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A reappraisal of the *Pleurotus eryngii* complex – New species and taxonomic combinations based on the application of a polyphasic approach, and an identification key to *Pleurotus* taxa associated with *Apiaceae* plants

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ABSTRACT

The *Pleurotus eryngii* species-complex comprises choice edible mushrooms growing on roots and lower stem residues of *Apiaceae* (umbellifers) plants. Material deriving from extensive sampling was studied by mating compatibility, morphological and ecological criteria, and through analysis of ITS1-5.8S-ITS2 and IGS1 rRNA sequences. Results revealed that *P. eryngii sensu stricto* forms a diverse and widely distributed aggregate composed of varieties *elaeoselini*, *eryngii*, *ferulae*, *thapsiae*, and *tingitanus*. *Pleurotus eryngii* subsp. *tuoliensis* comb. nov. is a phylogenetically sister group to the former growing only on various *Ferula* species in Asia. The existence of *Pleurotus nebrodensis* outside of Sicily (i.e., in Greece) is reported for the first time on the basis of molecular data, while *P. nebrodensis* subsp. *fossulatus* comb. nov. is a related Asiatic taxon associated with the same plant (*Prangos ferulacea*). Last, *Pleurotus ferulaginis* sp. nov. grows on *Ferulago campestris* in northeast Italy, Slovenia and Hungary; it occupies a distinct phylogenetic position accompanied with significant differences in spore size and mating incompatibility *versus* other *Pleurotus* populations. Coevolution with umbellifers and host/substrate specificity seem to play key roles in speciation processes within this fungal group. An identification key to the nine *Pleurotus* taxa growing in association with *Apiaceae* plants is provided.

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Introduction

The *Pleurotus eryngii* species-complex (*Basidiomycota*, *Agaricales*) constitutes the only group of the genus *Pleurotus* whose members are associated with plants of the family *Apiaceae* by developing a facultatively biotrophic mode of growth (Hilber 1982; Joly *et al.* 1990; Zervakis & Balis 1996). Basidiomes of host/substrate-specific populations grow on plant roots or lower parts of stems singly or in small groups from September until June. The distribution range of this complex covers a rather well-defined zone in the Old World ranging from Morocco to the Netherlands in the west and extends to China eastwards, comprising most parts of south-central Europe and many regions of the Middle East and west Asia (Hilber 1982; Zervakis *et al.* 2001).

The significance of these fungi as choice edibles is growing fast (Chang 2005) and today they are among the highest priced cultivated mushrooms. In addition, several studies assessed their potential in a wide range of applications, e.g. exploitation of various lignocellulosic residues as cultivation substrates (Philippoussis *et al.* 2001; Rodriguez Estrada *et al.* 2009; Zervakis *et al.* 2013) and the subsequent use of spent material as animal feed (Kwak *et al.* 2008; Okano *et al.* 2007), production of biomass with valuable dietetic and medicinal properties (Chen *et al.* 2012; La Guardia *et al.* 2005; Synytsya *et al.* 2009), biological pretreatment of lignocellulosics for ethanol production (López-Abelairas *et al.* 2013), biodegradation of industrial effluents and toxic pollutants (Gómez-Toribio *et al.* 2009; Ntougias *et al.* 2012; Koutrotsios & Zervakis 2014), etc.

Accurately identified biological material with well-understood phylogenetic relationships within and among closely related taxa are essential prerequisites for mushroom strain selection, breeding, and exploitation. In the past, taxonomic studies for members of the *P. eryngii* complex were mainly based on host-specificity and anatomy often accompanied by mating compatibility data (Hilber 1982; Lewinsohn *et al.* 2002; Mou *et al.* 1987; Venturella *et al.* 2000, 2002; Zervakis & Balis 1996), and led to the confirmation of the status of *P. eryngii* var. *eryngii* (DC.: Fr.) Quél. and var. *ferulae* Lanzi, or the establishment of several new varieties under *P. eryngii*: var. *tuoliensis* C.J. Mou, var. *elaeoselini* Venturella *et al.*, var. *thapsiae* Venturella *et al.*, and var. *tingitanus* Lewinsohn *et al.* In addition to well-known taxa like *Pleurotus nebrodensis* (Inzenga) Quél. and *Pleurotus fossulatus* (Cooke) Sacc., other names were also introduced to accommodate related entities at specific or varietal level, e.g. *Pleurotus hadamardii* Costantin and *P. eryngii* var. *ferulaginis* Stropnik *et al.* (Gargano *et al.* 2011; Offner & Heim 1924; Pegler 1977; Venturella 2000; Stropnik *et al.* 1988).

However, in many cases delimitation of species proved to be problematic, while pertinent reports were often contradictory; hence, conclusions on the correct identity of several taxa and their affinities were ambiguous and difficult to rely on. Indicative examples are the confusion in naming the white-coloured forms of *Pleurotus* mushrooms growing on *Apiaceae* plants irrespectively of their geographic origin or host (Heim 1960; Hilber 1982; Saber 1997), the dubious taxonomic position of *Pleurotus* populations associated with *Laserpitium* spp. (Chinan & Venturella 2012; Joly *et al.* 1990), the simultaneous use of three different names (i.e., *P. eryngii* var. *tuoliensis*, *P. nebrodensis*, and *Pleurotus ferulae*) to describe the same taxon in China (Choi *et al.* 2009; Mao 2000; Mou *et al.* 1987; Zhang *et al.* 2005), the misapplication of names (e.g. *P. hadamardii*; Offner & Heim 1924), the conflicting arguments on the taxonomic status of the epithet 'nebrodensis' at either species or varietal level (Kawai *et al.* 2008; Rodriguez Estrada *et al.* 2010; Zervakis *et al.* 2012), the establishment of new taxa without adequate supporting evidence such as *Pleurotus himalayaensis* (Dhancholia 2013), etc.

Recently the use of molecular approaches shed some light into this perplexed situation by confirming the taxonomic status of varieties *eryngii*, *ferulae*, and *elaeoselini* (Rodriguez Estrada *et al.* 2010; Zervakis *et al.* 2001), by identifying 'Bai-Ling-Gu' (widely used in China to describe '*P. nebrodensis*' specimens) as *P. eryngii* var. *toluensis* (Kawai *et al.* 2008), and by studying the largely unexplored relevant material originating from Iran (Ravash *et al.* 2010). Nevertheless, significant discrepancies still exist regarding the phylogeny of this species complex since the examined material in previous studies represented only part of its known distribution, while data on the taxonomic status and phylogenetic relationships for and among 'key-taxa' such as *P. nebrodensis*, *P. fossulatus*, and *P. eryngii* var. *tuoliensis* (and in respect to *P. eryngii*) are still missing together with much needed information on other under-studied populations associated with *Ferulago* and *Laserpitium* associated plants.

In this study, sequences from the nuclear internal transcribed spacer (ITS1-5.8S-ITS2) and intergenic spacer (IGS1) regions of the ribosomal RNA repeat from 46 specimens of the *P. eryngii* complex, covering to a significant extent (its occurrence both in terms of geographic distribution and associated-plant diversity), were examined in conjunction with morphology, ecology, and compatibility data. Phylogenetic analysis was used to infer relationships within this *Pleurotus* group, to define evolutionary paths in accordance with biogeography and to provide insight about speciation processes for plant-associated populations.

Materials and methods

Biological material

Forty-six (46) specimens representing most of the known world-wide distribution range of *Pleurotus eryngii* species-complex populations were included in this study; in addition, this material was associated with different *Apiaceae* plants, i.e., *Prangos ferulacea*, *Elaeoselinum asclepium* subsp. *asclepium*, *Margotia gummifera*, *Eryngium amethystinum*, *Eryngium campestre*, *Eryngium maritimum*, *Peucedanum cervaria*, *Ferula assa-foetida*, *Ferula communis*, *Ferula feruloides*, *Ferula ovina*, *Ferula sinkiangensis*, *Ferula tingitana*, *Laserpitium latifolium*, *Laserpitium siler*, *Thapsia garganica*, and *Thapsia villosa*, in order to more accurately reflect the pertinent diversity. Details on the identity of the fungal material used appear in Table 1. No type specimens were included in the study; however, care was taken to ascertain a wide/diverse representation of the *Pleurotus* taxa examined. All material is deposited in the herbaria of the Laboratory of General and Agricultural Microbiology of the Agricultural University of Athens (ACAM) and of the