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Punctularia atropurpurascens in the Villa Ada urban Park in Rome, Italy

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Abstract

The wood-decay saprophytic fungus *Punctularia atropurpurascens*, a species normally growing in tropical and subtropical zones, has been observed on a marcescent cork oak stump, in a mixed wood composed of cork oaks, pines and holm oaks, in the Villa Ada urban Park in Rome, Italy. *P. atropurpurascens*, with its striking morphology and purplish colouration, is considered a rare species, also interesting for the biological properties of the chemical molecules it produces. Fungus identity was confirmed by DNA-based analysis.

Keywords: Punctularia atropurpurascens; Punctulariaceae; phylogeny; Villa Ada urban Park

Riassunto

Il fungo lignicolo saprofita *Punctularia atropurpurascens*, specie che cresce in zone tropicali e subtropicali, è stato rinvenuto su un ceppo marcescente di quercia da sughero, in un bosco misto di sughere, pini e lecci, all'interno del Parco cittadino di Villa Ada a Roma. *P. atropurpurascens*, con la sua morfologia appariscente e la colorazione violacea, viene considerata una specie rara, interessante anche per le proprietà biologiche delle molecole chimiche che produce. L'identità del fungo è stata confermata attraverso l'analisi del DNA.

Parole chiave: Punctularia atropurpurascens; Punctulariaceae; filogenesi; Parco urbano di Villa Ada

Introduction

The genus *Punctularia*, corticioid Basidiomycota in the family *Punctulariaceae*, contains two species: *Punctularia atropurpurascens* (Berkeley & Broome) Petch (Petch, 1916), synonym *P. subhepatica*, and *P. strigosozonata* (Schweinitz) P.H.B. Talbot. The family *Punctulariaceae* was created in 1964 by the mycologist Marinus Anton Donk in order to accommodate the genus *Punctularia*, and included in the Aphyllophorales group based upon microscopic characteristics (Donk, 1964). Successively, its taxonomic positioning was modified and the fungus was collocated into the Corticiales (Larsson,

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2007), having molecular analysis pointed out the distance between families *Punctulariaceae* and *Corticiaceae* in its narrowest sense (Hjortstam, 1995).

P. atropurpurascens is a purplish coloured, easily observable saprophytic fungus that grows on marcescent wood, in prevalence deciduous. Its fruiting body spreads on the growth substrate, its superior surface is fluffy, membranous, waxy, with a purplish-brownish, coloured, central zone. The fungus can also appear in the form of brown violet coloured crusts, with a whitish margin. An aqueous exudate in the form of reddish coloured droplets is often present. The lower surface is adherent to the growth substrate. The hymenium is found on the upper surface and it is distributed in a not uniform manner, in spots. At a microscopic level, spores (6.5-9×3.5-5µm) are ellipsoid, hyaline or brownish, generally described as smooth. Lipid bodies are observed within some spores, that are reported to have various functional roles in fungal cells (Wang *et al*., 2002; Narvaes da Rocha Campos *et al*., 2008; Brondz *et al*., 2004) as well as in mammalian cells (Ferretti *et al*., 1999). The hyphal system is monomitic, made up of only generative hyphae, which appear branched, septate and with clamp connections. Basidia produce four sterigma. Violet crystal encrustations are sometimes present within the hymenium, the conidia, produced through sporogenesis, are chained, subglobose or ellipsoid, with a violet brown colour (Martini, 2016).

The species is used for the isolation of several chemical compounds like 'Phlebiarubrone' and its hydroxylated derivatives, as well as the 'Phlebiakauranol' aldehyde, a diterpene with a kaurene core that is intermediate in the synthesis of gibberellins, molecules acting as hormones in fungi and plants (Dawson, 1968). All these components hold a range of biological activities, such as antifungal, antimicrobial and antitumoral (Anke *et al*., 1986).

The main role of shikimic acid, an important metabolic intermediate in plants, fungi and microorganisms, is to supply aromatic amino acids essential to the organisms. Furthermore, the shikimic acid pathway provides intermediate molecules for the biosynthesis of other aromatic compounds, that can be divided into simple benzene derivatives, diphenyl benzoquinones and related molecules specific to fungi, together with other particular compounds synthesised by fungi and superior plants (Turner, 1971). 'Phlebiarubrone' (4,7-diphenyl-1,3-benzodioxole-5,6-dione) (Fig. 1) is a molecule belonging to the diphenyl benzoquinones or terphenyl quinones and a red pigment initially isolated from the fruiting bodies of basidiomycetes, in particular from the mycelium of *Punctularia strigosozonata* (formerly *Phlebia strigosozonata*) (McMorris *et al*., 1963). This diphenyl benzoquinone molecule and its hydroxylated derivatives, that are violet pigments, have been observed also in *P. atropurpurascens* (Gill *et al*., 1987; Anke *et al*., 1984). The importance of these intermediate polycyclic terphenyls, isolated from fungi of the genus *Punctularia*, has been highlighted by other studies that noted their capacity to act as bioactive molecules, possessing cytotoxic, antibacterial, anti-inflammatory and antioxidant properties (Calì *et al*., 2003).

Interest in *P. atropurpurascens*, a species reported as subtropical, derives from the fact that, while being rather rare in Italy (there have been only a few reports from some zones of Sicily; Mondello, 2017), a specimen has been observed in one of the greater urban parks of Rome: Villa Ada Savoia.

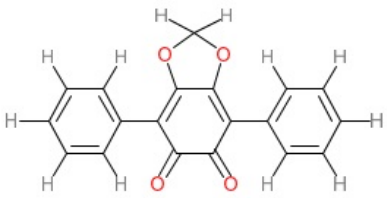

Fig. 1. 'Phlebiarubrone' chemical structure
Fig. 1. 'Phlebiarubrone' struttura chimica

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Materials and methods

Area of study

The Villa Ada urban Park (Fig. 2) consists of tree grooves for about 80% of its 160 hectares and the specific plant diversity, provided by both native and exotic species, makes it a mix of plots, consisting of monospecific or polyspecific stands. The dominant tree species is pine (*Pinus nigra* J.F. Arnold, *P. pinaster* W. Aiton, *P. pinea* L.), but groups of locusts (*Robinia pseudoacacia* L.), cedars (*Cedrus atlantica* (Endl.) Carrière, *C. deodara* (Roxb.) G. Don, *C. libani* A. Rich.), olive trees (*Olea europaea* L.), chestnut trees (*Castanea sativa* Mill.), walnut trees (*Juglans californica* S. Watson, *J. regia* L.) and yews (*Taxus baccata* L.) also grow in the meadows or on the borders. Monospecific woods are composed of holm oak (*Quercus ilex* L.) laurel (*Laurus nobilis* L.) or maple (*Acer sp*. L.), whereas polyspecific ones by oak species like pubescent oak (*Quercus pubescens* Willd.), cork oak (*Quercus suber* L.) and holm oak (*Quercus ilex* L.), or mixed conifer plots composed of redwood (*Sequoia sempervirens* (D. Don) Endl.), spruce (*Picea abies* (L.) H. Karst.) and cypresses (*Cupressus sempervirens* L.).

Fig. 2. A glimpse of the Villa Ada urban Park, an area of holm oaks and cork oaks.
Fig. 2. Uno scorcio del Parco cittadino di Villa Ada, una zona di lecci e sughere.

Morphological analysis

Microscope images of a fragment of the fungus were obtained by means of a digital camera AmScope MU500 mounted on an AmScope B490 microscope, operating at 800× and 2000× magnifications. Samples were analysed dry mount and wet mount in H_2O without dye.

Molecular analysis

DNA-based analysis was performed on a sample of the fungus at the University of Tartu in Estonia by Prof. U. Kõljalg and Dr. I. Saar, in the frame of the "Submit specimens for sequencing" service of the

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UNITE project. DNA extraction, PCR amplification and sequencing were as in Lindahl *et al*., 2013. In particular, a sequence of 915 base pairs was obtained that included the nuclear ribosomal internal transcribed spacer (ITS1) region, the 5.8S internal gene, the nuclear ribosomal internal transcribed spacer (ITS2) region, and a portion of the 28S Large Subunit LSU. This sequence was subjected to Basic Local Alignment Search Tool (BLAST 2.7.1+, Camacho *et al*., 2009) on the GenBank (Benson *et al*., 2018) and UNITE (Kõljalg *et al*., 2013) databases.

To create a dataset for phylogenesis, sequences from various fungi belonging to the *Punctulariaceae* were gathered from UNITE and GenBank databases and one sequence of *P. atropurpurascens* published on the MushroomObserver website. Three species having an increasing phylogenetic distance were chosen as outgroups to polarise the phylogenetic tree: *Vuilleminia comedens* (Nees) Maire from the Corticiales (same order), *Mycoacia fuscoatra* (Fr.) Donk and *Lepista sordida* (Schumach.) Singer from the Agaricomycetes (same class). Their sequences were obtained from GenBank (Tab. 1).

During further bioinformatic analysis, sequences were aligned with the MUSCLE algorithm (Edgar, 2004), utilising MEGA 7.0.26 software (Kumar *et al*., 2016) and then fine-tuned manually. For phylogenetic analyses, two methods were adopted: Maximum Likelihood and Bayesian inference. For Maximum Likelihood in PhyML 3.1 (Guindon *et al*., 2010), Nearest Neighbour Interchange (NNI) has been set up as topology research, initial trees were obtained applying BIONJ and Neighbour-Joining to a matrix of pairwise distances and HKY85 was used as the nucleotide substitution model. Otherwise for Bayesian inference, MrModeltest 2.3 (Nylander, 2004) suggested SYM+G as the best model for nucleotide evolution for all three regions (ITS1, 5.8S and ITS2). This model was applied in MrBayes 3.2.6 software (Ronquist *et al*., 2012): the Markow Chain Monte Carlo (MCMC) algorithm was run using four chains (three hot and one cold), setting a temperature of 0.2 and iterations for 2×10^6 generations on two trees with a diagnose frequency every 5,000 generations and a 25% burn-in. The final mean standard deviation of the separated frequencies was 0.004, indicating good convergence. The phylogram reporting the posterior probabilities that two branches belonged to the same clade is visualised with FigTree v1.4.3.

Results

Collection and morphological identification

The “Sughereta” is an area of the Villa Ada Savoia park of about 7 hectares composed of cork oaks with pine trees and holm oaks, in the centre of which a creek flows. Here, in October 2016, on a chopped cork oak stump, a *P. atropurpurascens* specimen proliferated, a showy wood-decay fungus, woolly, violet, more or less brightly coloured (Fig. 3). The spotted specimen was located laterally on the moss of the bark, on the top surface and, observed from above, on the bark and on areas of the bast, the cambium and the growth rings. The stump was photographed again in the autumn (Fig. 3a-b) of the following year, when *P. atropurpurascens* had a cottony morphology, feathery, with variable lilac tones, almost white in the most external parts and, in this period of maximum growth, presented reddish aqueous droplets, presumably containing the red pigment ‘Phlebiarubrone’. During the winter months, on the contrary, the fungus (Fig. 3c) spread out on the bark of the stump as a compact layer, was less woolly, with several zones dried out, and had an intense violet colour. During spring it appeared dry, dark-brown, adherent to the bark but partly powdered, and probably occurred on the inside of the stump (Fig. 3d). These observations indicate that the fungus undergoes strong morphological seasonal variations and, in any case, is persistent throughout the year on the colonised host.

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Fig. 3. Morphological characteristics of *P. atropurpurascens* in different seasonal periods (a, b: autumn; c: winter; d: spring, summer).
Fig. 3. Variazioni nella morfologia di *P. atropurpurascens* nei diversi periodi stagionali (a, b: autunno; c: inverno; d: primavera, estate).

Optical microscopic characteristics, performed on violet filaments, are consistent with what reported in the literature (Martini, 2016; Mondello, 2017). Hymenial structures with short branches, a monomitic hyphal system and chained conidia. The spores show an ellipsoid form and their walls appear frequently brownish; on the inside of some spores both lipid bodies and pinkish droplets are found (Fig. 4).

Identification of the fungus was not immediate. This because another species exists, very similar macroscopically, differing little from the morphological description of *P. atropurpurascens*: *Hypochnella violacea* Auersw. ex J. Schröt.. This latter species is also not very widespread, considered from rare to very rare. In the Czech Republic (Holec *et al*., 2006) and in The Netherlands (Arnolds *et al*., 2008) it is included in a list of threatened species, while in Italy it has been proposed for inclusion in the European Red List (Saitta *et al*., 2011).

The discrimination among the two species is not clear even at microscopic level. Differences between *P. atropurpurascens* and *H. violacea* have been described regarding spores (thin walls rather than thick, hyaline or brownish, no-amyloid in the first species, brownish violet and weakly amyloid in the

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other species); hyphae, with clamp connections occurring in the first and absent in the second; length of basidia (40-50 [-80] µm against less than 30 µm) (Hjortstam *et al*., 1987). In the various samples examined (Fig. 4), microscopy of the mycelium did not seem to be dissimilar from what is reported for both species and hyphae presented sporadic clamp connections. The spores showed an ellipsoid form, with a smooth wall, neither thin nor thick, with a prevalently brownish-violet colour.

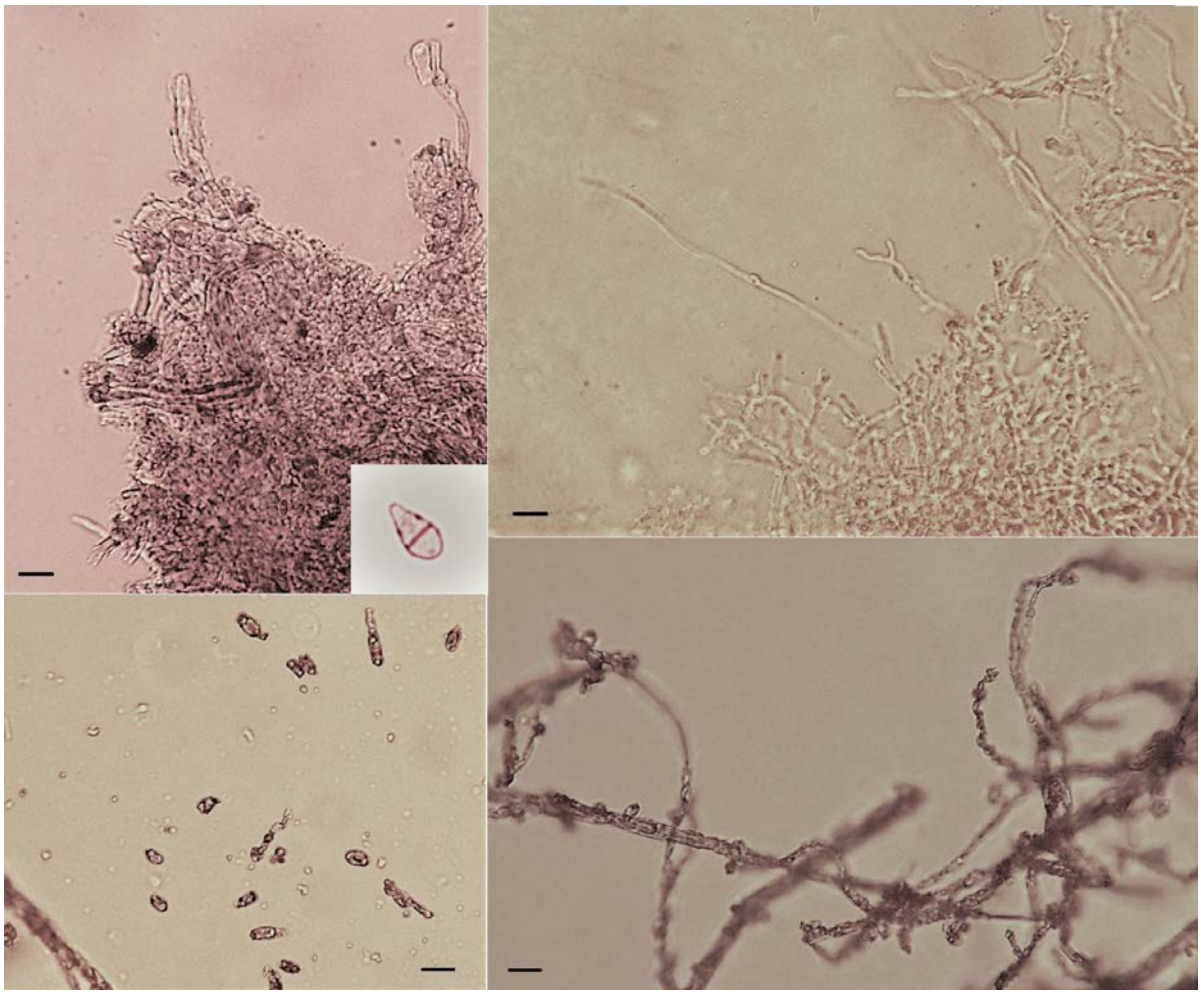

Fig. 4. *P. atropurpurascens* mycelium and spores. Bar length = 10 µm.
Fig. 4. Morfologia di micelio e spore di *P. atropurpurascens*. Lunghezza della barra = 10 µm.

Molecular identification

Molecular methods were applied to obtain a definitive identification. Ribosomal DNA sequencing is largely used for identification and systematics purposes. The target region was a fragment of the rRNA operon located between 18S and 28S rRNA genes, including the internal 5.8S rRNA gene and the hypervariable, flanking ITS1 and ITS2 spacers. This region provides superior taxon resolution compared to other popular DNA markers and it has been therefore proposed as the consensus primary fungal barcode (Schoch *et al*., 2012). The genomic sequence of *P. atropurpurascens* (UNITE acc. no. UDB034596) was subjected to a BLAST search for the highest homologies in the GenBank and UNITE databases, providing a positive identification of the fungus (similarity=99%; bit-score=1026; E-value=0; gaps=0%).
Based on this outcome, a dataset was created for further analysis, combining the sequences of various fungi belonging to the *Punctulariaceae*. Unfortunately, a sequence of *H. violacea* could not be introduced in this dataset because no sequence of this species has been deposited in neither database.

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The final dataset with the gaps added during the alignment process, resulted in a selection of 26 sequences with a length of 1334 base pairs (Tab. 1). For phylogenetic analysis, two methods were adopted: Maximum Likelihood and Bayesian inference. Both methods produced substantially the same tree (only the phylogram obtained through Bayesian inference is shown here) (Fig. 5). The sequence from the newly found fungus in the urban park in Rome finely fitted into the *P. atropurpurascens* clade. In detail, the sequence is identical to the sequence of a finding in California in a contiguous way along the latter's length of 432 base pairs, but dissimilar by 2 and 4 substitutions and 4 gaps from the sequences of the findings in Italy, which in turn are one substitution without gaps diverse from the American sequence.

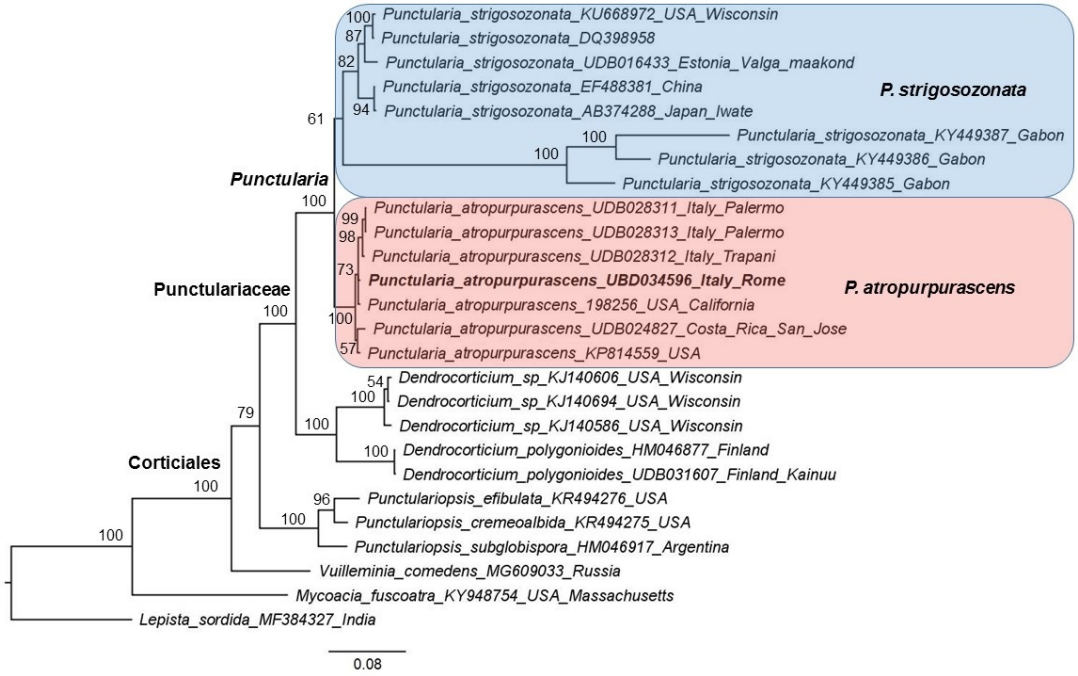

Fig. 5. Phylogenetic tree based upon alignment of the nuclear ribosomal ITS sequences reported in Table 1. In bold the newly sequenced sample.
Fig. 5. Albero filogenetico basato sull'allineamento delle sequenze del gene nucleare ITS indicate nella Tabella 1. In grassetto il nuovo esemplare sequenziato.

Discussion and conclusions

Before the present report, *Punctularia atropurpurascens* had been found only three times in Italy, all reports being from Sicily (Mazzaforno and Monte Pellegrino in the province of Palermo, Birribbaida in the province of Trapani; metadata from the UNITE database, acc. nos. UDB028311, UDB028313 and UDB028312 respectively). The occurrence of this fungus in an urban park in Rome is remarkable, considering it is regarded a tropical or subtropical species. This finding is thus the fourth observation of *P. atropurpurascens* in Italy and the first in the Latium region. What's important to notice, is that all reports were made in a ten-year time frame. This fungus importance lies not only in its rareness (Mondello, 2017), but also in the potential of its molecules and pigments such as 'Phlebiarubrone' and 'Phlebiakauranol' aldehyde, a characteristic common to other members of the of the genus *Punctularia*, holding different biological activities and great prospects for biomedical applications (Anke *et al*., 1986; Calì *et al*., 2003; Järvinen *et al*., 2016). For instance, in *E. coli* the antibacterial activity of 'Phlebiarubrone' has been correlated with its capacity to inhibit DNA transcription, translation and replication, thus it was supposed that its mechanism of action could be related to protein synthesis

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inhibition or DNA replication (Järvinen *et al*., 2016). Furthermore, some proteins that bind carbohydrates and glycoconjugates such as lectins, have been purified from the mycelium of *P. atropurpurascens*, in particular a new lectin with specificity to N-acetylglucosamine (Alborés *et al*., 2014). Lectins are implicated in several biological processes. For instance, in fungi they induced the formation of mycelium structures and fruiting bodies and facilitate the adherence of parasitic fungi to the host substrate. These fungal lectins have biological activity also in animals and humans, being involved in inflammation and in mechanisms of immune defence. On the other hand, articles studying the exploitation of basidiomycetes for their capacity to degrade xenobiotics like insecticides and pesticides, report that *P. atropurpurascens* is susceptible to the endosulfan insecticide and does not grow in presence of this compound (Rivero Machado *et al*., 2017).

DNA analysis finally identified the specimen unequivocally as *P. atropurpurascens*. It's striking that the macro- and microscopic differences with *H. violacea* are smaller than the morphological variations of the same specimen during its life cycle. Since in systematics they are placed in different genera, families and orders of the Agaricomycetes, we can hypothesise that the strong resemblance of these two species could be a case of evolutionary convergence. The morphological variations of *P. atropurpurascens* seem related with different biochemical mechanisms at various growth stages as well as with different environmental and climatic conditions. Moreover, the fruiting body's persistence on the oak stump in any period of the year made it possible to observe these variations with the seasonal changes. A certain seasonality seems to exist, probably as a function of humidity since the specimen anticipated the transformation of the fluffy stage to the crustose stage in the extremely dry spring of 2017 with respect to the more rainy spring of 2018.

The Villa Ada Park, due to its extent and variety of environments, turned out to be rich in mycological diversity. Indeed, many fungal genera and species were observed there, some of which are infrequent and therefore not much spotted in wild natural environments, like *Myriostoma coliforme* (Dicks.) Corda and *Podoscypha multizonata* (Berk. & Broome) Pat. (Knijn *et al*., 2017). The question arises, how these fungal species ended up growing in a city park; probably through the planting of trees originating from remote localities, or through the supply of soil and bark chips, as happened for *Leratiomyces ceres* (Cooke & Massee) Spooner & Bridge, an Australian species that has been conveyed in this way into England and hence in other European green spaces (Noordeloos, 2011), including the Villa Ada Park (Knijn *et al*., 2017). In this connection, it turns interesting that the sequence of the fungus is identical to an example from California and slightly different from findings of the same species in Sicily. The occurrence of this uncommon and important species in a green urban space, in areas to some extent neglected and degraded, poses a nontrivial aspect of the problem of the cure, conservation and requalification of Parks and their vegetation in large urban areas. Certainly, it is of the utmost importance that fungal biodiversity is included in whatsoever Urban Green Space Maintenance Plan.

Acknowledgements

We would like to thank Prof. U. Kõljalg and Dr. I. Saar of the University of Tartu in Estonia for sequencing a portion of the rDNA of the fungus. Furthermore, we thank both of them and Prof. T.W.M. Kuyper, of the University of Wageningen, The Netherlands, for valuable advice, and the reviewer of the manuscript for precious suggestions.

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Tab. 1. Sequences used in the phylogenetic analysis. In bold the newly sequenced sample. (*) The sequences are misidentified as *P. strigosozonata* in the UNITE database (Irja Saar, personal communication, 23/02/2018).
Tab. 1. Sequenze utilizzate per le analisi filogenetiche. In grassetto il nuovo esemplare sequenziato. (*) Le sequenze sono state erroneamente identificate come *P. strigosozonata* nel database UNITE (Irja Saar, comunicazione personale, 23/02/2018).

Species	Origin	DataBase	Accession	Declared sequence	Length
Dendrocorticium polygonioides	Finland	GenBank	HM046877	18S partial, ITS1, 5.8S, ITS2 partial	662 bp
Dendrocorticium polygonioides	Finland, Kainuu	UNITE	UDB031607	18S, ITS1, 5.8S, ITS2	596 bp
Dendrocorticium sp	USA, Wisconsin	GenBank	KJ140586	18S partial, ITS1, 5.8S, ITS2 partial	564 bp
Dendrocorticium sp	USA, Wisconsin	GenBank	KJ140606	18S partial, ITS1, 5.8S, ITS2 partial	509 bp
Dendrocorticium sp	USA, Wisconsin	GenBank	KJ140694	18S partial, ITS1, 5.8S, ITS2 partial	494 bp
Punctularia atropurpurascens	**Italy, Rome**	**UNITE**	**UDB034596**	**18S partial, ITS1, 5.8S, ITS2, 28S partial**	**915 bp**
Punctularia atropurpurascens	USA, California	Mushroom Observer	198256	ITS	432 bp
Punctularia atropurpurascens (*)	Italy, Palermo	UNITE	UDB028311	ITS1, 5.8S, ITS2	865 bp
Punctularia atropurpurascens (*)	Italy, Trapani	UNITE	UDB028312	ITS1, 5.8S, ITS2, LSU D1, LSU D2	1115 bp
Punctularia atropurpurascens (*)	Italy, Palermo	UNITE	UDB028313	ITS1, 5.8S, ITS2, LSU D1, LSU D2	1115 bp
Punctularia atropurpurascens	USA	GenBank	KP814559	18S partial, ITS1, 5.8S, ITS2, 28S partial	668 bp
Punctularia atropurpurascens	Costa Rica, San José	UNITE	UDB024827	ITS1, 5.8S, ITS2	653 bp
Punctularia strigosozonata	Japan, Iwate	GenBank	AB374288	18S, ITS1, 5.8S, ITS2, 28S	595 bp
Punctularia strigosozonata	?	GenBank	DQ398958	ITS1 partial, 5.8S, ITS2 partial	551 bp
Punctularia strigosozonata	China	GenBank	EF488381	18S partial, ITS1, 5.8S, ITS2, 28S partial	607 bp
Punctularia strigosozonata	USA, Wisconsin	GenBank	KU668972	18S partial, ITS1, 5.8S, ITS2, 28S partial	621 bp
Punctularia strigosozonata	Gabon	GenBank	KY449385	ITS1 partial, 5.8S, ITS2, 28S partial	599 bp
Punctularia strigosozonata	Gabon	GenBank	KY449386	ITS1 partial, 5.8S, ITS2, 28S partial	607 bp
Punctularia strigosozonata	Gabon	GenBank	KY449387	ITS1 partial, 5.8S, ITS2, 28S partial	607 bp
Punctularia strigosozonata	Estonia, Valga maakond	UNITE	UDB016433	ITS1, 5.8S, ITS2	564 bp
Punctulariopsis cremeoalbida	USA	GenBank	KR494275	18S partial, ITS1, 5.8S, ITS2, 28S partial	692 bp
Punctulariopsis efibulata	USA	GenBank	KR494276	18S partial, ITS1, 5.8S, ITS2 partial	599 bp
Punctulariopsis subglobispora	Argentina	GenBank	HM046917	18S partial, ITS1, 5.8S, ITS2, 28S partial	600 bp
Vuilleminia comedens	Russia	GenBank	MG609033	18S partial, ITS1, 5.8S, ITS2, 28S partial	658 bp
Mycoacia fuscoatra	USA, Massachusetts	GenBank	KY948754	18S partial, ITS1, 5.8S, ITS2, 28S partial	639 bp
Lepista sordida	India	GenBank	MF384327	ITS1, 5.8S, ITS2, 28S partial	691 bp

References

Alborés S, Mora P, Cerdeiras MP and Fraguas LF. (2014). Screening for lectins from basidiomycetes and isolation of *Punctularia atropurpurascens* lectin. Journal of Basic Microbiology, 54: 89-96.

Anke H, Casser I, Herrmann R and Steglich W. (1984). Neue Terphenylchinone aus Mycelkulturen von *Punctularia atropurpurascens* (Basidiomycetes). Zeitschrift für Naturforschung, 39c: 695-698.

Anke H, Casser I, Steglich W and Pommer EH. (1986). Antibiotics from basidiomycetes. 26. Phlebiakauranol aldehyde an antifungal and cytotoxic metabolite from *Punctularia atropurpurascens*. The Journal of Antibiotics, 40: 443-449.

DOI: https://doi.org/10.6092/issn.2531-7342/8349

Arnolds E and Veerkamp M. *Basisrapport Rode Lijst Paddenstoelen*. Ed. Nederlandse Mycologische Vereniging, Utrecht, (2008).

Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Ostell J, Pruitt KD and Sayers EW. (2018). GenBank. Nucleic Acids Research 46: D41-47.

Brondz I, Høiland K and Ekeberg D. (2004). Multivariate analysis of fatty acids in spores of higher basidiomycetes: A new method for chemotaxonomical classification of fungi. Journal of Chromatography B, 880: 303-307.

Calì V, Spatafora C and Tringali C. (2003). Polyhydroxy-*p*-terphenyls and related *p*-terphenylquinones from fungi: overview and biological properties. Studies in Natural Products Chemistry, 29J: 263-307.

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K and Madden TL. (2009). BLAST+: architecture and applications. BMC Bioinformatics, 10: 421-429.

Dawson JR. Gibberellins. In *The tetracyclic diterpenes vol 9.* 41-58. Ed. Pergamon Press, Oxford, (1968).

Donk MA. (1964). A conspectus of the families of Aphyllophorales. Persoonia, 3: 199-324.

Edgar RC. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research, 32: 1792-1797.

Ferretti A, Knijn A, Iorio E, Pulciani S, Giambenedetti M, Molinari A, Meschini S, Stringaro A, Calcabrini A, Freitas I, Strom R, Arancia G and Podo F. (1999). Biophysical and structural characterization of 1H-NMR-detectable mobile lipid domains in NIH-3T3 fibroblasts. Biochimica et Biophysica Acta, 1438: 329-348.

Gill M and Steglich W. Pigments of Fungi (Macromycetes). In: *Progress in the Chemistry of Organic Natural Products* 51: 18-19. Ed. Springer and Verlag, Vienna, (1987).

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W and Gascuel O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology, 59: 307-321.

Hjortstam K, Larsson KL and Ryvarden L. *The Corticiaceae of North Europe vol 1*. Ed. Fungiflora, Oslo, (1987).

Hjortstam K. (1995). Two new genera and some new combinations of corticioid fungi (Basidiomycotina, Aphyllophorales) from tropical and subtropical areas. *Mycotaxon*, 54: 183-193.

Holec J and Beran M. (2006). Red list of fungi (macromycetes) of the Czech Republic. Příroda, 24: 1-282.

Järvinen P, Nybond S, Marcourt L, Ferreira Queiroz E, Wolfender JL, Mettälä A, Karp M, Vuorela H, Vuorela P, Hatakka A and Tammela P. (2016). Cell-based bioreporter assay coupled to HPLC micro-fractionation in the evaluation of antimicrobial properties of basidiomycete fungus *Pycnoporus cinnabarinus*. Pharmaceutical Biology, 54: 1108-1115.

Knijn A and Ferretti A. *Funghi a Villa Ada*. Ed. Youcanprint, Lecce, (2017).

Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Dueñas M, Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout P, Larsson E, Lindahl BD, Lücking R, Martín MP, Matheny PB, Nguyen NH, Niskanen T, Oja J, Peay KG, Peintner U, Peterson M, Põldmaa K, Saag L, Saar I, Schüßler A, Scott JA, Senés C, Smith ME, Suija A, Taylor DL, Telleria MT, Weiß M and Larsson K-H. (2013). Towards a unified paradigm for sequence-based identification of Fungi. Molecular Ecology, 22: 5271-5277.

Kumar S, Stecher G and Tamura K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution, 33: 1870-1874.

Larsson KH. (2007). Re-thinking the classification of corticioid fungi. Mycological Research, 111: 1040-1063.

Lindahl BD, Nilsson RH, Tedersoo L, Abarenkov K, Carlsen T, Kjøller R, Kõljalg U, Pennanen T, Rosendahl S, Stenlid J, and Kauserud H. (2013). Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide. New Phytologist, 199: 288-299.

Martini E. (2016). *Punctularia atropurpurescens*. In Excerpts from Crusts and Jells, 88: 1-8.

DOI: https://doi.org/10.6092/issn.2531-7342/8349

McMorris TC and Ancbel M. (1963). Phlebiarubrone, a basidiomycete pigment related to polyporic acid. Tetrahedron Letters, 5: 335-337.

Mondello F. (2017). Micologia Messinese https://goo.gl/66mqYT.

Narvaes da Rocha Campos A, Dutra Costa M, Totola MR and Chaer Borges A. (2008). Total lipid and fatty acid accumulation during basidiospore formation in the ectomycorrhizal fungus *Pisolithus* sp. Revista Brasileira de Ciência do Solo, 32: 1531-1540.

Noordeloos ME. Strophariaceae s.l. In *Fungi Europaei* 13: 1–658. Ed. Candusso, Alassio, (2011).

Nylander JAA. (2004). MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Petch T. (1916). Revisions of Ceylon fungi (Part IV). Annals of the Royal Botanic Garden (Peradeniya). 6: 153-183.

Rivero Machado A, Niell S, Heinzen H, Cesio MV, Cerdeiras MP and Soubes M. (2017). Screening of native basidiomycete capable of degrading xenobiotics using endosulfan as a model. INNOTEC, 12: 27-33.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA and Huelsenbeck JP. (2012). MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. Systematic Biology, 61: 539-542.

Saitta A, Bernicchia A , Gorjón SP, Altobelli E, Granito VM, Losi C, Lunghini D, Maggi O, Medardi G, Padovan F, Pecoraro L, Vizzini A, Persiani AM. (2011). Biodiversity of wood-decay fungi in Italy. Plant *Biosystems*, 145: 958–968.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque, Chen W and Fungal Barcoding Consortium. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi*. Proceedings of the National Academy of Sciences, 109: 6241-6246.*

Turner WB. Metabolites derived from intermediates of the shikimic acid pathway. In *Fungal Metabolites*, 33-61. Ed. Academic Press, London. (1971).

Wang C, Xing J, Chin C and Peters JS. (2002). Fatty acids with certain structural characteristics are potent inhibitors of germination and inducers of cell death of powdery mildew spores. Physiological and Molecular Plant Pathology, 61: 151-161.