**Corynespora, Exosporium and Helminthosporium revisited – New species and generic reclassification**

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Abstract: Molecular phylogenetic analyses of a multigene matrix of partial nuSSU-ITS-LSU rDNA, rpb2 and tef1 sequences were performed to investigate the phylogenetic relationships of Corynespora, Exosporium and Helminthosporium species. Based on phylogenetic analyses and morphology, the genus Exosporium is synonymised with Helminthosporium, and the genus Corynespora is revealed as polyphyletic. Corynespora smithii is confirmed to be closely related to the generic type C. cassiicola and its morphology is described and illustrated. Exosporium tiliae, Corynespora caespitosa, C. endiaedrae, C. leucaendri and C. olivacea are recognised in Helminthosporium, and Splanchnonema quercicola and S. kalakadense are combined in Helminthosporium. Based on pure culture studies and DNA sequence data, Massaria heterospora and Massarinula italica are shown to be the sexual morphs of Helminthosporium tiliae and H. microsorum, respectively. European accessions of Splanchnonema quercicola are recognised to differ from the North American type and are described as Helminthosporium quercinum. The sexual morph of H. oligosporum is recorded and described for the first time. The generic type of Helminthosporium, H. velutinum, is epitypified with a recent collection from the type host, Fagus sylvatica. Based on sequence data, Helminthosporium genistae is recognised as a distinct species. Several species for which subterminal stromata have been reported are shown to be fungiculous on Diaporthales, the “stromata” representing aborted and transformed host stromata or conidiomata: H. caespitosum, H. microsorum, H. quercicola and H. quercinum on Coryneum spp.; H. hispanicum on conidiomata of Juglanconis juglandinaria; H. juglandinum on conidiomata of Diaporthus sp.; H. oligosporum and H. tiliae on Hercotheca tiliae. The newly described H. austriacum is fungicolous on Anmphisphaeria cf. miltipunctata (Xylariales).

Key words: Ascomycota, Dothideomycetes, Massarinaeaeae, Phylogenetic analysis, Pleosporales.

Taxonomic novelties: New species: Helminthosporium austriacum Voglmayr & Jaklitsch, Helminthosporium hispanicum Voglmayr & Jaklitsch, Helminthosporium juglandinum Voglmayr & Jaklitsch, Helminthosporium quercinum Voglmayr & Jaklitsch; New combinations: Helminthosporium endiaedrae (Crous & Summerell) Voglmayr & Jaklitsch, Helminthosporium kalakadense (Subram. & Sekar) Voglmayr & Jaklitsch, Helminthosporium leucaendri (Quaedv. et al.) Voglmayr & Jaklitsch, Helminthosporium quercicola (M.E. Barr) Voglmayr & Jaklitsch; Epitypifications (basionyms): Corynespora oligosporum Corda, Exosporium caespitosum Ellis & Barthol., Exosporium tiliae Link, Helminthosporium genistae Fr., Helminthosporium microsorum D. Sacc., Helminthosporium velutinum Link, Massaria heterospora G.H. Otth, Massarinula italica D. Sacc., Splanchnonema olivaceum Walr.

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INTRODUCTION

The genus *Helminthosporium* produces a conspicuous asexual morph, and its generic type, *H. velutinum*, is a well-known species of almost world-wide distribution and has been commonly recorded from various hosts. Most *Helminthosporium* species are considered to be saprobes of chiefly woody hosts (Luttrell 1964, Alcorn 1988), but one species, *Helminthosporium solani*, is an economically important pathogen of potatoes, as it is the causing agent of silver scurf disease of potato tubers (Errampalli et al. 2001).

The taxonomic history of the genus *Helminthosporium* is complex. About 740 taxa have been placed in *Helminthosporium* (http://www.indexfungorum.org, Dec. 2016), but most of these are not congeneric with the generic type. After detailed morphological analyses, the genus *Helminthosporium* was restricted to species having porogenous, diastosepate conidia with conidial scars consisting of simple, flat-ridged pores; conidia are acropereurogenously borne on septate, erect conidiophores which cease growth after the formation of terminal conidia (Ellis 1961, Luttrell 1963, 1964). However, Hughes (1958) considered the distinction between pleurogenous vs. acrogenous conidia unsuitable for generic classification and widened the generic circumscription to include also species with acrogenous conidia.

The latter were placed in the genera *Corynespora* and *Exosporium* by Ellis (1961) and Luttrell (1964), which was subsequently widely accepted.

Applying this restricted circumscription, numerous species pathogenic to hosts from the *Poaceae* were transferred from *Helminthosporium* to the genera *Bipolaris* (= *Cochliobolus*), *Curvularia* (= *Pseudocochliobolus*), *Exserohilum* (= *Setosphaeria*), and *Pyrenophora* (= *Drechslera*), which are all members of the *Pleosporaceae* (Sivanesan 1987, Hyde et al. 2013, Tanaka et al. 2015). Other species like *H. asterinum* were also shown to be only distantly related (Olivier et al. 2000). In molecular phylogenetic analyses, the generic type, *H. velutinum*, was revealed to belong to *Massarinaeaeae* (Kodueb et al. 2007, Hyde et al. 2013, Tanaka et al. 2015). However, only few additional *Helminthosporium* species have been sequenced so far. In the most extensive molecular phylogenetic account available for the genus, Tanaka et al. (2015) included four *Helminthosporium* species as well as three yet unnamed strains.

Based on extensive morphological investigations, Ellis (1961) synonymised numerous species with *H. velutinum*, and accepted 10 species in the genus. Subsequently, numerous additional species were described, and Siboe et al. (1999) listed 27 accepted species for *Helminthosporium*, providing a table
summarising their main diagnostic morphological characters. With the recent description of several new species mainly from China and Japan, the number of species currently accepted in Helminthosporium has risen to about 46 (Mycobank, data retrieved December 2016). Unfortunately, for most of these recently described species no sequence data are available.

There are few records of sexual morphs of Helminthosporium, and most are considered dubious as they have not been verified by sequence data. Hughes (1953) reported the production of a Helminthosporium asexual morph in a British ex-ascospore isolate of an unnamed Massaria species from Quercus, but he provided no morphological description of the sexual morph. Also from Quercus, Barr (1993) mentioned Helminthosporium cf. velutinum as presumed asexual morph of her Splanchnonema quercicola, but without a morphological description of the asexual morph, and the connection was not confirmed by pure culture studies. It is tempting to interpret the records of Hughes (1953) and Barr (1993) to represent the same or closely related species, considering that Splanchnonema species have been classified in Massaria until Shoemaker & LeClair (1975) acknowledged the fundamental differences between both genera. However, the lack of a description of the sexual morph by Hughes (1953) and of the asexual morph by Barr (1993) makes this little more than a guess. Subramanian & Sekar (1987) described Splanchnonema kalakadense as the sexual morph of H. velutinum based on pure culture studies. Recently, Tanaka et al. (2015) described a massarina-like sexual morph for H. massarinum based on pure culture and sequence data.

In the course of a survey on corticolous Dothideomycetes, several collections of splanchnonema-like fungi were made on various hosts, which were closely associated with helminthosporium-, corynespora- and exosporium-like asexual morphs. Pure culture as well as DNA sequence data from both sexual and asexual morphs revealed conspecificity of the associated morphs, and phylogenetic analyses revealed that they are all closely related to Helminthosporium velutinum. This prompted us to initiate a detailed morphological and molecular phylogenetic study of several Helminthosporium, Exosporium and Corynespora taxa, which resulted in the taxonomic revision presented here.

MATERIALS AND METHODS

Isolates

The isolates used in this study either originated from ascospores or conidia of new species or from culture collections. Details of the strains including NCBI GenBank accession numbers of gene sequences used to compute the phylogenetic trees are listed in Table 1. Strain acronyms other than those of official culture collections are used here primarily as strain identifiers throughout the work. Representative isolates have been deposited at the Westerdijk Fungal Biodiversity Centre, Utrecht, The Netherlands (CBS culture collection). Details of the specimens used for morphological investigations are listed in the Taxonomy section under the respective descriptions. The following culture of Pseudosplanchnonema phorcioides was sequenced but is not further treated here: Austria, Wien, Donaustadt, Lobau, Panozzalacke, on dead corticated twigs of Morus alba, 1 Apr. 2006, W. Jaklitsch [WU 38888, culture L16 (ex ascospore) = CBS 122935]. Herbarium acronyms are according to Thiers (2017). Freshly collected specimens have been deposited in the Fungarium of the Department of Botany and Biodiversity Research, University of Vienna (WU).

Morphology

Microscopic observations were made in tap water except where noted. Morphological investigations of sexual and asexual morphs were consistently done from material on natural substrates. Methods of microscopy included stereomicroscopy using a Nikon SMZ 1500 and Nomarski differential interference contrast (DIC) using a Zeiss Axio Imager.A1 compound microscope equipped with a Zeiss Axiocam 506 colour digital camera. Images and data were gathered using a Nikon DS-U2 digital camera and measured by using the NIS-Elements D v. 3.22.15 or Zeiss ZEN Blue Edition softwares. For certain images of ascomata and conidiomata the stacking software Zerene Stacker v. 2.30.5 was used. Measurement of the mean plus and minus the standard deviation of a number of measurements given in parentheses. Photography of culture plates was performed with a Nikon Coolpix 4500 camera.

Culture preparation, DNA extraction, PCR and sequencing

Single ascospore or conidium isolates were prepared and grown on 2 % malt extract agar (MEA), or on 2 % corn meal agar plus 2 % w/v dextrose (CMD).

Growth of liquid culture and extraction of genomic DNA was performed as reported previously (Voglmayr & Jaklitsch 2011, Jaklitsch et al. 2012) using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) or the modified CTAB method of Riethmüller et al. (2002).

The following loci were amplified and sequenced: the complete internally transcribed spacer region (ITS1-5.8S-ITS2) and a ca. 900 bp fragment of the large subunit nuclear ribosomal DNA (nuLSU rDNA), amplified and sequenced as a single fragment with primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990); a ca. 1.7–2.2 kb fragment of the small subunit nuclear ribosomal DNA (nuSSU rDNA) with primers SL1 (Landvik et al. 1997) and NS24mod (Voglmayr & Jaklitsch 2011); a ca. 1.2 kb fragment of the RNA polymerase II subunit 2 (rpb2) gene with primers fRPB2-5f and fRPB2-7r (Li et al. 1999) or dRPB2-5f and dRPB2-7r (Voglmayr et al. 2010); and a ca. 1.3–1.5 kb fragment of the translation elongation factor 1-alpha (tef1) gene containing introns 4 and 5 and part of the exon with primers EF1-728F (Carbone & Kohn 1999) and TEF1LLErev (Jaklitsch et al. 2005) or EF1-2218R (Rehner & Buckley 2005). PCR products were purified using an enzymatic PCR cleanup (Werle et al. 1994) as described in Voglmayr & Jaklitsch (2008). DNA was cycle-sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems, Warrington, UK) and the PCR primers; in addition, primers ITS4 (White et al. 1990) and ITS5 (Vilgalys & Hester 1990) were used for the ITS LSU and nuSSU rDNA regions. For tef1, the internal primers TEF1_INTF (forward; Jaklitsch 2009) and TEF1_INT2 (reverse; 5’ CCACTTNGNTGTGC-CATCTTRTT 3’) were used for cycle sequencing in certain
Table 1. Isolates and accession numbers used in the phylogenetic analyses. Isolates/sequences in bold were isolated/sequenced in the present study.

| Taxon                        | Strain    | Culture no. | Specimen no. | SSU   | LSU   | ITS   | rpb2  | tef1  | Notes  |
|------------------------------|-----------|-------------|--------------|-------|-------|-------|-------|-------|--------|
| *Byssothecium circinans*     | CBS 675.92| –           | –            | -     |       |       |       |       |        |
| *Corynespora cassicola*      | CBS 100822| –           | –            | -     |       |       |       |       |        |
| *C. cassissica*              | CCP       | –           | –            | -     |       |       |       |       |        |
| *C. smithii*                 | CABI 5649b| –           | –            | -     |       |       |       |       |        |
| *Cyclothyriella rubronotata* | TR        | CBS 121892  | WU 36862     | –     | –     | –     | –     | –     |        |
| *C. rubronotata*             | TR9       | CBS 141486  | WU 36858TT   | –     | –     | –     | –     | –     |        |
| *Helminthosporium aquaticum* | S-096     | MFLUCC 15-0357 | KUAS 89692TT | – | –     | –     | –     | –     |        |
| *H. austriacum*              | L132      | CBS 139924  | WU 38826TT   | –     | –     | –     | –     | –     |        |
| *H. daiberiaceae*            | H 4628 (= TS 36) | MAFF 243583 | HHUF 27971    | AB797231 AB807521 LC014555 – | –     | –     | –     | –     |        |
| *H. endiandrae*              | CPC 22194 | CBS 138902  | CBS H-21984TT | – | –     | –     | –     | –     |        |
| *H. genistae*                | L125      | –           | WU 38836     | –     | –     | –     | –     | –     |        |
| *H. hispanicum*              | L109      | CBS 136917  | WU 38843TT   | –     | –     | –     | –     | –     |        |
| *H. juglandinum*             | L101      | CBS 136912  | WU 38844     | –     | –     | –     | –     | –     |        |
| *H. leucadendri*             | CPC 19345 | CBS 135133  | CBS H-21323TT | – | –     | –     | –     | –     |        |
| *H. magnisporum*             | H 4627 (= TS 33) | MAFF 239278 | HHUF 27968TT  | AB797232 AB807522 AB811452 – | –     | –     | –     | –     |        |
| *H. massanimum*              | KT 1564   | CBS 139690 = JCM 13095 = MAFF 239605 | HHUF 29069TT | AB797234 AB807524 AB809629 – | –     | –     | –     | –     |        |
| *H. microsporum*             | L108      | CBS 136916  | WU 38863     | –     | –     | –     | –     | –     |        |
| *H. smithii*                 | L123      | –           | WU 38861     | –     | –     | –     | –     | –     |        |
| *L. juglandinum*             | L174      | –           | WU 38854     | –     | –     | –     | –     | –     |        |
| *L. leucadendri*             | L175      | –           | WU 38852     | –     | –     | –     | –     | –     |        |
| *L. rubronotata*             | L94       | –           | WU 38860     | –     | –     | –     | –     | –     |        |
| *L. smithii*                 | L95       | –           | WU 38860     | –     | –     | –     | –     | –     |        |

(continued on next page)
| Taxon                        | Strain | Culture no. | Specimen no. | SSU    | LSU    | ITS    | rpb2 | tef1 | Notes  |
|-----------------------------|--------|-------------|--------------|--------|--------|--------|------|------|--------|
|                            | L96    | CBS 136910  | WU 38850^IT | KY984427 KY984329 KY984390 KY984448 | A     |
| H. oligosporum              | L106   | –           | WU 38869     | –      | KY984330 KY984330 KY984391 KY984449 | C     |
|                             | L111   | –           | WU 38872     | –      | KY984331 KY984331 KY984392 | – C   |
|                             | L92    | CBS 136908  | WU 38867     | KY984428 KY984332 KY984393 KY984450 | C     |
|                             | L93    | CBS 136909  | WU 38864^IT | –      | KY984333 KY984333 KY984394 KY984451 | A     |
| H. quercinum                | –      | CBS 112393  | –            | –      | KY984334 KY984334 KY984395 KY984452 | C     |
|                             | L105   | –           | WU 38877     | –      | KY984335 KY984335 KY984396 | – C   |
|                             | L107   | CBS 136915  | WU 38880     | –      | KY984336 KY984336 KY984397 | – A   |
|                             | L159   | –           | WU 38879     | –      | KY984337 KY984337 KY984398 | – A   |
|                             | L170   | –           | WU 38878     | –      | KY984338 KY984338 KY984399 | – C   |
|                             | L90    | CBS 136921  | WU 38876^IT | KY984429 KY984339 KY984400 KY984453 | A     |
|                             | L91    | –           | WU 38767^IT  | KY984340 KY984340 KY984401 KY984454 | C     |
| H. solani                  | –      | CBS 365.75  | CBS H-13302  | KY984430 KY984341 KY984402 KY984455 | C     |
|                            | –      | CBS 640.85  | –            | KY984342 KY984342 KY984403 | – C   |
| Helminthosporum sp. yone 38| –      | MAFF 243857 | HHUF 29740   | AB797237 AB807527 NARO^3 | – AB80502 | C     |
| H. tiliae                  | L171   | –           | WU 38881     | –      | KY984343 KY984343 KY984404 KY984456 | C     |
|                             | L87    | CBS 136906  | WU 38884     | –      | KY984344 KY984344 KY984405 | – A   |
|                             | L88    | CBS 136907  | WU 38878^IT  | KY984441 KY984345 KY984406 KY984457 | A     |
| H. velutinum               | H 4626 (= TS 28) MAFF 243854 | HHUF 27966 | AB797240 AB807530 LC014556 | – AB80505 | C     |
|                            | H 4739 (= TS 58) MAFF 243855 | HHUF 28243 | AB797235 AB807525 LC014557 | – AB80501 | C     |
|                            | H 4743 (= TS 68) MAFF 243856 | HHUF 28248 | AB797236 AB807526 NARO^3 | – – C   |
|                             | L115   | CBS 136924  | WU 38891     | –      | KY984347 KY984347 KY984408 KY984458 | C     |
|                             | L116   | –           | WU 38887     | –      | KY984348 KY984348 KY984409 KY984459 | C     |
|                             | L117   | –           | WU 38885     | –      | KY984349 KY984349 KY984410 KY984460 | C     |
|                             | L126   | –           | WU 38894     | –      | KY984350 KY984350 KY984411 KY984461 | C     |
|                             | L127   | –           | WU 38889     | –      | KY984351 KY984351 KY984412 KY984462 | C     |
|                             | L131   | CBS 139923  | WU 38892^IT  | KY984352 KY984352 KY984413 KY984463 | C     |
|                             | L134   | –           | WU 38895     | –      | KY984353 KY984353 KY984414 | – C   |
|                             | L135   | –           | WU 38896     | –      | KY984354 KY984354 | – KY984464 | C     |
|                             | L136   | –           | WU 38888     | –      | KY984355 KY984355 | – KY984465 | C     |
|                             | L140   | –           | WU 38890     | –      | KY984356 KY984356 KY984415 | – C   |
|                             | L163   | –           | WU 38893     | –      | KY984357 KY984357 KY984416 | – C   |
|                             | L176   | –           | WU 38897     | –      | KY984358 KY984358 | – – C   |
|                             | L98    | –           | WU 38866     | KY984433 KY984359 KY984407 KY984466 | C     |
|                            | S-033  | MFLUCC 15-0423 | HKAS 83990  | KU697308 KU697304 KU697300 | – – C   |
|                            | S-076  | MFLUCC 15-0243 | HKAS 84000  | KU697309 KU697305 KU697301 | – – C   |
|                            | S-135  | MFLUCC 15-0428 | HKAS 84015  | KU697307 KU697303 KU697299 | – – C   |
|                            | yone 63 | MAFF 243858 | HHUF 29741 | AB797238 AB807528 NARO^3 | – AB80503 | C     |
|                            | yone 96 | MAFF 243859 | HHUF 30140 | AB797239 AB807529 LC014558 | – AB80504 | C     |
| Massarina cistii            | –      | CBS 266.62 = JCM 14140 | ZT (Hütter & Loeffler)^IT | AB797249 AB807539 LC014568 | – AB80514 A |
| M. eburnea                 | –      | CBS 473.64  | –            | AF164367 GU301840 AF383959 genome^2 | genome^2 | A     |
|                            | H 3953 | CBS 136967 = JCM 14422 | HHUF 26621 | AB521718 AB521735 LC014569 | – AB80517 A |
| Periconia byssoides         | H 4600 (= TS 29) MAFF 243872 | HHUF 28238 | AB797280 AB807570 LC014581 | – AB80546 C |
|                            | P. digitata | – | – | AB797271 AB807561 LC014584 | – AB80537 C |
|                            | –      | CBS 845.96 = JCM 14142 | – | AB797277 AB807567 LC014586 | – AB80543 C |
|                            | P. macrospina | – | CBS 135663, DSE 2036^1 | – | KP184080 KP184038 KP183999 genome^1,4 | genome^1,4 C |
|                            | P. pseudodigitata | KT 1395 CBS 136999 = JCM 13166 = MAFF 239676 | HHUF 29370^IT | AB797274 AB807564 LC014591 | – AB80540 A |
Prior to phylogenetic analyses, the approach of Wiens (1998) was applied to test for significant levels of localised incongruence among the markers used for the combined analyses, using the level of bootstrap support (Sung et al. 2007) as described in
data analysis

For phylogenetic analyses, combined matrices of ITS-LSU, SSU, rpb2 and tef1 sequences were produced. GenBank sequences of Massarinaceae and Periconiaceae were selected according to Taniaka et al. (2015) and supplemented with GenBank sequences from additional Corynespora and Helminthosporium species; some ITS sequences of Japanese strains not deposited in GenBank were downloaded via the Microorganisms Search System of the Genetic Resources Center (NARO), Tsukuba, Japan (http://www.gene.affrc.go.jp/). For some strains for which the whole genome data are available, sequences were retrieved from JGI-DOE (http://genome.jgi.doe.gov/).

Cyclothryriella rubronotata was selected as outgroup (Jaklitsch & Voglmayr 2016). All alignments were produced with the server version of MAFFT (www.ebi.ac.uk/Tools/mafft), checked and refined using BioEdit v. 7.0.9.0 (Hall 1999). Due to alignment problems, 67 nucleotide characters at the 5′ end of the ITS1 were excluded. For phylogenetic analyses, all sequence alignments were combined. For Periconia macropinosa, ITS, LSU and SSU rDNA GenBank sequences of strain CBS 135663 were combined with the rpb2 and tef1 sequences from the genome of strain DSE 2036, as both strains have identical ITS and LSU sequences (D. Knapp, unpublished data).

Maximum parsimony (MP) analyses of the combined matrices were performed using a parsimony ratchet approach. For this, a nexus file was prepared using PRAP v. 2.0b3 (Müller 2004), implementing 1 000 ratchet replicates with 25 % of randomly chosen positions upweighted to 2, which was then run with PAUP v. 4.0a151 (Swofford 2002). The resulting best trees were then loaded in PAUP and subjected to heuristic search with TBR branch swapping (MULTREES option in effect, steepest descent option not in effect). Bootstrap analyses with 1 000 replicates were performed using 5 rounds of replicates of heuristic search with random addition of sequences and subsequent TBR branch swapping (MULTREES option in effect, steepest descent option not in effect) during each bootstrap replicate, with each replicate limited to 1 million rearrangements. In all MP analyses molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data; the COLLAPSE command was set to minbrlen.

Maximum likelihood (ML) analyses were performed with RAxML (Stamatakis 2006) as implemented in raxmlGUI v. 1.3 (Silvestro & Michalak 2012), using the ML + rapid bootstrap setting and the GTR+GAMMA substitution model with 1 000 bootstrap replicates. The matrices were partitioned for the individual gene regions, and substitution model parameters were calculated separately for them.

RESULTS

Molecular phylogeny

For Helminthosporium genistiae, no tef1 sequences could be obtained due to the presence of paralogs. Of the 5 100 and 5 099 nucleotide characters of the two combined matrices used for the phylogenetic analyses, 1 336 and 1 315 are parsimony informative, respectively (408 and 401 of SSU-ITS-LSU, 485 of rpb2, 443 and 429 of tef1). Fig. 1 shows the phylogram of one of
Fig. 1. Phylogram showing one of 90761 MP trees 4603 steps revealed by PAUP from an analysis of the combined ITS-LSU-SSU-rpb2-tef1 matrix of Massarinaceae, Periconiaceae and Corynesporaccae, with Cyclothyriella rubronotata (Cyclothyriellaceae) selected as outgroup. MP and ML bootstrap support above 50 % are given at the first and second position, respectively, above or below the branches; within species bootstrap support is mostly not shown due to lack of space. Strain numbers are given following the taxon names; strains formatted in bold were sequenced in the current study. Helminthosporium taxa formerly classified in Caryaespora and Exosporium are marked green and blue, respectively.

| Taxon Name          | Bootstrap Support | Strain Number |
|---------------------|-------------------|---------------|
| Helminthosporium    |                   |               |
| velutinum L116      | 100/100           |               |
| velutinum L126      | 100/100           |               |
| velutinum L127      | 100/100           |               |
| velutinum L115      | 100/100           |               |
| velutinum L98       | 100/100           |               |
| velutinum L117      | 100/100           |               |
| velutinum L140      | 100/100           |               |
| velutinum L131      | 100/100           |               |
| velutinum L134      | 100/100           |               |
| velutinum L163      | 100/100           |               |
| velutinum MAFF 243854 | 100/100          |               |
| velutinum MAFF 243855 | 100/100          |               |
| velutinum MAFF 243856 | 100/100          |               |
| velutinum MFLUCC 15-0243 | 100/100     |               |
| velutinum MFLUCC 15-0423 | 100/100     |               |
| velutinum MFLUCC 15-0424 | 100/100     |               |
| velutinum MFLUCC 15-0425 | 100/100     |               |
| velutinum MFLUCC 15-0426 | 100/100     |               |
| Helminthosporium    |                   |               |
| solani CBS 365.75   | 100/100           |               |
| solani CBS 640.85   | 100/100           |               |
| Helminthosporium    |                   |               |
| oligosporum L106    | 100/100           |               |
| oligosporum L92     | 100/100           |               |
| oligosporum L93     | 100/100           |               |
| oligosporum L111    | 100/100           |               |
| oligosporum L171    | 100/100           |               |
| Helminthosporium    |                   |               |
| australiacum L132   | 100/100           |               |
| australiacum L169   | 100/100           |               |
| australiacum L177   | 100/100           |               |
| australiacum L137   | 100/100           |               |
| Helminthosporium    |                   |               |
| aquadulcem MFLUCC 15-0357 | 100/100 |               |
| Helminthosporium    |                   |               |
| endiandrae CBS 138462 | 100/100       |               |
| massarium CBS 136560 | 100/100         |               |
| massarium MAFF 239604 | 100/100       |               |
| Helminthosporium    |                   |               |
| microsorum L108     | 100/100           |               |
| microsorum L175     | 100/100           |               |
| microsorum L174     | 100/100           |               |
| microsorum L96      | 100/100           |               |
| microsorum L94      | 100/100           |               |
| microsorum L123     | 100/100           |               |
| microsorum L85      | 100/100           |               |
| juglandinum L101    | 100/100           |               |
| juglandinum L102    | 100/100           |               |
| juglandinum L97     | 100/100           |               |
| juglandinum L118    | 100/100           |               |
| Helminthosporium    |                   |               |
| quercinum CBS 112393 | 100/100         |               |
| quercinum L90       | 100/100           |               |
| Helminthosporium    |                   |               |
| quercinum L91       | 100/100           |               |
| quercinum L105      | 100/100           |               |
| Helminthosporium    |                   |               |
| quercinum L107      | 100/100           |               |
| quercinum L159      | 100/100           |               |
| Helminthosporium    |                   |               |
| dalgae CBS 243853   | 100/100           |               |
| Helminthosporium    |                   |               |
| hispanicum CBS 139660 | 100/100       |               |
| helminthosporium    |                   |               |
| massarinum CBS 139660 | 100/100       |               |
| Helminthosporium    |                   |               |
| microsorum CBS 266.62 | 100/100       |               |
| Massaria eubumae CBS 139697 | 100/100 |               |
| Massaria eubumae CBS 473.64 | 100/100 |               |
| Stagonospora        |                   |               |
| pseuoprefecta CBS 120236 | 100/100 |               |
| Stagonospora        |                   |               |
| tainanensis MAFF 243860 | 100/100 |               |
| Stagonospora        |                   |               |
| perfecta MAFF 239603 | 100/100        |               |
| Stagonospora        |                   |               |
| paludosa CBS 130588 | 100/100           |               |
| Periconia           |                   |               |
| igniana CBS 845.98  | 100/100           |               |
| Periconia           |                   |               |
| macrospora CBS 135663 | 100/100       |               |
| Periconia           |                   |               |
| hyssosoldes MAFF 243872 | 100/100     |               |
| Periconia           |                   |               |
| pseudodigetata CBS 139699 | 100/100 |               |
| Periconia           |                   |               |
| digitata CBS 510.77 | 100/100           |               |
| Massaria            |                   |               |
| eubumae CBS 139697  | 100/100           |               |
| Massarinae          |                   |               |
| Corynespora         |                   |               |
| smithii L126        | 100/100           |               |
| Corynespora         |                   |               |
| smithii L130        | 100/100           |               |
| Corynespora         |                   |               |
| smithii L133        | 100/100           |               |
| Corynespora         |                   |               |
| smithii L139        | 100/100           |               |
| Corynespora         |                   |               |
| cassicola CBS 100822 | 100/100        |               |
| Cyclothyriella      |                   |               |
| rubronotata CBS 121892 | 100/100       |               |
| Cyclothyriella      |                   |               |
| rubronotata CBS 141486 | 100/100       |               |

Helminthosporium

Massarinaceae

Corynesporaceae

Cyclothyriellaceae (outgroup)
90 761 MP trees of 4 603 steps revealed from the analyses of the combined matrix containing all Helminthosporium accessions for which at least ITS and LSU sequences are available. Tree topologies of all MP trees were identical, except for minor topological differences within species. The backbone of the ML tree revealed by RAxML was similar to the MP strict consensus tree; it differed in a basal position of Periconia byssoides in the Periconia clade, a sister group relationship of Stagonospora perfecta and S. paludosa, an interchanged position of Byssothecium cicinans and Pseudosplanchnonema phorcioides and H. aquaticum being placed between them; a sister group relationship of H. endiandrae to H. leucadendri; a slightly different position of H. massarinum; and a sister-group relationship of H. juglandinum to the H. dalbergiae-H. magnisporum-H. quercinum clade (not shown).

The MP analyses of the combined matrix containing all Helminthosporium accessions, for which at least ITS, LSU and rpb2 sequences are available, revealed 145 MP trees of 4 310 steps (not shown). The best ML tree (InL = −2661.191) revealed by RAxML is shown as Fig. 2. The strict consensus tree of all 145 MP trees was fully compatible with Fig. 1, and it was similar to Fig. 2, except for slightly different topologies within Stagonospora.

In the MP and ML analyses of both matrices, most basal nodes received high support (Figs 1, 2). Our molecular phylogenetic analyses confirm previous investigations (Kodsiëb et al. 2007, Hyde et al. 2013, Tanaka et al. 2015) that the genus Helminthosporium belongs to the Massarinaceae. The genus Corynespora is revealed as polyphyletic. While the generic type Helminthosporium here recognised in analyses (C. caespitosa, C. endiandrae, C. leucadendri and C. olivacea (H. oligosporum); marked green in Fig. 1) are revealed to belong to the genus Helminthosporium. All species here recognised in Helminthosporium are contained in a monophyletic clade, which does not receive support in the analyses of the comprehensive combined matrix (Fig. 1). Remarkably, after removal of the Helminthosporium accessions lacking the rpb2, bootstrap support for the Helminthosporium clade strongly rises to 78 % and 91 % in the MP and ML analyses, respectively, and several additional nodes within the Helminthosporium clade received significantly higher support as well, especially in the ML analyses (Fig. 2). After exclusion of H. leucadendri for which only short rpb2 and tef1 sequences are available, the Helminthosporium clade becomes highly supported even in both analyses (94 % MP and 98 % ML bootstrap support; Fig. 2), whereas support for the other nodes is comparable to the analysis including H. leucadendri (not shown).

Within Helminthosporium, neither the species with corynespore-like nor with helminthosporium-like asexual morphs are closely related, but are rather interspersed (Figs 1, 2). The corynespore-like Helminthosporium oligosporum and the exosporium-like H. tiliae, both fungalous on Hecespora tiliae on Tilia spp., are sister species with maximum support, and closely related to the corynespora-like H. caespitosa (Figs 1, 2). The fungalous Helminthosporium species form three clades (Fig. 2). Two clades consist of species growing on old stromata or conidiomata of Diaporthales, whereas H. austriacum, which grows on effete ascomata of Amphisphaeria (Xylariales), is sister species to H. genistae, which is saprobic on Fabaceae (Fig. 2).

Culture characteristics

Culture images of nine studied Helminthosporium species grown on MEA and CMD are shown in Fig. 3. Detailed culture descriptions are given under the respective species.

Taxonomy

Corynespora Güssov, Z. PflKrankh. PflSchutz 16: 10. 1906.

Type species: Corynespora mazei Güssov, Consp. Regni Veget. (Leipzig) 16: 13. 1906.

Corynespora smithii (Berk. & Broome) M.B. Ellis, Mycol. Pap. 65: 3. 1957. Fig. 4.

Basionym: Helminthosporium smithii Berk. & Broome [as ‘Helmisporium’], Ann. Mag. nat. Hist., Ser. 2 7: 97. 1851.

Sexual morph unknown. Colony on natural substrate effuse, dark brown or black, velvety or spongy, forming small to widely effused patches up to more than 10 cm long. Mycelium partly superficial, partly immersed in the substrate, composed of branched, septate, subhyaline to brown, smooth-walled, 2–7 μm wide hyphae. Stromata partly superficial, partly immersed, brown, irregular in shape and often extending over large areas, pseudoparenchymatous, composed of cells (5.5–) 7.5–11.5(–15.0) μm diam (n = 44). Conidiophores 110–370 μm long, 7–12 μm wide at the base, 8–8.5 μm near the apex, arising singly or more often in dense tufts from superficial hyphae or from cells of the stromata, erect or ascending, simple, straight or flexuous, pale brown to dark brown, septate, with up to four successive cylindrical proliferations. Conidia (140–) 170–246(–350) × (9–)11.5–16(–19.5) μm (n = 61), with a 6–7.5 μm wide blackish-brown scar at the base, formed singly or in a short chain through a wide pore at the apex of the conidiophore, often with proliferation through the apical pore and formation of another conidium at the apex of the proliferation, almost cylindrical but usually slightly and gradually tapering towards the rounded apex and more abruptly towards the truncate base, straight or slightly curved, smooth, subhyaline to golden brown, 7–45-distoseptate, with angular lumina; wall up to 5.5 μm thick.

Habitat and host range: Saprobic on dead twigs and trunks of various woody plants.

Distribution: Europe (UK, Austria).

Typification: Lectotype of Helminthosporium smithii, here designated: UK, England, Dorset, Wareham Wood, on dead bark and wood of Ilex aquifolium, 10 Apr. 1850, W. Smith, ex Herb. Berk. (K/M) 233768; MBT376657. Same place, without date, W. Smith, ex herb. C.E. Broome [K/M] 233767, isotype.

Specimens examined: Austria, Niederösterreich, Wölflersdorf, Marchgraben, on Hippocrepis nema, 9 Oct. 2013, H. Voglmayr [WU 38820, culture L120 (ex conidium)]; Wien, Döbling, Kahlenberg, on Fagus sylvatica, 16 Nov. 2013, W. Jakitsch [WU 38821, culture L130 (ex conidium)]; Wien, Ottakring, Wilhelminenberg, on Fagus sylvatica, 24 Nov. 2013, H. Voglmayr [WU 38822,
Fig. 2. Phylogram of the best ML tree (lnL = -26619.1191) revealed by RAxML from an analysis of the reduced ITS-LSU-SSU-rpb2-tef1 matrix of Massarinaceae, Periconiaceae and Corynesporacaeae, with Cyclothyriella rubronotata (Cyclothyriellaceae) selected as outgroup. The matrix contains only Helminthosporium accessions for which at least ITS, LSU and rpb2 sequences are available. MP and ML bootstrap support above 50 % are given at the first and second position, respectively, above or below the branches; within species bootstrap support is mostly not shown. Bootstrap support for the Helminthosporium clade in bold marked by an asterisk (*) give results of analyses of the same matrix after the exclusion of H. leucadendri (for detailed explanation see text). Strain numbers are given following the taxon names; strains formatted in bold were sequenced in the current study.
We here provide a description modified from Ellis (1957) for comparison with *Helminthosporium*, because *C. smithii* is also found on woody substrates, sometimes in close association with *Helminthosporium velutinum*. Although the porogenous

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**Fig. 3.** *Helminthosporium* cultures at 22 °C. A, B. *H. austriacum* (L169). C, D. *H. caespitosum* (L141). E, F. *H. genistae* (L142). G, H. *H. juglandinum* (L118). I, J. *H. microsorum* (L96). K, L. *H. oligosporum* (L93). M, N. *H. quercinum* (L170). O, P. *H. tiliae* (L171). Q, R. *H. velutinum* (L115). S, T. *H. velutinum* (L131). A, C, E, G, I, K, M, O, Q, S. On CMD. B, D, F, H, J, L, N, P, R, T. On MEA. A. After 32 d. B. After 43 d. C, D, G–L, O–T. After 4 wk. E, F. After 3 wk. M, N. After 25 d.
distoseptate conidia with a dark brown scar and the conidiophores share morphological similarities to some Helminthosporium species as defined here, *C. smithii* is not closely related to *Helminthosporium* but forms a separate distant clade together with the generic type, *C. cassiicola*, which is currently classified as family Corynesporascaceae. *Corynespora smithii* is characterised by proliferating conidiophores and conidia, a feature which it shares with *C. cassiicola*. *Corynespora smithii* has been described from *Ilex aquifolium*; sequences from a culture from the type host match those obtained from *Fagus sylvatica* and *Hippocrepis emerus*, confirming a wide host range of the species given by *Ellis* (1957).

**Helminthosporium** Link, Mag. Gesell. naturf. Freunde, Berlin 3(1–2): 10. 1809.

**Synonym:** *Exosporium* Link, Mag. Gesell. naturf. Freunde, Berlin 3(1–2): 9. 1809.

Type species: *Helminthosporium velutinum* Link.

**Sexual morph** where known massaria- or splanchnonema-like. *Pseudostromata* formed in the upper bark, usually well-developed, dark (reddish) brown, pseudoparenchymatous, of thick-walled dark brown cells; margin composed of dark brown, verrucose hyphae; less commonly rudimentary and composed of thin-walled, smooth brown hyphae. *Ascomata* immersed in pseudostromata or upper bark, variably elevating the latter, singly or in small groups, large, ca. 300–1 000 μm diam (including wall of the pseudostroma), globose to depressed globose, often strongly depressed, dark brown to black. *Peridium* pseudoparenchymatous. *Ostioles* central, inconspicuous, not protruding above the cortical surface. *Hamathecium* consisting of numerous filiform, septate, branched, anastomosing, narrow pseudoparaphyses usually embedded in a gel matrix. *Asci* clavate or fusoid, containing 8 ascospores in irregularly biseriate arrangement, rarely 4 in uniseriate arrangement. *Ascospores* mostly large, hyaline or first hyaline to pale brown and turning medium to dark brown at full maturity, fusoid, broadly fusoid, subellipsoid, obovoid, less commonly oblong, asymmetric, with 1 eccentric primary septum and often with transverse or oblique.
distosepta, less commonly ring-like thickenings, in one or both parts, rarely with a longitudinal distoseptum in the larger part, strongly constricted at the primary septum, slightly or not constricted at the secondary distosepta, with subacute to rounded end cells; wall hyaline or brown, smooth or verrucose, sometimes with longitudinal striae; with granular to guttulate contents; each part surrounded by a thick gelatinous sheath.

**Habitat and host range:** Saprobe, rarely parasitic on plants, or fungicolous.

**Distribution:** Cosmopolitan, mainly known from Europe and USA.

**Colony** on natural substrate conspicuous, effuse to punctiform and hairy, or pulvinate, brown to black. *Myceiulum* immersed in the substrate. *Stromata* usually present. **Conidiophores** arising solitarily or in fascicles from substrate hyphae or stroma cells, erect, simple, straight or flexuous, brown, single-, few- to many-celled, with a well-defined small pore at the apex, commonly also with lateral pores beneath the upper septa, ceasing growth with the formation of a terminal conidium, usually not proliferating. Conidia formed singly (rarely in short chains), subhyaline to brown, obclavate, obpyriform to lageniform, commonly rostrate, distoseptate, usually with a distinct dark brown to black scar at the base. Cultures on MEA and CMD in most species slow-growing (fast in *H. quercinum* and *H. velutinum*), white, shades of brown or grey, rarely orange on MEA (*H. austriacum*), sometimes (*H. austriacum* and *H. tiliae*) with pigment diffusing into agar; odour in most species unpleasant. Culture images of nine studied *Helminthosporium* species are shown in Fig. 3.

**Note:** The genera *Helminthosporium* and *Exosporium* were described in the same publication ([Link 1809]). Fries (1832) synonymised *Exosporium* with *Helminthosporium*, placing *Exosporium tiliae*, the generic type, in *Helminthosporium*, which is therefore to be used as sanctioned name. We provide an emended generic description of *Helminthosporium* here to include also some species formerly classified in *Corynespora* and *Exosporium*, and to appropriately consider the sexual morphs newly linked to several species.

**Helminthosporium austriacum** Voglmayr & Jaklitsch, sp. nov. MycoBank MB821196. Fig. 5.

**Etymology:** Referring to its occurrence in Austria.

**Sexual morph** unknown. Colony on natural substrate effuse, black, hairy, up to more than 10 cm long. *Myceiulum* mostly immersed, at the surface forming small stroma-like aggregations of dark brown pseudoparenchymatous cells (6.5–) 8.7–12.5 (–14.0) μm diam (n = 30). **Conidiophores** 275–700 (–920) μm long, 11.5–19 μm wide at the base, tapering to 7–11 μm near the apex, arising solitarily or in fascicles from the stroma cells, erect, simple, straight or flexuous, thick-walled, sub-cylindrical, smooth, brown to dark brown, paler near the apex, with well-defined small pores at the apex and laterally beneath the upper 1–12 septa. *Conidia* (30–) 35–48 (–97) × (10.0–) 13.7–16.5 (–19.8) μm (n = 198), tapering to 4.5–6.0 μm at the distal end, with a blackish-brown 3–6 μm wide scar at the base, obpyriform to lageniform, straight or curved, smooth, pale brown, (4–) 5–7 (–10)-distoseptate, with angular lumina; wall up to 4.5 (–) 6 μm thick.

**Culture characteristics:** Culture L169: On CMD colony radius ca. 16 mm after 1 mo at 22 °C. Colony yellow-green, turning dull yellowish brown, centre nearly black, yellowish pigment diffusing into agar (Fig. 3A); odour sweetish or unpleasant (“chemical”). On MEA colony radius 14 mm after 1 mo at 22 °C. Colony thick, dense, zonate, orange, centre whitish to pale greenish, reverse with black and pale orange zones (Fig. 3B).

**Habitat and host range:** On dead corticated twigs and trunks of *Fagus sylvatica* and *Acer campestrae*: fungicolous on old ascocoma of *Amphisphaeria* cf. *millepunctata*.

**Distribution:** Europe; only known from Austria.

**Holotype:** Austria, Wien, Döbling, Kahlenberg, on dead corticated twigs of *Fagus sylvatica*, 16 Nov. 2013, W. Jaklitsch (WU 38826, ex-holotype) culture CBS 139924 = L132 (ex conidium); MBBT376640).

**Other specimens examined** (all on corticated dead twigs or trunks): Austria, Kärnten, St. Margareten im Rosental, Zabre, on *Fagus sylvatica*, 28 Dec. 2013, W. Jaklitsch [WU 38825, culture L137 (ex conidium)]; Wien, Ottaking, Wilhelminenberg, on *Fagus sylvatica*, 4 Dec. 2016, H. Voglmayr [WU 38827, culture CBS 142388 = L169 (ex conidium)]; Niederösterreich, Mannersdorf, Naturpark Wüste, on *Acer campestrae*, 11 Feb. 2017, H. Voglmayr & I. Greilhuber [WU 38828, culture L177 (ex conidium)].

**Notes:** *Helminthosporium austriacum* is well characterised by its small, distinctly lageniform conidia in combination with *Amphisphaeria* cf. *millepunctata*, mostly on *Fagus sylvatica*. These hosts are shared with the polyphagous *H. velutinum*, with which it can co-occur. *Helminthosporium austriacum* is apparently fungicolous as all four collections were associated with old ascocoma of *Amphisphaeria* cf. *millepunctata*. Also the orange colony colour seems to be characteristic, at least among the studied species. *Helminthosporium austriacum* has conidia of similar length as *H. mauritanicum* and *H. acaciae* ([Ellis 1961]); however, in the latter they are of different shape and significantly narrower (8–13 μm, mean 11.1 μm, and 10–14 μm, mean 12 μm, in *H. mauritanicum* and *H. acaciae*, respectively, vs. (10.0–) 13.7–16.5 (–19.8) μm, mean 15.1 μm, in *H. austriacum*). In addition, *H. mauritanicum* and *H. acaciae* occur on different hosts in (sub)tropical areas.

**Helminthosporium caespitosum** (Ellis & Barthol.) S. Hughes [as ‘*Helmisporium caespitosum’*], Canad. J. Bot. 36: 775. 1958. Fig. 6.

**Basionym:** *Exosporium caespitosum* Ellis & Barthol. [as ‘*cespitosum’*], J. Mycol. 8(4): 178. 1902.

**Synonyms:** *Corynespora caespitosa* (Ellis & Barthol.) M.B. Ellis [as ‘*cespitosa’*], Mycol. Pap. 87: 39. 1963.

*Corynespora* bramleyi M.B. Ellis, Mycol. Pap. 76: 34. 1960.

**Sexual morph** unknown. Colonies on natural substrate forming conspicuous dark red brown, scattered or crowded conidiomata. *Myceiulum* immersed, growing in aborted stromata or conidiomata of *Coryneum* below the periderm. *Conidiomata* 0.3–1.7 (–3.6)
mm wide (n = 63), 250–650 μm high (n = 30), superficial, stromatic, erumpent through the periderm, pulvinate to discoid, sometimes confluent, circular to ellipsoid, often irregularly lobed, internally composed of loose branched hyphae tending to be more compacted towards the surface. Conidiophores densely crowded, arising from the outer cell layer of the conidiomata, erect, simple, straight or curved, obpyriform, 0–2 septate, medium to dark reddish brown, (21–)27–37(–44) μm long, (11.2–)12.2–14.5(–16.5) μm wide (n = 80), with a swollen apex and a single conspicuous apical pore bearing the single conidium. Conidia (67–)82–109(–119) × (22.0–)27.3–35.5(–40.5) μm (n = 173), tapering to 3.5–9 μm at the distal end, with a 2.5–6 μm wide, dark brown to black scar at the base, broadly ellipsoid to obclavate, sometimes rostrate, straight or slightly
curved, with coarse scale-like flat verrucae, medium to dark reddish brown, paler toward the apex, (3–)6–10-distoseptate with angular lumina; wall up to 8 µm thick.

**Culture characteristics:** Culture L141: On CMD colony radius ca. 22 mm after 4 wk at 22 °C. Colony white turning dull brownish from the centre, dense, thin, aerial hyphae inconspicuous or lacking (Fig. 3C); odour strong, unpleasant. On MEA colony radius 20 mm after 4 wk at 22 °C. Colony roundish, surface velvety, covered by a white dense flat mat of aerial hyphae, reverse yellowish (Fig. 3D); odour strong, unpleasant.

**Habitat and host range:** On dead corticated twigs of *Betula* spp.: fungicolous on old stromata of *Coryneum lanciforme*.
**Distribution:** North America, Northern Europe; widespread but uncommon.

**Typification:** **USA,** Michigan, Mackinac Island, on dead birch limbs, 10 Jul. 1889, E.T. Harper 452 (NY 00928681, holotype).  
**Epitype,** here designated: **Canada,** Québec, Gatineau Park, Pints Lake, on dead corticated branches of *Betula* sp., without date, S.J. Hughes & W. Gams (CBS-H 000713; **ex-epitype** culture CBS 484.77; MBT376641).

**Other specimens examined** (all on dead corticated twigs of *Betula* spp.): **Norway,** Prov. Aust-Agder, Froland kommun, Ytre Lauvrak, on *Betula pendula,* 3 Oct. 2014, H. Voglmayr [WU 38829, culture L151 (ex conidium)].  
**Poland,** Ruciane-Nida, Niedźwiedzi Róg, on *Betula pubescens,* 19 Jul. 2015, H. Voglmayr & I. Greilhuber [WU 38830, **UK,** England, West Yorkshire, Brighouse, on *Betula pubescens,* 18 Apr. 2014, C.S.V. Yeates [WU 38831, culture L.141 (ex conidium)].

**Notes:** In the original description, the incorrect spelling “cespitosum” was used, which is here corrected to “caespitosum,” in accordance with MycoBank. *Helminthosporium caespitosum* is well characterised by its host (*Betula* spp.) and its large, dark red-brown conidiomata superficially resembling immature stromata of *Hypoxylon.* Conspicuity of North American and European accessions was confirmed by sequence data. A Canadian collection housed at Westerdijk Institute is chosen as the **epitype;** this collection was misidentified by sequence data. A **com** & Jaklitsch, *Helminthosporium endiandrae* (Crous & Summerell) Voglmayr & Jaklitsch, **comb. nov.,** MycoBank MB821197.

**Basionym:** *Corynespora endiandrae* Crous & Summerell, in Crous et al., Persoonia 33: 229. 2014.

**Holotype:** **Australia,** New South Wales, Nightcap National Park, S28.33.918 E153.20.228, on leaves of *Endiandra introrsa* (Lauraceae), 9 Mar. 2013, B.A. Summerell (CBS H-21984; **ex-holotype** culture CPC 22184 = CBS 138902).

**Notes:** *Corynespora endiandrae* is not closely related to the generic type of *Corynespora,* *C. cassicola,* but embedded within the *Helminthosporium* clade (Fig. 1). Also morphologically it fits the genus *Helminthosporium* as re-defined here in its non-proliferating conidiophores. In contrast to most other *Helminthosporium* species, *H. endiandrae* grows on leaves. For detailed descriptions and illustrations see Crous et al. (2014).

**Helminthosporium genistae** Fr. [as ‘Helmispornium’], Syst. mycol. (Lundae) 3(2): 360. 1832. Fig. 7.

**Sexual morph** unknown. Colony on natural substrate effuse, black, hairy. *Myccelium* immersed, at the substrate surface forming stroma-like aggregations of subgalline to dark brown pseudoparenchymatous cells (4.5–6.0–11.8–22.8 μm diam (n = 71). *Conidiophores* (155–)280–460(–560) μm long (n = 112), 15–23 μm wide at the base, tapering to 10.5–15 μm near the apex, arising usually in fascicles from stroma cells, simple, straight or flexuous, thick-walled, sub-cylindrical, smooth, brown to dark brown, with well-defined small pores at the apex and laterally beneath the upper 1–7 septa. *Conidia* (41–)51–73(–93) × (10.5–)12.7–15.8(–17.5) μm (n = 98), gradually tapering to 3–6.5(–8) μm at the distal end, with a 2–5 μm wide, blackish-brown to black scar at the base, straight or flexuous, obclavate to rostrate, smooth-walled, pale golden brown to brown, 5–12-distoseptate, with angular lumina; wall up to 6.5 μm thick.

**Culture characteristics:** Colony L142: On CMD colony radius ca. 10 mm after 4 wk at 22 °C. Colony white, dense, thick, zonate after exposure to light; aerial hyphae inconspicuous or lacking (Fig. 3E); odour unpleasant (cabbage-like). On MEA colony radius 9 mm after 4 wk at 22 °C. Colony with a slightly uneven margin, thick, with a white dense mat of aerial hyphae containing large drops; reverse yellow, brown in the centre (Fig. 3F); odour strong, unpleasant.

**Habitat and host range:** Saprobic on dead twigs of various fabaceous shrubs from the tribe Genisteeae.

**Distribution:** Europe (France, Italy, Spain); apparently common in the mediterranean to submediterranean region.

**Typification:** **France,** on dead twigs of *Cytisus scoparius,* J.B. Mougeot, ex Herb. E. Fries (UPS: BOT: F-783304, holotype).  
**Epitype,** here designated: **France,** Côte-d’Or (21), Vieux-Château, on dead corticated twigs of *Cytisus scoparius,* 15 Apr. 2014, A. Gardiennet, A.G. 14089 [WU 38832; **ex-epitype** culture CBS 142597 = L142 (ex conidium); MBT376642).

**Other specimens examined** (all on dead corticated twigs): **Greece,** Crete, Chania, SW Lakki, on *Chamaecytisus creticus,* 5 Jun. 2015, H. Voglmayr & W. Jaklitsch [WU 38833, culture L173 (ex conidium)].  
**Italy,** Lazio, Viterbo, Bomarzo, Monte Casoli, on *Cytisus scoparius,* 17 Oct. 2013, H. Voglmayr & W. Jaklitsch [WU 38834, culture CBS 139922 = L129 (ex conidium)]; Viterbo, Gradoli, il Purgarorio, on *Cytisus scoparius,* 13 Oct. 2013, H. Voglmayr & W. Jaklitsch [WU 38835, culture CBS 139921 = L128 (ex conidium)]; Viterbo, Norchia, on *Cytisus scoparius,* 14 Oct. 2013, H. Voglmayr & W. Jaklitsch [WU 38836, culture L125 (ex conidium)].  
**Portugal,** Sintra, Castelo dos Mouros, on *Cytisus cf. striatus,* 16 Feb. 2017, H. Voglmayr & W. Jaklitsch [WU 38899].  
**Spain,** Andalucía, Cádiz, Alcalá de los Gazules, El Picacho, on *Cytisus baeticus,* 1 Apr. 2014, W. Jaklitsch [WU 38837, culture L143 (ex conidium) = CBS 139927]; Huelva, Castano de Robledo, on *Ulex parviflorus,* 8 Apr. 2014, W. Jaklitsch [WU 38838, culture L147 (ex conidium)]; Huelva, Castano de Robledo, on *Cytisus striatus,* 8 Apr. 2014, W. Jaklitsch [WU 38839, culture CBS 139929 = L148 (ex conidium)]; Jaén, Otíñar, La Cañada, on *Cytisus fontanesii,* 12 May 2014, W. Jaklitsch [WU 38840, culture CBS 139930 = L149 (ex conidium)]; Jimena, Montes de Jimena, Puerto Galis, on *Calicotome villosa,* 4 Apr. 2014, W. Jaklitsch [WU 38841, culture CBS 139928 = L144 (ex conidium)]; Málaga, Cortes de la Frontera, La Sauceda, on *Cytisus baeticus,* 4 Apr. 2014, W. Jaklitsch [WU 38842, culture L145 (ex conidium)]; Canarias, La Gomera, Alto de Garajonay, on *Chamaecytisus proliferus,* 21 Mar. 2016, H. Voglmayr (WU 35976).
Notes: Helminthosporium genistae is morphologically similar to the polyphagous H. velutinum, which is also found on the type host, Cytisus scoparius, and it has been synonymised with the latter by Ellis (1961). However, sequence data reveal H. genistae as a distinct taxon. Culture morphology and growth rates also differ substantially between the species. The type collection of H. genistae preserved in the Fries herbarium at UPS has been collected by Mougeot, presumably in eastern France, and sequence data from a recent French collection confirm that the species occurs in this area. Due to the morphological similarities with H. velutinum (in absence of cultures), which also occurs on the type host, and due to the depauperate type collection which is not sent out for study, we here epitypify H. genistae with a recent collection for which sequence data and a culture are available.
Helminthosporium hispanicum Voglmayr & Jaklitsch, sp. nov. MycoBank MB821198. Fig. 8.

Etymology: Referring to Spain, where the type has been collected.

Sexual morph unknown. Colony on natural substrate punctiform, black, hairy, 140–700 μm diam. Mycelium mostly immersed, towards the surface forming stroma-like aggregations of light to dark brown pseudoparenchymatous cells (6.3–) 9.5–15.0(–20) μm diam (n = 80). Conidiophores 130–540 μm long, 13–22.5 μm wide at the base, tapering to 8–15 μm near the apex, arising solitarily or in small groups from the stroma cells, erect, simple, straight or flexuous, thick-walled, sub-cylindrical, smooth, dark to blackish brown, paler near the apex, with well-defined small pores at the apex and rarely laterally beneath the upper 1–2 septa. Conidia 69–99(–130) × (17–) 18–21(–24) μm (n = 20), tapering to 5.5–8 μm at the distal end, with a blackish-brown 4–6 μm wide scar at the base, obclavate, straight or flexuous, thin-walled, smooth, pale brown, (4–) 6–11(–14)-distoseptate, with angular lumina; wall up to 7 μm thick.

Habitat and host range: On dead corticated twigs of Juglans regia: fungicolous on old conidiomata of Juglanconis juglandina.

Distribution: Only known from the type collection in Asturias (Spain).

Fig. 8. Helminthosporium hispanicum (WU 38843, holotype). A, Two conidiomata in face view. B, C. Conidiophores with apical conidia in side view. D. Conidiophores. E, F, H. Conidiophore apices with apical pore (E, arrow) and apical young (F) and mature conidia (H, arrow). G. Conidiophore base and stroma cells. I–O. Conidia (vital in I–N, dead in O). All in water; except G, O in 3 % KOH. Scale bars: A = 200 μm; B, C = 100 μm; D = 20 μm; E–O = 10 μm.
**Holotype**: Spain, Asturias, Selviolla, on dead corticated twigs of Juglans regia, 1 Jun. 2013, W. Jaklitsch & H. Voglmayr [WU 38843; ex-holotype culture CBS 136917 = L109 (ex conidium); MBT376644].

**Notes**: Helminthosporium hispanicum grows on Juglans regia, a host which is also colonised by H. juglandinum, H. juglandis and the polyphagous H. velutinum; for comparison see notes under H. juglandinum below. Both H. hispanicum and H. juglandinum are fungicolous but colonise different hosts: H. hispanicum grows on old conidiomata of Juglanconis juglandina and H. juglandinum on conidiomata of a Diaporthe sp.

**Helminthosporium juglandinum** Voglmayr & Jaklitsch, sp. nov. MycoBank MB821199. Fig. 9.

**Etyymology**: Referring to its growth on Juglans spp.

Sexual morph unknown. Colonies on natural substrate discrete, punctiform, 0.3–1 mm wide, sometimes confluent, usually in large groups, blackish brown. Mycelium immersed, growing in aborted conidiomata of Diaporthe sp., the latter becoming transformed into distinct column-like, 0.3–1.1 mm wide and 200–450 μm high stromata below the periderm. Conidiophores (175–)215–325(–455) μm long (n = 120), 11–23 μm wide at the base, 8.5–14 μm wide near the slightly inflated apex, fasciculate, arising from the upper cells of the stroma, erect, simple, straight or flexuous, thick-walled, sub-cylindrical, smooth, brown to dark brown, darker to black at the apex, the latter with a well-defined apical pore. Conidia (69–)89–145(–205) μm × (15.0–)16.5–20.0(–25.0) μm (n = 83), tapering to 4.5–10 μm at the distal end, with a 3.5–7 μm wide blackish-brown scar at the base, rostrate, straight or flexuous, thin-walled, smooth, pale brown, (5–)9–17(–20)-distoseptate, with angular lumina; wall up to 12 μm thick.

**Culture characteristics**: Culture L118: On CMD colony radius 15 mm after 4 wk at 22 °C. Colony dense, thin, with brown and bluish zones eventually turning black, irregular whitish margin; surface resinous due to condensed excretions of shimmery organic compounds (Fig. 3G); odour unpleasant (“chemical”). On MEA colony radius 14 mm after 4 wk at 22 °C. Colony irregularly lobate, reddish brown, with a white to rosy mat of aerial hyphae at the margin, reverse rosy-brown (Fig. 3H); odour weak, fruity.

Habitat and host range: On dead corticated twigs of Juglans regia: fungicolous on conidiomata of Diaporthe sp.

**Distribution**: Europe (Austria, Italy).

**Holotype**: Austria, Niederösterreich, Gießhübl, on dead corticated twigs of Juglans regia, 1 Sep. 2013, H. Voglmayr [WU 38845; ex-holotype culture CBS 136922 = L118 (ex conidium); MBT376645].

**Other specimens examined** (all on dead corticated twigs of Juglans regia except where noted): Austria, Kärnten, St. Margareten im Rosental, Wogradra, 30 Dec. 2012, W. Jaklitsch [WU 38844, culture L101 (ex conidium) = CBS 136912]; Niederösterreich, Orth/Donau, on Juglans nigra, 26 Jan. 2013, H. Voglmayr & I. Greilhuber [WU 38846, culture L102 (ex conidium)] = CBS 136913; Orth/Donau, on Juglans nigra, 19 May 2013, H. Voglmayr & W. Jaklitsch [WU 38847]; Mühlleiten, on Juglans nigra, 4 Dec. 2016, H. Voglmayr & I. Greilhuber [WU 35975]. Italy, Toscana, Grosseto, Pitigliano, 23 Oct. 2012, W. Jaklitsch & H. Voglmayr [WU 38848, culture L97 (ex conidium) = CBS 136911]; Grosseto, Sovana, 23 Oct. 2012, W. Jaklitsch & H. Voglmayr [WU 38849].

**Notes**: Helminthosporium juglandinum appears to be the most common of the three species known on Juglans in Europe and is apparently confined to that host. Another species, Helminthosporium juglandis, has been described from Juglans in China (Zhao & Zhao 2012), but it clearly differs by much narrower conidia (10–12.7 μm). Helminthosporium hispanicum is morphologically highly similar to Helminthosporium juglandinum but differs by growth on old conidiomata of Juglanconis juglandina and by sequence data.

The description and illustrations of Exosporium stylobatum Curzi & Barbaini (1927), described in Italy from Juglans regia, closely resemble H. juglandinum. However, no original material could be obtained for investigation, and ITS (JQ044428) and LSU (JQ044447) sequences from the ex-type culture (CBS 160.30) are almost identical to those of Massarina corticola, which is not a member of Massarinaeae but of the distantly related Amorosiaeae (Thambugala et al. 2015). Interestingly, we isolated Massarina corticola from Juglans regia close to a colony of H. juglandinum, but the connection with Exosporium stylobatum remains obscure. In the light of these discrepancies, and due to the fact that three similar Helminthosporium species are known from Juglans in Europe (H. hispanicum, H. juglandinum and H. velutinum), Exosporium stylobatum remains a mystery, and it should be considered a nomen dubium.

**Helminthosporium kalakadense** (Subram. & Sekar) Voglmayr & Jaklitsch, comb. nov. MycoBank MB821200.

**Basionym**: Splanchnonema kalakadense Subram. & Sekar, Kavaka 15(1–2): 89. 1989 [1987].

**Holotype**: India, Tamil Nadu, Tirunelveli, Kalakad, Sengaltheri Forest, on dead unidentified twig, 24 Aug. 1980, G. Sekar (IMI 324680).

**Notes**: Ex-ascospore isolates of S. kalakadense produced a helminthosporium-like asexual morph closely resembling Helminthosporium velutinum (Subramanian & Sekar 1987). The morphological features of its sexual morph match the splanchnonema-like sexual morphs recorded for Helminthosporium in the present study. Although no sequence data are available, there is no doubt that the species belongs to Helminthosporium. However, we do not consider it to be conspecific with H. velutinum, for which no sexual morph is known and which differs by wider conidia [13–15 vs. (11–)14–18.5(–25) μm in H. velutinum]. Therefore we combine S. kalakadense in Helminthosporium here.

**Helminthosporium leucadendri** (Quaedvl. et al.) Voglmayr & Jaklitsch, comb. nov. MycoBank MB821201.

**Basionym**: Corynespora leucadendri Quaedvl. et al., Stud. Mycol. 75: 382. 2013.
Fig. 9. Helminthosporium juglandinum. A. Colony in face view. B. Punctiform conidiomata in face view. C. Conidiomata in side view with column-like subcortical stromata representing transformed conidiomata of Diaporthe. D, E. Conidiophores, in E with young apical conidium. F–I. Conidiophore apices with apical conidia in H, I. J. Thick-walled stroma cells in section. K. Conidiophore base (arrow) and stroma cells. L. Conidiophores on stroma in section. M–G1. Vital conidia. All in water. A–C, G, P, Q, S, T, V–Y, B1–D1, F1, G1. WU 38845 (holotype); D, Z, E1. WU 38844; E, F, J–L. WU 35975; H, I, M–O, R. WU 38847; U, A1. WU 38848. Scale bars: A = 5 mm; B = 500 μm; C = 100 μm; D, E = 20 μm; F–K, M–G1 = 10 μm; L = 50 μm.
Holotype: South Africa, Western Cape Province, Helderberg Nature Reserve, on leaves of Leucadendron sp. (Proteaceae), 14 Aug. 2000, S. Lee (CBS H-21323; ex-holotype culture CBS 135133 = CPC 19345).

Notes: Corynespora leucadendri is not closely related to the generic type of Corynespora, C. cassiicola, but was revealed as a member of the Helminthosporium clade (Tanaka et al. 2015, Figs 1, 2). Also morphologically it fits the genus Helminthosporium as re-defined here in its non-proliferating conidiophores. In contrast to most other Helminthosporium species, H. leucadendri grows on leaves. For detailed descriptions and illustrations see Quaedvlieg et al. (2013).

Helminthosporium microsorum D. Sacc. [as ‘Helmisporium’], Malpighia 12: 219. 1898. Figs 10, 11.

Synonym: Massarinula italica D. Sacc., Malpighia 12: 207. 1898.

Sexual morph. Pseudostromata formed in the upper bark, well-developed, dark reddish brown, pseudoparenchymatous, of thick-walled dark brown cells (5.2–9.0–14.5–17.5) × (3.8–) 5.5–9.2(–13.3) μm (n = 90); margin composed of dark brown, coarsely verrucose hyphae (16–23–37–41) × (5.2–) 5.5–8.0(–9.0) μm (n = 19). Ascomata immersed in pseudostromata, distinctly elevating the bark, singly or sometimes in small groups, 425–713 μm diam (n = 20), 136–320 μm high (n = 10) (including pseudostromatal margin), strongly depressed, entirely filled by pure white hymenium, peridium (10.6–13.8–24.5–(30.2) μm thick (n = 27), pseudoparenchymatous, of pale to medium brown cells (3.4–)4.1–6.3–8.4(–) μm wide (n = 34). Ostioles central, (86–)123–234(–262) μm long, (86–)95–159(–178) μm wide (n = 7). Hamathecium of f liform, septate, branched, anastomosing, 1.8–3.5 μm wide pseudo-paraphyses, extending and filling the ostiole. Asci (154–) 169–206(–218) × (36–)39–47(–49) μm (n = 15), clavate, containing 8 irregularly biseriate ascospores. Ascospores (35–) 43–61(–86) × (16–)18–21.5(–25.5) μm, l/w = (1.9–) 2.2–3.1(–4) (n = 140), hyaline to subhyaline, turning light brown at maturity, dark brown after e jection, subellipsoid to obovoid, rarely fusoid, asymmetric, 1-septate, with few to numerous ring-like thickenings of the inner wall giving the inner wall an irregular wavy outline, at full maturity sometimes developing into thin transverse distosepta, strongly constricted at the primary septum, with usually rounded, rarely subacute end cells; length of larger hemisphere/total length of ascospore = (0.52–)0.57–0.62(–0.66), mean = 0.59 (n = 110); wall smooth, hyaline, at maturity light brown; the contents granular, sometimes with a large and several smaller guttules per cell; each hemisphere surrounded by a thick gelatinous sheath. Asexual morph. Colonies on natural substrate punctiform, black, hairy, usually in patches. Mycelium immersed, growing in aborted stromata or conidiomata of Coryneum below the periderm. Conidiophores (96–)167–383(–564) μm long (n = 55), (11.5–) 12.5–15.8(–17.2) wide at the base (n = 28), (8.8–) 10.2–12.0(–13.5) μm wide near the apex (n = 27), fasciculate, arising from the upper cells of the stromata, simple, flexuose, cy lindrical, dark brown, smooth-walled, septate, with a pore at the apex and often 1–2 lateral pores beneath the upper 1–2 septa. Conidia (85–)93–121(–141) × (16–)17–20(–22) μm (n = 25), tapering to 5–9 μm at the distal end, with a 5–7.5 μm wide blackish-brown to black scar at the base, arising terminally and sometimes laterally through pores or thin areas in the conidiophore wall, obclavate, pale to golden-brown, smooth-walled, 7–11(–17)-distoseptate, with angular lumina; wall up to 5.7 μm thick. 

Culture characteristics: Culture L96: On CMD colony radius ca. 10 mm after 4 wk at 22 °C. Colony whitish to pale yellowish, dense, thick, aerial hyphae inconspicuous or lacking (Fig. 3); odour strong, chemical to fruity or rancid. On MEA colony radius up to 21 mm after 4 wk at 22 °C. Colony margin irregular, dense, whitish, with long white aerial hyphae, reverse pale yellowish, centre nearly black (Fig. 3J); odour strong, unpleasant (rancid-fruity). Habitat and host range: On dead corticated twigs of Quercus spp. (confirmed for Q. brachyphylla, Q. cerris, Q. cocculfera, Q. ilex, Q. macrolepis, Q. suber): fungicolous on old stromata or conidiomata of Coryneum sp.

Distribution: With certainty only known from Europe (Croatia, England, Greece, Italy, Portugal, Spain).

Tyification: Lectotype of Helminthosporium microsorum, here designated: Italy, Padova, Orto Botanico, on branches of Quercus ilex, Jun. 1897, D. Saccardo, Mycotheca italiana 194 (K[M) 233086l; MBT376646]. Lectotype of Massarinula italica, here designated: D. Saccardo, Contribuzione alla micologia veneta e modenese, Malpighia 12, 1898, tav. VII, Fig 3a–d (iconotype); MBT376647. Epitype of Helminthosporium microsorum and of Massarinula italica, here designated: Italy, Toscana, Grosseto, Pitigliano, on dead corticated twigs of Quercus ilex, 23 Oct. 2012, H. Voglmayr & W. Jaklitsch [WU 38850; ex-epitype culture CBS 136910 = L96 (ex ascospore); MBT376648, MBT376649].

Other specimens examined (all on dead corticated twigs of Quercus ilex except where noted): Croatia, Istria, Rovign, 14 May 2015, H. Voglmayr (WU 38851). Greece, Crete, between Lakki and Omalos, on Quercus cocculfera, 5 Jun. 2015, H. Voglmayr & W. Jaklitsch (WU 38852, culture L175 (ex conidium)); Omalos, on Quercus cocculfera, 5 Jun. 2015, H. Voglmayr & W. Jaklitsch (WU 38853); NE Askifou, on Quercus cocculfera, 6 Jun. 2015, H. Voglmayr & W. Jaklitsch (WU 38854, culture L174 (ex ascospore)); Pananiana, 4 Jun. 2015, H. Voglmayr & W. Jaklitsch (WU 38855); Rethymno, Kaloniktis, on Quercus macrolepis, 7 Jun. 2015, H. Voglmayr & W. Jaklitsch (WU 38856); Rethymno, Palelimnos, on Quercus cocculfera, 7 Jun. 2015, H. Voglmayr & W. Jaklitsch (WU 38857); Crete, Chania, Zounaki, on Quercus brachyphylla, 4 Jun. 2015, H. Voglmayr & W. Jaklitsch (WU 35974). Italy, Padova, without date, P.A. Saccardo, in Briosi & Cavara, Fungi parasitici 332 [K[M) 233087I, PADJ], Padova, Orto Botanico, 6 Apr. 2016, H. Voglmayr & W. Jaklitsch (WU 38858); Toscana, Pisa, Tirrenia, 30 Oct. 2015, W. Jaklitsch (WU 38859); Lazio, Viterbo, Bomarzo, La Pyramide, on Quercus cerris, 22 Oct. 2012, H. Voglmayr & W. Jaklitsch [WU 38860, culture L94 (ex ascospore), L95 (ex conidium)]; Viterbo, Vulci, 15 Oct. 2013, H. Voglmayr & W. Jaklitsch [WU 38861, culture L123 (ex conidium)]. Portugal, Sintra, Castelo dos Mouros, 16 Feb. 2017, H. Voglmayr & W. Jaklitsch (WU 35971); Sintra, M onserrate, on Quercus suber, 18 Feb. 2017, H. Voglmayr & W. Jaklitsch (WU 35972). Spain, Andalucía, Granada, SW Montefrio, 11 May 2014, W. Jaklitsch (WU 38862); Asturias, Pola de
Fig. 10. Helminthosporium microsorum, sexual morph. A–D. Ascomata in horizontal (A, B) and vertical (C, D) section, surrounded by well-developed pseudostromata. E. Three ostioles in face view. F, G. Peridium and pseudostroma in section. H. Pseudostroma with coarsely verrucose marginal hyphae in section. I. Coarsely verrucose marginal hyphae. J. Ascus with vital ascospores. K. Pseudoparaphyses. L. Immature ascospore. M–H1. Mature vital (sub)hyaline ascospores surrounded by gel sheath, eventually brown in age (X, Y, F1–H1). All in water, except F–I, in 3 % KOH. A, C–R, W–Y, WU 38860; B, S–V, WU 38850 (epitype); Z, WU 38852, A1–H1, WU 38854. Scale bars: A–E = 200 μm; F, H, J = 20 μm; G, I, K–H1 = 10 μm.
Notes: *Helminthosporium microsorum* and its sexual morph, *Massarinula italica*, were described and illustrated in the same publication (Saccardo 1898), but no connection was made between them. DNA sequence data from cultures obtained from sexual and asexual morphs revealed conspecificity of both morphs. At PAD, only a duplicate from Briosi & Cavara’s *Fungi parassitici* 332 is extant, which was collected at the type locality by P.A. Saccardo; no date is given but it has likely been collected after the description of the species. We therefore lectotypify *H. microsorum* with specimen K(M) 233086 distributed as part of D. Saccardo’s *Mycotheca italica* 194, which is cited in the protologue. As no collection of *Massarinula italica* appears to be extant in PAD, we select the illustrations in Saccardo (1898) as lectotype. For nomenclatural stability, we epitypify both names with the same recent collection containing the holomorph, for which a culture and DNA sequence data are available. *Helminthosporium microsorum* is a common species particularly on *Quercus ilex* in the Mediterranean. It grows on senescent stromata or conidiomata of *Coryneum* sp., which are commonly entirely filled and transformed by its hyphae; they have been mistaken for immersed stromata by Ellis (1961).

The ascospores from the Cretan collections from *Quercus cocifera* occasionally developed additional thin distosepta (see Fig. 10E1–H1) and were longer than those from the other collections [(49–)52–68 (–86) vs. (35–)42–50.5 (–54) μm], but as their ITS-LSU sequences were (almost) identical, this is considered to be within the range of the species.

*Helminthosporium oligosporum* (Corda) Hughes [as ‘*Helmisporium*’], Canad. J. Bot. 36: 775. 1958. Fig. 12.

*Basionym: Coryneum oligosporum* Corda, Icon. Fung. 5: 81. 1842.
Fig. 12. Helminthosporium oligosporum, sexual (A–V) and asexual (W–X1) morph. A. Three ostioles in face view. B–D. Ascomata in horizontal (B, C) and vertical (D) section, surrounded by a well-developed pseudostroma (B, D, showing fresh hydrated ascomata). E. Peridium and pseudostroma in section. F. Ascus with vital ascospores. G. Pseudoparaphyses. H–V. Mature vital ascospores surrounded by gel sheath; in M. showing germinating ascospore, in N. verruculose ascospore wall. W–Y. Conidiomata in face view. Z. Conidioma on old Hercospora tiliae stroma in vertical section. A1. Old stroma of Hercospora tiliae in section below conidioma. B1–D1. Conidiogenous cells with apical pore and young conidium (D1). E1–X1. Vital conidia (young in E1–H1, mature in H–X1). All in water. A, B, D, G–R, Y. WU 38866; C, E, F, S–V, P1, WU 38864 (epitype); W, X, B1–E1, H1–M1, O1, Q1–W1. WU 38867; Z, A1, F1–H1, WU 38870; NY, X1. WU 38872. Scale bars: A, X, Y = 500 μm; B–D, A1 = 200 μm; E, F = 20 μm; G–V, B1–X1 = 10 μm; W = 1 mm; Z = 300 μm.
Synonyms: Sporidesmium olivaceum Wallr., Fl. crypt. Germ. (Norimbergae) 2: 228. 1833, non Helminthosporium olivaceum Berk. & Ravenel, in Berkeley, Grevillea 3(no. 27): 102. 1875. Clasterosporium olivaceum (Wallr.) Sacc., Syll. fung. (Abellini) 4: 390. 1886.

Corynespora olivacea (Wallr.) M.B. Ellis, Mycol. Pap. 76: 32. 1960.

For additional synonyms, see Hughes (1958).

Sexual morph. Pseudostroma formed in the upper bark, well-developed, dark brown. Ascomata surrounded by pseudostroma, not to slightly elevating the bark and scarcely noticeable from outside, single, (580–)645–890(–1 045) μm diam (n = 32) (including pseudostromatal margin), globose to depressed globose, dark brown, peridium (including pseudostromatal margin) (38–)55–85(–93) μm thick (n = 36), pseudoparenchymatous, of medium to dark brown cells (4.2–)8.0–16.2(–22) μm (n = 52). Ostioles central, scarcely visible in surface view, not protruding above the cortical surface. Hamathecium of densely packed filiform, septate, branched, anastomosing and 2–4 μm wide pseudoparasyses embedded in a tough gel matrix. Ascii (202–)230–318(–376) × (34–)39.5–51.5(–54.5) μm (n = 21), clavate, containing 8 irregularly biseriate asciospores. Ascospores (44–)49–62(–70) × (11.5–)13.0–15.5(–18.5) μm, l/w = (2.6–)3.3–4.5(–5.9) (n = 129), light to medium brown, fusoid to ellipsoid, with angular lumina; wall up to 6.7 μm thick (n = 21), pseudoparenchymatous cells; length of larger hemisphere/total length of ascospore = (0.56–)0.60–0.67(–0.72), mean = 0.63 (n = 111); wall finely verruculose, brown; the contents granular; each hemisphere surrounded by a thick gelatinous sheath. Asexual morph. Colonies on natural substrate of conspicuous scattered or crowded dark brown to black conidiomata. Mycelium immersed, growing in aborted stromata or conidiomata of Hercesspora tiliae below the periderm. Conidiomata 0.1–2.8 mm wide, 70–960 μm high (n = 42), superficial, stromatic, erumpent through the periderm, hemispherical to pulvinate, sometimes confluent, circular, sometimes irregularly lobed, inside composed of loosely packed, compacted, branched, anastomosing and very thick-walled (up to 6 μm) hyphae, outside forming a brown to dark brown continuous layer of pseudoparenchymatous cells. Conidiophores (17–)22–35(–46) μm long, (8.0–)8.5–10.5(–11.5) μm wide (n = 23), densely crowded, arising from the outer conidiomatal cells, erect, simple, straight, cylindrical to slightly swollen at the apex, brown to dark brown, darker at the apex, 0–2 septate, smooth, with a single conspicuous apical pore bearing the single conidium. Conidia (37–)59–80(–124) × (14.8–)15.8–18.0(–20.0) μm (n = 111), tapering to 4–10.5 μm at the distal end, with a 4–8 μm wide dark brown to black scar at the base, obclavate, sometimes rostrate, straight or curved, smooth but occasionally wrinkled with age, pale brown to brown, paler toward the apex, 6–12(–16)-distoseptate, with angular lumina; wall up to 6 μm thick.

Culture characteristics: Culture L93: On CMD colony radius ca. 24 mm after 4 wk at 22 °C. Colony black, margin white (Fig. 3K); odour unpleasant. On MEA colony radius 22 mm after 4 wk at 22 °C. Colony whitish floccose by aerial hyphae, reverse yellowish (Fig. 3L); odour unpleasant.

Habitat and host range: On dead corticated twigs of Tilia spp.; fungicolous on aborted conidiomata and stromata of Hercesspora tiliae.

Distribution: Widespread in Europe and North America (Hughes 1983).

Typification: Holotype of Sporidesmium olivaceum: Germany, on rotten branches of Tilia, Herb. Wallroth (Wallroth genus no. 192, Wallroth species no. 1700) (STR 91001). Lectotype of Coryneum oligosporum, here designated: Czech Republic, S Praha, Zbraslav (Königsaal), on rotten branches of Corylus (re-identified as Tilia, based on bark anatomy), without date, Corda [PRM 155452; MBT376650]. Same data, ex herb Berkeley [K(M) 233686, IMI 74988, isotypes]. Epitope of Sporidesmium olivaceum and of Coryneum oligosporum, here designated: Austria, Niederöstereich, Heiligenkreuz, Kreuzweg, on dead corticated twigs of Tilia cordata. 14 Oct. 2012, H. Voglmayr & I. Greilhuber [WU 38864; ex-epitipe culture CBS 136909 = L93 (ex ascospore); MBT376651, MBT376652].

Other specimens examined (all on dead corticated twigs): Austria, Niederöstereich, Heiligenkreuz, Kreuzweg, on Tilia cordata, 13 Jan. 2013, H. Voglmayr & I. Greilhuber (WU 38865); ibid., 8 Dec. 2016, H. Voglmayr & I. Greilhuber (WU 38866); St. Corona/Öschpf, on Tilia platyphyllos, 14 Oct. 2012, H. Voglmayr & I. Greilhuber [WU 38867, culture CBS 136908 = L92 (ex conidium)]; Steiermark, Graz, Botanical Garden of the University, on Tilia platyphyllos, 15 Oct. 2012, C. Scheuer (WU 38868); Wien, Floridsdorf, Marchfeldkanalweg, on Tilia cordata, 14 Oct. 2012, W. Jaklitsch [WU 38869, culture L106 (ex conidium)]; Währing, Türcenchantzpark, on Tilia platyphyllos, 16 May 2013, H. Voglmayr (WU 38870). France, Côte-d’Or (21), Véronnes, on Tilia spp., 20 Nov. 2013, A. Gardiennet, A.G. 13220 (WU 38871). Spain, Asturias, Pola de Somiedo, on Tilia platyphyllos, 3 Jun. 2013, H. Voglmayr & W. Jaklitsch [WU 38872, culture L111 (ex conidium)]; Pola de Somiedo, Saliencia, on Tilia platyphyllos, 2 Jun. 2013, H. Voglmayr & W. Jaklitsch (WU 35977).

Notes: The description of the asexual morph has been modified from Hughes (1983). Helminthosporium oligosporum has been commonly known as Corynespora olivacea. Classification in Corynespora goes back to Ellis (1960), who found that the conidiophore “sometimes proliferates through the apical pore and forms another conidium at the apex of the proliferation”. However, neither Luttrell (1963) nor Hughes (1983), who investigated about 70 collections from North America and Europe, observed a regular proliferation of conidiophores, which concurs with our observations. According to the results of molecular phylogeny, Hughes (1958) was correct in placing the species in Helminthosporium. As in Helminthosporium the epithet olivaceum is preoccupied by a different species (Helminthosporium olivaceum Berk. & Ravenel), the next available name, Coryneum oligosporum Corda, was combined in Helminthosporium by Hughes (1958). The host for the latter was given as Corylus, but bark and wood anatomy of the type specimen (PRM 155452) reveals it as Tilia. To stabilise the connection between both names, we here epitypify Sporidesmium olivaceum and Coryneum oligosporum.
with a recent collection for which cultures and DNA data are available from the sexual and asexual morphs.

According to our knowledge, the sexual morph is here described for the first time. Sister group relationship to \textit{H. tiliae}, which also occurs on \textit{Tilia} spp. and which is the generic type of \textit{Exosporium} (see below), received maximum support.

Like its close relative \textit{H. tiliae}, \textit{H. oligosporum} consistently grows on aborted conidiomata or stromata of \textit{Hercospora tiliae}. On the same twigs we observed apparently healthy stromata close to infected ones bearing conidiomata of \textit{H. oligosporum}, in which aborted host perithecia were still visible (see Fig. 12A). However, most host stromata are completely filled and transformed by hyphae of \textit{H. oligosporum}, but usually the diagnostic black line delimiting the stromata of \textit{Hercospora tiliae} is still visible. The true nature of these structures has not been correctly recognised before; they have been interpreted as subperidermal stromata by Ellis (1960) and Hughes (1983).

**Helminthosporium quercicola** (M.E. Barr) Voglmayr & Jaklitsch, comb. nov. MycoBank MB821202. Fig. 13.

**Basionym:** Splanchnonema quercicola M.E. Barr, Mycotaxon 49: 140. 1993.

**Sexual morph. Ascomata** immersed in the upper bark, strongly elevating the bark, towards the apex sometimes covered by a weakly developed, rudimentary pseudostroma of thin-walled, light elevating the bark, towards the apex sometimes covered by a

**Ascomata** are described as \textit{H. quercinum} from European collections, which

**Notes** 

**Helminthosporium quercicola** is so far only known from the type collection. As the material is very sparse, we based our observations and illustrations primarily on the permanent slides included in the type, which had already partly dried out. The description given above is modified from that of Barr (1993) and the comprehensive unpublished notes of R.A. Shoemaker attached to the type (as NY 00914610), and was supplemented with our own observations. As also the asexual morph is sparse and only few conidia were seen, conidial measurements likely do not represent the full range.

**Helminthosporium quercicola** was described as Splanchnonema quercicola by Barr (1993) from Quercus cf. reticulata collected in Arizona (USA), and \textit{Helminthosporium cf. velutinum} was mentioned as its presumed asexual morph, but not described in detail. In an extensive unpublished note attached to the type specimen (NY 00914610), R.A. Shoemaker compared \textit{S. quercicola} with European material (IMI 19472) for which Hughes (1953) demonstrated by pure culture studies the connection of a massaria-like sexual morph with an unidentified \textit{Helminthosporium} species. Shoemaker concluded that the European material was morphologically distinct by larger ascomata with thicker walls as well as different ascospore ornamentation and therefore not conspecific with \textit{S. quercicola}. He also doubted that a helminthosporium-like asexual morph was present on the type specimen of \textit{S. quercicola}, and interpreted the conidiophores as setae. As he found conidia of \textit{Coryneum} in microscopic mounts of conidiophores from the type specimen, but no \textit{Helminthosporium} conidia, he concluded that Barr (1993) mistook ascomatal setae mixed with \textit{Coryneum conidia} as \textit{Helminthosporium cf. velutinum}.

Unfortunately, no material from North America was available for DNA sequencing. Re-investigation of the type collection confirmed morphological differences of the sexual morph of \textit{Helminthosporium quercicola} from European collections, which are described as \textit{H. quercinum} below (for details, see notes below). However, we do not agree with the conclusion of Shoemaker that setae were misidentified as conidiophores by Barr (1993). Although the type collection contains only a sparse sexual as well as asexual morph, and most conidiophores are in a very young stage before conidiation, a few conidiophores bearing young conidia were found during a thorough search under the stereomicroscope (see Fig. 13W, X). In addition, a conidiophore fragment showing a typical lateral pore as well as a few typical helminthosporium-like conidia were found in a microscope mount of old conidiophores (see Fig. 13Z–C1). Like in the European \textit{H. microsorum} and \textit{H. quercinum}, \textit{H. quercicola} apparently grows on old conidiomata of \textit{Coryneum}, which explains the presence of \textit{Coryneum} conidia in the microscope mounts mentioned by Shoemaker.
**Helminthosporium quercinum** Voglmayr & Jaklitsch, sp. nov.

**Etymology:** Referring to its growth on *Quercus* spp.

**Sexual morph.** Pseudostromata formed in the upper bark, well-developed, dark reddish brown, pseudostroma wall 65–180 μm wide (n = 14), pseudoparenchymatous, of thick-walled dark brown cells (4.0–)6.7–10.5(–12.3) μm wide (n = 30), at the margin and...
Fig. 14. Helminthosporium quercinum, sexual morph. A, B. Ostioles. C–H. Ascomata in horizontal (C–F) and vertical (G, H) section, surrounded by a well-developed pseudostroma and subiculum (E–H). I, J. Peridium and pseudostroma in section. K. Peridium of ascoma basis in section. L. Pseudostroma with verrucose marginal cells.
on top surrounded by dark brown, distinctly verrucose, (4.5–)4.8–6.5(–7.0) μm wide subicular hyphae. Ascomata surrounded by pseudostroma, distinctly elevating the bark, single or in small groups, 570–910 μm diam (n = 24), 365–540 μm high (n = 10) (including pseudostromatal margin), strongly depressed, often entirely filled by pure white hymenium, peridium 18–60 μm thick at the margin (n = 12), 22–40 μm at the base (n = 11), pseudoparenchymatous, of pale to medium brown cells (4.0–)

### Notes

- M–O: Verrucose subicular hyphae. P. Ascus with vital ascospores. Q. Pseudoparaphyses. R–M1. Mature ascospores surrounded by gel sheath (R–J1. vital, K1–M1. dead; in K1–M1 showing widely disposed striae in the ascospore wall). All in water, except I–K, N, O, L1 in 3% KOH. A–E, G–N, P; X–Cl, K1, M1. WU 38876 (holotype); F, O, L1. IMI 219012; Q. WU 38874; R, S. WU 38879; T–W. WU 38878; D1–J1. WU 38880. Scale bars: A = 500 μm; B = 200 μm; C, J = 100 μm; F = 50 μm; G, I–Z = 10 μm; H = 20 μm.
Other specimens examined (all on dead corticated twigs of Quercus): Austria, Burgenland, Purbach, Purbacher Heide, on Quercus pubescens, 4 Feb. 2017, H. Voglmayr & I. Greilhuber (WU 38873); Niederösterreich, Bisamberg S Hagenbrunn, on Quercus cerris, 5 Feb. 2017, H. Voglmayr & W. Jaklitsch (WU 38874); Mödling, Kalenderberg, on Quercus petraea, 11 Feb. 2013, H. Voglmayr & I. Greilhuber (WU 38875); Unterzögersdorf, on Quercus robur, 25 Apr. 2013, H. Voglmayr & I. Greilhuber [WU 38877, culture L105 (ex conidium)]; Greece, Crete, Rethymno, Kaloniktis, on Quercus brachyphylly, 7 Jun. 2015, H. Voglmayr & W. Jaklitsch [WU 38878, culture L170 (ex conidium)]; Portugal, Sintra, Monserate, on Quercus faginea, 17 Feb. 2017, H. Voglmayr & W. Jaklitsch (WU 35973); Spain, Andalucia, Guadalajara, Aurión, on Quercus faginea, 8 Apr. 2015, W. Jaklitsch [WU 38879, culture L159 (ex conidium)]; Asturias, Viscas, on Quercus petraea, 4 Jun. 2013, H. Voglmayr & W. Jaklitsch [WU 38880, culture CBS 136915 = L107 (ex conidium)]; U.K., England, Surrey (VC: 17), Esher, West End Common, map grid EQ1263, on dead, attached twigs of Quercus robur, 25 Jan. 2004, B.M. Spooner [K(M)121279!]; Devon, Steps Bridge, on dead, corticated twigs of Quercus sp., 15 Sep. 1947, ex herb. C.O.C. Chesters No. 948, 949 (IMI 19472; recently re-numbered IMI 219012).

Notes: Helminthosporium quercinum is morphologically similar to H. quercicola from the USA, under which name European collections have been identified and recorded (Tello Mora 2015, Osieck & Koopmans 2016). However, H. quercinum differs from H. quercicola in distinctly larger ascomata embedded in a well-developed stroma surrounded by a subicularium of verrucose hyphae and in more distinct but shorter and less densely disposed striae of the ascospore wall. Therefore, we consider the European material to represent a distinct species, which is described here.

Hughes (1953) experimentally proved the connection of a massaria-like sexual morph with an unnamed Helminthosporium species by pure culture studies of a British collection from Quercus, but it was never formally described. Based on a detailed morphological comparison, R.A. Shoemaker concluded that the collection studied by Hughes was not conspecific with the North American H. quercicola (unpubl. notes in the type collection of H. quercicola; see notes above). A re-investigation of the material investigated by Hughes (IMI 19472; recently re-numbered IMI 219012) by us showed that it fully matches H. quercinum, except for somewhat shorter conidiophores (40–150 μm vs. 80–330 μm in the other collections studied). As the ascoma, ascospore and conidium characters fully agree with our sequenced collections, we identify this material as H. quercinum. Under the name Splanchnonema quercicola, Osieck & Koopmans (2016) recorded and illustrated the sexual morph from the Netherlands; the holomorph of a Spanish collection is illustrated by Tello Mora (2015).

The asexual morph of H. quercinum is very similar to that of H. microsorum but differs by conidiophore aggregations, which are distinctly punctiform in H. microsorum and more effuse in H. quercinum. In addition, the conidiophores of H. quercinum are shorter. Both species usually grow tightly associated with old Coryneum stromata or conidiomata on oaks, but usually on different oak (and Coryneum) species.

Culture CBS 112393, which was isolated as endophytic mycelium from Fagus sylvatica in Italy and deposited as Corynespora proliferata, was revealed to represent H. quercinum in our phylogenetic analyses (Fig. 1). Culture CBS 112393 was evidently misidentified as Corynespora proliferata, which was described from dead wood of Fagus sylvatica but differs substantially from H. quercinum according to the original description (Loerakker 1975).
**Helminthosporium tiliae** (Link) Fr. [as ‘Helmisporium’], Syst. mycol. (Lundae) 3(2): 360. 1832. Fig. 16.

Basionym: Exosporium tiliae Link, Mag. Gesell. naturf. Freunde, Berlin 3(1−2): 10. 1809.

Synonym: Massaria heterospora G.H. Otth, Mitt. naturf. Ges. Bern: 49. 1868.

**Sexual morph.** Pseudostromata weakly developed, rudimentary. Ascomata (345−)422−572−(722) μm diam (n = 29), (257−)270−370−(398) μm high (n = 9), depressed subglobose, immersed in the upper bark, slightly elevating the bark, single, dark brown, peridium (28−)33−56−(76) μm thick (n = 36), pseudo-parenchymatous, cells (6−)9−16−(21) μm long (n = 53), dark brown, black in KOH. Ostioles central, not protruding above the bark surface. *Hamathecium* of densely packed filiform, septate, branched, anastomosing, 1.5−3.5 μm wide pseudoparaphyses embedded in a tough gel matrix. Asci (185−)206−245−(257) × (38−)40−48−(52) μm (n = 30), clavate or fusoid, usually containing 8 irregularly biseriate, rarely 4 uniseriate ascospores. Ascospores (39−)47−58−(73) × (15.0−)17.7−21.2(−23.5) μm, l/w = (2.0−)2.4−3.1−(3.8) (n = 146), first light brown, becoming dark brown at full maturity, obvoid to fusoid, distinctly asymmetric, first 1-septate, developing 2−3 transverse, sometimes oblique distosepta in the larger and 1−(2) in the smaller hemisphere, rarely with a longitudinal septum in the larger hemisphere, strongly constricted at the primary septum, slightly or not constricted at the secondary distosepta, with subacute to rounded end cells; length of larger hemisphere/total length of ascospore = (0.50−)0.57−0.63−(0.66), mean = 0.60 (n = 134); wall finely verrucose, brown; the contents granular; each hemisphere surrounded by a thick gelatinous sheath. *Asexual morph.* Colonies on natural substrate discrete, punctiform, blackish brown. Mycelium immersed, growing in aborted stromata or conidiomata of *Hercospora tiliae* below the peridium, at the substrate surface forming stroma-like aggregations of light to dark brown pseudo-parenchymatous cells. *Conidiophores* (68−)79−133−(150) μm long (n = 35), 9−15 μm wide at the base, 8−12 μm wide near the slightly inflated apex, fasciculate, arising from the upper cells of the stroma, simple, straight or flexuous, cylindrical, finely verrucose, brown to dark brown, very dark brown to black at the apex, 3−6 septate, forming one to several conidia. *Conidia* (57−)74−111−(122) × (13.5−)13.7−19.0(−24.5) μm (n = 20), tapering to 6−9.5 μm at the distal end, with a 5−7 μm wide, blackish-brown to black scar at the base, straight or curved, obclavate to rostrate, smooth-walled, pale to golden brown, 7−18−(25) distoseptate, with angular lumina; wall up to 6.5 μm thick.

**Culture characteristics:** Culture L171: On CMD colony radius ca. 16 mm after 4 wk at 22 °C. Colony dull grey-brown, turning black from the centre, with whitish margin and a pale rosy halo around the colony, with long white aerial hyphae (Fig. 3O); odour indistinct. On MEA colony radius 10 mm after 4 wk at 22 °C. Colony thick, dense, silvery-grey with small black dots and white margin, reverse brownish (Fig. 3P); odour indistinct to pleasant.

**Habitat and host range:** On dead corticated twigs of *Tilia* spp.: fungicolous on aborted conidiomata and stromata of *Hercospora tiliae*.

**Distribution:** Widespread in Europe.

**Typification:** Holotype of *Exosporium tiliae*: Germany, without place, date and collector, on dead twigs of *Tilia* sp. (B 700016453!). Holotype of *Massaria heterospora*: Switzerland, Bern, on dead twigs of *Tilia cordata*, without date, G. Otth 16 (B 700014746!). Epitope of *Exosporium tiliae* and *Massaria heterospora*, here designated: Austria, Oberösterreich, Raab, Wetzbach, on dead corticated branches of *Tilia platyphyllos*, 8 Sep. 2012, H. Voglmayr [WU 38882; ex-epitope cultures CBS 136907 = L88 (ex ascospore), L89 (ex conidium); MBT376654 and MBT376655, respectively].

**Other specimens examined** (all on dead corticated twigs of *Tilia*): Austria, Kärnten, St. Margareten im Rosental, Wograda, on *Tilia cordata*, 22 Aug. 2012, W. Jaklitsch [WU 38884, culture CBS 136906 = L87 (ex ascospore)]; Niederösterreich, Mayerling, on *Tilia sp.*, 8 Dec. 2016, H. Voglmayr & I. Greilhuber [WU 38881, culture L171 (ex conidium)]; Oberösterreich, Raab, Wetzbach, on *Tilia platyphyllos*, 11 May 2013, H. Voglmayr (WU 38883).

**Notes:** *Helminthosporium tiliae* is the type of the genus *Exosporium* and has been commonly known as *Exosporium tiliae*. No sexual morph was known for *H. tiliae*, and *Massaria heterospora* is here proven to be its sexual morph. A recent holomorph collection, for which cultures and sequence data are available, is here designated as epitope to firmly establish the connection between the sexual and asexual morphs. Sister group relationship to *H. oligosporum*, also growing on *Tilia* spp. and formerly classified within *Corynespora* (see above), received maximum support.

Like its close relative *H. oligosporum*, *H. tiliae* consistently grows on aborted conidiomata or stromata of *Hercospora tiliae*. Usually the host stromata and conidiomata are fully transformed and filled with brown hyphae of *H. tiliae*, but occasionally host perithecia are still recognisable. However, the diagnostic black line delimiting the stromata of *Hercospora tiliae* is usually well seen. Growth on *Hercospora* is here reported for the first time; the infected transformed host stromata and conidiomata have been erroneously interpreted as immersed stromata by Ellis (1961).

**Helminthosporium velutinum** Link [as ‘Helmisporium’], Mag. Gesell. naturf. Freunde, Berlin 3(1−2): 10, tab. 1:9. 1809. Fig. 17.

**Sexual morph unknown.** Colony on natural substrate effuse, black, hairy. Mycelium immersed, on the substrate surface forming stroma-like aggregations of light to dark brown pseudo-parenchymatous cells (5.0−)6.5−11.0−(19.8) μm diam (n = 58). *Conidiophores* (163−)340−698−(960) μm long (n = 247), 14−26 μm wide at the base, tapering to 8−12 μm near the apex, arising solitarily or in fascicles from the stroma cells, simple, straight or flexuous, cylindrical, thick-walled, smooth, brown to dark brown, with well-defined small pores at the apex and laterally beneath the upper 1−12 septa. *Conidia* (42−)56−89−(142) × (11−)14.3−18.5−(24.7) μm (n = 351), gradually tapering to (3.5−)5−8 μm at the distal end, with a 1.4−3.7 μm wide, blackish-brown to black scar at the base, straight or flexuous, obclavate to rostrate, smooth-walled, pale golden brown to brown, 6−18 distoseptate, with angular lumina; wall up to 4.5 μm thick.

**Culture characteristics:** Cultures L115 and L131: On CMD colony radius (20−)28−45 mm after 4 wk, i.e. sometimes mycelium
Fig. 16. Helminthosporium tiliae, sexual (A–U) and asexual (V–K1) morph. A. Three ostioles in face view. B–D. Ascomata in horizontal (B, C) and vertical (D) section. E. Peridium in section. F. Ascus with dead ascospores. G. Pseudoparaphyses. H–U. Mature vital (H–O) and dead (P–U) ascospores surrounded by gel sheath (N, Q–U). V. Conidiomata in face view. W. Conidioma on old Hercospora tiliae stroma in vertical section. X. Old stroma of Hercospora tiliae in section below conidioma. Y–A1. Conidiophores
(culture L115) filling a centrally inoculated 90 mm diam Petri dish entirely at 22 °C. Colony more or less circular, dense, brown with yellow tint or olivaceous fading to colourless or whitish at the margin, with short or long and dense white aerial hyphae spreading from the centre (Fig. 3Q, S); odour indistinct to slightly unpleasant (cabbage-like). On MEA growth irregular, depending on the condition of the mycelium; colony radius e.g. 42 mm, i.e. reaching the plate margin at one side after 4 wk at 22 °C or ca. 20 mm after 4 wk. Colony roundish or irregular, dense, thick, with shades of grey plus white patches of dense tufts of white aerial and stroma cells (Y, Z) with apical pores (arrows). B1–K1. Vital (B1–H1) and dead (J1, K1) conidia. All in water, except R–U, A1, J1, K1. in 3 % KOH. A–Q, V–Y, WU 38882 (epitype); R–U, J1, B 700014746 (holotype of Massaria heterospora); Z, B1–H1. WU 38881; A1, K1. B 700016453 (holotype of Exosporium tiliae). Scale bars: A, B = 200 μm; C, D, W, X = 100 μm; E, F = 20 μm; G–U, Y–K1 = 10 μm; V = 500 μm.
hyphae with yellow to black drops of clear fluid or a dense flat white mat or a thick zonate white and brownish mat of aerial hyphae, sometimes with partial black marginal patches (Fig. 3R, T); odour indistinct or slightly unpleasant (cabbage-like).

Habitat and host range: Saprobic on various plants; usually on dead twigs of various trees and shrubs, sometimes on herba-
ceous stems.

Distribution: Widespread and common in temperate Eurasia and America, probably almost cosmopolitan.

Typification: Holotype of Helminthosporium velutinum: Germany, without place and date, on dead twigs of Fagus syl-
vatica (B 700016457!). Epitype, here designated: Austria, Wien, Döbling, Kahlenberg, on dead corticated twigs of Fagus syl-
vatica, 16 Nov. 2013, W. Jaklitsch [WU 38892; ex-epitype culture L131 (ex conidium) = CBS 139923; MBT376656].

Other specimens examined (all on dead corticated twigs): Austria, Kärnten, St. Margareten im Rosental, Dullach, Drau-
Auen, on Euonymus europaeus, 10 Aug. 2013, W. Jaklitsch & H. Voglmayr [WU 38885, culture L117 (ex conidium)]; St. Margareten im Rosental, Fern-Wograda, on Juglans regia, 16 Nov. 2012, W. Jaklitsch [WU 38886, culture L98 (ex conidium)]; ibid., on Juglans regia, 10 Aug. 2013, H. Voglmayr & W. Jaklitsch [WU 38887, culture L116 (ex conidium)]; St. Margareten im Rosental, Gupf, on Corylus avellana, 8 Nov. 2013, W. Jaklitsch [WU 38888, culture L136 (ex conidium)]; Niederösterreich, Krems, Egelsee, on Genista tinctoria, 27 Oct. 2013, W. Jaklitsch & H. Voglmayr [WU 38889, culture L127 (ex conidium)]; Krems, Senftenberg, on Cytisus scoparius, 15 Feb. 2014, H. Voglmayr & W. Jaklitsch [WU 38890, culture L140 (ex conidium)]; Oberösterreich, St. Willibald, Aichet, on Sambucus nigra, 16 Aug. 2013, H. Voglmayr [WU 38891, culture CBS 136924 = L115 (ex conidium)].

Germany, Hessen, Rheingau, Lorch, on Cytisus scoparius, 3 Apr. 2015, W. Jaklitsch [WU 38893, culture L163 (ex conidium)].

Italy, Lazio, Viterbo, Norchia, on Acer campestre, 14 Oct. 2013, H. Voglmayr & W. Jaklitsch [WU 38894, culture L126 (ex conidium)].

Spain, Canarias, Tenerife, Los Batanes, on Prunus lusitanica, 17 Dec. 2013, W. Jaklitsch [WU 38895, culture L134 (ex conidium)]; La Gomera, El Cedro, on Gesnouinia arborea, 24 Mar. 2016, H. Voglmayr [WU 38897, culture L176 (ex conidium)].

Sweden, Skåne, NE Helsingborg, Kropp parish, Vasatorp, 3.8 km SSW of the church, 56°03’13” N, 12°46’47” E, on Ribes rubrum, 27 Oct. 2013, S.-A. Hanson [WU 38896, culture L135 (ex conidium)].

Notes: Helminthosporium velutinum is by far the most commonly recorded and best-known species of the genus. Remarkably, it has been recorded world-wide from a wide range of woody and herbaceous substrates; more than 100 hosts are listed in Farr & Rossman (2016). Numerous species have been synonymised with H. velutinum by Ellis (1961) based on morphology, but it is uncertain whether all of them are conspecific. Based on sequence data, one of these putative synonyms, H. genistae, is here shown to represent a distinct species, which is apparently confined to fabaceous hosts (see above). Although some of the literature records may represent misidentifications, polyphagous nature of H. velutinum has been confirmed by sequence data in the present study, and GenBank sequences confirm presence of this species in Europe, Asia and the Americas.

The holotype specimen in B is in good condition; however, considering the presence of cryptic species within Helminthosporium, we designate an epitype of H. velutinum from the original host, Fagus sylvatica, for which a culture and sequence data are available, to stabilise the application of the name.

DISCUSSION

In the molecular phylogenetic analyses (Figs 1, 2), several species previously classified in Corynespora and Exosporium are revealed as closely related to Helminthosporium, and are thus here recognised as members of the genus Helminthosporium. Although revealed as monophyletic, the genus Helminthosporium receives no significant bootstrap support in the extended combined matrix, and also the relationships within Helminthosporium remain partly unsupported (Fig. 1). This significantly changes after removal of the accesses for which no rpb2 sequences are available, and the Helminthosporium clade becomes moderately to highly supported (78 % MP and 91 % ML bootstrap support for the Helminthosporium clade; Fig. 2), and the same is also observed in several additional nodes within Helminthosporium. After exclusion of H. leucadenstri, for which only comparatively short rpb2 and tef1 sequences are available, the Helminthosporium clade becomes highly supported in both analyses (94 % MP and 98 % ML bootstrap support; Fig. 2). This once again demonstrates that the ribosomal genes commonly provide insufficient phylogenetic resolution on the generic to familial level, challenging the common practice of generic and familial reclassification solely based on phylogenies of ribosomal genes. The results of our phylogenetic analyses also show that inclusion of accessions in multi-
gene analyses, for which only ITS and/or LSU sequences are available, can be problematic, as the low phylogenetic signal can result in significantly decreasing overall phylogenetic resolution. At least the rpb2 should be sequenced and included in phylo-
genetic analyses of ascomycetes in general and dothideomy-
cetes in particular, as this marker usually significantly increases the phylogenetic resolution. Also the tef1 locus, which has been included in the multi-gene analyses of Massarineae by Tanaka et al. (2015), and which has been shown to resolve well in many ascomycete lineages, is a marker of good resolution at the generic level and below, if the introns are included; however, as it contains paralogs in Helminthosporium genistae it could not be obtained for all species.

The results of the molecular phylogenetic analyses necessi-
tate a critical re-evaluation of the morphological characters traditionally considered to be diagnostic for Corynespora and Helminthosporium. The main character used for distinction be-
 tween Corynespora and Helminthosporium by Ellis (1961) and Luitrel (1963, 1964), acrogenous vs. acropleurogenous con-
idiation, is shown to be insignifi-
cant in a phylogenetic context. Already Luitrel (1964) pointed out that another character used by Ellis (1961), i.e. percurrent (Corynespora, Exosporium) vs. non-
percurrent (Helminthosporium) conidiophore proliferation, does not separate the genera. On the one hand, percurrent prolif-
eration has been observed in H. velutinum by Luitrel (1964), whereas on the other hand Luitrel (1963) and Hughes (1983) could not confirm regular percurrent proliferation in Helmintho-
sporium oligosporum, which was classified in Corynespora by Ellis (1961). Considering that numerous species described in
Corynespora have not been critically studied, it is likely that several of them belong to Helminthosporium. The present investigations significantly extend the knowledge of sexual morphs of Helminthosporium. Based on pure culture and sequence data, Tanaka et al. (2015) described a massariniform sexual morph for Helminthosporium massarinum, but challenged the few previous reports of massarinia- and splanchonemiform sexual morphs for Helminthosporium. Based on pure culture and sequence data as well as herbarium studies, we here experimentally confirm sexual morphs for four Helminthosporium species, three of which are splanchonemiform-like. In addition, our morphological re-investigation of the type specimen of Splanchnonema quercicola revealed that Barr (1993) was correct reporting the associated asexual morph to be helminthosporium-like. Although the North American S. quercicola is morphologically very close to the massariniform sexual morph reported by Hughes (1953) for an unnamed Helminthosporium species from Europe, our extensive investigations of the material of Hughes as well as of freshly collected material revealed several morphological differences. Therefore, we consider the European records attributed to S. quercicola to represent a distinct species, which is here described as H. quercinum. Massaria heterospora, a little-known and recorded splanchonemiform sexual morph is here proven to be connected with Helminthosporium tiliae, and an apparently undescribed splanchonemiform sexual morph is reported for H. oligosporum for the first time. In addition, we show that Massarinula italica is the sexual morph of H. microsorum. In light of this experimental evidence, we consider it fully justified to combine Splanchnonema quercicola and S. kalakadense, for which helminthosporium-like asexual morphs have been reported (Subramanian & Sekar 1987, Barr 1993) but for which no sequence data are available, in Helminthosporium. In this context, it may also be worth mentioning that Pseudosplanchnonema phorcioides, another splanchonemiform-like species lacking a helminthosporium-like asexual morph, is closely related to Helminthosporium (Figs 1, 2).

With the exception of H. solani, a plant parasite on Solanum, most species of Helminthosporium have been reported as sap- robes of plants, usually from woody substrates. With the transfer of several Corynespora species parasitic on leaves, the genus now contains additional plant pathogens. In addition, the current detailed investigations revealed that some well-known Helminthosporium species are fungicolous, mostly on Diaporthales, and the prominent subperidermal stromata of e.g. H. caesipilosum, H. oligosporum and H. tiliae actually represent transformed host stromata or conidiomata. However, it is unknown whether these Helminthosporium species are parasitic or saprobic on their fungal hosts.

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75

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