IJM - Italian Journal of Mycology
ISSN 2531-7342 - Vol. 53 (2024): 75 - 84
Journal homepage: https://italianmycology.unibo.it/

Research article

First report of *Akanthomyces dipterigenus* (Hypocreales: Cordycipitaceae) in the Iberian Peninsula

Paula Caride[1], Josefina Garrido[1], Mauro Rivas-Ferreiro[2], Iria Villar[1], Marisa Castro[3]

[1]Environmental Biology research group (BA2), Department of Ecology and Animal Biology, University of Vigo, Vigo, Spain
[2]Centro de Investigación Mariña (CIM), Department of Biochemistry, Genetics and Immunology, University of Vigo, Vigo, Spain
[3]MycoGalicia Plantae S.L. Head Researcher, Tui, Spain

Corresponding author e-mail: paula.estevez.caride@uvigo.gal

ARTICLE INFO
Received 26/01/2024; accepted 17/05/2024
https://doi.org/10.6092/issn.2531-7342/18993

Abstract
Akanthomyces dipterigenus is a species of entomopathogenic fungus (Hypocreales: Cordycipitaceae) that has undergone several reclassifications in recent decades. While it has been utilized for biological control, information on its distribution and ecology remains limited. Furthermore, this species has only recently been reported in continental European countries. Our research provides the first report of *A. dipterigenus* in the Iberian Peninsula. Fungal isolation was achieved via a modified baiting technique featuring *Rhopalosiphum padi* aphids. Results suggest that the isolation methodologies and ecological traits of the fungus may either aid or hinder its detection. Additional research is necessary to assess its distribution throughout the Peninsula and neighbouring European countries, and to examine its potential as a biological control agent.

Keywords
Aphid, bait, entomopathogenic, fungi, isolation, *Lecanicillium*

Introduction

Akanthoymces dipterigenus (Petch) Spatafora, Kepler, Zare and B. Shrestha (*Cephalosporium dipterigenum* Petch), belongs to a notable group of entomopathogenic fungi. Previously, it was classified in the Postrata section W. Gams of the genus *Verticillium* News, which includes anamorphs of both Hypocreales and Phyllacorales fungi, many of which are plant pathogens. Following numerous analyses of this section (Zare et al., 2000; Zare and Gams, 2001a,b; Sung et al., 2001), *Lecanicillium* W. Gams and Zare was established as a new genus. This entailed the inclusion of several entomopathogenic and mycopathogenic Clavicipitacean species in it. Among these was *Verticillium lecanii* (Zimm.), which was renamed and later split into three distinct species based on

genetic, morphological and ecological features (Zare and Gams, 2001a,b): *L. lecanii* (Zimm.) Zare & W. Gams, *L. muscarium* (Petch) Zare & W. Gams, and *L. longisporum* (Petch) Zare & W. Gams (detailed in Table 1). In the literature on *A. dipterigenus*, *L. longisporum* is one of the most frequently cited synonyms. Further investigations support this reclassification, as outlined by Kouvelis et al. (2008), Sung et al. (2007), and Kepler et al. (2017). The latter author prioritized the name *Akanthomyces* Lebert, resulting in the renaming of many species, including their specific epithets such as *A. muscarius* (Petch) Spatafora, Kepler & B. Shrestha and *A. dipterigenus*.

Despite *A. dipterigenus*'s ability to infect aphids, the majority of the existing biological control literature focuses on *A. lecanii* and *A. muscarius*, both of which have commercialized strains such as Koppert's Mycotal, a microbiological pesticide. It is believed that Vertalec, also from Koppert Co., may have included *A. dipterigenus* strains at some point (Zare and Gams, 2003b). Moreover, despite Zare and collaborators efforts, numerous studies persist in using obsolete nomenclature or incorrectly identifying *A. lecanii*, *A. muscarius* and *A. dipterigenus*. Insufficient morphological descriptions, particularly concerning size of conidia and conidiophores, colony appearance, habitat, or strain sequencing, impede the revision of these articles.

Table 1 – Characteristics of the three species complex based on Zare and Gams (2001b, 2003a,b,c). Note that the size of the conidia is significant.

Akanthomyces lecanii	**Colony characteristics** (PDA, 10 days and 24 ºC incubation) Size: 15–25 mm Morphology: compact, yellowish-white, with deep yellow reverse **Microscopical characteristics** Phiallides: short and aculeate 11–20(30) × 1.4–1.8 µm Conidia: Short and ellipsoidal 2.5–3.5(4.2) × 1–1.5 µm **Host** Hemiptera: Aleyrodidade, Coccidae, Diaspididae, Pseudoccocidae
A. dipterigenus	**Colony characteristics** (PDA, 10 days and 24 ºC incubation) Size: 10–30 mm Morphology: raised, white becoming sulphur-yellow, reverse cream to pale yellow **Microscopical characteristics** Phiallides: long and sub-aculeate 20–40 × (1)2–2.7 µm, with globose heads present Conidia: long, ellipsoidal to oblong-oval, mostly 1-celled and rarely 1-septate, 5.3–10.7 × 1.5–2.5 µm **Host** Hemiptera: Aphididae, Coccidae
A. muscarius	**Colony characteristics** (PDA, 10 days and 24 ºC incubation) Size: (14) 25–30 mm Morphology: compact white, reverse cream to pale yellow or uncoloured. **Microscopical characteristics** Phiallides: sub-aculeate (15)20–35 × 1–1.7 µm Conidia: ellipsoid to sub-cylindrical (2)2.5–5.5(6) × 1–1.5(1.8) µm **Host** Wide spectrum: insect-pathogenic, plant-pathogenic and fungal-pathogenic

Akanthomyces dipterigenus has been documented in various countries across Asia, including China (Wang et al., 2024), Sri Lanka (Zare et al., 2000), and India (Ramanujam et al., 2011), as well as in the Americas, such as Brazil (Lopes et al., 2023), Mexico (Berlanga-Padilla et al., 2018), Peru (Zare and Gams, 2001b), Uruguay (Rivas et al., 2013), Venezuela, and the United States of America (Kouvelis et al., 2008). The species has been recorded in various European countries, including the United Kingdom (Zare et al., 2000; Kouvelis et al., 2008), Estonia (Tedersoo et al., 2014) and the Czech Republic (Lebert, 1858).

Studies conducted in the Iberian Peninsula have only identified related species, such as *A. lecanii* in Spain (Olivares-Bernabeu and López-Llorca, 2002; Asensio et al., 2003; Díaz et al., 2009). Additionally, Portuguese researchers have also reported *A. muscarius* (Soares et al., 2022), *L. aphanocladii* Zare & W. Gams, *L. dimorphum* (J.D. Chen) Zare & W. Gams (Sharma et al., 2018), *L. saksenae* (Kushwaha) Kurihara & Sukarno, and *L. psalliotae* (Treschew) Zare & W. Gams (Trovão et al., 2013; Soares et al., 2022). Some of these *Lecanicillium* species are not closely related to *A. dipterigenus* and remain unclassified in the Cordicypitaceae (Kepler et al., 2017).

To our knowledge, there is no previous record of *A. dipterigenus* in the Iberian Peninsula, hence this particular study represents the first official report of this species. However, there is a possibility that samples #28 and #48 from Asensio et al. (2003) could be consistent with *A. dipterigenus*, as the conidial sizes range up to 10.6 ± 0.28 µm and 11 ± 0.79 µm respectively. Nonetheless, without access to genetic data, ecological information, or colony characteristics, it is not possible to confirm these suppositions.

Materials and Methods

Sampling was carried out in the Province of Pontevedra in Galicia, a temperate Atlantic region of NW Spain (Fig. 1), at 10 crop and orchard sites (Table 2). This was repeated every 1.5 months during a 2.5-year study from 2020 to 2022.

Soil samples were collected from the top 15 cm of soil using a disinfected shovel. For each replicate, five subsamples were randomly taken, placed in a plastic bag, and refrigerated until insect baiting (Lacey, 2012). The soil was then sifted through a 2 mm mesh and 30 mL of soil was added to 9 cm Petri dishes. To prevent fungal damage, the soils were neither dried nor heated before the experiments. To promote fungal growth and potential infection, the soil moisture level was elevated to approximately 70% relative humidity by adding 3 to 10 mL of distilled water, depending on the initial soil dampness.

Living colonies of *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae) were maintained in a laboratory, growing on *Tritricum durum* L. pots at a temperature of 20 ºC and under a 16:8 h photoperiod. To isolate fungal strains from soil, we performed a modified bait method (Zimmermann, 1986). Twenty individuals, including nymphs and female alata and aptera, were carefully placed onto Petri dishes containing the soil using a delicate brush. Four to five *T. durum* leaves were added, joined at the base, and covered with aluminum foil and humid paper to ensure both plant survival and aphid nourishment. Every other day, the plates sealed with Parafilm® were dropped straight downward onto a surface 10 cm below, leading to the majority of the aphids dropping from the leaves unharmed onto the soil (a phenomenon commonly observed when the plates are rotated). Throughout the duration of the experiment (1 week), every infected individual was sampled and examined under a

microscope. After the fungal specimens had been identified, a small quantity of hyphal mass was inoculated onto PDA plates.

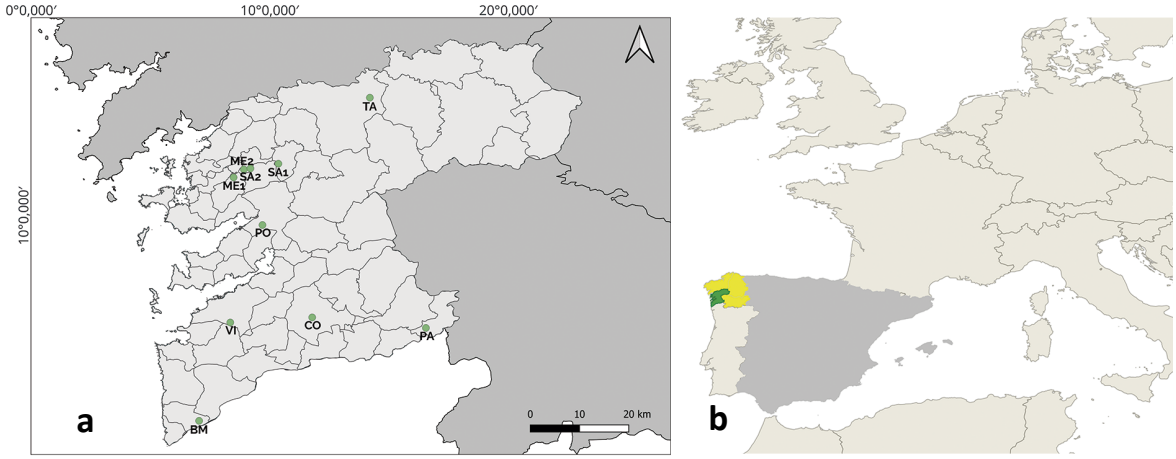

Fig. 1 – a. Sampling point locations in Pontevedra Province. b. Pontevedra Province (Highlighted in green), Galicia Autonomous Region (highlighted in yellow), Spain.

Table 2 – Summary of the sampling points.

Site	Cordinates	Elevation (m)	Crop management
BM	N41º55'39.1" W8º47'50.8"	18	Ecological
ME1	N42º33'37" W8º36'13.0"	167	Ecological
ME2	N42º32'31.7" W8º41'16.3"	66	Conventional
CO	N42º10'53.5" W8º31'16.0"	51	Ecological
PA	N42º09'19.7" W8º14'39.6"	224	Ecological
PO	N42º24'22.6" W8º38'33.3"	32	Conventional
SA1	N42º32'39.9" W8º40'01.9"	86	Conventional
SA2	N42º31'21.8" W8º42'46.9"	62	Conventional
TA	N42º42'54.4" W8º23'10.3"	325	Ecological
VI	N42º10'4.24" W8º43'16.6"	272	Conventional

Following the exclusion of any contaminants, the plates were incubated at 25 ºC without exposure to light. Mycelium samples from each strain were analyzed by the Genomic Services of the University of Vigo (CACTI). The total DNA from each sample was purified using the PureLink Genomic DNA Mini Kit (Invitrogen) protocol. The ITS region was amplified by polymerase chain reaction (PCR) using the primer pair ITS1-ITS4 (Irinyi et al., 2016). The reaction conditions were as follows: an initial denaturation at 96 °C for 5 min, followed by 35 cycles of 96 °C for 35 s, annealing at 50 °C for 35 s and 72 °C for 60 s, with a final extension at 72 °C for 7 min. PCR was carried out using Horse Power Taq DNA Polymerase (Canvax) following the manufacturer's instructions. The ITS amplicon bands were observed on 2% agarose gels. The PCR reactions were purified with ExoSAP-IT Express (Applied Biosystems), and DNA sequencing reactions were performed in both

directions using the same primers as the PCR reaction (ITS1-ITS4). The magnetic beads (Mag-Bind Seq DTR, Omega Bio-tek) were used to purify the products, which were then resolved using the SeqStudio Genetic Analyser (Applied Biosystems) at the CACTI. The consensus sequences were obtained by assembling the direct and reverse traces with SEQSCAPE v. 2.5 (Applied Biosystems) and MEGA software. The sequences were compared to the GenBank datasets using the Basic Local Alignment Search Tool (BLAST). A phylogenetic analysis was carried out using ITS sequences available in GenBank and published in taxonomic reviews of genus *Akanthomyces* (Manfrino et al., 2022, Liu et al., 2024, Wang et al., 2024) and our newly generated sequences; all sequences were trimmed using ITSx (Bengtsson-Palme et al., 2013), an alignment was carried out using MAFFT v7 online server (Katoh et al., 2019) with default settings, and the Maximum Likelihood (ML) analysis was carried out IQTree v2.1.3 online server (Trifinopoulos et al., 2016) using a partitioned analysis (three partitions: ITS1, 5.8S, ITS2) and 1,000 non-parametric bootstrap replicates to calculate support at the nodes. DNA sequences newly generated in this study were deposited in GenBank with accession numbers OR793230 and OR793231.

Results and Discussion

BLAST analysis showed up to a 99.82% similarity to *A. dipterigenus* isolates (MT457650). The ML phylogenetic tree (Supplementary Figure S1) also shows clustering between our sequences and the type specimen of *A. dipterigenus* (strain CBS126.27), as well as other *A. dipterigenus* sequences from GenBank. Ecological traits (host and environment), morphological characteristics of conidia, conidiophores and colony morphology supported this ID (Zare and Gams, 2001b; Zare 2003b).

Colonies on PDA at 25 ºC reached a diameter of 13.5–16.5 mm after 10 days, displaying a white-cream color and a slightly raised appearance (Fig. 2a). The reverse of the colony presents a cream color (Fig. 2b). Phialides with tapering nodes, usually consisting of 3–4 conidiogenous cells (Fig. 2c), measure 25–40 × 1.5–2 µm. The formation of globose heads (Fig. 2d), which merge numerous conidia, is very frequent, especially when the sample is handled with care. Conidia are notably oblong, except for the smallest ones which tend towards an ellipsoid shape (Figs 2c, 2d). Few have a single septum, and their sizes range from 8–13.5 × 1.5–2.5 µm.

Baiting with *R. padi* (n = 1205 replicates) yielded *A. dipterigenus* from 46.1% of the samples, along with *Conidiobolus* spp. (Entomophthorales: Ancylistaceae) from 1.5%, and *Metarhizium* sp. (Hypocreales: Clavicipitaceae) from 0.1%. The discovery of *A. dipterigenus* in all studied locations and seasons implies that this fungus is a ubiquitous entomopathogen in Galicia. Further sampling is necessary to confirm its presence in other temperate Atlantic regions, like the rest of the Peninsula. The effects of habitat type, crop management and soil characteristics on the fungus require evaluation (Quesada-Moraga et al., 2007; Sun et al., 2008; Jabbour and Barbercheck, 2009; Sharma et al., 2018; Uzman et al., 2019; Hallouti et al., 2020).

While earlier research in the peninsula identified species closely related to *A. dipterigenus* (Olivares-Bernabeu and López-Llorca, 2002; Asensio et al., 2003; Díaz et al., 2009; Soares et al., 2022), our capacity to review them is constrained by the absence of morphological data and genetic studies in these works. It is imperative for authors to utilize the most up-to-date nomenclatures and incorporate morphological and genetic information into their publications. This enables subsequent revisions and taxonomical studies.

Taxonomic history of the species and morphological similarities to other *Akanthomyces* may have led to the underestimation of this species in Europe, in favour of *A. lecanii* or even *A. muscarius*. Also, the methods applied in previous studies might have played a relevant role in the lack of detection of this species. For instance, the order Hemiptera has the greatest diversity of entomopathogenic fungi ever documented (Araújo and Huhges, 2016; López-Lastra and Lecuona, 2019). However, this group is rarely used as bait except for field screenings (Feng et al., 1990; Nielsen et al., 2003; Scorsetti et al., 2007). In contrast, *Galleria mellonella* L. (Lepidoptera: Pyralidae) and *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) are commonly used as experimental organisms, and it is likely that they have different affinities to fungal pathogens (Ali-Shtayeh et al., 2002; Meyling and Eilenberg, 2006; Quesada-Moraga et al., 2007; Mora et al., 2016; González-Baca et al., 2019). The collection of samples throughout all seasons or over several years is seldom observed in most biodiversity research (Meyling and Eilenberg, 2006; Scorsetti et al., 2007; Jabbour and Barbercheck, 2009; Meyling et al., 2011; Mora et al., 2016; Kovač et al., 2021). While the utilization of specific media for prevalent fungi, notably *Metarhizium* spp. or *Beauveria bassiana* (Balsamo) Vuillemin. (Hypocreales: Cordycipitaceae), could impede the retrieval of other species (Medo and Cagáň, 2011; Kovač et al., 2021). Several authors suggest the utilization of combined isolation methodologies and interdisciplinary approaches to avoid these issues (Jaronski, 2008; Sharma et al., 2018; Bueno-Pallero et al., 2020).

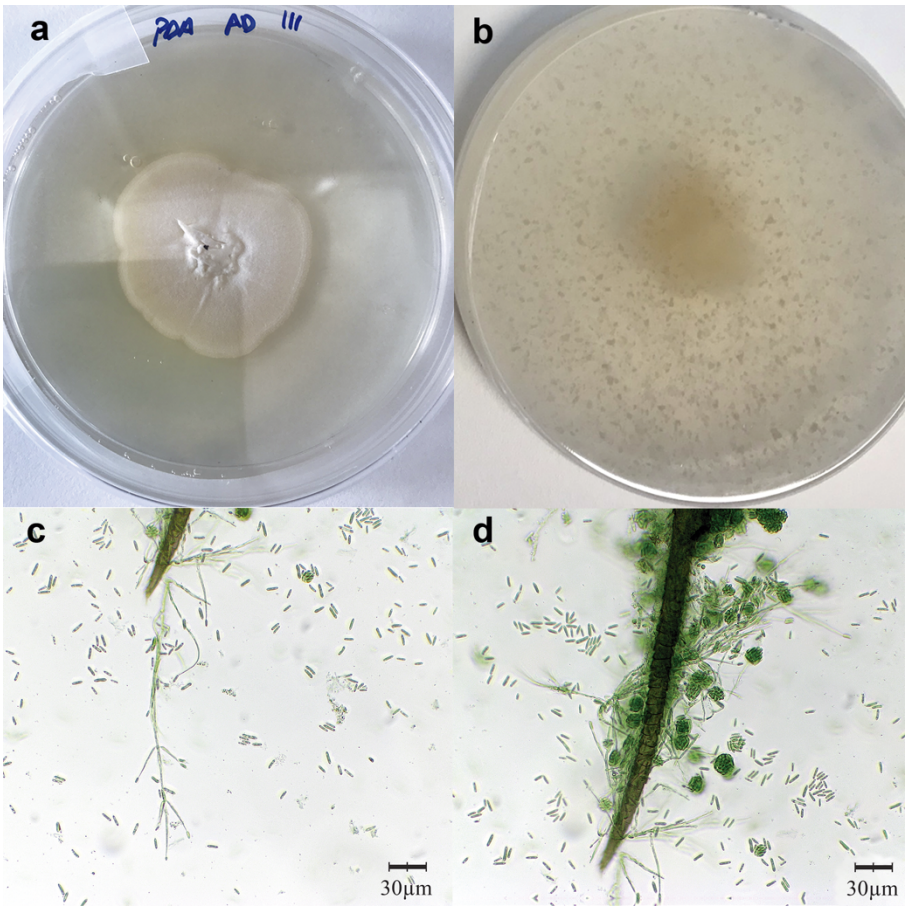

Fig. 2 – a) *A. dipterigenus* mycelium aspect after 20-day culture on PDA at 25 ºC, without light; b) Reverse of a PDA colony after 10 days of incubation; c) *R. padi* antenna covered by *A. dipterigenus* hyphae, showering phiallides and conidia; d) Another antenna showing globose heads formed by conidia.

In conclusion, it is essential that additional research on indigenous fungi is conducted to improve commercialized entomopathogenic strains. The investigation of less prevalent or new pathogenic fungi could benefit biological control programmes, particularly through the integration of strains that are already adapted to specific climates or ecosystems (Quesada-Moraga et al., 2023), or through their potential to become endophytic in crops (Nicoletti and Becchimanzi, 2020).

Conclusion

Akanthomyces dipterigenus was discovered in the Iberian Peninsula for the first time through this study. The species was frequently found in all locations and seasons investigated in NW Spain, using *Rhopalosiphum padi* as a bait insect. More research is needed to establish the extent of the species in the Peninsula. Previous taxonomic issues and isolation methods may have underestimated the presence of this species in Europe. Further research on native species should be carried on in order to improve biological control programmes.

Acknowledgements

Mauro Rivas-Ferreiro was funded by the Spanish Ministry of Education [grant number FPU20/02280]. We would like to thank the Environmental and Animal Biology Research Group (BA2) and the Universidade de Vigo for partially funding the study of this article. We are grateful to the CACTI (U. Vigo) for its genomic services, especially to Sebastián Comesaña. Also, to Hugo Fernández-Ricón (MycoGalicia Plantae) for his photographic assistance, and to Claudia Cristina López-Lastra (National Univeristy of La Plata) for her kind assistance. We are also grateful for the cooperation of the landowners, including C.I.F.P. A Granxa, Comunidade de Montes de Couso, Horsal Sociedade Cooperativa Galega, Mazás de Tabeirós and Misión Biológica de Galicia (CSIC).

References

Ali-Shtayeh MS, Mara'i AB, Jamous RM (2002) Distribution, occurrence and characterization of entomopathogenic fungi in agricultural soil in the Palestinian area. Mycopathologia 156(3):235–44 http://doi.org/10.1023/a:1023339103522

Araújo JPM, Hughes DP (2016) Diversity of entomopathogenic fungi: which groups conquered the insect body? In: Advances in Genetics, vol. 94 (Lovett B, Leger RJS, eds). Academic Press, Elsevier, pp 1–39. https://doi.org/10.1016/bs.adgen.2016.01.001

Asensio L, Carbonell T, López-Jiménez JA, López-Llorca LV (2003) Entomopathogenic fungi in soils from Alicante province [Spain]. Spanish Journal of Agricultural Research 1(3):37. https://doi.org/10.5424/sjar/2003013-33

Bengtsson-Palme J, Veldre V, Ryberg M, Hartmann M, Branco S, Wang Z, Godhe A, Bertrand Y, De Wit P, Sánchez-García M, et al. (2013) ITSx: Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for use in environmental sequencing. Methods in Ecology and Evolution 4:914-919. https://doi.org/10.1111/2041-210X.12073

Berlanga-Padilla AM, Ayala-Zermeño MA, Gallou A, Serna-Domínguez MG, Montesinos-Matías R, Arredondo-Bernal HC (2018) Report of the sexual stage of *Lecanicillium longisporum* in *Melanaphis sacchari.* Scientia Fungorum 47:73–77. https://doi.org/10.33885/sf.2018.47.1195

Bueno-Pallero FA, Blanco-Pérez R, Vicente-Díez I, Rodríguez-Martín AA, Dionísio L, Campos-Herrera R (2020) Patterns of occurrence and activity of entomopathogenic fungi in the Algarve (Portugal) using different isolation methods. Insects 11:352. https://doi.org/10.3390/insects11060352

Díaz B, Oggerin M, Lastra CCL, Rubio VJ, Fereres A (2009) Characterization and virulence of *Lecanicillium lecanii* against different aphid species. Biocontrol 54(6):825–835. https://doi.org/10.1007/s10526-009-9218-9

Feng M, Johnson JB, Kish LP (1990) Survey of entomopathogenic fungi naturally infecting cereal aphids (Homoptera: Aphididae) of irrigated grain crops in southwestern Idaho. Environmental entomology 19(5):1534–1542. https://doi.org/10.1093/ee/19.5.1534

González-Baca G, Venegas-Barrera CS, González-Gaona OJ, Vargas-Madriz H, Jiménez-Gómez MA, Pérez E, Ausencio A, Domínguez A (2019) Abundancia y distribución de hongos entomopatógenos en diferentes localidades y ambientes del sur de Tamaulipas. Revista Mexicana de Ciencias Agrícolas 10(3). https://doi.org/10.29312/remexca.v10i3.1550

Irinyi L, Lackner M, Sybren de Hoog G, Meyer W (2016) DNA barcoding of fungi causing infections in humans and animals. Fungal Biology 120(2):125–136.

Hallouti A, Ait Hamza M, Zahidi A, Ait Hammou R, Bouharroud R, Ait Ben Aoumar A, Boubaker H (2020) Diversity of entomopathogenic fungi associated with Mediterranean fruit fly (*Ceratitis capitata* (Diptera: Tephritidae)) in Moroccan Argan forests and nearby area: impact of soil factors on their distribution. BMC Ecology 20:64. https://doi.org/10.1186/s12898-020-00334-2

Jabbour R, Barbercheck ME (2009) Soil management effects on entomopathogenic fungi during the transition to organic agriculture in a feed grain rotation. Biological Control 51(3):435–443. https://doi.org/10.1016/j.biocontrol.2009.08.004

Jaronski S (2008) Soil ecology of the entomopathogenic Ascomycetes: a critical examination of what we (think) we know. In: Use of Entomopathogenic Fungi in Biological Pest management (Ekesi S, Maniania NK, eds). Research Signpost, Trivandrum, India, pp 91–144.

Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20(4):1160–1166. https://doi.org/10.1093/bib/bbx108

Kepler RM, Luangsa-Ard JJ, Hywel-Jones NL, Quandt CA, Sung GH, Rehner SA, Aime MC, Henkel TW, Sanjuan T, Zare R et al. (2017) A phylogenetically-based nomenclature for *Cordycipitaceae* (*Hypocreales*). IMA Fungus 8(2):335–353. https://doi.org/10.5598/imafungus.2017.08.02.08

Kouvelis VN, Sialakouma A, Typas MA (2008) Mitochondrial gene sequences alone or combined with ITS region sequences provide firm molecular criteria for the classification of *Lecanicillium* species. Mycological research 112(7):829–844. https://doi.org/10.1016/j.mycres.2008.01.016

Kovač M, Tkaczuk C, Pernek M (2021) First report of entomopathogenic fungi occurrence in forest soils in Croatia. Forests 12:1690. https://doi.org/10.3390/f12121690

Lacey LA (2012) Manual of techniques in invertebrate pathology, 2nd edition. Academic Press, London, UK.

Lebert H (1858) Ueber einige neue oder unvollkommen gekannte Krankheiten der Insekten, welche durch Entwicklung niederer Pflanzen im lebenden Körper enstehen. Zeitschrift für wissenschaftliche Zoologie 9:439–453.

Liu SL, Wang XW, Li GJ, Deng CY, Rossi W, Leonardi M, Liimatainen K, Kekki T, Niskanen T et al. (2024) Fungal diversity notes 1717–1817: taxonomic and phylogenetic contributions on genera and species of fungal taxa. Fungal Diversity 124:1–216. https://doi.org/10.1007/s13225-023-00529-0

Lopes RB, Souza TA, Mascarin GM, Souza DA, Bettiol W, De Souza HN, Faria M (2023) *Akanthomyces* diversity in Brazil and their pathogenicity to plant-sucking insects. Journal of invertebrate pathology 200:107955. https://doi.org/10.1016/j.jip.2023.107955

López-Lastra CCL, Lecuona RE (2019) Micopatologia de artrópodos. Ediciones INTA, Buenos Aires, Argentina.

Manfrino R, Gutierrez A, Diez del Valle F, Schuster C, Ben Gharsa H, López Lastra C, Leclerque A (2022) First description of *Akanthomyces uredinophilus* comb. nov. from hemipteran insects in America. Diversity 14:1118. https://doi.org/10.3390/d14121118

Medo J, Cagáň L (2011) Factors affecting the occurrence of entomopathogenic fungi in soils of Slovakia as revealed using two methods. Biocontrol 59(2):200–208. https://doi.org/10.1016/j.biocontrol.2011.07.020

Meyling NV, Eilenberg J (2006). Occurrence and distribution of soil borne entomopathogenic fungi within a single organic agroecosystem. Agriculture, Ecosystems and Environment 113:336–341. https://doi.org/10.1016/j.agee.2005.10.011

Meyling NV, Thorup-Kristensen K, Eilenberg J (2011) Below- and aboveground abundance and distribution of fungal entomopathogens in experimental conventional and organic cropping systems. Biological Control 59:180–186. https://doi.org/10.1016/j.biocontrol.2011.07.017

Mora MAE, Rouws JRC, Fraga ME (2016) Occurrence of entomopathogenic fungi in Atlantic forest soils. Microbiology discovery 4(1):1.

Nicoletti R, Becchimanzi A (2020) Endophytism of *Lecanicillium* and *Akanthomyces*. Agriculture 10:205. https://doi.org/10.3390/agriculture10060205

Nielsen C, Hajek AE, Humber RA, Bresciani J, Eilenberg J (2003) Soil as an environment for winter survival of aphid-pathogenic Entomophthorales. Biocontrol 28(1):92–100. https://doi.org/10.1016/s1049-9644(03)00033-1

Olivares-Bernabeu CM, López-Llorca LV (2002) Fungal egg-parasites of plant-parasitic nematodes from Spanish soils. Revista iberoamericana de micologia 19(2):104–110. https://www.reviberoammicol.com/2002-19/104110.pdf

Quesada-Moraga E, González-Mas N, Yousef-Yousef M, Garrido-Jurado I, Fernández-Bravo M (2023) Key role of environmental competence in successful use of entomopathogenic fungi in microbial pest control. Journal of Pest Science 97:1–15 https://doi.org/10.1007/s10340-023-01622-8

Quesada-Moraga E, Navas-Cortés JA, Maranhao EAA, Ortiz-Urquiza A, Santiago-Álvarez C (2007) Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. Mycological research 111:947–966. https://doi.org/10.1016/j.mycres.2007.06.006

Ramanujam B, Balachander M, Roopa G, Rangeshwaran R, Karmakar P (2011) ITS sequencing of Indian isolates of *Lecanicillium* species. Journal of Biological Chemistry 25(4):337–341.

Rivas F, Núñez PI, Jackson TA, Altier N (2013) Effect of temperature and water activity on mycelia radial growth, conidial production and germination of *Lecanicillium* spp. isolates and their virulence against *Trialeurodes vaporariorum* on tomato plants. BioControl 59(1):99–109. https://doi.org/10.1007/s10526-013-9542-y

Scorsetti AC, Humber RA, García JJ, López-Lastra CC (2007) Natural occurrence of entomopathogenic fungi (*Zygomycetes*: *Entomophthorales*) of aphid (*Hemiptera*: *Aphididae*) pests of horticultural crops in Argentina. BioControl 52:641–655. https://doi.org/10.1007/s10526-006-9045-1

Sharma L, Oliveira I, Torres L, Marques G (2018) Entomopathogenic fungi in Portuguese vineyards soils: suggesting a '*Galleria-Tenebrio*-bait method' as bait-insects *Galleria* and *Tenebrio* significantly underestimate the respective recoveries of *Metarhizium (robertsii)* and *Beauveria (bassiana).* MycoKeys 38:1–23. https://doi.org/10.3897/mycokeys.38.26790

Soares F, Trovão J, Portugal A (2022) Phototrophic and fungal communities inhabiting the Roman cryptoporticus of the national museum Machado de Castro (UNESCO site, Coimbra, Portugal). World Journal of Microbiology and Biotechnology 38:157. https://doi.org/10.1007/s11274-022-03345-x

Sun, B, Yu H, Chen AJ, Liu X (2008) Insect-associated fungi in soils of field crops and orchards. Journal of Crop Protection 27(11):1421–1426. https://doi.org/10.1016/j.cropro.2008.07.010

Sung GH, Hywel-Jones NL, Sung JM, Luangsa-Ard JJ, Shrestha B, Spatafora JW (2007) Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. Studies in Mycology 57:5–59. https://doi.org/10.3114/sim.2007.57.01

Sung GH, Spatafora JW, Zare R, Hodge KT, Gams W (2001) A revision of *Verticillium* sect. Prostrata. II. Phylogenetic analyses of SSU and LSU nuclear rDNA sequences from anamorphs and teleomorphs of the Clavicipitaceae). Nova Hedwigia 72(3–4):311–328 https://doi.org/10.1127/nova.hedwigia/72/2001/311

Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R, Villarreal-Ruiz L, Vasco-Palacios AM, Thu PQ, Suija A et al. (2014) Fungal biogeography. Global diversity and geography of soil fungi. Science 346(6213):1256688. https://doi.org/10.1126/science.1256688

Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Research 44(W1):W232–W235. https://doi.org/10.1093/nar/gkw256

Trovão J, Mesquita N, Paiva DS, Paiva de Carvalho H, Avelar L, Portugal A (2013) Can arthropods act as vectors of fungal dispersion in heritage collections? A case study on the archive of the University of Coimbra, Portugal. International biodeterioration & biodegradation 79:49–55. https://doi.org/10.1016/j.ibiod.2012.10.015

Uzman D, Pliester J, Leyer I, Entling MH, Reineke A (2019) Drivers of entomopathogenic fungi presence in organic and conventional vineyard soils. Applied Soil Ecology 133:89–97. https://doi.org/10.1016/j.apsoil.2018.09.004

Wang Y, Wang Z, Luo R, Souvanhnachit S, Thanarut C, Dao V, Yu H (2024) Species diversity and major host–substrate associations of the genus *Akanthomyces*. Research Square https://doi.org/10.21203/rs.3.rs-2907259/v1

Zare R, Gams W (2001a) A revision of *Verticillium* sect. Prostrata. III. Generic classification. Nova Hedwigia 72:47–55. https://doi.org/10.1127/nova.hedwigia/72/2001/329

Zare R, Gams W (2001b) A revision of *Verticillium* section Prostrata. IV. The genera *Lecanicillium* and *Simplicillium* gen. nov. Nova Hedwigia 73:1–50.

Zare R, Gams W (2003a) *Lecanicillium lecanii*. IMI Descriptions of Fungi and Bacteria 1565:1–3. https://doi.org/10.1079/DFB/20056401565

Zare R, Gams W (2003b) *Lecanicillium longisporum.* IMI Descriptions of Fungi and Bacteria 1566:1–2. https://doi.org/10.1079/dfb/20056401566

Zare R, Gams W (2003c) *Lecanicillium muscarium.* IMI Descriptions of Fungi and Bacteria 1567:1–6. https://doi.org/10.1079/dfb/20056401567

Zare R, Gams W, Culham A (2000) A revision of *Verticillium* sect. Prostrata I. Phylogenetic studies using ITS sequences. Nova Hedwigia 71(3–4):465–480. https://doi.org/10.1127/nova/71/2000/465

Zimmermann G (1986) The '*Galleria* bait method' for detection of entomopathogenic fungi in soil. Journal of Applied Entomology 102(1–5):213–215. https://doi.org/10.1111/j.1439-0418.1986.tb00912.x