Marine fungi from Mira river salt marsh in Portugal

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Summary The occurrence of fungi in the Mira salt marsh, Portugal was investigated for 12 months. Baits of *Spartina maritima* stems were exposed to permanent or temporary submersion at the upper and lower limits of the intertidal zone. The baits were observed for fruit bodies and spores directly and after incubation in moist chambers.
Twenty six marine species were identified (17 Ascomycota, two Basidiomycota and seven mitosporic fungi). Twenty four are new records for Portugal. *Nia globospora* Barata & Basílio was published as a new species. Species were characterized with respect to frequency of occurrence, colonization capability and substrate succession. The diversity and similarity indexes of the fungi under different conditions were determined.

Key words Baits, Biodiversity, Marine fungi, Salt marsh

Approximately 444 taxa of filamentous fungi are recognized as obligate or facultative marine fungi able to grow and reproduce in saline or brackish water environments. The majority are Ascomycota (360 species). Basidiomycota (ten species) and mitosporic fungi (74 species) are less abundant [6]. The most widely accepted definition of marine fungi is that of Kohlmeyer and Kohlmeyer [9], "obligate marine fungi are those that grow and sporulate exclusively in marine or estuarine habitat; facultative marine fungi are those from a freshwater or terrestrial milieu, which are able to grow and possibly also sporulate in a marine environment". Thus, marine fungi are not a taxonomically, but an ecologically and physiologically defined group. They are saprotrophs, symbionts or parasites on plants or animals. All are microscopic with the exception of the largest (4-5 mm diameter) marine ascomycetes and basidiomycetes. Saprobic fungi are important decomposers of cellulose, in the form of driftwood, pilings, mangrove roots, and marsh plants. In marine and estuarine environments fungi are predominantly in the intertidal zone where they can colonise the substrata or hosts.

Salt marshes are among the most productive ecosystems of the world. It is recognised that most of the productivity of the salt marshes is in the form of grasses which are decomposed by fungi and bacteria and the plant nutrients are recycled within the marsh [4]. Senescent halophytes (e.g. *Juncus*, *Salicornia*, *Spartina*) in salt marshes are inhabited by diverse ascomycetes and mitosporic fungi, although rarely basidiomycetes. The lower part, ca. 40 cm on top of the rhizome, is inhabited by marine organisms (e.g. algae, barnacles, and fungi). Above this zone are salt-tolerant fungi, while the uppermost part of the culms harbours terrestrial species [4].

One of the methods used to study the occurrence of marine fungi and to obtain pure isolates is baiting [12]. Baits can be made of various materials such as mangrove wood, mangrove leaves, mangrove seedlings, herbaceous stems of plants, and other lignocellulosic substrates. Seasonal species composition and fungal population abundance can be determined. A succession of fungal fruit bodies have been observed: which were classified into early, intermediate and late colonisers using this method [8].

The species composition, abundance of fungal populations and the patterns of succession associated with *Spartina maritima* baits exposed in the intertidal zone of a salt marsh in the Mira river were studied and are reported herein.

Materials and methods

The study site selected for this survey was a small salt marsh located in the centre of the estuary of the river Mira in Portugal (37° 43' N 08° 45' W; UTM 29 SNB2273).

The bait was portions of the herbaceous stems of the halophyte *S. maritima* (Curtis) Fernald. Young plants were collected to prepare the baits. The top portions of the stems were cut in to 8.5 cm lengths. Baits were divided into groups of five and each group was placed inside a nylon net bag (15x15 cm) and sterilised with gamma radiation (30KGy) [3]. The bags were then placed inside a cylindrical net box (60 baits per box). Seven boxes were placed in the salt marsh from January 1995 to February 1996. Two boxes were submerged in front of the salt marsh and one in the river mouth. The other ones were placed at the upper (two boxes) and lower (two boxes) limits of the intertidal zone. The baits were subjected to permanent or temporary submersion because of tides at the intertidal zone of

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marsh. Ten baits were recovered from each location with intervals of four to six weeks.

Baits were observed with a dissecting microscope to detect fruiting bodies and spores of marine fungi. They were kept in moist chambers and re-examined monthly for fungi for eight months. The more detailed morphology was studied using light microscopy (Leitz laborlux S, with Normaski) with sea-water solution as the mounting medium. The fungi were identified according to [9,10]. The physicals parameters recorded were: pH, salinity and temperature of water of the sampling sites [4].

After the identification of fungi the following parameters were determined: Number of occurrence of each taxa (N.O.= number of baits where the taxon was detected); frequency of occurrence of all taxa (F.O.= number of baits colonized by a specific taxon divided by the total number of baits multiplied by 100); average number of fungi per bait (= total number of fungal specimens divided by the total number of baits) [11]; the diversity of the mycota detected on baits exposed to different conditions in the marsh, based on Species richness (S), Shannon diversity index (H´) and on Evenness (E) [7]; the similarity of the mycota detected on baits based on Sorenson similarity index (Cs) [1]; time needed to differentiate mature fruit bodies (t.n.d.f.) for each taxon (t.n.d.f. = number of months that the baits remained in the study site until the observation of mature fruit bodies directly after collection of the baits). The taxa were classified based in their capability of colonising the substrate as: early colonisers (t.n.d.f. ≤ three months); intermediate colonisers (three months < t.n.d.f. > six months) and late colonisers (t.n.d.f. ≥ six months) [3]. The taxa were also classified based in their frequency of occurrence as very frequent (≥ 20%), frequent (10-20%) and infrequent (≤ 10%) [11].

Results

The dates of placing the baits and the last dates of collection are presented in table 1.

The temperature of water varied from 12 to 24 °C, the minimum and maximum pH was 6.6 and 8. The salinity ranged between 2.57 psu and 31.81 psu.

There were identified 21 taxa from baits exposed in 19/01/95 (13 Ascomycota, one Basidiomycota and seven mitosporic fungi). There were four very frequent taxa, *Lulworthia* sp. (88.6%), *Natantispora retorquens* (33.6%), *Zalerion varium* (27.9%) and *Cirrenalia macrocephala* (22.9%). Frequent taxa were a Pyrenomycete (18.6%) and *Byssothecium obiones* (14.3%). The other 15 taxa were infrequent (Table 2). In 5/08/95, there were 15 taxa (12 Ascomycota, one Basidiomycota and two mitosporic fungi). There were two very frequent taxa, *Lulworthia* sp. (86.7%) and *Natantispora retorquens* (41.1%). Frequent taxa were *Aniptodera chesapeakensis* (18.9%) and *Lignicola laevis* (12.2%). The other 11 taxa were infrequent (Table 3). The Basidiomycota *Nia globospora* was publish previously as a new species [2].

The average number of fungi per bait was (a) 1.2 for permanent submersion, and (b) 3.2 and 3.7 for baits exposed on the intertidal zone of the marsh at the lower and upper limits respectively in winter (Table 2). The average number of fungi per bait was 1.9 for baits permanently submerged in summer (5/08/95) (Table 3).

Table 4 demonstrates the diversity of the mycota, associated to baits exposed in winter (19/01/95), to different conditions in the marsh. The highest values for species richness, Shannon index and Evenness were obtained for baits exposed in the intertidal zone of the marsh, being the values of Shannon index and Evenness for the lower limit of this zone slightly superior when comparing with upper limit values.

Table 5 shows the diversity of the mycota, associated with the baits exposed to permanent submersion in summer. Species richness value was equal to 15, the values for Shannon index and Evenness were 1.72 and 0.63 respectively.

Table 6 shows the values for Sorenson similarity index (Cs). This index was designed to equal 1 in cases of complete similarity and 0 if the sites are dissimilar and have no species in common. In present survey Sorenson similarity index was higher for the mycota detected at the lower and at the upper limits of intertidal zone (0.82), which means the similarity of the two mycota on the two sites was high. When comparing the similarity of the mycota detected on baits exposed to three places in the marsh in winter, 14 taxa were common to baits exposed at the lower and upper limits of the intertidal zone (Table 6). There were four taxa common to baits exposed at the lower limit of the intertidal zone and that were permanently submerged. Cs in this case was 0.29, which means that the similarity of the two mycota was low. Finally, three taxa were common to baits exposed to permanent submersion and those exposed at the upper limit of the intertidal zone. Cs was 0.21 which means that the similarity of the two mycota was low.

The taxa were classified based on their capability of colonising the substrate as early, intermediate and late colonisers. Early colonisers were: *N. retorquens* (t.n.d.f. = 1-3 months), *Lulworthia* sp. (t.n.d.f. = 3-4 months) and the unidentified Pyrenomycete (t.n.d.f. = 3 months). Intermediate colonisers were *Phialorophorophoma litoralis* (t.n.d.f. = 4 months). *Lignincola laevis* (t.n.d.f. = 4-8 months) was an intermediate/late coloniser. The late colonisers were *Dictyosporium pelagicum* (t.n.d.f. = 6 months), *B. obiones* (t.n.d.f. = 6-8 months), *Tirispora unicaudata* (t.n.d.f. = 6 months), *Halosarpheia* sp.I (t.n.d.f. = 7 months), *N. globospora* (t.n.d.f. = 7 months), *A. chesapeakensis* (t.n.d.f. = 7- 8 months) and *Nia vibrissa* (t.n.d.f. = 10 months) (Tables 7 and 8).

Lulworthia sp., *N. retorquens* (very frequent species), and *Phoma* sp. (infrequent taxon) were the only taxa detected on baits exposed to all conditions in the salt marsh (Table 2). There were 11 taxa identified only on baits that were exposed to the intertidal zone of the salt marsh at both limits. *N. vibrissa* was the fungus detec-

Table 1. Dates of placing and recovering the baits in Mira salt marsh.

	Permanent submersion	Intertidal zone upper limit	Intertidal zone lower limit
Dates of placing the baits	19/01/95 (winter) 5/08/95 (summer)	19/01/95 (winter)	19/01/95 (winter)
Last dates of recovered the baits	31/10/95 9/02/96	28/06/95	28/06/95

Table 2. Percent of frequency of occurrence of marine fungi on *Spartina maritima* baits exposed on three places of Mira salt marsh (period of experimental assay 19/01/95 - 31/10/95).

Taxa	Permanent submersion N.O. (60 baits)	Temporary submersion Lower limit N.O. (40 baits)	Temporary submersion Upper limit N.O. (40 baits)	N.O. (140 baits)	F.O. %
Very Frequent					
Lulworthia sp.	54	35	35	124	88.6
Natantispora retorquens	9	20	18	47	33.6
Zalerium varium•	0	16	23	39	27.9
Cirrenalia macrocephala	0	10	22	32	22.9
Frequent					
Unidentified Pyrenomycete•	0	9	17	26	18.6
Byssothecium obiones	0	10	10	20	14.3
Infrequent					
Lignicola laevis	0	9	1	10	7.1
Sphaerulina oraemaris	0	6	3	9	6.4
Panrobis (viscos) viscosus	0	5	1	6	4.3
Sphaerulina albispiculata•	0	1	5	6	4.3
Dictyosporium pelagicum•	0	1	4	5	3.6
Nia vibrissa•	5	0	0	5	3.6
Tirispora unicaudata	0	0	3	3	2.1
Phaeosphaeria spartinicola•	0	2	1	3	2.1
Phoma sp.•	1	1	1	3	2.1
Corollospora maritima•	1	1	0	2	1.4
Trichocladium constrictum•	0	1	1	2	1.4
Halosarpheia unicaudata	0	1	0	1	0.7
Phaeosphaeria macrosporidium•	0	0	1	1	0.7
Leptosphaeria pelagica•	0	1	0	1	0.7
Phialophorophoma litoralis	0	0	1	1	0.7
Total number specimens	70	129	147	346	
Average number of fungi per bait	1.2	3.2	3.7	2.5	
Marine taxa identified	5	17	17	21	

•Taxa identified exclusively in winter.
N.O. = number of baits where the taxon was detected.
F.O. = number of baits colonized by a specific *taxon* divided by the total number of baits multiplied by 100. Very frequent (⩾ 20%); frequent (10-20%) and infrequent (⩽ 10%).
Average number of fungi per bait = total number of fungal specimens divided by the total number of baits.

Table 3. Percent of frequency of occurrence of marine fungi on *Spartina maritima* baits exposed in Mira salt marsh (period of experimental assay 5/08/95 - 9/02/96).

Taxa	Permanent submersion N.O. (90 baits)	F.O. %
Very Frequent		
Lulworthia sp.	78	86.7
Natantispora retorquens	37	41.1
Frequent		
*Aniptodera chesapeakensis**	17	18.9
Lignicola laevis	11	12.2
Infrequent		
*Nia globospora**	6	6.7
Byssothecium obiones	5	5.6
Phialophorophoma litoralis	4	4.4
Tirispora unicaudata	3	3.3
*Ceriosporopsis halima**	2	2.2
Cirrenalia macrocephala	1	1.1
Halosarpheia sp.I*	1	1.1
Halosarpheia unicaudata	1	1.1
Panrobis viscosus	1	1.1
*Phaeosphaeria neomaritima**	1	1.1
Sphaerulina oraemaris	1	1.1
Total number specimens	169	
Average number of fungi per bait	1.9	
Marine taxa identified	15	

*Taxa identified exclusively in summer.
N.O. = number of baits where the *taxon* was detected.
F.O. = number of baits colonized by a specific *taxon* divided by the total number of baits multiplied by 100.
Very frequent (⩾ 20%); frequent (10-20%) and infrequent (⩽ 10%).
Aveage number of fungi per bait = total number of fungal specimens divided by the total number of baits.

Table 4. Species richness, Shannon index and Evenness of fungi collected on baits exposed in Mira salt marsh (period of experimental assay 19/01/95 - 31/10/96).

Places on marsh where baits were exposed	Species Richness (S)	Shannon Index (H')	Evenness (E)
Permanent submersion	5	0.77	0.48
Temporary submersion (Lower limit of intertidal zone)	17	2.27	0.8
Temporary submersion (Upper limit of intertidal zone)	17	2.22	0.78
Σ	21	2.18	0.71

Table 5. Species richness, Shannon index and Evenness of fungi collected on baits exposed in Mira salt marsh (period of experimental assay 5/08/95-9/02/96).

Places on marsh where baits were exposed	Species Richness (S)	Shannon Index (H')	Evenness (E)
Permanent submersion (In front salt marsh)	8	1.47	0.71
Permanent submersion in a anchorage near river mouth	13	1.76	0.69
Σ	15	1.72	0.63

Table 6. Comparison of the mycota detected on baits exposed to three different conditions in Mira salt marsh, based on the Sorenson similarity index (period of experimental assay 19/01/95 - 31/10/96).

Places on marsh where baits were exposed	Number of species in common	Sorenson Index (Cs)
Intertidal zone (lower and upper limits)	14	0.82 h
Lower limit of intertidal zone and permanent submersion	4	0.29 l
Upper limit of intertidal zone and permanent submersion	3	0.21 l

(h = high: l = low)

Table 7. Time taken for fungi to differentiate mature fructifications on *Spartina maritima* baits exposed to different conditions in Mira salt marsh (period of experimental assay 19/01/95 - 31/10/96).

Taxa	Permanent submersion (t.n.d.f.)	Temporary submersion Lower limit (t.n.d.f.)	Temporary submersion Upper limit (t.n.d.f.)
Baits placed in the salt marsh in winter	(d.o.)	(d.o.)	(d.o.)
Ascomycota			
Natantispora retorquens	3	3	3
Lignincola laevis	-	-	4
Lulworthia sp.	3	4	4
Byssothecium obiones	-	6	6
Unidentified Pyrenomycete	-	3	3
Basidiomycota			
Nia vibrissa	10	-	-
Mitosporic fungi			
Dictyosporium pelagicum	-	-	6

(d.o.) = data based on direct observation of baits.
(t.n.d.f.) = number of months that the baits stays in the study site until the observation of mature fruit bodies directly after collection of the baits.

Table 8. Time needed for fungi to differentiate mature fructifications on *Spartina maritima* baits exposed to permanent submersion in Mira salt marsh (period of experimental assay 5/08/95 - 9/02/96).

Taxa	Permanent submersion in front salt marsh (t.n.d.f.)	Permanent Submersion in an anchorage near river mouth (t.n.d.f.)
Baits placed in the salt marsh in summer	(d.o.)	(d.o.)
Ascomycota		
Aniptodera chesapeakensis	7	8
Natantispora retorquens	1	3
Halosarpheia sp.I	7	-
Lignicola laevis	-	8
Lulworthia sp.	3	3
Byssothecium obiones	-	8
Tirispora unicaudata	-	6
Basidiomycota		
Nia globospora	7	-
Mitosporic fungi		
Phialophorophoma litoralis	-	4

(d.o.) = data based on direct observation of baits.
(t.n.d.f.) = number of months that the baits stays in the study site until the observation of mature fruit bodies directly after collection of the baits.

ted only on baits subjected to permanent submersion. *Corollospora maritima* was identified on two baits, one was exposed to permanent submersion and the other was located at the lower limit of the intertidal zone, subject to the rotation of the tides. The other five taxa were observed on baits exposed at the lower and at the upper limit of the intertidal zone (Table 2).

Discussion

These results demonstrated that the number of occurrence of some taxa varied with the submersion period of baits and also that the majority of the reproductive structures of taxa were from the intertidal zone of the salt marsh (Table 2). This zone of the salt marsh was the place with the higher values for the following parameters: (a) average number of fungi per bait and species richness (Table 2), (b) Shannon diversity index and (c) Evenness (Table 4).

Based on data obtained by direct observation of baits the taxa were classified in early, intermediate and late colonisers (Tables 7 and 8). For some taxa the conditions of permanent or temporary submersion dictated the time needed to differentiate fruit bodies, increasing or decreasing this time (Table 7). T.n.d.f varied from taxon to taxon and the same taxon may have different t.n.d.f. depending on the environmental condition to which it was subjected (e.g. *Lulworthia* sp., Tables 7 and 8).

Gessner and Goos [5] studied the fungi from decomposing *S. alterniflora* located on a marsh in southern Rhode Island, USA. One of the methods was baiting. the baits were made with dried portions of grass, and they were confined inside nylon bags, exposed on a salt marsh and in an adjacent tidal creek. The direct microscopic examination yielded seven marine Ascomycota, *C. maritima* (marsh), *Haligena spartinae* (marsh), *Leptosphaeria discors* (= *B. obiones*) (marsh and adjacent tidal creek), *Leptosphaeria pelagica* (marsh), *Nais inornata* (marsh), *Remispora hamata* (= *Ceriosporopsis hamata*) (marsh and adjacent tidal creek) and *Sphaerulina pedicellata* (= *Buergenerula spartinae*), and two mitosporic fungi *Monodictys pelagica* and *Phoma* sp. There were four common taxa to this and the present surveys *C. maritima*, *L. discors* (= *B. obiones*), *L. pelagica* and *Phoma* sp. The present survey yielded a higher number of marine fungi (26) when comparing with the Gesssner and Goss survey (9). These authors did not incubate the baits in moist chambers, after the examination of the substrate. In this way they have detected only the fungi with fructifications on baits at the dates of recovery and they did not identify mycelial forms. In present survey 14 (ten Ascomycota and four mitosporic fungi) of the total species identified were detected only after a period of incubation in moist chamber.

In conclusion, results of the present research suggest that baits of *S. maritima* stems was a good substrate for the collection of marine fungi. A period of incubation of baits in moist chambers was necessary to permit the differentiation of spores or fruit bodies by the mycelia. *N. vibrissa* presented the higher value for t.n.d.f. The intertidal zone of the salt marsh was the most favourable place for baits to be colonizer by a high diversity of marine fungi.

References

1. Abdel-Wahab MA, El-Sharouny HM. Ecology of subtropical mangrove fungi with emphasis on *Kandelia candel* mycota. In: Hyde KD (Ed.). Fungi in marine environments. Fungal diversity research series 7. Hong Kong, Fungal Diversity Press, 2002: 247–265.
2. Barata M, Basílio MC, Baptista-Ferreira JL. *Nia globospora* a new gasteromycete on baits of *Spartina maritima* in Portugal. Mycol Res 1997; 101: 687-690.
3. Barata M. Fungos marinhos superiores associados a *Spartina maritima* em estuários da costa Portuguesa. Ph.D. Thesis. Faculty of Sciences, University of Lisbon, Portugal 1997.
4. Barata M. Fungi on the halophyte *Spartina maritima* in salt marshes. In: Hyde KD (Ed.) Fungi in marine environments. Fungal diversity research series 7. Hong Kong, Fungal Diversity Press, 2002: 179-193.
5. Gessner RV, Goos RD. Fungi from decomposing *Spartina alterniflora*. Can J Bot 1973; 51: 51-55.
6. Hyde KD, Sarma VV, Jones EBG. Morphology and taxonomy of higher marine fungi. In: Hyde KD, Pointing SB (Eds.) Marine mycology-A practical approach. Fungal diversity research series 1. Hong Kong, Fungal Diversity Press, 2000: 172-204.
7. Maria GL, Sridhar KR. Richness and diversity of filamentous fungi on woody litter of mangroves along the west coast of India. Current Science 2002; 83: 1573-1580.
8. Pointing SB, Fryar SC, Hyde KD, Mouzouras R. Assessment of wood decay, fungal interactions and colonization. In: Hyde KD, Pointing SB (Eds.) Marine mycology-A practical approach. Fungal diversity research series 1. Hong Kong, Fungal Diversity Press, 2000: 113-136.
9. Kohlmeyer J, Kohlmeyer E. Marine mycology-the higher fungi. New York, Academic Press, 1979.
10. Kohlmeyer J, Kohlmeyer BV. Illustrated key to the filamentous higher marine fungi. Bot Mar 1991; 34: 1-61.
11. Tan TK, Leong WF, Jones EBG. Succession of fungi on wood of *Avicennia alba* and *A. lanata* in Singapore. Can J Bot 1989; 67: 2686-2691.
12. Vrijmoed LLP. Isolation and culture of higher filamentous fungi. In: Hyde KD, Pointing SB (Eds.) Marine mycology-A practical approach. Fungal diversity research series 1. Hong Kong, Fungal Diversity Press, 2000: 1-20.