Asterodiscus and Stigmatodiscus, two new apothecial dothideomycete genera and the new order Stigmatodiscales

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Abstract During a survey on corticolous Dothideomycetes, several collections with ascospores matching the genera Asteromassaria and Stigmatomassaria (Pleomassariaceae, Pleosporales) were revealed from dead corticated twigs of Acer, Carpinus and Tamarix. Closer morphological examination showed that their ascomata were apothecial, with a hamathecium consisting of septate, branched paraphyses, which are apically swollen at maturity. Several collections were cultured and sequenced, and a Blast search of their nuc 28S rDNA sequences revealed dothideomycetous affiliation, but without a close match to a specific family or order. Phylogenetic analyses of a multigene matrix containing a representative selection of Dothideomycetes from four genes (nuc 18S rDNA, nuc 28S rDNA, rpb2 and tef1) revealed placement within Dothideomycetes but without a supported familial or ordinal affiliation. Based on the phylogenetic analyses and morphological investigations, the new genera Asterodiscus and Stigmatodiscus, with the two new species A. tamaricis and S. enigmaticus, are described and illustrated, and placed in the new family Stigmatodiscaceae and new order Stigmatodiscales.

Keywords Ascomycota · Dothideomycetes · New species · New genus · New family · New order · Phylogenetic analysis · Taxonomy

Introduction

During a survey on corticolous ascomycetes in Istria, Croatia in September 2010, we collected a dothideomycetous species from dead corticated twigs of Carpinus orientalis which showed ascospores with a striking resemblance to Stigmatomassaria pupula (Pleomassariaceae, Pleosporales; see Barr 1982, under Splanchnonema pupula). They were first considered to represent poorly developed, aberrant ascomata of Stigmatomassaria, but detailed morphological examination showed that the ascomata were clearly apothecial, with ascomata opening at maturity and having septate paraphyses with apical swellings at their free ends. Sequence data obtained from cultures of ascospores left no doubt that they do not belong to Stigmatomassaria or even Pleosporales. NCBI Blast searches confirmed dothideomycetous affinities, but without indication of a closer relative. Subsequently, we collected and cultured the fungus several times from Acer monspessulanum, A. campestre and A. sempervirens from various European countries.

In summer 2013, we found another dothideomycetous species on corticated dead twigs of Tamarix spp. with ascospores matching the genus Asteromassaria (Pleomassariaceae, Pleosporales; see Barr 1982), but again with apothecial ascomata having apically swollen paraphyses. Sequence data obtained from pure cultures revealed a close phylogenetic relationship to the Stigmatomassaria-like apothecial dothideomycete.

These fungi could not be identified upon consultation of the taxonomic literature, and no genera or families offered
themselves for an appropriate taxonomic placement. Therefore, they are described in the new genera Asterodiscus and Stigmatodiscus, which are placed in a new family and order within Dothideomycetes, according to the results of multigene phylogenetic analyses and detailed morphological investigations.

Materials and methods

Morphological observations

Hand sections of ascomata were made using a razor blade and mounted in water on a microscope slide, gently torn apart with a preparation needle when necessary and covered with a cover slip. Slides were examined and photographed using a Zeiss Axio Imager.A1 (Zeiss, Jena, Germany) microscope equipped with a Zeiss AxioCam ICc3 or Axiocam 506 colour digital camera. Measurements are reported as maxima and minima in parentheses and the mean plus and minus the standard deviation given in parentheses. For falcate conidia, the shortest distance between both ends (i.e. diameter) was measured. The specimens were deposited in the fungarium of the University of Vienna (WU).

Pure culture isolation

Mature ascomata on corticated twigs were horizontally cut using a sterile razor blade, the apothecia separated from the surrounding host tissue, transferred in a sterile drop of water on a microscope slide, torn apart with a forceps to release the ascospores from asci and pipetted on a 2 % malt extract agar (MEA) plate supplemented with 200 mg/L penicillin G and streptomycin sulphate (Sigma-Aldrich, St. Louis, MO). Germinated ascospores were then transferred to 2 % MEA plates, which were sealed with laboratory film and incubated at room temperature. Cultures were deposited at CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands (CBS).

DNA extraction, PCR and sequencing

Growth of liquid cultures and extraction of genomic DNA was done according to Voglmayr and Jaklitsch (2011), either using the modified CTAB extraction protocol of Riethmüller et al. (2002) or the DNeasy Plant Mini Kit (QiAgen GmbH, Hilden, Germany). The following loci were used for identification and phylogenetic analyses: The complete ITS region and D1 and D2 domains of 28S nuc rDNA region (ITS-28S) were amplified using the primers V9G (de Hoog and de and Gerrits van den Ende 1998) and LR5 (Vilgalys and Hester 1990). The 18S nuc rDNA region was amplified with primers SL1 (Landvik et al. 1997) and NS24mod (Voglmayr and Jaklitsch 2011). A ca 1.1 kb fragment of the RNA polymerase II subunit 2 (rpb2) gene was amplified using the primer pair fRPB2-5f and fRPB2-7cr (Liu et al. 1999). A ca 1.3 kb fragment of translation elongation factor 1-α (tef1) gene was amplified with the primers EF1728F (Carbone and Kohn 1999) and TEF1LLErev (Jaklitsch et al. 2005). The latter fragment includes the fourth and the fifth intron and a significant portion of the last large exon. A ca. 0.8 kb fragment of the β-tubulin (tub2) gene was amplified with primers T1HV (5′ CANMATGCCGAGATYGTAAYGT 3′) and BtHV2r (5′ CATCATRCGRTCNGGAACCT 3′), which were newly developed for Dothideomycetes. T1HV is a modified version of primer T1 (O’Donnell and Cigelnik 1997), whereas BtHV2r is upstream from T222 (O’Donnell and Cigelnik 1997); the primers were developed from genome sequences of representative Dothideomycetes downloaded from the Joint Genome Institute (JGI, http://jgi.doe.gov/). PCR products were purified using an enzymatic PCR cleanup (Werle et al. 1994) as described in Voglmayr and Jaklitsch (2008). DNA was cycle-sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems, Warrington) and the PCR primers; in addition, the following primers were used: ITS-28S region: ITS4 (White et al. 1990), LR3 (Vilgalys and Hester 1990); SSU: NS1088 (Kauff and Lutzoni 2002). Sequencing was performed on an automated DNA sequencer (ABI 3730xl Genetic Analyzer, Applied Biosystems).

Phylogenetic analyses

To reveal the phylogenetic position of the two new genera, a matrix of aligned nucleotide sequences from four different phylogenetic markers (18S, 28S, rpb2 and tef1), and 219 representatives of Dothideomycetes and Eurotiomycetes, including two species from Lecanoromycetes as outgroup, was produced. The matrix of Boehm et al. (2015) was downloaded from TreeBASE (www.treebase.org; submission No. S16,151) and served as the matrix basis after removal of a few sequences. Sequences of six additional taxa were downloaded from the GenBank nucleotide database. These, together with the newly generated sequences, were manually aligned to the matrix of Boehm et al. (2015), or realigned with the server version of MAFFT (www.ebi.ac.uk/Tools/mafft). The resulting alignments were subsequently checked and refined using BioEdit version v. 7.0.4.1 (Hall 1999). For alignment of rpb2, first the alignment of Boehm et al. (2015) was translated into a protein matrix which was then re-aligned with the new protein sequences using MAFFT, and the respective new DNA sequences were then manually aligned to the original rpb2 nucleotide matrix using the protein alignment as reference. Prior to phylogenetic analyses, the approach of Wiens (1998) was applied to test for significant levels of localized incongruence among the four genes, using the level of bootstrap support (Sung et al. 2007). For this, the 80 %
maximum likelihood (ML) bootstrap consensus trees for each individual partition were compared using the same parameters as for the combined analysis given below, but using 100 fast bootstrap replicates. No significant topological conflicts were observed between the bootstrap trees of 18S, 28S, rp2 and tef1, indicating the absence of significant incongruence and combinatoriality of the matrices (Wiens 1998). The resulting combined sequence matrix contained 5721 nucleotide positions from four genes (1653 from 18S, 1230 from 28S, 1875 from rp2, 963 from tef1). GenBank accession numbers of newly generated sequences are given in Table 1.

For detailed phylogenetic analyses within the new family, the ITS-28S, rp2 and tub2 sequences of all accessions sequenced were aligned and combined in a single three-loci matrix; for GenBank accession numbers see Table 1. This three-loci matrix contained 3657 nucleotide positions (1654 from ITS-28S, 1168 from rp2 and 835 from tub2).

For maximum likelihood (ML) analyses and Bayesian inference (BI) of the multi-gene analysis of *Dothideomycetes*, unique model parameters were applied for each marker and codon (where applicable), with the dataset divided in eight partitions according to Schoch et al. (2009a). The general time reversible model, assuming a proportion of invariant sites and gamma-distributed substitution rates (GTR + I + G), was selected as best model for all four genes (LSU, SSU, rp2 and tef1) by Modeltest 3.6 (Posada and Crandall 1998) under the Akaike Information Criterion (AIC) and was implemented in the subsequent ML and BI analyses. The matrix of *Stigmatodiscaceae* was divided in three partitions according to the three loci.

For ML analyses, fast tree searches were done with RAxML (Stamatakis 2006) as implemented in raxmlGUI 1.3 (Silvestro and Michalak 2012), using the GTR+CATI substitution model. 500 fast bootstrap replicates were computed using the GTRCATI substitution model.

Bayesian analyses were performed on the large matrix of *Dothideomycetes* with the computer program MrBayes (version 3.2.2; Huelsenbeck and Ronquist 2001). Three parallel runs of four incrementally heated, simultaneous Markov chains were performed over 10 million generations, of which every 1000th tree was sampled in each run, using the GTR + I + G model for all eight partitions. The first 4000 of the 10,000 trees sampled were discarded, and a 90% majority rule consensus of the remaining trees was computed to obtain posterior probabilities (PP).

Maximum parsimony (MP) analyses were performed with PAUP v. 4.0a142 (Swofford 2002) only for the small three-loci matrix of *Stigmatodiscaceae*, using 1000 replicates of heuristic search with random addition of sequences and subsequent TBR branch swapping (MULTREES option in effect,
steepest descent option not in effect). All molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data; the COLLAPSE command was set to NO. Bootstrap analysis with 1000 replicates was performed in the same way, but using 10 rounds of random sequence addition and subsequent TBR branch swapping during each bootstrap replicate.

**Results**

**Molecular phylogeny**

No *tef1* sequences could be obtained for *Stigmatodiscus*, despite various attempts using different primer sets, polymerases or PCR protocols; therefore, this gene could only be included.

Fig. 1 Simplified phylogram showing the best RAxML maximum likelihood tree (lnL = −137,324.200031) obtained from the combined multigene (LSU, SSU, *rpb2*, *tef1*) matrix of 219 taxa including major orders in Dothideomycetes, Arthoniomycetes and Eurotiomycetes, with two members of Lecanoromycetes (*Cladonia caroliniana*, *Flavoparmelia caperata*) selected as outgroup according to Boehm et al. (2015). Ordinal and familial classification follows Hyde et al. (2013) and Boehm et al. (2015). *Stigmatodiscus* and *Asterodiscus* form a highly supported clade representing a distinct order within Dothideomycetes. Except for Stigmatodiscales, all lineages were collapsed to ordinal or familial level. ML bootstrap support above 50 % and Bayesian posterior probabilities above 0.9 are given above or below the branches.
for Asterodiscus in the multi-gene analyses of Dothideomycetes. Of the 5721 nucleotide positions included in the Dothideomycetes matrix, 2595 were parsimony informative (488 from 18S, 507 from 28S, 1198 from rpb2, 402 from ref1). The best ML tree revealed by the RAxML analysis of the matrix including representatives of all major orders and families of Dothideomycetes, Arthoniales, Trypetheliales and Eurotiomycetes is shown as phylogram in Fig. 1, with the orders/families collapsed to enable a better overview. As observed in previous studies on Dothideomycetes (e.g. Schoch et al. 2009a, 2009b, Hyde et al. 2013; Wijayawardene et al. 2014; Boehm et al. 2015), many of the deeper nodes receive insignificant or low internal support, whereas many of the orders and families are highly supported. Asterodiscus and Stigmatodiscus form a distinct highly supported clade within Dothideomycetes and are contained in a clade with Monoblastiales, Dyfrolymcytale and Acrosporale, but only with low ML bootstrap support (65 %). Sister group relationship of the Asterodiscus-Stigmatodiscus clade to the Monoblastiales-Dyfrolymcytale clade receives low ML bootstrap support as well (52 %), which means that a statistically well-supported close relationship to other orders within Dothideomycetes remains obscure at this time.

Of the 5721 nucleotide positions included in the three-loci matrix of Stigmatodiscaceae, 476 were parsimony informative (148 from 28S-ITS, 208 from rpb2, 120 from tub2). The MP analysis revealed 12 MP trees of score 580; in the strict consensus tree, all nodes lacking MP bootstrap support collapsed to a polytomy (data not shown). Figure 2 shows the best ML tree of the detailed phylogenetic analysis of Stigmatodiscaceae revealed by RAxML, which is fully compatible with the MP strict consensus tree. Within Stigmatodiscus enigmaticus, some sequence variability was observed between the various accessions included in the analyses. The accessions from Crete on Acer sempervirens were set apart as sister group to the other accessions with moderate (ML) to high (MP) support. There is no further grouping according to hosts or geographic origins, and the lack of morphological differentiation indicates that only a single Stigmatodiscus species is involved. The sequences of all three Asterodiscus accessions from Austria, France and Italy were highly similar to identical.

**Taxonomy**

**Stigmatodiscaceae Voglmayr & Jaklitsch, ord. nov.**
MycoBank MB 815325.

Type family: *Stigmatodiscaceae* Voglmayr & Jaklitsch.

*Ascomata* apothecoid, embedded in cortex of dead twigs, without distinct margin nor excipulum. *Hamatectium* paraphysate; paraphyses septate, unbranched or rarely branched and anastomosing above, with free apical ends, covered by an epithecium. *Asci* bitunicate, fissitunicate, 1-, without a ring. *Ascospores* large, slightly to strongly asymmetric, hyaline to brown, multisepitate with one euseptum and several distosepta, wall surrounded by gel coating. *Conidiomata* in nature immersed, peridermal, pycnidial.

**Stigmatodiscaceae Voglmayr & Jaklitsch, fam. nov.**
MycoBank MB 815326.

Type genus: *Stigmatodiscus* Voglmayr & Jaklitsch.

Ascomata apothecoid, embedded in cortex of dead twigs, dark brown to black, without distinct margin. *Hamatectium* paraphysate; paraphyses septate, unbranched or rarely branched and anastomosis above, with swollen free apical ends, embedded in a rubber-like gel matrix and covered by an epithecium. *Hymenial gel* I-. *Asci* sequentially produced over a long time, bitunicate, fissitunicate, 1-, broadly fusoid to saccone, with thin ectl- and thick endotunica, apically with wide ocular chamber, without a ring. *Ascospores* slightly to strongly asymmetric, distal part larger than proximal part, hyaline to brown, 1-euseptate in early stages, developing 2 additional distosepta, wall surrounded by gel coating. *Conidiomata* in nature immersed, peridermal, pycnidial.

**Asterodiscus** Voglmayr, Gardiennet & Jaklitsch, gen. nov.
MycoBank MB 815329, Facesoffungi number: FoF 01656.

**Etymology:** Referring to the striking similarity of its ascospores to those of Asteromassaria.
Type species: *Asterodiscus tamaricis* Voglmayr, Gardiennet & Jaklitsch.

*Ascomata* embedded in cortex of dead twigs, initially covered by bark, lifting the bark as a black bump, globose-depressed to broadly pyriform, apically covered by brown tissue of *textura intricata*, at maturity apothecioid, breaking open in the centre with irregular radial cracks; finally a depressed disc becoming fully exposed with age, sometimes confluent with neighbouring discs, dark brown to black, without distinct margin. *Hamathecium* of hyaline, septate paraphyses, often branched and Anastomosing above, with distinctly swollen free apical ends, embedded in a rubber-like gel matrix and covered by an amorphous matrix forming an epithecium. *Hymenial gel* I-. *Asci* forming sequentially and slowly, bitunicate, fissitunicate, 1-, variable in shape from broadly fusoid to saccate, with thin ecto- and thick endotunic; apex with a wide ocular chamber, without ring. *Ascospor es* large, 30–50 μm long, asymmetric, distal part slightly larger than the proximal part, ends rounded to subacute, hyaline to light brown with age, first 1-septate, developing 2 additional distosepta and becoming 3-septate, distinctly constricted at the septa, secondary septa with large pores; wall thick, smooth after one month at room temperature, first whitish, turning dark brown after ejection, ends mostly rounded, distal ends sometimes subacute, surrounded by a thick gelatinous sheath, wall smooth to finely verrucose, the contents granular, with a large, strongly refractive guttule per cell.

*Conidiomata* on the natural substrate not observed, on 2 % MEA acer cular, superficial, aggregated, ca 200–450 μm wide, conidiophores densely aggregated, branched. *Conidiogenous cells* phialidic, (7.2–)10.2–15.3–(20.4) × (1.8–)2.1–3.2–(3.8) μm (*n* = 30), lageniform. *Conidia* irregularly sinuously curved to falcate, (9.4–)10.6–13.6–(17.4) × (1.0–)1.2–1.6–(2.1) μm (*n* = 70), hyaline, smooth. *Cultures* very slow-growing, with uneven margins, colony on 2 % MEA reaching 32 mm diam after one month at room temperature, first whitish, turning dark brown, with whitish aerial hyphae in the centre, reverse first hazelnut brown, then black; on PDA colony light brown, cottony, with the centre covered by whitish mycelium, reverse hazelnut brown with dark brown centre.

*Holotype*: AUSTRIA, Wien, Landstrasse, Botanical Garden of the University (HBV), on *Tamarix tetrandra*, 1 Aug 2013, H. Voglmayr (WU 35906, ex-type culture CBS 136919 = L114).

*Additional specimens examined*: AUSTRIA, Wien, Landstraße, Botanical Garden of the University (HBV), on *Tamarix tetrandra*, 7 May 2015, H. Voglmayr (WU 35924). CROATIA, Istra, Rovinj, Kamp Amarun, on *Tamarix sp.*, 15 May 2015, H. Voglmayr, M. & I. Greilhuber (WU 35921, culture L161). FRANCE, Bourgogne, Côte-d’Or (21), Issur-Tillé, route de Dijon, on *Tamarix gallica*, 25 Apr 2015, A. Gardiennet AG15034 (WU 35907). Lux, on *Tamarix gallica*, 21 Jul 2013, R. Rousseaux & A. Gardiennet AG13137 (WU 35908, culture L113 = CBS 136918). Véronnes, on *Tamarix gallica*, 16 Apr 2015, A. Gardiennet AG15029 (WU 35909). ITALY, Lazio, Viterbo, Vetralla, on *Tamarix latifolia* (28 Apr 2015).

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**Fig. 3** *Asterodiscus tamaricis*, sexual morph. **a, b** Ascomata erumpent from bark in face view. **c** Ascomata in transverse section, showing the hymenium with asci. **d-h** Vertical sections of ascomata embedded in bark; **d, e** closed young ascomata showing the hamathecial threads connected with apical dark brown tissue of *textura intricata*; **f-h** apothecioid ascomata with paraphyses and asci embedded in a gel matrix stained bluish (**f, g**) to olivaceous (**h**) below the epithecium. **i, j** Olivaceous to dark brown epithecium of young ascomata forming a *textura intricata* and young paraphyses below; **i** detail from **d**. All in water. Sources: **a, c, h** WU 35908; **b** WU 35907; **d-g, i, j** WU 35924. Scale bars: **a** = 1 mm, **b** = 250 μm, **c** = 100 μm, **d-h** = 50 μm, **i, j** = 10 μm.
Ascomata apothecoid, embedded in cortex of dead twigs, initially covered by bark, later becoming exposed through irregular cracks, eventually becoming fully exposed with age, dark brown to black, without distinct margin. Hamathecium of hyaline, septate paraphyses, unbranched or rarely branched and anastomosing above, with distinctly swollen free apical ends, embedded in a rubber-like gel matrix and covered by an amorphous matrix forming an epithecium. Hymenial gel 1-Ascii sequentially produced over a long time, bitunicate, fissitunicate, 1-, variable in shape from broadly fusoid to saccate, with thin ecto- and thick endotunica, apically with wide ocular chamber, without ring. Ascospores large, 45–80 µm long, asymmetric, distal part distinctly larger than the proximal part, proximal end rounded, distal end rounded to subacute, brown, 1-euseptate in early stages, developing 2 additional distosepta and becoming 3-septate, distinctly con- to subacute, brown, 1-euseptate in early stages, developing 2 additional distosepta and becoming 3-septate, distinctly constricted at the septa, surrounded by a thick gelatinous sheath, wall distinctly verruculose, dark brown, the contents granular, often with a large and several smaller guttules per cell.

Conidiomata on the natural substrate associated with ascomata, immersed, peridermal, pycnidal, depressed, unilocular, of circular to irregular shape, opening in irregular bark cracks, 200–500 µm diam, wall thin, inconspicuous, ca. 10–15 µm, composed of hyaline cells. Ostiole dark brown. Conidiogenous cells phialidic, cylindrical, (11.2–)13.0–17.3(–21.7) × (1.5–)2.0–3.0–(3.7) µm (n = 40). Conidia falcate, (9.3–)13.0–17.0–(18.7) × (1.0–)1.3–1.7–(2.0) µm (n = 99), not germinating on 2 % MEA. On 2 % MEA forming superficial, aggregated, 250–400 µm wide acervuli, conidiogenous cells and conidia similar to those on the natural substrate. Conidiophores branched. Conidiogenous cells (13.5–)14.3–25.2(–30) × (1.1–)1.5–2.5 µm (n = 10). Conidia (9.0–)12.7–19.7–(24.2) × (1.0–)1.1–1.5–(1.8) µm (n = 40). Cultures on 2 % MEA very slow-growing, reaching 14 mm diam after one month at room temperature, dark grey to black, at age with whitish aerial hyphae in the centre, reverse first brown, then black. Holotype: AUSTRIA, Wien, Landstraße, Botanical Garden of the University (HBV), on Acer monspessulanum, 14 Oct. 2010, H. Voglmayr (WU 35914, ex-type culture CBS 132036 = L69).

Additional specimens examined: AUSTRIA, Niederösterreich, Gumpoldskirchen, near Richardshof, on Acer campestre, 24 May 2015, H. Voglmayr & I. Greilhuber (WU 35931). Wien, Donaustadt, Lobau, Panozzalacke, on Acer campestre, 4 Feb. 2012, H. Voglmayr (WU 35913, culture L84). Landstraße, Botanical Garden of the University (HBV), on Acer monspessulanum, 7 May 2015, H. Voglmayr (WU 35925). CROATIA, Istria, Krmica, on Carpinus orientalis, 25 Sep. 2010, H. Voglmayr & W. Jaklitsch (WU 35915, culture L68). St. Golaš, on Carpinus orientalis, 31 Oct 2010, H. Voglmayr (WU 35916, culture L71 = CBS 131997). CZECH REPUBLIC, Morava, Lednice, Arboretum, on Acer monspessulanum, 9 Oct 2010, H. Voglmayr & W. Jaklitsch (WU 35917, culture L64). FRANCE, Provence-Alpes-Côte d’Azur, Dept. Alpes-de-Haute-Provence (04), Priéuré de Ganagobie, on Acer monspessulanum, 30 Jul 2011, H. Voglmayr (WU 35918,
Phylogenetic analyses place Stigmatidiscus within Dothideomycetes (Fig. 1), but their closest relatives remain obscure and unresolved. A weakly support relationship to Monoblastiales, Dyrofomycetales and Acerospermales is only revealed in ML analyses, and these orders are morphologically and ecologically highly distinct (Hyde et al. 2013; Pang et al. 2013).

Morphologically, the two new genera share a unique combination of characters within Dothideomycetes: they have apothecial ascomata embedded in host tissue lacking an excipulum, saccate fissitunicate asci, large, 3-septate ascospores with an excipular euscepta and two additional distoesta, being surrounded by a large gelatinous sheath. Ascospores of Stigmatidiscus resemble those of Stigmatomassaria, but are colorless, apothecial ascomata embedded in host tissue lacking a perithecial excipulum and two additional distoesta, being surrounded by a large gelatinous sheath. A few species of Lichenothelia have brown asymmetric ascospores with one euscepta and two distosepta surrounded by a large gelatinous sheath (Hawksworth 1981). However, the ascomata of Lichenothelia are quite unlike those of Stigmatidiscus: they are superficial and perithecioid when young, becoming apothecial (leciodeine) at maturity with an excipulum composed of pseudoparenchymatous tissue (Hawksworth 1981; Øvstedal and Lewis Smith 2001). In addition, in Lichenothelia species with 3-septate ascospores these are much smaller (less than 30 μm) and commonly become submurmiform. Ascospores similar to those of Asterodiscus can also be found in other groups, in particular in the Arthropyriniaceae of the Pleosporales, but ascocarps of members of this family do not open to become apothecial (Hyde et al. 2013). Within Trypeteliaceae, Architrypephelium and Ornatoyprenis have similar ascospores of the same size as observed in Stigmatidiscus, but they are lichenized and pyrenocarpous (Aptroot 1991). In Xylolpezia (Sherwood-Pike and Boise 1986), where ascocarps are first immersed and enclosed, asci are functionally unistome and the ascospores are eucoytic. The only order of the Dothideomycetes comprising exclusively discomycetous fungi is the Patellariaceae. However, all representatives of this order form black, drought-tolerant, more or less superficial apothecia, and their ascospores are eucoytic, except in Lirellodiscus (Kutorga and Hawksworth 1997; Yacharoen et al. 2015). All lineages discussed above for which sequence data are available are phylogenetically distant from Stigmatidiscus (Fig. 1).

Although ascospores of the Stigmatidiscus are highly similar to those of the genera Asteromassaria and Stigmatomassaria, the fungi of the present study are phylogenetically remote from these. Asteromassaria and Stigmatomassaria are both contained within Pleomassariaceae, Pleosporales (unpubl. data), whereas the fungi of the present study form a highly supported clade within Dothideomycetes, which is of uncertain phylogenetic affinity (Fig. 1). In addition, ascomata of Asteromassaria and Stigmatomassaria are perithecioid pseudothecia typical of
Pleomassariaceae (Barr 1982). Acknowledging their profound phylogenetic and morphological distinctness, classification within two new genera in a new family and order is fully justified.

The genera Asterodiscus and Stigmatodiscus are similar in their ascomata traits. The main differences concern the ascospore shape and colouration, analogous to the closely related genera Asteromassaria and Stigmatomassaria. Asterodiscus has hyaline, only slightly asymmetric ascospores which become brownish only after ejection, whereas Stigmatodiscus has dark brown, asymmetric ascospores with the distal part distinctly larger than the proximal part.

Ecologically, species of Asterodiscus and Stigmatodiscus are characterised by growth on recently dead corticated twigs still attached to the trees. While Asterodiscus appears to be confined to Tamarix spp., Stigmatodiscus has been primarily observed on various Acer species (A. campestre, A. monspessulanum, A. sempervirens) but also on the unrelated Carpinus orientalis. Asterodiscus tamaricis and Stigmatodiscus enigmaticus are widely distributed in Central and Southern Europe and have been commonly observed.

Fig. 6 Stigmatodiscus enigmaticus, asexual morph. a Conidioma (pycnidium) immersed in periderm. b Detail of pycnidium with densely aggregated conidiogenous cells and conidia. c–e Phialides producing conidia. f Falcate conidia. g, h Acervular conidiomata on MEA showing conidial drops. All in water. Sources: c, d, f–g, WU 35922; a, b, e. WU 35925. Scale bars: a = 100 μm. b = 20 μm. c–f = 10 μm. g, h = 400 μm.
during the current study on various *Tamarix* spp. and *Acer monspessulananum*, respectively. Occurrence on exposed twigs primarily in submediterranean climate indicates a high level of drought tolerance. It is remarkable that they have remained undetected up to date on these widely distributed hosts, demonstrating once again the need of detailed biodiversity studies on corticolous ascomycetes especially in Central and Southern Europe (e.g. Voglmayr and Jaklitsch 2008, 2011; Jaklitsch and Voglmayr 2014; Voglmayr et al. 2014; Jaklitsch et al. 2012; Jaklitsch et al. 2013, 2014, 2015; Galán et al. 2015; Checa et al. 2015).

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