Lichens or endophytes? The enigmatic genus *Leptosillia* in the *Leptosilliaceae* fam. nov. (*Xylariales*), and *Furfurella* gen. nov. (*Delonicicolaceae*)

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**Abstract** Based on DNA sequence data, the genus *Leptosillia* is shown to belong to the *Xylariales*. Molecular phylogenetic analyses of ITS-LSU rDNA sequence data and of a combined matrix of SSU-ITS-LSU rDNA, *rpB1*, *rpb2*, *tef1* and *tub2* reveal that the genera *Cresporhaphis* and *Liberyomyces* are congeneric with *Leptosillia*. *Coelosphaeria fusariospora*, *Leptorhaphis cinctula*, *Leptorhaphis quercus* f. *macrospora*, *Leptorhaphis piniola*, *Leptorhaphis wien-kampii*, *Liberyomyces pistaciae*, *Sphaeria muelleri* and *Zignoëlla slaptonensis* are combined in *Leptosillia*, and all of these taxa except for *C. fusariospora*, *L. piniola* and *L. pistaciae* are epitypified. *Coelosphaeria fusariospora* and *Cresporhaphis rhinoa* are lectotypified. *Liberyomyces macrosporus* and *L. salicifilus*, which were isolated as phloem and sapwood endophytes, are shown to be synonyms of *Leptosillia macrospora* and *L. wienkampii*, respectively. All species formerly placed in *Cresporhaphis* that are now transferred to *Leptosillia* are revealed to be non-lichenized. Based on morphology and ecology, *Cresporhaphis chibaeensis* is synonymised with *Raphidiocytis trichosposella*, and *C. rhinoa* is considered to be unrelated to the genus *Leptosillia*, but its generic affinities cannot be resolved in lack of DNA sequence data. Phylogenetic analyses place *Leptosillia* as sister taxon to *Delonicicolaceae*, and based on morphological and ecological differences, the new family *Leptosilliaeaceae* is established. *Furfurella*, a new genus with the three new species, *F. luteostilata*, *F. nigrescens* and *F. stromatica*, growing on dead branches of Mediterranean fabaceous shrubs from tribe *Genistaeae*, is revealed to be the closest relative of *Delonicicola* in the family *Delonicicolaceae*, which is emended. ITS DNA sequence data retrieved from GenBank demonstrate that the *Leptosilliaeaceae* were frequently isolated or sequenced as endophytes from temperate to tropical regions, and show that the genus *Leptosillia* represents a widely distributed component of endophyte communities of woody plants.

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**INTRODUCTION**

The monotypic genus *Leptosillia*, based on *L. notha*, was post-humously described by Höhnell (1928) in a manuscript edited by J. Weese, with *Harpagrostoma notha* as its asexual morph. As the genus name suggests, *Leptosillia* was considered to be closely related to the diaporthalean genus *Sillia*. Oddly enough, it was, however, classified in *Botryosphaeriaceae* (*Melanoposidaceae*), which was probably added by J. Weese. Since its original description, *Leptosillia notha* has apparently never been recorded again, although it is growing on bark of *Acer pseudoplatanus*, which is a common and widespread tree in many parts of Europe. Due to the vague original description and the lack of illustrations, its systematic placement could so far not be critically evaluated, and the few references in the literature made it even more mysterious. Hawksworth (in Erisson & Hawksworth 1987) noted that the type of *Leptosillia* was based on a specimen of *Cryptospora (= Sillia) cinctula* distributed by Rehm (*Ascomyceten, no. 2047; Rehm 1913*), and after studying a slide of the type at F H, the fungus was tentatively referred to *Valsaceae*. However, it is unclear how Hawksworth came to that conclusion, as the original description of *L. notha* was based on a German collection made by H. Diedicke, and neither in the original description nor on the labels of the type collection, neither *Cryptospora (= Sillia) cinctula* nor Rehm’s *Ascomyceten* are mentioned. This misapplication was perpetuated in the latest edition of the Dictionary of the Fungi (Kirk et al. 2008), and *Leptosillia* is currently placed in *Valsaceae* in Index Fungorum (http://www.indexfungorum.org/Names/Names.asp; accessed in Feb. 2019).

In the course of an ongoing research project on phylogenetic of *Diaporthales*, the first author successfully recollected *Leptosillia notha* to clarify its systematic affiliation by morphology and DNA sequence data. We also collected, cultured and sequenced a small pyrenomycete from the corky bark strips of *Ulmus minor*, which we identified as *Cresporhaphis ulmi* (Calatayud & Aguirre-Hudson 2001). To our surprise, the ITS-LSU rDNA sequences of *Leptosillia notha* and *Cresporhaphis ulmi* turned out to be highly similar, raising the question whether both are congeneric. Nucleotide BLAST searches of the ITS also revealed a high similarity to *Liberyomyces*, an endophytic coelomycetous asexual morph genus of xylarialean affinities that was isolated from the inner bark and sapwood of *Salix* and *Ulmus* species (Pažoutová et al. 2012). In addition, we collected several specimens of a pyrenomycete with a yellow scurf and valsa-like ascospores on dead branches of fabaceous Mediterranean shrubs, which could not be identified but later turned out to be closely related to the isolates mentioned above as well. The monotypic genus *Delonicicola*, which was recently described from seed pods of *Delonix regia* in Thailand (Perera...
et al. 2017), also showed high sequence similarities to our isolates. This prompted us to recollect several other Cresporella species. These were isolated in pure culture; the morphology of their sexual and asexual morphs was studied and their ecology was investigated to ascertain if these are truly lichenised as previously postulated. In addition, multi-gene analyses were performed with a matrix of SSU-ITS-LSU, rpb1, rpb2, tef1 and tub2 sequences to reveal their phylogenetic affiliation, to clarify genus, species and family boundaries and to settle their taxonomy in a polyphasic approach.

**MATERIALS AND METHODS**

**Sample sources**

All isolates included in this study originated from ascospores of freshly collected specimens on bark of living or recently dead branches or trunks; typical habitats of *Leptosilis* species are illustrated in Fig. 1. 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 (CBS-KNAW), Utrecht, The Netherlands. Details of the specimens used for morphological investigations are listed in the Taxonomy section under the respective descriptions. Herbarium acronyms are according to Thiers (2018), and citation of exsiccate follows Triebel & Scholz (2018). 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. Methods of microscopy included stereomicroscopy using a Nikon SMZ 1500 equipped with a Nikon DS-U2 digital camera or a Keyence VHX-6000 system, 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 the NIS-Elements D v. 3.22.15 or Zeiss ZEN Blue Edition software. For certain images of ascomata the stacking software Zerene NIS-Elements D v. 3.22.15 or Zeiss ZEN Blue Edition software. For certain images of ascomata the stacking software Zerene (Zerene Systems LLC, Richland, WA, USA) was used. Measurements are reported as maxima and minima in parentheses and the range representing the mean plus minus the standard deviation of a number of measurements given in parentheses.

**Culture preparation, DNA extraction, PCR and sequencing**

Ascospore isolates were prepared and grown on 2 % corn meal dextrose agar (CMD; CMA; Sigma, St Louis, Missouri; supplemented with 2 % (w/v) D(+)-glucose monohydrate) or 2 % malt extract agar (MEA; 2 % w/v malt extract, 2 % w/v agar-agar; Merck, Darmstadt, Germany). Growth of liquid cultures 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).

The following loci were amplified and sequenced: the complete internal transcribed spacer region (ITS1-5.8S-ITS2) and a c. 900–1200 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 c. 1.2 kb fragment of the RNA polymerase II subunit 1 (rpb1) gene with primers RPB1-Af (Stiller & Hall 1997) and RPB1-6R1asc (Hofstetter et al. 2007); a c. 1.2 kb fragment of the RNA polymerase II subunit 2 (rpb2) gene with primers FRPB2-5f and FRPB2-7cr (Liu et al. 1999) or dRPB2-5f and dRPB2-7r (Voglmayr et al. 2016a); a c. 1.3–1.5 kb fragment of the translation elongation factor 1-alpha (tef1) gene with primers EF1-728F (Carbone & Kohn 1999) and TEF1LLErev (Jaklitsch et al. 2005) or EF1-2218R (Rehner & Buckley 2005); and a c. 1.6 kb fragment of the beta tubulin (tub2) gene with primers T1 and T22 (O’Donnell & Cigelnik 1997) or T1D and T22D (Voglmayr et al. 2019). PCR products were purified using an enzymatic PCR cleanup (Welte 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), LR22R-A (Voglmayr et al. 2012) and LR3 (Vilgalys & Hester 1990) were used for the ITS-LSU region, TEF1_INTF (Jaklitsch 2009) and TEFD_IR (Voglmayr et al. 2018) for tef1, and BtHVf (Voglmayr & Mehrabi 2018) and BtHVR v2 (Voglmayr et al. 2016b) for tub2. Sequencing was performed on an automated DNA sequencer (3730xl Genetic Analyzer, Applied Biosystems).

**Data analysis**

Following the results of nucleotide BLAST searches of ITS and LSU sequences generated during the present study, a phylogenetic analysis was performed with an ITS-LSU rDNA sequence matrix of a representative selection of *Xylariales*. Taxon and sequence selection was based on Jaklitsch et al. (2016b), with some recent additions (Perera et al. 2017, Voglmayr et al. 2018, Wendt et al. 2018). For rooting the tree, LSU sequences of four taxa of Sordariomycetes (*Calosphaeria pulchella*, *Chaeothelaria inunera*, *Diaporthe eres*, *Ophiostoma pilleum*) were included as outgroups. For detailed investigations of species relationships and delimitation within and between the genera and families, a combined matrix of five loci (partial SSU-ITS LSU rDNA, rpb1, rpb2, tef1 and tub2) was produced. Four taxa of Sordariomycetes (*Calosphaeria pulchella*, *Caudospora taleola*, *Juglanconis juglandina*, *Lasiosphaeria ovina*) were selected as outgroup taxa; due to alignment issues, their ITS and tef1 introns were not included in the matrix. The GenBank accession numbers of sequences used in these analyses are given in Table 1. For some strains for which whole genome data are available, sequences were retrieved from JGI-DOE (http://genome.jgi.doe.gov/).

Sequence alignments for phylogenetic analyses were produced with server versions of MAFFT (www.ebi.ac.uk/Tools/mafft or http://mafft.cbrc.jp/alignment/server1), checked and refined using BioEdit v. 7.2.6 (Hall 1999). For tef1 and ITS-LSU rDNA, the localpair and for tub2 the globalpair options were selected for performing fast Fourier transform (FFTS), with a gap open penalty of 1.0 for tef1 and tub2, for all other markers, the default settings were used. Poorly aligned and gappy regions were removed from the ITS and the introns of tef1 and tub2, and the terminal intron of the rpb2 was entirely removed. The final ITS-LSU matrix used for phylogenetic analyses contained 1345 and the combined five loci data matrix 7052 nucleotide characters; viz. 1626 of SSU-ITS-LSU, 1210 of rpb1, 1104 of rpb2, 1516 of tef1 and 1596 of tub2. 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 analysis, using the level of bootstrap support (Sung et al. 2007) as described in Jaklitsch & Voglmayr (2014). For this, the 70 % maximum parsimony (MP) bootstrap consensus trees calculated for each individual partition, using the same parameters as given below, were compared. Except for some nodes within the same species, no topological conflicts were observed between these bootstrap trees of the various genes, indicating the absence of significant incongruence and combinability of the five loci (Wiens 1998).
Maximum likelihood (ML) analyses were performed with RAxML (Stamatakis 2006) as implemented in raxmlGUI v. 1.5 (Silvestro & Michalak 2012), using the ML + rapid bootstrap setting and the GTRGAMMA substitution model with 1 000 bootstrap replicates. The matrix was partitioned for the different gene regions included in the combined multilocus analyses.

Maximum parsimony (MP) analyses were performed with PAUP v. 4.0a163 (Swofford 2002). All molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data; the COLLAPSE command was set to MINBRLEN. For the ITS-LSU matrix, first a parsimony ratchet approach was used. For this, nexus files were prepared using PRAP v. 2.0b3 (Müller 2004), implementing 1 000 ratchet replicates with 25 % of randomly chosen positions upweighted to 2, which were then run with PAUP. In a second step, the best trees obtained by the parsimony ratchet

![Fig. 1 Typical habitats of the Leptosillia species sampled; arrows denoting ascomata on cork wings (b, j), bark furrows (d, h) or bark scales (f). a–b. Leptosillia acerina on branches of Acer campestre; c–d. Leptosillia macrospora on bark of living trunks of Quercus robur; e–f. Leptosillia muelleri on bark of living trunks of Acer pseudoplatanus; g–h. Leptosillia wienkampii on bark of living trunks of Salix sp.; i–j. Leptosillia slaptonensis on branches of Ulmus minor.](image)
Table 1  Isolates and accession numbers used in the phylogenetic analyses. Isolates/sequences in bold were isolated/sequenced in the present study.

| Taxon                        | Isolates | Host | Type | Substrate/Isolation source | Country | GenBank accession no. | References |
|------------------------------|----------|------|------|----------------------------|---------|-----------------------|------------|
| Acrocordia occults           | RS9 = CBS 140500 | E    |      |                            |         | KT049893              |            |
| Amphibambusa bambusabiozika | MFUCC 11–0617 | H    |      |                            |         | KF744433              |            |
| Amphisphaeria umbra          | HKUCC 994 | E    |      |                            |         | AF006905              |            |
| Annonolygosporon truxatum    | CBS 140778 | E    |      |                            |         | KY610419              |            |
| Anostosma decipiens         | CD = CBS 133221 | H |      |                            |         | MC777465              |            |
| Anostosmaeella rubroata      | MFUCC 16-0479 | E    |      |                            |         | KF533545              |            |
| Arthrinium acrocinum        | CBS 133609 = NRRL 25634 | H    |      |                            |         | KF144836              |            |
| Arthrinium phragmitis       | CBS 135458 | H    |      |                            |         | KF144099              |            |
| Arthrinium saccharicolor     | CBS 83.71 | H    |      |                            |         | KF144222              |            |
| Barmaelia rhamnicola        | BR = CBS 142772 | E    |      |                            |         | MF488690              |            |
| Bartalinia rubicola         | MFLUCC 11–0617 | H |      |                            |         | KP744474              |            |
| Basiseptospora fallax       | PSC = CBS 129020 | H    |      |                            |         | AF009805              |            |
| Beltrania rhombica          | CPC 27482 | E    |      |                            |         | KY610419              |            |
| Beltrania spinulatae         | CBS 137974 | H    |      |                            |         | AF009805              |            |
| Bolwigia cinerea            | ATCC 28063 | H    |      |                            |         | MC777465              |            |
| Calibeca saucourus           | CBS 136.62 | E    |      |                            |         | KG005961              |            |
| Calosphaeria pulchella       | CBS 115999 | H    |      |                            |         | KG005961              |            |
| Camillea obularia           | CCA 135458 | H    |      |                            |         | MC777465              |            |
| Cordersia amygdalinus        | CBS 124266 | H    |      |                            |         | MG095980              |            |
| Creosphaeria sarcaspirae     | CBS 124266 | H    |      |                            |         | MG095980              |            |
| Crouatia scala              | CBS 124266 | H    |      |                            |         | MG095980              |            |
| Delonicicola smalense        | MFUCC 15-0670 | H    |      |                            |         | MC777465              |            |
| Delonicicola sp.            | MYCO-ARIZ SNP360 | H    |      |                            |         | MC777465              |            |
| Dimorphodia chamaecyparissiae | CBS 113277 | H    |      |                            |         | MC777465              |            |
| Diaporthe eres              | CBS 100373 | H    |      |                            |         | MC777465              |            |
| Distria disciformis          | CBS 197.49 | H    |      |                            |         | MC777465              |            |
| Entosordaria perfidiosa     | CBS 142773 | H    |      |                            |         | MC777465              |            |
| Eutypa lata                  | UCR-EL1   | H    |      |                            |         | MC777465              |            |
| Fusarium oxysporum           | CBS 100373 | H    |      |                            |         | MC777465              |            |
| Fusarium luteosoralis        | CE = CBS 143620 | H    |      |                            |         | MC777465              |            |
| Fusarium nigrescens         | CE = CBS 143621 | H    |      |                            |         | MC777465              |            |
| Fusarium stromaticum        | CE = CBS 144409 | H    |      |                            |         | MC777465              |            |
| Graphophysa platystomum     | CBS 270.87 | H    |      |                            |         | MC777465              |            |
| Hymenoscyphus hyoplasticus   | LH = CBS 140410 | E    |      |                            |         | MC777465              |            |
| Hypoxylon busii             | UME 31430 |       |      |                            |         | MC777465              |            |
| Taxon | Strain1 | Host2 | Type3 | Substrate / Isolation source | Country | GenBank accession no. | References2 |
|-------|---------|-------|-------|--------------------------------|---------|----------------------|------------|
| Hypoxylon fragiforme | MUCL 1264 | E | | | | KC477229 | – | KM186296 | – | KM271282 |
| Idriella kunata | CBS 204.56 | H | | | | KP869544 | KP858981 | – | | |
| Juglanonk niglandina | CBS 133343 | | | | | – | KY427149 | KY427199 | KY427218 | KY427234 |
| Kretschmaria doestula | CBS 163.93 | | | | | KC477237 | KF610458 | – | KY624227 | – | KY271251 |
| Lasiosphaeria orina | CBS 958.72 | | | | | – | – | AY587846 | genome | genome | genome | |
| Lasiosphaeria paeonae | CBS 120506 | | | | | JF440976 | | | | | |
| Leptotropa fuckelii | LEF = CBS 140409 | N | | | | KT496902 | KT496902 | MK523263 | MK523308 | MK523300 | MK523337 |
| Leptosphaeria asaxina | CRA | A. campestris | bark | Austria | | MK527848 | MK527848 | – | MK523281 | MK523309 | MK523338 |
| CR A1 | CBS 143939 | A. campestris | E | bark | | MK527849 | MK527849 | MK523264 | MK523282 | MK523310 | MK523339 |
| CR A2 | | | | | | MK527850 | MK527850 | – | MK523283 | MK523311 | MK523340 |
| CR A3 | | | | | | MK527851 | MK527851 | – | MK523284 | MK523312 | MK523341 |
| Leptosphaeria macrospora | CCF 4028 | Ulmus laevis | H | living bark/sapwood tissue | Czech Republic | FR715522 | FR715522 | – | FR715509 | – | FR715498 | Pažoutová et al. (2012) |
| CR M1 | | Que rusc robur | bark | Germany | | MK527852 | MK527852 | – | MK523285 | MK523313 | MK523342 |
| CR M2 | CBS 184367 | Que rusc petraea | E | bark | Austria | MK527853 | MK527853 | MK523265 | MK523286 | MK523314 | MK523343 |
| CR M4 | | Que rusc robur | bark | Austria | | MK527854 | MK527854 | – | MK523287 | MK523315 | MK523344 |
| CR M7 | | Que rusc robur | bark | Germany | | MK527855 | MK527855 | – | MK523288 | MK523316 | MK523345 |
| Leptosphaeria muellei | CM | A. pseudoplatanus | bark | Austria | | MK527856 | MK527856 | – | MK523289 | MK523317 | MK523346 |
| CR M3 | CBS 184368 | A. pseudoplatanus | E | bark | | MK527857 | MK527857 | MK523266 | MK523290 | MK523318 | MK523347 |
| CR M6 | | A. pseudoplatanus | bark | Austria | | MK527858 | MK527858 | – | MK523291 | MK523319 | MK523348 |
| Leptosphaeria pataceae | ISP 1641 1989 = CBS 128196 | Pataca varia | H | canker tissue (parasite) | Italy | MH798901 | MH798901 | MK523267 | MK791334 | MK791335 | Vitale et al. (2018) |
| ISP 2105 | Pataca varia | H | canker tissue (parasite) | Italy | FR681904 | – | | | | | |
| ISP 2106 | Pataca varia | H | canker tissue (parasite) | Italy | FR681905 | – | | | | | |
| Leptosphaeria cladosporioides | CRU 1 | CBS 184369 | Ulmus minor | bark | Austria | MK527859 | MK527859 | – | MK523292 | MK523321 | MK523349 |
| CR U2 | | Ulmus minor | bark | Austria | | MK527860 | MK527860 | – | MK523293 | – | MK523350 |
| CR U3 | | Ulmus minor | bark | Austria | | MK527861 | MK527861 | – | MK523294 | – | MK523351 |
| Nad = CBS 145296 | Nad = CBS 145296 | Ulmus minor | E | bark | China | MK527862 | MK527862 | MK523268 | MK523294 | MK523322 | MK523351 |
| Leptosphaeria sp. A23 | Annona squamosa | endophyte | India | | | EF488447 | – | | | | unpublished |
| AWB8 | A. squamosa | living wood tissue | India | | | JX443559 | – | | | | |
| PPM 0003 | Calocedrus macrocarpa var. formosana | living host tissue | Taiwan | | | KO272618 | KO272618 | – | | | |
| PPM 0024 | Calocedrus macrocarpa var. formosana | living host tissue | Taiwan | | | KO242164 | KO242164 | – | | | |
| E6820C | Cassia prunifolia | living stem tissue | Ecuador | | | H217861 | – | | | | |
| VegaE7-79 | Coffee arabica | living petiole tissue | USA (Hawaii) | | | EU009996 | – | | | | |
| OTU 173 | Coffea sp. | leaf disk tissue | Puerto Rico | | | KT328745 | – | | | | |
| INPA 01538 | Erythrophyllum macrophyllum | living host tissue | Costa Rica | | | KJ264202 | – | | | | |
| CX | Eugenia uruguayensis | leaf petiole tissue | Uruguay | | | KU212866 | – | | | | |
| MX 17 | Hevea brasiliensis | living sapwood tissue | Mexico | | | JQ905737 | – | | | | |
| MX 194 | Hevea brasiliensis | living sapwood tissue | Mexico | | | JQ905738 | – | | | | |
| MX 229a | Ilex quysosia | living host tissue | Ecuador | | | JN642178 | – | | | | |
| HS 25 | living unidentified plants | living host tissue | China | | | KY498633 | – | | | | |
| MIB 07 | Madhuca indica | living bark tissue | India | | | JN60409 | – | | | | |
| clone OTU_F75_R46 | Notococcus fusca | living leaves | New Zealand | | | MF976713 | – | | | | |
| E15610E | Paeoniocela odorata | living stem tissue | Ecuador | | | KJ026133 | – | | | | |
| E 11-3111 | Ulmus macrocarpa | living bark/sapwood tissue | China | | | FJ026139 | – | | | | |
| M36 | unknown | unknown (mangrove) | unknown | | | KT306540 | – | | | | |
| E14625A | Viroa calophylla | living stem tissue | Ecuador | | | KM096934 | – | | | | |
| Leptosphaeria wirkenii | AK60 09 | Ulmus laevis | living bark/sapwood tissue | Czech Republic | | FR715513 | FR715513 | – | | | |
| CCF 4020 | Ulmus laevis | H | living bark/sapwood tissue | Czech Republic | | FR715515 | FR715515 | – | | | |
| CCF 4021 | Ulmus laevis | H | living bark/sapwood tissue | Czech Republic | | FR715519 | FR715519 | – | | | |
| CCF 4022 | Ulmus laevis | H | living bark/sapwood tissue | Czech Republic | | FR715523 | FR715523 | – | | | |
| CCF 4023 | Ulmus laevis | H | living bark/sapwood tissue | Czech Republic | | FR715525 | FR715525 | – | | | |
| CCF 4024 | Ulmus laevis | H | living bark/sapwood tissue | Czech Republic | | FR715520 | FR715520 | – | | | |

References:
1. Indicates the strain number.
2. Indicates the strain origin.
3. Indicates the type of isolation.
4. Indicates the substrate and isolation source.
5. Indicates the country of isolation.
6. Indicates the GenBank accession number.
7. Indicates the references cited for each strain.
Table 1 (cont.)

| Taxon | Strain1 | Host2 | Type3 | Substrate / Isolation source | Country | GenBank accession no. | References |
|-------|---------|-------|-------|-------------------------------|---------|-----------------------|------------|
|       |         |       |       |                               |         |                       |            |
|       |         |       |       |                               |         |                       |            |
|       |         |       |       |                               |         |                       |            |

1 Abbreviations: ATCC: American Type Culture Collection, Manassas, VA, USA; BRIP: Queensland Plant Pathology Herbarium, Brisbane, Australia; CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CCF: Culture collection of the Dept. of Botany, Charles University, Prague, Czech Republic; CIP: Culture collection of Pedro Crous, housed at CBS; DAOM: Canadian National Mycological Herbarium, Ottawa, Canada; HKUCC: The University of Hong Kong Culture Collection, Hong Kong, China; ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; INBio: Instituto Nacional de Biodiversidad, Costa Rica; ISIPale: Culture collection of the Consiglio per la Ricerca in Agricoltura e l’Analisi dell’Economia Agraria, Roma, Italy (CREA-DC); MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MP: Culture collection of Martiná Ritlóvá, Department of Taxonomy, Institute of Botany of the Czech Academy of Sciences, Pruhonice, Czech Republic; MUCL: BCCM/MUCL Agro-food & Environmental Fungal Collection, Louvain-la-Neuve, Belgium; MYCO-ARIZ: Gilbertson Mycological Herbarium, University of Arizona, Tucson, USA; NRRL: Agricultural Research Service Culture Collection, Peoria, IL, USA; STMA: Culture collection of Mark Stadler, Helmholtz-Zentrum für Infektionsforschung, Braunschweig, Germany; UCR: University of California, Riverside, USA; UME: Herbarium of the Department of Ecology and Environmental Science, Umeå University, Umeå, Sweden.

2 Hosts and References only given for GenBank sequence accessions within the Deloniicolaceae–Leptosilliaceae clade.

3 Ex-holotype strain of Liberomyces saliciphilus.

4 Sequence retrieved from genome deposited at JGI-DOE (http://genome.jgi.doe.gov/).
Fig. 2. Phylogram of the ML tree (lnL = -19 991.4865) revealed by RAxML from an analysis of the ITS-LSU rDNA matrix of selected Xylariales, showing the phylogenetic position of Furfurella and Leptosillia. Strain/culture numbers or GenBank accession numbers are given following the taxon names; for the endophyte isolates, the host is given in brackets. ML and MP bootstrap support above 50% are given at the first and second position, respectively, above or below the branches. Accessions in bold were isolated and sequenced in the present study; those in green were generated in endophyte studies, those in red represent plant pathogens, and those in blue were isolated from ascomata growing on dead plant tissues (bark, wood, seed pods).
analyses were loaded in PAUP and subjected to heuristic search using TBR branch swapping (MULTREES option in effect, steepest descent option not in effect). MP analysis of the combined multilocus matrix was done using 1 000 replicates of heuristic search with random addition and subsequent branch swapping during each bootstrap replicate; in addition, each replicate was limited to 1 million rearrangements in the ITS-LSU matrix.

RESULTS

Molecular phylogeny

Of the 1 345 characters included in the ITS-LSU analyses, 516 were parsimony informative. The best ML tree (lnL = -19 991.4865) revealed by RAxML is shown as Fig. 2. MP analyses revealed 4 598 MP trees 4 041 steps long (not shown). Most of the tree backbone was identical in all MP trees; differences were mainly present within the clade containing the Amphisphaeriaceae, Apiosporaceae, Beltraniaeae, Melogrammataceae, Phlogicylindriaceae, Pseudomassariaceae, Sporocadaceae and Vialaeaceae (AABMPPSV clade; not shown).

![Phylogram of the ML tree](image-url)

Fig. 3 Phylogram of the ML tree (lnL = -84 566.4626) revealed by RAxML from an analysis of the combined SSU-ITS-LSU-rpb1-rpb2-tele-tub2 matrix of selected Xylariaceae, showing the phylogenetic position of Furfurella and Leptosillia. ML and MP bootstrap support above 50 % are given at the first and second position, respectively, above or below the branches. Strain/culture numbers are given following the taxon names; accessions in bold were isolated and sequenced in the present study.
The MP strict consensus tree was mostly compatible with the ML tree; notable exceptions were a placement of the Conioceciaceae-Microdochiaeae clade basal to the AABMPPSV clade; an interchanged position of the Hypoxylaceae with the Barmaeliaeae-Graphostromataceae clade, and within the Leptosillia clade, a position of the Calocedrus macrolepis endophyte as sister to Leptosillia macrospora and of the Nothofagus fusca endophyte as basal to the clade containing, amongst various endophyte accessions, Leptosillia pistaciae, L. macrospora, L. slaptonensis and L. wienkampii (not shown). The clade containing Delonicicola, Furfurella, Leptosillia and numerous unclassified endophytes received high support in both analyses (96 % ML, 97 % MP), and the clade containing Delonicicola, Furfurella gen. nov. and the Phoradendron endophyte medium (89 % ML) and high (96 % MP) support. The Leptosillia clade, however, was resolved as monophyletic only in the ML analyses, where it received moderate support (78 %); besides the six Leptosillia species, this clade contained numerous ITS sequence accessions of endophytes from various geographic areas and hosts, which were scattered throughout the clade (Fig. 2). In the strict consensus of the MP trees, three subclades were placed in a polytomy:

i. the Delonicicola clade;
ii. a highly supported Leptosillia acerina–L. muelleri clade (including various endophyte isolates); and
iii. a weakly supported clade containing the residual Leptosillia species plus the rest of endophyte isolates (not shown).

Of the 7 052 characters included in the combined five locus analyses, 3 093 were parsimony informative (476 from SSU-ITS-LSU, 613 from rpb1, 579 from rpb2, 656 from tef1 and 769 from tub2). The best ML tree (lnL = -84 566.4626) revealed by RAxML is shown as Fig. 3. The MP analysis revealed 6 MP trees 19 319 steps long (not shown); tree topologies of all MP trees were identical except for slightly different positions of Calceomyces lacunosus. Tree topologies of the MP trees were similar to the ML tree, except for a sister group relationship of Diatrypaceae and Lopadostomataceae, a basal position of Requienella to the other Xylariaceae s.l., a sister group

![Cultures on CMD at 15–17 °C](image)

- a. Furfurella nigrescens (CE); b. Furfurella stromatica (CE4); c–e. Leptosillia acerina (c, e: CRA1, d: CRA); f–h. Leptosillia macrospora (f: CRM1, g: CRM4, h: CRM2); i–k. Leptosillia muelleri (i: CRM3, j: CRM6); l–m. Leptosillia slaptonensis (l: CRU1, m: CRU2); n–p. Leptosillia wienkampii (n: CRW, o: CRW1, p: CRU).
  - a, c, e–g, i, k–l, n–o. Surface view; b, d, h, j, m, p. Reverse. All after 58 d; except b after 27 d and e, k after 7.5 mo.
relationship of Graphostromataceae to Xylariaceae s.str., and an interchanged position of Microdochium and Calceomycetes in some of the MP trees (not shown). In both analyses, the clade containing Delonicicola, Furfurella and Leptosillia and the Delonicicola-Furfurella subclade received maximum support (Fig. 3), while the Leptosillia subclade was highly supported (99 % ML, 93 % MP). Given the marked morphological differences (see below) and the highly supported phylogenetic subdivision in the multigene analyses, the new family Leptosilliaeae is established for the genus Leptosillia.

Culture characteristics
Culture images of two Furfurella and five Leptosillia species grown on CMD are shown in Fig. 4. Detailed culture descriptions are given under the respective species.

TAXONOMY

Delonicicoleae R.H. Perera et al., emend. Voglmayr & Jaklitsch
Type genus. Delonicicola R.H. Perera et al., Cryptog. Mycol. 38: 334. 2017.

Family of Xylariales. Pseudostromata variable, from conspicuously pulvinate to virtually absent, immersed in host tissue, erumpent to rarely superficial, variously coloured, ranging from yellowish, brown to black; visible as raised, dark spots on the host surface, as black, more or less elevated patches on wood or erumpent through bark, occasionally covered by bright turquoise, yellow to yellow-green scurf. Ascomata perithecial, immersed in pseudostroma, aggregated, globose, subglobose to conical or irregular, subhyaline to pale brown, with an apical ostiole. Peridium subhyaline to medium brown, KOH-, of textura angularis or prismatic. Ostioli papillate. Hamathecium composed of hyaline, septate or asperate, unbranched or occasionally branched paraphyses. Ascii arising from the base or margins of the ascomata, clavate to cylindrical, straight, curved to sinusous, thin-walled, containing 8 biseriately arranged ascospores, inamyloid and without a distinct apical apparatus. Ascospores ellipsoid or allantoid, equilateral or inequilateral, asperate or septeate, not constricted at the septa, hyaline, thin-walled, smooth, with rounded apices, without appendages or gelatinous sheath. Asexual morph unknown.

Notes — Furfurella can be easily discriminated from its closest relative, Delonicicola, by its large, allantoid, asperate ascospores, a bright yellow, yellow-green to turquoise scurf on the stroma and ostioli, a medium brown ascoma wall, and by growth on dead branches of Mediterranean fabaceous shrubs from tribe Genisteae.

In all species, the ascospore contours are only faintly seen in asci mounted in water, but become distinct in KOH and Lugol. Ascospores and asci shrink considerably in water to ensure comparability of the data.

Furfurella luteostiolata Voglmayr & Jaklitsch, sp. nov. — MycoBank MB829926; Fig. 5
Etymology. Referring to the yellow scurf around its ostioli.

Holotype. GREECE, Crete, Chania, Omalos, 920 m a.s.l., N35.37”E23.897”, in bark of thin dead branches of Genista acanthoclada, soc. Microthyrium sp., Diaportha sp., 5 June 2015, W. Jaklitsch & H. Voglmayr (WU 39987; ex-holotype culture CBS 143620 = CE3).

Pseudostromata immersed in the woody substrate and erumpent through the bark, reduced mostly to the region around the apical parts of the ascomata and covered by a bright sulphur yellow scurf, slightly blackening the bark surface around the erumpent stroma. Ascomata perithecial, c. 200–250 µm diam, embedded in bark or wood, solitary or in groups of up to 5, irregularly subglobose to pyriform, horizontally compressed when dry, with a central apical ostiole; perithecial content dull brown, waxy when dry. Peridium 16–26 µm thick, brown, KOH-, becoming hyaline towards the centrum, pseudoparenchymatous to prosenchymatous, consisting of thin- or thick-walled, hyaline to brown, isodiametric to elongated cells forming a textura angularis or prismatic. Ostioli variously developed, from inconspicuous and not protruding to long cylindrical and protruding; ostiolar canal with c. 1 µm wide hyaline periphyses embedded in a gelatinous matrix. Hamathecium composed of elongate, hyaline, septate, occasionally branched, basally broad and apically tapering paraphyses. Ascii arising from the base and the margins of the ascomata, sequentially produced; fusoid, clavate to cylindrical, straight, curved or sinusous, thin-walled, with marginal fissurate dehiscence, containing 8 biseriately or fasciculately arranged ascospores, without a stipe and an apical apparatus, inamyloid but appearing bitunicate with a distinct ocular chamber in Lugol after treatment with 3 % KOH. Ascospores allantoid, asperate, hyaline, thin-walled, smooth, with rounded apices, without appendages or gelatinous sheath. Asexual morph unknown.

Notes — Furfurella can be easily discriminated from its closest relative, Delonicicola, by its large, allantoid, asperate ascospores, a bright yellow, yellow-green to turquoise scurf on the stroma and ostioli, a medium brown ascoma wall, and by growth on dead branches of Mediterranean fabaceous shrubs from tribe Genisteae.

In all species, the ascospore contours are only faintly seen in asci mounted in water, but become distinct in KOH and Lugol. Ascospores and asci shrink considerably in Lugol, therefore measurements were done in water to ensure comparability of the data.

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Pseudostromata immersed in the woody substrate and erumpent through the bark, reduced mostly to the region around the apical parts of the ascomata and covered by a bright sulphur yellow scurf, slightly blackening the bark surface around the erumpent stroma. Ascomata perithecial, c. 200–250 µm diam, embedded in bark or wood, solitary or in groups of up to 5, irregularly subglobose to pyriform, horizontally compressed when dry, with a central apical ostiole; perithecial content dull brown, waxy when dry. Peridium 16–26 µm thick, brown, KOH-, becoming hyaline towards the centrum, pseudoparenchymatous to prosenchymatous, consisting of thin- or thick-walled, hyaline to brown, isodiametric to elongated cells forming a textura angularis or prismatic. Ostioli variously developed, from inconspicuous and not protruding to long cylindrical and protruding; ostiolar canal with c. 1 µm wide hyaline periphyses embedded in a gelatinous matrix. Hamathecium composed of elongate, hyaline, septate, occasionally branched, basally broad and apically tapering paraphyses. Ascii arising from the base and the margins of the ascomata, sequentially produced; fusoid, clavate to cylindrical, straight, curved or sinusous, thin-walled, with marginal fissurate dehiscence, containing 8 biseriately or fasciculately arranged ascospores, without a stipe and an apical apparatus, inamyloid but appearing bitunicate with a distinct ocular chamber in Lugol after treatment with 3 % KOH. Ascospores allantoid, asperate, hyaline, thin-walled, smooth, with rounded apices, without appendages or gelatinous sheath. Asexual morph unknown.

Pseudostromata immersed in the woody substrate and erumpent through the bark, reduced mostly to the region around the apical parts of the ascomata and covered by a bright sulphur yellow scurf, slightly blackening the bark surface around the erumpent stroma. Ascomata perithecial, c. 200–250 µm diam, embedded in bark or wood, solitary or in groups of up to 5, irregularly subglobose to pyriform, horizontally compressed when dry, with a central apical ostiole; perithecial content dull brown, waxy when dry. Peridium 16–26 µm thick, brown, KOH-, becoming hyaline towards the centrum, pseudoparenchymatous to prosenchymatous, consisting of thin- or thick-walled, hyaline to brown, isodiametric to elongated cells forming a textura angularis or prismatic. Ostioli variously developed, from inconspicuous and not protruding to long cylindrical and protruding; ostiolar canal with c. 1 µm wide hyaline periphyses embedded in a gelatinous matrix. Hamathecium composed of elongate, hyaline, septate, occasionally branched, basally broad and apically tapering paraphyses. Ascii arising from the base and the margins of the ascomata, sequentially produced; fusoid, clavate to cylindrical, straight, curved or sinusous, thin-walled, with marginal fissurate dehiscence, containing 8 biseriately or fasciculately arranged ascospores, without a stipe and an apical apparatus, inamyloid but appearing bitunicate with a distinct ocular chamber in Lugol after treatment with 3 % KOH. Ascospores allantoid, asperate, hyaline, thin-walled, smooth, with rounded apices, without appendages or gelatinous sheath. Asexual morph unknown.
l/w = (3.1–)3.8–4.8(–5.3) (n = 75), allantoid, aseptate, hyaline, thin-walled, smooth, with rounded apices, without appendages or gelatinous sheath. Asexual morph unknown.

Culture characteristics — On CMD colony radius 32 mm after 23 d at 22 °C. Colony whitish, very dense, turning cream with age, with abundant white aerial mycelium in the centre.

Habitat & Host range — Only known from corticated dead branches of *Genista acanthoclada*.

Distribution — Only known from the type collection in Crete (Greece).

Notes — *Furfurella luteostiolata* differs from the other two known *Furfurella* species by its broader and stouter ascospores and by the bright sulphur yellow scurf around the ostioles.
Fig. 6 Furfurella nigrescens. a–d. Black pseudostromata in wood or bark (a, b. with yellow-green scurf; c. clypeus-like black discoloration of bark); e, h. transverse section through pseudostromata and perithecia with dull orange, waxy perithecial contents; f. bright sulphur yellow scurf; g. black protruding ostioles laterally covered by yellow-green scurf; i. transverse section of perithecial wall and pseudostroma (bottom left); j. vertical section of pseudostroma embedded in bark with single perithecium and clypeus-like black discoloration of bark surface; k. section of peridium and pseudostroma (bottom); l. septate paraphyses; m–o. asci; p–x. ascospores. All in water, except l, o in Lugol after KOH pre-treatment, n in 3 % KOH (a–b, e–g, m–n, p–r: WU 39990 (holotype); c, h–l, o, s–v: WU 39992; d, w, x: WU 39991). — Scale bars: a–c, e = 300 µm; d, f = 500 µm; g–j = 100 µm; k–x = 10 µm.
embedded in bark or wood, solitary or aggregated in groups, lenticular, subgloboso to pyriform, horizontally compressed when dry, with a central apical ostiole. *Peridium* 11–22 µm thick, light brown, KOH+, becoming hyaline towards the centrum, pseudoparenchymatous to prosenchymatous, consisting of thin-walled, light brown, isodiometric to elongated cells 5–11 x 1–3.5 µm forming a textura angularis to prismatica, becoming hyaline towards the centrum; perithecial contents dull orange, waxy when dry. **Ostioles** either flat, non-protruding, or distinctly cylindrical to conical and projecting up to 200 µm, 80–100 µm wide, black, of thick-walled, dark brown cells with narrow lumen; when protruding ostiole laterally covered by a sulphur yellow to yellowish green scurf. *Hamathecium* composed of elongate, hyaline, sepalate, occasionally branched paraphyses up to 5.5 µm wide at the base, gradually tapering to 1.3 µm towards the distal ends. Ascii (64–)72–89–(99) x (10.5–)11.5–13.2–(14.5) µm (n = 35), fusoid, clavate to cylindrical, straight or slightly curved, thin-walled, with fissurate dehiscence, containing 8 ascosporae biseriately arranged or in two fascicles, without a stipe and an apical apparatus, inamylloid. Ascospores variable in length, (18–)20–29–(35) x (4.0–)4.5–5.2–(6.0) µm, l/w = (3.7–)4.2–5.9–(7.2) µm (n = 157), aseptate, asperate, hyaline, thin-walled, smooth, with rounded apices, without appendages or gelatinous sheath. **Asexual morph** unknown.

**Culture characteristics** — On CMD colony radius up to 34 mm after 29 d at 22 °C, covering almost the entire plate. Colony whitish, dense, thin, becoming yellowish brown to brown from the centre, with white aerial mycelium in the centre; odour sweetish.

**Habitat & Host range** — On dead branches of *Calicotome villosa* and *Chamaecytisus cetrarios*. Additional specimen examined. *S. Tello S.T.15031803 (WU 39994; culture CE5).*

**Notes** — Compared to the other two species of the genus, *Furfurella nigrescens* is more inconspicuous as its scurf is less prominent and sometimes even entirely absent. However, it is distinctly blackening the host surface, ranging from circular and clypeus-like around single ascomata in bark to extensive irregular patches around aggregated ascomata embedded in wood. In addition, its cultures develop a bright yellow aerial mycelium on CMD (Fig.4a).

**Furfurella stromatic** Voglmayr & Jaklitsch, sp. nov. — MycoBank MB829928; Fig. 7

**Etymology.** Referring to the well-developed pseudostromata.

**Holotype.** *S. Tello S.T.15031803 (WU 39994; culture CE4)*. — in host decorticated branch of *Genista cinierea*, 29 Feb. 2016, S. Tello S.T.29023610 (WU 39993; ex-holotype culture CBS 144409 = CE4).

**Pseudostromata** conspicuous, 0.25–2.1 mm long, 0.15–1.2 mm wide, pulvinate, superficial on wood or erumpent through bark cracks, exterior black and covered by a bright sulphur yellow, yellowish green to turquoise scurf, interior light brown. **Ascomata** perithecal, 240–460 µm diam, c. 250–280 µm high, embedded in a pseudostroma, gregarious in groups up to 25, subgloboso, globose to pyriform, horizontally compressed when dry, with a central apical ostiole; perithecial content dull orange, waxy when dry. **Peridium** 21–29 µm thick, light brown, KOH-, becoming hyaline towards the centrum, pseudoparenchymatous to prosenchymatous, consisting of thin-walled, light brown, isodiometric to elongated cells 2–11.5 x 1–3.5 µm forming a textura angularis, becoming hyaline towards the centrum. **Ostioles** cylindrical to conical, protruding above stromata up to 250 µm, 80–160 µm wide, black, laterally covered by a sulphur yellow to yellowish green scurf. *Hamathecium* composed of elongate, hyaline, sepalate, occasionally branched paraphyses up to 5 µm wide at the base, gradually tapering to 1.7 µm towards the distal ends, deliquescent at maturity, Ascii (78–)89–122–(139) x (10.7–)11.3–13.5–(14.5) µm (n = 28), clavate to cylindrical, usually slightly curved, thin-walled, with fissurate dehiscence, containing 8 biseriately arranged ascosporae, without a stipe and an apical apparatus, inamylloid, easily detached at maturity. Ascospores variable in length, (23–)29–38–(47) x (3.7–)4.7–5.5–(6.5) µm, l/w = (5.1–)5.7–7.1–(8.1) (n = 103), aseptate, asperate, hyaline, thin-walled, smooth, with rounded apices, without appendages or gelatinous sheath. **Asexual morph** unknown.

**Culture characteristics** — On CMD colony radius 40 mm after 29 d at 22 °C, covering almost the entire plate. Colony whitish, dense, thin, becoming yellowish brown to brown from the centre, with white aerial mycelium in the centre; odour sweetish.

**Habitat & Host range** — On dead branches of *Genista cinitrea*.

**Distribution** — Only known from southern Spain (Andalucia).

**Additional specimen examined.** *S. Tello S.T.15031803 (WU 39994; culture CE5).*

**Notes** — *Furfurella stromatic* is well distinct from the other known species of the genus by its conspicuous elongate pulvinate pseudostromata containing up to 25 perithecia with distinctly protruding black ostioles. This overall appearance, and in particular the fact that the ascii become easily detached at maturity, the deliquescent paraphyses and the allantoid hyaline ascosporae, point towards a placement of this fungus in the *Diasporthales*. Similar cases of misleading morphological evidence for taxa phylogenetically recently reclassified in *Xylariales* include e.g., *Melogramma* (previously classified in *Diasporthales*; Jaklitsch & Voglmayr, 2012), *Arthrocladia* and *Requierella* (previously classified in *Pyrenulales*; Jaklitsch et al. 2016b) and *Strickeria* (previously classified in *Dothideomycetes*; Jaklitsch et al. 2016b). Of all three *Furfurella* species, *F. stromatic* has the most conspicuous bright yellow to yellowish green scurf.

**Key to species of Furfurella**

1. Pseudostromata conspicuous, erumpent to superficial, pulvinate, exterior black and covered by a bright sulphur yellow, yellowish green to turquoise scurf ............... 2. *F. stromatic*
2. Pseudostromata inconspicuous, reduced to virtually absent, mostly in host tissue ........................................... 2
3. Pseudostromata concentrated around the erumpent ostioles, at margins covered by bright sulphur yellow scurf, ascospores (5.5–)6.5–7.5–(8.2) µm wide ............ *F. luteostiolata*
4. Pseudostromata embedded in substrate, not to slightly elevating but blackening the substrate surface, ascospores (3.7–)4.7–5.5–(6.5) µm wide ............ *F. nigrescens*

**Leptosillia** Voglmayr & Jaklitsch, fam. nov. — MycoBank MB829929

**Etymology.** Referring to the name of the type genus.

**Type genus.** *Leptosilla* Höhn.

**Family of Xylariales.** Ascomata perithecal, superficial to partly immersed in bark, scattered, gregarious or confluent, black, sometimes collapsed, with a central apical ostiolar papilla. *Peridium* melanized, KOH-, of textura angularis or prismatica.
Fig. 7 Furfurella stromatica. a–e. Pseudostromata covered by bright yellow-green scurf and erumpent through bark (a–d) or superficial on wood (e); f. transverse section through pseudostroma and perithecia; g. side view of pulvinate stroma on wood, with protruding ostioles; h. vertical section of pseudostroma and peritheciophora with orange, waxy perithecial content; i. vertical section of pseudostroma and perithecia, with yellow scurf dissolving in KOH; j. black erumpent ostioles laterally covered by bright yellow-green scurf; k. section of perithecial wall and pseudostroma (lower half); l–m. asci; n–y. ascospores. All in water, except i, l in 3 % KOH, m in Lugol after KOH pre-treatment (a–d, f, i–j, l, t–y: WU 39993 (holotype); e, g–h, k, m–s: WU 39994). — Scale bars: a–f, i–j = 300 µm; g–h 100 µm; k–y = 10 µm.
Ostioles papillate, sometimes sulcate, base of the ostiolar canal sometimes with hyaline periphyses. *Hamathecium* composed of hyaline, septate, occasionally branched paraphyses embedded in a gelatinous matrix. *Asci* arising from the base of the ascomata, sequentially produced; clavate to cylindrical, curved to sinuous, thin-walled, containing 8 bi-, triseriately or fasciculately arranged ascospores, inamyloid and without a distinct apical apparatus. *Ascospores* ranging in shape from nearly straight, falcate, lunate, sinuous, sigmoid to hook-shaped, aseptate or septate, not constricted at the septa, hyaline, thin-walled, smooth, with rounded to subacute apices, without appendages or gelatinous sheath. *Conidiomata* pycnidial, superficial to partly immersed in bark, globose to pyriform, black, scattered, aggregated or confluent, uniseriately or irregularly plurilocular. *Peridium* more or less melanized, of *textura globulosa* to *angularis*. *Conidiophores* short, hyaline, arising from the inner layer of the peridium. *Conidiogenous cells* cylindrical to lageniform. *Conidiogenesis* either enteroblastic-phialidic or holoblastic with sympodial proliferation, both types sometimes found within the same conidioma. *Conidia* commonly of two types according to their formation, allantoid, falcate or filiform, aseptate, hyaline, thin-walled.

Notes — *Leptosiliaceae* is closely related to *Deloniocolaceae*, from which it differs significantly by semi-immersed to superficial, black ascomata and, when present (L. *muelleri*), by different stroma structure.

**Leptosillia** Höhn., Mitt. Bot. Inst. Tech. Hochsch. Wien 5: 111. 1928

Synonyms. *Cresporhaphis* M.B. Aguirre, Bull. Brit. Mus. ×. Schulzer & Sacc., Rev. Mycol. (Toulouse) 6 (no. 22): 70. 1884.

Type species. *Leptosilla notha* Höhn., a synonym of *L. muelleri* (Duby) Voglmayr & Jaklitsch.

**Ascomata** perithelial, 100—400 µm diam, superficial to partly immersed in bark, scattered singly, gregarious or confluent, black, smooth, sometimes collapsed, (sub)globose to pyriform, with a central apical ostiole. *Peridium* melanized, KOH-, becoming subhyaline towards the centrum, pseudoparenchymatous to prosenchymatous, consisting of thick-walled, dark brown, isodiametric to elongated cells forming a *textura angularis* or *prismatica*. *Ostioles* papillate, sometimes sulcate; base of the ostiolar canal sometimes with hyaline periphyses. *Hamathecium* composed of elongate, hyaline, filiform, septate, occasionally branched paraphyses embedded in an inamyloid gelatinous matrix; in some species with hyaline, refractive, dextrinoid granular exudates turning amber-red in Lugol. *Asci* arising from the base of the ascomata, sequentially produced; clavate to cylindrical, curved to sinuous, thin-walled, containing 8 bi-, triseriately or fasciculately arranged ascospores, with a short stipe, without a distinct apical apparatus, inamyloid but a narrow, short pore visible in Lugol. *Ascospores* from nearly straight, hooked, falcate, lunate, sinuous to sigmoid, aseptate or up to 11-septate, not constricted at the septa, hyaline, thin-walled, smooth, with rounded to subacute apices, without appendages or gelatinous sheath.

**Conidiomata** pycnidial, superficial to partly immersed in bark, globose to pyriform, black, smooth, scattered, aggregated or confluent, uniseriately or irregularly plurilocular. *Peridium* light to dark brown, continuous, composed of thin-walled, more or less isodiametric cells, forming a *textura globulosa* to *angularis*. *Conidiophores* short, hyaline, thin-walled, smooth, branched up to three times, arising from the inner layer of the peridium. *Conidiogenous cells* cylindrical to lageniform. *Conidiogenesis* either enteroblastic-phialidic and bearing usually curved, filiform, sometimes narrowly falcate conidia, or holoblastic with sympodial proliferation and bearing allantoid to falcate conidia; in some species both types of conidiogenous cells and conidia produced in the same conidioma. *Conidia* allantoid, falcate, lunate or filiform, aseptate, hyaline, thin-walled, smooth.

Notes — *Leptosillia* was posthumously described (Höhnel 1928) in a manuscript edited by J. Weese, based on a holomorph specimen collected on bark of Acer *pseudoplatanus* in Germany. While Höhnel is given as the author of this publication, it is not clear which additions were provided by Weese.

The comment of Hawksworth (in Eriksson & Hawksworth 1987) that the type of *Leptosillia* was based on a specimen of *Sillia cinctula* distributed by Rehm in his Ascomyceten no. 2047 is erroneous. Rehm’s Ascomyceten no. 2047 of Cryptospora (= *Sillia cinctula*) represents a North American collection from Castanea dentata (Rehm 1913), which conforms to the original description of that species and has nothing to do with *Leptosillia*.

All *Cresporhaphis* species currently accepted in Index Fungorum (accessed Feb. 2019) are here combined in *Leptosillia* except *C. chibaensis* and *C. rhosta*; for further details see below. Although no DNA data are available for *C. fusariospora* and *C. pinicola*, their morphology and habitat support inclusion in the genus.

**Leptosillia acerina** (Rehm) Voglmayr & Jaklitsch, comb. nov. — MycoBank MB829930; Fig. 8

Basionym. *Leptorphaphis acerina* Rehm, Ber. Naturhist. Vereins Augsburg 26: 51. 1881.

Synonyms. *Cresporhaphis* acerina (Rehm) M.B. Aguirre, Bull. Brit. Mus. (Nat. Hist.), Bot. 21 (2): 147. 1991.

*Metasphaeria robergia* Schulzer & Sacc., Rev. Mycol. (Toulouse) 6 (no. 22): 70. 1884.

Typification. GERMANY. Bayen, Franken, near Sugenheim, young deciduous forest, on cortex of living branches of Acer campestre, 1870, H. Rehm, Ascomyc. no. 197 (S-L1668, lectotype of *Leptorphaphis acerina* selected by Aguirre-Hudson 1991; K(M) 111821, W 1923-1578, W 2009-00424: isotype).

*Leptosillia acerina* (Rehm) Voglmayr & Jaklitsch, comb. nov. — MycoBank MB829930; Fig. 8

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*Leptosillia acerina* (Rehm) Voglmayr & Jaklitsch, comb. nov. — MycoBank MB829930; Fig. 8

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Synonyms. *Cresporhaphis* acerina (Rehm) M.B. Aguirre, Bull. Brit. Mus. (Nat. Hist.), Bot. 21 (2): 147. 1991.

*Metasphaeria robergia* Schulzer & Sacc., Rev. Mycol. (Toulouse) 6 (no. 22): 70. 1884.

Typification. GERMANY. Bayen, Franken, near Sugenheim, young deciduous forest, on cortex of living branches of Acer campestre, 1870, H. Rehm, Ascomyc. no. 197 (S-L1668, lectotype of *Leptorphaphis acerina* selected by Aguirre-Hudson 1991; K(M) 111821, W 1923-1578, W 2009-00424: isotype).

*Leptosillia acerina* (Rehm) Voglmayr & Jaklitsch, comb. nov. — MycoBank MB829930; Fig. 8

Basionym. *Leptorphaphis acerina* Rehm, Ber. Naturhist. Vereins Augsburg 26: 51. 1881.
H.	Voglmayr	et	al.: Leptosilliaceae fam.	nov.	and	Furfurella
gen.	nov.

Conidia

Conidiophores and conidiogenous cells similar to those from natural substrate. *Conidia* (18–)20–25(–28) × (2.0–)2.3–2.7(–3.0) μm, l/w = (7.0–)8.1–10.2(–12.5) (n = 54), falcate to lunate, asep-
Fig. 9  a–y. Leptosillia fusariospora (GZU 000335714, isotype). a–c. Perithecia on bark (a, c. horizontally collapsed and cupulate); d–e. side view of perithecia with apical papilla; f. peridium in section; g–j. asci (g. with paraphysis, j. in Lugol after KOH pre-treatment); k, l. ascus tips (l. in Lugol after KOH pre-treatment); m–y. ascospores; arrows denoting septa. — z–h1. Leptosillia aff. fusariospora (NY 00270482). z. a1. Cupulate perithecia on bark in side (z) and surface (a1) view; b1–h1. ascospores. All in 3 % KOH, except where noted. — Scale bars: a–e, z–a1 = 100 µm; f–j = 10 µm; k–y, b1–h1 = 5 µm.
tate, hyaline, thin-walled, smooth, with subacute tapering ends, containing numerous guttules especially towards the ends.

Habitat & Host range — Only known from cork wings and outgrowths (the rhytidome) of living or dead branches of *Acer campestre*.

Distribution — Europe; known from Austria, Croatia and Germany (Ellis & Everh. 1991, this study).

Additional specimens examined (all on cork wings of branches of *Acer campestris*), Austria, Niederösterreich, SE Gaaden, Am Tenneberg, 29 Jan. 2017, H. Voglmayr & I. Greilhuber (WU 39996, culture CRA3); Mödling, Richardshof, 5 Nov. 2016, H. Voglmayr & I. Greilhuber (WU 39997, culture CRA2); Pfaffstätten, near Heberberg, 23 Apr. 2016, H. Voglmayr (WU 39998, culture CRA).

Notes — *Leptosillia acerina* is well characterised by its host, *Acer campestre*. It has so far been only found on cork wings of young living or recently dead branches, which are formed by young trees in open stands. Although the species has apparently not been recorded since the late 19th century and was only known from the type localities of the heterotypic synonyms (*Aguirre-Hudson 1991*), the current observations in eastern Austria indicate that, at least in Central Europe, it may be rather common in suitable habitats. This species has most likely been overlooked in mycological field studies.

*Leptosillia fusariospora* (Ellis & Everh.) Voglmayr & Jaklitsch, comb. nov. — MycoBank MB829931; Fig. 9

Basionym. *Coelosphaeria fusariospora* Ellis & Everh., J. Mycol. 4: 65. 1888.

Synonym. *Leiosphaerella fusariospora* (Ellis & Everh.) M.E. Barr, Myco-taxon 46: 62. 1993.

Typification. USA, Kansas, on bark of (living)? cottonwood trees (*Populus deltoides*), soc. *Teichospora kansensis*, without place and date. G. Egeling, comm. J.W. Eckfeldt, in Ellis & Everhart, N. Amer. Fungi Ser. 2 no. 1957 (NY 00883560, lectotype of *Coelosphaeria fusariospora* here selected, MBT 385917; GZU 00035714, K(M) 252230, K(M) 252231, NY 00883561, NY 00883562, NY 00883563 isotypes).

Ascomata perithecial, superficial to basally immersed in bark, (135–)160–205–(230) µm diam (n = 30), 90–190 µm high, black, shiny, smooth, scattered singly to gregarious, subglobose to hemispherical, circular from above, commonly horizontally collapsed and then cupulate, with a distinct central apical papilla c. 30–55 µm wide, 30–50 µm high. *Peridium* continuous, dark brown, becoming light brown to hyaline towards the centrum, 17–40 µm thick, of *textura angularis* composed of thick-walled, isodiametric to elongated cells 4–15 × 2–4 µm with dark brown walls, becoming thin-walled and subhyaline towards the centrum. *Hamathecium* composed of hyaline, smooth, thin-walled, septate, occasionally branched, 1.7–3.5 µm wide paraphyses embedded in an amylloid gelatinous matrix; paraphyses not observed. *Asci* (46–)51–68–(83) × (7.0–)8.0–9.5–(10.3) µm (n = 50), unitunicate, cylindrical, straight, curved to sinuous, thin-walled, containing 8 ascospores arranged biseriately or (n = 50), unitunicate, cylindrical, straight, curved to sinuous, thin-walled, containing 8 ascospores arranged biseriately or uniseriately, mostly fusoid to falcate but lacking strongly curved to hooked ends (Fig. 9b–h1), and also the ascospores are distinctly superficial (Fig. 9z, a1) and somewhat larger ((183–)206–276–(361) µm diam (n = 60)). The specimen may therefore represent a distinct *Leptosillia* species, but fresh collections and sequences are necessary for a detailed evaluation.

The treatment of *Coelosphaeria fusariospora* by Barr (1993) is confusing: first she combined it in *Leiosphaerella*, but a few pages later she considered the species to be conspecific with *Crepensorhaphis rhoina*. Our detailed re-examination of type specimens of *Coelosphaeria fusariospora* and *Crepensorhaphis rhoina* did not confirm this synonymy, but revealed them as two different, unrelated species. While asci, ascospores and also the corticolous ecology of *Coelosphaeria fusariospora* are in full agreement with *Leptosillia*. *Crepensorhaphis rhoina* differs by an amyloid apical ascus ring, mostly fusoid to curved ascospores of irregular shapes and by growth on dead wood. The latter is therefore not considered to be congeneric with *Leptosillia* (see notes under *C. rhoina* below).

*Leptosillia macrospora* (Eitner) Voglmayr & Jaklitsch, comb. & stat. nov. — MycoBank MB829932; Fig. 10

Basionym. *Leptorhaphis quercus* f. macrospora Eitner, Jahresber. Schles. Ges. Vaterl. Cult. 78: 25. 1901 ‘1900’.

Synonyms. *Crepensorhaphis macrospora* (Eitner) M.B. Aguirre, Bull. Brit. Mus. (Nat. Hist.), Bot. 21 (2): 149. 1991.

*Liberyomyces macrosporus* Pažoutová et al., Mycologia 104: 201. 2012.

Typification. Poland, Silesia, Nimptsch, Klein Ellugh, on Quercus robur, 12 Apr. 1892, E. Eitner (W 19701, lectotype of *Leptorhaphis quercus* f. macrospora selected by Aguirre-Hudson 1991). – Austria, Niederösterreich, Schönfeld, Wacholderheide NE of the golf course, on bark of living trunks of *Quercus petraea*, 5 May 2016, H. Voglmayr & I. Greilhuber (WU 39999, epitype of *Leptorhaphis quercus* f. macrospora here designated (MBT 385918); ex epitype culture CRM2 = CBS 143627).

Notes — *Leptosillia fusariospora* is well characterised by an ascospore shape similar to macroconidia of *Fusarium*, from which its species epithet was derived. It is similar to the European *L. acerina* in its horizontally collabent, cupulate ascocoma and has asceptate ascospores of similar size; however, it differs by differently shaped ascospores occasionally becoming uniseptate at maturity, different hosts and distribution (North America vs Europe). Unfortunately, no cultures and DNA sequences are available for *L. fusariospora*, but both the morphological characteristics and the ecology of the species match with the genus *Leptosillia*, into which it is therefore combined.

Numerous copies of the type collection were distributed as Ellis & Everhart, N. Amer. Fungi Ser. 2 no. 1957, but to our knowledge no lectotype has yet been selected. In NY, where the Ellis collection is kept, there are four collections corresponding to the protologue, one (NY 00883560) labelled as holotype, two, bearing the label of Ellis & Everhart, N. Amer. Fungi Ser. 2 no. 1957 (NY 00883561, NY 00883562), as isotypes, and one (NY 00883563) without a type label but with the same data given for the other collections, but with a collection date Oct. 1887. The latter also represents an ascopte as it was collected ahead of the publication of the taxon, and it includes an original note with exactly the same ascospore and ascus measurements as given in the protologue. Based on preservation, the isotype specimen NY 00883560 of the Ellis collection is here selected as lectotype. Most of the isotype specimens investigated also contain ascomata of *Teichospora kansensis*.

The collection from *Celtis occidentalis* distributed as *Coelosphaeria fusariospora* in Ellis & Everhart, N. Amer. Fungi Ser. 2 no. 3016 is here only tentatively attributed to the species. It has distinctly longer ascospores ((28–)33–41–(44) × (2.2–)2.5–2.9–(3.1) µm, l/w = (10.8–)12.1–15.3–(16.7) (n = 25)) of a different shape, being narrowly fusiform to falcate but lacking strongly curved to hooked ends (Fig. 9b1–h1), and also the ascocoma are distinctly superficial (Fig. 9z, a1) and somewhat larger ((183–)206–276–(361) µm diam (n = 60)). The specimen may therefore represent a distinct *Leptosillia* species, but fresh collections and sequences are necessary for a detailed evaluation.

The treatment of *Coelosphaeria fusariospora* by Barr (1993) is confusing: first she combined it in *Leiosphaerella*, but a few pages later she considered the species to be conspecific with *Crepensorhaphis rhoina*. Our detailed re-examination of type specimens of *Coelosphaeria fusariospora* and *Crepensorhaphis rhoina* did not confirm this synonymy, but revealed them as two different, unrelated species. While asci, ascospores and also the corticolous ecology of *Coelosphaeria fusariospora* are in full agreement with *Leptosillia*. *Crepensorhaphis rhoina* differs by an amyloid apical ascus ring, mostly fusoid to curved ascospores of irregular shapes and by growth on dead wood. The latter is therefore not considered to be congeneric with *Leptosillia* (see notes under *C. rhoina* below).
Fig. 10 Leptosillia macrospora. a–d. Perithecia on bark; note the stellate or sulcate structures on the apical papillae; e–f. asci in 3% KOH; g. ascus tip in Lugol; h. paraphyses; i–t. vital ascospores; arrows denoting septa; u–v. pycnidia and conidial drops in culture (CMD, isolation plate, 7 d); w–y. conidiophores, conidiogenous cells and conidia from pycnidia on natural substrate; z–f1. conidia from pycnidia in culture (CMD, 6 mo). All in water, except where noted (a–b, i–l, n–p, w–f1: WU 39999 (epitype); c–f: WU 40004; g–h, m, q–t: WU 40000; u–v, g1–r1: CRM2). — Scale bars: a–b, u = 200 µm; c–d, v = 100 µm; e–f, h–t, w–y = 10 µm; g, z–r1 = 5 µm.
Ascomata perithecial, half-immersed in bark to superficial, (170–)200–255–(275) µm wide (n = 46), (210–)220–270–(280) µm high (n = 6), black, smooth, scattered singly, sometimes gregarious, pyriform, circular from above, sometimes laterally collapsed, with a central apical papilla laterally enlarged by stellate or sulcate structures on the surface. Peridium continuous, dark brown, becoming hyaline towards the centrum, 20–30 µm thick, of a textura angularis composed of thin-walled, isodiametric to elongated cells 4–8 µm diam with subhyaline to dark brown walls. Hamathecium composed of hyaline, smooth, thin-walled, septate, occasionally branched, 1.5–3.3 µm wide paraphyses embedded in an inamyloid gelatinous matrix; paraphyses not observed. Asci (94–)104–120–(135) × (8.8–)9.5–11.5–(13.0) µm (n = 46), unitunicate, clavate to cylindrical, curved, thin-walled, containing 8 ascospores arranged in fascicles, with a short stipe, inamylloid and without a distinct apical apparatus. Ascospores (53–)65–93–(109) × (3.0–)3.5–4.5–(5.0) µm, l/w = (12.8–)16.1–24.2–(31.3) (n = 82), variable in shape from sinuous, sigmoid, semicircular to hook-shaped, at maturity 1–3 septate, hyaline, thin-walled, smooth, with narrowly rounded ends, multiguttulate when vital.

Pycnidia scattered on bark, black, similar to ascomata except for smaller size, c. 100–150 µm diam. Peridium continuous, dark brown, c. 10 µm thick, composed of few layers of thin-walled dark brown cells 3–5.5 µm diam. Conidiophores short, reduced, hyaline, smooth, branched up to two times, composed of short, cylindrical to almost isodiametric cells arising from the inner wall of the pycnidium. Conidiogenous cells (2.0–)7.3–12.0–(17.5) × (0.9–)1.9–2.5 (µm) (n = 83), enteroblastic, phialidic, lageniform to cylindrical, hyaline, smooth, arranged in dense terminal whorls. Conidia (16–)18–23–(24) × (0.8–)0.9–1.1–(1.2) µm, l/w = (16.3–)17.6–23.7–(26.1) (n = 20), filiform, curved, asetapetate, hyaline, thin-walled, smooth, containing few guttules when vital.

Culture characteristics and asexual morph in culture — Colony on CMD at 16 °C reaching 38–56 mm diam after 58 d; variable in colour and growth depending on the strain, greyish brown when vital. Conidiophores consist of thin-walled cells. Conidia (75–90 (sensu Hirayama & Tanaka 2011), related to species of the genus Pseudosagedia, and in particular with P. leptoconidia, but in this the ascospores are at least 7-septate, the ascomata present an additional involucrum over the exciple, and the thallus is clearly lichenized with Trentepohlia. Berger & Brietemezhofer (2000) reported the species from Austria growing on Tilia cordata (Donautal, Oberösterreich, Berger 9578). We requested the material for study, but instead we received another collection from the same area, also labelled as Creporhaphis macrospora, but growing on Malus sp. (Berger 12951). Examination of this voucher revealed a fungus with filiform, multiseptate ascospores (75–90 × 3.5 µm) but with distinct fissitunicate asci. This collection is probably a new species of the genus Lophistoma (sensu Hirayama & Tanaka 2011), related to Lophistoma subcutanea (see Huhndorf 1992: 503–504; Fig. 2), which is also found on bark of Rosaceae but has smaller ascospores (25–29 × 3–3.5 µm).

Based on sequence data and morphology, Liberomyces macrospora represents the asexual morph of Leptosilia macrospora, and is therefore a synonym of the latter. The description of the asexual morph in pure culture was modified from the description of Pažoutová et al. (2012), and that of the pycnidia from natural substrate was adapted from the description in Aguirre-Hudson (1991). In the present study, pycnidia on the natural substrate could be found in only one occasion, and the pycnidium investigated only produced enteroblastically formed, filiform conidia; however, their size agrees well with those recorded from culture and given in Aguirre-Hudson (1991).

Leptosilia muelleri (Duby) Voglmayr & Jaklitsch, comb. nov. — MycoBank MB829933; Fig. 11, 12

Basionym. Sphaeria muelleri Duby, in Rabenhorst, Klotzsch. Herb. Vivum. Mycol., Edn 2: no. 642. 1858.

Synonyms. Creporhaphis muelleri (Duby) M.B. Aguirre, Bull. Brit. Mus. (Nat. Hist.), Bot. 21: 151, 1962. Leptosphaeria muelleri (Duby) Auerw., in Gonnnermann & Rabenhorst, Mycol. Eur. Pyren., 5–6: f. 12, f. 167. 1869.

Psilosphaeria muelleri (Duby) Cooke (as ‘mulleri’), Grevillea 16 (no. 78): 50. 1887.

Zignoëlla muelleri (Duby) Sacc. & Traverso, Syll. Fung. (Abellini) 20: 1170. 1911.

Cyspora muelleri (Sacc.) Died., Krypt.-Fl. Brandenburg (Leipzig) 9: 545. 1914.

Harpostroma nothum (Sacc.) Höhn. (as ‘notha’), Mitt. Bot. Inst. Tech. Hochsch. Wien 5: 112. 1928.
Fig. 11  *Leptosillia muelleri*, sexual morph. a–i. Single and confluent perithecia on bark in surface (a–f) and side (g–i) view; j. vertical section through pseudo-stroma with perithecia; k. strongly dextrinoid granular hamathelial exudates in Lugol after KOH pre-treatment; l–m. asci; n. ascus tip in Lugol; o. paraphyses; p–g1. ascospores (p–t. dead, u–g1. vital). All in water, except where noted (a, e, j, p–t: FH 00304540 (holotype of *Leptosillia notha*); b–c, f–g, l, o, u, y–z: WU 40005 (epitype); d, h, n, v–x: WU 40006; i, m, a1–b1: WU 40007; k, c1–g1: WU 40006). — Scale bars: a–b = 500 µm; c–e = 200 µm; f–l = 100 µm; j = 50 µm; k–n = 10 µm; o–g1 = 5 µm.
Ascomata perithecial, embedded in a pseudostroma, emerging from cracks on the surface of bark scales, (100–)140–210(–260) µm diam (n = 67), black, matt, smooth, rarely scattered singly and pyriform, but usually confluent and then irregular in shape and c. 1 mm long, immersed in bark to half of their height, not collapsing, with an indistinct to distinct central apical papilla. Peridium continuous, of a textura angularis, composed of an outer dark brown to black, 10–30(–45) µm thick layer of thin-walled isodiametric cells 2.5–5.5 µm diam with dark brown walls, forming a pseudostroma surrounding the inner wall, and an inner, 12–20 µm thick subhyaline to pale brown layer corresponding to the perithecium wall of (sub)hyaline to

![Image of Leptosillia muelleri](image-url)

**Fig. 12** a–d1. *Leptosillia muelleri*, asexual morph. a–c. Pycnidia on CMD isolation plates (a–b, 7 d; c, 37 d); d–h. conidiophores, conidiogenous cells and conidia from pycnidia on natural substrates; i–k. conidiophores, conidiogenous cells and conidia from pycnidia in pure culture (i, k. CMD 8 d; j. CMD 19 d); l–r, z–d1. conidia from natural substrate (l–n. dead; o–r, z–d1. vital); s–y. vital conidia from pycnidia in culture on CMD (s–u. 19 d; v–y. 8 d). All in water, except d, l–n from permanent slide (a–b, i, v–x: CRM3 (ex-epitype culture); c, j, s–u: CRM; d, l–n: FH 00304540 (holotype of *Leptosillia notha*); e–h, o–r, z–d1: WU 40005 (epitype); k, y: CRM6). e1. *Septoria notha* (holotype, PAD) — Scale bars: a = 1 mm; b–c = 200 µm; d–k = 10 µm; l–d1 = 5 µm; e1 = 3 mm.
light brown cells similar to those of the outer layer but slightly smaller and sometimes radially compressed changing into a textura prismatica. Hamathecium composed of hyaline, smooth, thin-walled, septate, occasionally branched, 1–3 μm wide paraphyses embedded in an inamyloid gelatinous matrix; with hyaline, refractive, strongly dextrinoid granular exudates turning amber-red in Lugol; periphyses not observed. Asci (65–)75–100(–107) × (7.2–)8.0–9.5(–11.3) μm (n = 25), uniloculate, cylindrical, slightly curved to sinuous, thin-walled, containing 8 bi- or triseriately arranged ascospores, with a short stipe, without a distinct apical apparatus, inamyloid but a narrow, short pore visible in Lugol. Ascospores (20–)25–33(–38) × (2.0–)2.8–3.7–(4.7) μm, l/w = (5.6–)7.7–10.1–(12.0) (n = 98), fusoid, lunate to falcate, aseptate, hyaline, thin-walled, smooth, with subacute to narrowly rounded tapering ends, when vital containing 2–3 large and numerous small guttules especially towards the ends.

Pychnidium on bark usually confluent, black, practically indistinguishable from ascomata. Conidiophores short, hyaline, smooth, simple or irregularly branched, arising from the inner wall of the pychnidium. Conidiogenous cells (6.8–)8.0–15.2(–27.5) × (1.2–)1.6–3.6(–5.3) μm (n = 72), lageniform to cylindrical, of two types interspersed within the same pychnidium: a) holoblastic with sympodial proliferation, bearing falcate conidia; b) enteroblastic, phialidic, bearing narrower, filiform conidia. Conidia of two types: a) holoblastic, 21–27(–32) × 2.0–2.5(–3.0) μm, l/w = (8.8–)9.5–11.8(–13.0) (n = 27), falcate, hyaline, smooth, with narrowly rounded ends, with few small guttules when vital; b) enteroblastic, (19–)23–28(–31) × (0.8–)0.9–1.2(–1.4) μm, l/w = (16.5–)19.9–29.0(–34.6) (n = 33), filiform, curved to semi-circular.

Culture characteristics and asexual morph in culture — Colonies on CMD at 16 °C reaching 50–55 mm diam after 58 d; first white, turning cream to greyish brown in the centre, with white woolly aerial mycelium, reverse cream, dark greyish brown in the periphery; textura angularis. Colony on straw agar 50–55 mm diam after 58 d; first white, turning cream to greyish brown in the centre, with white woolly aerial mycelium, reverse cream, dark greyish brown in the periphery; textura angularis-prismatica.

Notes — The holotype of Leptosilla notha, a holomorphic collection, from the Höhnel herbarium deposited in FH morphologically resembles our recent collections and the type of the earlier name Sphaeria muelleri. Remarkably, in the conidiomata observed on the natural substrate of the epitype two types of conidia are present: falcate and filiform ones, which are formed holoblastically and enteroblastically, respectively. However, in pure culture only holoblastical multiguttulate conidia were found; these were somewhat wider than those observed on the natural substrate. In a permanent mount of conidiomatal sections attached to the holotype, only phialides with filiform conidia were seen, with conidial sizes only slightly wider (22(–)26–31(–34) (1.3–)1.4–1.7(–1.8) μm (n = 25)); this, however, may be due to the mounting medium. To preserve the holotype, we did not make new preparations of the asexual morph.

Diederie (1915) identified the asexual morph on the holotype collection of Leptosilla notha as Septoria notha and recombined the species as Cystosporina notha. Subsequently, Höhnel (1928) established the monotypic genus Harpochroma for the latter, but challenged the conspecificity with Saccardo’s Septoria notha. We agree that this conspecificity is doubtful. The type specimen of Septoria notha is extant in PAD, and although it could not be microscopically investigated, no structures resembling Leptosilla notha were seen on the specimen under the stereomicroscope. Also the ecology does not quite fit, as the substrate is a thin, corticated branch of c. 6 mm diam (Fig. 12e1), while L. notha is confined to bark scales of old living trunks. In the original description (Saccardo 1880), the host of Septoria notha is erroneously given as Acer platanoides; it is here re-identified as Acer pseudoplatanus based on bark and wood characters of the type specimen. This is in line with the fact that Saccardo (1880) assumed a connection with Diaportha hystrix, a species commonly known from Acer pseudoplatanus but not from A. platanoides (Wehmeyer 1933).

Leptosilla pinicola (Samp.) Voglmayr & Jaklitsch, comb. nov. — MycoBank MB829934; Fig. 13

Basionym. Leptothaphis pinicola Samp., Bolm Soc. Broteriana, Coimbra, sér. 2: 2. 163. 1924 (1923).
Synonym. Cresporhaphis pinicola (Samp.) M.B. Aguirre, Bull. Brit. Mus. (Nat. Hist.), Bot. 21: 152. 1991.

Typification. PORTUGAL, Estremadura, Sierra de Sintra, Castelo dos Mouros, on bark of Pinus sp., 11 Apr. 1943, C. Tavares (LISU 511, neotype designated by Aguirre-Hudson 1991; UPS L-074953, isotype).

Ascomata perithecial, superficial on bark, (150–)180–230 (–280) μm wide (n = 32), black, shiny, smooth, scattered singly, sometimes gregarious, globose to hypophorm, circular from above, with an indistinct central apical papilla. Peridium continuous, dark brown, becoming hyaline towards the centrum, c. 20 μm thick, of textura angularis composed of thick-walled, isodiametric to slightly elongated cells 3.5–5.5 μm diam with dark brown walls, towards the centrum becoming a textura angularis-prismatica of thinner-walled pale brown to subhyaline cells. Hamathecium composed of hyaline, smooth, thin-walled, septate, occasionally branched, 1.5–2.3 μm wide paraphyses embedded in an inamyloid gelatinous matrix; periphyses of hyaline, smooth, thin-walled, 1.5–2 μm wide hyphae. Asci (74–)78–(95) × (9.5–)9.8–11.0(–11.7) μm (n = 24), uniloculate, cylindrical to fusoid, usually slightly curved, thin-walled, containing 8 ascospores arranged in fascicles, with a short stipe, inamyloid and without a distinct apical apparatus. Ascospores (35–)44–58 (–65) × (1.8–)2.4–3.0(–3.5) μm, l/w = (14.1–)16.2–21.7 (–23.7) (n = 20), acicular, often slightly curved, 5–11-septate, not constricted at the septa, hyaline, thin-walled, smooth.

Notes — Only two collections from the type locality in Portugal (Sintra, near Lisbon; Aguirre-Hudson 1991), dating back to the first half of the 20th century, are confirmed here as belonging to Leptosilla pinicola. Unfortunately, the species could not be recollected by the first author despite extensive search on the bark of various pine species at and near the type locality. Despite the lack of fresh material for sequencing, morphologically the species fits well in the genus Leptosilla. The current
description and illustrations are based on the isoneotype specimen from UPS, with few additions from Aguirre-Hudson (1991).

The species (as Cresporhaphis pinicola) has been cited from Austria by Berger et al. (1998) from bark of Prunus avium, and from Lithuania by Motiejūnaitė (2007) from bark of Berberis sp. Re-examination of the latter has confirmed that the material (K(M) 117899) is not conspecific with the type of Leptosillia pinicola because the ascospores are longer and more slender (62–78 × 2–3 μm), and arranged in a single fascicle in the ascus. This collection might yet represent a new species of Leptosillia, but DNA studies will be needed to confirm this. It is also unlikely that the material recorded from Austria is conspecific to L. pinicola due to the unrelatedness of the host, but we had no opportunity to study the collection.

**Leptosillia pistaciae** (Voglmayr et al.) Voglmayr, *comb. nov.* — MycoBank MB829935

*Basionym. Liberomyces pistaciae* Voglmayr et al., *Mycocent.* 40: 41. 2018.

Notes — In the current phylogenetic analyses this recently described serious canker pathogen of pistachio (*Pistacia vera*) is placed within *Leptosillia*, which necessitates a generic transfer. So far, no sexual morph is known for this species. For morphological description, illustrations and pathogenicity, see Vitale et al. (2018).

**Fig. 13** *Leptosillia pinicola* (UPS L-074953, isoneotype). a–f. Single and gregarious perithecia on bark in surface (a–d) and side (e–f) view; g. peridium in section; h. paraphyses; i–k. asci (k in Lugol); l–o. ascospores. All in 3 % KOH, except where noted. — Scale bars: a = 1 mm; b–c = 200 μm; d–f = 100 μm; g, l–o = 5 μm; h–k = 10 μm.
Fig. 14 Leptosillia slaptonensis. a–d. Perithecia on bark in surface (a–b) and side (c–d) view; e–f. asci (f. in Lugol); g. paraphyses; h–m. o–t. vital ascospores; arrows denoting septa; n. pale, translucent pycnidia in culture (CMD, isolation plate, 42 d); u–v. conidiophores, conidiogenous cells and conidia from pycnidia on natural substrate; w–a1. conidia from natural substrate; b1–c1. conidiophores and conidiogenous cells from pycnidia in culture (CMD, isolation plate, 42 d); d1–j1. conidia from pycnidia in culture (CMD, isolation plate; d1. 20 d; e1–j1. 40 d). All in water, except where noted (a–f, h–j: WU 40010 (epitype); g, o–p: WU 40012; k–m: WU 40014; q–a1: WU 40015; n, b1–c1, e1–j1: CRU3; d1: CRU2). — Scale bars: a–d = 100 μm; e–g, u–v, b1–c1 = 10 μm; h–m, o–t, w–a1, d1–j1 = 5 μm; n = 400 μm.
Ascomata perithecial, superficial to partly immersed in bark, (115–)145–190–(250) μm diam (n = 77), black, shiny, smooth, scattered singly to aggregated and occasionally confluent, pyriform, circular from above, commonly laterally collapsed, with a central apical papilla. Peridium continuous, c. 25–30 μm thick, a texture angularis of thin-walled, isodiametric or somewhat elongated dark brown cells 6–10 μm diam with dark brown walls, becoming paler towards the centre. Hamathecium composed of hyaline, thin-walled, septate, occasionally branched, 2–4 μm wide paraphyses embedded in an inamyloid gelatinous matrix; periphyses 2–3 μm wide, unbranched, thin-walled, smooth. Ascii (67–)79–98–(114) × (9.5–)10.2–12.3–(14.5) μm (n = 64), uniloculate, clavate to fusiform, curved, thin-walled, containing 8 ascospores arranged in two fascicles, with a short stipe, inamyloid and without a distinct apical apparatus; with fuscate dehiscence. Ascospores (31–)37–46–(55) × (3.2–)3.5–4.0–(4.8) μm, l/w = (7.4–)9.4–12.6–(14.0) (n = 116), falcate, 1- or 3-septate, hyaline, thin-walled, smooth, with narrowly to broadly rounded ends, multiguttulate when vital. 

Ascocarps scattered on bark, black, practically indistinguishable from ascocarps except for slightly smaller size. Conidiophores short, hyaline, smooth, branched up to two times, arising from the inner wall of the pycnidium. Conidiogenous cells (5.7–)7.3–10.0 (–13.5) × (1.4–)1.6–2.2–(3.1) μm (n = 75), holoblastic with sympodial proliferation, lageniform to cylindrical, hyaline, smooth, disposed in dense terminal whorls of up to 5. Conidia (15–)19–23–(25) × (1.5–)1.7–2.2–(2.7) μm, l/w = (7.4–)9.3–12.0–(14.0) (n = 90), falcate to lunate, asceptate, hyaline, thin-walled, smooth, with narrowly rounded ends, containing small guttules when vital. 

Culture characteristics and asexual morph in culture — on CMD at 16°C reaching 45–51 mm diam after 58 d; first cream, turning dark grey brown to black in the centre, with sparse aerial mycelium mostly in the centre, margin even, reverse medium to dark grey brown at least in the centre. Pycnidia (230–)250–370–(410) μm diam (n = 10), partly immersed to almost superficial, pale whitish translucent, aggregated to confluent, opening by irregular apical rupitides. Conidiophores and conidiogenous cells similar to those from the natural substrate but less regular and more variable in shape; often producing a single conidium; sympodial conidiation rarely seen. Conidia (13–)15–23–(29) × (2.1–)2.3–2.7–(3.1) μm, l/w = (4.5–)6.0–9.4–(12.6) (n = 50), similar to those from the natural substrate but more irregular and variable in shape, varying from allantoid, falcate to sigmoid, asceptate, rarely becoming 1-septate, hyaline, thin-walled, smooth, with mostly broadly rounded ends, sometimes containing numerous guttules especially towards the ends.

Habitat & Host range — Only known from cork wings and outgrowths of living or dead branches of Ulmus minor. 

Distribution — Europe; known from Austria, UK, Spain (Cannon 1997, Calatayud & Aguirre-Hudson 2001, this study).
Fig. 15 Leptosillia wienkampii. a–d. Perithecia on bark in surface (a–b) and side (c–d) view; note the sulcate structures on the apical papillae (c–d); e–f. asci (f. in Lugol); g. paraphyses; h–t. vital ascospores; u. strongly dextrinoid granular hamathecial exudates in Lugol after KOH pre-treatment; v. pycnidia and conidial drops in culture (CMD, 16 d); w–z, g1. conidiophores, conidiogenous cells and conidia from pycnidia on natural substrate; a1–f1. conidiophores, conidiogenous cells and conidia from pycnidia in culture (CMD, isolation plate, 40 d). All in water, except where noted (a, g, r: WU 40021; b: WU 40018; c, s–t, w–z, g1: WU 40020; d–e, h–p: WU 40017; f, q: WU 40016 (epitype); u: WU 40023; v, a1, e1: CRW3; b1–d1, f1: CRW1). — Scale bars: a–b, v = 200 µm; c–d = 100 µm; e–f, u = 10 µm; g–f, w–g1 = 5 µm.
Notes. Based on sequence data and morphology, *Libero-
myces saliciphilus* represents the asexual morph of *Leptosilla wienkampii*, and is therefore a synonym of the latter. Most of the description of the asexual morph in pure culture was based on our own observations, with a few additions from the description of *Libero/myces saliciphilus* by Pažoutová et al. (2012). In the present study, pycnidia on natural substrate could be found on only two specimens, and they produce holoblastically formed allantoid conidia matching those from pure culture. When de-
scribing *L. saliciphilus*, Pažoutová et al. (2012) recorded only the holoblastically formed conidia from pycnidia in pure culture; yet, in some of our pure cultures, both holoblastically and enteroblastically formed conidia were occasionally produced within the same pycnidium. Remarkably, Aguirre-Hudson (1991) recorded pycnidia on the natural substrate with enteroblastically produced, cylindrical to filiform conidia 20–25 μm × 1 μm in size, indicating that on the natural substrate the two different conidial types may be formed in different pycnidia.

**Key to accepted species of Leptosilla with sexual morphs**

| 1. Ascospores aseptate, occasionally 1-septate | 2. Ascospores consistently 1- to multisepate |
| 2. On bark of Acer spp.; only known from Europe; ascospores always aseptate | 3. On bark of other hosts; in Europe or North America; ascospores occasionally 1-septate |
| 3. Ascemata commonly confluent, in a pseudostroma, not collapsed; on bark scales of mature trunks of Acer pseudo-platanus; on the natural substrate conidia of two types (enteroblastic-filiform, holoblastic-falcate) formed within the same pycnidium | 4. Ascospores falcate to lunate, with broadly rounded ends; ascemata not horizontally collapsed, with an apical papilla laterally slightly enlarged by stellate or sulate structures; ascsti strongly sinuous; on various broadleaf trees (mostly *Salix* and *Ulmus* spp.) in Europe | L. wienkampii |
| 4. Ascospores straight to slightly curved, usually with distinctly hooked, narrowly rounded ends (similar to *Fusarium macro-
conidia*); ascemata commonly horizontally collapsed and cu-
pulate, with an apical papilla without stellite or sulate struc-
tures; asci straight, curved to slightly sinuous; on *Populus deltoides* and (probably) *Celtis occidentalis* in North Amer-
ica | L. fusariospora |
| 5. Ascospores multisepate; on bark of trunks of *Pinus* | L. pincola |
| 5. Ascospores 1–3-septate; on various broadleaf trees | 6. Ascospores 50–110 μm long; on trunks of *Quercus* spp. | L. macrospora |
| 6. Ascospores 30–55 μm long; on cork wings and outgrowths of branches of *Ulmus minor* | L. slaptotenosis |

**EXCLUDED CRESPORPHAPHIS SPECIES**

Based on morphology and ecology, the following two species are considered not to be congeneric with *Crespophaphis*, and they are therefore not transferred to *Leptosilla*.

*Crespophaphis rhoina* M.E. Barr, Mycotaxon 46: 64; 1993; Fig. 16

Replaced synonym. *Sphaeria rhoina* Ellis & Everh., J. Mycol. 1 (7): 92; 1885, non *Sphaeria rhoina* Schwein., Trans. Amer. Philos. Soc., New Series 4 (2): 218. 1832 '1834'.

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**Additional specimens examined.** AUSTRIA, Niederösterreich, Baden, Hele-
nental, on bark of living trunk of *Salix fragilis*, 23 Apr. 2016, H. Voglmayr & I. Greilhuber (WU 40017; culture CRW1); Hohenau, Marchaun near sugar refinery, on bark of living trunk of *Ulmus laevis*, 5 June 2016, H. Voglmayr & I. Greilhuber (WU 40018; culture CRM5); Neunkirchen, Mollram, on bark of living trunk of *Ulmus minor*, 1 Nov. 2018, H. Voglmayr & I. Greilhuber (WU 40019; culture CRW3); Puchberg am Schneeberg, Sonnleiten, Wasser-
fallweg, on bark of living trunk of *Salix sp.*, 24 Nov. 2018, H. Voglmayr & I. Greilhuber (WU 40023); Steiermark, Arching, riverine forest of the Enns adjacent to Pürschtachener Moor, on bark of living trunk of *Ulmus glabra*, 26 May 2016, H. Voglmayr & I. Greilhuber (WU 40020; culture CRU); ITALY, Sicily, Graniti, Casa delle Monache, on bark of living trunk of *Ulmus minor*, 16 June 2016, H. Voglmayr & W. Jaklitsch (WU 40021; culture CRW2); UK, England, Surrey, Kew, Royal Botanic Gardens, Lake (NW side of), on bark crevices of *Populus isosaericara*, 20 Aug. 2007, M.B. Aguirre-Hudson & T. Koko (KMU 154239); South Essex, VC18, Southend-on-Sea, Chalkwall Park, on pond, on bark furrows of *Salix sp.*, 1 July 2014, P.M. Earland-Bennett (K(M) 199631); ibid., Southchurch Park, by lake in park, on bark furrows of *Salix sp.*, 5 June 2014, P.M. Earland-
Bennett (K(M) 199632).
Fig. 16 Cresporhaphis rhoina. a–d. Horizontally collapsed, cupulate perithecia on wood in surface (a–c) and side (d) view; e. ascoma in vertical section; f. peridium in section; g–h, k. asci and paraphyses (k. in Lugol after KOH pre-treatment); i–j, l–m. ascus tips (l–m. in 3 % KOH followed by Lugol, showing the shallow amyloid apical ring); n. paraphyses tips; o–z. ascospores. All in 3 % KOH, except where noted (a–b, d–f, i–j: GZU 000335638 (isotype); c, g–h, k–w: GZU 000335637 (isotype); x–z: NY 00875175 (lectotype)). — Scale bars: a = 400 µm; b–c = 200 µm; d = 100 µm; e = 50 µm; f–h, k, o–z = 10 µm; i–j, l–n = 5 µm.
Typification. USA, New Jersey, Gloucester Co., Newfield, weather-beaten dead wood of Rhus copallinum, May 1885, without collector, in Ellis & Everhart, N. Amer. Fungi Ser. 2 no. 1669 (NY 00875175, lectotype of Sphaeria rhoina Ellis & Everh. (MTB 385924) here selected; GZU 00335637, GZU 00335638, isotypes).

Ascomata perithecial, superficial on dead wood, (140–)170–300(–460) μm diam (n = 101), 60–140 μm high (n = 20), black, matt, smooth, scattered to gregarious, lenticular, horizontally collapsed and distinctly cupulate when dry, circular from above, with or without a small central apical papilla. Peridium continuous, of a textura angularis, 23–38 μm thick, composed of an outer blackish brown, 12–28 μm thick layer of very thick-walled, more or less isodiametric cells with dark brown walls, and an inner brown, 5–20 μm thick layer of brown, elongate, thin-walled cells 5–19 × 2–5 μm. Hamathecium composed of hyaline, smooth, thin-walled, septate, mostly unbranched, 1.5–3 μm wide paraphyses embedded in an inamyloid gelatinous matrix; paraphyses not observed. Ascii (63–)74–87(–102) × (6.3–)7.7–9.0(–10.0) μm (n = 49), uniloculate, fusiform, straight to curved, thin-walled, containing 8 irregularly biseriately arranged ascospores, with a short stipe and a small, shallow, amyloid, c. 1.8 μm wide and 0.5 μm high apical ring. Ascospores (19–)23–30(–39) × (2.5–)2.8–3.2(–3.5) μm, I/w = (6.7–)7.5–10.1(–13.4) (n = 91), variously shaped from straight and fusiform, falcate, hook-shaped to sinuous, aseptate, hyaline, thin-walled, smooth, with narrowly rounded to subacute ends, containing few guttules. Asexual morph unknown.

Notes — Barr (1993) established Cresporhaphis rhoina as a new name for Sphaeria rhoina Ellis & Everh., a later homonym of Sphaeria rhoina Schwein. Based on similar ascomata, ascii and ascospores, Barr (1993) considered C. rhoina to be closely related to the generic type, C. wienkampii. The ascii were described as uniloculate with a shallow inamyloid apical ring. However, re-examination of the type collection showed the presence of a small but distinct amyloid apical ring in Lugol after KOH pre-treatment, which indicates xylariaceous affinities but excludes the species from Cresporhaphis. Also the growth on dead wood differs from all confirmed species of Cresporhaphis, which are all corticulous. It is therefore not congeneric with Cresporhaphis (and accordingly Leptosilla), but its morphological characters are insufficient to allow a well-founded generic reclassification within Xylariales.

The synonymy of C. rhoina and Coelosphaeria fusariospora proposed by Barr (1993) could not be confirmed after re-examination of type material of both species; the latter lacks a distinct amyloid apical ascus ring and has ascospores and a corticulous ecology in line with Leptosilla, into which it is thus combined (see also notes of L. fusariospora above).

Numerous copies of the type collection were distributed as Ellis & Everhart, N. Amer. Fungi Ser. 2 no. 1669, but to our knowledge no lectotype has been selected. The specimen from NY bears the original notes; the ascospore size range is somewhat smaller than in the copies from GZU and exactly matches the range (20–25 μm) given in the original description in Ellis & Everhart (1885). In all other characters, the specimens from NY and GZU fully agree. Therefore, the investigated isotype specimen from NY is here selected as lectotype.

**Rhaphidicyrtis trichosporella** (Nyl.) Vain., Acta Soc. Fauna Fl. Fenn. 49: 217. 1921

Synonym. Cresporhaphis chibaensis H. Harada, Lichenology 12: 32. 2014.

**Holotype of Cresporhaphis chibaensis. JARHE, Honshu, Chiba-ken, Inzaishi, Muzai, 15 m elev., on trunk of Alinus japonica, 4 Dec. 2007. H. Harada 25172 (CBM-FL-23891).**

For descriptions and illustrations of Cresporhaphis chibaensis, see Harada (2014).

Notes — Cresporhaphis chibaensis differs substantially in several respects from the generic type and the other confirmed species of Cresporhaphis: It is clearly lichenised with a distinct crustose lichen thallus, has perithelial ascomata with lateral, slightly sunken ostioles not situated on an apical papilla, a hamathecium composed of very thin, at least apically anastomosing threads and very long, filiform ascospores with numerous septa. It is therefore not considered to be related to the Cresporhaphis species here transferred to Leptosilla. Harada (2014) failed to note the hamathecial gel Lugol’s iodine reaction in the protologue of the species. Nevertheless, and considering the other morphological features, this species is a later synonym of Rhaphidicyrtis trichosporella, a species known from various substrates in Northern Europe, including Alinus sp. (Ekman et al. 2013).

**DISCUSSION**

**Molecular phylogeny**

The molecular phylogenetic analyses confirm a close relationship of all Cresporhaphis species for which DNA data are available with the type species of Leptosilla, and further, these are closely related to Delonicicola. Within this clade, two highly supported lineages are evident in the multigene analyses: the Delonicicola-Furfurella subclade and the Leptosilla subclade, which we recognise as two distinct families, Delonicicolaceae and Leptosillicaceae, based also on marked morphological differences between those genera. In the ITS-LSU rDNA analyses, the Leptosillicaceae are resolved only in the ML analyses (Fig. 2) but not in the MP analyses. This shows that, within Xylariales, the ITS-LSU alone does not always resolve generic and family affiliations well, which is also known from previous studies (e.g., Voglmayr & Yule 2006, Jaklitsch & Voglmayr 2012, Jaklitsch et al. 2016b). This conflict is, e.g., also seen in the Pseudomassariaceae, a morphologically and ecologically well-characterised family, which is also monophyletic in the ML analyses, albeit with low support (Fig. 2), but not resolved in the MP analyses. Insufficient phylogenetic resolution may be the result of rearrangements and length differences of the ITS, causing problems in producing a reliable alignment, in combination with insufficient phylogenetically informative and/or homoplastic characters. Therefore, multigene phylogenies are necessary for an improved phylogenetic resolution within Xylariales (Voglmayr et al. 2018, Wendt et al. 2018).

**Classification**

The taxa here classified in Leptosilla are a case example how the historical divide of the mycological and lichenological communities led to multiple separate, independent descriptions of the same species within different classification frames, and how this also influenced the hypotheses about their ecology. Being bark inhabitants, most of the species here classified as Leptosilla were first encountered and described by lichenologists, and based on ascospore and ascocarp characters, most of them were originally placed in the heterogeneous genus Leptorhaphis. In her detailed monograph, Aquirre-Hudson (1991) confined Leptorhaphis to bark saprotrophs with affinities to Arthopyreniaceae (Dothideomycetes), and she transferred putatively lichenised species with thin-walled, uniloculate asci, true paraphyses and perithelial ascomata to the new genus Cresporhaphis, which she tentatively classified within the Trichosporhaeliares (Sordariomycetes). This classification was mostly accepted up to date (e.g., Lücking et al. 2017), but challenged in Jaklitsch et al. (2016a) who considered this placement...
doubtful. However, there was consensus that its phylogenetic placement required further detailed studies. Until our present study, the then monotypic genus *Leptosillia* was classified within the *Diaporthales* (Kirk et al. 2008), with a presumed familial affiliation to the *Valisaceae* (Index Fungorum, accessed Feb. 2019). This classification was primarily based on the original description (Höhnel 1928), which hypothesised a close relationship to the diaporthalean genus *Sillia*, and was perpetuated in Eriksson & Hawksworth (1987). However, after its description the taxon was never recorded again, and the original material was never critically re-examined. Therefore, it is not surprising that no connection of the little-known *Leptosillia notha* was ever made to species classified in *Creporthaphis*, and later *Creporthaphis*.

As a result of our study, the comparison of the type specimens of *Creporthaphis muelleri* and *Leptosillia notha* confirmed them to represent the same species, requiring a name change to *L. muelleri*, based on priority. As the genus *Creporthaphis* has a different generic type species, *C. wienkampii*, the question arises whether the two genera should be kept separate or classified within the same genus, which in the latter case should be *Leptosillia* due to priority. The results of the phylogenetic analyses (Fig. 1, 2) revealed both options as tenable, as the *L. acerina*- *L. muelleri* and *L. macrospora*- *L. uliptenosis*- *L. wienkampii* lineages formed two distinct subclades within the *Leptosilliaeae*. However, after critical consideration we prefer a classification of all species under a single genus *Leptosillia*, as we did not find any morphological or ecological characters diagnostic for the two lineages. In addition, if *Creporthaphis* were maintained, also *L. pistaciae* would need another generic name, as would several other lineages now only known as endophyte isolates. It would also be impossible to genericise the place *L. fusariospora* and *L. pinicola*, which morphologically belong to *Leptosilliaeae* but for which no DNA sequence data are available. All these arguments favour a classification within a single genus.

When describing *Delonicicola* and *Delonricicolaceae*, Perera et al. (2017) also established a new order *Delonicicicolales*. However, in their phylogenetic analyses the placement of *Delonicicicolaceae* as sister group to *Xylariacea* did not receive statistical support. In our phylogenetic analyses of the ITS-LSU matrix the *Delonicicicolaceae-Leptosilliaeae* clade was embedded within *Xylariaceae* (Fig. 2), while in the multi-gene analyses a sister group relationship to the other *Xylariales* was highly supported (Fig. 3). However, the latter analyses contain only a small subset of *Xylariaceae*, as most xylariaceous lineages lack multigene sequence data. Considering these uncertainties, we do not accept a separate order *Delonricicolales* here.

**Morphology of the asexual morph**

Pycnidal asexual morphs were produced in culture in all *Leptosillia* species investigated so far. The asexual morph of the genus *Leptosillia* is remarkable by the common presence of two morphologically different types of conidia, which are also differently produced, i.e., enteroblastic phialidic and holoblastic with sympodial proliferation. In several species, these two types have been observed within the same conidiomata (e.g., *L. macrospora*, *L. muelleri*, *L. wienkampii*), but apparently both types are not always produced. For instance, Pažoutová et al. (2012) observed two types in *L. wienkampii*, but only a single type in *L. macrospora*, while in our investigations it was the other way round. Therefore, it cannot be excluded that both types are also formed in species for which so far only a single type has been observed (*L. acerina*, *L. uliptenosis*). Interestingly, pycnidia were commonly produced in the isolation plates, while in several species only few or no pycnidia were formed after subculturing. Apart from the species treated in our manuscript, holoblastically formed falcate conidia have been reported by Kolařík et al. (2012) for one of the endophyte isolates (*VegaE4-79 from Coffea arabica*).

In our fresh collections, pycnidia were rarely seen on the natural substrate; however, as they are very similar to ascomata except for their smaller sizes, they could have been overlooked. In these, two conidial types have only been observed in *L. muelleri*, while in the other species either the enteroblastic phialidic (*L. acerina*, *L. macrospora*) or the holoblastic type with sympodial proliferation (*L. uliptenosis*, *L. wienkampii*) was present. So far, no asexual morphs were observed for the species only known from herbarium specimens, *L. fusariospora* and *L. pinicola*.

**Ecology**

Based on the association with corticolous algae on bark, most of the species here classified as *Leptosillia* were commonly considered to be facultatively lichenised, which may be due to the fact that they were mainly studied by lichenologists. When establishing the genus *Creporthaphis*, a synonym of *Leptosillia*, Aguirre-Hudson (1991) described the thallus as crustose, smooth to pulvulent, greyish white and immersed in the bark but associated with an unidentified globose chlorococccoid photobiont. In the notes to the various species included, she described them as ‘probably lichenised’, and later, Calatayud & Aguirre-Hudson (2001) considered *Creporthaphis ulmi* as not lichenised. Detailed investigations of numerous fresh specimens collected during the present study as well as of herbarium specimens did not confirm the presence of a lichen thallus in the former *Creporthaphis* species here reclassified in *Leptosillia*. Although under certain environmental conditions the ascomata may be associated with chlorococccoid algae, this association is not constantly observed and entirely missing in some collections of all species examined. In addition, all species studied in fresh condition germinate and grow easily in pure culture. Therefore, the current investigations do not support that the former *Creporthaphis* species are lichenised, with the exception of the recently described *Creporthaphis chibaensis*, which, however, is not considered to be congeneric with the type of *Creporthaphis* but conspecific with the lichen *Rhaphidicyrtis trichosporellae*.

The publication of Pažoutová et al. (2012) shed a new light on the ecology of *Leptosillia*. They isolated and described two asexual morph species, *Libermes macrosporus* and *L. salicophilus*, as endophytes from phloem and sapwood of various, usually symptomless broadleaf trees. In our investigations, morphology and sequence data revealed the former to be synonymous with *Leptosillia macrospora* and *L. wienkampii*, respectively. This points to a primary ecology of *Leptosillia* as endophyte, which is also in line with the formation of ascomata on bark of living trees, and further supported by the numerous ITS GenBank accessions of endophytes from various hosts and geographic origins which are embedded within the *Leptosilliaeae* clade (Fig. 2). Therefore, this indicates that the *Leptosilliaeae* comprise widespread and important components of the endophyte communities of woody hosts, and they may harbour numerous undescribed species especially in understudied tropical and subtropical areas. It is interesting that *Leptosillia pistaciae*, a recently described canker pathogen of *Pistacia vera* (Vitale et al. 2018), is also embedded within the *Leptosilliaeae* clade, which indicates that pathogenicity may have secondarily evolved from an endophytic lifestyle. However, it also cannot be excluded that some of the strains isolated as endophytes may actually represent latent pathogens.
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