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Standard Paper

Long sigmoid and twisted ascospores in the genus *Harpidium*: *H. longisporum* sp. nov., a synopsis of the genus and a key to the species

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Abstract

Harpidium longisporum is proposed as a new species. It is characterized by an areolate, mainly black thallus with trebouxioid algae, K+ blue-purple pigmented parts, pycnoascocarps forming aspicilioid apothecia, with moniliform paraphyses, unitunicate-rostrate, thick-walled asci and long sigmoid, lunate to falcate or irregularly curved and twisted ascospores, growing on steps of a vertical, intermittently moist, gneiss rock face. The genus *Harpidium* now comprises four species worldwide and, based on the selected specimens, a genus synopsis, a comparative table and a key to the species are included.

Key words: *Harpidiaceae*, lichens, new species, Spain, taxonomy

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Introduction

Harpidium Körb. is a poorly known crustose lichen genus, with trebouxioid algae, growing in rain tracks on siliceous rocks. Originally established as monospecific (Körber 1855), with *Harpidium rutilans* Körb. as the type species, it was first collected in southern Poland, extending its known distribution, years later, to the Mediterranean region and dry-warm areas of Central Europe (e.g. Szatala 1930; Tavares 1949; Sancho & Crespo 1983; van den Boom 1992; Kossowska & Fabiszewski 2004; Hafellner & Türk 2016; Nimis *et al.* 2018; Nimis & Martellos 2020). Most recently and based on morphological and anatomical characters, two new species of *Harpidium* were described: *Harpidium nashii* Scheid. (Schultz *et al.* 2000), from Santa Cruz Island (California, USA) and Baja California (Mexico); *H. gavilaniae* Amo de Paz *et al.* (Lumbsch *et al.* 2011), from Namaqualand (South Africa). Therefore, three allopatric species comprise this genus (Wijayawardene *et al.* 2017).

Schultz *et al.* (2000) include a brief account of the knowledge of the genus (but see also Henssen *et al.* (1987)). *Harpidium* was firstly placed in *Lecanoraceae* (Zahlbruckner 1926), and later in *Harpidiaceae* together with *Euopsis* Nyl. (Hafellner 1984). This genus has two species containing cyanobacteria as photobiont, with *Euopsis granatina* (Sommerf.) Nyl. also having green algae (Büdel & Henssen 1988; Jørgensen 2007). Henssen *et al.* (1987) ranked both genera in *Lichinaceae*, although currently, the phylogenetic position of *Harpidium* is unclear (Lücking *et al.* 2017; Wijayawardene *et al.* 2020).

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In the course of fieldwork in a siliceous inselberg near the city of Madrid (Cerro de San Pedro, Spain), a student collected material, close to rain tracks on siliceous rocks, of an unidentified crustose, areolate, black lichen, similar in appearance to species of *Lichinaceae*. I had the opportunity to study the morphology of three well-developed samples of this material and, to my knowledge, it represents a novel undescribed species in *Harpidium*.

Harpidium longisporum is therefore proposed as a new species, based on morphological and anatomical characters and is contrasted, in a synoptic way, with the other three worldwide species known in the genus. A species key is also provided.

Materials and Methods

The study is based on 27 herbarium specimens, including seven types, from G, MA, MAF, UPS and VAL. Morphological and anatomical examinations were performed under a Nikon SMZ-1500 stereomicroscope and a Nikon Eclipse-80i microscope, with bright field and differential interference contrast (DIC). Images were captured with a microscope mounted Nikon DS-Ri2 digital camera. Observations and measurements of anatomy, ascospores and conidia were made using thin hand-cut sections mounted in water. Sizes of areoles and apothecia were made with a Leitz-Wetzlar ×8 scale magnifying lens. Extreme values were noted between brackets when they represented no more than 5% of the readings. When possible, at least 30 spores and conidia from different thalli were measured at ×1000 magnification; only developed spores lying outside the asci were measured. Considering the spore shape of treated species (sigmoid to falcate, cf. Kirk *et al.* (2008)), the length was measured in its original form, from end to end and the width at its widest perimeter; number of spores and conidia readings (*n*) is given in parentheses.

Zerene Stacker software (https://zerenesystems.com) was used to combine successive different focal image levels. Current mycological terminology is used, following Kirk *et al.* (2008). The species data in Table 1 and in the key, text and images are based on observations from the material examined in this study, including the type specimens. Standard reagents for coloured spot test reactions (Orange et al. 2010) were used: 10% KOH (K), commercial bleach solution (C), Lugol's iodine (I) and two or three crystals of para-Phenylenediamine freshly dissolved in a few drops of ethanol (Pd). Thin-layer chromatography (TLC) was carried out following Orange *et al.* (2010), except for *Harpidium nashii*, with TLC solvent system C (200 ml toluene/30 ml acetic acid) and silica gel 60 F254 aluminum sheets (Merck, Darmstadt) being used.

Taxonomy

Harpidium Körb.

Syst. Lich. Germ. (Breslau) **2**, 157 (1854) [1855]; type species: *Harpidium rutilans* Körb.

Scheidegger & Schultz (2004) provided a complete genus description, to which the following data are incorporated from the last two described species (*Harpidium gavilaniae* and *H. longisporum*): 1) thallus surface dull, black, grey, brown-red to copper red, sometimes with purple tinges; 2) photobiont a trebouxioid green alga up to 16 µm wide; 3) paraphyses moniliform, up to 6 µm in the apices; 4) ascospores simple, colourless, sigmoid, reniform, allantoid, lunate, falcate to irregularly curved and twisted, without halo, 7–35 × 2.5–7 µm; 5) conidia cylindrical to ellipsoid, 2.5–4 × 1–1.5 µm; 6) all pigmented parts of thallus (including epihymenium) K+ blue-purple; 7) known from Europe, western North America and western South Africa; 8) growing in seasonally humid and sunny sites on siliceous rocks of mainly arid to subhumid areas with Mediterranean or temperate climate regimes.

Notes. The genus *Harpidium* is well defined, uniform in morphological and chemical characteristics and all included species have a similar structure, with hemiangiocarpous pycnoascocarps forming apothecia (Scheidegger & Schultz 2004). In contrast to other crustose lichens, *Harpidium* species can be distinguished in the field (with a hand lens) by their copper red to brown-red pigment tinges (K+ blue-purple) on a black, grey or brown background thallus-surface colour.

Differential characters among species are limited to those included in Table 1 and in the key.

The genus, for now, is restricted to Europe, western North America and western South Africa, and grows primarily on acid rocks mainly in the Mediterranean climate regions of these areas.

The closely related genus *Euopsis*, together with *Harpidium*, was re-established in the family *Harpidiaceae* under *Pezizomycotina incertae sedis*, forming, interestingly, a clade apparently not closely related to *Lichinomycetes* or any other known class in the *Ascomycota* (Lucking *et al.* 2017; Wijayawardene *et al.* 2017).

The gaps in *Harpidium* species knowledge, and also in the family, are many and require new collections and analysis.

Harpidium gavilaniae Amo de Paz *et al.*

In Lumbsch *et al.*, *Phytotaxa* **18**, 64, fig. 14A & B (2011); type: South Africa, Northern Cape, Namaqualand, 57 km east of Springbok, Kangna's Farm, 29°34′58″S, 18°20′29″E, 1036 m, succulent karoo desert on quartzite creek area, on quartzite rock, 5 June 2005, *A. Crespo*, *P. K. Divakar*, *D. L. Hawksworth* & *G. Amo de Paz* SA–80b (MAF–Lich. 16488—holotype!; 16489—isotype!).

(Fig. 2E)

Known only from the type locality in South Africa (Lumbsch *et al.* 2011, fig. 14A & B), with an arid Mediterranean climate regime, it is apparently very rare but perhaps under-recorded owing to limited lichenological explorations. No lichen substances were detected by TLC. Based on my observations of the specimens studied, the distinguishing characteristics of *Harpidium gavilaniae* are summarized in Table 1.

Harpidium longisporum V. J. Rico sp. nov.

MycoBank No.: MB 841482

Similar to *Harpidium rutilans*, but differs in developing a non-effigurate thallus, wider and mainly black to dark brown areoles with copper red and purple tinges, apothecia sometimes indistinguishable from thallus surface and longer, narrower, sigmoid, lunate, falcate to irregularly curved and twisted ascospores, 18–35 × 2.5–4 µm.

Type: Spain, Madrid, Guadalix de la Sierra, Sierra de San Pedro, ladera N del Pico de San Pedro, 40°44′18″N, 03°42′44″W, 1160 m, sobre ortogneis glandular, en escalones de pared vertical ±humedecida y con orientación NW, 6 February 2011, *C. Llorente* 227 (MAF-Lich. 23603—holotype; 23604, 23605—isotypes).

(Figs 1, 2A–C)

Thallus crustose, areolate, continuous or in groups of a few areoles, with ±regular distance between areoles, uneven in shape and not effigurate, up to 6 cm diam., without hypothallus, fragile when moist. *Areoles* (0.1–)0.5–1.5 mm diam. and up to 0.9 mm high, rounded to elongate (up to 1.5 mm long) or angular, entire to sometimes fissured to divided, fastened to the substratum along its entire underside, surface flat to moderately dotted-rugose, dull, mostly black to dark brown with irregular copper, brown-red or purple tinges (observed with a hand lens), flanks marked and angled or gently descending to the substratum and concolorous with surface. *Anatomy*: heteromerous to indistinctly stratified in section, depending on its thickness, with a paraplectenchymatous general arrangement of hyphae mainly formed by round cells; *upper cortex* paraplectenchymatous, 12.5–27 µm high, structured in 2–5 rows of rounded cells 5–12 µm wide and with walls up to 3 µm thick, upper cells with pigmented walls forming a dark brown to copper red hood with occasional purple tinge, extending down the lateral sides to the dark lower stratum of the medulla, without a clear epinecral layer; *photobiont layer* discontinuous, up to 75 µm high, with trebouxioid unicellular green algae, up to 16 µm wide, in a paraplectenchymatous hyphal structure, now and then algal groups in the medulla and also under the apothecia; *medulla* compact, of paraplectenchymatous to seldom irregularly arranged fungal hyphae, greyish to translucent, 200–350(–800) µm high; a true lower cortex is lacking, but attached to the substratum is a dark brown to copper red gelatinous basal layer, of variable thickness and intermixed with ±paraplectenchymatous hyphae. *Symbiotic propagules* not observed.

Ascomata apothecia aspicilioid, with pycnoascocarp development type, ±frequent, rounded to irregular, up to 0.4 mm wide,

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Table 1. Distinguishing morphological characters observed of worldwide *Harpidium* species. All information is derived from material examined during this study unless otherwise noted (*). All species without lichen substances (TLC).

	H. gavilaniae	*H. longisporum*	*H. nashii* (*)	*H. rutilans*
Thallus (crustose, saxicolous, silicicolous)	areolate, partly effigurate; areoles separated, irregular and sometimes elongate to lobulate especially towards the margins	areolate, non-effigurate; areoles separated, irregular and rarely elongate, non-lobulate	squamulose; squamules in small groups or mainly scattered, rounded and minute umbilicate	areolate, partly effigurate or forming small effigurate rosettes; areoles crowded to separated, irregular and sometimes elongate to lobulate especially towards the margins
Areoles/ squamules/ lobule dimensions	areoles 0.3–0.6(–0.8) mm diam., elongate to lobulate areoles 0.4–1.3 mm long	areoles (0.1–)0.5–1.5 mm diam., elongate areoles up to 1.5 mm long	squamules 0.5–1.2(–1.6) mm diam.	areoles 0.2–0.9 mm diam., elongate to lobulate areoles 0.5–0.9(–1.1) mm long, sometimes with furcate ends
Colour (dull)	copper red to dark brown-red, with purple tinges	mostly black to dark brown, with occasional copper to brown-red tinges	copper red to dark brown-red, with grey and purple tinges	grey, brown-red to copper red, with purple tinges
Apothecia (pycnoascocarp, aspicilioid, flat)	conspicuous, up to 0.5 mm diam., disc dark brown	sometimes inconspicuous, up to 0.4 mm diam., disc black	conspicuous, up to 0.4 mm diam., disc dark brown	conspicuous, up to 0.4 mm diam., disc dark brown
Ascospores (simple and colourless)	reniform to irregularly allantoid, at times twisted tips, (8–)9–12(–13) × (3.5)4–6.5(–7) µm (n = 30)	sigmoid, lunate to falcate or irregularly curved and twisted, 18–35 × 2.5–4 µm (n = 40)	reniform, allantoid to lunate, at times twisted tips, 7.5–10 × 5–7.5 µm	reniform, allantoid to lunate, rarely shortly sigmoid, twisted, (9–)9.5–15(–17) × (3–)3.5–6(–6.5) µm (n = 60)
Known distribution (on siliceous rocks)	South Africa (Namaqualand)	central Spain (Madrid)	western North America (California and Baja California)	Mediterranean and dry central Europe

(*) Measurements and observations of the *H. nashii* specimen examined in this study overlap with those of Schultz *et al.* (2000), so the original description data are included here.

sometimes indistinguishable from thallus surface, without margin or with a very shallow thalline margin surrounded by the vegetative plectenchyma of the areole, one to several per areole; ascomata development begins with ascogonia formed beneath pycnidia and trichogynes protruding into the pycnidial cavity to the outside. *Disc* at first ±punctiform, later plane, flat and open to slightly concave, sometimes slightly rugose, concolorous with thallus, now and then inconspicuous. *Exciple* paraplectenchymatous, reduced, 8–18 µm wide, hyaline with dark brown to copper red pigments in its upper and medium part. *Epihymenium* dark brown to copper red, sometimes with purple tinges, 9–21 µm high; *hymenium* hyaline, 62–95 µm tall; *paraphyses* mainly simple and seldom branched and anastomosing, moniliform in last 2–6(–7) cells, apices 5–8 µm wide and with dark brown to copper red pigmented walls and surrounding hood; *subhymenium* and *hypothecium* undifferentiated, variable in thickness but up to 100 µm together, hyaline, of ±rounded up to 15 µm ±gelatinized cells. *Asci* 8-spored, unitunicate-rostrate, thick walled, with a collar-like amyloid inner layer that expands outwards (rostrum) to eject spores when mature. *Ascospores* simple, colourless, sigmoid, lunate to falcate or irregularly curved and twisted, also irregular in thickness, 18–35 × 2.5–4 µm (n = 40).

Pycnidia frequent, laminal, immersed, punctiform (producing a dotted-rugose outer appearance of areolae) to invisible, globose, up to 180 µm wide; wall hyaline, ostiole surroundings dark brown to copper red. *Conidia* simple, colourless, ellipsoidal to cylindrical, 2.5–4 × 1–1.5 µm (n = 30). *Ascogonia* frequently formed beneath pycnidia, developing trichogynes that cross to the surface.

Chemistry. Thallus and medulla, I−, C−, Pd−, UV−. Cortex, epihymenium, dark stratum of medulla, and all pigmented parts in the thallus K+ blue-purple under microscope. Hymenium I+ blue to vine red, KI+ blue. TLC: no lichen substances detected.

Etymology. The specific epithet refers to the length of mature ascospores, longer in this species than in others of the genus.

Habitat and distribution. So far, *Harpidium longisporum* is known only from the type locality in Madrid (central Spain), growing on steps of a vertical, intermittently moist gneiss rock face exposed to sun. It is a subhumid open place with rock walls, in the supramediterranean belt of the Mediterranean region, where the typical potential forest is dominated by *Quercus pyrenaica* (Rivas-Martínez 1987). The species was collected beside *Aspicilia intermutans* (Nyl.) Arnold, *Buellia aethalea* (Ach.) Th. Fr., *Catillaria atomarioides* (Müll. Arg.) H. Kilias, *Circinaria hoffmanniana* (S. Ekman & Fröberg ex R. Sant.) A. Nordin, *Lecania* sp., *Rhizocarpon geographicum* (L.) DC., *Rinodina milvina* (Wahlenb.) Th. Fr. and in some portions covered by *Stigonema* sp., *Xanthoparmelia pulla* (Ach.) O. Blanco *et al.* and *X. tinctina* (Maheu & Gillet) Hale.

Notes. This is a very rare species, and has not been collected since, despite years of searching in similar localities in Spain. Thus future collections may change the morphological description and will provide fresh material for further study.

Harpidium longisporum is easily separated from the other species in the genus by its spore shape and size, and also by the structure and colour of its thallus areoles (see Table 1 and the species key).

A sample was reported as *Harpidium* cf. *rutilans* from Yosemite NP (California, USA) by Hutten *et al.* (2013). The

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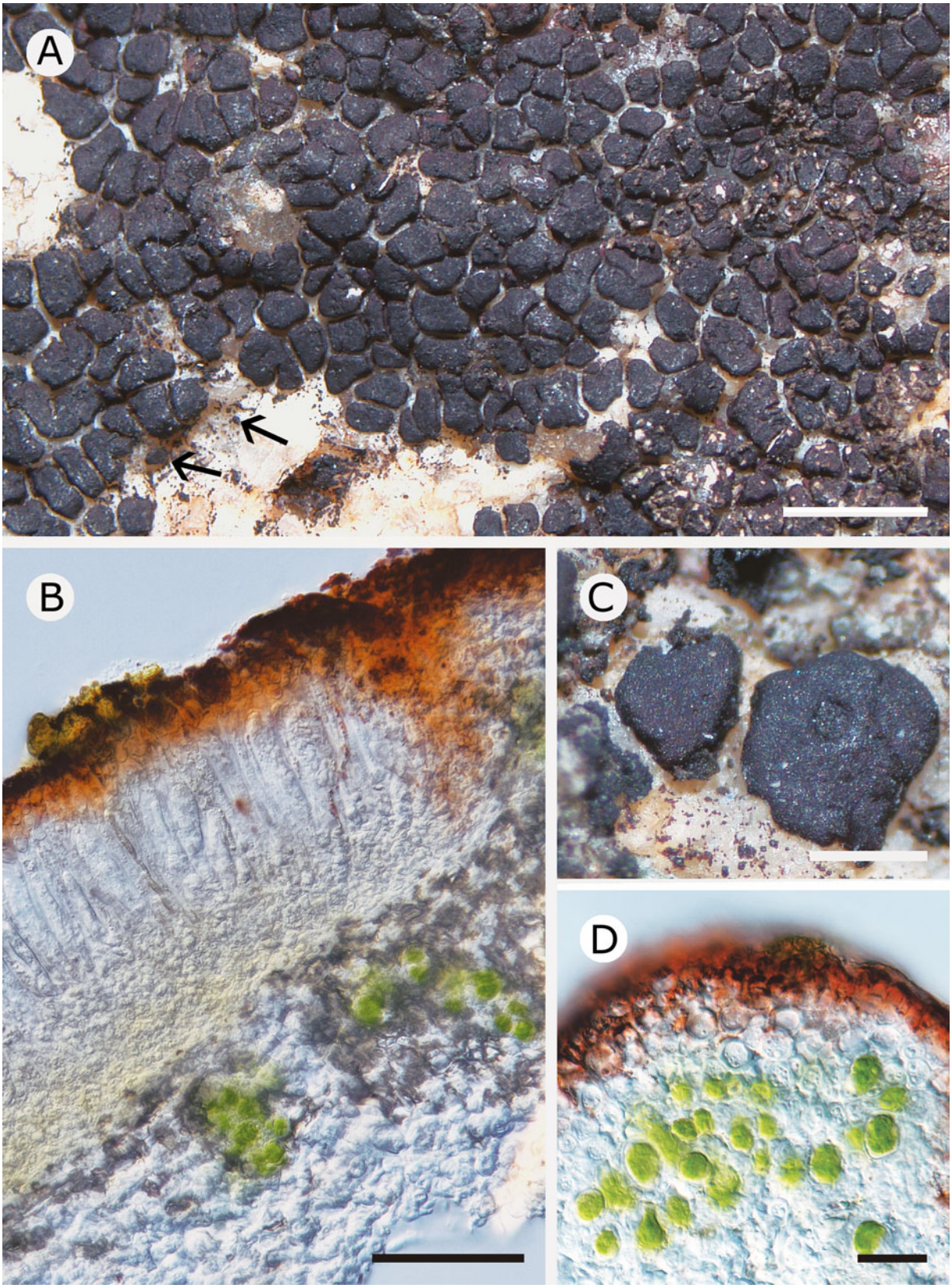

Fig. 1. *Harpidium longisporum* (holotype, MAF-Lich. 23603). A, habitus, showing minute new areoles (arrows) together with threads of *Stigonema* sp.. B, section of apothecium, in water, DIC. C, areole with apothecium. D, section of thallus, in water, DIC. All individual photographs are composites of stacked images. DIC = differential interference contrast microscopy. Scales: A = 2 mm; B = 50 μm; C = 0.5 mm; D = 20 μm. In colour online.

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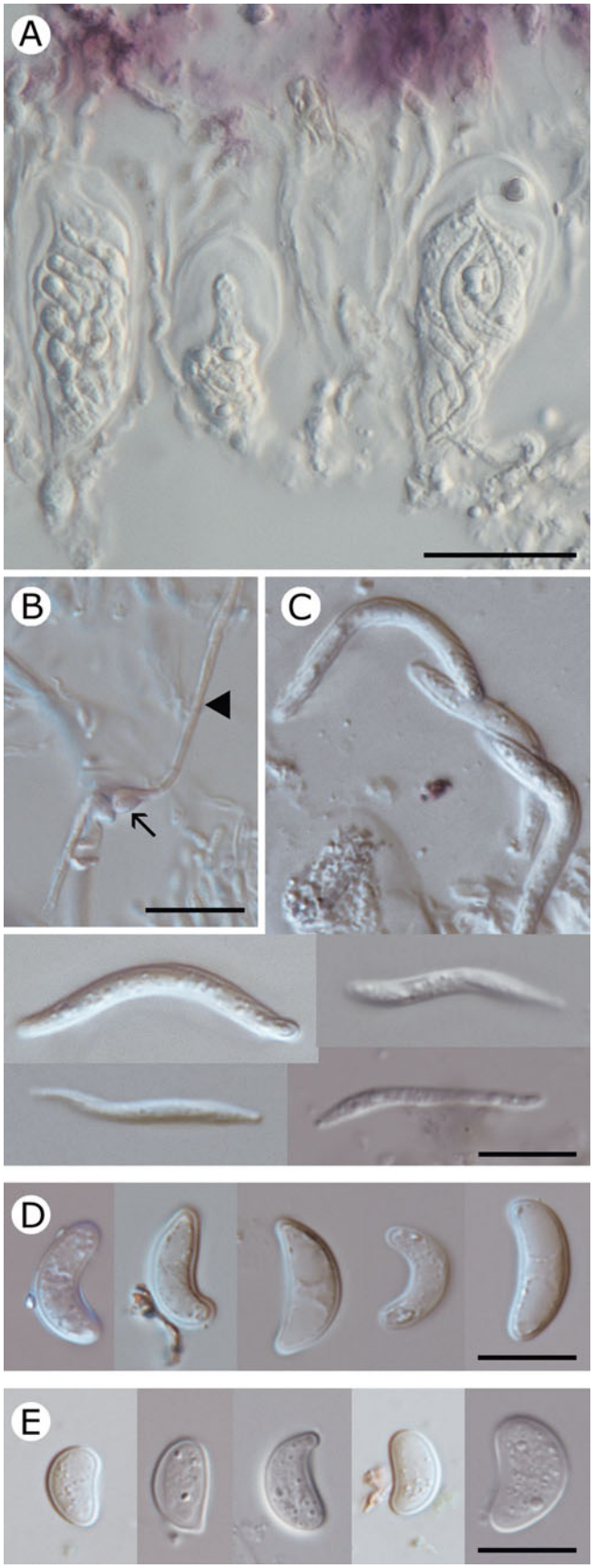

limited specimen was kindly studied morphologically by M. Schultz (Hamburg, in litt.) and he noted that it is close to *Harpidium longisporum* with shorter falcate ascospores and some differences in thallus development; but in conclusion more well-developed material and molecular analyses are needed.

According to my observations, this species has two ways to enlarge. A small number of areoles were seen with short fractures dividing them and forming new areoles, both in the margins and thallus centre. It is also common to see regularly separated, very small young areoles at the thallus margins (Fig. 1A), but neither a hypothallus layer nor any type of hypha has been observed interconnecting areoles or penetrating the rock to attach and join it. Each areole is attached to the substratum through a dark brown to copper red gelatinous basal layer in the medulla, similar to that developed in *Harpidium rutilans* (Henssen *et al.* 1987) and in some *Lichinaceae* growing on hard rocks (Moreno & Egea 1991; Schultz & Büdel 2002). These indicate that the new areoles at the thallus margins must have arisen from ascospore germination and the capture of suitable algae on the rock substratum.

Lichenicolous fungi were not observed, just like in the other species of the genus, but sometimes the areoles of *Harpidium longisporum* are completely covered by threads of *Stigonema* sp., indicating that it is seasonally covered by seeping water.

Harpidium nashii Scheid.

In Schultz *et al.*, *Bryologist* **103**, 802, figs 1 & 2 (2000); type: Mexico, Baja California, Ensenada, northern Vizcaino Region of the Sonoran Desert, 10 km north of Cataviña, 29°47′N, 114°45′W, 570 m, granite boulder area, 6 January 1989, *C. Scheidegger* (G 00291742—holotype! [scanty material]; UPS L125885—isotype! [scanty material]).

Known only from a small number of localities in western North America with an arid Mediterranean climate regime (Schultz *et al.* 2000, fig. 2; Knudsen & Kocourková 2012), it is perhaps overlooked but certainly rare because there are only a small number of collections. The material studied is scanty, only one apothecium was used to see ascospores, unfortunately only four not well-developed spores were seen, and no images were obtained. No lichen substances were detected by Scheidegger & Schultz (2004). Distinguishing characters observed in studied specimens of *Harpidium nashii* overlap with those in the original description (Schultz *et al.* 2000), as is noted in Table 1.

Specimens examined. **Mexico:** *Baja California*: Ensenada, north Vizcaino Region of the Sonoran Desert, 1 km north of Cataviña, 29°43′N, 114°40′W, 560 m, arroyo with *Washingtonia* (*Brusera*, *Idria*, *Lophocereus schottii*, etc.), batholitic granite boulder area, 1989, *C. Scheidegger* (UPS L125886).

Fig. 2. A–C, *Harpidium longisporum* (holotype, MAF-Lich. 23603). A, section of hymenium with asci, in K, DIC. B, water squash preparation of pycnidium, showing ascogonium (arrow) and trichogyne (arrowhead), DIC. C, ascospores, in water, DIC. D, ascospores of *Harpidium rutilans* (MAF-Lich. 23606, 23610), in water, DIC. E, ascospores of *Harpidium gavilaniae* (holotype, MAF-Lich. 16488), in water, DIC. Individual photographs in A and C are composites of stacked images. DIC = differential interference contrast microscopy. Scales: A = 20 µm; B–E = 10 µm. In colour online.

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Harpidium rutilans Körb.

Syst. Lich. Germ. (Breslau) **2**, 157, Taf. I, fig. 8a & b (1854) [1855].—*Acarospora rutilans* (Körb.) Hue, *Nouv. Arch. Mus. Hist. Nat., ser. 5*, **1**, 121 (1909).—*Lecanora rutilans* (Körb.) Nyl., *Actes Soc. Linn. Bordeaux* **21**, 326, 461 (1857), nom. illegit., Art. 53.1, non *Lecanora rutilans* (Ach.) Ach. 1810.—'*Zeora rutilans*' Flot., *Uebersficht der Arbeiten und Veränderungen der Schlesischen Gesellschaft für Vaterländische Kultur im Jahre 1849*, 122 (1850), nom. nud., Art. 38.2; ind. loc.: 'An schroffen Felswänden im Vorgebirge (namentlich gern an Quarzadern im Granit), klafterweite Streifen bewässerter Stellen bedeckend: im Hirschberger Thale auf dem Kynast, dem Popelstein bei Gotschdorf, dem Prudelberge und den Hertelbergen bei Hirschberg (*Fw. Kbr.*)'; type: Poland, Schlesien, *G. W. Körber* s. n. (Leiden (Naturalis Biodiversity Center, Netherlands) L 0056641—holotype [?, sic], n. v., temporarily unavailable).

(Fig. 2D)

The type species of *Harpidium* is known from Europe in Austria (Hafellner & Türk 2016; Nimis *et al.* 2018), Bulgaria (Szatala 1930; Mayrhofer *et al.* 2005), Czech Republic, Italy (van den Boom 1992; Nimis & Martellos 2020), France (Roux *et al.* 2020), Romania, Poland (Körber 1855, Taf. I, fig. 8a & b; Kossowska & Fabiszewski 2004), Portugal (Tavares 1949), Spain and Switzerland. It grows on open, step surfaces of siliceous rocks with periodical water seepage after rain, both in the Mediterranean region and in dry-warm areas of temperate Central Europe, and is perhaps overlooked but not frequent. Roux *et al.* (2020) consider it a very rare species in France, but of international interest and critically endangered. The type material probably needs an adequate typification but is temporarily unavailable. No lichen substances were detected by TLC. Based on observations of specimens examined in this study, the distinguishing characteristics of *Harpidium rutilans* are summarized in Table 1 and in the key. Concerning *Harpidium* cf. *rutilans* from Yosemite NP (California, USA) reported by Hutten *et al.* (2013), see 'Notes' under *Harpidium longisporum*.

Specimens examined. **Czech Republic:** *South Moravia*: in valle fluvii Jihlavka prope pagum Mohelno, 300 m, ad saxa serpentinica, 1971, *A. Vězda* [Vězda, *Lich. Sel. Exs.* no. 1065] (UPS L24794).—**Romania:** *Caras-Severin*: im Szvetnik-Thale beim Dorfe Mehadia, Comitat Krassó-Szöreny in Ungarn, an der Südseite eines grossen Porphyrblockes, 1885, *H. Lojka* [Arnold, *Lich. Exs.* no. 1108b] (UPS L169771, L169772); *ibid*., Valle Szvetnik, Mehadia, *Lojka*, det. *O. Szatala* (originally labeled as 'Hungary', MA-Lichen 869).—**Spain:** *Castellón*: Chóvar, Puerto de Eslida, 30SYK2916, 580 m, comunidades de *Acarospora hilaris*, 1991, *V. Calatayud* (VAL-Lich. 1957, MAF-Lich. 23611); Almonacid, cerca del Pico Espadán, 30SYK2420, 920 m, areniscas ortocuarcíticas, 1992, *V. Calatayud* (VAL-Lich. 2110); Alfondeguilla, Pico Femella, 30SYK3415, 600 m, areniscas ortocuarcíticas, 1990, *V. Calatayud* (VAL-Lich. 1609); Azuebar, Barranco Mosquera, 30SYK2517, 620 m, areniscas ortocuarcíticas, 1991, *V. Calatayud* (VAL-Lich. 1965); *ibid*., 1992, *V. Calatayud* [Vězda, *Lich. Rar. Exs.* no. 45] (UPS L26013); *ibid*., (MAF-Lich. 23610). *Valencia*: Serra, El Garbí, 30SYJ29, 570 m, 1992, *V. Calatayud* (VAL-Lich. 2145). *Zaragoza*: Embid de la Ribera, desfiladero de río Jalón, 520 m, sobre cuarcitas compactas, en superficie vertical alejada del suelo, 1983, *E. Barreno*, *A. Crespo*, *L. G. Sancho*, *A. G. Bueno* & *V. J. Rico* (MAF-Lich. 2432); *ibid*., 41°24′51″N, 01°36′45″W, *V. J. Rico* & *E. Barreno* (MAF-Lich. 23606); *ibid*., 41°25′06″N, 01°35′46″W, 513 m, matorral bajo sobre cuarcitas, 2005, *A. Gutiérrez* & *G. Amo de Paz* (MAF-Lich. 16487); *ibid*., *G. Amo de Paz* (MAF-Lich. 23607, 23608, 23609).—**Switzerland:** *Valais*: Ausserberg, E of Trogdoref, Wasserleita, near the chapel, 46°18′N, 07°51′E, Grid 633:129.4, 1040 m, on rocks on the sun-exposed steep S slope, 1987, *R. Moberg* (UPS L9195).

Key to *Harpidium* species

1 Ascospores long sigmoid, lunate to falcate or irregularly curved and twisted, 18–35 × 2.5–4 µm; thallus areolate, dull, mostly black to dark brown with irregular copper to brown-red or purple tinges; central Spain . . . **Harpidium longisporum**
Ascospores reniform, allantoid, lunate to falcate or occasionally shortly sigmoid and twisted, 7.5–15(–17) × (3–)3.5–7.5 µm; thallus dull, grey, copper to brown-red with purple tinges 2

2(1) Thallus of rounded, scattered umbilicate squamules; ascospores reniform, allantoid to lunate, 7.5–10 × 5–7.5 µm; USA and Mexico . . . . . **Harpidium nashii**
Thallus areolate, continuous or forming thalli of few areoles, sometimes distinctly effigurate with sporadically or regularly elongate to lobulate marginal areoles, areoles affixed over all its lower surface 3

3(2) Ascospores reniform to irregularly allantoid, (8–)9–12(–13) × (3.5–)4–6.5(–7) µm; thalli sometimes incompletely effigurate; South Africa . . . . . **Harpidium gavilaniae**
Ascospores reniform, allantoid to lunate, (9–)9.5–15(–17) × (3–)3.5–6(–6.5) µm; thalli sometimes forming small effigurate rosettes; Mediterranean and temperate dry central Europe . . . . . **Harpidium rutilans**

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