

БИОРАЗНООБРАЗИЕ, СИСТЕМАТИКА,
ЭКОЛОГИЯ

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***XYLARIA PUTAMINUM (XYLARIACEAE, ASCOMYCOTA),
A RARE MEDITERRANEAN SPECIES: FIRST RECORD IN ITALY***

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Xylaria putaminum is reported for the first time in Italy. The species was described from Northern Africa and this Italian record represents the second one in Europe. The ascocarps were collected in the Natural Reserve of “Capo Rama” (Sicily) on senescent fruits of *Olea europaea*. Macro- and micromorphological descriptions are provided and new information on the geographic distribution and molecular data of this species has now been increased.

Keywords: fungal diversity, ITS, *Olea*, phylogeny, Sicily

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INTRODUCTION

The genus *Xylaria* Hill ex Schrenk (*Xylariaceae, Ascomycota*) includes 670 saprotrophic inter- and intra-specific taxa (www.mycobank.org) and it is worldwide distributed, especially in tropical and subtropical regions (Kirk et al., 2008; Fournier et al., 2011). Only 16 species of *Xylaria* have been reported in Italy (Onofri et al., 2005; Saitta et al., 2011; Venturella et al., 2011) and three of those were collected in Sicily (Venturella et al., 2000, 2001). *Xylaria putaminum*, described by Bertault (1984) for Morocco, has been recently reported for the first time in Europe, in Spain (Sánchez Iglesias, 2016). Therefore, the distributional area, considering the exclusive substrate of growth (on *Olea europaea* L. fruits), should be considered the Mediterranean basin, as also supported by Fournier (2014) and Sánchez Iglesias (2016).

The substrate of growth for *Xylaria* species is mainly represented by wood, litter, dead fruits, leaves, seeds, animal dung (Rogers et al., 2005; Hsieh et al., 2010). Many *Xylaria* species are host-specific, especially when they grow on seeds and fruits (Rogers, 1979; Læssøe, Lodge, 1994; Xu, 1999; San Martín et al., 2001; Rogers et al., 2002). However, the identification can be sometimes problematic since only theleomorphic stromata bearing fertile perithecia can provide enough information for a correct identification (Fournier, 2014).

Since this species has been recorded only in few areas in the world, and, to the best of our knowledge, not before in Italy, the present study aims to increase the knowledge on its geographical distribution and molecular data.

MATERIALS AND METHODS

Study site. The study site is located in the Natural Reserve of “Capo Rama” (38°8'31.43"N, 13°4'10.20"E, Fig. 1) in the locality Terrasini – Province of Palermo, in Sicily (Italy). The sampling site, placed at 34 m a.s.l., was an olive grove dominated by the presence of *O. europaea* with a relevant accumulation of leaves and fruits. Underneath the olive trees we observed the presence of several ruderal species of plants, being *Urtica membranacea* Poir., *Oxalis pes-caprae* L. and *Lobularia maritima* (L.) Desv the most dominant.

The climate of the area is ascribed to the Mediterranean pluviseasonal oceanic type (Rivas-Martinez 2008). The mean monthly temperature is 25.4°C in August and 11.6°C in January; while, the monthly rainfall varies from 85 mm in December to 2 mm in July (www.climate-data.org).

Survey and Species Identification. The *Xylaria putaminum* ascocarps were collected on senescent fruits of *Olea europaea* in Autumn 2016 (October–December).



Fig. 1. Geographic localization of the study site and the world distribution of *Xylaria putaminum*. Localities of collection are marked with a black circle.

The identification and description of the ascomata were based on the macro- and microscopic analysis on fresh and dried specimens. Fresh material was dried in an air dehydrator at 40°C for 30 hours. Microscopic observations and measurements of at least 50 ascospores, 20 asci and 20 paraphyses for each dried specimen, were made using an OPTIKA B-500Tpl microscope and OPTIKAM Pro 5 camera, after rehydrating sections in Congo Red.

The specimens were described following Bertault (1984). Nomenclature and author abbreviations were used in accordance with Bertault (1984) and MycoBank (<http://www.mycobank.org>).

All voucher specimens are deposited at Herbarium of Natural History Museum, University of Tartu, Estonia (TU).

Molecular analysis. Genomic DNA was extracted in a lysis buffer [0.8M Tri-HCl, 0.2M $(\text{NH}_4)_2\text{SO}_4$, 0.2% w/v Tween-20] (Soil BioDyne, Tartu, Estonia) using proteinase K method (100 μl lysis buffer and 2.5 μl proteinase K; incubation at 56°C for 24 h and at 98°C for 15 min). The universal primers ITS5 and ITS4 (White et al. 1990) were used to recover the full ITS that covers roughly 1000 bp. PCR was carried out in 25 μl containing 0.5 μl of each primer, 5 μl FirePol Mastermix (Solis BioDyne, Tartu, Estonia), 1 μl of 10× diluted DNA template and sterilized distilled wa-

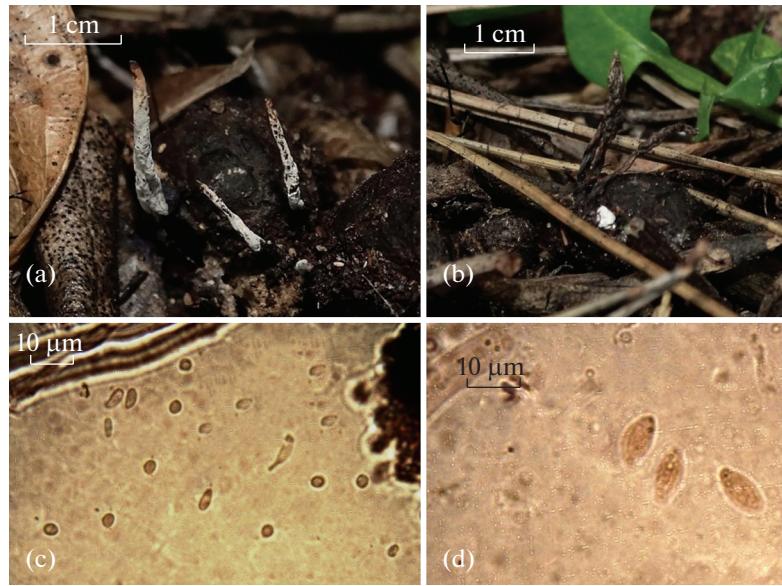


Fig. 2. *Xylaria putaminum*: anamorphic stromata (a); teleomorph stromata (b); conidiospores (c); ascospores (d).

ter. For amplifying the ITS region, PCR conditions consisted of 15 min at 95°C, 35 cycles of 30 sec. at 95°C, 30 sec at 55°C, 1 min at 72°C, and 10 min at 72°C. Amplification success was checked with 1% agarose gel via electrophoresis. PCR products were cleaned using Exo-SAP enzymes GE Healthcare (Freiburg, Germany) using incubation at 37°C for 45 min and at 85°C for 15 min. PCR products were sequenced in Macrogen Inc. (Amsterdam, The Netherlands) using the PCR primers. Quality check and manual editing was performed using Sequencher 5.1 (Gene Codes Corporation, Ann Arbor, USA). The obtained sequence has been deposited to the NCBI GenBank database under the accession number MW750754.

Phylogenetic analysis. The sequence obtained by amplification of the ITS gene was used as a query in BLAST. Highly similar sequences were found through BLASTN searching the nucleotide collection database. Sequences assigned to *Xylaria* species were extracted and aligned using MUSCLE version 3.8.21_2 (Edgar, 2004). The alignment was curated using trimAl version 1.4.1 (Capella-Gutiérrez et al., 2009) and a tree was inferred with FastTree version 2.1.10_1 (Price et al., 2009). To ensure the validity of the results, both the EMBL-EBI Sequence Analysis Tools (<https://www.ebi.ac.uk/services>; Madeira et al., 2019) and the NGPhylogeny analysis tools (<https://ngphylogeny.fr/>; Lemoine et al., 2019) were used to execute the analytical workflows. The final tree was visualized using the iTol web tool version 5 (<https://itol.embl.de/>; Letunic, Bork, 2019).

RESULTS

As a result of the macro- and microscopic examination, and molecular analysis, the species *Xylaria puta-*

minum was identified. The detailed morphological characteristics of the specimens are provided below.

Taxonomy

Xylaria putaminum Maire et Durieu (Fig. 2).

Stromata anamorphic: erected, 0.5–3 × 10–30 mm. Stipes are sterile, fibrillose, blackish with violet hues, 5–20 × 0.5–3 mm, often expanded over the substrate as rhizomorphic. Apical stromata surface is whitish due to numerous conidiophores, which are large, spatuliform and bifurcate. Stipe up to 1/3 or 2/3 of the total stroma length.

Stromata teleomorphic: fusiform, erected, fibrose, 18–39 × 2–5 mm, often unbranched, sometimes with the enlarged, spatuliform or bifurcate apex. Stipes are 5–20 × 0.5–1.4 mm, often sinuous, rarely bifurcate; the surface is longitudinally striate, fibrillose, brown or brown-reddish, sometimes with violet hues, with an enlarged base in correspondence of the substrate of growth. Clavulae are fertile, clearly larger than the stipe, fusiform, narrower at the apex, 8–16 × 0.9–3.5 mm. Apical stromata surface have greyish hues, are brown to black, and are longitudinally striate. Asci are cylindrical and measure (100) 120–150 × (5) 6–7 (9) μm. Paraphyses are abundant, filiform, acuminate, longer than asci, hyaline. Ascospores are ellipsoid, (8) 10–11 (12) × (3) 4–5 μm, smooth, often guttulate, with broadly rounded ends. Conidiospores are amygdaliform, hyaline, and measure (4) 5 (6) × 2 (3) μm.

Material examined: Italy, Sicily, Terrasini, Natural Reserve of Capo Rama, 31.10.2016 (TU124298), 12.11.2016 (TU124299), 01.12.2016 (TU124300), 20.12.2016, 38°8'31.43"N, 13°4'10.20"E – 34 m. a.s.l. On senescent fruits of *Olea europaea* (TU124830).

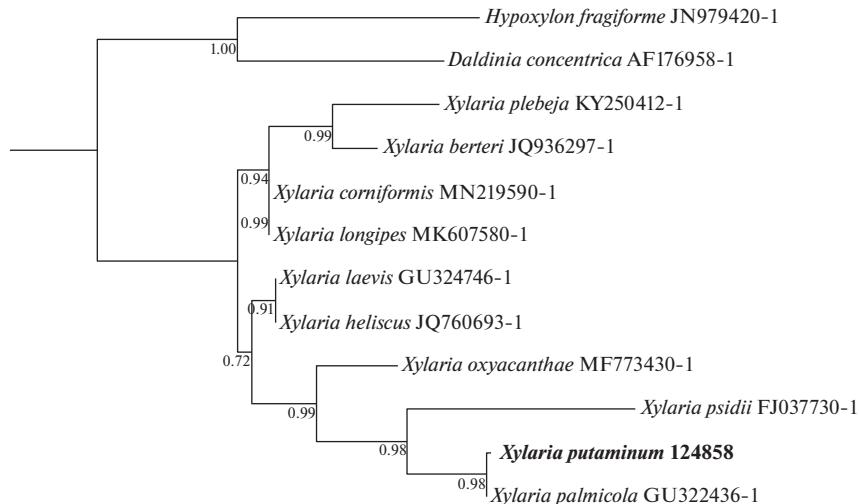


Fig. 3. Phylogenetic relationship of *Xylaria putaminum* with other *Xylaria* species, as inferred from ITS nrDNA sequence data. *Hypoxylon fragiforme* and *Daldinia concentrica*, two species commonly found on dead and decaying wood, were used as outgroups. Taxon names are followed by the GenBank accession numbers of the sequences. Bootstrap values are indicated on the tree nodes.

Phylogeny

The phylogenetic relationship of *Xylaria putaminum* with other *Xylariales* is illustrated in Fig. 3. *X. putaminum* and *X. palmicola* form a monophyletic clade with high support value.

DISCUSSION

Xylaria putaminum was collected in Africa (in Algeria), and macromorphological described for the first time in 1920, as reported by Sánchez Iglesias (2016). Later, this species was re-collected (in 1960) in Morocco (Bertault 1984) and it was given a complete morphological description.

Taxonomically, this species belongs to *Fusiformes*-group because fusoid habitus of its stromata with acute and sterile apex (Bertault, 1984). *X. putaminum* is well differentiated from other species of *Fusiformes* by its exclusive substrate of growth: the senescent fruits of *Olea europaea*. Within the *Fusiformes*, *Xylaria bulbosa* (Pers.) Berk. et Broome differs from *X. putaminum* for the base of the stipe, typically thickened. *X. apiculata* Cooke has stromata that are brown-argillaceous; *X. carpophila* (Pers.) Fr. typically grows on decaying fruits of *Fagus sylvatica* L. while *X. oxyacanthae* Tul. et C. Tul. grows on fruits of *Crataegus* spp. The stromal thickness of both *X. scopiformis* (Mont. ex P. Joly) T. Schumach. and *X. pumila* (Fr.) Mont. is less than 2 mm, while that of *X. jruvensis* is 2.0–2.5 mm and of *X. cupressoides* Bertault up to 4 mm (Bertault, 1984; Fournier, 2014). In the context of olive-inhabiting xylarias, it is worth noting the species *X. oleagina* Thum. described growing on olive fruits and collected in Dalmatia (Thumen, 1883).

Current references on the geographic distribution of *X. putaminum* point out its presence only in few areas in

the word: in Africa (in Morocco and in Algeria, Bertault, 1984) and in Europe (in Spain, Sánchez Iglesias, 2016). Hence, our finding increases the knowledge on the geographic distribution of this species and, to the best of our knowledge, this can be considered the first record in Italy since no other records are available (Onofri et al., 2005; Saitta et al., 2011; Venturella et al., 2011), and the second one in the European continent.

Ecologically, both the African and the Spanish samples were collected on senescent fruits of *Olea europaea* and *O. europaea* var. *sylvestris*, respectively. By our record it possible to confirm the preferential substrate of growth and to extend the distributional area of *Xylaria putaminum* of more than 1000 Km to the eastern part of the Mediterranean basin (Fig. 1). The distribution of this species is probably underestimated considering that *Olea europaea* grows in the entire Mediterranean basin (Fournier, 2014).

CONCLUSION

The collection and the identification by both morphological and molecular analysis of *Xylaria putaminum* allow us to expand our knowledge on the geographic distribution of this species. In addition, the collection of a rare fungal species in Sicily, confirms previous studies on the presence of a high macrofungal diversity in this region (Onofri et al., 2005; Saitta et al., 2011, 2017; Venturella et al., 2011). Future studies should be addressed to investigate less or never studied areas in order to increase our knowledge on the macrofungal species diversity and distribution.

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REFERENCES

- Bertault R. *Xylaires d'Europe et d'Afrique du nord*. Bull. Soc. Mycol. France. 1984. V. 100 (2). P. 139–175.
- Capella-gutiérrez S., Silla-Martínez J.M., Gabaldón T. Tri-mAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics. 2009. V. 25. P. 1972–1973.
<https://doi.org/10.1093/bioinformatics/btp348>
- Edgar R.C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004. V. 32. P. 1792–1797.
<https://doi.org/10.1093/nar/gkh340>
- Fournier J. Update on European species of *Xylaria*. 2014.
<http://www.ascofrance.fr/uploads/xylaria/201406.pdf>
- Fournier J., Flessa F., Peršoh D. et al. Three new *Xylaria* species from southwestern Europe. Mycol. Progress. 2011. V. 10. P. 33–52.
<https://doi.org/10.1007/s11557-010-0671-8>
- Hsieh H.M., Lin C., Fang M. et al. Phylogenetic status of *Xylaria* subgenus *Pseudoxylaria* among taxa of the subfamily *Xylarioideae* (*Xylariaceae*) and phylogeny of the taxa involved in the subfamily. Molec. Phylogen. Evol. 2010. V. 54 (3). P. 957–969.
<https://doi.org/10.1016/j.ympev.2009.12.015>
- Læssøe T., Lodge D.J. Three host specific *Xylaria* species. Mycologia. 1994. V. 86 (3). P. 436–446.
<https://doi.org/10.1080/00275514.1994.12026431>
- Lemoine F., Correia D., Lefort V. et al. NGPhylogeny.fr: new generation phylogenetic services for non-specialists. Nucleic Acids Res. 2019. V. 47. P. 260–265.
<https://doi.org/10.1093/nar/gkz303>
- Letunic I., Bork P. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. Nucleic Acids Res. 2019. V. 47. P. 256–259.
<https://doi.org/10.1093/nar/gkz239>
- Madeira F., Park Y.M.V., Lee J. et al. The EMBL-EBI search and sequence analysis tools APIs. 2019. V. 47. P. 636–641.
<https://doi.org/10.1093/nar/gkz268>
- Onofri S., Bernicchia A.R., Filippello V. et al. Checklist dei funghi italiani. Carlo Delfino Editore, 2005.
- Price M.N., Dehal P.S., Arkin A.P. FastTree: computing large minimum evolution trees with profiles instead of a distant matrix. Molec. Biol. Evol. 2009. V. 26. P. 1641–1650.
<https://doi.org/10.1093/molbev/msp077>
- Rivas Martinez S. Global Bioclimatics. 2008.
http://www.globalbioclimatics.org/book/bioc/global_bioclimatics-2008_00.htm
- Rogers J.D. *Xylaria magnoliae* sp. nov. and comments on several other fruit-inhabiting species. Can. J. Bot. 1979. V. 57. P. 941–945.
<https://doi.org/10.1139/b79-115>
- Rogers J.D., Ju Y.M., Lehmann J. Some *Xylaria* species on termite nests. Mycologia. 2005. V. 97. P. 914–923.
https://www.zobodat.at/pdf/Sydotia_54_0091-0097.pdf
- Rogers J.D., San Martín F., Yu Y.M. A reassessment of the *Xylaria* on *Liquidambar* fruits and two taxa of *Magnolia* fruits. Sydotia. 2002. V. 54 (1). P. 91–97.
- Saitta A., Bernicchia A., Gorjón S.P. et al. Biodiversity of wood-decay fungi in Italy. Plant Biosystems. 2011. V. 145 (4). P. 958–968.
<https://doi.org/10.1080/11263504.2011.633114>
- Saitta A., Losi C., Ambrosio E. First record of *Phlebia marginatae* (*Polyporales, Basidiomycota*) in Italy. Nova Hedwigia. 2017. V. 105 (1–2). P. 37–41.
- San Martín F., Lavín P., Rogers J.D. Some species of *Xylaria* (*Hymenomycetes, Xylariaceae*) associated with oaks in Mexico. Mycotaxon. 2001. V. 79. P. 337–360.
- Sánchez Iglesias F. *Xylaria putaminum* (*Ascomycota, Xylariaceae*) en Sevilla, suroeste de la Península Iberica. Rev. Catal. Microl. 2016. V. 37. P. 65–73.
- Thumen F. Die Pilze des Oelbaumes. Beschreibung sammllicher bis Ende des Jahres 1883 bekannt gewordener, auf dem Oel- oder Olivenbaum, *Olea sativa* Lin., Vorkommender Pilze. Bollettino della Societa Adriatica di scienze naturali in Trieste. 1883. V. 8. P. 215–244.
<https://www.biodiversitylibrary.org/item/46069#page/255/mode/1up>
- Venturella G., Altobelli E., Bernicchia A. et al. Fungal biodiversity and in situ conservation in Italy. Plant Biosystems. 2011. V. 145 (4). P. 950–957.
<https://doi.org/10.1080/11263504.2011.633115>
- White T.J., Bruns T., Lee S. et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics – PCR protocols: a guide to methods and application. 1990. V. 18. P. 315–322.
<https://msafungi.org/wp-content/uploads/2019/03/February-2013-Inoculum.pdf>
- Xu A.S. A new species of *Xylaria*. Mycosistema. 1999. V. 18. P. 137–140.

Редкий средиземноморский вид *Xylaria putaminum* (Xylariaceae, Ascomycota): первая находка в Италии

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Статья посвящена первой находке в Италии аскомицета *Xylaria putaminum*. Этот вид был описан в Северной Африке, и обсуждаемая находка является второй в Европе. Аскомы были собраны в природном заповеднике “Капо Рама” (Сицилия) на старых плодах маслины *Olea europaea*. Приведены макро- и микроморфологические описания этого редкого вида, а также обсуждается новая информации о его географическом распространении и молекулярно-филогенетической позиции.

Ключевые слова: маслина, разнообразие грибов, филогения, Сицилия, ITS