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TAXONOMIC DESCRIPTION

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Revealing the hidden diversity of marine fungi in Portugal with the description of two novel species, *Neoascochyta fuci* sp. nov. and *Paraconiothyrium salinum* sp. nov.

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Abstract

Fungi are ubiquitous organisms with a wide distribution in almost all ecosystems, including marine environments. Coastal and estuarine ecosystems remain poorly unexplored as fungal habitats, potentially harbouring a hidden diversity with important ecological roles. During an extensive survey of marine fungi in coastal and estuarine Portuguese environments, a collection of 612 isolates was obtained from water, algae, sponges and driftwood. From these, 282 representative isolates were selected through microsatellite-primed PCR (MSP-PCR) fingerprinting analysis, which were identified based on DNA sequence data. The collection yielded 117 taxa from 38 distinct genera, which were identified using DNA sequence analysis. Overall, fungal community composition varied with host/substrate, but the most abundant taxa in the collection were *Cladosporium cladosporioides*, *Penicillium terrigenum*, *Penicillium brevicompactum* and *Fusarium equiseti/incarnatum* complex. The occurrence of a high fungal diversity harbouring novel species was disclosed. Through a multilocus phylogeny based on ITS, *tub2* and *tef1*-α sequences, in conjunction with morphological and physiological data, we propose *Neoascochyta fuci* sp. nov. and *Paraconiothyrium salinum* sp. nov.

INTRODUCTION

The marine environment is an inexhaustible resource for the isolation of unexploited microorganisms with unique characteristics, in particular marine fungi [1].

Over time, different definitions have been given to marine fungi and it remains controversial [2]. Marine fungi were first defined based on their physiological characteristics, such as the requirement for seawater (salinity ≥30%) to grow [3, 4]. However, the definition generally quoted is that proposed by Kohlmeyer and Kohlmeyer [5]. These authors restricted the term marine fungi to two major ecological groups: (a) obligate, those that grow and sporulate exclusively in a marine or estuarine habitat; and (b) facultative, those from freshwater or terrestrial milieus that are able to grow and sporulate in marine environments. Recently, other authors have replaced Kohlmeyers' definition by using a broader definition, overcoming the issue related to marine-derived isolates [2], referring to fungi isolated from marine or marine-related habitats or substrates [6]. In this context, Pang *et al.* [7] reviewed the use of the terms 'marine fungi' and 'marine-derived fungi' and proposed a wide-ranging definition of a marine fungus, including any fungus that is able to grow and/or sporulate (on substrata) in marine environments; those that form symbiotic relationships with other marine organisms; or those that have adapted and evolved or also are metabolically active in marine environments. We will use this definition from this point forward.

Currently, the total diversity of marine fungi is estimated to be 10 000–12 500 species [8, 9] with 1257 species described so far, distributed in 539 genera, 74 orders, 168 families, 20 classes and five phyla [2]. Marine fungi represent less than 1% of described fungal species [10, 11] and are particularly poorly

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Keywords: fungal diversity; *Didymellaceae*; *Didymosphaeriaceae*; phylogeny; taxonomy.
Abbreviations: AFSW, autoclaved filtered saline water; BI, Bayesian inference; CMG, culture collection of Micael Gonçalves, housed at Department of Biology, University of Aveiro, Portugal; MEA, malt extract agar; ML, maximum-likelihood; MP, maximum-parsimony; MSP-PCR, microsatellite-primed PCR; MUM, Micoteca da Universidade do Minho; MUM-H, Micoteca da Universidade do Minho-Herbário; NJ, neighbour-joining; OA, oatmeal agar; PDA, potato dextrose agar; PP, posterior probability.
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The GenBank/EMBL/DDBJ accession numbers of strain CMG 47/MUM 19.41 are ITS = MN053014; *tub2* = MN066618 and of strain CMG 49/MUM 19.91 are ITS = MN369540; *tub2* = MN380479; *tef-1*α = MN380481.
Two supplementary tables and two supplementary figures are available with the online version of this article.

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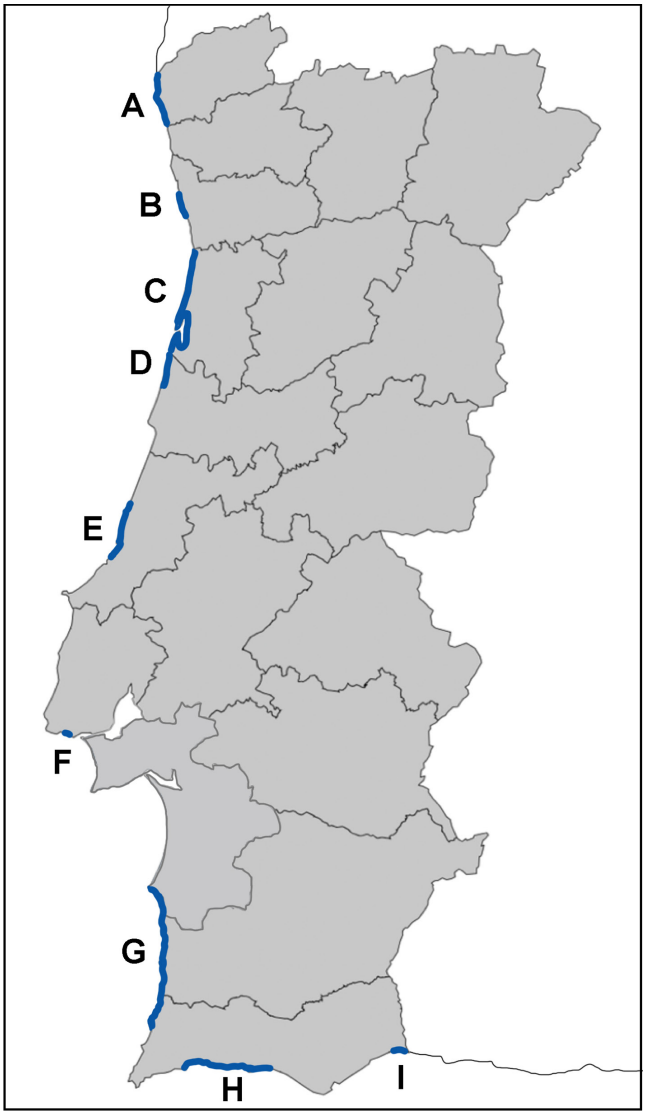

Fig. 1. Location of sampling sites in the current study. A, Viana do Castelo; B, Matosinhos; C, Aveiro; D, Mira; E, Nazaré; F, São Pedro do Estoril; G, Sines, H/I, Algarve. For more rigorous localizations, see Table 1.

characterized. They have been isolated from a wide range of organic and inorganic substrata such as marine mangrove plants, macroalgae/seaweed, drift- and intertidal wood, marine animals (corals, sponges, nematodes, etc.), sediments, and coastal and open-ocean water columns [7, 12]; they can occur as saprobes, endobionts, parasites and mutualists [2]. Also, they play a major role in nutrient recycling and in the regulation of energy flow in marine ecosystems [2].

Marine fungi are distributed throughout the world and certain fungi are found only in certain geographical regions, such as the tropics, subtropics, and temperate or polar waters [13–15]. Culture-dependent studies with morphology-based analyses have retrieved fungi from marine environments [1, 16–18]. Recently, advances in high-throughput sequencing technologies have enabled research on marine fungi using culture-independent methods on deep-sea and benthic sediments [19, 20], hydrothermal vents [21–23], oxygen-deficient environments [24, 25], global surface waters [26, 27] and coastal habitats [28, 29].

In this context, coastal marine environments, despite being known as part of highly productive ecosystems, remain poorly investigated regarding the biodiversity of their mycobiota. Some diversity studies are available, in particular regarding algicolous [1, 30–32] and lignicolous fungi [33, 34], sponges [35], sediments and seawater [26, 28, 29] and marine animals [36].

The diversity of fungi from Portuguese marine environments is poorly known. To address this knowledge gap, an extensive survey of the fungal diversity associated with various substrates/hosts across coastal and estuarine environments in Portugal was performed, using culture-dependent methods. A robust identification was allowed based on microsatellite-primed PCR (MSP-PCR) fingerprinting to group isolates according to their genetic fingerprinting patterns and then sequencing of DNA markers of representative isolates of the collection, such as internal transcribed spacer (ITS), beta-tubulin gene (*tub2*) and translation elongation factor 1 alpha (*tef1-α*). Here we also report the morphological, cultural and phylogenetic characterization of two novel fungal species in the genera *Paraconiothyrium* (*Didymosphaeriaceae*) and *Neoascochyta* (*Didymellaceae*).

METHODS

Collection and isolation

Water (3 litres at 2 m deep), algae – mainly *Fucus* and *Ulva* species (n=60) – and driftwood (n=14) were collected from various Portuguese coastal beaches, while sponges (n=15) and water were collected from different sites in the estuary of Ria de Aveiro, Portugal (Fig. 1, Table 1). Samples were placed in sterile plastic containers and maintained at 4 °C until fungal isolation. Water samples were vacuum-filtered with sterile 0.22 µm cellulose membranes (Merck). Then, the membranes were vigorously washed in 10 ml of autoclaved filtered saline water (AFSW). Aliquots of 100 µl from each water sample were spread onto potato dextrose agar (PDA) containing 3% sea salts (Sigma-Aldrich). Algae, sponges and driftwood samples were washed with AFSW, cut into small pieces and placed on PDA with 3% sea salts. Streptomycin and tetracycline, at final concentrations of 100 mg l^{-1}, were added to PDA medium to inhibit the growth of bacteria. Five replicates of PDA plates were used for each sample. The plates were incubated at 25 °C and examined daily to observe the growth of fungal hyphae. Distinct fungal colonies were then transferred to new PDA plates with sea salts for further isolation and purification.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fresh mycelium of cultures growing on PDA according to Möller *et al.* [37]. MSP-PCR

Table 1. List of the sampling sites in this study

C, coastal; E, estuary.

Locality code	Locality name	Origin	GPS coordinates	Sampling date
A	Viana do Castelo	C	41° 41' 57" N 8° 51' 22" W	08/10/17
		C	41° 44' 07" N 8° 52' 26" W	08/10/17
		C	41° 46' 50" N 8° 52' 17" W	08/10/17
		C	41° 48' 50" N 8° 52' 03" W	08/10/17
		C	41° 51' 05" N 8° 52' 03" W	08/10/17
B	Matosinhos	C	41° 10' 39" N 8° 41' 46" W	08/10/17
		C	41° 13' 05" N 8° 43' 00″ W	08/10/17
		C	41° 11' 43" N 8° 42' 35" W	08/10/17
C	Barra	C	40° 38' 38" N 8° 44' 57" W	12/09/17
		C	40° 38' 37" N 8° 44'58"W	12/09/17
		C	40°38'29"N 8°45' 00" W	12/09/17
		C	40° 38' 32" N 8° 45' 25" W	12/09/17
		C	40° 38' 30" N 8° 45' 27" W	12/09/17
		C	40° 38' 11" N 8° 44' 55" W	16/09/17
	Cortegaça	C	40° 56' 16" N 8° 39' 33" W	15/10/17
		C	40° 56' 39" N 8° 39' 31" W	15/10/17
	Costa Nova	C	40° 37' 10" N 8° 45' 14" W	16/09/17
		C	40° 36' 55" N 8° 45' 18" W	16/09/17
	Esmoriz	C	40° 57' 46" N 8° 39' 14" W	15/10/17
	Espinho	C	41° 00' 07" N 8° 38' 51" W	15/10/17
		C	41° 00' 35" N 8° 38' 52" W	15/10/17
	Vagueira	C	40° 33' 33" N 8° 46' 17" W	16/09/17
	Ria de Aveiro	E	40° 37' 48" N 8°43'58"W	26/09/18
		E	40°39 '33" N 8° 43' 27" W	26/09/18
		E	40° 40' 38" N 8° 42' 20" W	26/09/18
		E	40° 43' 00" N 8° 42' 04" W	26/09/18
		E	40° 38' 54" N 8° 44' 23" W	26/09/18
D	Areão	C	40° 31' 7" N 8° 47' 3" W	16/09/17
	Mira	C	40° 29' 22" N 8° 47' 34" W	16/09/17
E	Marinha Grande	C	39° 46' 21" N 9° 01' 39" W	30/09/17
	Nazaré	C	39° 35' 26" N 9° 04' 37" W	30/09/17
		C	39° 35' 49" N 9° 04' 22" W	30/09/17
	Paredes da Vitória	C	39° 42' 19" N 9° 03' 05" W	30/09/17
		C	39° 42' 01" N 9° 02' 56" W	30/09/17
	S.Pedro de Moel	C	39° 45' 21" N 9° 01' 57" W	30/09/17
F	S.Pedro do Estoril	C	38° 41' 28" N 9° 21' 55" W	01/10/17
		C	38° 41' 32" N 9° 22' 03" W	01/10/17

Continued

Table 1. Continued

Locality code	Locality name	Origin	GPS coordinates	Sampling date
		C	38° 41' 33" N 9° 22' 05" W	01/10/17
		C	38° 41' 33" N 9° 22' 18" W	01/10/17
G	Sines	C	37° 55' 31" N 8° 48' 21" W	19/10/17
		C	37° 57' 15" N 8° 52' 01" W	19/10/17
	Porto Covo	C	37° 49' 44" N 8° 47' 28" W	19/10/17
	Vila Nova de Milfontes	C	37° 43' 18" N 8° 47' 25" W	19/10/17
	Costa Vicentina	C	37° 17' 41" N 8° 51' 59" W	19/10/17
		C	37° 26' 25" N 8° 47' 58" W	19/10/17
H	Lagos	C	37° 05' 48" N 8° 40' 06" W	19/10/17
	Albufeira	C	37° 04' 32" N 8° 18' 32" W	19/10/17
	Portimão	C	37° 07' 08" N 8° 33' 01" W	19/10/17
I	Vila Real de Santo António	C	37° 09' 54" N 7° 24' 04" W	19/10/17

fingerprinting with the $(GTG)_5$ primer was used for molecular typing of all isolates, following Alves *et al.* [38]. Briefly, analysis of the genetic fingerprinting patterns was performed with GelCompar II software (Applied Maths). The Pearson correlation coefficient was applied, and cluster analysis was performed using the UPGMA algorithm. The resulting dendrograms were analysed in order to obtain groups of isolates with at least 85% similarity. This cut-off was determined so that patterns that were known to be equal would be considered to be in the same cluster. Representative isolates of each group were randomly selected and subjected to PCR amplification of the ITS region of the rDNA (using primers ITS1 and ITS4 [39]) as described by Alves *et al.* [40]. For isolates whose ITS region was not sufficient for identification, additional molecular markers were used for each taxonomic group, such as beta-tubulin (*tub2*) using a combination of Bt2a/T1 and Bt2b primers [41, 42] and translation elongation factor 1 alpha (*tef1-α*) with EF1-688F and EF1-2218R primers [43, 44] with the cycling conditions previously described by Lopes *et al.* [45]. The amplified PCR fragments were purified with the NZYGelpure kit (NZYTech) before sequencing at GATC Biotech. The nucleotide sequences were analysed with FinchTV v.1.4.0 (Geospiza). A BLASTN search against the nucleotide collection (nr/nt) database using the ITS, *tub2* and *tef1-α* sequences was carried out to determine the closest matching sequences. Information from the representative isolates was used to provide a taxonomic affiliation to all isolates from the collection.

Four isolates (CMG 47, CMG 48, CMG 49 and CMG 50) could not be affiliated to any of the currently known species and probably represented undescribed species. Thus, the closest related sequences were added to the sequence alignment to determine the taxonomic affiliation, and morphological characterizations were performed. Sequences were aligned with CLUSTALX v. 2.1 [46], using the following parameters: pairwise alignment parameters (gap opening=10, gap extension=0.1) and multiple alignment parameters (gap opening=10, gap extension=0.2, transition weight=0.5, delay divergent sequences=25 %). Alignments were checked and edited with BioEdit Alignment Editor v.7.2.5 [47]. Phylogenetic analyses were done with MEGA v.7.0 [48]. All gaps were included in the analyses. MEGA v.7.0 was also used to determine the best substitution model to be used to build the maximum-likelihood (ML) tree. ML analyses was performed on a neighbour-joining (NJ) starting tree automatically generated by the software. Nearest-Neighbour-Interchange (NNI) was used as the heuristic method for tree inference with 1000 bootstrap replicates. Maximum-parsimony (MP) analysis were performed with PAUP v. 4.0b10 [49]. All characters were unordered and of equal weight, and gaps were treated as missing data. The heuristic search option with 100 random taxon additions and subtree pruning and regrafting (SPR) method as the branch-swapping algorithm were applied. Bayesian inference (BI) was performed using Mr. Bayes v. 3.0b4 [50]. Four Markov chain Monte Carlo (MCMC) chains were set to run for 10 million generations, sampling every 100th generation for a total of 10 000 trees. The first 1000 trees were eliminated from further analysis. The remaining were used to generate a majority-rule consensus tree and calculate the posterior probabilities (PP). Trees were visualized with TreeView [51]. The sequences generated in this study were deposited in GenBank and taxonomic novelties in MycoBank. The alignment and tree were deposited in TreeBase (S24639, S25049).

Morphology and growth studies

The new species identified through phylogenetic analyses were observed with a Nikon SMZ1500 stereoscopic microscope and a Nikon Eclipse 80i microscope equipped with differential interference contrast. Fungal structures were

mounted in 100% lactic acid. Photographs and measurements were taken with a Nikon DSRi1 camera and the NIS-Elements D program (Nikon). Colony characters and pigment production were registered after 5 and 7 days of growth on PDA, malt extract agar (MEA) and oatmeal agar (OA) incubated at 25 °C for *Neoascochyta* sp. and *Paraconiothyrium* sp., respectively. Colony colours (obverse and reverse) were assessed according to the colour charts of Rayner [52]. Morphological descriptions were based on cultures sporulating on OA and PDA/pine needles, after 2 months of incubation at 25 °C, for *Neoascochyta* sp. and *Paraconiothyrium* sp., respectively.

Temperature growth studies were performed for the new species described. A 5-mm-diameter plug was taken from the margin of an actively growing colony (7 days old) and placed in the centre of PDA, MEA and OA plates. Three replicate plates per isolate were incubated at 10, 15, 20, 25, 30 and 35 °C in the dark. Colony diameter was measured after 5 days and 1 week for *Neoascochyta* sp. and *Paraconiothyrium* sp, respectively.

To evaluate the growth requirements for sea salts, the new species was cultured in PDA with 3% (w/w) sea salts. Three replicate plates per isolate were incubated at 25 °C for 5 and 7 days in the dark. After incubation the diameter of the colonies was measured and compared.

RESULTS

Diversity of fungal isolates

This study addressed for the first time the diversity of the fungal species in coastal marine environments from Portugal and in the estuary of the Ria de Aveiro (Table 1). A total of 525 fungal isolates were obtained from seawater (*n*=283), algae (*n*=214) and driftwood (*n*=28) from Portuguese beaches, while 89 fungal isolates were collected from saline water (*n*=24) and sponges (*n*=65) in the estuary of the Ria de Aveiro. Molecular typing of the collection using MSP-PCR yielded 282 representative isolates for which sequences of the ITS rRNA region were obtained. BLASTN searches against the nucleotide collection (nr/nt) database unambiguously affiliated the isolates from Portuguese coastal beaches (Fig. 2) and estuary (Fig. 3) to 31 and 23 distinct genera, respectively. Details of each species found are given in Tables S1 and S2. The *Penicillium* species from Portuguese coastal beaches described previously [53] were included here in the analysis of fungal diversity on coastal marine environments from Portugal.

Among the different taxa identified, the majority of fungal isolates belonged to the genera *Cladosporium* (39.2%, *n*=206), *Penicillium* (31.4%, *n*=165) and *Fusarium* (8.8%, *n*=46), in the samples from Portuguese coastal beaches, and *Trichoderma* (19.3%, *n*=19), *Penicillium* (15.9%, *n*=14), *Verticillium*/*Gloeotinia* (11.4%, *n*=10) and *Fusarium* (10.2%, *n*=9) in the samples from the Ria de Aveiro estuary. When analysing the collection of isolates from coastal beaches, 64 different fungal species were found in seawater, 46 in algae and 17 in driftwood (Fig. 4a; Table S1, available in the online version of this article). However, fewer samples of driftwood were analysed, which could justify the lower diversity found in these samples. Likewise, 12 different species were found in saline water and 42 in sponges (Fig. 4b, Table S2).

Phylogenetic analysis

BLASTN searches against the NCBI nucleotide database using the ITS sequences for isolates CMG 47 and CMG 48 identified the closest matches as *Neoascochyta paspali* [GenBank accession no. MH861378; similarities 503/506 (99%), 2 gaps], *Phoma* sp. (GenBank accession no. FJ228201; similarities 501/504 (99%), no gaps] and Fungal sp. strain OTU23 (GenBank accession no. KT923242; similarities 502/506 (99%), 2 gaps]. Beta-tubulin was also sequenced to confirm the phylogenetic placement within the genus *Neoascochyta*. The highest similarities, using the *tub2* gene sequence, were to *N. paspali* [GenBank accession no. FJ427158; similarities 338/344 (98%), no gaps], *N. soli* [GenBank accession no. KY742363; similarities 330/334 (99%), no gaps] and *N. paspali* (GenBank accession no. GU237640; similarities 328/334 (98%), no gaps]. Therefore, sequences (ITS+*tub2*) of CMG 47 and CMG 48 were aligned with those of several related *Neoascochyta* species (Table 2). Alignment of the ITS+*tub2* comprised 31 sequences (including the outgroup), and there was a total of 1001 positions in the final dataset. In the ML phylogenetic tree (Fig. 5), the novel isolates clustered in a clade that received high (97%) bootstrap support with high PP values (0.98) within the genus *Neoascohyta* with a close relationship with *N. soli* and *N. paspali*.

Regarding isolates CMG 49 and CMG 50, the closest matches for the ITS sequence retrieved various hits, of which those with the highest sequence similarity belonged to unidentified isolates of the family *Didymosphaeriaceae*, such as *Paraphaeosphaeria* sp. [GenBank accession no. FJ770071; similarities 563/564 (99%), no gaps], *Pleosporales* sp. [GenBank accession no. KP263116; 539/541 (99%), 1 gap) and *Paraphaeosphaeria* sp. (GenBank accession no. MH383206; 537/539 (99%), 1 gap]. The closest match of an identified species was *Paraconiothyrium cyclothyrioides* [GenBank accession no. MH383206; 535/565 (95%), 7 gaps] and *P. estuarinum* [GenBank accession no. MH383206; 532/565 (94%), 7 gaps]. ITS sequences were aligned separately with those of related genera/species of *Didymosphaeriaceae* (Table 2) to identify the species that are closest to our isolates, before performing a multilocus phylogenetic analysis. Additional molecular markers using *tub2* and *tef1-α* gene sequences were used to confirm the phylogenetic placement within the genus *Paraconiothyririum*. The closest hit using the *tub2* gene sequence was *P. hakeae* [GenBank accession no. KY979920; 509/604 (84%), 14 gaps] and using the *tef1-α* gene sequence was *Austropleospora keteleeriae* [GenBank accession no. MK360045; 892/920 (97%), no gaps], *Cylindroaseptospora siamensis* [GenBank accession no. MK360048; 891/920 (97%), no gaps] and *Pseudopithomyces entadae* [GenBank accession no. MK360083; 890/920 (97%), no gaps]. Thus, the alignment of the ITS, ITS+*tub2* and ITS+*tef1-α* contained 54, 34 and 28 sequences (including the outgroup), and there were a total of 836, 1317 and 1761 positions in the final dataset, respectively.

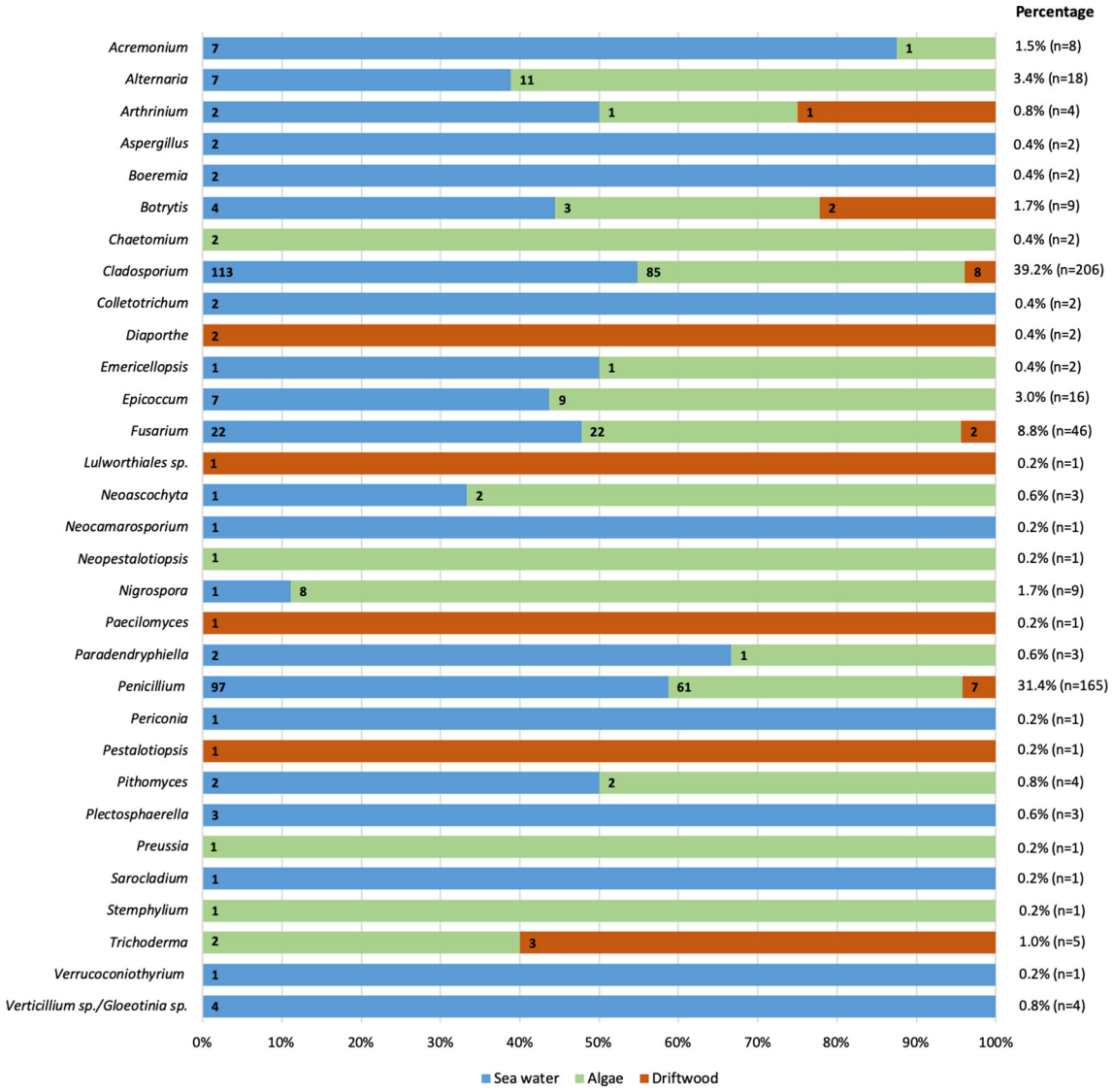

Fig. 2. Distribution of the 31 genera by host or substrate from samples of Portuguese coastal beaches.

In all ML phylogenetic trees (Fig. 6; Figs S1 and S2), all novel isolates clustered in a clade that received high (74, 97 and 85%) bootstrap support with high PP values (0.96, 0.84 and 1.00) within the family *Didymosphaeriaceae* with a close relationship to the genus *Paraconiothyrium* (ITS+*tub2*, ITS+*tef1-α*, Figs S2 and Fig. 6) with the following *p*-distances of nucleotide sites with *P. estuarinum* and *P. cyclothyrioides*: ITS+*tub2*=0.066–0.080) and ITS+*tef1-α*=0.032.

These two novel lineages in the genera *Neoascochyta* and *Paraconiothyrium* are phylogenetically well delimited and are clearly distinct from other closely related species described so far and therefore are proposed here as novel species.

Description of *Neoascochyta fuci* M. Gonçalves & A. Alves sp. nov

Neoascochyta fuci (fu'ci. N.L. gen. n. *fuci* from *Fucus*) Fig. 7.

Typus. Portugal, Lumiar Beach, Viana do Castelo (41° 44' 07" N 8° 52' 26" W), isolated from *Fucus* sp., 8 October 2017, M. Gonçalves, deposited in the MUM Herbarium (holotype: a dried culture sporulating, MUM-H 19.41; ex-type living culture, MUM 19.41=CMG 47). GenBank accession numbers for DNA sequences derived from ex-type: ITS=MN053014; *tub2*=MN066618.

Micromorphology. On OA, conidiomata pycnidial, aggregated or solitary, globose to sub-globose, dark brown, immersed on the agar rarely superficial, with a single ostiole. Conidiomata wall pseudoparenchymatous, composed of thick layers of isodiametric cells with brown pigmentation. Conidiogenous cells not recorded. Conidia fusoid to cylindrical, sometimes ellipsoidal with rounded apex, smooth-walled, hyaline, aseptate (mean±SD=7.6±0.6×3.0±0.2 µm, *n*=100), with several

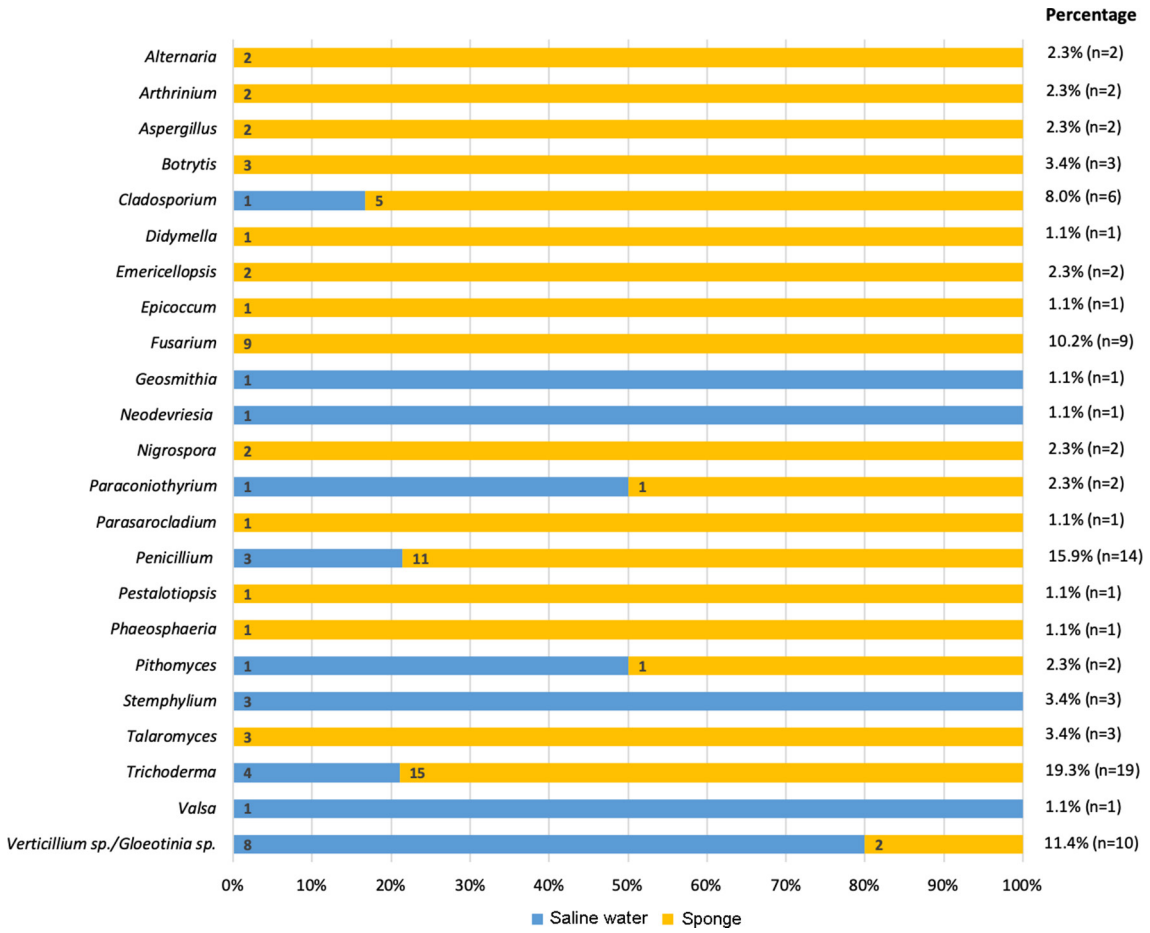

Fig. 3. Distribution of the 23 genera by host or substrate from samples of the estuary of the Ria de Aveiro.

polar guttules. Chlamydospores not observed. Sexual morph unknown.

Colony characteristics. Colonies flat with fluffy and aerial mycelium. PDA 25 °C, 5 days: colonies growing to 80 mm in diameter, obverse, margin regular, white; reverse olivaceous-black. No differences were observed in terms of colony diameter when grown in PDA with and without the addition of 3% sea salts. MEA 25 °C, 5 days: colonies growing to 77 mm in diameter, obverse, margin regular, white; reverse brown vinaceous to olivaceous, from the centre to the margins. OA 25 °C, 5 days: colonies growing to 55 mm in diameter, obverse, irregular white mycelium; reverse violaceous black. At 35 °C, there was no growth in any of the media tested.

Known distribution. Portugal.

Habitat. Fucus sp.

Additional specimens examined. Portugal, Lumiar Beach, Viana do Castelo (41° 44' 07" N 8° 52' 26" W), isolated from *Fucus* sp., M. Gonçalves, living culture CMG 48. GenBank accession numbers: ITS=MN053015; *tub2*=MN066619.

MycoBank: MB831828

Notes: Neoascochyta fuci clustered with *N. soli* (LC 8165) and *N. paspali* (CBS 560.81) in a distinct clade in this genus. Micromorphologically, they differ in conidial morphology and dimensions. Conidia of *N. paspali* are obclavate-ovoid to ellipsoidal, without guttules and are 5.5–8.5(–11)×2.5–4 µm. Conidia of *N. soli* are ellipsoidal to oblong, with two to several polar guttules and are 7–10×3–4 µm, while conidia of *N. fuci* are fusoid to cylindrical, sometimes ellipsoidal with rounded apex, straight and slightly larger and thinner than *N. soli* and of 7.6±0.6×3.0±0.2 µm. The phylogenetic tree demonstrates that *N. fuci* is phylogenetically distinct from *N. soli* and *N. paspali*. *Neoascochyta fuci* differs from *N. paspali* in four and six nucleotide positions in ITS and *tub2*, respectively, and in four nucleotide positions in the two-loci sequences from *N. soli*.

Description of *Paraconiothyrium salinum* M. Gonçalves & A. Alves sp. nov

Paraconiothyrium salinum (sa.li'num. N.L. neut. n. *salinum* from saline water) Fig. 8.

Typus. Portugal, Ria de Aveiro (40° 40' 38" N 8° 42' 21" W), isolated from unknown sponge, 26 September 2018,

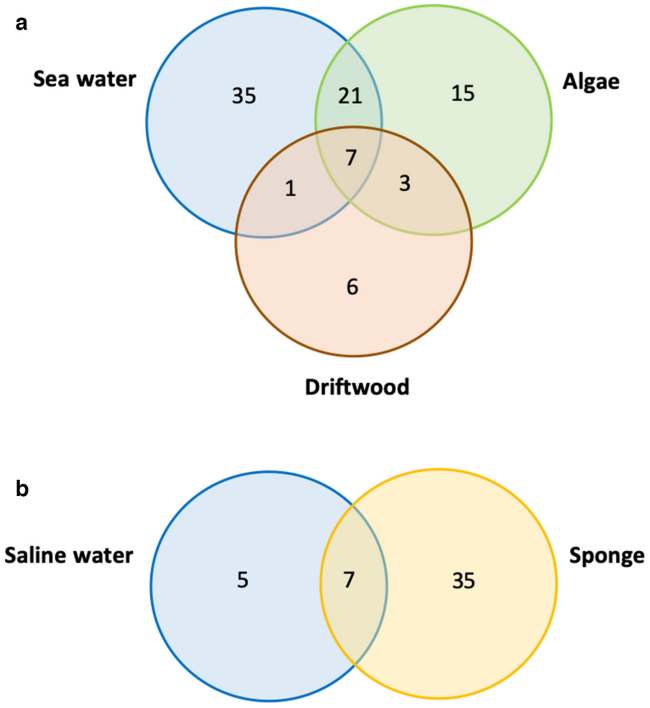

Fig. 4. Venn diagram of the number of fungal species isolated from (a) coastal beaches and (b) estuary of the Ria de Aveiro.

M. Gonçalves, deposited in the MUM Herbarium, (holotype: a dried culture sporulating on pine needles AVE-F-6; ex-type living culture, MUM 19.91=CMG 49). GenBank accession numbers for DNA sequences derived from ex-type: ITS=MN369540*; tub2*=MN380479; *tef-1α*=MN380481.

Micromorphology. On PDA, mycelium smooth, wide hyphae (mean±SD=1.6±0.3 µm, *n*=50). Hyphae thick-walled, smooth, aseptate rarely septate, hyaline to yellowish brown. On pine needles, conidiomata eustromatic, aggregated or solitary, irregularly globose or flattened, black, with merging cavities without ostioles. Conidiomata wall pseudoparenchymatous, composed of thick layers of isodiametric cells and irregular cells with yellowish brown pigmentation. Conidiogenous cells ampulliform to subcylindrical, hyaline, phialidic, producing smooth, ellipsoidal or subcylindrical conidia. Conidia straight, with rounded apices at both ends, sometimes with one or two smalls polar guttules, and with thin and smooth walls, yellowish brown and aseptate (mean±SD=3.7±0.3×1.2±0.2 µm, *n*=100). Chlamydospores not observed. Sexual morph unknown.

Colony characteristics. PDA 25 °C, 1 week: colonies growing to 50 and 44 mm in diameter with and without 3% sea salts, respectively, obverse olivaceous near the centre becoming lighter towards the borders; reverse brown vinaceous in the centre and yellowish white at periphery. MEA 25 °C, 1 week: colonies growing to 34 mm in diameter, obverse and reverse greenish olivaceous near the centre becoming lighter towards the borders. OA 25 °C, 1 week: colonies growing to 44 mm in diameter, obverse and reverse greenish olivaceous near the centre with fluffy aerial white mycelium at periphery. At 35 °C, there was no growth in any of the media tested.

Know distribution. Portugal.

Habitat. Saline water and sponge.

Additional specimens examined. Portugal, Ria de Aveiro (40° 40' 38" N, 8° 42' 21" W), isolated from saline water, M. Gonçalves, living culture CMG 50. GenBank accession numbers: ITS=MN369541; *tub2*=MN380480; *tef-1α*=MN380482.

MycoBank: MB832768

Notes: Paraconiothyrium salinum is phylogenetically distinct and forms a sister clade to *P. estuarinum* (CBS 109850), *P. cyclothyrioides* (CBS 972.95) and *P. thysanolaenae* (MFLU 11-0142) being phylogenetically closely related to *P. estuarinum*. Micromorphologically, they differ in conidia size, morphology and colour. Conidia of *P. estuarinum* are (3–)3.2–4(–6)×1.4–1.7(–2) µm, straight or slightly curved and are olivaceous or yellowish brown while the conidia of *P. salinum* are not as wide, 3.7±0.3×1.2±0.2 µm, straight and yellowish brown. Also, *P. salinum* differs from *P. estuarinum* in 33 and 68 nucleotide positions in ITS and *tub2*. No *tef-1α* sequence is available for *P. estuarinum*.

DISCUSSION

Studies on the diversity of fungi around the world have been increasing, especially those focusing on the production of natural products for biotechnological applications. Despite efforts made to understand fungal diversity, the abundance and ecological function of fungal communities in marine environments in many regions and on different substrata have yet to be explored [54, 55].

In the present work, through the use of cultivation-dependent methods, several species of filamentous fungi were recovered from different substrates. We found that each habitat – estuarine and coastal – hosted distinct fungal communities. Studies on marine fungi have already shown that there are clearly differences of taxa between ecosystems [56]. Jeffries *et al.* [28] showed strong partitioning of fungal community composition between estuarine, coastal and oceanic samples, revealing that each marine environment represents a distinct fungal habitat hosting discrete communities. Estuarine and ocean samples form clearly separated clusters, and coastal samples emerge as a transitional zone between them. Some authors have indicated that these variations in fungal communities are controlled by salinity, temperature, oxygen and nutrient patterns, suggesting that marine fungi respond to environmental gradients, playing a role in marine nutrient cycles. Moreover, factors such as temporal, spatial and environmental contexts and anthropogenic pressure can influence the fungal interactions and their distribution [55].

The phylum Ascomycota was the most common taxon across all sites and as shown in other studies this phylum dominates marine environments [9, 57]. The most frequent species found in our samples were generally common soil-associated fungal

Table 2. List of isolates used in this study

Species	Family	Strain	Host/Substrate	Country	GenBank accession no.		
					ITS	*tub2*	*tef1-α*
Alloconiothyrium aptrootii	*Didymosphaeriaceae*	CBS 980.95*	Soil	Papua New Guinea	JX496121	JX496460	–
		CBS 981.95	Soil	Papua New Guinea	JX496122	JX496461	–
Austropleospora archidendri	*Didymosphaeriaceae*	CBS 168.77*	*Archidendron bigeminum*	Myanmar	MH861045	JX496388	–
Austropleospora keteleeriae	*Didymosphaeriaceae*	MFLUCC 18-1551*	*Keteleeria fortunei*	China	MK347802	–	MK360045
Bimuria novae-zelandiae	*Didymosphaeriaceae*	CBS 107.79*	Soil	New Zealand	MH861181	–	DQ471087
Coniothyrium palmarum (Outgroup)	*Leptosphaeriaceae*	CBS 400.71*	*Chamaerops humilis*	Italy	AY720708	KT389792	DQ677903
Cucurbitaria berberidis (Outgroup)	*Cucurbitariaceae*	CBS 130007*	*Berberis vulgaris*	Austria	MH865620	LT717676	MF795846
Cylindroaseptospora siamensis	*Didymosphaeriaceae*	MFLUCC 17-2527*	*Leucaena* sp.	Thailand	MK347760	–	MK360048
Didymocrea sadasivanii	*Didymosphaeriaceae*	CBS 438.65	Soil	India	DQ384103	–	–
Didymocrea leucaenae	*Didymosphaeriaceae*	MFLUCC 17-0896*	*Leucaena* sp.	Thailand	MK347721	–	MK360052
Kalmusia italica	*Didymosphaeriaceae*	MFLUCC 13-0066*	*Spartium junceum*	Italy	KP325440	–	–
Kalmusia longispora	*Didymosphaeriaceae*	CBS 582.83*	*Arceuthobium pusillum*	Germany	MH861658	JX496436	–
Kalmusia variispora	*Didymosphaeriaceae*	CBS 121517*	Grapevine	Syria	JX496030	JX496369	–
Karstenula rhodostoma	*Didymosphaeriaceae*	CBS 691.94	*Frangula alnus*	Sweden	LC014559	–	AB808506
Leptosphaeria doliolum (Outgroup)	*Leptosphaeriaceae*	CBS 505.75*	*Urtica dioica*	Netherlands	JF740205	JF740144	–
Laburnicola centaureae	*Didymosphaeriaceae*	MFLUCC 13-0601*	*Centaurea* sp.	Italy	KX274239	–	–
Laburnicola hawksworthii	*Didymosphaeriaceae*	MFLUCC 13-0602*	*Laburnum* sp.	Italy	KU743194	–	–
Laburnicola muriformis	*Didymosphaeriaceae*	MFLUCC 16-0290*	*Laburnum anagyroides*	Italy	KU743197	–	KU743213
Letendraea cordylinicola	*Didymosphaeriaceae*	MFLUCC 11-0150	*Cordyline* sp.	Thailand	KM213996	–	–
		MFLUCC 11-0148*	*Cordyline* sp.	Thailand	KM213995	–	–
Montagnula bellevaliae	*Didymosphaeriaceae*	MFLUCC 14-0924*	*Bellevalia romana*	Italy	KT443906	–	KX949743
Montagnula cirsii	*Didymosphaeriaceae*	MFLUCC 13-0680*	Thistle	Italy	KX274242	–	KX284707
Montagnula opulenta	*Didymosphaeriaceae*	UTHSC DI16-208	–	USA	LT796834	LT796914	LT797074
Montagnula saikhuensis	*Didymosphaeriaceae*	MFLUCC 16-0315*	Forest soil	Thailand	KU743209	KU743216	–
Neoascochyta adenii	*Didymellaceae*	CBS 142108*	*Adenium obesum*	Thailand	KY173423	KY173607	–
Neoascochyta argentina	*Didymellaceae*	CBS 112524*	*Triticum aestivum*	Argentina	KT389524	KT389822	–
Neoascochyta cylindrispora	*Didymellaceae*	CBS 142456*	Human tissue	USA	LT592963	LT593032	–
Neoascochyta desmazieri	*Didymellaceae*	CBS 297.69*	*Lolium perenne*	Germany	KT389508	KT389806	–
		CBS 758.97	Hay	Norway	KT389509	KT389807	–
		CBS 247.79	*Gramineae*	Austria	KT389507	KT389805	–

Continued

Table 2. Continued

Species	Family	Strain	Host/Substrate	Country	GenBank accession no.		
					ITS	*tub2*	*tef1-α*
Neoascochyta europaea	*Didymellaceae*	CBS 820.84*	*Hordeum vulgare*	Germany	KT389511	KT389809	–
		CBS 819.84	*Hordeum vulgare*	Germany	KT389510	KT389808	–
Neoascochyta exitialis	*Didymellaceae*	CBS 812.84	*Hordeum vulgare*	Germany	KT389517	KT389815	–
		CBS 811.84	*Secale cereale*	Germany	KT389516	KT389814	–
		CBS 389.86	*Triticum aestivum*	Switzerland	KT389515	KT389813	–
		CBS 113693	*Allium* sp.	Sweden	KT389513	KT389811	–
		CBS 110124	*Triticum* sp.	Netherlands	KT389512	KT389810	–
Neoascochyta fuci	***Didymellaceae***	**CMG 47/MUM 19.41***	***Fucus* sp.**	**Portugal**	**MN053014**	**MN066618**	–
		CMG 48	***Fucus* sp.**	**Portugal**	**MN053015**	**MN066619**	–
Neoascochyta graminicola	*Didymellaceae*	CBS 816.84	*Hordeum vulgare*	Germany	KT389523	KT389821	–
		CBS 815.84	*Hordeum vulgare*	Germany	KT389522	KT389820	–
		CBS 586.79	*Hordeum vulgare*	Belgium	KT389521	KT389819	–
		CBS 447.82	*Triticum aestivum*	Germany	KT389520	KT389818	–
		CBS 301.69	*Lolium multiflorum*	Germany	KT389519	KT389817	–
		CBS 102789	*Lolium perenne*	New Zealand	KT389518	KT389816	–
Neoascochyta paspali	*Didymellaceae*	CBS 560.81*	*Paspalum dilatatum*	New Zealand	FJ427048	FJ427158	–
		CBS 561.81	*Lolium perenne*	New Zealand	GU237889	GU237640	–
		ICMP 6614	*Paspalum dilatatum*	New Zealand	KT309957	KT309539	–
		ICMP 6819	*Dactylis glomerata*	New Zealand	KT309992	KT309572	–
		ICMP 6615	*Lolium perenne*	New Zealand	KT309958	KT309540	–
Neoascochyta soli	*Didymellaceae*	LC 8165*	Soil	China	KY742121	KY742363	–
		LC 8166	Soil	China	KY742122	KY742364	–
Neoascochyta tardicrescens	*Didymellaceae*	CBS 689.97*	Hay	Norway	KT389526	KT389824	–
Neoascochyta triticicola	*Didymellaceae*	CBS 544.74*	*Triticum aestivum*	South Africa	GU237887	GU237488	–
Neokalmusia brevispora	*Didymosphaeriaceae*	KT2313*	*Sasa kurilensis*	Japan	LC014574	–	AB539113
		KT1466	*Sasa* sp.	Japan	LC014573	–	–
Neptunomyces aureus	*Didymosphaeriaceae*	MUM 19.38/CMG 10A*	*Gracilaria gracilis*	Portugal	MK912119	MK934131	MK947998
		CMG 11	*Enteromorpha* sp.	Portugal	MK912120	MK934132	MK947999
		CMG 12	*Ulva* sp.	Portugal	MK912121	MK934133	MK948000
		CMG 13	*Enteromorpha intestinalis*	Portugal	MK912122	MK934134	MK948001
		CMG 14	*Enteromorpha* sp.	Portugal	MK912123	MK934135	MK948002
Paracamarosporium fagi	*Didymosphaeriaceae*	CBS 140008*	*Fagus sylvatica*	Germany	KR611886	–	–
Paracamarosporium fungicola	*Didymosphaeriaceae*	CBS 113269*	Resupinate polypore fungus	Albania	JX496020	JX496359	–

Continued

Table 2. Continued

Species	Family	Strain	Host/Substrate	Country	GenBank accession no.		
					ITS	*tub2*	*tef1-α*
Paracamarosporium hawaiiense	*Didymosphaeriaceae*	CBS 120025*	*Sophora chrysophylla*	USA	JX496027	JX496366	–
Paracamarosporium psoraleae	*Didymosphaeriaceae*	CPC 21632*	*Psoralea pinnata*	South Africa	KF777143	–	–
Paraconiothyrium babiogorense	*Didymosphaeriaceae*	CBS 128292	*Huperzia selago*	Poland	MH864845	–	–
Paraconiothyrium brasiliense	*Didymosphaeriaceae*	CBS 100299*	*Coffea arabica*		AY642531	JX496350	–
Paraconiothyrium cyclothyrioides	*Didymosphaeriaceae*	CBS 972.95*	Soil	Papua New Guinea	JX496119	JX496458	–
		UTHSC:DI16-265	Human	USA	LT796859	LT796939	LT797099
		UTHSC:DI16-327	Human	USA	LT796884	LT796964	LT797124
		UTHSC:DI16-279	Human	USA	LT796864	LT796944	LT797104
		UTHSC:DI16-252	Human	USA	LT796852	LT796932	LT797092
		UTHSC:DI16-218	Human	USA	LT796839	LT796919	LT797079
Paraconiothyrium estuarinum	*Didymosphaeriaceae*	CBS 109850*	Sediment from estuarine habitat	Brazil	MH862842	JX496355	–
Paraconiothyrium fuckelii	*Didymosphaeriaceae*	CBS 653.85	*Picea abies*	Germany	MH861909	JX496443	–
		CBS 764.71B	Man	Netherlands	MH860341	JX496451	–
		CBS 584.69	Root of gymnosperm	Denmark	MH859378	JX496437	–
		CBS 797.95	Rubus sp.	Netherlands	JX496113	JX496452	–
Paraconiothyrium fuscomaculans	*Didymosphaeriaceae*	CBS 116.16	*Malus* sp.	USA	MH854649	–	–
Paraconiothyrium magnoliae	*Didymosphaeriaceae*	MFLUCC 10-0278*	*Magnolia liliifera*	Thailand	KJ939280	–	–
Paraconiothyrium nelloi	*Didymosphaeriaceae*	MFLU 14-0813*	*Spartium junceum*	Italy	KP711360	–	–
Paraconiothyrium rosae	*Didymosphaeriaceae*	MFLU 151115*	*Rosa canina*	Italy	MG828932	–	–
Paraconiothyrium salinum	*Didymosphaeriaceae*	**CMG 49/MUM 19.91***	**Sponge**	**Portugal**	**MN369540**	**MN380479**	**MN380481**
		CMG 50	**Saline water**	**Portugal**	**MN369541**	**MN380480**	**MN380482**
Paraconiothyrium thysanolaenae	*Didymosphaeriaceae*	MFLU 11-0142*	*Thysanolaena maxima*	Thailand	KP744453	–	–
Paramassariosphaeria anthostomoides	*Didymosphaeriaceae*	CBS 615.86*	*Cerastium uniflorum*	Switzerland	MH862005	–	–
Paramassariosphaeria clematidicola	*Didymosphaeriaceae*	MFLUCC 16-0172*	*Clematis vitalba*	Italy	KU743206	–	–
Paraphaeosphaeria angularis	*Didymosphaeriaceae*	CBS 167.70*	*Saccharum officinarum*	Brazil	MH859539	JX496386	–
Paraphaeosphaeria michotii	*Didymosphaeriaceae*	CBS 340.86	*Phragmites australis*	France	MH861961	JX496418	–
Paraphaeosphaeria minitans	*Didymosphaeriaceae*	CBS 111750	*Sclerotinia sclerotorium*	Italy	JX496017	JX496356	–

Continued

Table 2. Continued

Species	Family	Strain	Host/Substrate	Country	GenBank accession no.		
					ITS	*tub2*	*tef1-α*
Pseudocamarosporium africanum	*Didymosphaeriaceae*	CBS 121166*	*Prunus persica*	South Africa	JX496029	JX496368	–
Pseudocamarosporium corni	*Didymosphaeriaceae*	MFLUCC 13-0541*	*Cornus sanguinea*	Italy	KJ747048	–	–
Pseudocamarosporium cotinae	*Didymosphaeriaceae*	MFLUCC 14-0624*	*Cotinus coggygria*	Russia	KP744460	–	–
Pseudocamarosporium lonicerae	*Didymosphaeriaceae*	MFLUCC 13-0532*	*Lonicera* sp.	Italy	KJ747047	–	–
Pseudocamarosporium pteleae	*Didymosphaeriaceae*	MFLUCC 17-0724*	*Ptelea trifoliata*	Russia	MG828950	–	MG829233
Pseudopithomyces chartarum	*Didymosphaeriaceae*	UTHSC 04-678	*Homo sapiens*	USA	HG518060	–	–
		UTHSC 03-2472	*Homo sapiens*	USA	HG518059	–	–
Tremateia arundicola	*Didymosphaeriaceae*	MFLUCC 16-1275*	Herbaceous stem	UK	KX274241	–	KX284706
Tremateia guiyangensis	*Didymosphaeriaceae*	GZAAS01*	Herbaceous stem	China	KX274240	–	KX284705
Xenocamarosporium acaciae	*Didymosphaeriaceae*	MFLUCC 17-2432	*Leucaena* sp.	Thailand	MK347766	–	MK360093

CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CMG: Culture collection of Micael Gonçalves, housed at Department of Biology, University of Aveiro, Aveiro, Portugal; CPC: Culture collection of Pedro Crous, housed at CBS; MFLU: Herbarium of Mae Fah Luang University; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MUM: Culture collection hosted at Center for Biological Engineering of University of Minho, Braga, Portugal; UTHSC: Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio, Texas, USA. Ex-type strains are marked with an asterisk. Sequences generated in this study are shown in bold.

orders, including mainly Hypocreales, Pleosporales, Eurotiales, Capnodiales, Xylariales and Helotiales. These results are in line with previous studies, in which the genera *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Fusarium*, *Penicillium, Phoma* and *Trichoderma* are commonly found in marine environments [2]. Some unique taxonomic groups previously reported in coastal marine environments, such as Chytridiomycota (chytrids) [28, 55], were not found in this study. However, Hyde *et al*. [14] reported that using classical culture-dependent studies, marine fungi localized at coastals environments are predominantly allied to the phyla Ascomycota and Basidiomycota. In fact, evidence of chytrids in the marine environments based on culture-dependent studies is scarce. By contrast, in high-throughput sequencing studies, Chytridiomycota have been described as the most abundant fungal group [58–60] that may play an important role in aquatic food webs in the ocean [61, 62]. Among the isolates from driftwood substrates found in our survey, one isolate was identified as *Lulworthia* sp. belonging to the order Lulworthiales that comprises marine ascomycetes [63] which are commonly associated with wood substrates [64]. Previous studies in Portuguese waters have also shown the presence of Lulworthiales species in standing plants and baits of *Spartina maritima* [65] and wood of *Pinus pinaster* and *Fagus sylvatica* [64].

Among the taxa identified, Dothideomycetes (mostly Pleosporales) was the major group found in our collection. It has been reported that marine Dothideomycetes may have evolved recently in the sea from terrestrial species, many of them maintaining an active mechanism of spore dispersal [66–69] and developing morphological and physiological characteristics that allowed them to adapt to marine conditions. Recently, Gonçalves *et al*. [70] described new Dothideomycetes species: *Neptunomyces aureus*, isolated from healthy tissues of macroalgae such as *Enteromorpha* sp., *Gracilaria gracilis* and *Ulva* sp.; *Neocamarosporium aestuarinum* [71] isolated from saline water or in association with *Halimione portulacoides*; and *Verrucoconiothyrium ambiguum* [72] also isolated from seawater. Garzoli *et al*. [1] reported, for the first time, some Didymosphaeriaceous species in *Padina pavonica* collected in the Mediterranean Sea such as *Paraconiothyrium variabile*, *Paraphaeosphaeria neglecta* and more eight unidentified Didymosphaeriaceous species. Also, *Paraphaeosphaeria michotii* was reported in *Phragmites australis* typically found in wetlands [73]. Particularly noteworthy and in addition to Dothideomycetes, Gonçalves *et al*. [53, 74] and Crous *et al*. [75] identified novel marine fungal species within lineages that are well known from terrestrial habitats, such as *Penicillium lusitanum* (Eurotiomycetes) isolated from seawater, three species of *Parasarocladium* (Sordariomycetes) isolated from

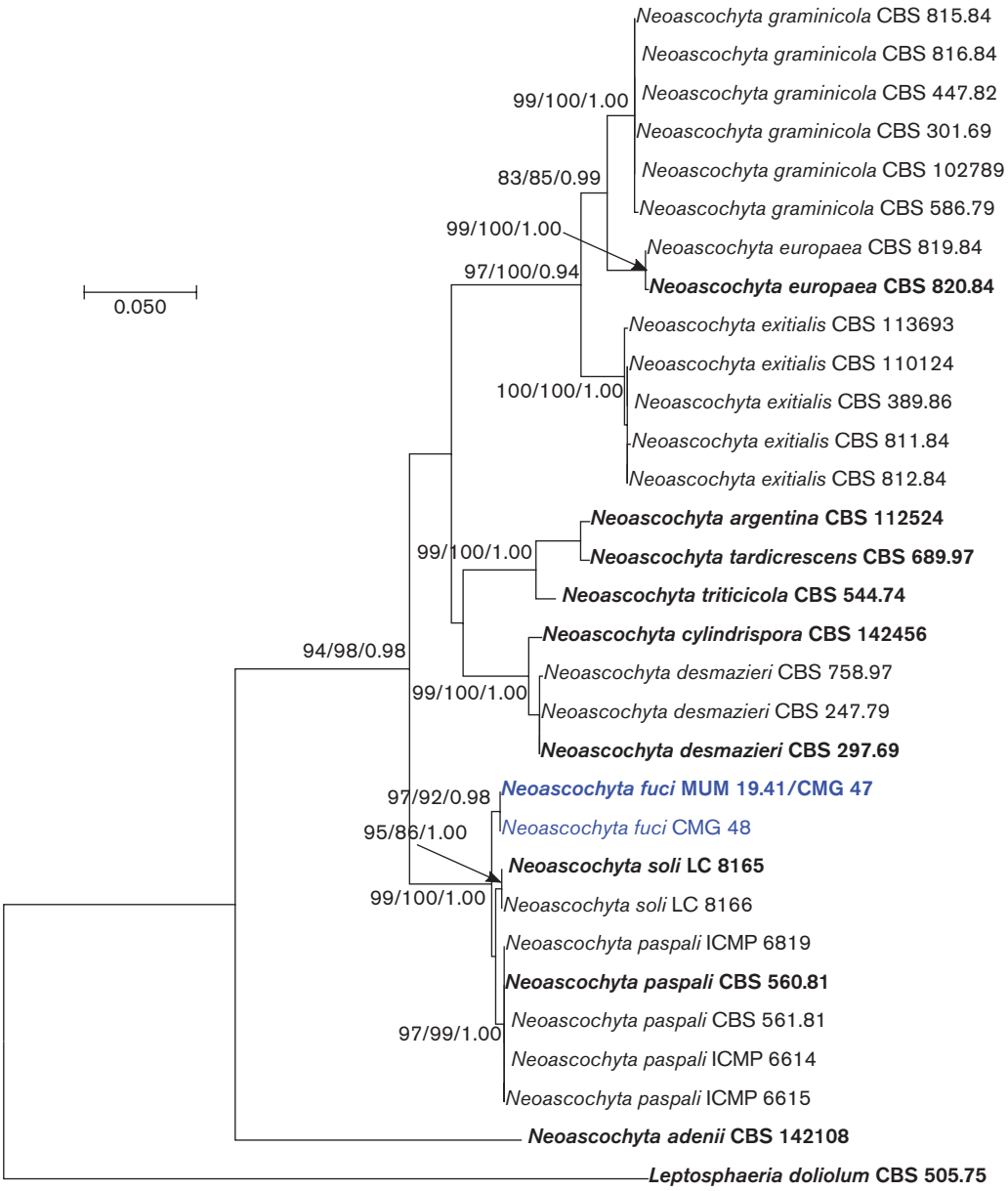

Fig. 5. Phylogenetic relationships of *Neoascochyta* species based on combined ITS and *tub2* sequence data and inferred using the ML method under the Kimura two-parameter model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site and rooted to *Leptosphaeria doliolum* (CBS 505.75). Bootstrap values (≥70%) and posterior probabilities (≥0.94) are shown at the nodes (ML/MP/BI). Ex-type strains are in bold and the new taxa proposed from the current study are in blue.

saline water, macroalgae and sponge, three species of *Emericellopsis* (Sordariomycetes) also isolated from macroalgae and *Trichoderma aestuarinum* (Sordariomycetes) isolated from saline water.

In the current study, two novel Dothideomycetes species are described, namely *Paraconiothyrium salinum* and *Neoascochyta fuci*. *Paraconiothyrium salinum* is introduced in the genus *Paraconiothyrium* based on phylogenetic and morphological analyses. The genus *Paraconiothyrium* was introduced by Verkley *et al.* [76] to accommodate four species, *P. estuarinum*, *P. brasiliense*, *P. cyclothyrioides* and *P. fungicola*. Currently, the genus comprises 24 species listed in the Index Fungorum (2020) and Mycobank databases but there are some species that have already been transferred to other genera such as: *P. fungicola*, *P. hawaiiense* and *P. africanum*, now belonging to the genus *Paracamarosporium*; *P. minitans*, now as *Paraphaeosphaeria*; *P. archidendri*, now as *Austropleospora*; and other species. In fact, the taxonomic affiliation of *Paraconiothyrium* species is confusing, with contrasting differences at the phylogenetic and morphological level. Liu *et al.* [77] reported that the morphological characters of *Paraconiothyrium* species are variable. *Paraconiothyrium*

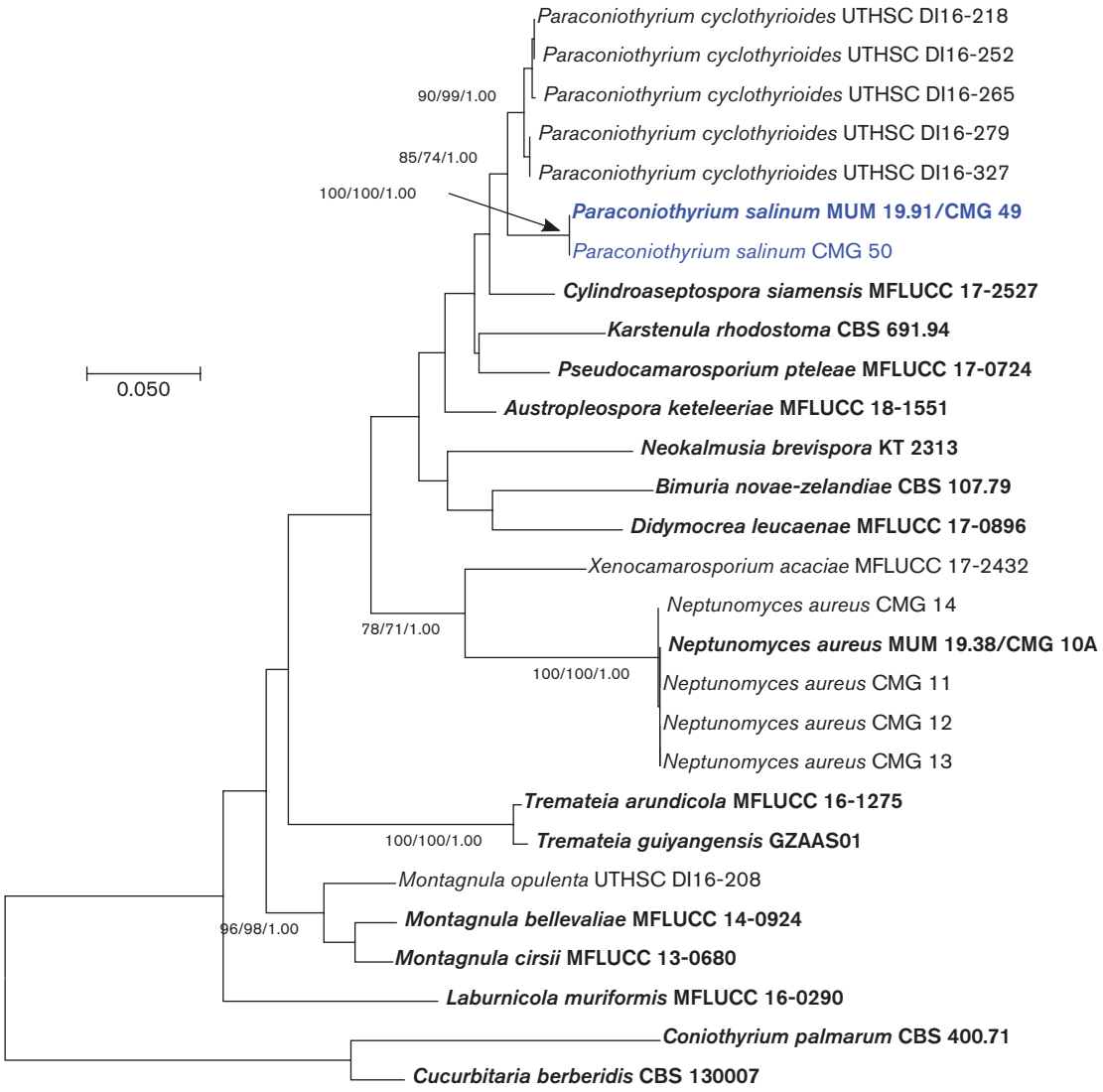

Fig. 6. Phylogenetic relationships of *Didymosphaeriaceae* species based on ITS and *tef1*-α sequence data and inferred using the ML method under the Tamura–Nei model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site and rooted to *Cucurbitaria berberidis* (CBS 130007) and *Coniothyrium palmarum* (CBS 400.71). Bootstrap values (≥70%) and posterior probabilities (=1.00) are shown at the nodes (ML/MP/BI). Ex-type strains are in bold and the new taxa proposed from the current study are in blue.

sensu stricto is typified by *P. estuarinum* isolated from sediments of an estuarine environment. The conidiomata can be eustromatic and rarely pycnidial, the conidiogenous cells are phialidic, sometimes percurrent, and the conidia are smooth-walled, aseptate sometimes one-septate, hyaline to brown at later stages of development [78]. Our novel species fits well within this concept, sharing similarities with *P. estuarinum* but with differences in conidia size, morphology and colour. The phylogenetic analyses also provide strong evidence that *P. salinum* belongs in the genus *Paraconiothyrium*, where it forms a sister clade to *P. estuarinum* (CBS 109850), *P. cyclothyrioides* (CBS 972.95) and *P. thysanolaenae* (MFLU 11–0142) with high bootstrap support. However, there were no *tub2* and *tef1-α* sequence data available for most species described as *Paraconiothyrium* and therefore the phylogenetic analyses presented did not encompass all known species in *Paraconiothyrium* and many of them have been redefined at the genus level. Also, according to our phylogenetic analyses other species described as *Paraconiothyrium*, such as *P. fuckelii* (CBS 653.85, CBS 764.71B, CBS 584.69, CBS 797.95), *P. rosae* (MFLU 15–1115) and *P. babiogorense* (CBS 128292) may represent a separate genus, but this needs to be confirmed by further studies. Also, there are slight differences in conidia morphology. For example, the conidia of *P. fuckelii* are subglobose to ellipsoid or obovoid, rarely more cylindrical, initially hyaline becoming olivaceous-brown [76]. Two other described *Paraconiothyrium* species, *P. nelloi* (MFLU 14–0813) and *P. fuscomaculans* (CBS 116.16), fit in the genus

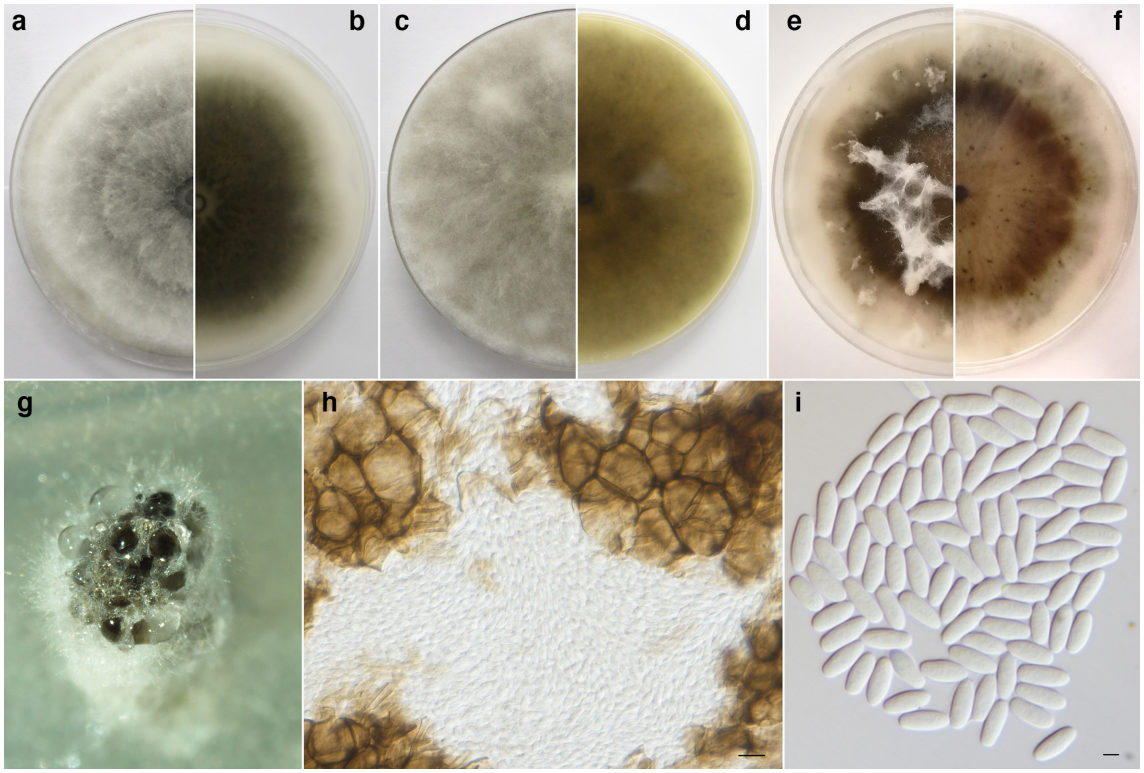

Fig. 7. *Neoascochyta fuci* (MUM 19.41). (a, b) Colony after 5 days at 25 °C on PDA (obverse and reverse). (c, d) Colony after 5 days at 25 °C on MEA (obverse and reverse). (e, f) Colony after 5 days at 25 °C on OA (obverse and reverse). (g) Conidiomata after 2 months at 25 °C on OA. (h) Section of conidiomata. (i) Conidia. Bars, 10 µm (h); 2.5 µm (i).

Kalmusia. Conidia of *P. nelloi* are globose to obovate, thick-walled, smooth-walled, one-celled, initially hyaline becoming dark brown with polar guttules [77]. For example, conidia of *Kalmusia spartii* are initially hyaline becoming light brown and also contain polar guttules [77]. Multi-gene phylogenies including other species of the family *Didymosphaeriaceae* and morphological analyses are needed to evaluate the redisposition of *Paraconiothyrium*-like species.

Most species of *Paraconiothyrium* described until now have been found in association with plants or soil with the exception of *P. estuarinum* (isolated from estuarine sediment) and some strains of *P. cyclothyrioides* from the UTHSC collection (isolated from superficial tissue in clinical patients) reported by Valenzuela-Lopez *et al.* [79]. These authors confirm the data from two previous clinical reports in which *P. cyclothyrioides* was found in immunocompromised patients. In general, species of *Paraconiothyrium* are regarded as ubiquitous soil fungi and are being used in antibiotic production, and as biocontrol agents and bioremediators [78, 80]. Here we described a novel species that occurs as endophytes or epiphytes on healthy unidentified sponges and in estuarine water. Höller *et al.* [35] reported some *Coniothyrium*-like species from 16 species of sponges and concluded that each sponge hosted a specific fungal community regardless of their location. Interestingly, GenBank information of the strains with ITS closest matches of MUM 19.91=CMG 49 shows that these strains were also isolated from the marine environment, more specifically from *Gelliodes carnosa* (marine sponge) (*Paraphaeosphaeria* sp., GenBank: FJ770071), marine sponge (*Pleosporales* sp., GenBank: KP263116), mangrove sediments (*Paraphaeosphaeria* sp., GenBank: KY827359) and *Haliclona caerulea* (marine sponge) (*Paraphaeosphaeria* sp., GenBank: DQ092522). This suggests that marine sponges and marine/estuarine environments could harbour a fungal diversity hotspot of the genus *Paraconiothyrium* or didymosphaeriaceous species that deserves further investigation.

With 13 *Neoascochyta* species described to date and the majority of them appearing to have some host preference, i.e. they can be found in association with various Poaceae plant species, this study reports for the first time a novel *Neoascochyta* species isolated from the macroalgae *Fucus* sp. Although most of members of the family *Didymellaceae* are plant-associated fungi [81], a few species have been isolated from other substrates, including from marine environments, such as *Ascochyta salicorniae* isolated from green alga *Ulva* sp. [82] and *Didymella aquatica*, the first *Didymella* species known from water [81]. Also, *D. eucalyptica*, *Ascochyta herbicola* and *V. ambiguum* have been reported from water [72, 81, 83]. Thus, three different groups based on conidial morphology are evident in *Neoascochyta: N. dactylidis*, *N. europaea*, *N. exitialis* and *N. graminicola* with one-septate conidia; *N. argentina*, *N. cylindrispora*, *N. desmazieri*,

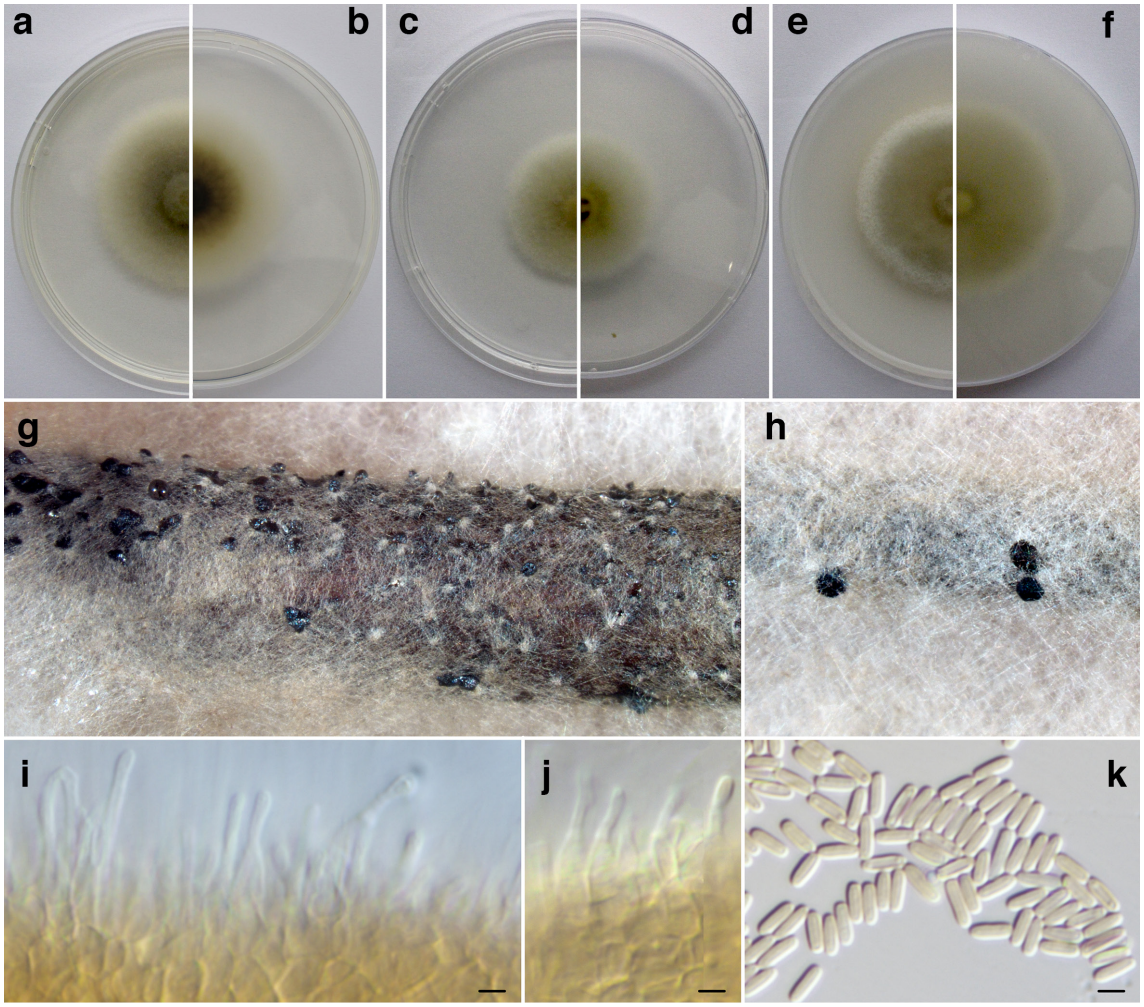

Fig. 8. *Paraconiothyrium salinum* (MUM 19.91). (a, b) Colony after 1 week at 25 °C on PDA (obverse and reverse). (c, d) Colony after 1 week at 25 °C on MEA (obverse and reverse). (e, f) Colony after 1 week at 25 °C on OA (obverse and reverse). (g, h) Conidiomata after 2 months at 25 °C on pine needles. (i, j) Conidiogenous cells. (k) Conidia. Scale bars, 2.5 µm (i–k).

N. rosicola, *N. tardicrescens* and *N. triticicola* with mainly one-septate conidia but occasionally aseptate; and *N. paspali* and *N. soli* with aseptate conidia. Morphologically, the novel species described, *N. fuci*, fits within the last group but differs in conidia size. Phylogenetically it is clear that it has a close relationship with *N. soli* and *N. paspali*.

As mentioned above, species belonging to *Paraconiothyrium* and *Neoascochyta* are typically found in terrestrial environments. We described here two novel species isolated from the marine environment. Marine fungi face substantial challenges and require additional adaptations, high salinity being the most obvious stressor in the marine compartment, leading to osmotic and ionic stress [54]. Our results showed that both species, *P. salinum* and *N. fuci*, can be classified as slightly halophilic because they can grow equally well in the presence and absence of 3% sea salts. Although they can tolerate salinity, many marine fungi are not typically halophilic because they do not show a preference for salinity [54]. Thus, the ecological relevance of these species and the functional interactions with their hosts is still unknown.

This study has provided a snapshot of the diversity of marine mycobiota present in coastal and estuarine sites in Portugal. We have opened new challenges for further progress towards uncovering marine fungal diversity by exploring new habitats/substrates. In the future, this knowledge will be useful to elucidate potential ecological roles of these microorganisms, allowing the identification of novel natural products with applications, such as antibacterial and anticancer properties, and degradation and metabolization of polymers or hydrocarbons, which may be used in bioremediation processes.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

References

1. Garzoli L, Poli A, Prigione V, Gnavi G, Varese GC. Peacock's tail with a fungal cocktail: first assessment of the mycobiota associated with the brown alga *Padina pavonica*. *Fungal Ecol* 2018;35:87–97.
2. Jones EBG, Pang K-L, Abdel-Wahab MA, Scholz B, Hyde KD *et al*. An online resource for marine fungi. *Fungal Divers* 2019;96:347–433.
3. Johnson TW, Sparrow FK. *Fungi in Oceans and Estuaries*. Weinheim, Germany: J. Cramer; 1961. p. 685.
4. Tubaki K. Studies on the Japanese marine fungi, lignicolous group (III), algicolous group and a general consideration. *Annual Report of the Institute for Fermentation Osaka* 1969;4:12–42.
5. Kohlmeyer J, Kohlmeyer E. *Marine Mycology: The Higher Fungi*. London: Academic Press; 1979. p. 704.
6. Jensen PR, Fenical W. Secondary metabolites from marine fungi. *Fungal Diver Res Ser* 2002;7:293–315.
7. Pang K-L, Overy DP, Jones EBG, Calado M, Burgaud G *et al*. 'Marine fungi' and 'marine-derived fungi' in natural product chemistry research: toward a new consensual definition. *Fungal Biol Rev* 2016;30:163–175.
8. Jones EBG. Are there more marine fungi to be described? *Bot Mar* 2011;54:343–354.
9. Jones EBG, Pang K-L. Tropical aquatic fungi. *Biodivers Conserv* 2012;21:2403–2423.
10. Kis-Papo T. Marine fungal communities. In: Dighton J, Wjits JF, Oudemans P (eds). *The fungal community, its organisation and role in the ecosystem*, 3rd edn. Boca Baton: CRC Press; 2005. pp. 61–92.
11. Richards TA, Jones MDM, Leonard G, Bass D. Marine fungi: their ecology and molecular diversity. *Ann Rev Mar Sci* 2012;4:495–522.
12. Raghukumar S. *Fungi in coastal and oceanic marine ecosystems*. New York: Springer; 2017.
13. Hyde KD. Frequency of occurrence of lignicolous marine fungi in the tropics. In: Moss ST (ed). *The biology of marine fungi*. Cambridge: Cambridge Univ Press; 1986. pp. 311–322.
14. Hyde KD, Jones EBG. Marine mangrove fungi. *Mar Ecol* 1988;9:15–33.
15. Schmit JP, Shearer CA. A checklist of mangrove associated fungi. *Mycotaxon* 2003;80:423–477.
16. Burgaud G, Hué NTM, Arzur D, Coton M, Perrier-Cornet J-M *et al*. Effects of hydrostatic pressure on yeasts isolated from deep-sea hydrothermal vents. *Res Microbiol* 2015;166:700–709.
17. Damare S, Raghukumar C. Fungi and macroaggregation in deep-sea sediments. *Microb Ecol* 2008;56:168–177.
18. Rédou V, Navarri M, Meslet-Cladière L, Barbier G, Burgaud G. Species richness and adaptation of marine fungi from deep-subseafloor sediments. *Appl Environ Microbiol* 2015;81:3571–3583.
19. Nagahama T, Takahashi E, Nagano Y, Abdel-Wahab MA, Miyazaki M. Molecular evidence that deep-branching fungi are major fungal components in deep-sea methane cold-seep sediments. *Environ Microbiol* 2011;13:2359–2370.
20. Nagano Y, Nagahama T, Hatada Y, Nunoura T, Takami H *et al*. Fungal diversity in deep-sea sediments – the presence of novel fungal groups. *Fungal Ecol* 2010;3:316–325.
21. Burgaud G, Le Calvez T, Arzur D, Vandenkoornhuyse P, Barbier G. Diversity of culturable marine filamentous fungi from deep-sea hydrothermal vents. *Environ Microbiol* 2009;11:1588–1600.
22. Le Calvez T, Burgaud G, Mahé S, Barbier G, Vandenkoornhuyse P. Fungal diversity in deep-sea hydrothermal ecosystems. *Appl Environ Microbiol* 2009;75:6415–6421.
23. Xu W, Guo S, Pang K-L, Luo Z-H. Fungi associated with chimney and sulfide samples from a South Mid-Atlantic Ridge hydrothermal site: distribution, diversity and abundance. *Deep Sea Research Part I* 2017;123:48–55.
24. Jebaraj CS, Forster D, Kauff F, Stoeck T. Molecular diversity of fungi from marine oxygen-deficient environments (odes). *Prog Mol Subcell Biol* 2012;53:e208:189.
25. Wang Y, Zhang WP, Cao HL, Shek CS, Tian RM *et al*. Diversity and distribution of eukaryotic microbes in and around a brine pool adjacent to the Thuwal cold seeps in the red sea. *Front Microbiol* 2014;5:37.
26. Richards TA, Leonard G, Mahé F, Del Campo J, Romac S *et al*. Molecular diversity and distribution of marine fungi across 130 European environmental samples. *Proc Biol Sci* 2015;282:2015–2243.
27. Stern RF, Picard KT, Hamilton KM, Walne A, Tarran GA *et al*. Novel lineage patterns from an automated water sampler to probe marine microbial biodiversity with ships of opportunity. *Prog Oceanogr* 2015;137:409e420:409–420.
28. Jeffries TC, Curlevski NJ, Brown MV, Harrison DP, Doblin MA *et al*. Partitioning of fungal assemblages across different marine habitats. *Environ Microbiol Rep* 2016;8:e238:235.
29. Picard KT. Coastal marine habitats harbor novel early-diverging fungal diversity. *Fungal Ecol* 2017;25:1–13.
30. Garzoli L, Gnavi G, Varese GC, Picco AM. Mycobiota associated with the rhodophyte alien species *Asparagopsis taxiformis* (Delile) Trevisan de Saint-Léon in the Mediterranean Sea. *Mar Ecol* 2015;36:e968:959–968.
31. Gnavi G, Garzoli L, Poli A, Prigione V, Burgaud G *et al*. The culturable mycobiota of *Flabellia petiolata*: first survey of marine fungi associated to a Mediterranean green alga. *PLoS One* 2017;12:e0175941.
32. Vohník M, Borovec O, Kolaříková Z, Sudová R, Réblová M. Extensive sampling and high-throughput sequencing reveal *Posidoniomycesatricolor* gen. et sp. nov. (*Aigialaceae*, Pleosporales) as the dominant root mycobiont of the dominant Mediterranean seagrass *Posidoniaoceanica*. *MycoKeys* 2019;55:59.
33. Azevedo E, Caeiro MF, Rebelo R, Barata M. Biodiversity and characterization of marine mycota from Portuguese waters. *Anim Biodivers Conserv* 2011;34:205–215.
34. Garzoli L, Gnavi G, Tamma F, Tosi S, Varese GC *et al*. Sink or swim: updated knowledge on marine fungi associated with wood substrates in the Mediterranean sea and hints about their potential to remediate hydrocarbons. *Prog Oceanogr* 2015;137:140–148.
35. Höller U, Wright AD, Matthee GF, Konig GM, Draeger S *et al*. Fungi from marine sponges: diversity, biological activity and secondary metabolites. *Mycol Res* 2000;104:1354–1365.
36. Godinho VM, de Paula MTR, Silva DAS, Paresque K, Martins AP *et al*. Diversity and distribution of hidden cultivable fungi associated with marine animals of antarctica. *Fungal Biol* 2019;123:507–516.
37. Möller EM, Bahnweg G, Sandermann H, Geiger HH. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acids Res* 1992;20:6115–6116.
38. Alves A, Phillips AJL, Henriques I, Correia A. Rapid differentiation of species of *Botryosphaeriaceae* by PCR fingerprinting. *Res Microbiol* 2007;158:112–121.
39. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal genes for phylogenies. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds). *PCR Protocols: A guide to methods and applications*. California: Academic Press; 1990. pp. 315–322.
40. Alves A, Correia A, Luque J, Phillips A. *Botryosphaeria corticola*, sp. nov. on *Quercus* species, with notes and description of *Botryosphaeria stevensii* and its anamorph, *Diplodia mutila*. *Mycologia* 2004;96:598–613.
41. Glass NL, Donaldson GC. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl Environ Microbiol* 1995;61:1323–1330.
42. O'Donnell K, Cigelnik E. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Mol Phylogenet Evol* 1997;7:103–116.

43. Alves A, Crous PW, Correia A, Phillips AJL. Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. *Fungal Divers* 2008;28:1–13.
44. Rehner SA. Primers for elongation factor 1-α (EF1-α). 2001. http://www.nacse.org/yfaaberg/aftol/EF1primer.pdf.
45. Lopes A, Phillips AJL, Alves A. Mating type genes in the genus *Neofusicoccum*: mating strategies and usefulness in species delimitation. *Fungal Biol* 2017;121:394–404.
46. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 1997;25:4876–4882.
47. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Sym Ser* 1999;41:95–98.
48. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
49. Swofford DL. PAUP*: Phylogenetic analysis using parsimony (* and other methods). Version 4.0. Sinauer Associates, Sunderland, Massachusetts, 2000.
50. Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 2003;19:1572–1574.
51. Page RD. TreeView: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* 1996;12:357–358.
52. Rayner RW. *A Mycological Color Chart*. Kew: Commonwealth Mycological Institute; 1970.
53. Gonçalves MFM, Santos L, Silva BMV, Abreu AC, Vicente TFL *et al*. Biodiversity of *Penicillium* species from marine environments in Portugal and description of *Penicillium lusitanum* sp. nov., a novel species isolated from sea water. *Int J Syst Evol Microbiol* 2019;69:3014–3021.
54. Gladfelter AS, James TY, Amend AS. Marine fungi. *Curr Biol* 2019;29:R191–R195.
55. Grossart H-P, Van den Wyngaert S, Kagami M, Wurzbacher C, Cunliffe M *et al*. Fungi in aquatic ecosystems. *Nat Rev Microbiol* 2019;17:339–354.
56. Taylor JD, Cunliffe M. Multi-year assessment of coastal planktonic fungi reveals environmental drivers of diversity and abundance. *ISME J* 2016;10:2118–2128.
57. Jones EBG, Sakayaroj J, Suetrong S, Somirithipol S, Pang K-L. Classification of marine Ascomycota, anamorfic taxa and Basidiomycota. *Fungal Diver* 2009;35:1–203.
58. Comeau AM, Vincent WF, Bernier L, Lovejoy C. Novel chytrid lineages dominate fungal sequences in diverse marine and freshwater habitats. *Sci Rep* 2016;6:30120.
59. Hassett BT, Gradinger R. Chytrids dominate Arctic marine fungal communities. *Environ Microbiol* 2016;18:2001–2009.
60. Hassett BT, Ducluzeau A-LL, Collins RE, Gradinger R. Spatial distribution of aquatic marine fungi across the Western Arctic and sub-arctic. *Environ Microbiol* 2017;19:475–484.
61. Amend A, Burgaud G, Cunliffe M, Edgcomb VP, Ettinger CL *et al*. Fungi in the marine environment: open questions and unsolved problems. *mBio* 2019;10:e01189–18.
62. Kagami M, Miki T, Takimoto G. Mycoloop: chytrids in aquatic food webs. *Front Microbiol* 2014;5:166.
63. Kohlmeyer J, Spatafora JW, Volkmann-Kohlmeyer B. Lulworthiales, a new order of marine Ascomycota. *Mycologia* 2000;92:453–458.
64. Azevedo E, Barata M, Marques MI, Caeiro MF. *Lulworthia atlantica*: a new species supported by molecular phylogeny and morphological analysis. *Mycologia* 2017;109:287–295.
65. Calado MdaL, Carvalho L, Pang K-L, Barata M. Diversity and ecological characterization of sporulating higher filamentous marine fungi associated with *Spartina maritima* (Curtis) Fernald in two Portuguese salt marshes. *Microb Ecol* 2015;70:612–633.
66. Jones EBG, Suetrong S, Sakayaroj J, Bahkali AH, Abdel-Wahab MA, Boekhout T *et al*. Classification of marine Ascomycota, Basidiomycota, Blastocladiomycota and Chytridiomycota. *Fungal Divers* 2015;73:1–72.
67. Liu Y, Singh P, Liang Y, Li J, Xie N *et al*. Abundance and molecular diversity of thraustochytrids in coastal waters of southern China. *FEMS Microbiol Ecol* 2017;93:89.
68. Liu J-K, Hyde KD, Jeewon R, Phillips AJL, Maharachchikumbura SSN *et al*. Ranking higher taxa using divergence times: a case study in Dothideomycetes. *Fungal Divers* 2017;84:75–99.
69. Vijaykrishna D, Jeewon R, Hyde KD. Molecular taxonomy, origins and evolution of freshwater ascomycetes. *Fungal Divers* 2006;23:351–390.
70. Gonçalves MFM, Vicente TFL, Esteves AC, Alves A. *Neptunomyces aureus* gen. et sp. nov. (*Didymosphaeriaceae*, Pleosporales) isolated from algae in Ria de Aveiro, Portugal. *MycoKeys* 2019;60:31–44.
71. Gonçalves MFM, Aleixo A, Vicente TFL, Esteves AC, Alves A. Three new species of *Neocamarosporium* isolated from saline environments: *N. aestuarinum* sp. nov., *N. endophyticum* sp. nov. and *N. halimiones* sp. nov. *Mycosphere* 2019;10:608–621.
72. Gonçalves MFM, Silva BMV, Esteves AC, Alves A. *Verrucoconiothyrium ambiguum* sp. nov., a novel species isolated from sea water, and affiliation of the genus *Verrucoconiothyrium* to the family *Didymellaceae*. *Int J Syst Evol Microbiol* 2019;69:3769–3776.
73. Eriksson O. On graminicolous pyrenomycetes from Fennoscandia I. Dictyosporous species (339-380). II. Phragmosporous and scolecosporous species (381-440). III. Amerosporous and didymosporous species (441-466). *Arkiv før Botanik* 1967;6:339–466.
74. Gonçalves MFM, Vicente TFL, Esteves AC, Alves A. Novel halotolerant species of *Emericellopsis* and *Parasarocladium* associated with macroalgae in an estuarine environment. *Mycologia* 2020;112:154–171.
75. Crous PW, Wingfield MJ, Lombard L, Roets F, Swart WJ *et al*. Fungal planet description sheets: 951-1041. *Persoonia* 2019;43:223–425.
76. Verkley GJM, Dukik K, Renfurm R, Göker M, Stielow JB. Novel genera and species of coniothyrium-like fungi in *Montagnulaceae* (Ascomycota). *Persoonia* 2014;32:25–51.
77. Liu JK, Hyde KD, Jones EBG, Ariyawansa HA, Bhat DJ *et al*. Fungal diversity notes 1–110: taxonomic and phylogenetic contributions to fungal species. *Fungal Divers* 2015;72:1–197.
78. Verkley GJM, da Silva M, Wicklow DT, Crous PW. *Paraconiothyrium*, a new genus to accommodate the mycoparasite *Coniothyrium minitans*, anamorphs of *Paraphaeosphaeria*, and four new species. *Stud Mycol* 2004;50:323–335.
79. Valenzuela-Lopez N, Sutton DA, Cano-Lira JF, Paredes K, Wiederhold N *et al*. Coelomycetous fungi in the clinical setting: morphological convergence and cryptic diversity. *J Clin Microbiol* 2017;55:552–567.
80. Wanasinghe DN, Phukhamsakda C, Hyde KD, Jeewon R, Lee HB *et al*. Fungal diversity notes 709–839: taxonomic and phylogenetic contributions to fungal taxa with an emphasis on fungi on *Rosaceae*. *Fungal Divers* 2018;89:1–236.
81. Chen Q, Hou LW, Duan WJ, Crous PW, Cai L. *Didymellaceae* revisited. *Stud Mycol* 2017;87:105–159.
82. Osterhage C, Kaminsky R, König GM, Wright AD. Ascosalipyrrolidinone a, an antimicrobial alkaloid, from the obligate marine fungus *Ascochyta salicorniae*. *J Org Chem* 2000;65:6412–6417.
83. Chen Q, Jiang JR, Zhang GZ, Cai L, Crous PW. Resolving the *Phoma* enigma. *Stud Mycol* 2015;82:137–217.