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A new species of *Stigmidium* (*Mycosphaerellaceae*, Ascomycetes) from western North America

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The new lichenicolous fungus *Stigmidium hesperium* is described from *Caloplaca* species from western North America.

Key words: California, lichenicolous fungi, Mexico, *Mycosphaerella*, systematics, taxonomy.

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Lichenikolní houba *Stigmidium hesperium* je popsána z *Caloplaca coralloides* ze západu Severní Ameriky.

INTRODUCTION

Stigmidium Trevis. is a genus of ascomycetes comprising at least 89 binominals (Robert et al. 2009, Etayo 2008, Zhurbenko 2009, Kocourková and Knudsen 2009) and is currently included in the *Mycosphaerellaceae* (Lumbsch and Huhndorf 2007). The generic type is *S. schaereri* (A. Massal.) Trevis., a lichenicolous species occurring on sterile thalli of *Solorina* species. Most species of *Stigmidium* are lichenicolous, though Aptroot (2006), using a broad concept of the genus, has recently added *S. parasiticum* (G. Winter) Aptroot, parasitic on the fungus *Godronia fuliginosa*, *S. ascophylli* (Cotton) Aptroot, parasitic on the brown seaweed *Pelvetia canaliculata*, and *S. apophlaeae* (Kohlm.) Aptroot, parasitic on the red alga *Apophlaea lyalli*.

In *Mycosphaerellaceae*, the genus *Stigmidium* in the strict sense is distinguished by perithecioid ascomata with punctiform ostioles with a hamathecium of periphyses, with periphysoids, and hyaline 1-septate ascospores (rarely turning

brown in a few species) (Roux and Triebel 1994, Calatayud and Triebel 2003). The periphyses may be described as external periphyses when they form a distinct fringe around the ostiole of filaments derived from the perithecial wall (Roux and Triebel 1994). Internal periphyses line only the ostiole passage. The distinction between external and internal periphyses may be an important taxonomic character in the segregation of some species currently included in *Stigmidium* s. l. which lack distinct external periphyses as well as in the taxonomy of other genera such as the lichenicolous *Neolamya* (Kocourková 2009). Periphysoids are short filaments originating from the wall of the upper cavity of the perithecia and are never attached to the bottom of the cavity. They are referred to as pseudoparaphyses of which two types are recognized (Roux and Triebel 1994) but generally called periphysoids by many authors. Type A periphysoids are narrow and two-celled and characterize the group that Roux and Triebel (1994) considered as *Stigmidium* s. str., based on generic type *S. schaereri*. Type B periphysoids are long and relatively large, made up of 3–5 more or less equal cells, and characterize the "*S. placynthii* group." Type B periphysoids are especially hard to observe and best seen in younger perithecia and may disintegrate when the ascomatal cavity is filled with mature asci. For instance, *S. squamariae* (B. de Lesd.) Roux & Triebel was considered to have no periphysoids (Roux and Triebel 1994) but further material allowed the discovery of Type B periphysoids (Roux and Triebel 2005). *Stigmidium* species usually lack interascal filaments. The *Stigmidium psorae* group has interascal filaments, may or may not have ostiolar periphyses, and lacks periphysoids, and should eventually be segregated from the genus (Calatayud and Triebel 2003). Some species have been transferred to or described in *Stigmidium*, in which no hamathecium has been observed, for instance *S. cladoniicola* Zhurbenko & Diederich (Zhurbenko and Diederich 2008).

Due to a lack of standardized morphological nomenclature coupled with differences in taxonomic descriptions and generic concepts of *Stigmidium* by different authors, many *Stigmidium* species have overlapping ascospore and ascomata size, forcing keys to be structured according to hosts (Hawksworth et al. 2008, Ihlen and Wedin 2008) rather than by individual morphological characters.

New species of *Stigmidium* are sometimes described from only the holotype or a few additional specimens. Some characters may not have been observed at all because of the lack of material, as for instance in the case of periphysoids in *S. squamariae*. Or the authors could not gauge the full range of variation within a taxon. For instance, *S. epistigmellum* (Nyl. ex Vouaux) Kocourk. & K. Knudsen was described from a single specimen (Vouaux 1912). When we were able to study over twenty specimens and compare them with the holotype, the ascospore size given by Vouaux was found to be on the low end of the natural variation of ascospore size found in the species because the ascospores in the holotype were predominately immature (Kocourková and Knudsen 2009).

Coupled with divergent concepts of the genus is a host-based taxonomy of *Stigmidium*: "Species concepts in the genus *Stigmidium* Trevis. require a thorough re-appraisal as the taxonomy is currently largely host-based." (Hawksworth 1986). It is possible that there may be some species of *Stigmidium* which are morphologically indistinguishable and are actually conspecific and are generalists that occur on multiple species in different genera or families. But so far species of *Stigmidium* appear to be host specific on the family, genus, or specific levels.

Despite the taxonomic problems involved in the morphological study of *Stigmidium*, one must treat host selection as a primary character in symbiont genera, whether species are generalists or have narrower host ranges. The symbiont-host relationship is developed over evolutionary time and may be indicative of significant cryptic differences that are accessible to molecular analysis and possibly expressed in morphological characters not valued by current taxonomic description or originally observed in the type specimens. We believe that this is especially true in the study of *Stigmidium*, but also in other poorly understood lichenicolous genera, such as *Endococcus*.

It is obvious that the genus *Stigmidium* is in need of a comprehensive revision, based on the excellent work of Roux and Triebel (1994) and rooted in a developing knowledge of the family *Mycosphaerellaceae*. There are approximately 50–60 species or more in the genus that need to be revised.

In this paper we describe a new species of *Stigmidium* distinguished by Type B periphysoids, *S. hesperium*, which is lichenicolous on several species of *Caloplaca*. We will compare it with some species that are similar in morphological characters as well as with all species previously known from the same host genus, *Caloplaca*.

MATERIALS AND METHODS

Specimens were supplied by Paul Diederich, Pieter van den Boom, and Thomas Nash III (ASU).

Specimens have been examined using standard microscopical techniques. Hand-made sections were studied in water, 10 % KOH [K] and lactophenol cotton blue (LPB). Amyloid reactions were tested in Lugol's iodine 1 % with [I] and with pre-treatment with 5 % KOH [K/I]. Chromatic reactions were tested with 1 % solution of brilliant cresyl blue (BCr). Ascospore and asci measurements were made in water with an accuracy of 0.5 µm and given in the form "(minimum–) mean minus standard deviation–mean–mean plus standard deviation (–maximum)" and followed by the number of measurements (n); the length/breadth ratio of ascospores is indicated as l/b and given in the same way.

Macro- and microphotographs were taken with a digital camera Olympus C5050 on Olympus SZX 9 Stereomicroscope and Olympus BX 50 (to × 1250) fitted with a Nomarski differential interference contrast.

RESULTS

Stigmidium hesperium Kocourk., K. Knudsen & Diederich, **sp. nov.** Figs. 1–7.

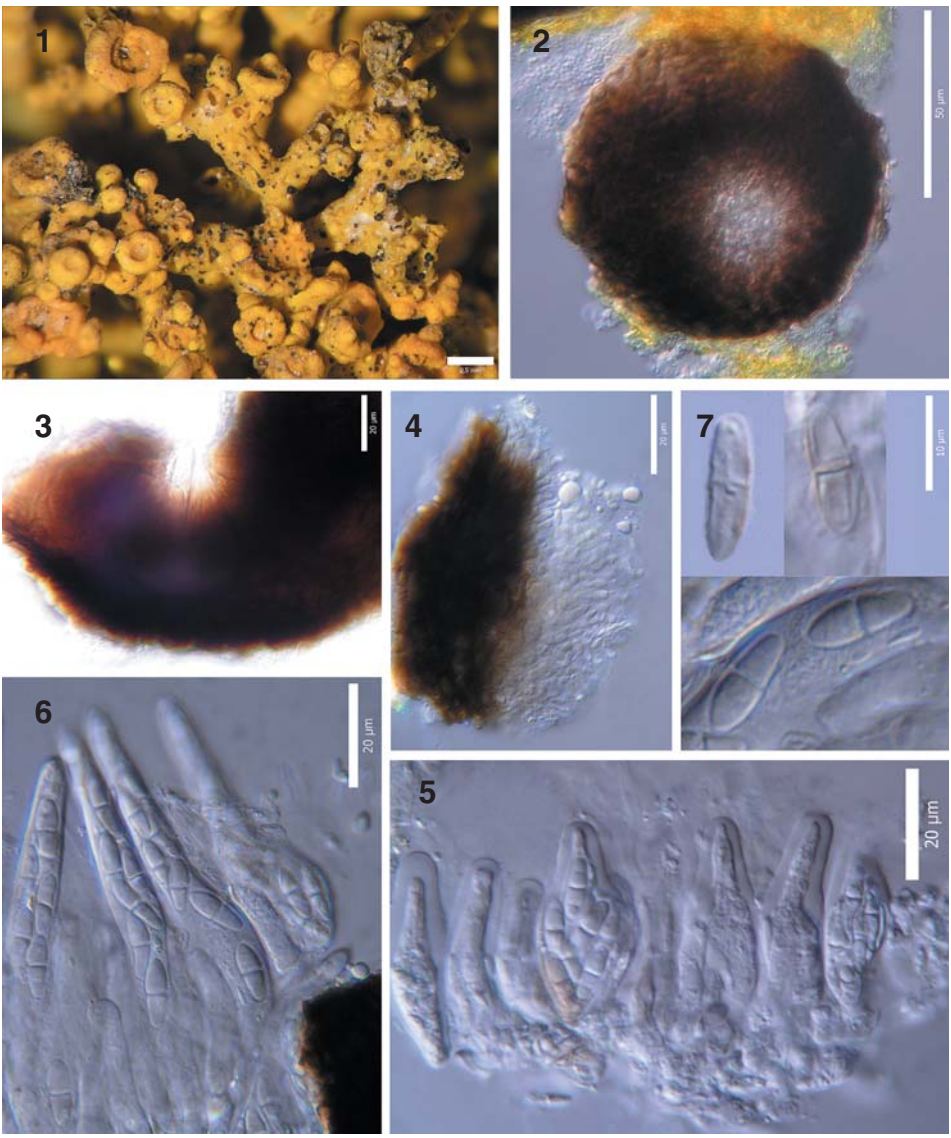
(MycoBank MB513340)

Fungus lichenicola, in thallis lichenum speciei *Caloplacae* parasiticus. Ascomata globosa ad subglobosa, semi-immersa ad sessilia, 40–90(–110) µm alta, (40–)55–80(–100) µm lata; hamathecium cum periphysibus exterioribus, 11–15 µm longis, 1,5–2,0 µm latis, et periphysoidibus typo B, hyphis inter ascos nullis, gelatina hymeniale I–, K/I–. Asci saccati, 6–8-spori, cum ascosporis irregulariter dispositis; endoascus apicaliter truncatus vel cum ascosporis maturis, denique "nasse" formatis, (28,5–)30,0–34,85–40,0(–44) × 12,0–14,0–16,0(–16,5) µm. Ascosporae 1-septatae, obiter cum pseudoseptis, hyalinae, sed usitate fuscae in hypermaturitate; non constrictae vel aliquot constrictae, cellulae aequales, cum cellula inferiore aliquanto angustiore; non-halonatae, non ornamentatae, (12,5–)13,0–13,75–14,5(–15,5) × (–3,5)4,0–4,5–5,0(–5,5) µm (n=20), l/b (2,5–)2,7–3,1–3,5(–3,6). Conidiomata non observata.

Type. U.S.A.: California, Monterey Co., along the coast S of Asilomar, China Rock, alt. 5 m a.s., saxicolous, on coastal rock, on *Caloplaca coralloides*, 19 July 2008, coll. P. Diederich 16787 & D. Ertz (BR, holotype; PRM 915594, hb. Diederich, isotypes; other isotypes are planned to be distributed in Hafellner, Lichenicolous Biota).

Etymology. The name, which means "western", refers to the region where the species was originally discovered in the western North America in the United States and Mexico.

Description. Vegetative hyphae not observed. Ascomata perithecioid, black, globose to subglobose, ostiolate, 40–90(–110) µm high, (40–)55–80(–100) µm wide, usually half-immersed to sessile on the host apothecia or thallus, causing bleaching and erosion of the host. Ascomatal wall dark brownish-black in upper 1/5, lighter brown or reddish brown in middle or lower 2/5–4/5, sometimes hyaline in lower 2/5–1/5, 10–15 µm thick in optical section, of 2–3 cell layers, of textura angularis, cells mostly 3–6 × 5–6 µm, sometimes variously shaped. Hamathecium of external periphyses sensu Roux and Triebel (1994), not as distinct as in some species, 11–15 µm long, 1.5–2.0 µm wide. Pendent periphysoids (type B) sensu Roux and Triebel (1994) originating from the upper wall of the ascomatal cavity, hyaline, branched or not, 12–18 µm long, mostly 2.0 µm wide, septate, formed of 3–5 cells, cells ca. 3 µm long, best observed in young ascomata. Interascal filaments lacking. Hymenial gel I–, K/I–, BCr– to BCr+ light violet. Asci originating from the lower wall of the ascomatal cavity, fissitunicate, saccate, the apex of endoascus distinctly truncate when ascospores are immature, eventually forming a distinct nasse when ascospores are mature, 6–8-spored, with ascospores irregularly arranged, (28.5–)30.0–34.85–40.0(–44) × 12.0–14.0–16.0(–16.5) µm, n = 10, I–, exoascus BCr–, endoascus BCr+ dark blue. Ascospores 1-septate, but occasionally with additional pseudosepta, hyaline, but occasionally turning brown when overmature, not constricted or slightly constricted at the



Stigmidium hesperium

Fig. 1. Infected thallus of *Caloplaca coralloides*. Scale = 0.5 mm.
Fig. 2. Ascoma. Scale = 50 µm.
Fig. 3. Ostiolum and external periphyses. Scale = 20 µm.
Fig. 4. Pendant periphysoids type B. Scale = 20 µm.
Fig. 5. Immature asci and mature asci with ascospores. Scale = 20 µm.
Fig. 6. Asci releasing ascospores. Scale = 20 µm.
Fig. 7. Mature ascospores and overmature brownish ascospore. Scale = 10 µm.

septum, cells usually equal or slightly unequal in length, lower cell usually slightly narrower, not halonate, without ornamentation, (12.5–)13.0–13.75–14.5(–15.5) × (3.5–)4.0–4.5–5.0(–5.5) µm (n = 20), l/b (2.5–)2.7–3.1–3.5(–3.6); ascospore wall BCr– to Bcr+ pale violet. Conidiomata not seen.

Substrate and ecology. On apothecia and thallus of *Caloplaca coralloides* (Tuck.) Hulting, *C. rosei* Hasse, *C. texana* Wetmore & Kärnefelt, and an undetermined *Caloplaca* species in Mexico, pathogenic, eventually eroding and bleaching the thallus and apothecia.

Distribution. Currently known from Monterey County on the central Californian coast (USA), and Baja California Sur (Mexico) in western North America.

Other specimens examined

MEXICO: Baja California Sur: 21 km NE Route 2 along secondary road to San Francisco de la Sierra, 27° 32' 30"N, 113° 5' 53"W, 500 m, on *Caloplaca texana* with no visible damage to host, 31 Dec. 1997, T. Nash 40012 (ASU); ibid., on *Caloplaca* species, T. Nash 40013 (ASU); east ridge crest of Guadalupe Mountains, along secondary road between Mission Guadalupe and San Juan de la Pila, 26° 53' 54" N, 112° 26' 7" W, 1200 m, on *Caloplaca* species, 3 Jan. 1998, T. Nash 39896 (ASU).

USA: California, Monterey Co.: Point Lobos State Reserve, Allen Memorial Grove, Cypress Grove Trail, on north vertical granite outcrops, 36° 21' 2" N, 121° 57' 00" W, 25 m, on *Caloplaca coralloides* on vertical north side of granite outcrop, 23 July 2002, P. van den Boom 29349 & 29370 (hb. van den Boom); Point Lobos Reserve, Sea Lion Point, overlooking Sea Lion Cove, 36° 31' 07" N, 121° 57' 14" W, on *Caloplaca coralloides*, 3 m, 14 May 2009, K. Knudsen 11130 (UCR); Pacific Grove, Ocean View Blvd, east of lighthouse near Esplanade Park, 36° 38' 1" N, 121° 55' 05" W, 10 m, on north side of vertical granite outcrop, 22 July 2002, P. van den Boom 29209 (hb. van den Boom); Pacific Valley, Los Padres National Forest, 35° 56' 42" N, 121° 28' 43" W, 20 m, on *Caloplaca rosei* on rock outcrop on ocean bluff, 12 May 2009, K. Knudsen 11069.1 (hb. Etayo, UCR).

DISCUSSION

The presence of pendent Type B periphysoids places *Stigmidium hesperium* in the *S. placynthii* group (Roux and Triebel 1994) in which we currently recognize five species including the one described in this paper (Roux and Triebel 1994, 2005; Roux and Navarro-Rosinés 1994; Kocourková and Knudsen 2009). *Stigmidium placynthii* Cl. Roux & Nav.-Ros. on *Placynthium nigrum* has shorter, narrower and halonate ascospores than *S. hesperium* [(7.5–)9.5–11.1–12.0(–13.5) × 3.0–3.3–3.5(–4.0)] (Roux and Triebel 1994). *Stigmidium epistigmellum* (Nyl. ex Vouaux) Kocourk. & K. Knudsen on maritime *Caloplaca* species has longer and narrower ascospores than *S. hesperium* [(14.5–)15.8–17.45–19.1(–21.5) × (3.5–)3.9–4.2–4.7(–5.0) µm] (Kocourková and Knudsen 2009). *Stigmidium squamariae* on *Lecanora* species has halonate ascospores, shorter and slightly narrower than *S. hesperium* [(8.5–)9.0–11.8–13.5(–14.0) × (3.0–)3.5–4.0–4.5 µm] (Roux and Triebel 1994). *Stigmidium clauzadei* Cl. Roux & Nav.-Ros. on *Verrucaria nigrescens* and *V. viridula* has infrequently halonate ascospores that are longer and often wider

than *S. hesperium* [(10–)12.5–15.1–17.5(–19.5) × (4.5–)5.0–5.5–6.5(–7.5)] (Roux and Navarro-Rosinés 1994). The small differences in ascospores size are an important morphological expression of phylogenetic distance. In the oldest descriptions of *Stigmidium*, in which ascospores were not statistically calculated, ascospore ranges are too broad and these species need to be revised.

There is no need to compare *S. hesperium* (with type B periphysoids) with the 21 species of *Stigmidium* with type A periphysoids (Roux and Triebel 1994; Roux et al. 1995, 1998; Calatayud and Triebel 2001, 2003; Triebel and Cáceres 2004; Zhurbenko and Triebel 2009), as these differ in morphology of periphysoids, having two unequal cells compared to the 3–5 usually equal cells of Type B periphysoids. Nor is it necessary to compare *S. hesperium* with species without an observed hamathecium or with interascal filaments.

In addition to *S. epistigmellum*, two other species are currently known from *Caloplaca* species. *Stigmidium cerinae* Cl. Roux & Triebel described from bryophilous *Caloplaca stillicidiorum* differs from *S. hesperium* especially in having Type A periphysoids. *Stigmidium johnii* Halici & D. Hawksw. was recently described from a saxicolous *Caloplaca* in Turkey, and has a hamathecium of only internal periphyses (Halici, pers. comm.; Halici and Hawksworth 2007). Apparently the species lacks periphysoids, but if Type B periphysoids were present, they may have been hard to observe, and the description is based only on the holotype, limiting the number of observations, and no new specimens have been collected yet. Nonetheless, *S. johnii* has slightly longer and definitely wider ascospores than *S. hesperium* [(12.5–)13.0–16.5 × 5.0–6.5 µm].

Though *S. epistigmellum* and *S. hesperium* occur in maritime habitats in California and Mexico, so far they share only one host in common, *Caloplaca rosei* (Kocourková and Knudsen 2009).

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