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ORIGINAL ARTICLE

Three new species of *Stigmatodiscus* from Mallorca (Spain)

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Abstract
During a survey on corticolous Dothideomycetes in Mallorca, several collections with ascomata, asci, and ascospores matching the genus *Stigmatodiscus* (Stigmatodiscales, Dothideomycetes) were revealed, which did not fit any described species. Therefore, these collections were cultured and sequenced, and a multigene matrix of four loci (nuc18S-ITS-28S rDNA, *rpb2*, *tef1* and *tub2*) was produced. Based on the results of the phylogenetic analyses of this matrix and of morphological investigations, three new species (*Stigmatodiscus labiatus*, *S. oculatus*, and *S. pinicola*) are described and illustrated, *Asterodiscus* is synonymised with *Stigmatodiscus* and the new combination *S. tamaricis* is proposed. A key to all currently known species of *Stigmatodiscus* is provided.

Keywords Ascomycota · Dothideomycetes · Multigene phylogenetic analysis · Stigmatodiscaceae · Taxonomy · 3 new species · 1 new combination

Introduction

During a study of corticolous ascomycetes of Mallorca, the second author made several collections of dothideomycetes with hysteriform to apothecial ascomata embedded in host tissue and lacking an excipulum, which showed a character combination of branched, septate, apically swollen paraphyses with dark brown incrustation, saccate, bitunicate asci, and large, brown, asymmetric, one- or three-septate ascospores with an excentric euseptum and eventually two additional distosepta with large pores, each hemispore part being surrounded by a gelatinous sheath. These characters resembled the recently described genus *Stigmatodiscus* (Voglmayr et al. 2016, 2017), but the Mallorcan collections did not match any described species. Therefore, they were isolated it in pure culture; DNA sequence data of nuc18S-ITS-28S rDNA, *rpb2*, *tef1*, and *tub2* were generated for phylogenetic analyses; and detailed morphological examinations were conducted. As a result, three new species of *Stigmatodiscus* were revealed, which are here described and illustrated.

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Materials and methods

Morphological observations

Stereomicroscopy illustrations were captured either with a Keyence VHX-6000 system or with a Nikon SMZ 1500 stereomicroscope equipped with a Nikon DS-U2 digital camera. For certain images of ascomata, the stacking software Zerene Stacker version 1.04 (Zerene Systems LLC, Richland, WA, USA) was used. Hand sections of ascomata and conidiomata were made using a razor blade and mounted in water or 3% KOH 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 506 colour digital camera. Measurements were done with the Keyence VHX-6000, NIS-Elements D v.3.0 or Zeiss ZEN Blue Edition software packages and are reported as maxima and minima in parentheses and the range representing the mean plus and minus the standard deviation of a number of measurements given in parentheses. 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 to a sterile drop of water on a microscope slide, torn apart with a forceps to release the ascospores from asci, which were 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 or 2% corn meal agar (CMA, Sigma-Aldrich) supplemented with 2% *w/v* dextrose (CMD) plates, which were sealed with laboratory film and incubated at 16 or 22 °C. Cultures were deposited at the Westerdijk Fungal Biodiversity Centre, Utrecht, The Netherlands (CBS culture collection).

DNA extraction, PCR and sequencing

Growth of liquid cultures and extraction of genomic DNA was done according to Voglmayr and Jaklitsch (2011), using the DNeasy Plant Mini Kit (QIAgen GmbH, Hilden, Germany). The following sequence regions were used for identification and phylogenetic analyses: the complete nucITS region and D1 and D2 domains of nuc28S rDNA region (ITS-LSU) were amplified using the primers V9G (de Hoog and Gerrits van den Ende 1998) and LR5 (Vilgalys and Hester 1990). The nuc18S rDNA region (SSU) was amplified with primers SL1 (Landvik et al. 1997) and NS24mod (Voglmayr and Jaklitsch 2011). A ca 1.2 kb fragment of the RNA polymerase II subunit 2 (*rpb2*) gene was amplified using the primers fRPB2-5f and fRPB2-7cr (Liu et al. 1999). A ca 1.3–1.7 kb fragment of translation elongation factor 1-α (*tef1*) gene was amplified with the primers EF1-728F (Carbone and Kohn 1999) and TEF1-LLErev (Jaklitsch et al. 2005) or EF1-2218R (Rehner and Buckley 2005), and a ca 0.8 kb fragment of the beta tubulin (*tub2*) gene with primers T1 (O'Donnell and Cigelnik 1997) or T1HV and BtHV2r (Voglmayr et al. 2016). 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-LSU region: ITS4 (White et al. 1990), LR2R-A (Voglmayr et al. 2012) and 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 new isolates produced in the present study, a matrix of aligned nucleotide sequences from the four different phylogenetic markers (SSU-ITS-LSU, *rpb2*, *tef1* and *tub2*) was produced. GenBank sequences of four taxa (*Anisomeridium ubianum* and *Megalotremis verrucosa* from Monoblastiales, *Dyfrolomyces rhizophorae* from Dyfrolomycetales and *Palawania thailandense* from Palawaniaceae) were selected as outgroup according to Voglmayr et al. (2017) and the results of BLAST searches. Sequences were aligned with the server version of MAFFT (www.ebi.ac.uk/Tools/mafft) and subsequently checked and refined using BioEdit version v. 7.0.9.0 (Hall 1999). For alignment of *rpb2*, the alignment was translated into a protein matrix and the gap positions corrected according to the codons. The combined sequence matrix contained 6723 nucleotide positions (1770 from SSU, 1514 from ITS-LSU, 1167 from *rpb2*, 1417 from *tef1*, 855 from *tub2*). GenBank accession numbers of the sequences included in the phylogenetic analyses are given in Table 1.

Maximum likelihood (ML) analyses were performed with RAxML (Stamatakis 2006) as implemented in raxmlGUI 1.3 (Silvestro and Michalak 2012), using the ML + rapid bootstrap setting and the GTRGAMMA substitution model with 1000 bootstrap replicates. The matrix was partitioned for the individual gene regions, and substitution model parameters were calculated separately for them.

Maximum parsimony (MP) analyses were performed with PAUP v. 4.0a161 (Swofford 2002), 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 5 rounds of random sequence addition and subsequent TBR branch swapping during each bootstrap replicate.

Bootstrap support below 70% was considered low, between 70 and 90% medium/moderate and above 90% high.

Results

Molecular phylogeny

For *S. pinicola*, no *tef1* could be obtained. Of the 6723 nucleotide positions, 1016 were parsimony informative (64 from SSU, 310 from ITS-LSU, 258 from *rpb2*, 231 from *tef1* and 153 from *tub2*). The parsimony analyses revealed 27 MP trees 2398 steps long, one of which is shown as phylogram in Fig. 1. Tree backbone of the 27 MP trees was identical, except for minor differences within *S. enigmaticus*. The best tree revealed by RAxML (-ln = 20,909.518) was fully compatible with the MP strict consensus tree.

Table 1 Isolates and GenBank accession numbers of sequences used in the phylogenetic analyses; those in bold were isolated/sequenced in the present study

Taxon	Origin	Host	Voucher	Type[1]	Isolate	GenBank accession numbers[2]				
						SSU	ITS-LSU	*rpb2*	*tef1*	*tub2*
Anisomeridium ubianum	Fiji		Lumbsch 19845j		MPN94	GU327682	GU327709[3]	–	JN887421	–
Dyfrolomyces rhizophorae	Hawaii, Oahu				JK 5456A	GU479766	GU479799[3]	–	GU479860	–
Megalotremis verrucosa	Colombia		Luecking 26,316		MPN104	JN887383	GU327718[3]	–	JN887426	–
Palawania thailandense	Thailand	*Dypsis lutescens*	MFLU 16–1872	H	MFLUCC 14–1121	KY086495	KY086493[3]	KY086496	–	–
Stigmatodiscus enigmaticus	Austria, Wien	*Acer campestre*	WU 35913		L84	–	KU234114	KU234127	**MH756082**	KU234146
S. enigmaticus	Austria, Wien	*Acer monspessulanum*	WU 35914	H	L69 = CBS 132036	KU234130	KU234108	KU234121	**MH756078**	KU234140
S. enigmaticus	Croatia, Istria	*Carpinus orientalis*	WU 35915		L68	–	KU234107	KU234120	**MH756077**	KU234139
S. enigmaticus	Croatia, Istria	*Carpinus orientalis*	WU 35916		L71 = CBS 131997	–	KU234109	KU234122	–	KU234141
S. enigmaticus	Czech Republic, Morava	*Acer monspessulanum*	WU 35917		L64	KU234129	KU234106	KU234119	–	KU234138
S. enigmaticus	France, Alpes-de-Haute-Provence	*Acer monspessulanum*	WU 35918		L76 = CBS 132037	–	KU234111	KU234124	–	KU234143
S. enigmaticus	France, Var	*Acer monspessulanum*	WU 35919		L75	–	KU234110	KU234123	**MH756079**	KU234142
S. enigmaticus	Greece, Crete	*Acer sempervirens*	WU 35911		L82	–	KU234112	KU234125	**MH756080**	KU234144
S. enigmaticus	Greece, Crete	*Acer sempervirens*	WU 35912		L83	KU234131	KU234113	KU234126	**MH756081**	KU234145
S. enigmaticus	Italy, Lazio	*Acer campestre*	WU 35920		L122	–	KU234104	KU234118	–	KU234137
S. labiatus	**Spain, Mallorca**	***Quercus* sp.**	**WU 39973**	**H**	**AP6516 = CBS 144700**	**MH756065**	**MH756065**	**MH756074**	**MH756083**	**MH756089**
S. labiatus	**Spain, Mallorca**	***Quercus coccifera***	**WU 39980**		**AP141216**	–	**MH756066**	–	–	–
S. oculatus	**Spain, Mallorca**	***Populus canadensis***	**WU 39975**		**AP10816**	**MH756067**	**MH756067**	**MH756075**	**MH756084**	–
S. oculatus	**Spain, Mallorca**	***Cistus albidus***	**WU 39976**		**AP231016B**	–	**MH756068**	–	**MH756085**	–
S. oculatus	**Spain, Mallorca**	***Olea europaea***	**WU 39974**	**H**	**AP161116 = CBS 144701**	–	**MH756069**	–	**MH756086**	**MH756090**
S. oculatus	**Spain, Mallorca**	***Pistacia lentiscus***	**WU 39977**		**AP171116**	–	**MH756070**	–	**MH756087**	**MH756091**
S. oculatus	**Spain, Mallorca**	***Globularia alypum***	**WU 39978**		**AP311216**	–	**MH756071**	–	**MH756088**	**MH756092**
S. oculatus	**Spain, Mallorca**	***Globularia alypum***	**WU 39978**		**AP311216A**	–	**MH756072**	–	–	–
S. pinicola	**Spain, Mallorca**	***Pinus halepensis***	**WU 39979**	**H**	**AP21916B = CBS 144702**	**MH756073**	**MH756073**	**MH756076**	–	**MH756093**
S. pruni	Austria, Niederösterreich	*Prunus spinosa*	WU 35945	H	L167 = CBS 142598	KX611110	KX611110	KX611109	KX611111	**MH756094**
S. tamaricis	Austria, Wien	*Tamarix tetrandra*	WU 35906	H	L114 = CBS 136919	KU234128	KU234101	KU234116	KU234133	KU234135
S. tamaricis	France, Bourgogne	*Tamarix gallica*	WU 35908		L113 = CBS 136918	–	KU234100	KU234115	KU234132	KU234134
S. tamaricis	Italy, Lazio	*Tamarix* sp.	WU 35910		L124	–	KU234102	KU234117	–	KU234136

[1] H holotype

[2] Sources of GenBank sequences: Nelsen et al. (2009, 2011), Suetrong et al. (2009), Mapook et al. (2016), Voglmayr et al. (2016, 2017)

[3] Only LSU available

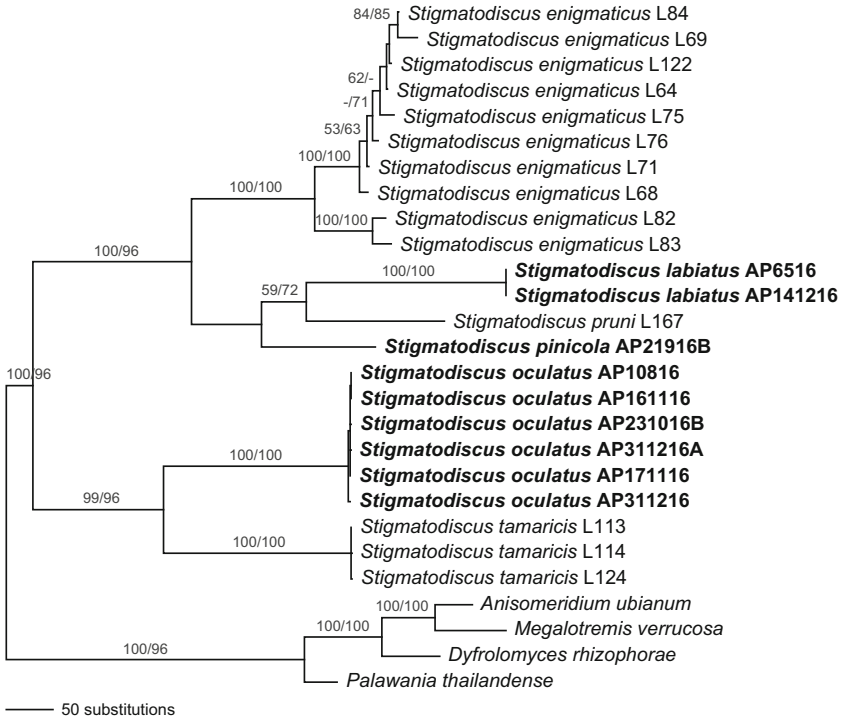

Fig. 1 Phylogram showing one of 27 MP trees 2398 steps long obtained from an MP analysis of the combined multigene matrix of nucSSU-ITS-LSU rDNA, *rpb2*, *tef1* and *tub2* from *Stigmatodiscus*. MP and ML bootstrap values above 50% are given at first and second position, respectively, above the branches. The isolates of the new species described in the present study are formatted in bold

In the MP and ML analyses, the Stigmatodiscales were highly supported, and all *Stigmatodiscus* species for which more than one accession was sequenced received maximum support. Within *Stigmatodiscus*, the newly described *Stigmatodiscus oculatus* formed a highly supported clade with *Asterodiscus tamaricis*, and this clade was revealed as sister group to the other *Stigmatodiscus* species with high support. The newly described *S. labiatus* clustered with *S. pruni* with low (59% MP) to medium (72% ML) support, and the newly described *S. pinicola* was placed as sister species to the *S. pruni*-*S. labiatus* clade without support. The clade containing *S. enigmaticus*, *S. labiatus*, *S. pinicola* and *S. pruni* received maximum (MP) or high (96% ML) support, but within this clade, the sister group relationship of *S. enigmaticus* to the *S. pinicola*-*S. pruni*-*S. labiatus* subclade was unsupported.

Culture characteristics

Culture images of the three new *Stigmatodiscus* species grown on CMD are shown in Fig. 2. Detailed culture descriptions are given under the respective species.

Taxonomy

Stigmatodiscus labiatus Voglmayr & Pintos, sp. nov. Figs. 3, 4.
MycoBank MB 827487.

Etymology: Referring to the lip-shaped ascomata.

Ascomata hysteriform, scattered, rarely gregarious or confluent, corticolous, erumpent through the periderm, in face view (275–)380–1000(–1610) μm long, (145–)200–350(–570) μm wide ($n = 51$), with sides consisting of usually two, rarely three black lips (peridium), (45–)90–170(–200) μm wide ($n = 50$), with a narrow central slit, usually closed when dry and not exposing the blackish elongated disc. Bark tissues not visibly altered, no black line visible in bark or wood. Peridium coriaceous, pseudoparenchymatous, black, up to 130 μm thick at the apex, 17–31 μm thick at the sides, almost absent to 10 μm thick at the base, composed of small angular to rounded, dark brown, thick-walled cells 4–8 μm diam. Hamathecium composed of hyaline, septate, filiform branched paraphyses 1.7–3 μm wide, embedded in a tough hymenial gel, simple, not anastomosing, 82–130 μm long, longer than asci, swollen at their apices up to 4 μm and incrusted with dark brown granules forming an epithecium, neither staining blue in Lugol nor in Melzer's reagent after pre-treatment with 3% KOH. Asci (67–)78–103(–108) × (41–)43–59(–71) μm ($n = 18$), bitunicate, broadly ellipsoid to globose, almost sessile, with a distinct apical chamber, thick-walled, typically containing 8 irregularly bi- to triseriate ascospores, very stable, fissitunicate dehiscence not observed. Ascospores (34.5–)38–43(–47.5) × (13.8–)15.5–17.5(–19.3) μm, l/w = (2.2–)2.3–2.6(–2.8) ($n = 84$), brown, asymmetric, broadly fusiform, straight, 1-septate, strongly constricted at the septum, each hemispore surrounded by a separate gelatinous sheath quickly dissolving in water, upper cell slightly broader, with broadly rounded ends and distinctly constricted in the middle with a ring-like thickening inside; wall finely verruculose, brown, the contents granular, usually with a large and several smaller guttules per cell.

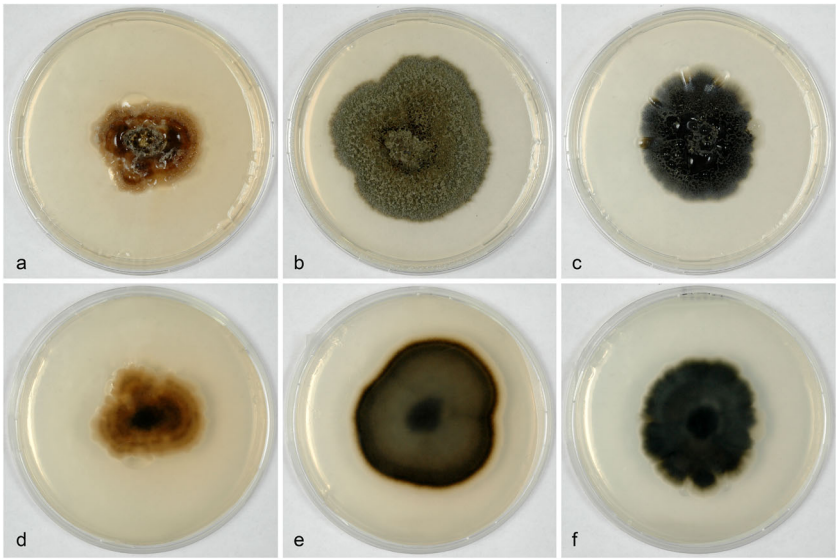

Fig. 2 *Stigmatodiscus* cultures on CMD after 42 days at 16 °C; **a**–**c** in face view, **d**–**f** reverse. **a**, **d** *S. labiatus*; **b**, **e** *S. oculatus*; **c**, **f** *S. pinicola*. Sources: **a**, **d** AP6516; **b** AP231016; **c**, **f** AP21916b; **e** AP311216

Conidiomata on the natural substrate associated with ascomata, visible as minute black dots 80–160 μm in diam, immersed, peridermal, pycnidial, unilocular, of circular shape, opening with a central ostiole, 100–200 μm diam, marginal wall thin, ca. 10 μm, composed of subhyaline to light brown cells, wall around ostiole ca. 22–50 μm thick, composed of dark brown cells. Conidiophores short, branched up to three times. Condiogenous cells phialidic, cylindrical, (8.5–)9.0–12.3(–15.5) × (1.5–)1.7–2.3(–2.7) μm ($n = 50$). Conidia (14–)17–20(–22) × (0.9–)1.1–1.4(–1.7) μm ($n = 36$), hyaline, falcate to semicircular, aseptate.

Cultures slow-growing, with uneven lobed margins, colony on CMD reaching 42 mm diam after 42 days at 16 °C, first whitish, turning medium to dark red brown, with sparse lighter brown aerial mycelium in the centre, reverse zonate, dark brown in the centre, with medium and dark red brown concentric zones towards the margins, entire culture black after 6 months. No conidiomata seen in pure culture.

Habitat: on dead corticated braches of *Quercus* and *Rhamnus alaternus*.

Distribution: only known from Mallorca (Spain).

Holotype: Spain, Mallorca, Es Capdellà, Finca Son Marti, on corticated dead branches of *Quercus* sp., 6 May 2016, A. Pintos AP6516 (WU 39973), ex holotype culture CBS 144700.

Additional specimens examined: Spain, Mallorca, Calvià, Finca Pública Es Galatzó, on corticated dead branches of *Quercus coccifera*, 14 Dec. 2016, A. Pintos AP141216 (WU 39980); Esporlas, on dead corticated branches of *Rhamnus alaternus*, A. Pintos, 20 Aug. 2018 AP20818.

Notes: The hysteriform ascomata and 1-septate ascospores of *S. labiatus* are similar to the closely related *S. pruni*, but the latter has distinctly smaller ascospores (26–35 × 11–14 μm vs. 35–48 × 14–19 μm in *S. labiatus*), and it occurs on a different host, *Prunus spinosa*. In addition, the mature ascospore cells of *S. labiatus* are more distinctly constricted in the middle and have a ring-like thickening inside the wall.

Stigmatodiscus oculatus Voglmayr & Pintos, sp. nov. Fig. 5.

MycoBank MB 827488.

Etymology: Referring to its eye-shaped ascomata.

Ascomata hysteriform, scattered to gregarious, corticolous, erumpent through the periderm, commonly arranged in parallel to the branch axis, in face view (160–)280–510(–880) μm long, (110–)180–300(–380) μm wide ($n = 96$), with sides consisting of usually two, rarely three black lips (peridium) not in mutual contact, with a slit-like to almost circular central opening exposing the blackish elongated to broadly oval disc. Bark tissues not visibly altered, no black line visible in bark or wood. Peridium coriaceous, pseudoparenchymatous, dark brown to black, up to 120 μm thick at the apex, 38–55 μm thick at the sides, almost absent to 20 μm thick at the base, composed of small angular to rounded cells 3–10 μm diam. Hamathecium composed of hyaline, septate, filiform branched paraphyses 1.7–2.5 μm wide, embedded in tough hymenial gel, simple, not anastomosing, 82–123 μm long, longer than asci, swollen at their apices up to 5.5 μm and incrusted with dark brown granules forming an epithecium, neither staining blue in Lugol nor in Melzer's reagent after pre-treatment with 3% KOH. Asci 72–84(–90) × (29–)35–49 μm ($n = 10$), bitunicate, clavate to pyriform, almost sessile, with a distinct apical chamber, thick-walled, typically containing 8 irregularly bi- to triseriate ascospores, very stable, fissitunicate dehiscence not observed. Ascospores (25.5–)27.5–31(–33) × (9.5–)10.5–12.0(–12.5) μm, l/w = (2.3–)2.5–2.7(–2.9) ($n = 63$), brown, asymmetric, broadly fusiform, straight, first 1-septate, developing 2 additional distosepta and becoming 3-septate with age, strongly constricted at the primary septum, weakly at secondary septa,

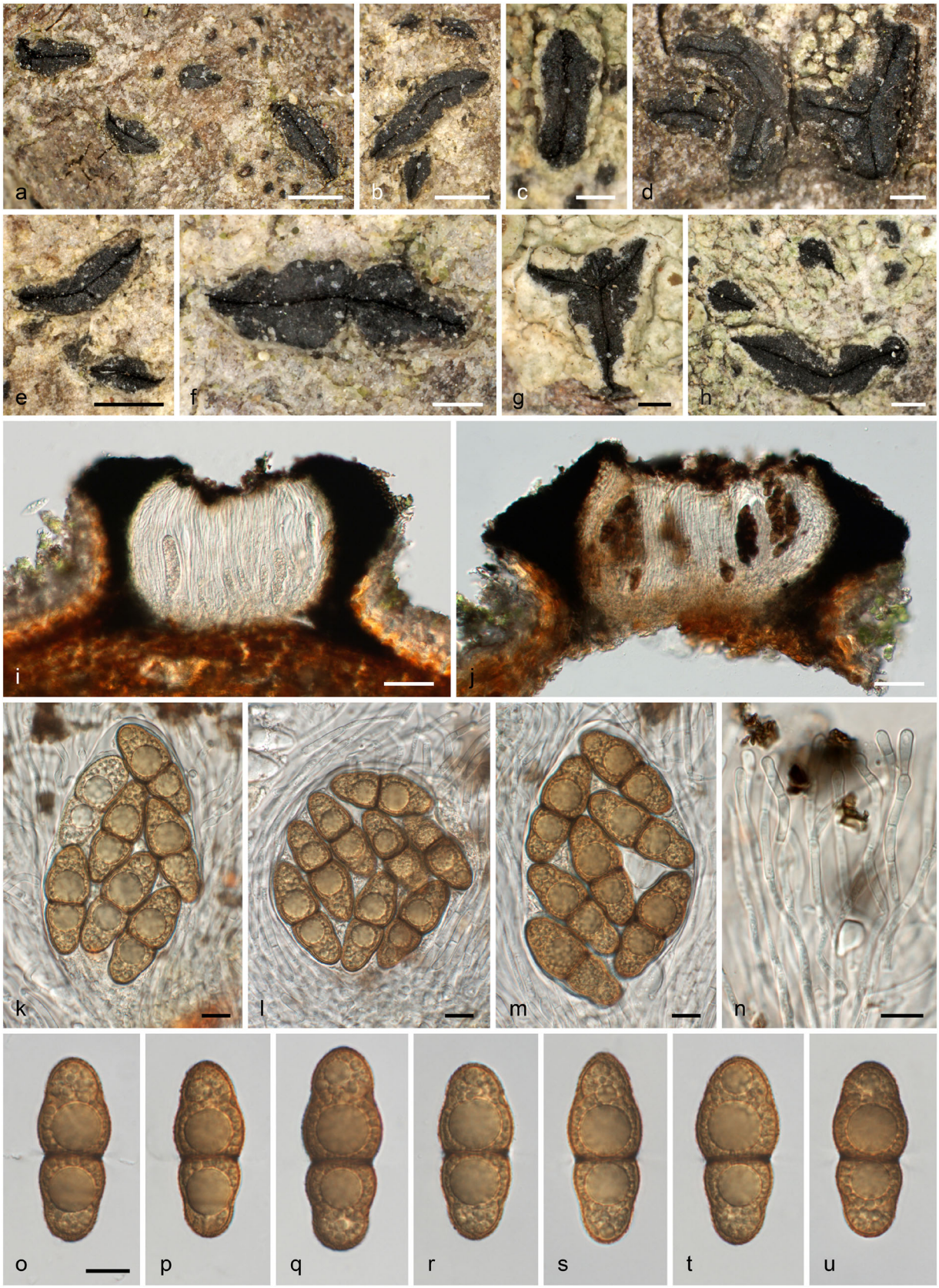

Fig. 3 *Stigmatodiscus labiatus*, sexual morph. **a–h** Ascomata erumpent from bark in face view. **i**, **j** Vertical sections of ascomata embedded in bark. **k–m** Asci. **n** Apically inflated septate paraphyses, covered by an dark brown amorphous incrustation. **o–u** Vital ascospores. All in water. Sources: **a–h**, **l**, **o–u** WU 39973 (holotype); **i–k**, **m**, **n** WU 39980. *Scale bars*: a, **b**, **e** 500 μm; **c**, **d**, **f–h** 200 μm; **i**, **j** 50 μm; **k–u** 10 μm

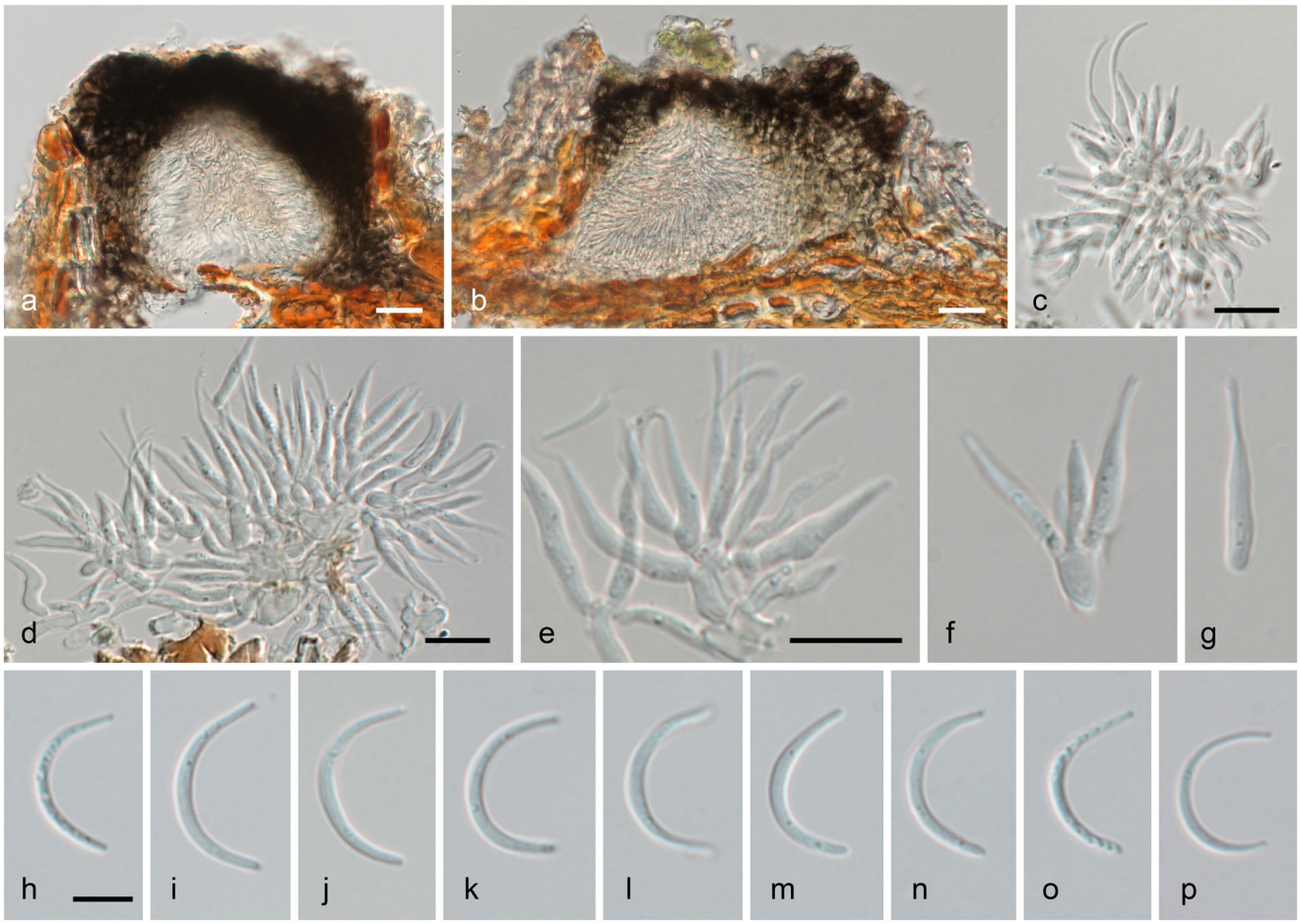

Fig. 4 *Stigmatodiscus labiatus*, asexual morph. **a**, **b** Conidiomata (pycnidia) immersed in periderm in vertical section. **c–e** Conidiophores with densely aggregated phialides. **f**, **g** Phialides. **h–p** Falcate to semicircular conidia. All in water, except **a**, **c–g**, **p** in 3% KOH. Sources: **a**, **c–g**, **p** WU 39980; **b**, **h–o** WU 39973 (holotype). *Scale bars*: **a**, **b** 20 μm; **c–g** 10 μm; **h–p** 5 μm

secondary septa with large pores, each hemispore surrounded by a separate gelatinous sheath quickly dissolving in water, upper hemispore slightly broader, with broadly rounded ends; wall finely verruculose, brown, the contents granular, often with a large and several smaller guttules per cell.

Conidiomata on the natural substrate and in pure culture not observed.

Cultures slow-growing, with uneven margins, colony on CMD reaching 58 mm diam after 42 days at 16 °C, first whitish, soon turning dark olive brown, with abundant woolly surface mycelium, reverse dark brown to black.

Habitat: on dead corticated braches of various Mediterranean trees and shrubs.

Distribution: only known from Mallorca (Spain).

Holotype: Spain, Mallorca, Campos, Sa Ràpita, on corticated dead branches of *Olea europaea*, 16 Nov. 2016, A. Pintos AP161116 (WU 39974), ex holotype culture CBS 144701.

Additional specimens examined: Spain, Mallorca, Puig de Ros, on dead corticated branches of *Populus canadensis*, 10 Aug. 2016, A. Pintos AP10816 (WU 39975); Calvià, Playa Portals Vells, on dead corticated branches of *Cistus albidus*, 23 Oct. 2016, A. Pintos AP231016B (WU 39976); Campos, Sa Ràpita, on dead corticated branches of *Pistacia lentiscus*, 17 Nov. 2016, AP171116 (WU 39977), Calvià, Portals Vells, on dead corticated branches of *Globularia alypum*, 31 Dec. 2016, AP311216 and AP311216A (WU 39978).

Notes: *Stigmatodiscus oculatus* is evidently polyphagous as it has been found on corticated twigs of various shrubs and trees. Within the *Stigmatodiscus* species with four-celled ascospores, it is well distinct by ascospores smaller than 33 × 12.5 μm.

Stigmatodiscus pinicola Voglmayr & Pintos, sp. nov. Figs. 6 and 7.

MycoBank MB 827489.

Etymology: Referring to its growth on *Pinus*.

Ascomata hysteriform to apothecioid, scattered, corticolous, initially covered by bark, erumpent through the periderm, in face view (205–)255–410(–600) μm long, (145–)180–285(–375) μm wide ($n = 30$), with sides consisting of usually 2–3 black lips (peridium) not in mutual contact,

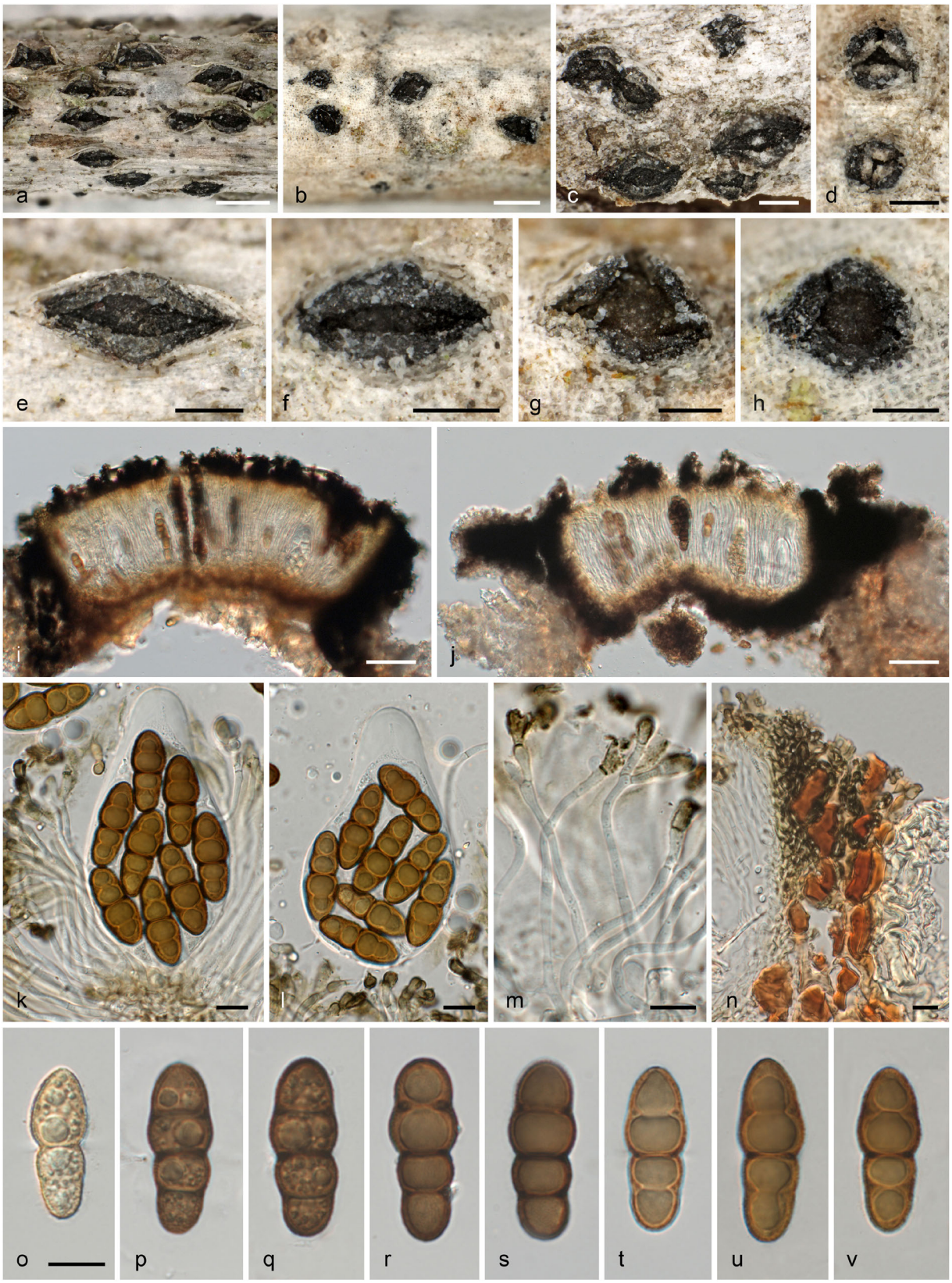

Fig. 5 *Stigmatodiscus oculatus*, sexual morph. **a–h** Ascomata erumpent from bark in face view. **i**, **j** Vertical sections of ascomata embedded in bark. **k**, **l** Asci. **m** Apically inflated septate paraphyses, covered by a dark brown amorphous incrustation. **n** Ascoma margin in transverse section. **o–v** Ascospores (**o** immature, **p–v** mature; **o–q** vital **r–v** dead). All in water, except **k–n**, **u**, **v** in 3% KOH. Sources: **a**, **c**, **e**, **k–n**, **u**, **v** WU 39977; **b**, **d**, **f–i**, **r–t** WU 39974 (holotype); **j**, **o–q** WU 39975. *Scale bars*: **a**, **b** 500 μm; **c–h** 200 μm; **i**, **j** 50 μm; **k–v** 10 μm

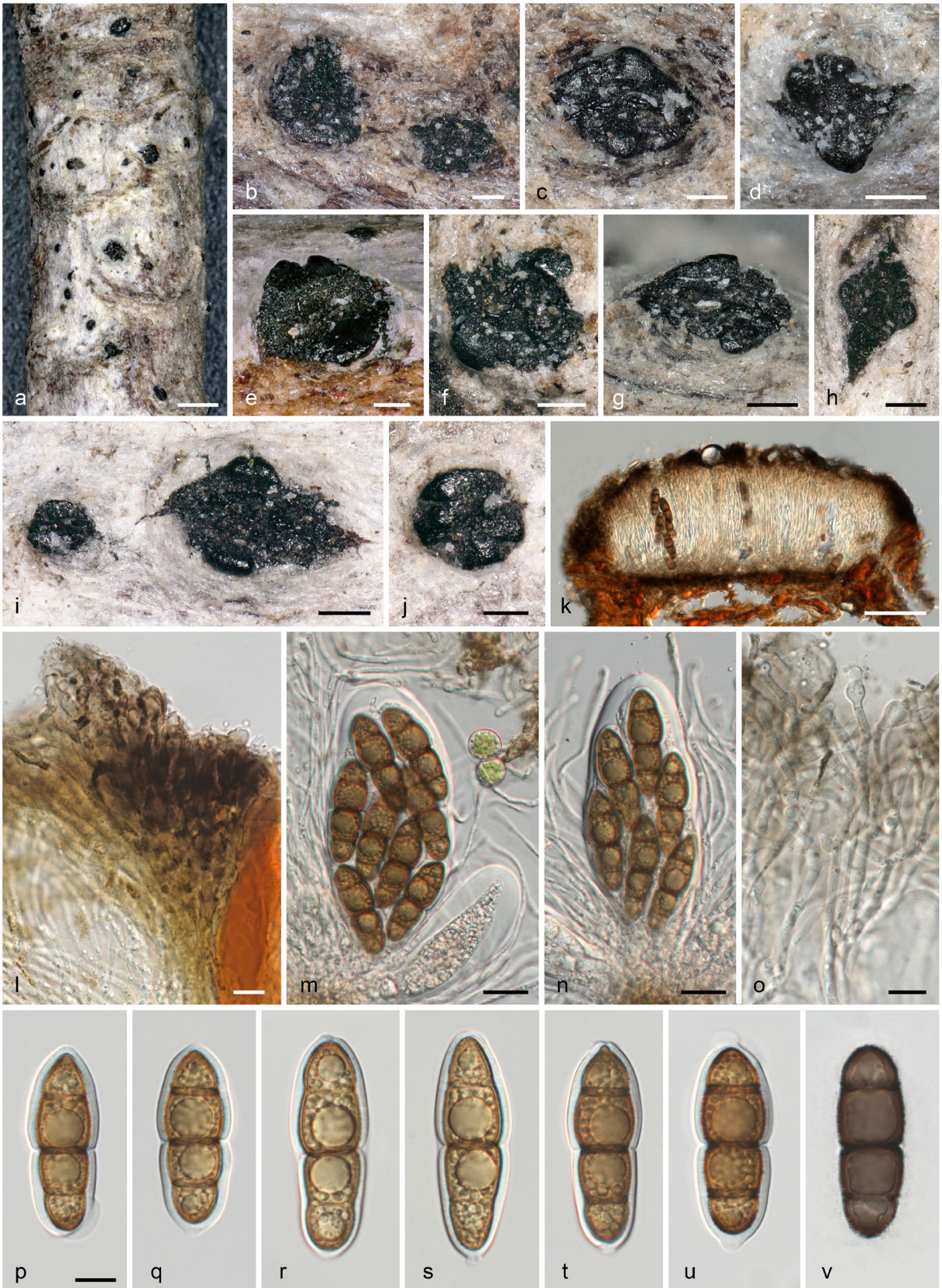

Fig. 6 *Stigmatodiscus pinicola*, sexual morph (WU 39979, holotype). **a–j** Ascomata erumpent from bark in face view. **k** Vertical section of ascoma. **l** Ascoma margin in transverse section. **m, n** Asci. **o** Apically inflated septate paraphyses, covered by an dark brown amorphous incrustation. **p–v** Ascospores (**p–u** vital, **v** dead). All in water, except **l**, **o**, **v** in 3% KOH. *Scale bars*: **a** 500 μm; **b–k** 100 μm; **m**, **n** 20 μm, **l**, **o–v** 10 μm

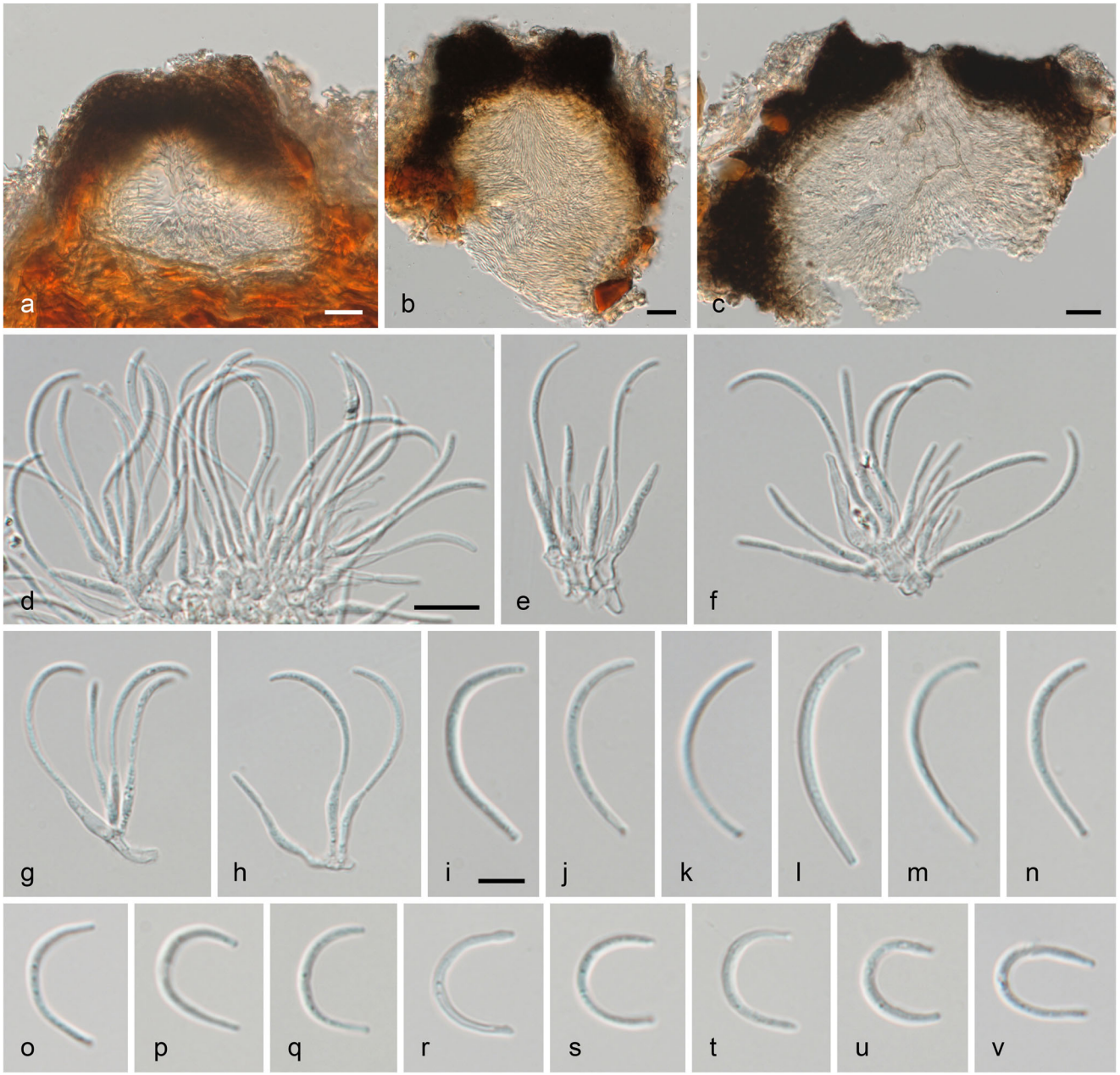

Fig. 7 *Stigmatodiscus pinicola*, asexual morph (WU 39979, holotype). **a–c** Conidiomata (pycnidia) immersed in periderm in vertical section; in **c** through ostiole. **d–h** Conidiophores with densely aggregated phialides. **i–v** Falcate to semicircular conidia. All in water, except **b**, **c** in 3% KOH. *Scale bars*: **a–c** 20 μm; **d–h** 10 μm; **i–v** 5 μm

with a slit-like to circular central opening exposing the blackish elongated to circular disc. Bark tissues not visibly altered, no black line visible in bark or wood. Peridium coriaceous, pseudoparenchymatous, dark brown, up to 70 μm thick at the apex, 15–25 μm thick at the sides, almost absent to 20(–30) μm thick at the base, composed of small angular to rounded brown cells 3–9 μm diam. Hamathecium composed of hyaline, septate, filiform branched paraphyses 1.5–3.5 μm wide, embedded in tough hymenial gel, simple, not anastomosing, ca. 150–220 μm long, longer than asci, swollen at their apices up to 7 μm and incrusted with dark brown granules forming an epithecium, neither staining blue in Lugol nor in Melzer's reagent after pre-treatment with 3% KOH. Asci (104–)111–135(–138) × 47–54(–57) μm ($n = 10$), bitunicate, broadly fusiform to ellipsoid, almost sessile, with a distinct apical chamber, thick-walled, typically containing 8 irregularly bi- to triseriate ascospores, very stable, fissitunicate dehiscence not observed. Ascospores (40.5–)43.5–50(–52.5) × (13.5–)14.5–16.8(–18.0) μm, l/w = (2.6–)2.8–3.2(–3.5) ($n = 45$), brown, asymmetric, fusiform, straight, first 1-septate, developing 2 additional distosepta and becoming 3-septate with age, strongly constricted at the primary septum, weakly at secondary

septa, secondary septa with large pores, each hemispore surrounded by a separate gelatinous sheath, upper hemispore slightly broader, with subacute to rounded ends, end cells lighter brown at maturity; wall finely verruculose, brown, the contents granular, often with a large and several smaller guttules per cell.

Conidiomata on the natural substrate associated with ascomata, visible as minute black dots 110–200 μm in diam, immersed, peridermal, pycnidial, clypeate, unilocular, of circular shape, opening with a central ostiole, 140–230 μm diam, 130–210 μm high, marginal wall ca. 14–25 μm thick, of light brown cells, wall around ostiole ca. 30–60 μm thick, composed of brown cells. Ostiole dark brown, ca. 20–25 μm wide. Conidiophores short, branched up to two times. Condiogenous cells phialidic, cylindrical, (9.5–)12.0–17.2(–21.2) × (1.9–)2.2–2.9(–3.0) μm ($n = 30$). Conidia (18–)19–25(–28) × 1–1.6 μm ($n = 30$), hyaline, falcate to semicircular, aseptate.

Cultures slow-growing, with uneven margins, colony on CMD reaching 49 mm diam after 42 days at 16 °C, first whitish, then turning black, with sparse grey aerial mycelium, reverse black.

Habitat: on dead corticated braches of *Pinus halepensis*.

Distribution: only known from Mallorca (Spain).

Holotype: Spain, Mallorca, Es Capdellà, Castell Son Claret, on corticated dead branches of *Pinus halepensis*, 21 Sep. 2016, A. Pintos AP21916B (WU 39979), ex holotype culture CBS 144702.

Notes: *Stigmatodiscus pinicola* is well characterised by the small apothecioid-hysteriform ascomata with usually circular outline and indistinct lips and by its host, *Pinus halepensis*. *Stigmatodiscus enigmaticus* and *S. tamaricis* also have four-celled ascospores of similar size but have different hosts; in addition, *S. enigmaticus* differs by larger ascomata (0.4–1.5 vs. 0.2–0.4(–0.6) mm) which are surrounded by bark flaps, and *S. tamaricis* by ascospores long remaining (sub)hyaline and by paraphyses with emerald to deep blue amorphous incrustation (Voglmayr et al. 2016).

Stigmatodiscus tamaricis (Voglmayr, Gardiennet & Jaklitsch) Voglmayr & Jaklitsch, comb. nov.

MycoBank MB 827490.

Basionym: *Asterodiscus tamaricis* Voglmayr, Gardiennet & Jaklitsch, Fungal Diversity 80: 276 (2016).

Specimen examined: Spain, Mallorca, Calvià, Magalluf, on corticated dead branches of *Tamarix* sp., 9 Sep. 2016, A. Pintos AP9916A.

Notes: With the addition of *Stigmatodiscus oculatus*, *Asterodiscus tamaricis* becomes phylogenetically embedded within *Stigmatodiscus* and is therefore transferred to the latter. *Stigmatodiscus tamaricis* is widely distributed on *Tamarix* spp. in Central and Southern Europe, and the specimen cited above is the second record of the species for Mallorca; the first Mallorcan record was recently published in Siquier et al. (2018).

Key to the species of *Stigmatodiscus*

1. Ascospores at maturity with a primary septum, only very rarely developing two additional distosepta, brown; ascomata distinctly hysteriform.....2
   Ascospores at maturity with a primary septum and two additional distosepta, hyaline or brown; ascomata apothecioid or hysteriform.....3
2. Ascospores (26.5–)29–32.5(–34.5) × (10.8–)11.5–12.7(–13.8) μm; on *Prunus spinosa* .....*S. pruni*
   Ascospores (34.5–)38–43(–47.5) × (13.8–)15.5–17.5(–19.3) μm; on Mediterranean *Quercus* spp.....*S. labiatus*
3. Mature ascospores in vital asci hyaline to light brown, becoming dark brown after ejection, (33.5–)40–45(−49) × (12.8–)14.3–16.5(−17.7) μm; ascomata apothecioid, circular; paraphyses tips covered by an olivaceous, emerald to deep blue amorphous incrustation; on *Tamarix* spp. .....*S. tamaricis*
   Mature ascospores brown; paraphyses tips covered by a dark brown amorphous incrustation; on other hosts.....4
4. Ascospores (25.5–)27.5–31(–33) × (9.5–)10.5–12.0(–12.5) μm; ascomata hysteriform; polyphagous..... *S. oculatus*
   Ascospores larger than 40 × 13 μm, ascomata mostly circular, apothecioid 5
5. Ascomata 0.4–1.5 mm diam, surrounded by irregular bark flaps; ascospores (46–)54–64 (−73) × (16.5–)20.0–24.3(−32.5) μm; on *Acer* spp., *Carpinus orientalis*..... *S. enigmaticus*
   Ascomata 0.2–0.4(–0.6) mm diam, not surrounded by bark flaps; ascospores (40.5–)43.5–50(–52.5) × (13.5–)14.5–16.8(–18.0); on *Pinus halepensis*.....*S. pinicola*

Discussion

Voglmayr et al. (2016) established the two genera *Asterodiscus* and *Stigmatodiscus* within the new family and order Stigmatodiscaceae and Stigmatodiscales, respectively, primarily based on differences in ascomatal shape and hyaline vs. brown ascospores. The morphological boundaries and phylogenetic differences between the two genera were considered distinct enough for establishing two genera. This concept was already challenged by Voglmayr et al. (2017), who described *S. pruni*, another new species with brown but two-

celled ascospores, which differed substantially from the generic type, *S. enigmaticus*, by distinctly hysteriform ascomata.

The results of the current phylogenies necessitate a re-evaluation of the genus *Asterodiscus*, because it forms a highly supported clade with *Stigmatodiscus oculatus*. Whereas the ascospore shape and septation of *S. oculatus* are similar to *A. tamaricis*, the brown ascospores are indicative of *Stigmatodiscus*. Therefore, if the genus *Asterodiscus* were maintained, this would necessitate an emendation of the genus. However, considering the morphology of the new species described since the study of Voglmayr et al. (2016), the morphological differences between *Asterodiscus* and *Stigmatodiscus* seem insufficient to maintain them as distinct genera, which are therefore here synonymised.

None of the newly described species produced asexual morphs in pure culture; however, in *S. labiatus* and *S. pinicola*, an asexual morph was found tightly associated with the sexual morphs on the natural substrate. As conidia did not germinate on MEA or CMD, the connection with the sexual morphs could not be experimentally proven. However, we are confident that the associated asexual morphs belong to the respective species, as the morphology of their conidiomata as well as their conidial ontogeny, size, and shape fully match the asexual morph of *S. enigmaticus*, which was documented from natural substrate as well as pure cultures originating from ascospores (Voglmayr et al. 2016), proving the connection.

It is astonishing that within a small area, three new species of *Stigmatodiscus* could be found. It remains so far unclear whether the new species are endemic to Mallorca, or whether they co-occur with their widely distributed hosts in other regions the Mediterranean. This once again demonstrates that in the Mediterranean, the species diversity of corticolous ascomycetes is very incompletely studied, and that many species still await description (e.g. Voglmayr and Jaklitsch 2011; Jaklitsch and Voglmayr 2011, 2014; Voglmayr et al. 2016; Jaklitsch et al. 2014, 2015, 2018a, b; Galán et al. 2015; Checa et al. 2015). Considering that the Mediterranean is amongst the main biodiversity hotspots of the world (Myers et al. 2000), additional species of *Stigmatodiscus* are likely to be detected in this species-rich area.

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