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ISOLATION OF *Papulaspora halima* AND A NEW MORPHOLOGICAL VARIETY OF *Halosphaeria tubulifera* FROM SEAWATER OF POTTER COVE (KING GEORGE ISLAND, SOUTH SHETLAND ISLAND, ANTARCTICA)

(Aislamiento de ***Papulaspora halima*** *y de una nueva variedad morfológica de* ***Halosphaeria tubulifera*** *desde agua de mar de Potter Cove (Isla Rey Jorge /25 de Mayo, islas Shetland del Sur , Antártida))*

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Palabras clave: Antártida, ascomicetes, hifomicetes acuáticos, hongos marinos

ABSTRACT

Marine fungi ascribed to the ascomycetes and the hyphomycetes are infrequently reported for the Southern Ocean. For this reason, the main objective of the present work was to detect the presence of these fungi seawater of Potter Cove, King George (25 de Mayo) Island, South Shetland Island, Antarctica.

For this purpose marine fungi were grown on wood test panels, placed into plastic nets in the tidal zone, exposed to the Antarctic seawater for different periods of time, which ranged between 2 and 12 months.

As a result of this survey, we were able to recover and identify two marine fungi, ***Papulospora halima*** *(which represents the first report for this environment) and a new morphological variety of* ***Halosphaeria tubulifera***.

RESUMEN

Los ascomicetes e hifomicetes marinos están escasamente documentados para el océano Atlántico Sur. Por este motivo, el principal objetivo del presente trabajo fue detectar la presencia de dichos hongos en las agua marinas de la Potter Cove, en la isla Rey Jorge/25 de Mayo (islas Shetland del Sur , Antártida).

Para este propósito, los hongos marinos se desarrollaron en paneles de madera dentro de una red plástica en la zona tidal, expuestos al agua de mar antártica por diferentes períodos de tiempo que oscilaron entre 2 a 12 meses. Como resultado de este estudio, fuimos capaces de recuperar e identificar 2 hongos marinos, ***Papulospora halima*** *(que representa el primer reporte para este ambiente) y una nueva variedad morfológica de* ***Halosphaeria tubulifera***.

INTRODUCTION

Even though knowledge about the structure and function of the different Antarctic ecosystems has been increased with the successive scientific expeditions, which started at the beginning of the XX century, such knowledge continues being deficient in some areas. One of these areas is mycology. One of the consequences is the small number of references to the fungal components made in several reviews dealing with the Antarctic microbiota (Palmisano & Garrison 1993; White *et al.* 1993). However, at the present time, psychrotolerant fungi are reported as relevant sources of cold-enzymes, secondary metabolites and

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antimicrobial compounds with an enormous potential in the industrial and biotechnological field (Bennett 1998, Cowan *et al.* 2007). Marine fungi, in particular, play a key role in the energy flow from detritus to higher trophic levels in the marine ecosystems. However, at present; reports on diversity of marine fungi are predominantly focused on temperate and tropical waters. Even in these more accessible and previously studied environments, a significant fraction of the isolates represent new reports for the studied area (Prasannarai & Sridhar 2001, Pang *et al.* 2004) evidencing the lack of knowledge on biodiversity of marine fungi mentioned above. The Antarctic marine environment, then, remain practically unexplored for fungal biodiversity . Only two previous studies reported the presence and diversity of true marine fungi (excluding asco- and basidiomycetes yeasts) in such environments. Pugh & Jones (1986), using wood bait technique, reported the presence of ***Ceriosporopsis circumvestita*** (Kohlm.) Kohlm., ***C. halima*** Linder, ***C. tubulifera*** (Kohlm.) Kirk ex Kolm., ***Corollospora maritima*** Werdermann, ***Remispora maritima*** Linder , ***R. stellata*** Kohlm., ***Humicola alopallonella*** Meyers & Moore, ***Monodyctis pelagica*** (T.W. Johnson) E.B.G Jones, ***Zalerion maritimum*** Linder and a stysanus-like fungus, from South Orkney archipelago and South Georgia island. Grasso *et al.* (1997), using the same technique, have found ***Aniptodera chesapeakensis*** Shearer & M.A. Mill, ***Botryophialophora marina*** Lindner, ***Camarosporium palliatum*** Kohlm. & E. Kohlm, ***Cirrenalia macrocephala*** (Kohlm.) Meyers & R.T Moore, ***Halosarpheia*** Kohlm. & E. Kohlm., ***Lignincola laevis*** Höhnk, ***Monodictys*** sp., ***Thalassoascus cystoseirae*** (Ollivier) Kohlm., ***T. lessoniae*** Kohlm., ***Trichocladium achrasporum*** (Meyers & R.T Moore) M. Dixon ex Shearer & J.L. Crane (current name: ***Humicola alopallonella*** Meyers & R.T . Moore), ***T. constrictum*** I. Schmidt, ***Ulocladium*** sp., ***Zalerion maritima*** (Lindner) Anastasiou, ***Z. varia*** Cabello, Aramb. & Liggieri, and ***Zopfiella marina*** Furuya & Udagawa, in seawaters of Terra Nova Bay.

In previous studies, coordinated screenings were performed in different ecosystems from the Antarctic Peninsula by researchers from University Rovira i Virgili (URV), Reus, Spain and the Argentinean Antarctic Institute (IAA) in order to investigate the biology and taxonomy of Antarctic fungi. As a result of such collaboration it has been possible to describe a new genus, ***Antarctomyces*** (Stchigel *et al.,* 2001), and three new species of soil-borne ascomycetes: ***A. psychrotrophicus, Apiosordaria antarctica*** and ***Thielavia antarctica*** (Stchigel *et al.,* 2001; Stchigel *et al.,* 2003). More recently , we also presented new reports of micromycetes from soil and plant material (Stchigel *et al.,* 2005, 2008). In this work, the authors performed a preliminary study on the Antarctic marine mycobiota, which could play an important role as cellulose detritus decomposers and as seaweed pathogens.

Here, we present the finding of two fungi of marine origin, ***Papulaspora halima***, that represent a first report for Antarctic marine environments, and a new morphological variety of ***Halosphaeria tubulifera***.

MATERIAL AND METHODS

Wood test panels were exposed to seawater of Potter Cove (62°14'S, 58°40'W), near the scientific Argentinean «Jubany» station, in the 25 de Mayo (King George) Island, South Shetland Islands, Antarctica (Fig.1).

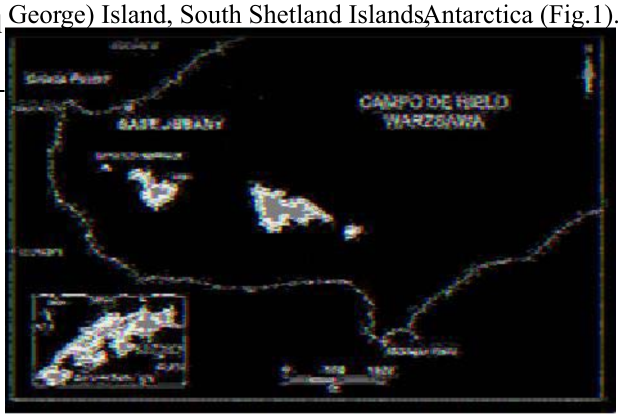

Fig. 1. Map of the sampled area

In order to improve a selective isolation of marine fungi, some wood panels were placed into plastic nets, which were tied with nylon ® cords and anchored to the ground (modified from Kohlmeyer & Kohlmeyer, 1979). Later, panels were exposed to the seawater at the intertidal level during a variable period of time (2–12 months). After exposure, the wood panels were stored at -20 ºC, sent to the IAA, and finally studied at the URV.

The wood panels exposed to seawater were first examined under a stereomicroscope to find sexual and asexual fungal reproductive structures. Once located, these structures were transferred to a mounting medium (water, lactic acid, Melzer's reagent and 0.1 % metilene blue water solution) using dissection needles. Measurements of the fungal structures were taken from the material mounted in water, and photomicrographs obtained using a light-field microscope (Leitz Dialux 20 EB). The panels, once the fungi had been identified, were dried and stored at the culture collection of the Faculty of Medicine, Reus, Spain (FMR).

RESULTS AND DISCUSSION

Two of the marine fungi species found growing on the wood test panels were not previously reported for Antarctica. The description and relevant biological data of these two isolates are presented and discussed below .

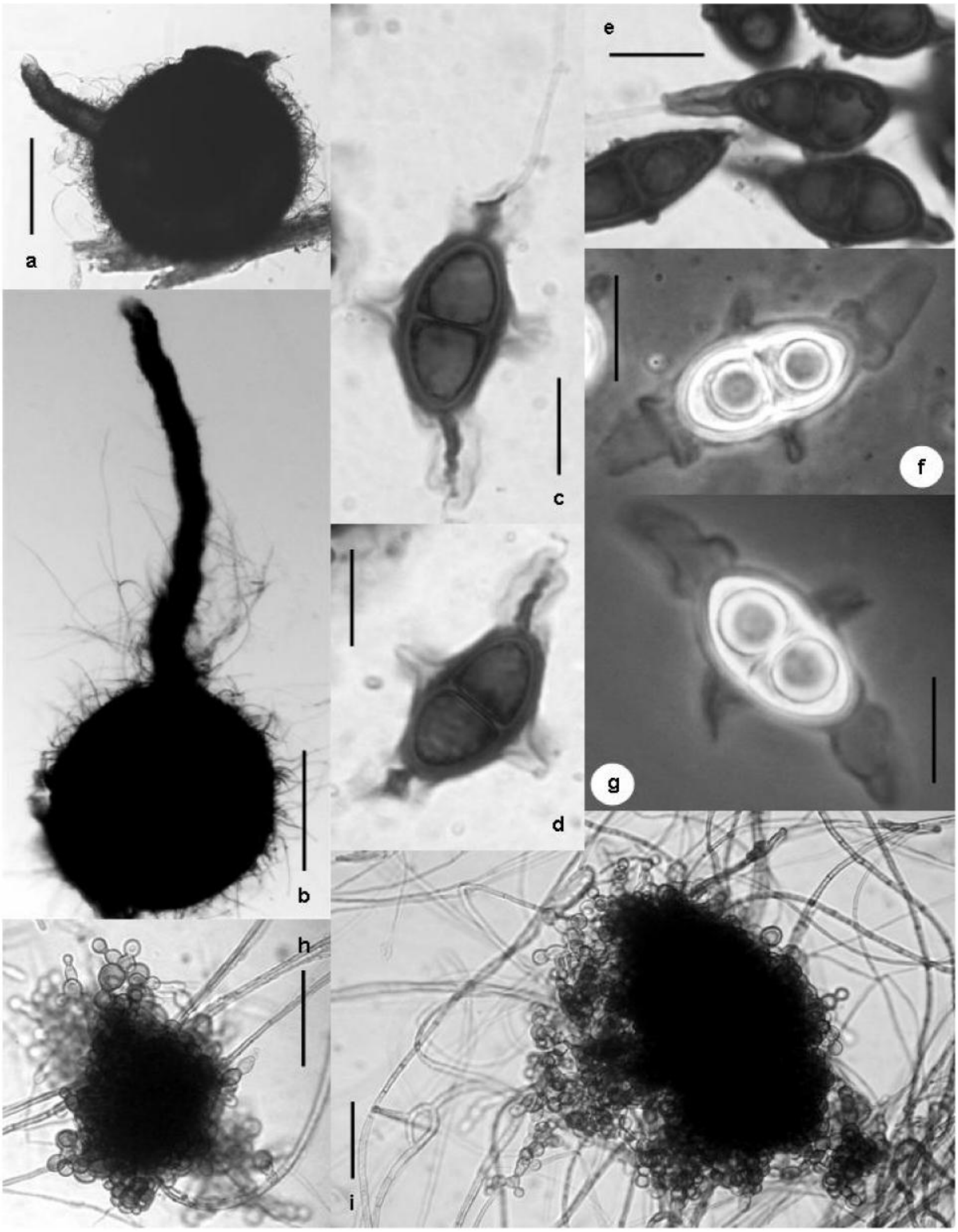

Fig. 2. *Halosphaeria tubulifera* var. *longicollea*, FMR 9389. a, b. Ascomata (LM). c-g. Ascospores showing the exosporic sheath and the viscous appendages (c-e, LM; f, g, Nomarski). *Papulaspora halima* FMR 9388. h, i. «Papulaspores» (LM). Bars: a, b, 200µm; c-g, 10µm; h, i, 50µm.

Halosphaeria tubulifera Kohlm. Nova Hedwigia. 2, 311. 1960. (Figura 2 a-g)

Ascomata superficial, scatted, ostiolate, with a spherical body (250–500 µm diam) and a cylindrical or irregular neck, coriaceous, black under reflected light, hairy; peridial wall up to 40 µm thick, of *textura angularis*, reddish brown to dark brown, composed of up to 10 layers of flattened cells, thin- to thick-walled; neck up to 1000 µm long (usually between 200 and 450 µm), 50-100 µm wide, dark brown to black, but usually much paler to the apex, composed by polygonal cells (similar to those of peridial wall); hairs regularly septate, pale brown to brown, paler to the apex, thin- to thick-walled, up to 200 µm long, 2-5 µm wide at the base, apex rounded; asci 8-spored, claviform, pedicelate, 60–80 x 15–25 µm, unitunicate, thin-walled and without apical structures, soon evanescent; two-celled ascospores, 15–20 x 9–10 µm (without the surrounding sheath), ellipsoidal, slightly constricted at the medial septum, hyaline, thick-walled, surrounded by an exosporic sheath, which forms a cylindrical to conical tubular structures at both extremes of the ascospore, containing inside a mucilaginous appendage, which is transformed in a gelatinous mass that is extruded out of the pores of the exosporic tubs, forming adhesive filaments; exosporium also form a ring, of 4–6 µm thick, that surrounds the septum.

Specimen: Antarctica, King George Island, Potter Cove, growing on a wood test panel submerged into seawater, 12 June 2002, col. L.M.A. Ruberto and W.P. Mac Cormack, id. A.M. Stchigel (FMR 9389).

Members of the order **Halosphaeriales** Kohlm. have a marine life cycle and are characterized by its membranous, ostiolated ascomata usually bearing necks (but some lacking openings), the production of unitunicate, thin-walled, evanescent asci usually without apical structures, the ascospores with appendages and sheaths to sail in the water and to attach to usually cellulosic substrates (Jones, 1995). This order contains 55 genera (Eriksson, 2006). However, the order **Halosphaeriales** is actually considered as a polyphyletic assemblage, showing several evolutionary lines (Spatafora *et al.,* 1998).

Halosphaeria tubulifera was previously described as growing on discomposing wood (ej. *Betula pubescens*, *Fagus sylvatica*, *Pinus sylvestris*, *Quercus* spp., *Salix* spp.), and reported in Canada, Denmark, Germany, Great Britain, Unit States of America, Ireland, and Sweden (Kohlmeyer & Kohlmeyer, 1979). For Antarctic marine environments, this fungus (as ***Cerisporopsis tubulifera***) was previously reported by Pugh & Jones (1986). Our specimen differs from the original and later descriptions as it has a longer neck (up to 1000 µm, vs. papillate or up to 165 µm) and hairy ascomata. Pugh and Jones (1986) did not mention morphological differences among their isolates and those recovered from other locations of the world.

Papulaspora halima Anastasiou, Nova Hedwigia 266, 266. 1963. (Figura 2 h-i)

Micelium regularly septate, branched, anastomosed; hyphae subhyaline to dark brown, 2–4 µm wide; papulaspores subglobose or irregular, superficial, 50–1000 µm diam, grayish-brown to nearly black, originated from chains of cells; cells 5–15 µm diam, spherical, ovoid, ellipsoidal, barrel-shaped or irregular, thin- to thick-walled, brown to grayish-brown, disarticulating when old.

Specimen: Antarctica, King George Island, Potter Cove, growing on a wood test panel submerged into seawater, 12 April 2002, col. L.M.A. Ruberto and W.P. Mac Cormack, id. M. Calduch and A.M. Stchigel (FMR 9388).

Papulaspora Preuss at present encompass already 40 cosmopolitan species. Their habitats are mostly decomposing plants and soil. Although no molecular studies were done yet, ***Papulaspora*** seem to be an assemblage of phylogenetically unrelated genera. However ***Hypomyces*** and ***Melanospora*** (belonging to the order **Hypocreales; Ascomycota**) are their only known sexual states (Domsch *et al.,* 1980; Storey, 2002).

Papulaspora halima grows on decomposing wood (ej. *Arbutus menziesii*, *Abies alba*, *Betula papyrifera*, *Fagus sylvatica*, *Larix decidua*, *Ochroma lagopus*, *Olea europaea*, *Pinus pinaster*, *Populus alba*, *Quercus* spp., *Tamarix aphylla*), and was reported previously for Canada, Italy, Japan, Thailand and USA (Kohlmeyer & Kohlmeyer, 1979; Chatmala *et al.* 2004).

The generic position of ***P. halima*** in ***Papulaspora*** is rather questionable, because their propagules are not composed by a central cluster of dark cells surrounded by a lighter spore coat, as occurs in other species of the genus (Weresub and LeClair, 1971; Kohlmeyer & Kohlmeyer, 1979). But ***P. halima*** also differs from two morphologically related genera, ***Minimedusa*** Weresub & Le Clair, in which case the «bulbils» (papulaspore-like propagules) arise from the aggregation of several lateral hyphae, and ***Myriococcum*** Fr., which produces dense masses of small-celled «bulbils» arising from one to several lateral branches coiled around the main branch (Weresub & LeClair, 1971).

Our specimen differs from previous descriptions because of the absence of the ring-like widenings at the septa.

The present study, although restricted, clearly reflects the lack of knowledge about the diversity of fungi (with the exception of the yeasts) in the marine Antarctic ecosystems, because both specimens, ***Halosphaeria tubulifera*** and ***Papulaspora halima***, were unreported until now for such geographical site.

The authors consider that the morphological differences between their specimens and the preceding reports would be caused by their adaptation to the Antarctic environments.

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