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Three new species of *Stigmidium* s. l. (lichenicolous ascomycetes) on *Acarospora* and *Squamarina*

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Abstract: *Stigmidium cartilagineae* Calatayud & Triebel (on the apothecial discs of *Squamarina cartilaginea*), a lichenicolous fungus belonging to *Stigmidium* s. str., is described as new. Two other species of *Stigmidium* s. l. with a net of branched and anastomosing, rudimentary interascal filaments are also described as new to science: *Stigmidium rouxianum* Calatayud & Triebel (on *Acarospora cervina*) and *S. squamarinicola* Calatayud & Triebel (on the thallus of *Squamarina* spp.). They are related to *Stigmidium psorae* and treated here as '*Stigmidium*' *psorae* group. To facilitate discussion of the distinguishing features of this group, a table comparing the terms referring to hamathecial hyphae in pyrenocarpous ascomycetes is presented.

Key words: hamathecial hyphae, isolichenan, lichenicolous fungi, lichens, *Sphaerellothecium*, '*Stigmidium*' *psorae* group.

Introduction

During our studies on *Stigmidium* Trevis. and morphologically similar genera, several new species have been discovered, three of which are described in this paper. One of these new taxa (host: *Squamarina cartilaginea*) matches the characters of typical *Stigmidium* species, is described here as *S. cartilagineae*. Although *Squamarina* species host several lichenicolous fungi (e.g. Navarro-Rosinés *et al.* 1994, 1995), *Stigmidium* species have not been previously reported on them. The most extensive keys to the numerous species of this genus are provided by Clauzade *et al.* (1989), and the species corresponding to *Pharcidia epicymatia* sensu Keissler or to *Stigmidium schaereri* auct. have been revised by Roux & Triebel (1994). Subsequently, several other species have been described (see Calatayud & Triebel 2001 for references), and three further species have been introduced by Etayo (2000, 2002). The other two new species described in this paper (*S. rouxianum* on *Acarospora cervina*, and *S. squamarinicola* on *Squamarina* spp.) are closely related to *S. psorae*. The '*Stigmidium*' *psorae* group shares features with *Stigmidium* s. str., but clearly differs from it by the occurrence of a net of long interascal filaments. These features of the hamathecium have an outstanding systematic value in ascomycetes and therefore, as already noted by Calatayud & Triebel (1999) for *S. psorae*, the placement of these three taxa in *Stigmidium* is most probably inappropriate.

As the naming of hamathecial filaments might lead to confusion especially in the groups of ascomycetes treated here, we have included a table that presents definitions and compares the different terms referring to the morphology and topology of hamathecial filaments with those based on ontogeny (Table 1). In the descriptions below both sets of terms have been used in parallel.

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TABLE 1. *Comparison of terms for hamathecial filaments of ascomycetes*

Definitions of terms	Terms without reference to ontogeny	Terms based on ontogeny
Filaments confined to the ostiolar canal	Ostiolar filaments	Periphyses
Filaments originating at the upper half of the cavity, short, pendant, never attached at the base of the cavity	Short pendant interascal filaments	Periphysoids Short pseudoparaphyses (type a* or type b‡)
Filaments between the asci, long, primarily or secondarily attached at the base of the cavity	Long interascal filaments	
(a) Primarily attached at the top and at the base of the cavity		Paraphysoids
(b) Primarily attached at the base of the cavity		Paraphyses s. str.
(c) Primarily attached at the top, in some cases secondarily attached at the base of the cavity		Long pseudoparaphyses

*Two-celled pseudoparaphyses with elongated end cell.

‡Three- to five-celled pseudoparaphyses with cells of more or less equal size; see Roux & Triebel (1994, fig. 6f,g).

Materials and Methods

The material is deposited at M, VAB—Lich. and in the private herbarium of V. Calatayud. It was examined with standard microscopic techniques. Photographs were taken with an Olympus SZH10 stereomicroscope and with an Olympus BX50 microscope provided with DIC, and drawings were made with the aid of a drawing tube. The material was mounted in water and in lactophenol cotton blue. The amyloid reactions were tested in iodine-potassium iodide solution (1%) [=Lugol's iodine solution], directly (I) and after a KOH pre-treatment (K/I). A 3% solution of Congo red (CR) was used to study the structure of the endoascus, and a 1% solution of Brilliant Cresyl blue (BCr) for testing meta-chromatic reactions of the endoascus, epispore and vegetative hyphae. All measurements were made in water. For the ascospore size, the range (given in non-italics and outside parenthesis) was calculated after manually rejecting 10% of the highest and the lowest values measured; the averages are in italics, and the extremes are given within parentheses. Abbreviations for institutional herbaria follow Holmgren *et al.* (1990), and those for exsiccata Triebel & Scholz (2001–2002).

The following specimens were examined for comparison with the three new *Stigmidium* species:

Stigmidium acetabuli Calatayud & Triebel: **Spain**: *Castilla-La Mancha*: Prov. Albacete, Sierra de Alcaraz, cerca del Puerto de las Crucetillas, *c.* 38°31′N, 2°24′W, *c.* 1300 m, (on *Pleurosticta acetabulum*), sobre *Prunus* sp., al lado de la carretera, 17 iv 2000, *V. Calatayud* (hb. Calatayud 41—isotypus).

Stigmidium epixanthum Hafellner: **Spain**: *Islas Canarias*: Tenerife: El Sauzal, [on *Acarospora* (*Xanthothallia*) *lavicola*], sobre roca volcánica, s.d., *V. Calatayud & E. Barreno* (VAB-Lich. 7853).

Stigmidium fuscatae (Arnold) R. Sant.: **Germany**: *Sachsen*: Regierungsbezirk Chemnitz,Vogtländisches Kuppenland, *c.* 8 km N of Plauen, NE of Rentzschmühlhäuser, (50°34′N 12°10′E), (on *Acarospora fuscata*), partly shady diabasic rocks, *c.* 340 m, 15 x 1996, *P. Scholz* (M, Triebel, *Microf. exs.* no. 207 sub *Stigmidium fuscatae*). *Bayern*: Mittelfranken, Brauner Jura eines Steinbruchs nördlich von Treuchtlingen, (on *Acarospora fuscata*), iv 1870, *F. Arnold* (M—holotypus).—**Austria**: *Burgenland*: Südburgenland, *c.* 2 km NW of Neusiedl by Güssing, (on *Acarospora fuscata*), on old tiles, 7 iv 1989, *J. Hafellner* (M, Santesson, *Fungi lichenicoli exs.* no. 196 sub *Stigmidium fuscatae*).—**Spain**: *Comunidad Valenciana*: Prov. València, Marines, alrededores del pueblo, U.T.M. 30SYK1102, (on *Acarospora fuscata*), sobre arenisca, 460 m, 2 ii 1998, *V. Calatayud* (hb. Calatayud 122).

Stigmidium neofusceliae Calatayud & Triebel: **Spain**: *Comunidad Valenciana*: Prov. Castelló, Almenara, La Frontera, U.T.M. 30SYK3405 (*c.* 39°45′18″N 0°16′6″W), (on *Neofuscelia pulla*), on sandstone, 230 m, 16 iv 1993, *V. Calatayud* (VAB—Lich. 7066—holotypus, M—isotypus).

Stigmidium squamariae (de Lesd.) Roux & Triebel: **Spain**: *Andalucía*: Prov. Cádiz, Jimena de la Frontera, Los Alcornocales, 36°22′N, 5°34′W, *c.* 425 m, (on *Lecanora muralis*), sobre roca silícea, 1 iv 2000, *V. Calatayud & J. Cerveró* (hb. Calatayud 121).

Taxonomy

Stigmidium cartilagineae Calatayud & Triebel sp. nov.

A specie *Stigmidium acetabuli* Calatayud & Triebel praecipue differt ascomatibus majoribus, 60–100 (–140) μm, ascis (4–)6–8-sporis, ascosporis leviter

minoribus et gelatina hymenii jodo violascente. In apotheciis lichenis *Squamarina cartilaginea* crescit.

Typus: Spain, Comunidad Valenciana, prov. Castelló, Els Ports, close to Castellfort, 40°31′N, 0°14′W [=U.T.M. 30TYK3489], *c.* 1150 m, on the apothecial discs of *Squamarina cartilaginea*, on calcareous rock, 16 January 2001, *V. Calatayud* (M–0031466—holotypus; hb. Calatayud 126—isotypus).

(Figs 1A–H, 4A & B)

Ascomata perithecioid, dark brown, globose or subglobose, 60–100(–140) µm in diam., scattered, immersed in the apothecial disc of the host or partly erumpent; the infection not producing any distinct alteration to the host. *Wall* pseudoparenchymatous, of *textura angularis*, upper third brown and lower part hyaline, *c.* 4–8 µm wide and with 1–2 layers of cells at the base. Cells of the wall ± polygonal, mostly 5–7 µm diam., or elongated (in superficial view of the ascomata), I−. Hymenial gel I+ violet, K/I+ violet or K/I−. External periphyses (sensu Roux & Triebel 1994) rather conspicuous. Pendant interascal filaments relatively long, 2(–3)-celled, not branched (pseudoparaphyses of 'type a', sensu Roux & Triebel 1994); cells 4–7 × 1–2 µm, the apical one sometimes slightly widened at the apex. *Asci* (4–)6–8-spored, *c.* 28–32 × 11–13 µm, wall thickened at the apex, clavate or saccate, sessile or shortly stipitate, ascospores distichously arranged in the asci, I−; endoascus BCr−, CR+ orange, sometimes less intense orange in the apical zone. *Ascospores* 1-septate, constricted at the septum, lower cell narrower and sometimes longer than the upper one, not pseudotetrablastic, colourless, with or without a gelatinous sheath, surface smooth, with 1–3 oil droplets per ascospore cell, ascospore wall BCr−, or outer part of the wall BCr+ blue in the oldest, brownish ascospores, 8–*9·9*–12(–14) × 3–*3·9*–4·5(–5·0) µm [length/width index: (1·8–)2·2–*2·5*–3(–3·5); *n* = 38]; the oldest ascospores sometimes pale brown and with a granulose surface.

Conidiomata not observed.

Vegetative hyphae colourless, I−, BCr−.

Host. On the apothecial discs of *Squamarina cartilaginea* (With.) P. James, without causing any apparent injury.

Etymology. The epithet '*cartilagineae*' refers to the host species.

Ecology and distribution. *Stigmidium cartilagineae* is known from the type collection, and a further collection from Macedonia. It is found on *Squamarina cartilaginea* growing on calcareous soils. Although the apothecial discs of the host were covered with abundant ascomata of the lichenicolous fungus, they continued to produce ascospores, and neither a change in colour nor change in the morphology of the infected parts was observed.

Remarks. *Stigmidium cartilagineae* is characterized by its brown, scattered ascomata, confined to the apothecial disc of the host, never invading the thallus nor the apothecial margin of the host. In this species, rather well-developed external periphyses (sensu Roux & Triebel 1994) are formed around the ostiole; the pendant interascal filaments are also quite distinctive, having mostly two cells [pseudoparaphyses of 'type a', according to the typology of Roux & Triebel (1994); periphysoids in the sense of other authors] although, in some cases, three cells have been observed. The asci have six or eight ascospores, rarely four; when there are less than eight, remnants of aborted ascospores are usually observed. Particularly remarkable for *S. cartilagineae* is the I+ violet reaction of the hymenial gel (contrasting with the I+ blue reaction of the surrounding hymenium of the host). This reaction may be caused by isolichenan (Common 1991), and has been reported so far in two other species of *Stigmidium*: *S. neofusceliae* Calatayud & Triebel (Calatayud & Triebel 1999), and *S. triebeliae* Etayo ('*S. triebelae*') (Etayo 2000). Two of the species of the '*Stigmidium*' *psorae* group, *S. psorae* and *S. squamarinicola*, also show this reaction. *Stigmidium squamarinicola* occurs on the same host genus, but clearly differs by having longer interascal filaments (probably

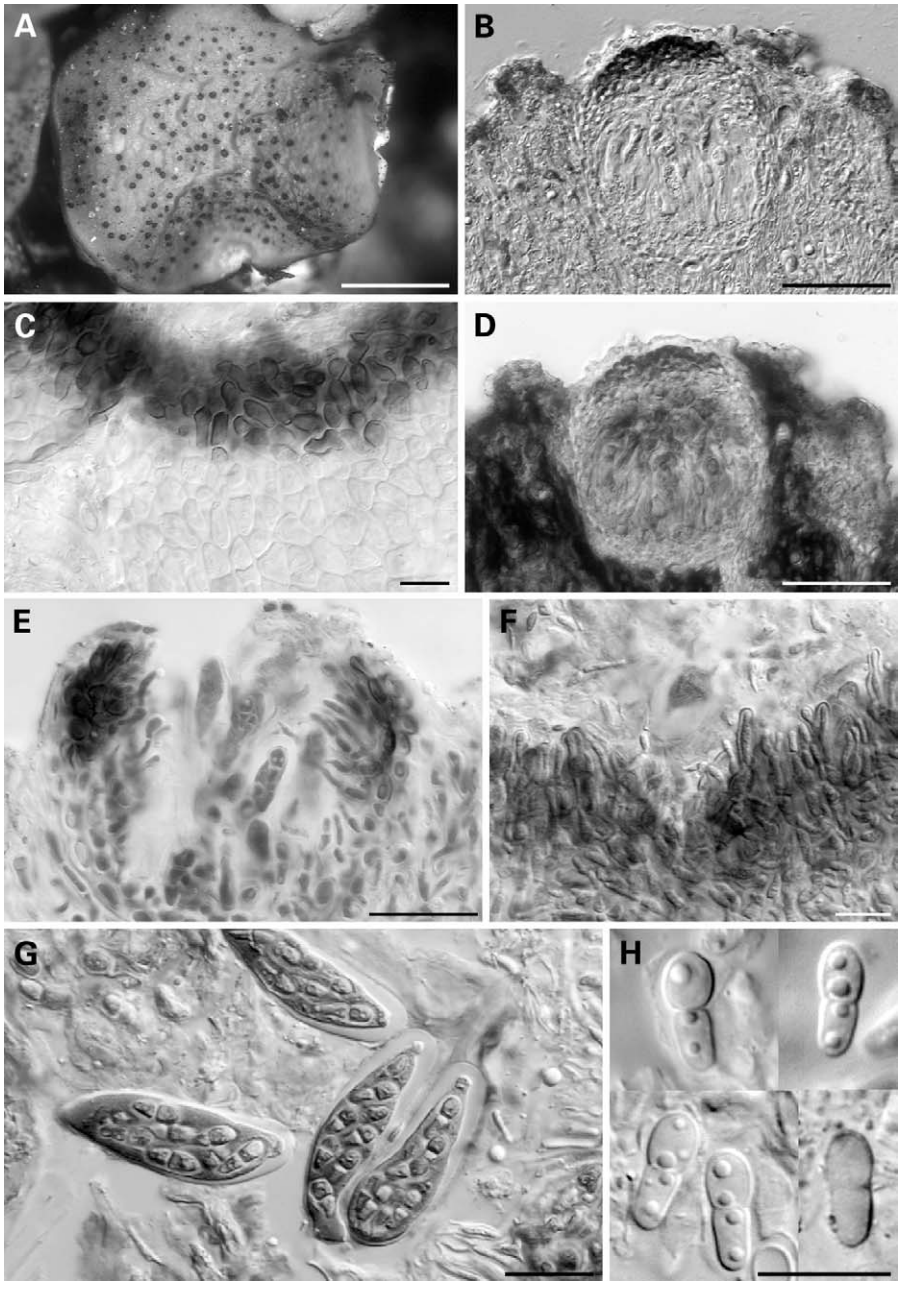

FIG. 1. *Stigmidium cartilagineae* (holotypus). A, habit; B, transverse section of an ascoma; C, wall of an ascoma in superficial view; D, transverse section of an ascoma after K/I, showing the violet reaction (dark in the picture) of the hymenial gel; E, pseudoparaphyses of 'type a' in lactophenol cotton blue; F, external periphyses, around the ostiole, squash preparation, after I; G, asci after K/I; H, ascospores, including an old, brown one. Scales: A = 1 mm; B & D = 50 μm; C = 10 μm; E = 25 μm; F–H = 10 μm.

TABLE 2. *Characters distinguishing* Stigmidium acetabuli, S. cartilagineae, *and* S. squamariae

Character	*S. acetabuli*	*S. cartilagineae*	*S. squamariae*
Effect on the apothecial discs	Not appreciable	Not appreciable	Partial or total darkening
Ascomata size (μm)	50–85 diam.	60–100(–140) diam.	60–75 × 40–55
Short pendant interascal filaments	Present	Present	Absent or perhaps very poorly developed
Hymenial gel	I−	I+violet	I−
Number of ascospores per ascus	8	(4–)6–8	8
Asci (μm)	20–30(–35) × 9–11	28–32 × 11–13	22–31 × 11–15
Ascospores (μm)	(7·5–)8–10.5(–11) × 3–4(–4·5)	8–12(–14) × 3–4·5(–5)	(8·5–)9–13(–14) × (3·5–)4–5(–6)
Host	*Pleurosticta acetabulum*	*Squamarina cartilaginea*	*Lecanora* gr. *muralis* and *L. polytropa*
Source	Calatayud & Triebel (2001)	Present paper	Roux & Triebel (1994)

paraphysoids), and notably larger ascospores. *Stigmidium neofusceliae* (host: *Neofuscelia* species) resembles *S. cartilagineae* in lacking pseudotetrablastic ascospores and metachromatic reactions in the endoascus and ascospores, but differs in a number of characters apart from its different host. It affects the host thallus, which becomes discoloured, not only the hymenial gel, but also the wall of the ascomata and the vegetative hyphae react I+ violet (according to Kocourkovà 2000 some specimens are I−); the asci are constantly 8-spored, and the ascospores are larger, 12–15(–15·5) × 5–6 (–7) μm. *Stigmidium triebeliae* is easily separated from *S. cartilagineae* by its vegetative hyphae that form a net of brown filaments spreading over the thallus of *Rimelia*, and by its BCr+ violet endoascus (Etayo 2000). As in the case of *S. cartilagineae*, several other species obligately grow in the hymenium of the host, for example *S. acetabuli* Calatayud & Triebel, *S. cerinae* Roux & Triebel, *S. collematis* Roux & Triebel, *S. congestum* (Körb.) Triebel, *S. lecidellae* Triebel, Roux & Le Coeur and *S. squamariae* (de Lesd.) Roux & Triebel (Roux & Triebel 1994; Roux *et al.*, 1995; Calatayud & Triebel 2001). The most similar of these species is *S. acetabuli* (host: *Pleurosticta acetabulum*), which has non-pseudotetrablastic ascospores of a similar size, and also lacks metachromatic reactions. It can be distinguished from *S. cartilagineae* by the I− reaction of the hymenial gel, smaller ascomata, slightly smaller ascospores, and constantly 8-spored asci (Table 2) (Calatayud & Triebel 2001). *Stigmidium acetabuli* should be referred to *Stigmidium* s. str., by virtue of its visible pendant interascal filaments (pseudoparaphyses of 'type a', sensu Roux & Triebel 1994), and compared with *S. collematis* (another taxon of this group restricted to the apothecial discs of the host and with non-pseudotetrablastic ascospores), to be consistent with the keys by Roux & Triebel (1994). However, a comparative study of *S. acetabuli* and *S. cartilagineae*, together with *S. squamariae*, has led us to conclude that these three species are probably very closely related, despite the apparent lack of visible pendant interascal filaments in *S. squamariae*, a feature regarded as a characteristic of the so-called *S. squamariae* group rather than *Stigmidium* s. str. (Roux & Triebel 1994). Although we could not detect any well developed filaments in *S. squamariae*, what seem to be incipient pendant filaments have been occasionally observed in some ascomata. Thus, the limits between both groups become more diffuse, although these three species have colourless vegetative hyphae (in *Stigmidium* s. str. they are usually pale brown), lack metachromatic reactions

(which frequently occur in *Stigmidium* s. str.), and have neither non-pseudotetrablastic nor elongated ascospores (in many species of *Stigmidium* s. str., they are pseudotetrablastic and relatively elongated). The main differences among these three species are the size of ascomata and ascospores, the reaction of the hymenial gel with I, the number of ascospores per ascus, and the host species (Table 2).

Additional specimen examined. **Macedonia**: Ohrid, supra lacum Ohridsko ezero prope vicum Elsani haud procul Peštani, (on *Squamarina cartilaginea*), in rupibus calcareis, supra terram et ad saxa, 980 m, 10 vii 1975, *A. Vězda* (M).

The 'Stigmidium' psorae group

Ascomata perithecioid, black. *Wall* pseudoparenchymatous, of *textura angularis*, brown. Ostiolar filaments (=periphyses) present or absent. Short pendant interascal filaments (=pseudoparaphyses sensu Roux & Triebel 1994; periphysoids sensu other authors) absent. *Interascal filaments* (most probably paraphysoids sensu Roux & Triebel 1994) long, up to 3 µm wide, branched and anastomosing, but usually rudimentary and compressed between the asci. *Asci* clavate or saccate, sessile or shortly stipitate, wall thickened at the apex and with a conspicuous ocular chamber with distinct nasse, bitunicate, dehiscence might occur by extension of the endoascus and gelification of the top of the exoascus (see Roux & Triebel 1994: 465), I−, with up to 8 spores. *Ascospores* hyaline, 1-septate, with a gelatinous sheath; the oldest ascospores sometimes brown, 1–3(–5)-septate, with a granulose surface.

Conidiomata producing bacilliform conidia.

Vegetative hyphae colourless.

Remarks. The '*Stigmidium*' *psorae* group comprises *S. psorae* and two related species described in this paper, *S. rouxianum* and *S. squamarinicola*. It shares many features with *Stigmidium* s. str., for example the ascomatal wall structure, the clavate or saccate, I− asci and mostly 1-septate hyaline ascospores, but differs in having long interascal hamathecial elements (most probably paraphysoids sensu Roux & Triebel 1994; see also Table 1). The hamathecium of many *Stigmidium* species has been studied in detail by Roux & Triebel (1994) in their revision of the species corresponding to *Pharcidia epicymatia* sensu Keissler or to *Stigmidium schaereri* auct. They found that *Stigmidium* species show short pendant interascal filaments formed by a basal cell and an elongated apical one (*Stigmidium* s. str.), filaments formed by 3–5 subequal cells (*S. placynthii* group), or filaments absent or at least not visible (*S. squamariae* group). All three groups have no long interascal filaments. In contrast, we found a net of branched and even anastomosing ± long interascal filaments in the three species of the '*Stigmidium*' *psorae* group. As the type of development of hamathecial elements is suggested to be very relevant from a systematic point of view, their interpretation is important. Within the '*Stigmidium*' *psorae* group they might be interpreted as filaments growing downwards to the base of asci, secondarily anastomosing and attaching to the subhymenium (pseudoparaphyses) or (more probably) as interascal filaments fixed primarily at the base and top of the ascoma cavity (paraphysoids) (see Table 1). The outer appearance of the filaments recalls certain genera of the *Dacampiaceae*. The genus *Sphaerellothecium*, for example, develops similar rudimentary paraphysoids. Its ascomata, however, are brown even at their lower part, and they are connected by a conspicuous net of brown vegetative hyphae. These hyphae, when growing superficially on the host thallus or apothecial discs, have a relatively thick and frequently sculptured wall. In the three species of the '*Stigmidium*' *psorae* group, the ascomata are frequently colourless below and the vegetative hyphae are also colourless. The recently described genus *Wernerella* (Navarro-Rosinés *et al.*, 1996, 1998), differs from *Sphaerellothecium* and *Stigmidium* in that its ascomata are initially perithecioid but finally apothecioid. In *Polycoccum* and *Pyrenidium* (*Dacampiaceae*), the net of interascal filaments is better developed and the ascospores are clearly brown. In our opinion, the three species of the '*Stigmidium*'

psorae group most likely represent a separate entity which might be regarded as a genus of the *Dacampiaceae* related to *Sphaerellothecium*. However, at present we consider it more prudent not to describe a new genus until a revision of the whole group including molecular phylogenetic studies is available. In addition, the type of development of the interascal elements and systematic significance of this as well as of other morphological characters involved should be studied more closely.

All species of the '*Stigmidium*' *psorae* group lack the metachromatic reactions (BCr+) of the endoascus, ascospore wall and hyphae; only in some old, brownish ascospores is the outermost part of the wall BCr+ blue. Up to now this reaction has been taxonomically useful only for species delimitation within *Stigmidium*. The same applies to the reaction of the endoascus with CR. All three species are CR+ orange (except in the apical zone, where it was CR− or less intense orange). This positive reaction of the endoascus with CR has also been reported for some *Stigmidium* species previously tested (Matzer 1996; Calatayud & Triebel 1999, 2001). Two species of the group, *S. squamarinicola* and *S. psorae*, eventually showed a I+ violet reaction of the hymenial gel. As discussed by Calatayud & Triebel (1999) and above (see remarks on *S. cartilagineae*), this reaction is not restricted to this group, as among pyrenocarpous lichenicolous fungi it also occurs in other taxa of *Stigmidium* and in *Polycoccum* (Calatayud & Atienza 2000).

Stigmidium psorae (Anzi) Hafellner

In Vězda, *Lich. sel. exsicc.* Fasc. 79 (no 1951–1975): 7 (1984)
For synonyms see Triebel (1989: 83).

Remarks. This species was described from Alpisella (Alpi Bormesi), from the squamules of *Psora decipiens* (Anzi 1868), and was transferred to *Stigmidium* by Hafellner (see Vězda 1984). It is a lichenicolous fungus commensalistic on *Psora* species: *P. crenata*, *P. decipiens* and *P. saviczii*. A more recent description and a drawing of the ascospores are given in Triebel (1989), and further illustrations and comments on the species are provided by Calatayud & Triebel (1999). The ascomata are immersed in the host thallus or erumpent, globose; in section the upper part is dark brown, and the lower part is medium or pale brown to almost colourless. The hymenial gel is frequently I+ violet, although this reaction may be absent in some specimens (Triebel 1989; Calatayud & Triebel 1999); the reaction probably depends on factors such as the amount of hymenial gel, age of the ascomata or time elapsed since the material was collected. The interascal filaments (although usually rudimentary) are branched and anastomosing, up to 3 µm wide. They have previously been observed by Vouaux (1912) [sub *Pharcidia psorae*], and more recently by Triebel (1989) and Calatayud & Triebel (1999). The asci are 8-spored, broadly cylindrical, clavate or ± saccate, and the endoascus is BCr−. The ascospores are mostly 1-septate, with or without a gelatinous sheath, colourless and with a BCr− wall, but finally they may become brown and 1–3-septate (rarely 4–5-septate), then reacting BCr+ blue in the outermost part of the wall. The vegetative hyphae are colourless. In Table 3, *S. psorae* is compared with the other two species of the '*Stigmidium*' *psorae* group, *S. rouxianum* and *S. squamarinicola*. It is clearly characterized by its larger asci and ascospores from the two other taxa.

Additional specimens examined. **Austria**: *Kärnten*: Hohe Tauern National Park, the Ankogel group, slope E of the Hagener hut towards the Greilkopf, NW of Mallnitz. 47°07′30″N, 13°05′45″E, alt. 2350–2450 m, [on *Psora decipiens*], on earth in crevices. 1989, *R. Türk & J. Hafellner* 23980 (M, Santesson, *Fungi Lichenicoli Exs.* no. 292). *Ost-Tirol*: Innergschlöß, N-Grat des Inneren Knorrkogels, alt. 2780 m., [on *Psora decipiens*], 31 viii 1988, *H. Wittmann* (M).—**Spain**: *Prov. Castelló*: Els Ports, *c.* 7 km SE of Cinctorres, Bnc. Cana, 40°33′N, 0°9′W, [on *Psora decipiens*], on carbonated soil, 900 m, 6 ii 2001, *V. Calatayud* (hb. Calatayud 124)

Stigmidium rouxianum Calatayud & Triebel sp. nov.

A specie *Stigmidium psorae* (Anzi) Hafellner praecipue differt ascosporibus minoribus, (14–)14·5–18(–21) ×

TABLE 3. *Characters distinguishing the species of the* 'Stigmidium' psorae *group*

Character	*S. psorae*	*S. rouxianum*	*S. squamarinicola*
Ascomatal diam. (μm)	(100–)125–150(–200)	80–200	85–120
Hymenial gel	I+violet or I−	I−	I+violet or I−
Number of ascospores per ascus	8	8	(4–)6–8
Asci (μm)	54–75(–80) × 19–25	55–70 × 17–21	38–54(–66) × 12–15(–18)
Ascospore size (μm)	(16–)17·5–22(–23·5) × (5–)5·5–7·5(–8)	(14–)14·5–18(–21) × (5–)6–7(–8)	(11–)13–15(–17) × 5–6·5(–7)
Ascospore gelatinous sheath	Sometimes present	Usually present	Sometimes present
Host	*Psora* species	*Acarospora cervina*	*Squamarina* species
Source	Triebel (1989)	Present paper	Present paper

(5–)6–7(–8) μm, et gelatina hymenii jodo non reagens. A specie *Stigmidium squamarinicola* Calatayud & Triebel praecipue differt ascomatibus et ascosporis majoribus, ascis 8-sporis, et gelatina hymenii jodo non reagens. In thallo raro apotheciisque lichenis *Acarospora cervina* crescit.

Typus: Spain, Aragón, Prov. Teruel, Sierra de Javalambre, La Puebla de Valverde, Escarbadero de los Lobos, U.T.M. 30TXK7043, *c.* 1700 m, on the thallus and apothecia of *Acarospora cervina*, on calcareous rocks, 20 September 1997, *V. Calatayud & P. L. Nimis* (M–0031464—holotypus; hb. Calatayud 123—isotypus).

(Figs 2A–L, 4E & F)

Ascomata perithecioid, black, globose, 80–200 μm diam., scattered or partly aggregated, largely immersed in host thallus, rarely in apothecia; the infection associated with slightly swollen areas in the host thallus. *Wall* pseudoparenchymatous, of *textura angularis*, half upper part dark brown, lower part hyaline or pale brown, *c.* 8–10 μm wide and with 3–4 layers of cells at the base. Cells of the wall ± polygonal, mostly 5–8 μm diam. (in superficial view of the ascomata), I−. Hymenial gel I−, K/I−. External periphyses (sensu Roux & Triebel 1994) sometimes conspicuous. Pendant interascal filaments (=pseudoparaphyses sensu Roux & Triebel 1994; periphysoids sensu other authors) absent. *Interascal filaments* (paraphysoids) long, relatively abundant, branched and anastomosing, with relatively short and 1–3 μm wide cells. *Asci* 8-spored, *c.* 55–70 × 17–21 μm, wall thickened at the apex and with a conspicuous apical chamber, clavate or saccate, sessile or shortly stipitate, ascospores distichously arranged in the asci, I−. *Ascospores* mostly hyaline, 1-septate, slightly constricted at the septum or not, obtuse, lower cell narrower than the upper one, usually with a gelatinous sheath *c.* 1–2 μm thick, surface smooth, not distinctly guttulate, ascospore wall BCr−, (14–) 14·5–*16·3*–18(–21) × (5–)6–*6·4*–7(–8) μm [length/width index: (1·8–)2·1–*2·5*–2·9(–3·2); $n = 49$]; old ascospores sometimes brown, 1–3-septate, constricted at the medium septum or not, with a granulose surface.

Vegetative hyphae colourless, I−.

Etymology. We dedicate this species to Claude Roux, in recognition of his outstanding contribution to the genus *Stigmidium* and related genera.

Host. Mostly on the thallus, but sometimes also on the apothecial discs of *Acarospora cervina* A. Massal. *Stigmidium rouxianum* is usually more abundant in the largest areoles of the host. Occasionally, the infected areoles are slightly deformed, so that it is possible that the fungus has a slightly damaging or adverse effect on the host. In section, the algal layer of the thallus of *Acarospora cervina* typically has a serrate shape, with zones where the algae are rather close to the surface ('crests') interrupted by other zones where the cortex extends

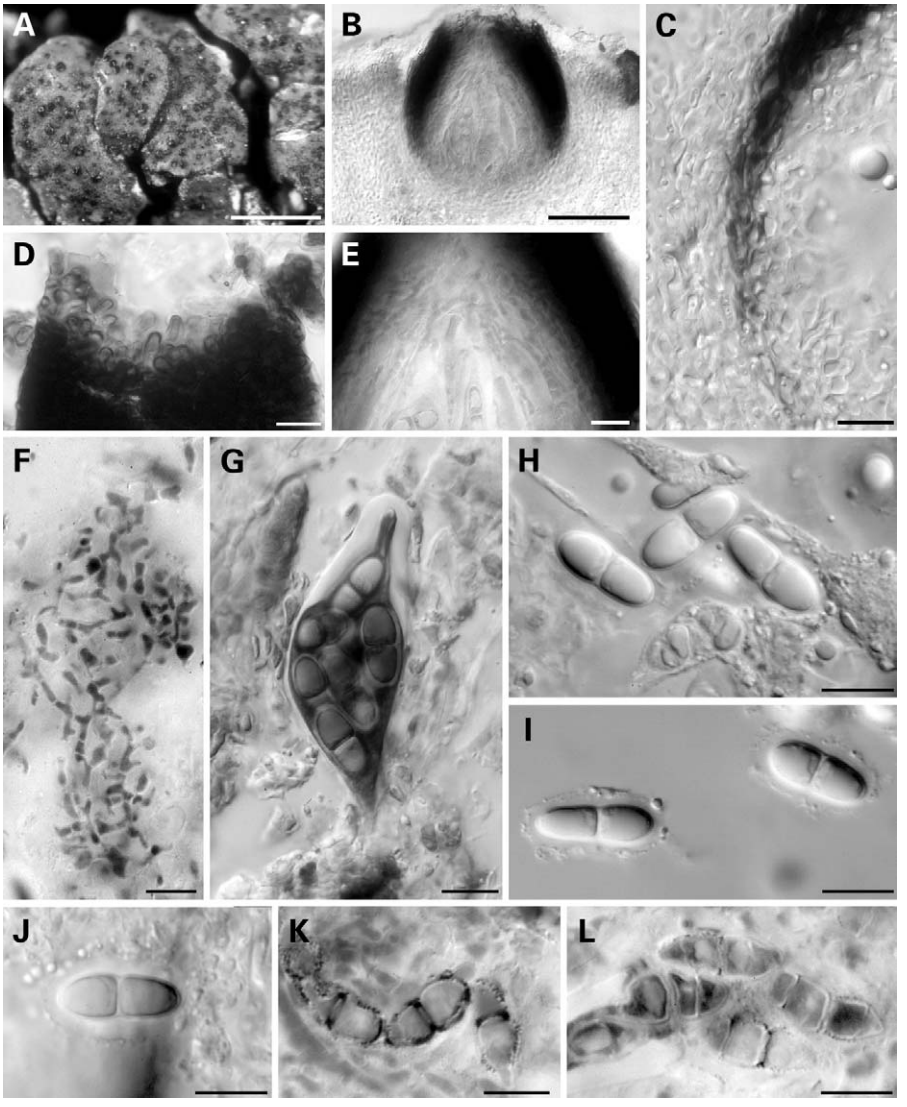

FIG. 2. *Stigmidium rouxianum* (holotypus). A, habit; B, transverse section of an ascoma; C, lateral wall of an ascoma; D, external periphyses, around the ostiole, squash preparation; E–F, interascal filaments in lactophenol cotton blue; G, an ascus in I; H–J, ascospores showing the gelatinous sheath, in water; K, old brown ascospores; L, old, brown 3-septate ascospores in lactophenol cotton blue. Scales: A = 1 mm; B = 50 μm; C–L = 10 μm.

downwards and the algal cells are more distant from the surface of the thallus ('valleys'). Interestingly, the ascomata of *S. rouxianum*, which develop in the upper part of the host thallus, are usually associated with such 'crests', where the algae are closer to the surface.

Ecology and distribution. The type collection of *Stigmidum rouxianum* is from Spain (Aragón, Prov. Teruel), at *c.* 1700 m, on calcareous rocks in a clearing of a *Pinus sylvestris* forest. In addition, three other collections from France, Italy, and Switzerland have been examined, all from the same host.

Remarks. In this species, the size of the ascomata and ascospores is intermediate between *S. squamarinicola* and *S. psorae* (Table 3). As in *S. psorae*, the asci are constantly 8-spored, and, at least in the material examined, the hymenial gel does not react after applying Lugol's solution, while an I+ violet reaction frequently occurs in the two other species of '*Stigmidium*' *psorae* group. Specimens of *S. rouxianum* with a very reduced net of interascal filaments might be confused with two other *Stigmidium* species occurring on *Acarospora*, *S. fuscatae* (Arnold) R. Sant. on *Acarospora* subgen. *Phaeothallia* and *S. epixanthum* Hafellner on *Acarospora* subgen. *Xanthothallia* (Hafellner *et al.* in press). In the material of *S. fuscatae* examined (including the holotype in M), the ascomata are abundant, mostly semi-immersed in the thallus, sometimes in the apothecia, *c.* 50–100 µm, centrum I−, with conspicuous pendant interascal filaments (pseudoparaphyses of 'type a'), 8-spored asci, 30–40 × 10–15 µm, BCr− and CR+ orange (apart from the apical region) endoascus, non-pseudotetrablastic ascospores, (8–)10–12 × 4–5(–5·5) µm, wall BCr−, usually with one large oil droplet per cell and without a gelatinous sheath. The vegetative hyphae are colourless. The much smaller size of the ascospores in this species allows an easy separation from *S. rouxianum*, and both taxa differ also in the ecology of their host species, *Acarospora fuscata* being a lichen growing on siliceous rocks and heavy metal rich boulders. The specimen examined of *Stigmidium epixanthum* on *Acarospora lavicola* has ascomata mostly inserted in the margins of the host areoles, prominent, with only the base immersed, *c.* 100–120 µm wide, wall dark brown, centrum I−, with conspicuous pendant interascal filaments (pseudoparaphyses of 'type a'), 8-spored asci, 45–60 × 13–15 µm, BCr− endoascus, non-pseudotetrablastic ascospores, 11–17 × 5–7 µm, wall BCr−, usually with one large oil droplet per cell. This species differs from *S. rouxianum* not only by its type of hamathecium but also by its prominent ascomata, inserted towards the margin of the host areoles, with darker ascomatal walls, and by its smaller ascomata, asci and ascospores. *Sphaerellothecium gowardii* Alstrup and M. Cole (on *Acarospora schleicheri*) has much smaller ascomata (40–60 µm diam.) connected to a net of superficial brown vegetative hyphae, and has larger ascospores (Alstrup & Cole 1998). *Zwackhiomyces argentinae* (Räsänen) D. Hawksw. & V. Atienza (on *Acarospora* sp.), according to the recent description given by Hawksworth & Atienza (1994) differs in a series of characters: sessile or half immersed ascomata, wall of the ascomata never hyaline at the base and with the pigment forming fine granules in the intercellular areas, and smaller ascospores (14–16 × 5–6 µm) with a finely verruculose epispore.

Additional specimens examined. **France**: *Alpes-Maritimes*: Sommet au N du Col de Valberg, [on *Acarospora cervina*], calcaires du Lias moyen, 1700 m, 17 iv 1954, ? *C. Roux* (scripsit) & *J. Poelt* (M).—**Italy**: *Trentino Alto–Adige*: Prov. Bolzano, Vintschgau, S-seitige Trockenhänge bei Laus, (on *Acarospora cervina*), 10 x 1953, *J. Poelt* (M).—**Switzerland**: *Kt. Wallis*: Burgberg Tourbillon in Sion, [on *Acarospora cervina*], 600 m, 1978, *V. John* 6886 (M).

Stigmidium squamarinicola Calatayud & Triebel sp. nov.

A specie *Stigmidium psorae* (Anzi) Hafellner praecipue differt ascomatibus minoribus, 85–120 µm diametro, ascosporibus minoribus, (11–)13–15(–17) × 5–6·5 (–7) µm, et ascis (4–)6–8-sporis. In thallis specierum diversarum generis *Squamarina* crescit.

Typus: Spain, Comunidad Valenciana, Prov. València, La Pobla de Vallbona, Pla dels Alchups, 39°38′N, 0°32′W [=U.T.M. 30SYJ1290], alt. 150 m, on the thallus of *Squamarina cartilaginea*, on carbonated clayey soil, 6 October 2000, *V. Calatayud* (M–0031465–holotypus; hb. Calatayud 125—isotypus).

(Figs 3A–H, 4C & D)

Ascomata perithecioid, dark brown, globose or subglobose, 85–120 µm diam., scattered, immersed in host thallus, the infection not producing bleaching nor any visible alteration to the host. *Wall* pseudoparenchymatous, of *textura angularis*, upper half dark brown, lower part usually hyaline, sometimes pale brown, *c.* 10–15 µm wide

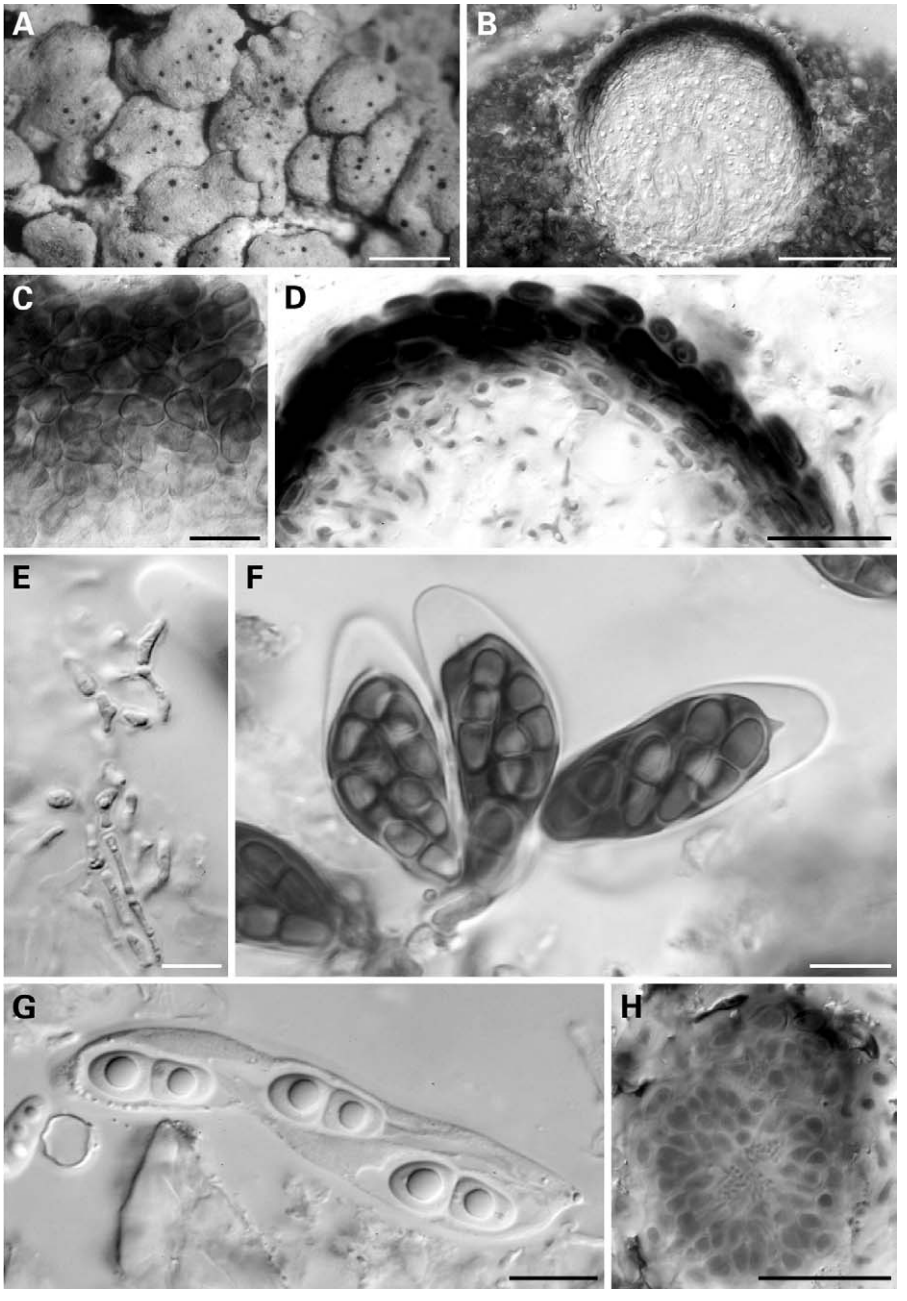

FIG. 3. *Stigmidium squamarinicola* (holotypus). A, habit; B, transverse section of an ascoma; C, wall of an ascoma in superficial view; D, interascal filaments in lactophenol cotton blue; E, detail of interascal filaments, after K/I; F, asci in I; G, ascospores; H, conidiogenous cells producing conidia in lactophenol cotton blue. Scales: A = 1 mm; B = 50 μm; C = 10 μm; D = 20 μm; E–G = 10 μm; H = 50 μm.

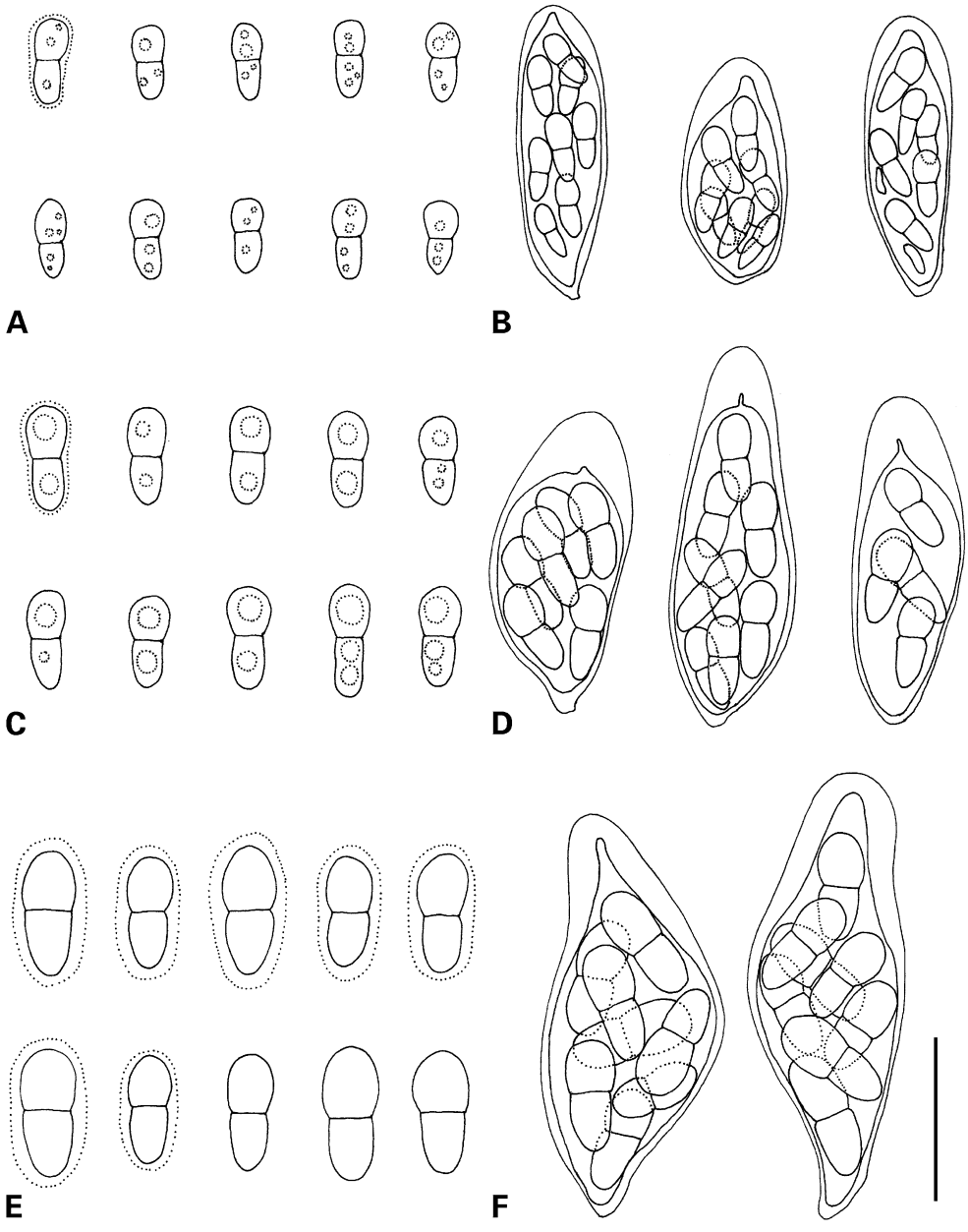

FIG. 4. *Sligmidium* species, asci and ascospores in water. A & B, *S. cartilagineae* (holotypus); A, ascospores, one of them showing the gelatinous sheath; B, asci, two with 8 ascospores and one with 6 viable and 2 aborted ascospores. C & D, *S. squamarinicola* (holotypus); C, ascospores, one of them showing the gelatinous sheath; D, asci, containing 4, 6 and 8 ascospores. E & F, *S. rouxianum* (holotypus); E, ascospores, most of them with a gelatinous sheath; F, two asci. Scale: 20 μm.

and with 3–4 layers of cells at the base. Cells of the wall ± polygonal, mostly 5–9 µm diam. (in superficial view of the ascomata), I− or partly I+ violet. Hymenial gel I+ violet, K/I+ violet or K/I−. External periphyses (sensu Roux & Triebel 1994) not conspicuous. *Interascal filaments* (paraphysoids) long but sometimes rather inconspicuous, branched and anastomosing, with relatively short and 1–2·5(–3) µm wide cells. *Asci* (4–)6–8-spored, *c.* 38–54 (–66) × 12–15(–18) µm, wall thickened at the apex and with a conspicuous apical chamber, clavate or ± saccate, sessile or shortly stipitate, ascospores distichously arranged in the asci, I−; endoascus BCr−. *Ascospores* 1-septate, constricted at the septum, lower cell narrower than the upper one, colourless or rarely brown when old, often with a gelatinous sheath, surface smooth, with 1(–2) large oil droplets per ascospore cell, ascospore wall BCr−, (11–)13–*14*–15(–17) × 5–*5·9*–6·5(–7) µm [length/width index: (2–)2·2–*2·4*–2·6(–3·2); n = 52].

Conidiomata rare (observed only once), subglobose, upper part brown, lower part colourless, *c.* 75 µm wide × 60 µm high; conidiogenous cells ampulliform or ellipsoid, *c.* 4–6 × 3–4 µm; conidia bacilliform, 3–4 × 0.5 µm.

Vegetative hyphae colourless, I−.

Host. On the thallus of *Squamarina cartilaginea* (With.) P. James, *S. gypsacea* (Sm.) Poelt and *S. lentigera* (Weber) Poelt without causing any apparent injury.

Etymology. The epithet '*squamarinicola*' refers to the host genus.

Ecology and distribution. *Stigmidium squamarinicola* has been collected on the thalli of *Squamarina*. It is probably a commensalistic or weakly parasitic fungus, since the infection does not seem to cause any visible injury to the host. *Stigmidium squamarinicola* is known from several localities in Spain, Israel, Switzerland, and perhaps France. In the type locality, several other species occurred on *Squamarina* species, including *Clypeococcum epicrassum, Lichenochora clauzadei* (Navarro-Rosinés *et al.* 1994), and *Lichenostigma rouxii* (Calatayud *et al.* 2002).

Remarks. This species is clearly characterized by being the taxon of the '*Stigmidium*' *psorae* group with the smallest ascomata, asci and ascospores (Table 3). It also differs from the other two species, *S. rouxianum* and *S. psorae*, by its frequently 6-spored asci (in the other species the asci are constantly 8-spored). In the material examined, 6-spored asci were the most abundant but, in the same ascomata, 8-spored asci were also frequent, and 4-spored asci were sometimes found. In *S. squamarinicola*, the I+ violet reaction in the hymenial gel is relatively inconspicuous, being usually restricted to the hymenial gel surrounding the interascal filaments and to small parts of the ascomatal wall. This reaction has also been observed in part of the material of *S. psorae* (see above). *Stigmidium cartilagineae*, also described in this paper from the same host genus, can be easily separated from *S. squamarinicola* by its occurrence only on apothecial discs of the host, by its different type of hamathecium, and by its clearly smaller asci and ascospores.

Additional specimens examined. **Israel**: Negev: near Ein-Ovdat, (on *Squamarina lentigera*), on calcareous soil, 23 ii 1964, *J. Fridman* (M).—**Switzerland or France**, ad rupes calcareas Jurassi in monte Chasseron, *Lesquereux*, et in Cebennis (les Cévennes), *Prost*, (on *Squamarina gypsacea*), no date (M, Mougeot, Nestler & Schimper, *Stirp. crypt. Vog.-Rhen.* no. 1148 sub *Lecanora smithii*).—**Spain**: *Andalucía*: Prov. Almería, zona de Tabernas, cerca de Los Yesos, 37°05′N, 2°17′W, *c.* 500 m, (on *Squamarina lentigera*], sobre suelo yesoso, 16 ix 2000 *V. Calatayd & C. Trescoli*, (hb. Calatayud 139). *Comunidad de Madrid*: Prov. Madrid, Aranjuez, Mar de Ontígola, *c.* 40°00′N, 3°37′W, alt. *c.* 500 m, (on *Squamarina lentigera*), sobre suelo rico en yeso, 28 vi 2001, *V. Calatayud* (hb. Calatayud 113). *Navarra*: Bàrdenas Reales, laguna de la Rada, suelo yesoso bajo pinar, 370 m, (on *Squamarina lentigera*), 14 iii 1998, *J. Etayo* (hb. Etayo 14963).—**Switzerland**: *Kt. Wallis*: ad terram apricam in Vallesia inf., (on *Squamarina lentigera*), no date, no collector (M, Schaerer, *Lich. Helv. exs.* Ed. I no. 343 sub *Parmelia crassa β. caespitosa*).

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