

Hormonema carpetanum sp. nov., a new lineage of dothideaceous black yeasts from Spain

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Abstract: Strains of an unnamed *Hormonema* species were collected from living and decaying leaves of *Juniperus* species, plant litter, and rock surfaces in Spain. Strains were recognized and selected based on their antifungal activity caused by the triterpene glycoside, enfumafungin, or based on morphological characteristics and ITS1-5.8S-ITS2 rDNA (ITS) sequence data. Examination of 13 strains from eight different sites demonstrated that they share a common set of morphological features. The strains have identical or nearly identical sequences of rDNA from ITS regions. Phylogenetic analyses of ITS region and intron-containing actin gene sequences demonstrated that these strains comprised a lineage closely allied to, but distinct from, *Hormonema dematioides*, and other dothideaceous ascomycetes with *Hormonema* anamorphs. Therefore, a new species, *Hormonema carpetanum*, is proposed and illustrated. The strains form a pycnidial synanamorph that resembles the coelomycete genus *Sclerophoma* in agar culture and on sterilized juniper leaves.

Taxonomic novelty: *Hormonema carpetanum* Bills, Peláez & Ruibal sp. nov.

Key words: *Dothideales*, endophyte, enfumafungin, *Kabatina*, *Rhizosphaera*, rock-inhabiting fungi, *Sclerophoma*, *Sydowia*.

INTRODUCTION

Enfumafungin is a hemiacetal triterpene glycoside that is produced in fermentations of a *Hormonema* sp. associated with living leaves of *Juniperus communis* L. (Liesch *et al.* 1998, Peláez *et al.* 2000, Schwartz *et al.* 2000). Enfumafungin is one, among several, new fungal triterpenoid glycosides that were discovered because of their potent *in vitro* antifungal activity. The mode of the antifungal action of enfumafungin and other fungal triterpenoid glycosides was determined to be inhibition of fungal cell wall glucan synthesis by their specific action on (1,3)- β -D-glucan synthase (Onishi *et al.* 2000, Peláez *et al.* 2000). Three enfumafungin-producing *Hormonema* Lagerb. & Melin strains from the province of Madrid were compared with ascomycetes that have *Hormonema* anamorphs, e.g., *Sydowia polyspora* (Bref. & Tavel) E. Müll., *Kabatina* R. Schneid. & Arx species, known *Hormonema* species, and other black yeast-like fungi, and were judged to represent an unnamed *Hormonema* species (Peláez *et al.* 2000). However, the *Hormonema* strains were not formally described as a new species.

During recent years, additional strains of the same *Hormonema* species were collected from living and decaying leaves of *Juniperus* species, plant litter, and rock surfaces in Spain (Table 1). Some of the strains were recognized and selected based on their antifungal activity caused by enfumafungin, while others were recognized based on morphological characteristics and ITS1-5.8S-ITS2 rDNA (ITS) sequence data. Examination of 13 strains from 8 different sites

demonstrated that they share a set of morphological features common to the enfumafungin-producing *Hormonema* sp. The strains have identical or nearly identical sequences of rDNA from ITS regions. Phylogenetic analyses of ITS region and actin gene sequences demonstrated that these strains comprised a lineage closely allied to, but distinct from, *Hormonema dematioides* Lagerb. & Melin, and other dothideaceous ascomycetes with *Hormonema* anamorphs. In this report we describe the set of isolates as a new species, *Hormonema carpetanum*, observe that the strains form a pycnidial synanamorph in agar culture, and present an expanded analysis of their molecular, morphological, and ecological characteristics.

MATERIALS AND METHODS

Isolates

Living cultures of *H. carpetanum* are maintained in the Merck Research Laboratories Microbial Resources Culture Collection in Rahway, New Jersey, U.S.A. (MRL), the Centro de Investigación Básica, Merck Sharp and Dohme in Madrid (CIBE), and unless indicated otherwise (Table 1). Reference strains of *Hormonema*, *Kabatina* and *Sydowia* species were obtained from the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

Table 1. Details pertaining to isolates of *Hormonema carpetanum* studied.

Isolate	Sequence accession No. (ITS, actin)	Geographic origin	Host or substratum
F131395	AY616203, AY616225	Circo de Gredos, Ávila, Spain	Living leaves, <i>Juniperus communis</i> subsp. <i>nana</i>
F138213	AY616200, AY616226	Sierra de Rubión, Covarrubias, Burgos, Spain	Living leaves, <i>Juniperus oxycedrus</i>
F059904 ¹	AY616210, AY616224	Navalquejigo, Madrid, Spain	Living leaves, <i>Juniperus communis</i>
F059461 ¹	AY616209, AY616223	Navalquejigo, Madrid, Spain	Living leaves, <i>Juniperus communis</i>
MF6167 ¹ , ATCC 74360, IMI 392072	AF182375, AY616222	Navalquejigo, Madrid, Spain	Living leaves, <i>Juniperus communis</i>
F154715	AY616207, AY616227	Trevélez, Granada, Spain	Leaf litter, <i>Cistus albidus</i>
F154786	AY616208, AY616228	Ossa de Montiel, Albacete, Spain	Leaf litter, <i>Juniperus sabina</i>
TRN24	AY616202, AY616220	Cancho Gordo, La Cabrera, Madrid, Spain	Granite
TRN25 ² , CBS 115712	AY616205, AY616217	Cancho Gordo, La Cabrera, Madrid, Spain	Granite
TRN31, IMI 392073	AY616206, AY616218	Cancho Gordo, La Cabrera, Madrid, Spain	Granite
TRN40, CBS 115713	AY616201, AY616221	Cancho Gordo, La Cabrera, Madrid, Spain	Granite
TRN201	AY616204, AY616219	La Tejera, Checa, Guadalajara, Spain	Sandstone
TRN278	AY616199, AY616216	Atazar, Madrid, Spain	Slate

¹Strains previously characterised in Peláez *et al.* (2000). ²Holotype and ex-type culture.

Morphology

All isolates were cultured in at least three different media to study their macro- and microscopic characters. The set of media used for characterization was based on their previous efficiency in the induction of sexual or asexual states, and included: potato-dextrose agar (PDA, Difco), oatmeal agar (OA, Difco), and Czapek-yeast extract agar (CYA) (Pitt & Hocking 1997). For some isolates, additional observations were made on cornmeal agar (Sigma-Aldrich) and potato-carrot agar (Gams *et al.* 1998). Colony diameter, texture, pigmentation, margin appearance, exudates, and colours in the descriptions were recorded after 2 wk at 22 °C, unless noted otherwise. Colour designations, e.g., 4F6–2, are from Kornerup and Wanscher (1978) and those in capital letters are from Ridgway (1912). Pycnidial conidiomata were induced in a subset strains by inoculating flasks of Sabouraud's maltose broth containing autoclaved fresh leaves of *Juniperus oxycedrus* L. with four 5-mm agar discs of mycelia and conidia. Flasks were incubated 5 d at 22 °C at 220 rpm on a rotary shaker. Mycelia-covered leaves were removed from liquid cultures with forceps and incubated on water agar. Additional observations on pycnidial conidiomata were made from 3- to 6-wk-old cultures on PDA and OA. Procedures for isolating strains from rock surfaces were described in Ruibal *et al.* (2005).

Microscopic features were evaluated by 1) observing structures mounted in 5 % KOH; 2) growing fungal colonies on cover glasses immersed in dilute malt extract agar (0.5 % malt extract Difco, 1.5 % agar) and supported on 2 % water agar. Colonies on cover glasses were fixed in 95 % ethanol, mounted in 5 % KOH, and photographed.

DNA extraction, amplification, DNA analyses

DNA extraction was performed by the methods described in Peláez *et al.* (1996). The first amplification of both internal transcribed spacers (ITS1 and ITS2) and the 5.8S gene of these isolates was per-

formed using primers 18S-3 (5'-GAT GCC CTT AGA TGT TCT GGG G-3') and ITS4A (Larena *et al.* 1999). To further test relationships at the inter- and intraspecific level, an intron-containing portion of the actin gene was amplified using primers ACT-512F and ACT-783R (Carbone & Kohn 1999).

Polymerase chain reactions were performed following standard procedures (5 min at 93 °C followed by 40 cycles of 30 s at 93 °C, 30 s at 53 °C and 2 min at 72 °C) with Taq DNA polymerase (Q-bioGene) following the procedures recommended by the manufacturer. Amplification products (0.1 µg/mL) were sequenced using the BigDye Terminators version 1.1 (Applied Biosystems, Foster City, CA) following the procedures recommended by the manufacturer. For all the amplification products, each strand was sequenced with the same primers used for the initial amplification. Separation of the reaction products by electrophoresis was performed in an ABI PRISM 3700 DNA Analyzer (Applied Biosystems, Foster City, CA). Partial sequences were assembled manually, and a consensus sequence was generated.

Sequence matching with public or proprietary databases was performed with BLAST2N and FastA (GCG WISCONSIN PACKAGE Version 10.3-UNIX, Accelrys Inc). A selection of the best matching sequences were aligned manually using GENE-DOC (Nicholas *et al.* 1997). Phylogenetic relationships were determined from the aligned sequences using PAUP version 4 (Swofford 2000). Nucleotide substitutions were treated as unordered, unweighted characters. Maximum parsimony trees were inferred using the heuristic search options with stepwise addition and the tree bisection reconnection algorithm. Data were resampled with 1000 bootstrap replicates by using the heuristic search option of PAUP. The percentage of bootstrap replicates that yielded greater than 50 % for each group was used as a measure of statistical confidence. Consensus trees were calculated using 50 % majority rule. Sequence alignments and phylogenetic trees were deposited in

TreeBase as accession numbers S1171, M2027 and M2028.

In addition to sequences of *Hormonema carpetanum* listed in Table 1, new sequences of the ITS1, ITS2 internal transcribed spacers and the 5.8S rDNA gene obtained in this study are: *Hormonema prunorum* (Dennis & Buhagiar) Herm.-Nijh. CBS 933.72 (AY616213), *Kabatina juniperi* R. Schneid. & Arx CBS 239.66 (AY616211), CBS 466.66 (AY616212). New sequences of actin gene fragment from *H. carpetanum* are listed in Table 1. In addition, the fragment was sequenced from *Sydowia polyspora* CBS 128.66 (AY616214) and *Kabatina juniperi* CBS 239.66 (AY616215).

The following sequences, largely derived from previous studies on dothideaceae fungi of the *Aureobasidium* and *Hormonema* complexes (de Hoog *et al.* 1999, Yurlova *et al.* 1999, Hambleton *et al.* 2003), were obtained from GenBank in order to supplement analyses of newly obtained sequences; *Hormonema dematioides* AJ278925, AJ278925, AJ278926, AJ278927, AJ278928, AJ278929, AJ278939, AF013228, AF462439, AY160202, *H. macrosporum* Voronin AJ244247, *Rhizosphaera kalkhoffii* Bubák AF01321, *Scleroconidioma sphagnicola* Tsuneda, Currah & Thormann AY220610, *Sclerophoma pythiophila* (Corda) Höhn. AF462438, *Sydowia polyspora* AJ244262.

RESULTS

Phylogenetic analysis

Sequencing of the rDNA of the 13 isolates of *H. carpetanum* revealed that the ITS1-5.8S-ITS2 gene fragment was 510 nucleotides long. The percentage homology among isolates of the *H. carpetanum* clade (Fig. 1) ranged between 99 to 100 %. The lengths of the actin gene fragments from *H. carpetanum* varied from 237 to 232 nucleotides among the 13 isolates. Percentages of homology of the actin gene fragments ranged from 92 to 100 % among the isolates, with isolate TRN278 having the highest sequence divergence (Fig. 2). To estimate intergeneric distances, the actin gene was also sequenced in *Sydowia polyspora* CBS 128.64 and *Kabatina juniperi* CBS 239.66. Attempts at amplification and sequencing of the actin gene fragment of *Kabatina juniperi* CBS 466.66 and *Hormonema prunorum* CBS 933.72 yielded chromatograms with poorly defined nucleotide sequences, suggesting the presence of more than one actin sequences within the same isolate (data not shown).

Morphology, blast search results, and alignments of the ITS1-5.8S-ITS2 rDNA clearly associated *H. carpetanum* with species classified among the *Dothideales*, especially a complex of fungi that have in common *Hormonema* conidial states (Peláez *et al.* 2000). Recognition and differentiation of *H. carpeta-*

num as a distinct and monophyletic lineage was well supported by parsimony analysis of both genes fragments (Figs 1, 2). In both single-gene genealogies, the isolates formed a discrete group supported in 90 % and 100 % of the bootstrap-resampled ITS1-5.8S-ITS2 and actin datasets, respectively (Figs 1, 2). Small infraspecific differences were evident among isolates of *H. carpetanum*, and infraspecific grouping varied depending on the gene fragment analysed.

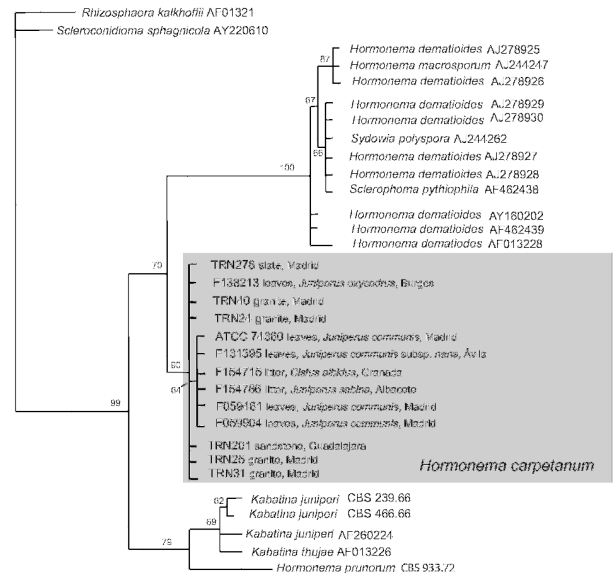


Fig. 1. Relationships of *Hormonema carpetanum* isolates and selected reference strains inferred by maximum parsimony consensus of aligned sequences of the ITS1-5.8-ITS2 rDNA. Statistical support (1000 bootstrap) values of > 50 % indicated at branch points. Tree parameters: total characters = 528, constant characters = 454, variable characters parsimony-uninformative = 23, variable characters parsimony-informative = 51, tree length = 103, consistency index (CI) = 0.777 and retention index (RI) = 0.932. Outgroup taxon: *R. kalkhoffii*.

Neither gene fragment grouped isolates of *H. carpetanum* according to their origins from living plants, plant litter, or rock surfaces (Figs 1, 2).

Isolates referable to *Sydowia polyspora* and its *H. dematioides* and *Sclerophoma pythiophila* synanamorphs also formed a distinct and well supported lineage parallel to that of *H. carpetanum* (Fig. 1). The infraspecific variation among the ITS1-5.8S-ITS2 dataset of *H. carpetanum* was similar to the differences observed among the same dataset from isolates referable to *Sydowia polyspora* and its synanamorphs (Fig. 1). The homology among isolates of the *S. polyspora*–*H. dematioides* clade ranged between 92 to 99.5 %. Pairwise comparisons of sequence homologies between strains of the *H. carpetanum* and the *S. polyspora*–*H. dematioides* clade ranged between 92 to 94 %, and most of the nucleotide differences between strains of the two clades were concentrated in the ITS2 region.

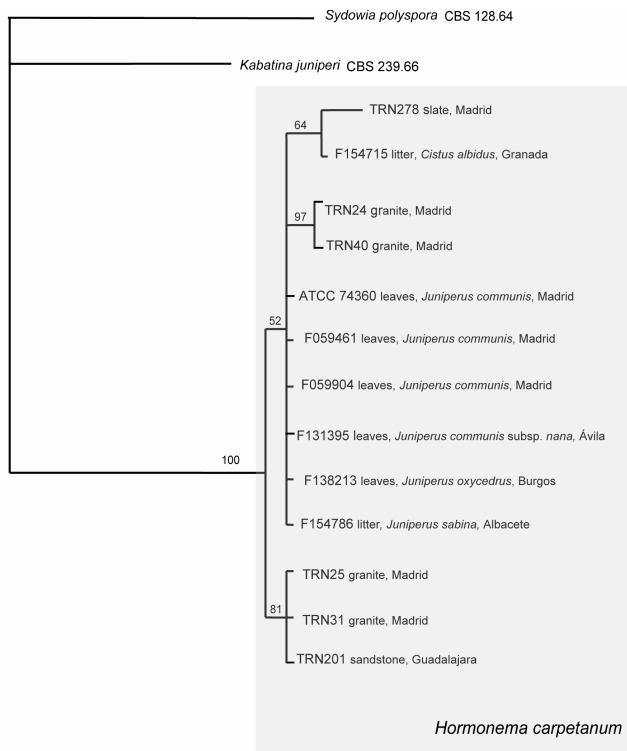


Fig. 2. Relationship of *Hormonema carpetanum* isolates and selected reference strains inferred by maximum parsimony consensus of aligned sequences of an intron-containing fragment of the actin gene. Statistical support (1000 bootstrap) values of > 50 % indicated at branch points. Tree parameters: total characters = 248, constant characters = 152, variable characters parsimony-uninformative = 78, variable characters parsimony-informative = 25, tree length = 142, consistency index (CI) = 0.915 and retention index (RI) = 0.750. Outgroup taxon: *S. polyspora*.

The relatively low bootstrap values within the *S. polyspora*–*H. dematioides* and *H. carpetanum* species clusters suggested that further delineation of isolates based on the ITS1-5.8S-ITS2 dataset alone is unreliable. However, some infraspecific grouping of *H. carpetanum* strains was apparent based on actin gene sequences (Fig. 2).

Taxonomic Part

Hormonema carpetanum Bills, Peláez & Ribal, **sp. nov.** MycoBank MB500039. Figs 3–21.

= *Hormonema* sp., Peláez *et al.*, Syst. & Appl. Microbiol. 23: 333. 2000.

Synanamorph: *Sclerophoma* sp.

Etymology: *Carpetanus* (Latin), in reference to the ancient Roman name for the Central Cordillera of Spain.

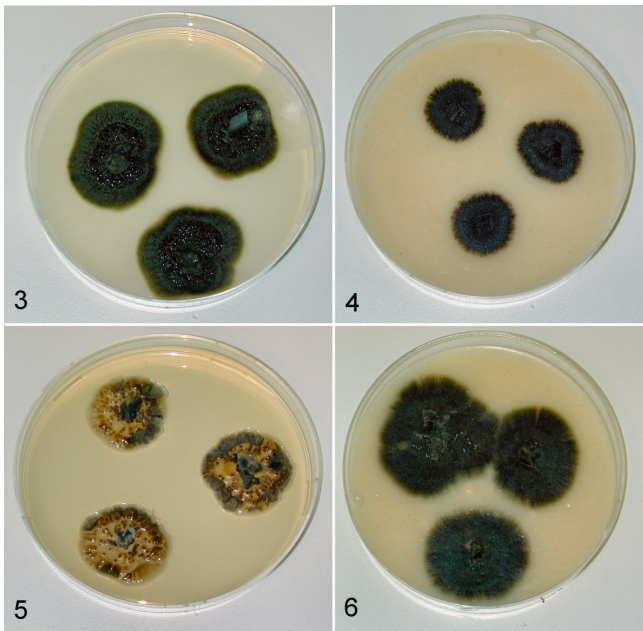
Species *H. dematioides* similis, sed conidiis maioribus et serie ex nucleicis acidis differt. Coloniae radiatim rugulosae in agaro avenae farinae vel tuberum solani dextrosato; constantes praecipue ex hyphis submersis radialiter exten-

sis; mycelio aereo absente vel exiguo, pycnidiis formatis in ca. 3–4 hebdomadis in vel sub agari superficie, plerumque cum massa humecta conidiorum e conidiogenesi myceliali accumulatorum in guttis vel filis radialibus olivaceo-nigris ad atris. Conidiophora absentia in mycelio. Cellulae conidiogenae holoblasticae, incorporatae, intercalares, mycelio similes. Conidia ad 7–14(–16) μm longa, ad 3–6 μm lata, hyalina ad pallide olivaceo-grisea. Conidiomata pycnidialia formata *in vitro*, similia generi *Sclerophoma*, ad 350 μm diam, globosa vel subglobosa, unilocularia, solitaria ad gregaria, olivaceo-brunnea ad atra, glabra vel setosa, initiata e massa stromatica cellularum isodiametricarum, strato tenui hypharum obiecta. Cellulae conidiogenae phialidicae, isodiametricae, 8–15 μm diam, unum vel plura conidia ex uno vel pluribus aegre distinctis ostioliis proferentes vel per conversionem ipsorum conidia praebentes. Conidia pycnidialia ellipsoidea ad late ellipsoidea, aliquando curvata vel constricta in parte media, glabra, attenuata ad basim, hyalina usque ad atrogriseo-brunnea, 8–14(–17) \times 4–6 μm .

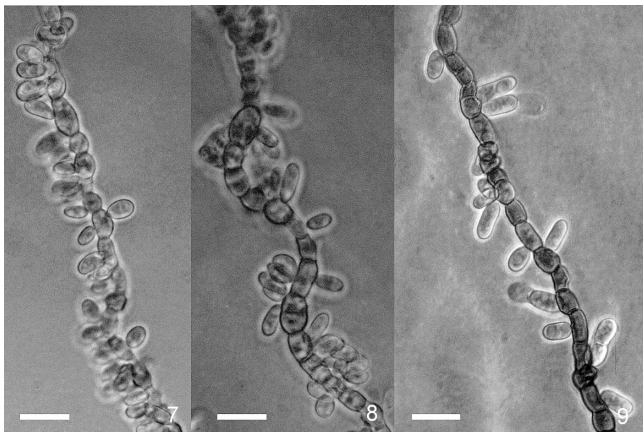
Inventa in foliis *Juniperi* speciei superficialiter disinfectis, plantarum residuis et superficie saxorum in Hispania centrali:

Holotype exsiccatum specimen herb. CBS H-13135, (CBS H-13136 **isotype**). Et cultura viva TRN 25 = CBS 115712.

Mycelium consisting of torulose, wide, often thick-walled, hyaline to dark olive to olive black, short-cylindrical to subglobose cells, often with hyphal cells longitudinally septate, in age sometimes developing groups of muriform cells, with individual cells up to 22 μm diam, generally sparsely branched, but sometimes becoming more branched with age. **Conidiophores** absent on mycelia. **Conidiogenous cells** holoblastic, integrated, intercalary, usually not differentiated from main axes of vegetative mycelium, occasionally arising from an undifferentiated lateral cell or filament, sometimes with a faint pore or slightly protruding annellate scar evident at the conidiogenous locus, often abundant along the entire radius of young extending hyphae, less so on older, contorted and melanized hyphae. **Conidia** up to 7–14(–16) μm long, up to 3–6 μm wide, aseptate, or rarely 1-septate in old cultures, usually ellipsoidal and tapered towards the base, but quite variable, occasionally pyriform or subglobose, smooth, often with one or more budding scars, hyaline to pale olivaceous-grey, often budding to produce 1 or 2 secondary conidia, accumulating in yeast-like masses along radial axes of vegetative hyphae.



Figs 3–6. *Hormonema carpetanum* in agar culture after two wks. 3. TRN40, on potato-dextrose agar. 4. TRN40, on oatmeal agar. 5. F059461, on Czapek yeast extract agar. 6. F059461, on oatmeal agar.



Figs 7–9. Mycelial conidiogenesis and conidia of *Hormonema carpetanum*. 7, 8. F131395, slide culture on dilute malt agar. 9. TRN 31, slide culture on dilute malt agar. Scale bars = 10 μ m.

Pycnidial conidiomata in agar or on sterilized leaves up to 350 μ m diam, globose to subglobose, unilocular, solitary to gregarious, rarely confluent in age, shiny, dark olive-brown to black, smooth, or with scanty 30–75 μ m long, basally up to 7 μ m wide setae; conidiomata initiating as stromatic masses of broadly cylindrical to isodiametric cells, enveloped by a thin layer of hyphal filaments, dehiscing by irregular erosion of upper layers, accumulating dark brown masses of moist conidia when mature. As basal stromatic cells of conidiomata accumulate and expand, upper layers of stromatic cells convert into conidiogenous cells. *Conidiogenous cells* phialidic,

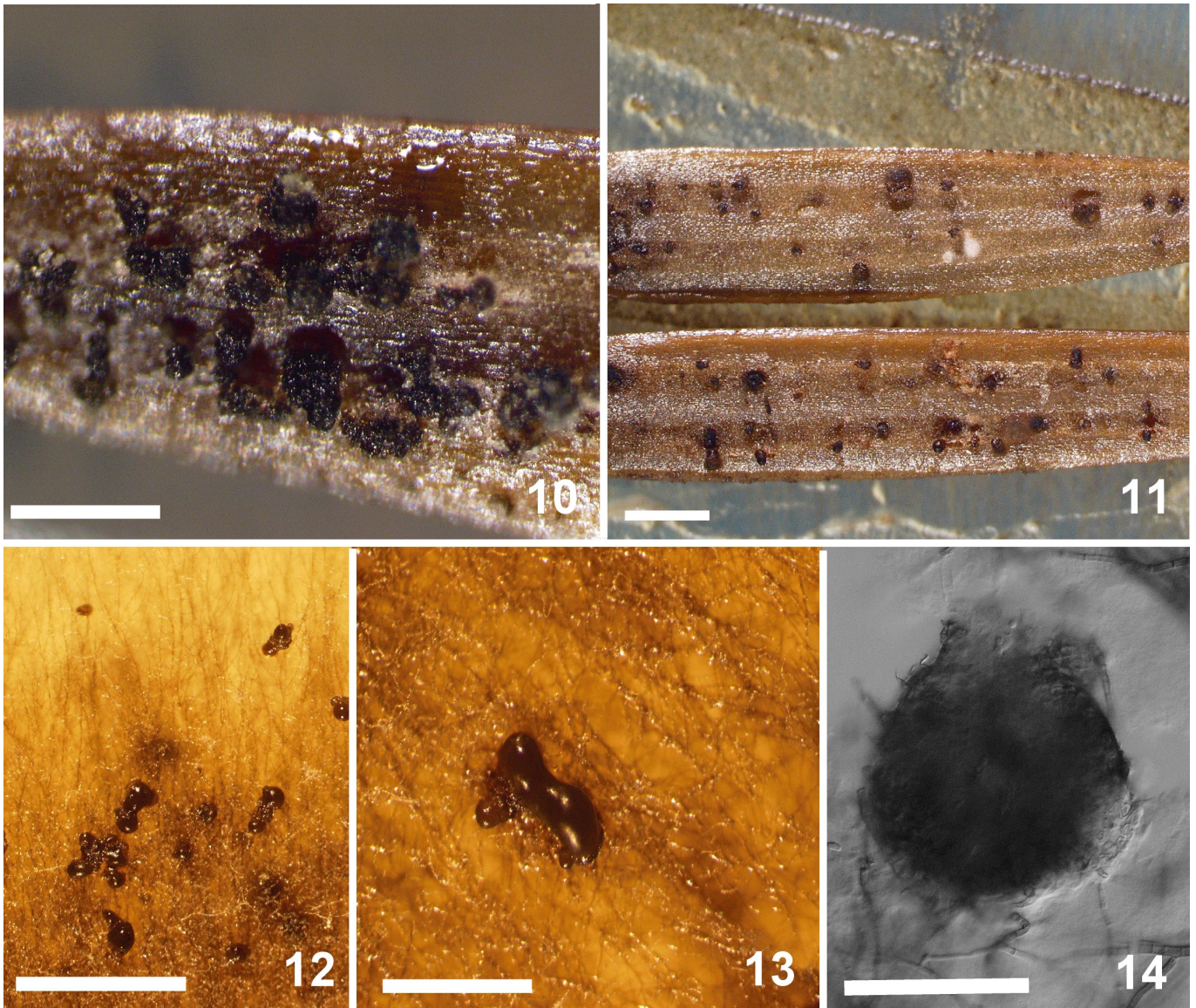
determinate, short cylindrical to isodiametric, 8–15 μ m diam, thin- to thick-walled, hyaline to pale, giving rise to one or more conidia, through one or more poorly defined openings or by conversion of the cell's internal contents directly into to a conidium, collapsing after conidia are released. *Pycnidial conidia* ellipsoid to broadly ellipsoid, sometime curved, indented on one side or constricted at the centre, smooth, usually tapered toward base, occasionally with eccentric basal scar, hyaline to dark greyish brown, 8–14(–17) \times 4–6 μ m.

Cultural characteristics: Colonies on PDA differed in radial extension depending on the isolate, ranging from 21–37 mm diam, with margin submerged, fimbriate, usually radially rugulose or slightly furrowed, often slightly depressed toward the centre, consisting predominantly of submerged, radially extending hyphae, shiny to moist, during the first few weeks, aerial mycelium absent to scant, but abundant aerial filamentous mycelium may emerge with prolonged incubation (>1 mo), in all strains scattered to gregarious pycnidia formed in about 3–4 wks at or below the agar surface, usually with masses of moist conidia from mycelial conidiogenesis accumulating in moist drops or in radial strands, pale olive-brown to dark olive, Yellowish Olive, Dark Olive, Dark Greenish Olive, 3F6–2, 4F6–2, or dark olive-grey, Dark Greyish Olive, Olivaceous-Black (1), 30F5–2, eventually becoming black. Colonies on OM ranged from 24–32 mm diam; depending on the isolate, submerged to appressed, radially furrowed, silky to shiny, moist, dark olive, olivaceous-black to black, in most strains scattered pycnidia formed at or below the agar surface in about 3–4 wks. Colonies on CYA 17–21 mm, raised, rugulose, with radially extending yeast-like hyphae, often with hyaline to buff watery zones or sectors mixed with dark olivaceous-black zones. Growth was poor on media with dilute starch as the primary carbon source, e.g., cornmeal agar or potato-carrot agar. No growth was observed at 37 °C.

Habitat: Isolated from surface-sterilized leaves of *Juniperus* species, plant litter, and rock surfaces.

Known distribution: Spain, from Burgos southward to Granada and from Ávila eastward to Guadalajara.

Specimens examined: Living cultures listed in Table 1. **Spain**, Madrid, La Cabrera, Cancho Gordo, isolated from surface of granite formation, Aug. 2001, dried **holotype** culture with mycelial conidia and pycnidia conidiomata and **ex-holotype** culture TRN25 = CBS 115712.



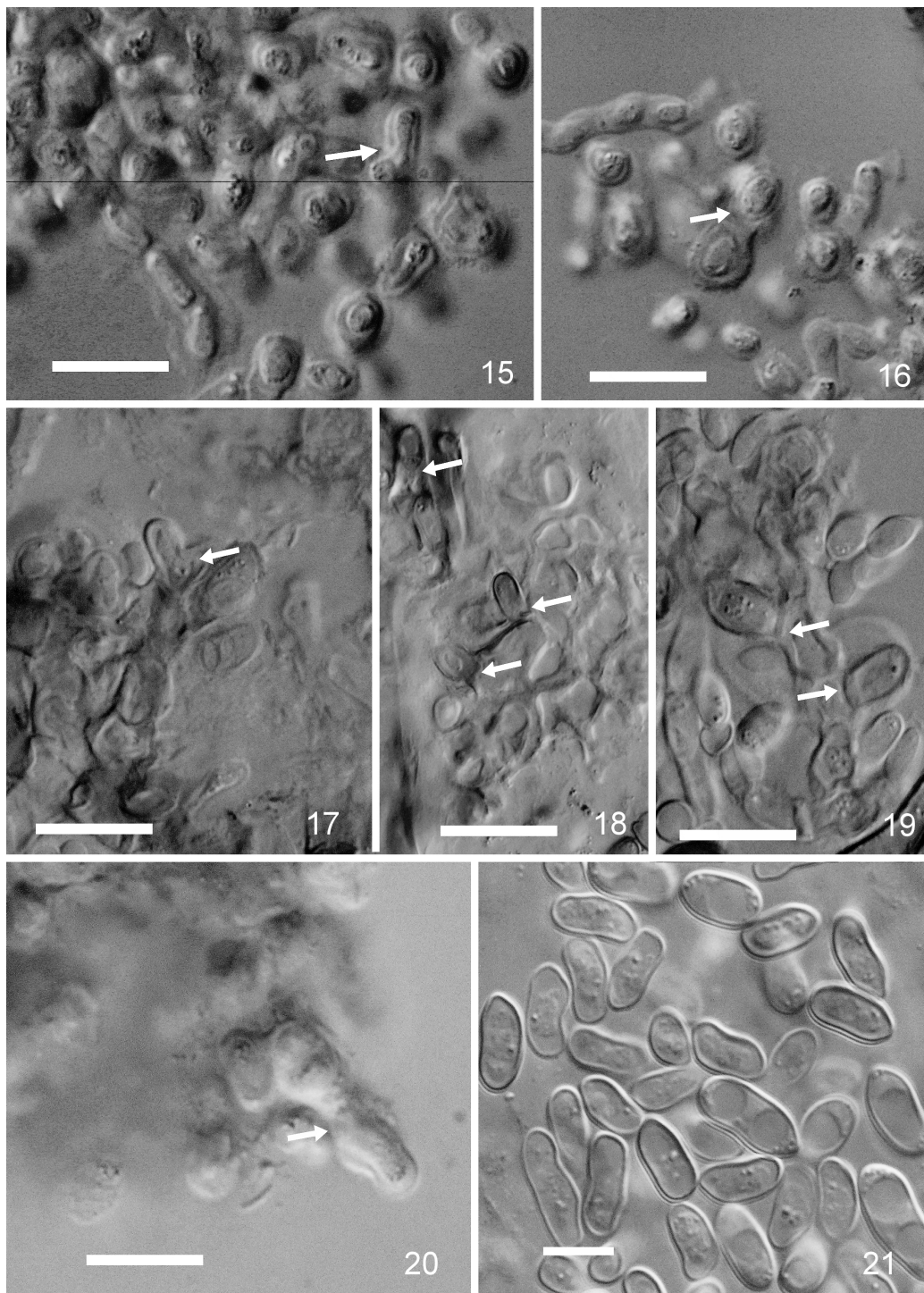
Figs 10–14. Pycnidia of *Hormonema carpetanum*. 10. ATCC 74360, pycnidia on autoclaved leaves of *Juniperus oxycedrus*. Scale bar = 500 μ m. 11. TRN31, pycnidia on autoclaved leaves of *J. oxycedrus*. Scale bar = 500 μ m. 12. TRN201, pycnidia from edge of oatmeal agar plate. Scale bar = 500 μ m. 13. TRN201, pycnidia from edge of oatmeal agar plate. 14. Scale bar = 250 μ m. TRN24, pycnidial initial from potato dextrose agar. Scale bar = 50 μ m.

DISCUSSION

The genus *Hormonema*, typified by *H. dematioides*, has been applied to melanized filamentous fungi that produce slimy, yeast-like conidia that are formed basipetally in a non-synchronous manner from one or few loci on cells of undifferentiated vegetative hyphae. Percurrent conidiogenous loci in *Hormonema* has served to distinguish the genus from similar fungi classified in *Aureobasidium* that produce conidia synchronously from the conidiogenous loci (Hermanides-Nijhof 1977, de Hoog & Yurlova 1994). The differences in modes of conidiogenesis and cluster analysis of ITS1 and ITS2 sequences clearly separated fungi with *Aureobasidium* anamorphs from those with *Hormonema* anamorphs (de Hoog *et al.* 1999, Yurlova *et al.* 1999). Furthermore, other studies based on ITS data have consistently

segregated strains of the *S. polyspora*–*H. dematioides* complex into well-supported clades (Yurlova *et al.* 1999, Hambleton *et al.* 2003). Peláez *et al.* (2000) used a combination of mycelial features, conidiogenesis, and ITS1-5.8S-ITS2 sequences to place the then unnamed *H. carpetanum* among dothideaceous fungi with *Hormonema* anamorphs. Our reanalysis of an expanded data set, which includes more isolates and more new sequences of related fungi from public databases, reconfirms the conclusion that the new species is a close, but distinct relative of *H. dematioides*, *Kabatina* species, *Rhizosphaera* species, and the recently described *Scleroconidioma sphagnicola* (Hambleton *et al.* 2003).

The pattern of synanamorphy of *H. carpetanum* is consistent with patterns of closely related fungi, viz. pycnidial conidiomata forming on plant surfaces, or sometimes in culture, and a mycelial conidial state (*Hormonema*) associated with vegetative growth.



Figs 15–21. Pycnidial conidiogenous cells and conidia of *Hormonema carpetanum*. 15. F131395, young stromatic cells and conidiogenous cell (arrow). 16. F131395, young stromatic cells and conidiogenous cell (arrow). 17. TRN201, stromatic cells and conidiogenous cell (arrow), note collapsed stromatic cells. 18. TRN201, stromatic cells and conidiogenous cells (arrows), note collapsed stromatic cells. 19. TRN201, stromatic cells and conidiogenous cells (arrows). 20. F131395, conidiogenous cell (arrow). 21. TRN31, pycnidial conidia produced on autoclaved leaves of *J. oxycedrus*. Scale bars = 10 µm.

In the preliminary description of *H. carpetanum* (Liesch *et al.* 1998, Peláez *et al.* 2000), pycnidial conidiomata were not mentioned probably because strains were not incubated for extended intervals on a variety of media. It is also possible that conidial masses from pycnidial initials were overlooked and confused with accumulated masses of dark conidia from vegetative hyphae. Sequence similarities to other species with pycnidial synanamorphs led us to suspect

that *H. carpetanum* should produce a pycnidial state, at least on host tissue. We were able to artificially induce pycnidia on autoclaved leaves of *J. oxycedrus* (Figs 10, 11). Once pycnidia were recognized on plant tissue, masses of stromatic cells at and below the agar surface were recognized as pycnidial initials (Fig. 14), and sporulating pycnidia were easily distinguished in 3–4 wk-old cultures on PDA and OA (Figs 12, 13). The development of both pycnidial conidiomata and

the *Hormonema* state of *Sydowia polyspora* have been observed *in vitro* (Sutton & Waterston 1970). In a monographic revision of the genus *Hormonema*, the possibility of *in vitro* production of acervuli and pycnidia by *Hormonema* species was acknowledged (Hermanides-Nijhof 1977), however, *in vitro* production of pycnidia by strains of *H. dematioides* was not described as a component of its *in vitro* life cycle.

Pycnidial development and morphology of *H. carpetanum* differs from that described in species of *Rhizosphaera*, *Kabatina*, *Scleroconidioma*, and *Sclerophoma*. *Rhizosphaera* species have discreet, phialidic conidiogenous cells, which are produced in intercalary or terminally on chains of cells arising from the pycnidial wall (Gourbière & Morelet 1979, Gourbière & Morelet 1980, Sutton 1980, Butin & Kehr 2000). In *Kabatina*, erect phialidic conidiogenous cells form on the upper layer of a stroma-like acervulus (Sutton 1980). In *Scleroconidioma*, the outer surface layer of stromatic conidiomata are converted into conidiogenous cells and produce conidia through percurrently proliferating cells or phialides (Tsuneda *et al.* 2000, Tsuneda *et al.* 2001). The conidiomata of *H. carpetanum* seem most similar to those of *Sclerophoma pythiophila* in which the cells of the stromatic tissue, consisting of a thick-walled *textura angularis*, function as conidiogenous cells. Descriptions of *S. pythiophila* describe the cells of the stromatic tissue which function as phialides. As conidiogenesis cells produce conidia, they accumulate in the upper regions of the conidiomata and are released by an irregular erosion of the conidiomatal wall or membrane (Sutton & Waterston 1970, Sutton 1980, Butin & Peredo 1986). In *H. carpetanum*, the stromatic cells of the pycnidia (Figs 15, 16) produce conidia blastically or internally (Figs 15–20). As the stromatic cells mature and collapse (Figs 17–20), conidia are released via erosion of the pycnidial membrane and accumulate as a moist mass (Figs 10, 12, 13). However, pycnidial conidial dimensions in *H. carpetanum* mostly are in the range of 8–14 × 4–6 µm, and therefore larger than those reported for *S. pythiophila* which have been recorded as 4–8 × 2–3 µm. Pycnidia of *S. pythiophila* are described as either multilocular or unilocular, while thus far *in vitro* only unilocular conidiomata have been observed in *H. carpetanum*. Furthermore, mycelial conidial dimensions in *H. carpetanum* often range up to 14 µm, while conidia of *H. dematioides* have been reported to be up to 12 µm (Hermanides-Nijhof 1977, de Hoog *et al.* 2000). We conclude that the pycnidial conidiomata of *H. carpetanum* could be accommodated in *Sclerophoma*, but we prefer not to formally name this state of the life cycle because it will likely be accompanied by the prevailing *Hormonema* state.

Tsuneda *et al.* (2004) described a new genus and species, *Endoconidioma populi* Tsuneda, Hambleton & Currah to accommodate a pycnidial fungus with a

closed peridium and pycnidial locule filled with conidiogenous cells that form conidia endogenously. A *Hormonema*-like synamorph is produced by the mycelia in culture. ITS sequence data indicated that the *E. populi* is a very close relative of *H. carpetanum*. Comparison of the morphological descriptions *E. populi* and *H. carpetanum* indicated the two are distinct and different fungi; Tsuneda *et al.* (2004) hypothesized, based on similarities in ITS sequences, that the yet undescribed *Hormonema* strain ATCC 74360 was congeneric with *Endoconidioma*.

Most fungi with *Hormonema* anamorphs described to date are associated with living plants (Hermanides-Nijhof 1977, Ramaley 1992, Middlehoven & de Hoog 1997, Tsuneda *et al.* 2000). Most notable among these fungi are a complex of *Rhizosphaera* and *Kabatina* species associated with needle diebacks in conifers (Gourbière & Morelet 1980, Sutton 1980, Martínez & Ramírez 1983, Butin & Kehr 2000), and *Sydowia polyspora* and its synanamorphs (Sutton & Waterston 1970, Sutton 1980, Butin & Peredo 1986). However, examination of the list of isolates of *H. carpetanum* (Table 1) suggests on one hand, a pattern of ecological specialization with regard to foliage of *Juniperus* species, yet on the other, the ability to at least survive on, and perhaps colonize, dead plant litter, and rock surfaces. Presumably the ability to survive in stone surfaces is related to heavily melanized vegetative cells or cell aggregates. Such cells could survive the high radiation and desiccation of these exposed environments, act as vegetative propagules, and perhaps propagate and disperse by budding yeast cells during favourable conditions. *Trimmatostroma abietis* (Butin *et al.* 1996), a fungus that grows epiphytically on living conifer needles and forms stromatic conidiomata on senescent needles of *Abies* and *Pinus* species, exhibits a similar pattern of distribution. The fungus has also been isolated from man-made and natural stone surfaces as well as being observed in certain traumatic human mycoses. The pattern of distribution is also reminiscent of that of *H. dematioides*, where the mycelial conidial state is frequently observed as an epiphyte and endophyte of various conifer species, isolated from dead conifer wood, and occasionally recovered from human and animal infections (Hermanides-Nijhof 1977, de Hoog *et al.* 2000).

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