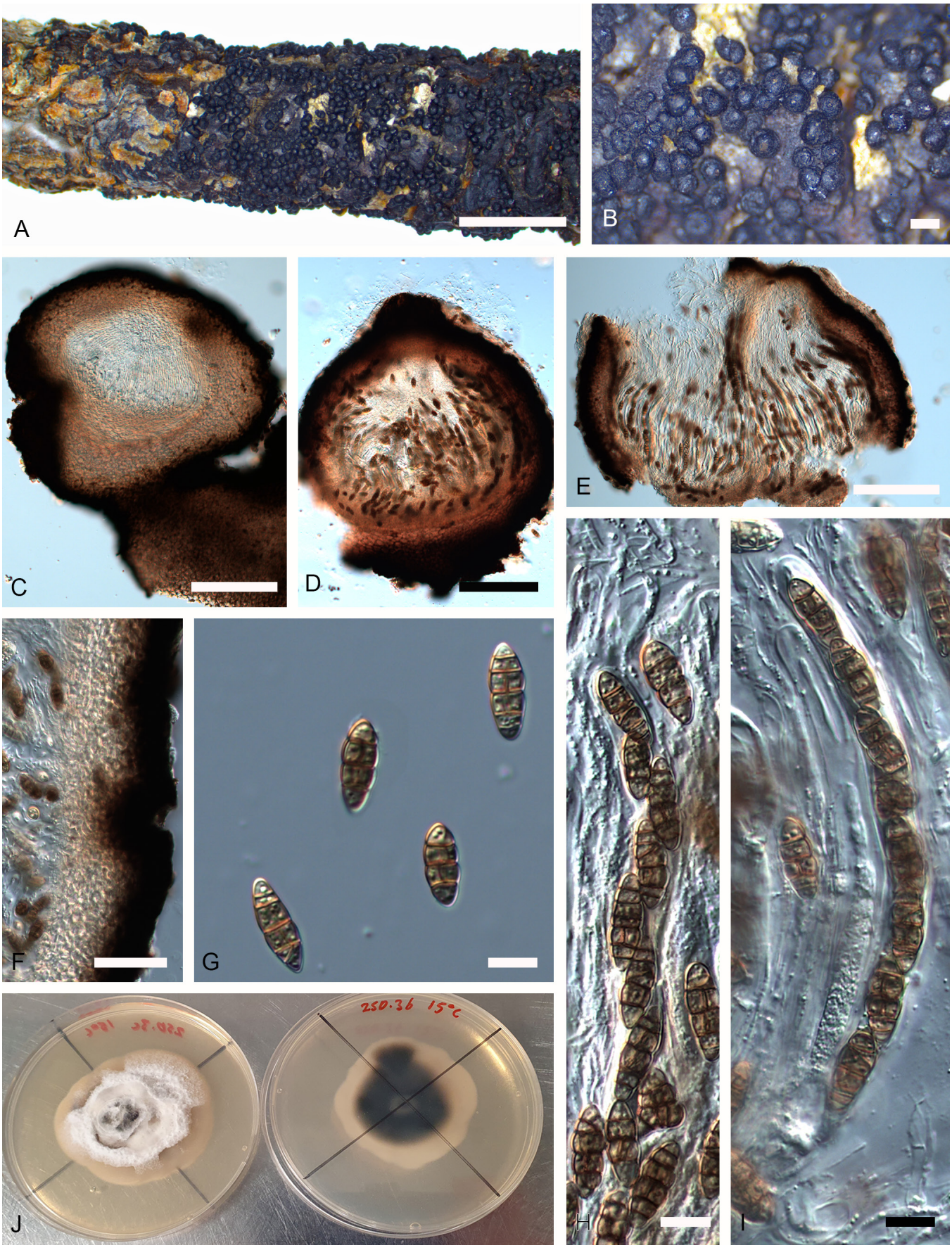

Fig. 9. *Cucurbidothis pithyophila* (SP23-19). **A.** Stroma on Scots pine branch. **B.** Ascomata. **C.** Section of developing ascoma. **D.** Section of ascoma with papillate ostiole. **E.** Section of ascoma showing dense pseudoparaphyses above the asci. **F.** Ascoma wall showing stroma and peridium. **G.** Ascospores (dictyosporous). **H, I.** Asci (in I. with pseudoparaphyses). **J.** Cultures from above (left) and below (right) on malt extract agar after 8 wk at 15 °C in the dark. Scale bars: A = 5 mm; B = 500 µm; C–E = 100 µm; F = 50 µm; G–I = 10 µm.

between the outer, or inner and outer, bark layers but otherwise not invading the host tissue, with some flaky bark overlaying the stroma surface, particularly at growing edges where there are less ascomata; raised, folded and undulating with cavities containing adelgids, loosely filled with very narrow hyaline filamentous material associated with the inner hyaline stromatal cells. In section, comprising a narrow outer stratum of brown melanised, occluded cells in a *textura angularis*; inwardly with mid brown thick cell walls and innermost a wide stratum with thick, hyaline and refractive cell walls. *Ascomata* covering the stroma, often crowded giving it a 'caviar' appearance, pseudothecial, black, coriaceous, smooth to granular, shiny, subglobose to globose, tightly clustered and sometimes misshapen, (250–)310–*345*–385(–420) µm diam., *n* = 30; stromatic, either only embedded at the base and almost entirely surrounded by stroma or embedded in the hyaline stratum up to a third of its height, with all strata covering the remaining upper parts; tightly clustered, ostiolate, often papillate, hyaline periphyses, often with a brown mass of released spores at the ostiole. *Peridium* formed inwardly of stroma, thin, comprising compressed layers of thin walled, hyaline cells, often difficult to observe, hymenium lining the base and sides of the ascoma. Wall of ascoma (stroma + peridium) 50–95 µm thick. *Cellular pseudoparaphyses*, narrow, septate, branching, numerous, forming a dense layer above the asci, anastomosing between and above asci (1.6–)2.2–*2.6*–3.0(–3.3) µm, *n* = 29. *Asci* cylindrical, 8-spored, numerous, bitunicate with a rounded apex and no apical apparatus visible, short stipitate, narrowing abruptly at the base to a distinct pedicel (124.5–)126.5–*136.5*–147.0(–165.0) × (8.5–)9.5–*10.5*–11.5(–13.5) µm, *n* = 19. *Ascospores* mid yellowish brown, fusiform, dictyosporus, euseptate, (3–)4–5(–6) -septate with 1 or 2 vertical septa mainly in the middle cells, but sometimes additionally in other cells, often oblique, constricted at septa, smooth, partially overlapping uniseriate (17.0–)18.0–*19.5*–21.0(–23.0) × (7.0–)7.5–*8.*0–9.0(–9.5) µm, *n* = 35; ascospore length/width = 2.4. Ascospores form germ tubes mainly from end cells.

Further material examined of asexual morph: **Great Britain**, Scotland, Highland, Rosehall, tree no. 4, 57.983238 -4.5583522, stroma at branch junction on small living branch of Scots pine (*Pinus sylvestris*) with both sexual and asexual morph present, associated with *Pineus pini*, Feb. 2023, *S. Green & M. Stanisz-Migal*, SP23-25A, E01515845. Fig. 10.

Conidiomata stromatic (sensu Sutton 1980), forming a single locule ('pycnidium'), tightly clustered between ascomata or sometimes dominating smaller stromata with ascomata lacking, variably sized (similar to ascomata) but often large and misshapen, densely packed with conidia, coriaceous, ostiolate with a pore, wall of conidioma as for ascoma (stroma + peridium) ca 50 µm thick. *Conidiophores* absent. *Conidiogenous cells* ampulliform to cylindrical, hyaline, enteroblastic forming phialides with indistinct collarettes, occasionally indeterminate with 1 or more proliferations, lining the entire cavity of the pycnidium, 7.0–*8.0*–10.0 × 3.0–*4.5*–6.0 µm, *n* = 6. *Conidia* subglobose to broadly ellipsoidal, smooth, thin-walled, mid yellow brown (3.5–)4.0–*4.5*–5.0(–5.5) × (2.5–)3.0–*3.5*–4.0(–4.5) µm, *n* = 69; conidia length/width = 1.3. Conidia germinate with a single germ tube.

Culture characteristics (*both morphs*): On MEA at 15 °C in the dark after 56 d, 40 mm diam. (0.7 mm/d). Culture derived from conidia slightly slower growing, 36 mm diam. (0.65 mm/d). Various shades of grey occurring often in concentric rings, often paler in the centre, felty, dense with an entire edge, quite tough in texture and difficult to cut, mostly circular growth, underside cream coloured becoming dark brown in the centre, and dark brown with a paler periphery with age. No guttation or pigments in the media. Sterile (Fig. 9).

Cucurbidothis parmeliarum (W. Phillips & Plowr.) Joanne E. Taylor & S. Green, ***comb. nov.*** MB 861558. Fig. 11.
Basionym: *Sphaeria parmeliarum* W. Phillips & Plowr., *Grevillea* **4**(no. 31): 124. 1876.
Synonyms: *Psilosphaeria parmeliarum* (W. Phillips & Plowr.) Cooke & Plowr., *Grevillea* **7**(no. 43): 84. 1879.
Leptosphaeria parmeliarum (W. Phillips & Plowr.) Sacc., *Syll. Fung*. (*Abellini*) **2**: 83. 1883.
Melanomma parmeliarum (W. Phillips & Plowr.) Cooke, *Grevillea* **16**(no. 78): 53. 1887.
Heptameria parmeliarum (W. Phillips & Plowr.) Cooke, *Grevillea* **18**(no. 86): 33. 1889.
Phaeospora parmeliarum (W. Phillips & Plowr.) Vouaux*, Bull. Soc. Mycol. France* **29**: 75. 1913.
Cucurbitaria pithyophila var. *cembrae* Rehm, *Ascomyceten*: no. 147. 1873.
Cucurbidothis pithyophila var. *cembrae* (Rehm) L. Holm, *Svensk Bot. Tidskr.* **61**: 454. 1967.
Curreya pithyophila var. *cembrae* (Rehm) Arx, in von Arx & van der Aa, *Sydowia* **36**: 3. 1983.

Type specimen: **Lectotype** (*lectotypus hic designatus*, MBT 10030113): **United Kingdom**, Gwynedd, Dolgellau, growing on *Parmelia saxatilis* on a living spruce fir, 22 Jun. 1875, coll. *W. Phillips* (Rev. *W.A. Leighton*). Plowright, Sphaeriacei Brit. [Cent. 3] no. 52, K-M001442086; **isolectotypes** MICH00015017 and NY02984691.

Stroma developing on a piece of bark from a thick branch or trunk, covering almost the whole fragmented specimen, black, overlayed by patches of thin, flaky brown bark layers, with some lichen thalli visible; folded and raised in places under which dried adelgids can be observed amongst white filamentous material. In section, comprising an outer stratum of occluded melanised cells in a *textura angularis*, a thin mid brown stratum inwardly with pigmentation restricted to the thick cells walls; cells walls of innermost stratum remain thick but become hyaline. *Ascomata* numerous, covering the stroma, clustered in tight groups, sometimes interspersed with small patches of bare stroma, pseudothecial, black, coriaceous, smooth to granular, somewhat shiny, subglobose to globose, sometimes misshapen, (360–)380–*430*–480(–520) µm diam., *n* = 25; stromatic, sometimes largely superficial and almost entirely surrounded by stroma and dislodging easily, but often partially embedded in the hyaline stratum at the base or up to a third of its height, with all strata covering the remaining upper parts; ostiolate with a pore, or infrequently a small papilla, and hyaline periphyses. *Peridium* formed inwardly of stroma, thin, comprising compressed layers of thin walled cells, pale brown becoming hyaline inwardly with the hymenium lining the base and less so the sides of the ascoma. Wall of ascoma (stroma + peridium)

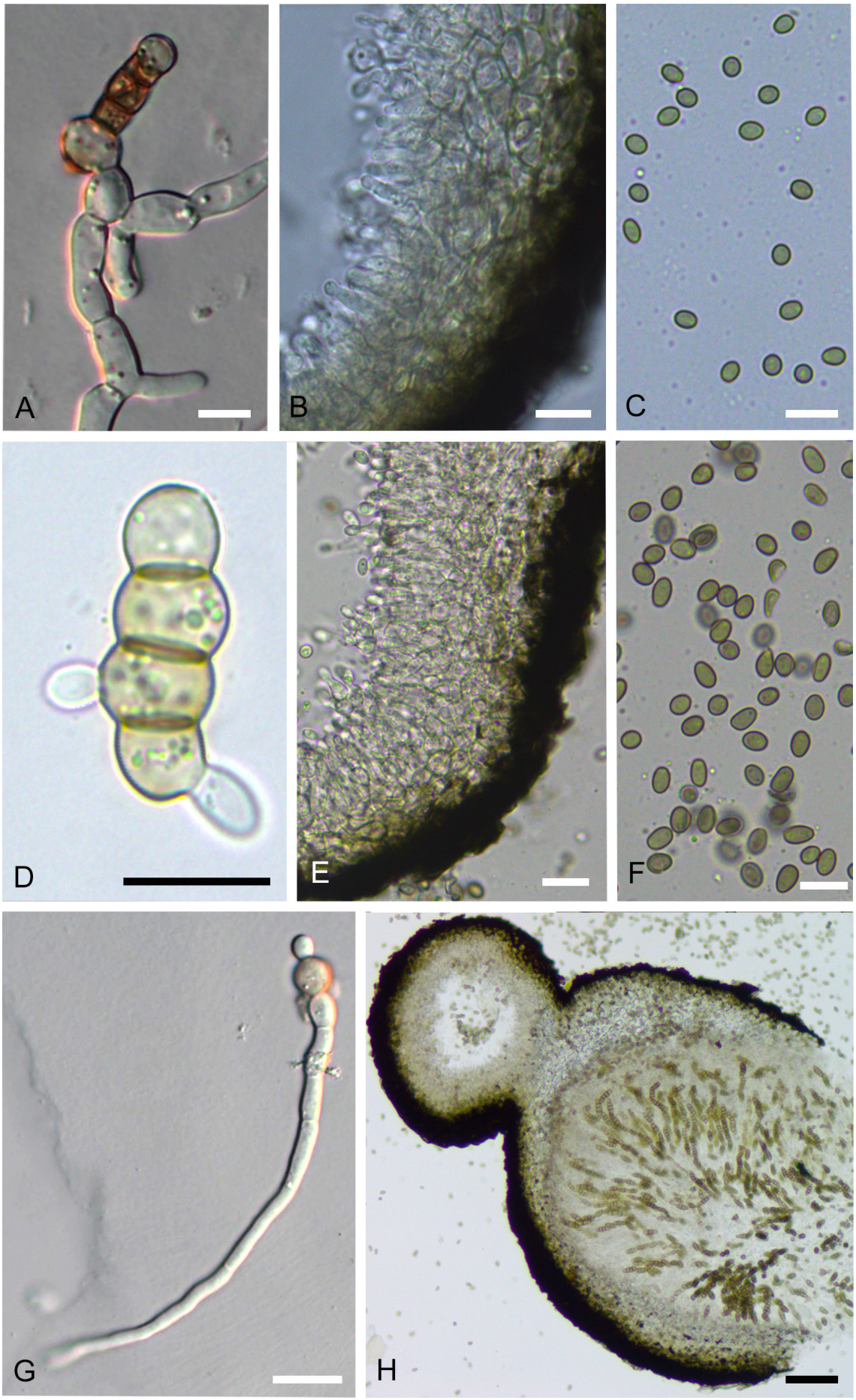

Fig. 10. Asexual morphs, germinating ascospores and conidia. **A–C.** *Cucurbidothis pithyophila*. **A.** Germinating ascospore. **B.** Conidioma wall (stroma + peridium) and conidiogenous cells. **C.** Conidia. **D–H.** *Cucurbidothis parmeliarum*. **D.** Germinating ascospore. **E.** Conidioma wall (stroma + peridium) and conidiogenous cells. **F.** Conidia. **G.** Germinating conidium. **H.** Section of ascoma and adjacent conidioma. A. SP23-53. B, C. SP23-25A D, H. SP23-52P. E, F. SP23-32A. G. SP23-72. Scale bars: A–G = 10 µm; H = 100 µm.

Fig. 11. *Cucurbidothis parmeliarum* (K-M001442086 – lectotype). **A.** Type specimen and herbarium packet. **B.** Detail of stroma and ascomata. **C.** Section of ascoma wall showing stroma and peridium. **D.** Dried adelgid (woolly aphid). **E.** Pseudoparaphyses. **F.** Ascus and pseudoparaphyses. **G.** Ascospores (phragmosporous). Scale bars: B, D = 500 µm; C = 50 µm; E–G = 10 µm.

92–116 µm thick. *Cellular pseudoparaphyses*, narrow, septate, branched, numerous, forming a dense layer above the asci, anastomosing between and above asci, 1.5–3 µm. *Asci* cylindrical, 8-spored, numerous, bitunicate with a bluntly rounded apex and no apical structures visible, short stipitate, narrowing to a distinct pedicel (132–)135–*140*–146(–150) × (9.0–)9.5–*10.0*–11.0(–12.0) µm. *Ascospores* mid yellowish brown, fusiform, phragmosporous, euseptate, 3–(4–5) transverse septa, slightly constricted at septa, basal cell sometimes slightly elongated, smooth, partially overlapping uniseriate (19.5–)21.0–*22.0*–23.0(–24.0) × (5.5–)6.5–*7.0*–7.5(–8.0) µm, *n* = 30; ascospore length/width = 3.2. *Conidiomata* not observed.

Notes: Holm (1967) states the host of the type is '*Picea abies*' (but is more likely to be Abies; unpublished data), and also records that he has observed this spore form on species of *Abies*, *Picea* and *Pinus* in Europe. The type material of *Sphaeria parmeliarum* was distributed as no. 52 in Plowright's exsiccatum Sphaeriacei Britannici, part III. Holm (1967) also observed an exsiccatum specimen in UPS, but none was located searching the catalogue; however, an online search revealed further isotypes including MICH00015017 where it cites that it was collected and determined by C.B. Plowright (Supplementary Material Fig. S2). A description from fresh material from Scotland is given, for the same reasons cited above for the *C. pithyophila*; and, as also discussed, the widespread geographic distribution and variety of *Pinaceae* hosts and associated adelgid species might indicate this is a species complex.

Further material examined of sexual morph: **Great Britain**, Scotland, Highland, nr Lochinver, Little Assynt, tree no. 7, 58.182123 -5.1350378, on small living branch of Scots pine (*Pinus sylvestris*) and associated with *Pineus pini*, 12 Jan. 2023, *Ewan Purser*, SP23-18, E01515835. GenBank: ITS, PV990051; *tef1-α*, PX680592; *tub2*, PX625930; *γ-actin*, PX680587; nuLSU, PX668389). Fig. 12.

Stroma black, coriaceous, encircling a small branch 3 mm diam., no visible branch junction, 18 × 5 mm, developing between the outer, or inner and outer, bark layers but otherwise not invading the host tissue, with some flaky bark remaining on the stroma surface, particularly at growing edges where there are less ascomata; raised, folded and undulating with cavities containing adelgids and loosely filled with very narrow hyaline filamentous material which is associated with the inner hyaline stromatal cells. In section, comprising a narrow outer stratum of brown melanised, occluded cells walls in a *textura angularis*, inwardly with mid brown thick cell walls and innermost a wide stratum with thick, hyaline and refractive cell walls *Ascomata* numerous, covering the stroma and giving it a 'caviar' appearance, leaving bare patches of stroma in places, pseudothecial, black, coriaceous, smooth, shiny, subglobose to globose, sometimes misshapen, (250–)315–*360*–405(–450) µm diam., *n* = 30, stromatic, either only embedded at the base and almost entirely surrounded by stroma or embedded in the hyaline stratum up to a third of its height, with all strata covering the remaining upper parts; tightly clustered, ostiolate with a pore or papilla, sparse periphysoids, often with a brown appearance at the ostiole. *Peridium* formed inwardly of the stroma, comprising layers of thin walled cells, darker outwardly, and thicker and hyaline inwardly with the hymenium lining the base of the ascoma. Wall of ascoma (stroma + peridium) ca 55–85 µm thick. *Cellular pseudoparaphyses* narrow, septate, branching, numerous, forming a dense layer above the asci, anastomosing between and above asci (1.0–)1.5–*2.5*–3.0(–4.0) µm, *n* = 38. *Asci* cylindrical, 8-spored, numerous, bitunicate with a rounded apex and no apical apparatus visible, narrowing gradually at the base to a distinct short pedicel (132.5–)145.5–*159.0*–172.5(–184.5) × (8.5–)9.5–*10.0*–10.5(–11.0) µm, *n* = 23. *Ascospores* mid yellowish brown, fusiform, phragmosporous, euseptate, (1–)3(–4)-septate, slightly constricted at septa, smooth, partially overlapping uniseriate (18.0–)19.5–*21.0*–23.0(–24.5) × (5.5–)6.5–*7.5*–8.0(–8.5) µm, *n* = 35; ascospore length/width = 2.9. Ascospores form germ tubes from each cell.

Further material examined of asexual morph: **Great Britain**, Scotland, Highland, Loch Maree, 57.630323 -5.3491698, on small living branch of Scots pine (*Pinus sylvestris*), only asexual morph present (identity confirmed through culture and DNA data), associated with *Pineus pini*, Feb. 2023, *E. Purser*, SP23-32A, E01515850. GenBank: ITS, PV990061; *tub2*, PX625934. Fig. 10.

Conidiomata stromatic (sensu Sutton 1980), forming a single locule ('pycnidium'), tightly clustered between ascomata or sometimes dominating smaller stroma with ascomata lacking, variably sized (similar to ascomata) but often large and misshapen, densely packed with conidia, coriaceous, ostiolate with a pore, wall of conidioma similar structure to ascoma (stroma + peridium) ca 50 µm thick. *Conidiophores* absent. *Conidiogenous cells* ampulliform to cylindrical, hyaline, enteroblastic forming phialides with indistinct collarettes, occasionally indeterminate with 1 or more proliferations, lining the entire cavity of the pycnidium, (6.0–)*8.5*(–12.5) × (2.5–)*3.5*(–7.0) µm, *n* = 12. *Conidia* ellipsoidal to slightly oblong, smooth, thin-walled, mid yellow brown (4.5–)6.0–*6.5*–7.5(–9.0) × (3.0–)4.0–*4.5*–5.0(–5.5) µm, *n* = 50; conidia length/width = 1.5. Conidia germinate with a single germ tube.

Culture characteristics (*both morphs*): On MEA at 15 °C in the dark after 56 d, 40 mm diam. (0.7 mm/d). Culture derived from conidia slightly faster growing 42 mm diam. (0.75 mm/d). Apricot coloured, often slightly paler towards the centre, becoming dark brown on the periphery with age, felty to woolly, medium dense with a somewhat diffuse edge, mostly circular growth, underside apricot becoming yellow brown in the centre, becoming dark brown on the periphery with age. No guttation or pigments in the media. Sterile (Fig. 12).

Notes: The two species differ in the morphology and dimensions of ascospores and conidia, ascus dimensions and culture characteristics. *Cucurbidothis parmeliarum* differs from the generic type, *C. pithyophila,* as it has longer, narrower ascospores ($\bar{x}$ 22 × 7 µm vs 19.5 × 7.5 µm; type specimens) and consistently produces phragmospores, but not dictyospores; conidia of *C. parmeliarum* are more ellipsoidal than subglobose as in *C. pithyophila* ($\bar{x}$ 6.5 × 4.5 µm vs 4.5 × 3.5 µm, conidia width/length 1.5 vs 1.3; UK specimens). The asci of *Cucurbidothis parmeliarum* are also

longer and narrower ($\bar{x}$ 159 × 10 µm vs 136.5 × 10.5 µm; UK specimens) and appear to form on the base of the ascomata, whereas those of *C. pithyophila* are formed on the base and lower sides. The cultures of both species have a similar mean daily growth rate ($\bar{x}$ = 0.7 mm/day; UK specimens) but those of *C. parmeliarum* are apricot coloured and those of *C. pithyophila* are various shades of grey and have a tougher texture. These morphological variations are reflected in clear genetic differences as outlined above and shown in Figs 5 & 6.

There has not been any critical discussion of these differences in the literature for '*Curreya pithyophila*' prior to this study. Holm (1967) noted the differences in the dimensions of the ascospore types and followed Rehm (1881)

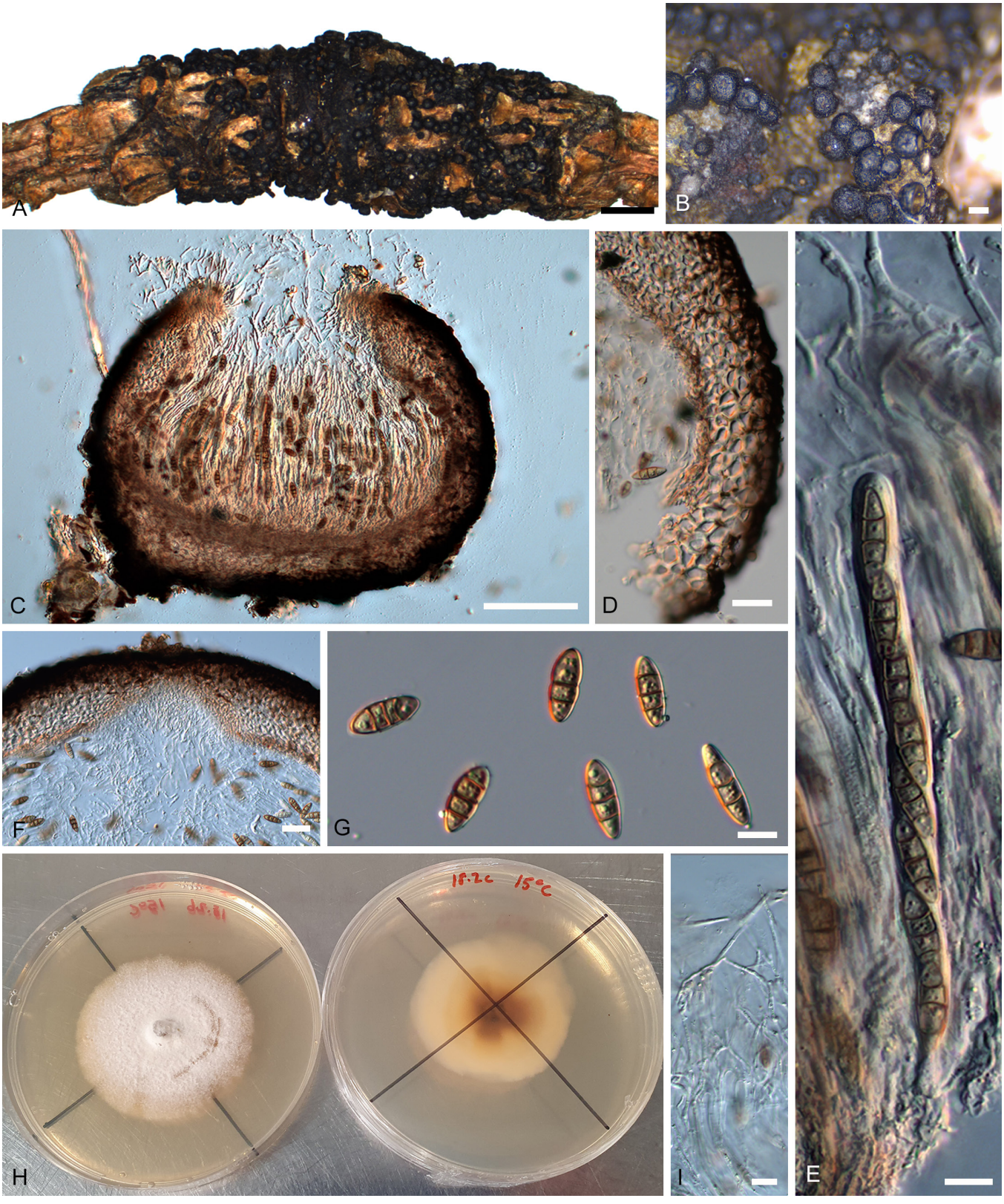

Fig. 12. *Cucurbidothis parmeliarum* (SP23-18). **A.** Stroma on Scots pine branch. **B.** Ascomata. **C.** Section of ascoma. **D.** Section of ascoma wall showing stroma and peridium. **E.** Ascus and pseudoparaphyses. **F.** Section of ostiole showing periphyses. **G.** Ascospores (phragmosporous). **H.** Cultures from above (left) and below (right), on malt extract agar after 8 wk at 15 °C in the dark. **I.** Pseudoparaphyses. Scale bars: A = 5 mm; B = 200 µm; C = 100 µm; D, F = 20 µm; E, G, I = 10 µm.

in recognising two formal varieties, whereas Casagrande (1969) recognised a single species and stated the description should be amended to include both ascospore forms. Casagrande (1969) was the only previous author to study isolates but ignored the corresponding ascospore-culture differences outlined above, and instead stated that cultures were very variable in morphology. Casagrande (1969) did report an example of fertile conidiomata being produced in culture by a phragmospore isolate of pine hosts.

Cucurbidothis shangrilana (V. Thiyag. *et al.*) Joanne E. Taylor & S. Green, ***comb. nov.*** MB 861559.
Basionym: *Alloleptosphaeria shangrilana* V. Thiyag. *et al.*, *Phytotaxa* **491**(1): 15. 2021.

Type specimen: **China**, Yunnan Province, Diqing Tibetan Autonomous Prefecture, Shangri-La, Jiantang (027°55'05.8''N, 099°36'33.4''E, 3234 m.a.s.l.), on dead wood of an unidentified dicotyledonous host, 14 Sep. 2018, *V. Thiyagaraja* (**holotype** HKAS 112210; **isotype** MFLU 21-0017).

Notes: The phylogenetic analyses in Figs 5 and 6 show that *Cucurbidothis* is nested in the *Leptosphaeriaceae*, in a clade with *Alloleptosphaeria* that is sister to *Leptosphaeria*, and that *Alloleptosphaeria shangrilana* is congeneric with *Cucurbidothis* (ML bootstrap support 97 %) into which it is therefore here combined. It should be noted that the type of *Alloleptosphaeria*, *A. italica*, is not included in the analysis as only nuLSU and ITS sequences were available (GenBank KT454722 and KT454714, respectively), and the sequences were short and would reduce the length of the alignment. However, in all phylogenies containing *A. italica*, it clusters with *A. iridicola* and close to *A. clematidis* (Thiyagaraja *et al.* 2021, Xu *et al.* 2022, Gao *et al.* 2023, Xu & Li 2025). Morphological comparison of the generic type, *A. italica*, and the other species in the genus, with *C. shangrilana* (Table 3) (Ariyawansa *et al.* 2015, Crous *et al.* 2018, Phukhamsakda *et al.* 2020, Thiyagaraja *et al.* 2021, Xu & Li 2025) also is in line with the molecular data. The ascomata of *Alloleptosphaeria* are immersed in the host tissue with a thin-walled peridium, composed of pseudoparenchymatous cells, forming a *textura angularis* and fusing outwardly with the host cells. Whereas ascomata of *Cucurbidothis shangrilana* are evidently immersed in an extensive stroma (this is not described in the protologue, although visible in provided images). Furthermore, the ascomata of *C. shangrilana* differ being at least twice the size of the other *Alloleptosphaeria* species and the asci of *C. shangrilana* are cylindrical rather than clavate, all of which are characteristic of *Cucurbidothis* (see generic descriptions in Supplementary Material Fig. S2). Re-examination of the type material of *Cucurbidothis shangrilana* would allow investigation of the extent of the stroma and the proximity with the host tissue and to search for evidence of adelgids (as although it is described on 'dead wood', the stroma might have formed on living tissue that subsequently died). In addition to the phylogenetic differences of *C. shangrilana* to the other species of *Cucurbidothis,* it differs from *C. parmeliarum* as it is dictyosporous, and from *C. pithyophila* in the larger ascospores ($\bar{x}$ = 27 × 8.5 µm) with more pointed cone-shaped ends, and larger asci ($\bar{x}$ = 160 × 12.5 µm) (Thiyagaraja *et al.* 2021).

DISCUSSION

A new and widespread outbreak of a cryptic stroma-forming fungus previously known as '*Curreya pithyophila'* is occurring on Scots pine in Scotland with a single finding in England. Based on a study of 76 specimens, with single spore isolates from 37 specimens, we show here that the outbreak is being caused by two closely related species which we assign to *Cucurbidothis pithyophila* and *C. parmeliarum*. These two fungi, in association with a woolly adelgid*,* are the primary causal agents in an epidemic of canker disease of Scots pine. Although our adelgid sequences matched both *Pineus pini* and *P. orientalis,* the former is regarded as the correct identification for an asexual strain that evolved from *P. orientalis* and is now anholocyclic, having lost both sexual reproduction and host alternation. Although *P. pini* and *P. orientalis* are named as separate species, they are essentially the same species, or closely related forms within a species complex (Havelka *et al.* 2019) with *P. pini* endemic on Scots pine in the UK.

The current outbreak of *C. pithyophila* and *C. parmeliarum* covers a widespread area of Scotland including the south-west (East Ayrshire and Dumfries and Galloway), east (Fife and Aberdeenshire), north-west (Highland) and the central belt (Midlothian). The UK records include the two previous outbreaks in Scotland in the early 1900s (McIntosh 1915) and 1960s (Murray & Parry 1969), as well as reports in Wales [in 1875, see Holm (1967) probably on *Abies*], north-east England (in 2004, FRDBI Record No.: 1221008; ABFGID 383090, on *Picea*) and in Devon (the present study, on *Pinus*). For such distinctive and visible symptoms, these reports are very sparse with only 10 records since 1970 from the BMS Fungal Records database and Fungus Conservation Trust CATE database (1977, 1980, 1986, 1996 and 2004). There are brief records in forest pathology (Peace 1962) and mycological textbooks (reported on *Abies* by Ellis & Ellis 1997), and on Fungi of Great Britain and Ireland (https://fungi.myspecies.info/) where it is reported as 'rare'.

It should be noted when discussing previous literature that the two *Cucurbidothis* species have been treated as a single species and, importantly, that it is possible that more (cryptic) species will be recognised when samples are investigated from other *Pinaceae* hosts at different locations associated with other adelgid species [see Casagrande (1969) for a list of host and adelgid species]. This will only be understood once sampling has been carried out elsewhere on a range of *Pinaceae* hosts or, as is currently ongoing, if herbarium specimens are investigated, preferably with DNA extractions for phylogenetic studies (unpublished data).

The UK isolates of *Cucurbidothis parmeliarum* and *C. pithyophila* have no close DNA sequence matches in the GenBank NCBI nucleotide database although there are sequences of misidentified strains of '*Curreya pithyophila*' available. Blast analyses in GenBank show that the LSU sequence of strain CBS 149.32 (GenBank DQ384102) is a member of the *Didymosphaeriaceae* (Valenzuela-Lopez *et al.* 2018), as is CBS 986.69 (GenBank MH871280), UTHSC DI16-357 (GenBank LN907500), IARI-RPF-1 (GenBank KF530860), IARI-RPF-17 (GenBank KF530856) and clone G-jav1-LSU1_OTU-0-043_307 (GenBank MF337705). Similarly, the ITS sequence of MAFF no. 410060 in NARO Genebank matches *Extremus* and *Paradevriesia* in the

Table 3. A comparison of the morphological characteristics of species in *Alloleptosphaeria*.

Species name	Host	Location	Life history	Stroma	Ascomata	Ascus morphology	Ascospore morphology	Asexual morph	Reference
Alloleptosphaeria clematidis	Dead stems of *Clematis subumbellata*	Thailand, Chiang Rai Province	Saprobic	None	Immersed in host tissue, visible as black spots, with a pseudoclypeus, solitary, scattered, uniloculate with apical ostioles, 237 × 160 µm	Cylindrical, short, bulbous pedicel, ocular chamber clearly visible when immature, ascospores partially overlapping uniseriate	Dictyospore, yellowish, broad fusiform, tapering towards the ends, round at both ends	Undetermined	Phukhamsakda *et al.* (2020)
A. iridicola	*Iris* sp., associated with leaf spots	UK, England	Pathogenic?	None	Immersed, globose, dark brown with central ostiole, 150–250 µm diam.	Narrowly ellipsoid, stipitate, ocular chamber, fasciculate, ascospores multiseriate	Phragmospore, pale brown, fusoid-ellipsoid, constricted at median septum, guttulate, finely roughened	Pycnidial, ostiolate; conidiogenous cells phialidic; conidia hyaline, subcylindrical to narrowly ellipsoid	Crous *et al.* (2018)
A. italica (Type)	Dead and hanging branches of *Clematis vitalba*	Italy, Forlì-Cesena Province	Saprobic	None	Immersed to semi-erumpent, solitary, scattered, globose or subglobose, papillate ostiole, 150–200 µm diam.	Cylindric-clavate to clavate, with a bulbous pedicel, thick-walled at the apex, with ocular chamber, ascospores overlapping 1–2 seriate	Phragmospore, yellowish brown, narrowly ovoid to clavate, 3-septate, constricted at the septa	Undetermined	Ariyawansa *et al.* (2015)
A. xanthoceratis	On dead stems of *Xanthoceras sorbifolium*	China, Jilin Province	Saprobic	None	Undetermined	Undetermined	Undetermined	Pycnidial, no distinct ostiole; conidiogenous cells phialidic; conidia hyaline, subcylindrical to narrowly ellipsoid	Xu *et al.* (2025)
Cucurbidothis shangrilana	Dead wood of an unidentified dicotyledonous host	China, Yunnan Province	Saprobic	Present (but not mentioned in description)	Semi-immersed to superficial, globose to subglobose, solitary or clustered, black, smooth, easily removed from the host substrate, apically ostiolate, 520–640 µm diam.	Cylindrical, short stipitate, ocular chamber, ascospores overlapping uniseriate	Dictyospore, dark brown, broadly fusiform, constricted at primary/median septum	Undetermined	Thiyagaraja *et al.* (2021)

Mycosphaerellales. In addition, strain CBS 955.68, listed in the CBS database as *Curreya pithyophila* from *Pinus cembrae* in Switzerland, is also not an isolate of this fungus since ITS sequences obtained for this isolate match *Sarea difformis* (*Sareales*), a pine inhabiting fungus (M. Stanisz-Migal, unpubl. data). None match the strains from Scots pine in Scotland herein and are all considered incorrectly identified. It should be noted that several references use sequences from the incorrectly identified strains (Berbee 1996, Kruys *et al.* 2006, Kruys & Wedin 2009, Vu *et al.* 2019).

Cucurbidothis is phylogenetically most similar to *Alloleptosphaeria,* and the new combination *Cucurbidothis shangrilana* (Thiyagaraja *et al.* 2021) is a 94–95 % match and therefore is sufficiently phylogenetically distant to be considered a different species, corresponding also with morphological differences. The fact that no correct DNA sequences exist in GenBank for these remarkably visible fungi illustrates just how obscure they have remained over the last two decades since the advent of routine DNA barcode sequencing. '*Curreya pithyophila*' is not even listed in recent comprehensive publications of fungi in Europe (Thompson 2013, Læssøe & Petersen 2019).

There have been three major studies on the biology of *Cucurbidothis* from several decades ago (Franz 1955, Casagrande 1969, Murray & Parry 1969), and these give us some understanding of this unusual relationship between the fungi and adelgids. There are two accounts of how a stroma initially develops. Franz (1955) studied the adelgids (*Adelges piceae*) with '*Cucurbitaria pithyophila*' (phragmospore form) on the main stem of fir (*Abies alba*) and noted that an adelgid colony, visible as a woolly mass on the bark, became covered by fungal stromata in the subsequent year. Whereas Murray & Parry (1969), described the initial stage of stroma formation by *C. pithyophila* on Scots pine, having observed an adelgid and eggs beneath a bark scale and the subsequent formation of a 'cone shaped' hollow stroma above them, which over time extends and forms ascomata. Murray & Parry (1969) suggested that the wool from the adelgids becomes packed to form cavities and noted that the adelgids were unable to escape. This 'capture' of the adelgids beneath the stroma enables them to escape predation (Franz 1955, Murray & Parry 1969). Casagranda (1969) gave a similar account of stroma formation as Murray & Parry (1969) and showed that the stroma can easily absorb moisture and expand, allowing extension of the stroma and its associated adelgid colonies.

There has not been any definitive conclusion on how the fungus feeds and the role of the adelgids in its nutrition. It was observed previously (Murray & Parry 1969) and in the present study, that when the branch dies on which stromata develop, then the adelgids and stromata die too, and the stromata dry up and peel away. This suggests that the fungus cannot survive without the adelgid colony. Both the present study and Murray & Parry (1969) also note that there is no fungal development in host tissues below stromata (Fig. 4). It could be assumed that the fungi thrive on the honeydew exuded by the adelgids, and possibly on the dead adelgids [an idea rejected by Casagrande (1969)]. Murray & Parry (1969) suggest that fungal development within the cavity is limited and further investigation on the origin of the woolly material in the cavity of the stroma is necessary (see Supplementary Material Fig. S4). The source of the fungal nutrition might be indicated by the carbon sources utilised. Based on experiments with different media, Casagrande (1969) showed that *Cucurbidothis* cannot utilise lignin constituents or cellulose, but does metabolise simple sugars. It was noted that the strains, regardless of provenance and spore form, behaved the same way on different media tested. It was speculated that these fungi gained nourishment from the adelgids but there was no explanation as to how (Casagrande 1969). It is hard to imagine how such large stromata could be maintained without sustenance from the adelgids.

A frequent observation in this study was the occurrence of both species of *Cucurbidothis* on Scots pine at the same sampling sites, on the same trees and even on the same branches, sometimes directly adjacent to each other (Fig. 2). It is quite remarkable that these two distinct species are so morphologically similar (at least macroscopically) and occupy the same unique ecological niche. Furthermore, despite differences in culture morphology, mainly in colour and texture of colonies, they both grow at the same rate, and cannot grow above 25 °C. Our results agree with Casagrande (1969) who reported that the optimal temperature for growth is 18–21 °C with no growth above 27 °C. A wider range of temperatures for ascospore and conidia germination (3–24 °C) was reported (Casagrande 1969), and no growth differences were reported between the ascospore forms.

All fungal species will have an optimum temperature for growth and many studies demonstrate fungal cardinal growth ranges for isolates (for example, see Sung *et al.* 2010, Hoa *et al.* 2023). The temperature characteristics of a species (i.e. maximum, minimum limits and the duration of growth and survival at their upper and lower limits) will influence their distribution (Torii *et al.* 2021). Temperature will be a key determinant in the distribution of these two *Cucurbidothis* species and will restrict their range both in latitude and altitude, despite the range of their *Pinaceae* hosts extending outside of these temperature limits. Further studies are necessary to investigate growth and survival of germinating spores of *Cucurbidothis parmeliarum* and *C. pithyophila* to enable modelling of potential distributions of these species and the likely impact of climate change.

The evolution of two separate species from an ancestral population requires an ecological or geographical barrier to gene flow, between the two sub-populations (Xu 2020). It is difficult to speculate on the observed distribution of these two distinct species in Scotland and how they have come to coexist so closely. Barr (1990) suggests that the dictyospore form originates from North America as only this form appears to have been recorded there, whereas the phragmospore form is of European origin. Murray & Parry (1969) observed only the dictyospore form (*C. pithyophila*) in the 1960s outbreak on plantation Scots pine in north-east Scotland. The report from the Perthshire outbreak in 1907 does not mention spore form but suggests that the affected plantation Scots pine were of foreign origin (McIntosh 1915). No phragmospore form of this fungus has been reported previously on Scots pine in the UK (only on *Abies*). Based on the evidence gained so far, it is possible that one, or both, of these fungi may represent recent introductions into the UK (Green *et al.* 2024). Studies of genomic variation within UK populations of *C. pithyophila* and *C. parmeliarum* may help to unravel the population structure and dynamics of these two species and indicate how long each population has been evolving for in the UK.

Further studies are required to determine the impact that this current *Cucurbidothis* epidemic is having on Scots pine. Franz (1955) suggests that for *Abies, Cucurbidothis* primarily infests unhealthy trees, although pathogenicity experiments were unsuccessful. McIntosh (1915) reported that the *Cucurbidothis* infestations resulted in branch killing of Scots pine, as did Murray & Parry (1969) who also concluded that the infestations had no economic significance being limited to the lower branches of Scots pine. Casagrande (1969) concluded that the association does not cause lasting damage to trees even when infestations reach their peak. However, the UK survey of Scots pine undertaken here has demonstrated that widespread fostering of damaging populations of *P. pini* beneath the stromata results in cambial damage. This provides wounded tissue for subsequent colonisation by secondary agents such as *Crumenulopsis sororia* (Green *et al.* 2024), a facultative wound pathogen of pine that causes perennating black cankers (Ennos & Swales 1987).

The factors promoting this current UK epidemic on a keystone native conifer species can only be speculated on. It may have been driven by introductions of new genotypes of *Cucurbidothis,* changing climatic patterns favouring the fungi, or increased adelgid populations. Although it is unclear what drives adelgid numbers, predation is known to have a controlling affect (Franz 1955, Murray & Parry 1969). The great increase in planting of Scots pine across Scotland as part of New Native Woodland Grant Schemes since the late 1980s (Newton *et al.* 2001) may also be a factor, particularly if trees planted on unsuitable sites are vulnerable to infestations. The dating of cankers through growth ring analysis at a range of sites may allow an approximation of when infection occurred and give an estimate of when the outbreak began. Knowing when and how the epidemic started will allow a better assessment of the future risks to Scots pine posed by these biologically fascinating fungi.

CONCLUSIONS

The primary agents responsible for the symptoms of cankering and crown dieback on Scots pine now prevalent across Scotland are two closely related species of *Cucurbidothis* (*C. pithyophila* and *C. parmeliarum*). These species are considered obscure in the literature, but appear to be locally common when occurring in outbreaks. Across Scotland, 76 samples were collected of living Scots pine branches bearing stromata and investigations showed that these fungi consistently associate with the Scots pine woolly adelgid, *Pineus pini*. It is *P. pini* which directly damages the tree through feeding, with feeding sites subsequently colonised by the wound pathogen *Crumenulopsis sororia,* which in turn causes the blackened cankers that are so disfiguring on Scots pine. Phylogenetic analyses have placed the *Cucurbidothis* species, and a third member (*C. shangrilana*), in the *Leptosphaeriaceae.* Further investigations are required to understand the drivers behind this current widespread outbreak of *Cucurbidothis* species and *P. pini* on Scots pine in Scotland.

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SUPPLEMENTARY MATERIAL

Fig. **S1. A–D.** Cankers caused by secondary agents (possibly *Crumenulopsis sororia*) on Scots pine in Scotland (Rothiemurchus, February 2023). **A.** Branches with *Cucurbidothis* stromata, and also cankers (arrowed). **B–D.** Branches with typical 'burst open' blackened cankers.

Fig. S2. Protologues and other information of all previous taxonomic literature on *Curreya pithyophila*, listed broadly in chronological order regardless of spore form as until now (2026), the two spore forms have been regarded as the same species.

Fig. S3. A. Alignment of phragmospore (SP23_18.1) and dictyospore (SP23_19.1) ITS sequences showing 6 differences highlighted in red bold across 603 base pairs. **B.** Alignment of phragmospore (SP23_18.1) and dictyospore (SP23_19.1) *tef1-α* sequences showing 11 differences highlighted in red bold across 242 base pairs. **C.** Alignment of phragmospore (SP23_18.1) and dictyospore (SP23_19.1) *tub*2 sequences showing 21 differences highlighted in red bold across 348 base pairs. **D.** Alignment of

phragmospore (SP23_18.1) and dictyospore (SP23_52D.1) γ-actin sequences showing 6 differences highlighted in red bold across 270 base pairs. **E.** Alignment of phragmospore (SP23_18.1) and dictyospore (SP23_19.1) nuLSU sequences showing 4 differences highlighted in red bold across 706 base pairs.

Fig. S4. A–C. Inner part of stroma stained with lactophenol cotton blue. **A.** Stroma inner layer of hyaline cells. **B–C.** Adelgid wool or fungal hyphae. Specimens collected in Whitewell, November 2023. Scale bars = 10 µm.

File S1. Sequence alignment matrix in FASTA format consisting of ITS and partial nuLSU and β-tubulin sequences used to generate Fig. 5.

File S2. Sequence alignment matrix in FASTA format consisting of ITS and partial nuLSU sequences used to generate Fig. 6.

Table S1. Complete specimen data including voucher information and GenBank Accession numbers.

Table S2. ITS, partial nuLSU and β-tubulin sequences from species of *Leptosphaeriaceae*, with *Didymella exigua* and *D. maydis* as outgroup taxa. Newly generated sequences in bold.

