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# Morphological and phylogenetic analyses of *Nia vibrissa*, a marine Basidiomycota collected in Portuguese waters

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## ABSTRACT

This study presents morphological and phylogenetic characterizations of *Nia vibrissa* specimens detected on *Fagus sylvatica* baits, after six months of incubation in moist chambers at the laboratory. The baits had been submerged at Cascais marina, Portugal, during a survey carried out in 2006–2008. Morphological observations evidenced differences in basidiocarp color and in the morphology of the peridial hairs, varying from straight to curved and with bifurcate to non-bifurcate ends. Morphological variability has often been reported, associated to the suggestion that *N. vibrissa* is a species complex. We addressed this subject through the evaluation of pairwise distances and phylogenetic analyses applying Bayesian and Maximum Likelihood methods, to multi-sequence alignments involving the large subunit (LSU) of the nuclear ribosomal DNA. Even though the six *N. vibrissa* isolates under analysis consistently clustered together with high support values and most of their pairwise distances ranged between 0 and 3%, one of them presents a higher distance value (4%) relative to other two isolates. These results recommend the need of further genetic evaluations, involving more isolates and gene regions, to answer the question addressed in this study: is *N. vibrissa* a species complex?

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## 1. Introduction

Basidiomycota are rare in marine environments where, according to Jones et al. (2015), 21 species were identified in 17 genera, including as last additions, four taxa from less saline waters (mangrove habitats): *Henningsomyces* spp., *Schizophyllum commune*, *Hyphoderma smabuci* and *Grammothele fuligo*.

Members of three genera from the family Marasmiaceae (*Nia*, *Calathella*, *Halocyphina*) are the most frequently detected in studies of marine mycology (Jones et al., 2015). In surveys carried out in Portugal, only species of the genus *Nia* were detected: *N. globispora* on *Spartina maritima* baits submerged in the estuary of Mira river (Barata et al., 1997; Barata, 2006) and on drift stems collected in sandy beaches (Sridhar et al., 2012) and also *Nia vibrissa* on baits of *S. maritima* submerged in Mira River (Barata, 2006) and baits of *Fagus sylvatica* submerged at Cascais marina (Azevedo et al., 2010, 2011).

*N. vibrissa* is widespread in marine environments colonizing a variety of submerged drift or intertidal woody substrates. This

species was originally detected on beech wood shavings submerged during several months at Biscayne Bay, Florida, having been classified as deuteromycete (Moore and Meyers, 1959). Later, Doguet (1967, 1968) observed basidia and clamp connections and Brooks (1975) demonstrated the existence of dolipore septa. These characters indicate that *N. vibrissa* is a homobasidiomycete, though not revealing its taxonomic position.

*N. vibrissa* produces minute, white, yellow, pink to dark yellow, superficial fruit bodies with peridial hairs, ovoid or ellipsoidal hyaline basidiospores with four lateral and one apical appendages (Kohlmeyer and Kohlmyer, 1979; Binder et al., 2001). This fungus differentiates reproductive structures in culture and Schimpfhauser and Molitoris (1991) reported the fact that some strains differentiate in culture either hairy or smooth fruit bodies with no peridial hairs.

Jones and Jones (1993), Binder et al. (2001) and Jones et al. (2009), based on the high morphological diversity observed on basidiocarp color and peridial hairs suggested the existence of a multi species complex for *N. vibrissa*. Since then, all authors agreed that further studies are required to address this subject.

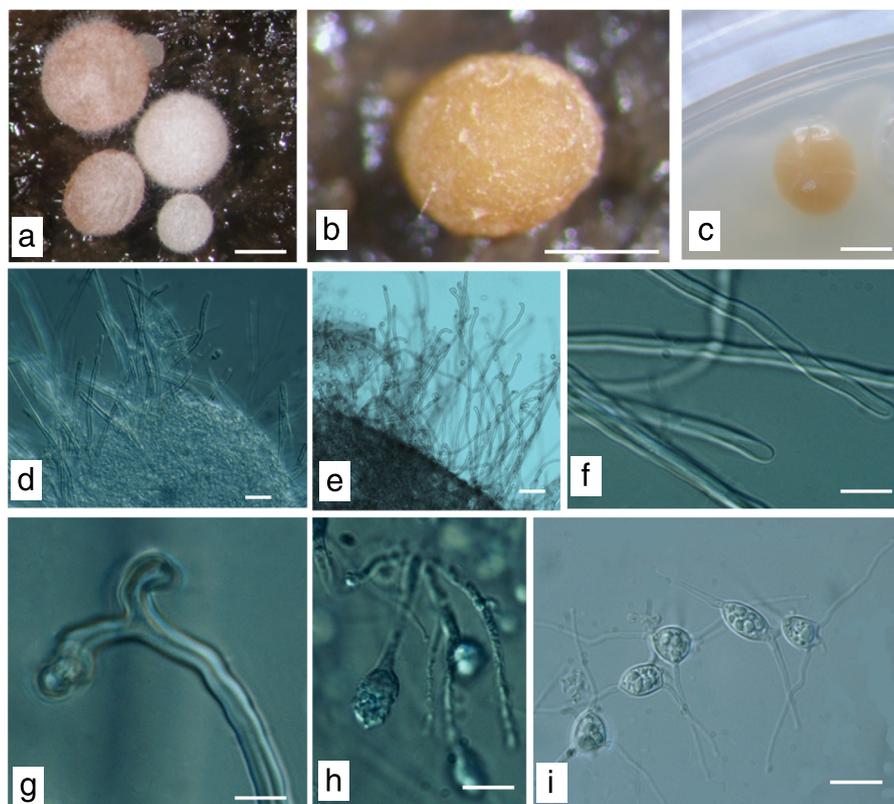
Based on analyses of partial sequences of nuclear (nuc) and mitochondrial (mt) small subunits (SSU) and large subunits (LSU) of ribosomal DNA (rDNA) regions, Binder et al. (2001) evaluated the phylogenetic position of *N. vibrissa*. These authors showed that *N.*

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**Fig. 1.** Morphological features of *Nia vibrissa*. a, b—Basidiocarps on *Fagus sylvatica* baits. c—Basidiocarp on culture medium. d, e—Basidiocarp wall with straight and curled peridial hairs. f—Straight peridial hairs. g—Bifurcated curled peridial hairs. h—Immature basidia. i—Basidiospores with appendages. Scale bars: a = 1 mm; b = 10 mm; c = 1 mm; d, e = 5  $\mu$ m; f, g, i = 10  $\mu$ m; h = 40  $\mu$ m.

*vibrissa* (one sequence) is a member of a named euagarics clade, clustering with the cyphelloid fungus *Henningsomyces candidus* (one sequence) whose basidiocarps are minute cup shaped forms.

Hibbet and Binder (2001) assessed the evolution of marine mushrooms based on the same regions referred above, including in their analyses four marine species (*Calathella mangrovei*, *Halocyphina villosa*, *N. vibrissa*, *Physalacria maipoensis*), one freshwater (*Limnoperdon incarnatum*) and 40 terrestrial species. This former study confirmed the placement of *N. vibrissa* in the euagarics clade. *N. vibrissa* (one sequence) and *H. villosa* (one sequence) clustered with *C. mangrovei* in a clade strongly supported by bootstrap value, all closely related with two terrestrial species (*Cyphellopsis anomala* and *Favolaschia intermedia*). These results were confirmed in LSU and combined LSU/5.8S phylogenetic analyses (Bodensteiner et al., 2004) where a well-supported group denominated *Nia* clade included three clades, one of them consisting of *N. vibrissa*, *H. villosa* and *C. mangrovei* (one sequence from each species). An identical *Nia* clade was obtained with a multilocus rDNA analysis, by Binder et al. (2006).

Marine fungi play diverse roles in ecosystems, allowing a number of biotechnological applications. This applies to isolates of *N. vibrissa*, evidencing involvement in wood decay (Schimpfhauser and Molitoris, 1991; Jones and Jones, 1993; Binder et al., 2001) and in the removal of hydrocarbon compounds of waters (Reyes et al., 2012).

Starting with a collection of morphologically distinct specimens of *N. vibrissa* detected on wood baits previously submerged at Cascais marina (Azevedo et al., 2010, 2011) and considering the availability of sequences of this fungus on databases, this work aims to evaluate the correspondence between morphological and genetic variability within *N. vibrissa*. Is *N. vibrissa* a species complex? This subject has been addressed by phylogenetic analyses

involving the LSU of the nuc rDNA region, to morphologically distinct representatives of this species, whose pairwise distances were also compared, for the same region.

## 2. Materials and methods

### 2.1. Morphological analysis

*Fagus sylvatica* baits collected at Cascais marina during a survey of our team (Azevedo et al., 2010, 2011) were subjected to indirect observations (after 5 mo of incubation in moist chambers at room temperature) under a stereomicroscope (Wild M8). Where basidiocarps of *N. vibrissa* were detected on the surface of the wood, fruit bodies were carefully removed with a needle and observed under the light microscope (Leitz Larbourz S with Normarski) in slides done with seawater as mounting media.

Microscopic characterizations of diagnose characters presented in Fig. 1 and Table 1, followed the current guidelines of marine mycology. Identifications were made using the dichotomous keys of Kohlmeyer and Kohlmyer (1979), Kohlmeyer and Volkmann-Kohlmyer (1991), Hyde and Sarma (2000), Jones et al. (2009). Photographs of microscopic characters were obtained with a Leica Wild MPS 52 camera using Fujichrome RTP-135, 64T Tungsten. Macroscopic features were obtained with a Nikon Coolpix 5000 camera and color annotations in macroscopical basidiocarp descriptions were based on Munsell soil color charts (<http://www.vcsu.edu/cmsfiles/327/b2fc4f5ebb.pdf>, last access: 8/17/2017).

The macromorphology of *N. vibrissa* colonies was performed following the procedures described by Sidrim and Moreira (1999), comprising the observation of color, rate of growth, margin and

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