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First Description of *Akanthomyces uredinophilus* comb. nov. from Hemipteran Insects in America

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Abstract: Filamentous fungi of the genera *Lecanicillium* and *Akanthomyces* (Ascomycota: Hypocreales: Cordycipitaceae) have been isolated from a variety of insect orders and are of particular interest as biological control agents for phloem-sucking plant pests. Three aphid- and whitefly-pathogenic fungal strains that had been isolated from naturally infected *Trialeurodes vaporariorum* and *Myzus persicae* in Argentina were assigned to the species *Lecanicillium uredinophilum* by combined analyses of morphology and ITS, LSU, EF1A, RPB1 and RPB2-based molecular taxonomy, giving rise to both the first description of this fungus from hemipteran insects and its first report from outside South-East Asia, especially from the American continent. A combination of phylogenetic reconstruction and analysis of pair-wise sequence similarities demonstrated that—reflecting recent changes in the systematics of Cordycipitaceae—the entire species *L. uredinophilum* should be transferred to the genus *Akanthomyces*. Consequently, the introduction of a new taxon, *Akanthomaces uredinophilus* comb. nov., was proposed. Moreover, extensive data mining for cryptic *A. uredinophilus* sequences revealed that (i) the fungus is geographically widely distributed, including earlier unrecognized isolations from further American countries such as the USA, Mexico, and Colombia, and (ii) entomopathogenic and mycoparasitic lifestyles are predominant in this species.

Keywords: *Akanthomyces* phylogeny; *Lecanicillium uredinophilum*; entomopathogenic fungi; mycoparasitic fungi; hemipteran insect; aphid; white fly; biocontrol; molecular taxonomy; Cordycipitaceae

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1. Introduction

The sap-sucking insects (Hemiptera) such as aphids and whiteflies have emerged as major agricultural pests with high economic relevance in numerous cropping systems [1,2]. These insects are exclusive phloem feeders and are among the most economically important pest insects of temperate agriculture [3]. Aphids and whiteflies are considered important pests on many crops, such as cotton, cucurbits, lettuce, potato, eggplant, pepper, soybean, sunflower, and tomatoes [3–5]. Aphids and whiteflies feed on plant nutrients essential for plant growth and development and induce phytotoxic effects by injecting saliva into the plant [6,7]. In addition to the impact of feeding, aphids also transmit plant viruses; more than 275, i.e., nearly 50% of all insect-borne plant viruses, are vectored by aphids [8,9].

The most prevalent approach to the management of hemipteran pests is the application of classical chemical insecticides [10,11]. However, insecticides commonly lose efficacy with the development of insecticide resistance, most notably in aphids and whiteflies [12–14].

Microbial control agents can serve as environmentally friendly components of integrated pest management (IPM) programs due to their selectivity, safety, and compatibility with other natural enemies [15]. Entomopathogenic fungi (EPF) are the most abundant group comprising app. 60% of all microbial insect pathogens [16]. Filamentous fungi are of particular interest as biological control agents for phloem-sucking plant pests since infection does not depend on the ingestion of fungal spores. Instead, upon topical contact with the host, fungal infection structures actively breach the integument, colonize the body cavity and kill the insect pest.

Systematically, one main group of fungal pathogens of aphids and whiteflies had traditionally been organized in the species *Verticillium lecanii*, i.e., as members of a taxonomic genus that mainly comprised pathogens of plants. In 2001, entomopathogenic and mycoparasitic "*Verticillium*" fungi were, on the basis of fungal morphology, ITS sequence comparisons and RFLP analyses, reorganized in the new genus *Lecanicillium* (Ascomycota: Hypocreales: Cordycipitaceae) with the type species *Lecanicillium lecanii* (Zimm.) Zare and W. Gams [17]. Until 2017, the genus *Lecanicillium* comprised almost 30 further species, including the originally defined "core species" *Lecanicillium longisporum*, *Lecanicillium muscarium* and *Lecanicillium attenuatum* [17], as well as the newly introduced entomopathogens *Lecanicillium sabanensis* [18] and *Lecanicillium pissodis* [19] and the mycoparasite *Lecanicillium uredinophilum* [20].

Lecanicillium lecanii was the first fungus studied and developed for use as a mycoinsecticide against aphids and other hemipteran pests in greenhouses [21]. To date, almost 15 *Lecanicillium*-based commercial preparations have been or are currently being developed to control various insect pests [22,23]. Among them, two commercial isolates of *L. muscarium*, Mycotal® and Vertalec®, were previously recommended to control whiteflies, especially under greenhouse conditions [22,23].

In 2017, part of the genus *Lecanicillium* comprising the type and core species was reassigned to the taxonomic genus *Akanthomyces* Lebert (Hypocreales: Cordycipitaceae) due to systematic priority considerations [24]. The genus *Akanthomyces*, established in 1858 with the type species *Akanthomyces aculeatus* [25], comprises fungal pathogens of insects and spiders [26–28]. Mains [29] amended and revised the genus *Akanthomyces* and characterized it by cylindrical synnemata covered by a hymenium-like layer of phialides producing one-celled catenulate conidia. Given the simplicity of the phenotypic characters and the overlap of the size and shapes of important diagnostic features, species in the genus *Akanthomyces* cannot be easily identified based on morphological criteria.

As a result of systematic molecular taxonomic studies leading to the synonymization of the genera *Lecanicillium* and *Akanthomyces* [24], the new taxonomic designations *Akanthomyces lecanii*, *Akanthomyces muscarius*, *Akanthomyces attenuatus*, *Akanthomyces sabanensis* and *Akanthomyces pissodis* were introduced for the respective *Lecanicillium* species, whereas the former species *Lecanicillium longisporum* was reorganized in the taxon *Akanthomyces dipterigenus* [24]. Moreover, molecular studies led to the recent introduction of several new species of *Akanthomyces* [27,28,30–33]. However, several previously recognized *Lecanicillium* species, e.g., *Lecanicillium psalliotae* have been demonstrated to be different from *Akanthomyces*, whereas for others, including *L. uredinophilum*, the systematic position with respect to *Akanthomyces* remains unresolved.

The species *L. uredinophilum* was introduced in 2015 to describe Korean isolates of a fungal mycoparasite attacking rust fungi. Morphologically, these parasitic fungi were characetrized by conspicuously long phialides and were shown to be genetically different from further *Lecanicillium* species, in particular from *L. longisporum*, by a multi-marker approach [20]. Subsequently, *L. uredinophilum* has been reported from an unidentified insect sampled in China [34] and from a Chinese caterpillar fungus (*Ophiocordyceps sinensis*) complex sampled in Tibet [35]. In the latter case, virulence of isolate QHLA for several insects, including aphids, has been demonstrated in laboratory bioassays.

Three fungal strains isolated from infected hemipteran insects sampled in Argentina that had been inconclusively characterized previously as *Lecanicillium lecanii sensu lato* [36] were demonstrated here to belong to the species *L. uredinophilum*. This study presents both the first description of this species from outside South-East Asia and the first report of natural infection of hemipteran insects by *L. uredinophilum*. The systematic position of *L. uredinophilum* with respect to *Akanthomyces* was analyzed using a multi-marker approach, and introduction of the taxon designation *Akanthomyces uredinophilus* comb. nov. is proposed. Moreover, the Genbank database was mined for cryptic data giving proof of unrecognized previous isolations of *L. uredinophilum* fungi. Taken together, these findings confirmed that both *A. uredinophilus* and its versatile entomopathogenic-mycoparasitic lifestyle are globally distributed and do not just represent a regional South-East Asian variety or adaptation. Moreover, systematic reorganization of *Lecanicillium uredinophilum* in the genus *Akanthomyces* corroborates that the entomopathogenic and mycoparasitic fungi formerly organized around the "core species" of the genus *Lecanicillium* remain phylogenetically tightly linked to each other as part of a presumably monophyletic group even after being transferred to a new genus. The identification of hemipteran-pathogenic *A. uredinophilus* fungi in America and especially in Argentina paves the way for the development of endemic biological control agents against these highly relevant agricultural pests.

2. Materials and Methods

2.1. Fungal Strains

Strains CEP 054 and CEP 057 were isolated from *Trialeurodes vaporariorum*, while CEP 108 was isolated from *Myzus persicae.* All three strains were previously isolated from La Plata, Buenos Aires province, Argentina (34°56′30.1″ S/58°04′53.7″ W). For isolation, the fungal spores were initially taken from insect cadavers on which the fungus had already sporulated, and fungi were grown on SDAY plates, i.e., Sabouraud dextrose agar (peptone: 10 g/L, dextrose: 20 g/L, agar 10 g/L) enriched with 1% yeast extract [37], supplemented with 25 ug/mL tetracycline to remove possible bacterial contamination. Several (2–5) rounds of sub-cultivation were performed to obtain single spore-derived colonies. Fungal isolates were routinely grown on SDAY agar at 24 °C in complete darkness for 15 d. Strains CEP 054, CEP 057 and CEP 108 were preserved as glycerol cryo-culture at −70 °C, on sterile filter paper, and freeze-dried in the CEPAVE Mycological Collection and deposited in the ARSEF Culture Collection (USDA-ARS, Ithaca, NY, USA) under accession numbers 7460, 7207 and 7462, respectively.

2.2. Morphological and Microscopic Characterization

Fungal species were identified based on both macroscopic and microscopic features. Morphological characterizations were made from in vitro cultures grown for 15 d at 24 °C on SDAY plates. Macroscopic features included the aspect, color and mycelium appearance of the fungal colonies. Microscope observations were made from fungal structures such as conidiophores, phialides and conidia. Fungal mycelia were mounted on glass slides and stained in lactophenol/cotton blue (0.01% w/v). Phialides and conidia were measured at magnifications of 400X using a model Axiostar Plus microscope (Zeiss, Germany). Furthermore, fungal structures were photographed with Moticam 3.0 MP Color Digital Camera (Motic, Xiamen, China). Semi-permanent slides were mounted, according to Humber [38]. Fungi were initially identified according to the taxonomic keys of Humber [38].

2.3. Virulence Bioassays

The virulence of three isolates, CEP054, CEP057 and CEP108, towards hemipteran insects was evaluated. The pathogenicity of fungal isolates CEP 057 and CEP 108 was tested on *M. persicae* apterous adults, as described in Manfrino et al. [34], while the activity of fungal isolates CEP 054 and CEP 057 was tested on *T. vaporariorum* nymphs according to Scorsetti et al. [39]. In all bioassays, the target insects were reared under laboratory conditions. For each isolate, 15 apterous adults of *M. persicae* and 25 fourth-instar nymphs of *T. vaporariorum* were used per replicate. The inoculation was performed by aspersion of suspensions of 1×10^7 conidia/mL in 0.01% (v/v) Tween 80 (sodium polysorbate). Controls were sprayed with solutions of 0.01% Tween 80. Three replicates and a control were performed for each isolate. The whole experiment was replicated twice. Cumulative mortality determined by the presence of mycelial masses was recorded daily over a period of 7–10 days [40]. Dead insects were removed daily and placed in plastic Petri dishes

(60 mm) with water agar (1%). Petri dishes were incubated at 25 °C for 3–5 days to allow for fungal development. Dead insects were mounted in lactophenol/cotton blue (0.01% *v*/*v*) to check for fungal infection.

2.4. DNA Extraction, PCR Amplification and Sequencing

DNA extraction was carried out according to Manfrino et al. [36] using the DNeasy Plant kit (Qiagen). Purified DNA was finally eluted in 100 μL elution buffer (10 mM Tris-Cl, pH = 8.5). The following phylogenetic markers were amplified from fungal DNA samples using GoTaq DNA polymerase (Promega) with the PCR primers and conditions indicated in Table 1: internal partial sequences of the gene encoding translation elongation factor 1-alpha (EF1A) (primer pair EF1A-983F/EF1A-2218R), of the genes encoding the largest (RPB1) and second-largest (RPB2) subunit of RNA polymerase II (primers RPB1Af/RPB1Cr and RPB2-5f/RPB2-7r, respectively), of the gene encoding the large (28S or LSU) ribosomal RNA (primers LR0R/LR5), as well as the complete ribosomal RNA operon internal transcribed spacer (ITS) region (ITS1–5.8S–ITS2) (primers ITS4/ITS5). The generalized PCR protocol employed for marker amplification consisted of one initial denaturation step of 95 °C for 2 min, 35 cycles of 30 s at 95 °C, 30 s at the primer-specific annealing temperature, and a 72 °C elongation step of amplicon-specific time, followed by a 5 min final elongation step at 72 °C. PCR product size was confirmed by agarose gel electrophoresis, and DNA was purified using a QIAquick® PCR purification kit (Qiagen, Germany). Additional primers used in combination with PCR primers for sequencing by StarSeq (Mainz, Germany) are indicated in Table 1. Raw sequence data were combined into a single consensus sequence for each fungal isolate and marker using version 11 of the MEGA software package [41]. Sequences were submitted to the GenBank database under accession numbers indicated in Table S1.

Table 1. PCR primers and parameters used in this study.

Primer Designation	Primer Sequence	Annealing Temperature (°C)	Elongation Time (s)	Reference
LR0R LR5	5′-GTACCCGCTGAACTTAAGC 5′-ATCCTGAGGGAAACTTC	58	120	[42]
ITS4 ITS5	5′-TCCTCCGCTTATTGATATGC 5′-GGAAGTAAAAGTCGTAACAAGG	52	120	[43]
EF1A-983F EF1A-2218R	5′-GCYCCYGGHCAYCGTGAYTTYAT 5′-ATGACACCRACRGCRACRGTYTG	52	120	[44]
EF1A-1567R	5′-ACHGTRCCRATACCACCSATCTT	sequencing primer		
EF1A-1577F	5′-CARGAYGTBTACAAGATYGGTGG	sequencing primer		this study
RPB1Af RPB1Cr	5′-GARTGYCCDGGDCAYTTYGG 5′-CCNGCDATNTCRTTRTCCATRTA	50	90	[45]
RPB2-5f RPB2-7r	5′-GAYGAYMGWGATCAYTTYGG 5′-CCCATRGCTTGYTTRCCCAT	50	120	[16]
RPB2-6f RPB2-6r	5′-TGGGGKWTGGTYTGYCCTGC 5′-GCAGGRCARACCAWMCCCCA	sequencing primer sequencing primer		[46]

2.5. Phylogenetic Analysis

Fungal strains and marker sequences employed as a reference for phylogenetic reconstruction are identified in Table S1. Marker sequences were aligned at the nucleotide sequence level using the CLUSTAL W function [47], as implemented in the MEGA 11 software package. For a comprehensive analysis of protein-encoding sequences, a “meta-gene” sequence generated by concatenation of markers EF1A, RPB1 and RPB2 was analyzed. Pairwise sequence similarity percentages were assessed from p-distance matrices calculated in

MEGA 11 from unfiltered nucleotide sequence data under pair-wise deletion of alignment gaps and missing data. Phylogenies were reconstructed using a p-distance matrix-based neighbor joining (NJ) method as implemented in MEGA 11. Tree topology confidence limits were explored in non-parametric bootstrap analyses over 1000 pseudo-replicates.

2.6. Database Mining for Cryptic Identifications of *L. uredinophilum*

Consensus sequences obtained from isolate CEP 057 for the five markers were used as queries in unfiltered BlastN searches across the Genbank database, allowing for up to 1000 similarity percentage sorted hits to be retained for analysis and applying a general 90% cut-off value for sequence coverage and the following marker specific sequence similarity cut-offs: ITS (98.0%), EF1A (94.0%), RPB1 (94.1%), RPB2 (94.0%), LSU (99.6%). Identified Genbank entries were aligned to the set of reference sequences (Table S1) and used for phylogenetic reconstruction in order to identify sequences clustering with references for *L. uredinophilum*.

3. Results

3.1. Morphological and Microscopic Characterization

Colonies grown on SDAY medium reaching 20–25 mm after 12 days at 24 °C, white to cream, velvety-cottony, irregular (Figure 1A), reverse pale yellow (Figure 1B). Asexual morph: Phialides gradually tapering towards the apex, 18.4–40.6 × 1.0–1.52 µm ($\bar{x}$ = 26.5 × 1.2, n = 25) produced singly or in whorls of up to 3–5 on prostrate hyphae (Figure 1C,D), secondary phialides produced from internode of original phialides. Conidia 1.9–4.7 × 1.3–2.6 µm ($\bar{x}$ = 3.3 × 1.8, n = 25), oval to cylindric, aseptate, smooth-walled, hyaline, aggregating in slimy head on the tip of phialides (Figure 1E). Sexual morph: undetermined.

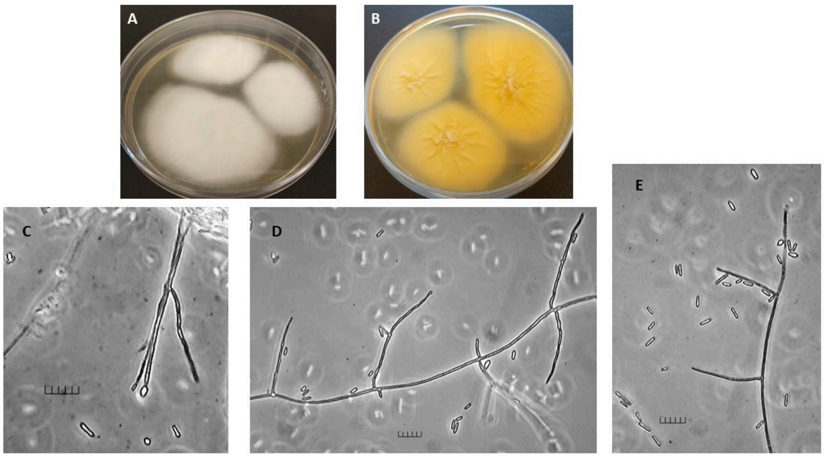

Figure 1. *Akanthomyces uredinophilus*. Upper (**A**) and reverse (**B**) view of cultures on SDAY after 12 days incubation. Verticillate phialides (**C**), phialides produced from prostrate hypha (**D**), phialides and conidia (**E**). Scale bar: 10 µm.

3.2. Virulence Bioassays

The three fungal isolates under study were pathogens to both *Trialeurodes vaporariorum* and *Myzus persicae*. The mortality caused by strains CEP 057 and CEP 054 when assayed against the original host, *T. vaporariorum*, was 52.6% ± 8.3 and 65.0% ± 23.0, respectively, at seven days post-treatment [39]. Against *M. persicae*, the mortality caused by CEP 57 and CEP 108 was 38.8% and 25.5%, respectively, at 10 days post-treatment [36]. Mortality in controls was 10%.

3.3. Molecular Taxonomic Identification

For the three fungal isolates under study, consistent consensus sequences were obtained for the five molecular taxonomic markers used. Comparisons with reference sequences gave rise to alignments comprising 685 bp (EF1A), 515 bp (RPB1), 792 bp (RPB2), 819 bp (LSU), and 470 bp (ITS), respectively, of these marker sequences. Concatenation of EF1A, RPB1 and RPB2 sequences thus gave rise to a meta-gene alignment comprising 1992 bp. Moreover, the ITS sequence of the *A. dipterigenus* type strain CBS 126.27 as well as the EF1A, RPB1, RPB2 and LSU sequences of the *A. attenuatus* type strain CBS 170.76 were determined for comparison. All new sequences reported in this study were submitted to the Genbank database under accession numbers indicated in Table S1. Pair-wise p-distances were calculated from alignments of ITS, LSU, and concatenated EF1A, RPB1 and RPB2 sequences (Tables S2 and S3). Alignments of the same markers were used to reconstruct the phylogeny of *Akanthomyces* fungi (Figures 2–4).

All marker sequences determined were at least 99.9% similar across the three Argentine isolates under study (Tables S2 and S3). In the EF1A-RPB1-RPB2 phylogeny (Figure 3), CEP 054, CEP 057 and CEP108 formed an independent branch within a 100% bootstrap-supported clade, uniquely comprising *L. uredinophilum* reference strains from Korea and China, including the specific type strain. Pair-wise sequence similarities across this presumed *L. uredinophilum* clade were ≥99.3%. Sequence similarities between the Argentine isolates and *Akanthomyces* reference strains outside this clade were ≤95.8% (as calculated for both the *A. muscarius* and *A. neocoleopterorum* type strains), thus giving rise to a supposed taxon gap of about 3.5%.

In the ITS phylogeny (Figure 2), the Argentine isolates formed a 91% bootstrap-supported clade together with *L. uredinophilum* references from China displaying 99.8% sequence similarity; no ITS sequence data were available for comparison for the *L. uredinophilum* type strain. Sequence similarities with reference strains outside this clade were ≤98.5% (for both the *A. lepidopterorum* and *A. neocoleopterorum* type strains). A qualitatively similar picture arose from the comparison of LSU sequences (Figure 4, Table S3).

In all reconstructed phylogenies, the respective presumed *L. uredinophilum* clade was found in a neighboring position to clades representing *Akanthomyces* species derived from former *Lecanicillium* core species. In the EF1A-RPB1-RPB2 tree, *L. uredinophilum* appeared most closely related to *A. muscarius*, *A. attenuatus*, *A. pissodis*, *A. neocoleopterorum*, and *A. lepidopterorum* (98% bootstrap support), with the over next neighbors being *A. lecanii* and *A. sabanensis*. Independent from the exact neighboring relationships, the *L. uredinophilum* clade appeared tightly integrated into the genus *Akanthomyces* as reorganized by Kepler et al. [24] for all three phylogenies.

These results from the molecular taxonomic analysis were consistent with both (i) the assignment of the three Argentine isolates CEP 054, CEP 057 and CEP108 to the taxonomic species *Lecanicillium uredinophilum* and (ii) the reorganization of this species in the genus *Akanthomyces*.

3.4. Database Mining for Cryptic Identifications of L. uredinophilum

Using the ITS sequence of isolate CEP 057 as a query for BlastN search across the Genbank nucleotide database, a total of 261 entries passed the 90% query coverage, and 98% pair-wise sequence similarity thresholds were applied. Out of these entries, 19 were found to associate with the presumed *L. uredinophilum* clade in phylogenetic reconstruction (Figure 2). Elimination of redundancies, e.g., entries from the same origin or study, led to the identification of 11 unrecognized descriptions of *L. uredinophilum*. Analogous database searches for the four other markers revealed two *L. uredinophilum*-associated LSU sequences (Figure 4); for both fungal specimens identified, ITS sequences were available in the database, but only one of these had already been identified in our ITS search. No further *L. uredinophilum*-associated entries were identified for the EF1A, RPB1 and RPB2 markers.

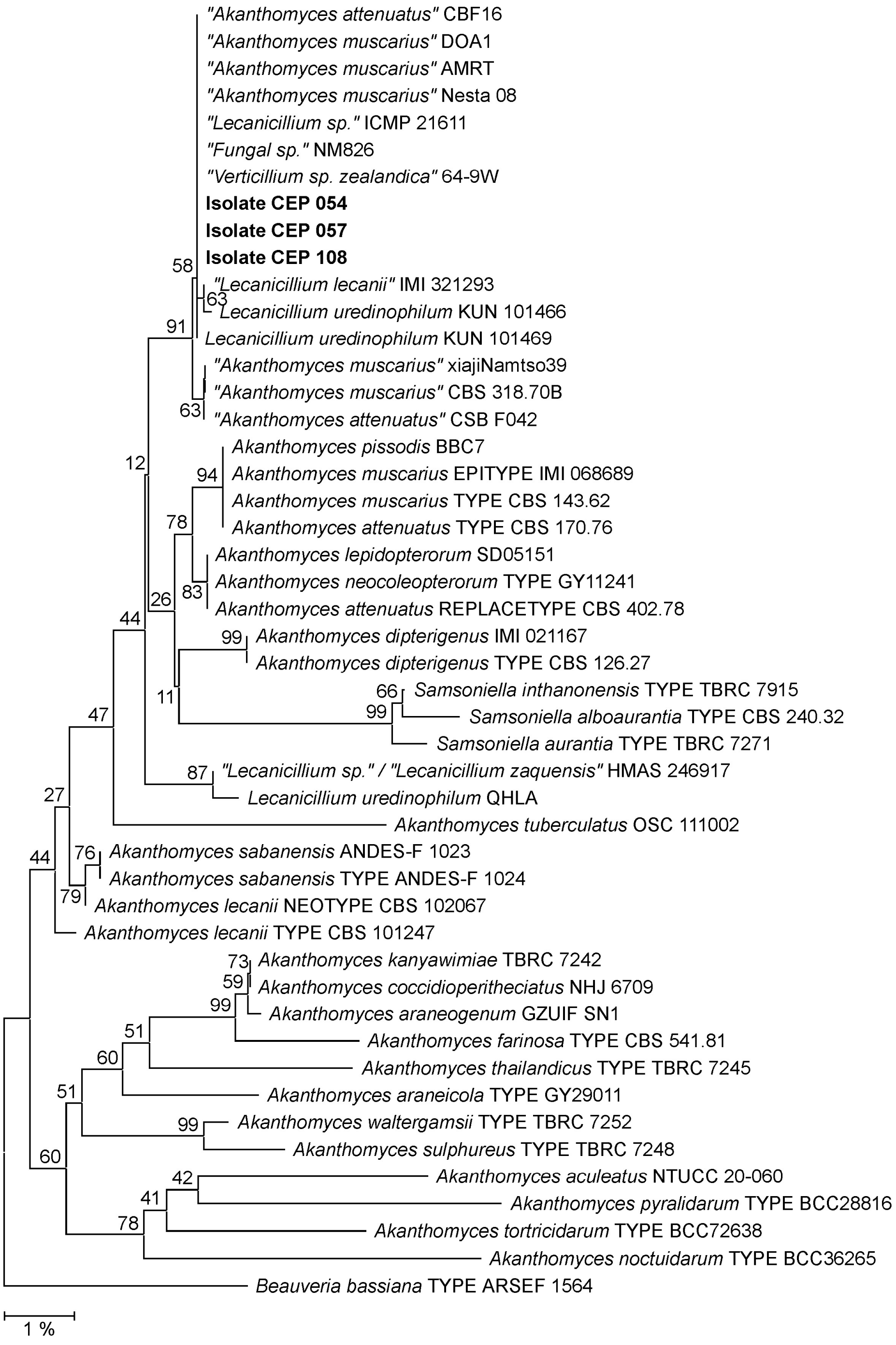

Figure 2. Neighbor joining (NJ) phylogeny of *Akanthomyces* fungi as reconstructed from ribosomal RNA operon internal transcribed spacer (ITS) sequences. Terminal branches are labeled by genus, species and strain designations; "TYPE" indicates specific type strains. Designations of fungal isolates from Argentina are shown in bold face; designations in quotation marks indicate non-reference sequences identified by data mining. Numbers on internal branches indicate bootstrap support percentages. The size bar corresponds to 1% sequence divergence with respect to phylogram branch lengths. The ITS sequence from the fungal entomopathogen *Beauveria bassiana* (Hypocreales; Cordycipitaceae) has been used as the outgroup.

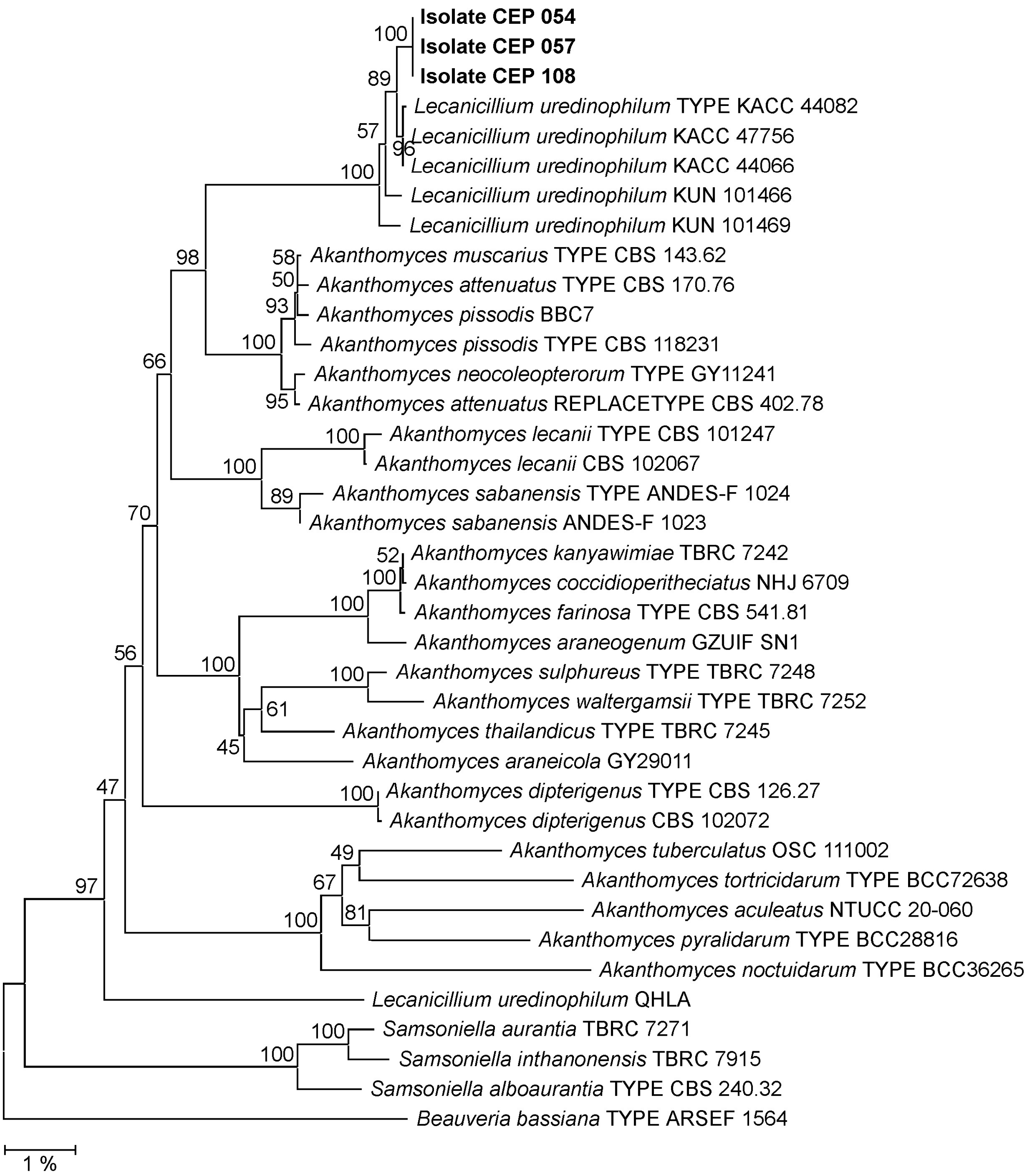

Figure 3. Neighbor joining (NJ) phylogeny of *Akanthomyces* fungi as reconstructed from concatenated EF1A, RPB1 and RPB2 nucleotide sequences. Terminal branches are labeled by genus, species and strain designations; "TYPE" indicates specific type strains. Designations of fungal isolates from Argentina are shown in bold face. Numbers on internal branches indicate bootstrap support percentages. The size bar corresponds to 1% sequence divergence with respect to phylogram branch lengths. A concatenation of orthologous sequences from the fungal entomopathogen *Beauveria bassiana* (Hypocreales; Cordycipitaceae) has been used as the outgroup.

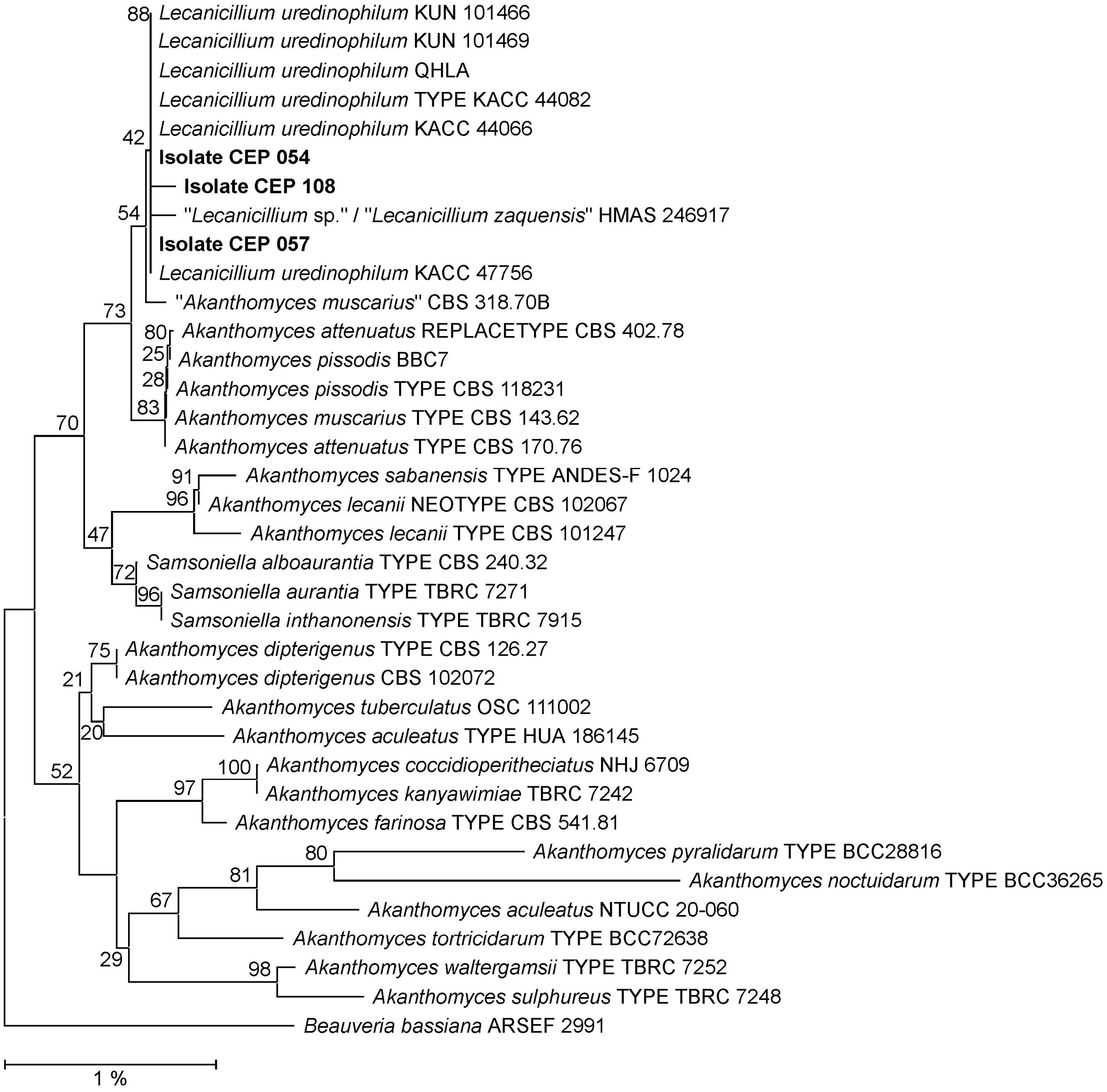

Figure 4. Neighbor joining (NJ) phylogeny of *Akanthomyces* fungi as reconstructed from ribosomal RNA large subunit (LSU) nucleotide sequences. Terminal branches are labeled by genus, species and strain designations; "TYPE" indicates specific type strains. Designations of fungal isolates from Argentina are shown in bold face; designations in quotation marks indicate non-reference sequences identified by data mining. Numbers on internal branches indicate bootstrap support percentages. The size bar corresponds to 1% sequence divergence with respect to phylogram branch lengths. The LSU sequence from the fungal entomopathogen *Beauveria bassiana* (Hypocreales; Cordycipitaceae) has been used as the outgroup.

The identified cryptic reports of *L. uredinophilum* sequence data (Table 2) spanned a wide range of geographic origins and isolation sources. Without being perceived as such, *L. uredinophilum* had previously been reported from South-East Asia (Tibet, China), America (Colombia, Mexico, USA), Africa (Kenya, South Africa), Europe and the Middle East (Germany, Turkey, Iran) and twice from New Zealand. Four reports associated the fungi described with insect hosts, three with basidiomycete fungi, one with the entomopathogenic ascomycete *Ophiocordyceps sinensis*, two with plant hosts, and one specimen had been isolated from a sweet water sample. At the moment of description, most specimens were assigned to existing *Lecanicillium* or *Akanthomyces* species different from *L. uredinophilum/A. uredinophilus*, but at least two reports discuss—at the level of Genbank entries—the introduction of a new fungal taxon to be termed "*Verticillium zealandica*" or "*Lecanicillium zaquensis*", respectively.

Table 2. Cryptic *Lecanicillium uredinophilum/Akanthomyces uredinophilus* associated Genbank entries.

Original Taxonomic Assignment	Strain Designation	Accession Number ITS	Accession Number LSU	Geographic Origin	Source/Natural Host	Reference
"*Verticillium zealandica*", "*Lecanicillium muscarium*"	64-9W	AF317540	n.a.	New Zealand	Passionwine hopper *Scolypopa australis* (Hemiptera)	[48]
"*Lecanicillium lecanii*"	IMI 321293	EF513008	n.a.	Colombia	*Hemileia vastatrix* (Basidiomycota; Uredinales) on coffee plant	[49]
"*Fungal* sp."	NM826	KJ867414	n.a.	California, USA	From leafs of California bay laurel (*Umbellularia californica*)	[50]
"*Akanthomyces attenuatus*"	CSB F042	KU574698	n.a.	Kenya	Endophyte of Native African Grass (*Brachiaria* spp.)	Genbank entry 2016
"*Lecanicillium* sp."	ICMP 21611	MF687199	n.a.	New Zealand	Octopus stinkhorn (*Clathrus archeri*) (Basidiomycota; Phallales)	Genbank entry 2017
"*Akanthomyces muscarius*"	CBS 318.70B	MH859686	MH871438	Germany	n.a.	[51]
"*Akanthomyces muscarius*"	Nesta 08	MN080299	n.a.	South Africa	*Hemileia vastatrix* (Basidiomycota; Uredinales) on coffee plant	Genbank entry 2019
"*Akanthomyces muscarius*"	AMRT	MW143523	n.a.	Iran	Asiatic rice borer *Chilo suppressalis* (Lepidoptera))	[52]
"*Akanthomyces muscarius*"	xiajiNamtso39	MZ544575	n.a.	Tibet, China	Water sample, Lake Nam	Genbank entry 2021
"*Akanthomyces attenuatus*"	CBF16	OL351559	n.a.	Mexico	Unidentified Thrips (Thysanoptera)	Genbank entry 2021
"*Akanthomyces muscarius*"	DOA1	OM397086	n.a.	Turkey	*Frankliniella occidentalis* (Thysanoptera)	Genbank entry 2022
"*Lecanicillium* sp.", "*Lecanicillium zaquensis*"	HMAS 246917 *	MT789698	MT789696	Tibet, China	Chinese caterpillar fungus *Ophiocordyceps sinensis* (Ascomycota; Hypocreales)	Genbank entry 2021

* Identified by LSU sequence as associated with *L. uredinophilum*, but in contradiction with ITS, EF1A and RPB1 sequence data.

4. Discussion

The molecular taxonomic analysis presented above firstly demonstrated that the *L. uredinophilum* type strain and further reference strains form a distinct, presumably monophyletic clade firmly located within the fungal species *Akanthomyces*, as reorganized by Kepler et al. [24]. In conclusion, following the example of the former core species of the genus *Lecanicillium*, the species *L. uredinophilum* should be reorganized into a new taxon to be named *Akanthomyces uredinophilus* comb. nov.; see the taxonomic description below. Moreover, three Argentine fungal isolates naturally infecting hemipteran insects were morphologically, microscopically and molecular-taxonomically characterized. The ITS, EF1A, RPB1, RPB2 and LSU marker-based molecular analysis firmly located strains CEP 054, CEP 057 and CEP 108 in the *L. uredinophilum*/*A. uredinophilus* clade.

Morphologically, the description of the isolates was consistent with the characteristics used to describe *L. uredinophilum*, i.e., being similar to the former *Lecanicillium* core species in having verticillate conidiophores, gradually tapering phialides, and ellipsoidal to oblong-oval aseptate conidia [17,20,29], but differing from *A. lecanii*, *A. dipterigenus*, *A. attenuatus* and *A. muscarius* by the longer phialides. Therefore, taking molecular and microscopic results together, isolates CEP 054, CEP 057 and CEP 108 were conclusively assigned to the new species *Akanthomyces uredinophilus.*

Former reports on *L. uredinophilum*, now *A. uredinophilus*, were exclusively from South-East Asia: Park et al. [20] introduced the species as a mycoparasite of rust fungi (Uredinales) in Korea, Wei et al. [34] described entomopathogenic *L. uredinophilum* isolates from China, and Meng et al. [35] concluded that one component (isolate QHLA) of a fungal entomopathogen complex from Tibet was *L. uredinophilum.* The Argentine strains characterized in this study represent the first report of this species from other parts of the world, especially from the American continent. Moreover, it is the first time the natural infection of hemipteran insects has been related to *L. uredinophilum.*

With respect to strain QHLA assigned by Meng et al. [35] to *L. uredinophilum*, the molecular-taxonomic analysis presented here has lent only weak support to this assignment. Whereas in the LSU phylogeny strain QHLA co-localized—under insufficient bootstrap support—with *L. uredinophilum* reference strains (Figure 4), it appeared unconnected to the *L. uredinophilum* clade in both the ITS and EF1A-RPB1-RPB2 trees (Figures 2 and 3). However, in all phylogenies, strain QHLA appeared tightly linked to a further fungal specimen, termed strain HMAS 246917. As strains QHLA and HMAS 246917 share both a rather specific source of isolation, i.e., the *Ophiocordyceps sinensis* complex, and their geographic origin, i.e., Qinghai province in Tibet, and have identical ITS and RPB1 marker sequences, one might expect them to stem from the same isolation event. However, for both specimens, LSU contradicts ITS, EF1A, RPB1 and RPB2 sequence data; a conclusive species level assignment is not possible on the basis of the currently available sequence data. There does not appear to date to be a formal description available for strain HMAS 246917, but the corresponding Genbank entries (MT789698, MT797809, MT797811) considered assignment to a new species to be termed "*Lecanicillium zaquensis*", an option much more in line with above molecular-taxonomy results than assignment to *L. uredinophilum*.

Beyond the above-mentioned explicit descriptions of fungal isolates, such as *L. uredinophilum*, data mining has revealed eleven cryptic reports of *A. uredinophilus* that had not been recognized as such (Table 2). Most authors of these cryptic reports assigned the fungus under study to either *A. muscarius* or *A. attenuatus*. Interestingly, the earliest identified report considered the introduction of a new species under the (invalidly published) designation "*Verticillium zealandica*" [48]. These cryptic reports demonstrated beyond the limited previously available knowledge that mycoparasitism and entomopathogenicity are the predominant, globally distributed ecophyiological lifestyles of *A. uredinophilus*. Moreover, together with earlier studies, including strains CEP 054, CEP 057 or CEP 108 [36,39], the reports contain valuable information with respect to the potential application of *A. uredinophilus* for biological control of insect pests or fungal phytopathogens in agriculture. The results of virulence bioassays, for instance, demonstrated that CEP 054, CEP 057 or CEP 108 were pathogens to aphids and whiteflies. Mortalities produced on whiteflies were higher than those on aphids, reaching a 65% mortality rate in *T. vaporariorum* and 38% in *M. persicae* [34,37]. Marshall et al. [48] tested the activity of *L. muscarium* isolates, revealed by data mining to be *A. uredinophilus*, against *Scolypopa australis* (Hemiptera: Ricaniidae) and showed that the isolates were highly pathogenic to *S. australis*. Moreover, a further ecophysiological trait linked here for the first time to *L. uredinophilum* or *A. uredinophilus*, namely endophytism, might open a future option for the development of respective biocontrol strategies.

Future attempts to tap into the potential of *A. uredinophilus* fungi, especially for hemipteran biocontrol, can rely on experience made with related *Akanthomyces* species. Wang et al. [53] found that *L. attenuatum* species showed high efficacy against nymphs and adults of *Acyrthosiphon pisum* Harris. Askary et al. [52] and Kim et al. [54,55] tested the activity of three *Lecanicillium* spp. isolates against aphids and *Sphaerotheca fuliginea* (the causal agent of cucumber powdery mildew). These authors observed that strains may have potential for development as a single microbial control agent effective against several plant diseases, pest insects and plant parasitic nematodes due to their antagonistic, parasitic and disease resistance-inducing characteristics. Broumandnia et al. [56] studied the potentials of four Iranian isolates of *A. lecanii* and *A. muscarius* to control *B. tabaci* on cucumber under laboratory conditions. The authors found that all isolates, especially *A. muscarius* (AGM5), exhibited appropriate potential as a biological control agent against *B. tabaci*. Lu et al. [57] tested the virulence of four strains assigned to *L. longisporum*, *L. attenuatum* and *A. lecanii* against *B. tabaci* and found that all isolates were pathogenic for this insect species. *Akanthomyces* fungi, including potentially versatile *A. uredinophilus*, should therefore be evaluated as powerful components of next-generation sustainable agriculture [58].

5. Conclusions

Molecular taxonomy demonstrated that the fungal species *Lecanicillium uredinophilum* has to be transferred to the genus *Akanthomyces*, giving rise to the new taxon combination *Akanthomyces uredinophilus*. Morphological and molecular-taxonomic identification of three hemipteran-associated fungal isolates from Argentina introduced a new host and a new country record to *A. uredinophilus*. Database mining for cryptic *A. uredinophilus* sequence reports revealed a wide geographic distribution of this fungus and its entomopathogenic or mycoparasitic lifestyles. Analysis of earlier reports and virulence bioassays with the Argentine strains against aphids and whiteflies indicated the potential of *A. uredinophilus* for the biological control of insect pests and fungal phytopathogens in agriculture.

6. Taxonomic Description

Akanthomyces uredinophilus (M.J. Park, S.B. Hong and H.D. Shin) Manfrino and Leclerque, **comb. nov.**

MycoBank MB814832

Basionym: Lecanicillium *uredinophilum* M.J. Park, S.B. Hong and H.D. Shin, *Mycotaxon* **130**: 997 (2015).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d14121118/s1: Table S1: Fungal strains and marker gene sequences used in this study. Table S2: Pair-wise p-distance values for aligned ITS (lower left triangle) and EF1A-RPB1-RPB2 (upper right) marker sequences. Table S3: Pair-wise p-distance values for aligned LSU marker sequences.

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Data Availability Statement: Sequence data analyzed in this study are publicly available from the Genbank database (https://www.ncbi.nlm.nih.gov) under nucleotide sequence accession numbers listed in Table 2 and Table S1 to this study.

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References

1. Van Emden, H.; Harrington, R. *Aphids as Crop Pests*; CABI Publishing: London, UK, 2007; p. 717.
2. Oliveira, M.R.V.; Henneberry, T.J.; Anderson, P. History, current status, and collaborative research projects for *Bemisia tabaci*. *Crop Prot.* **2001**, *20*, 709–723. [CrossRef]
3. Blackman, R.L. *Aphids on the Worlds Crops. An Identification and Information Guide*; John Wiley & Sons: New York, NY, USA, 2000.
4. Kunjwal, N.; Srivastava, R.M. Insect pests of vegetables. In *Pests and Their Management*; Omkar, Ed.; Springer Nature: Singapore; Pte Ltd.: Uttarakhand, India, 2018; pp. 163–221. [CrossRef]
5. Khan, I.A.; Wan, F.H. Life history of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) biotype B on tomato and cotton host plants. *J. Entomol. Zool. Stud.* **2015**, *3*, 117–121.
6. Henneberry, T.J.; Jech, L.F.; Hendrix, D.L.; Steele, T. *Bemisia argentifolii* (Homoptera: Aleyrodidae) honeydew and honeydew sugar relationships to sticky cotton. *Southwest. Entomol.* **2000**, *25*, 1–14. [CrossRef]
7. Chen, J.; McAuslane, H.J.; Carle, R.B.; Webb, S.E. Impact of *Bemisia argentifolii* (Homoptera: Auchenorrhyncha: Aleyrodidae) infestation and squash silverleaf disorder on Zucchini yield and quality. *J. Econ. Entomol.* **2004**, *97*, 2083–2094. [CrossRef]
8. Miles, P.W. Specific responses and damage caused by Aphidoidea. In *Aphids. Their Biology, Natural Enemies and Control*; Minks, A.K., Harrewijn, P., Eds.; Elsevier: Amsterdam, The Netherlands, 1989; Volume C, pp. 23–47.
9. Sylvester, E.S. Viruses Transmitted by Aphids. In *Aphids. Their Biology, Natural Enemies and Control*; Minks, A.K., Harrewijn, P., Eds.; Elsevier: Amsterdam, The Netherlands, 1989; Volume C, pp. 65–88.
10. Ragsdale, D.W.; Landis, D.A.; Brodeur, J.; Heimpel, G.E.; Desneux, N. Ecology and management of the soybean aphid in north America. *Annu. Rev. Entomol.* **2011**, *56*, 375–399. [CrossRef]
11. Bhatia, V.; Uniyal, P.L.; Bhattacharya, R. Aphid resistance in Brassica crops: Challenges, biotechnological progress and emerging possibilities. *Biotechnol. Adv.* **2011**, *29*, 879–888. [CrossRef]
12. Devonshire, A.L. Resistance of Aphids to Insecticides. In *Aphids, Their Biology, Natural Enemies and Control*; Minks, A.K., Harrewijn, P., Eds.; Elsevier: Amsterdam, The Netherlands, 1989; Volume C, pp. 123–139.
13. Moores, G.D.; Gao, X.; Denholm, I.; Devonshire, A.L. Characterization of insensitive acetylcholinesterase in insecticide-resistant cotton aphids, *Aphis gossypii* Glover (Homoptera: Aphididae). *Pestic. Biochem. Phys.* **1996**, *56*, 102–110. [CrossRef]
14. Foster, S.P.; Harrington, R.; Dewar, A.M.; Denholm, I.; Devonshire, A.L. Temporal and spatial dynamics of insecticide resistance in *Myzus persicae* (Hemiptera: Aphididae). *Pest Manag. Sci.* **2002**, *58*, 895–907. [CrossRef]
15. Lacey, L.A.; Shapiro-Ilan, D.I. Microbial control of insect pests in temperate orchard systems: Potential for incorporation into IPM. *Annu. Rev. Entomol.* **2008**, *53*, 121–144. [CrossRef]
16. Liu, Y.J.; Whelen, S.; Hall, B.D. Phylogenetic relationships among Ascomycetes: Evidence from an RNA Polymerase II subunit. *Mol. Biol. Evol.* **1999**, *16*, 1799–1808. [CrossRef]
17. Zare, R.; Gams, W. A revision of *Verticillium* section Prostrata. IV. The genus *Lecanicillium* and *Simplicillium* gen. nov. *Nova Hedwigia* **2001**, *73*, 1–50. [CrossRef]
18. Chiriví-Salomón, J.S.; Danies, G.; Restrepo, S.; Sanjuan, T. *Lecanicillium sabanense* sp. nov. (Cordycipitaceae) a new fungal entomopathogen of coccids. *Phytotaxa* **2015**, *234*, 63–74. [CrossRef]
19. Kope, H.H.; Leal, I. A new species of *Lecanicillium* isolated from the white pine weevil, *Pissodes strobi*. *Mycotaxon* **2005**, *34*, 331–340.
20. Park, M.J.; Hong, S.B.; Shin, H.D. *Lecanicillium uredinophilum* sp. nov. associated with rust fungi from Korea. *Mycotaxon* **2015**, *12*, 997–1005. [CrossRef]
21. Shah, P.A.; Pell, J.K. Entomopathogenic fungi as biological control agents. *Appl. Microbiol. Biotechnol.* **2003**, *61*, 413–423. [CrossRef] [PubMed]
22. De Faria, M.R.; Wraight, S.P. Mycoinsecticides and Mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. *Biol. Control.* **2007**, *43*, 237–256. [CrossRef]
23. Goettel, M.S.; Koike, M.; Kim, J.J.; Aiuchi, D.; Shinya, R.; Brodeur, J. Potential of *Lecanicillium* spp. for management of insects, nematodes and plant diseases. *J. Invertebr. Pathol.* **2008**, *98*, 256–261. [CrossRef]
24. Kepler, R.M.; Luangsa-ard, J.J.; Hywel-Jones, N.L.; Quandt, C.A.; Sung, G.H.; Rehner, S.A. A phylogenetically-based nomenclature for Cordycipitaceae (Hypocreales). *IMA Fungus* **2017**, *8*, 335–353. [CrossRef]

25. Lebert, H. Ueber einige neue oder unvollkommen gekannte Krankheiten der Insekten, welche durch Entwicklung niederer Pflanzen im lebenden Körper enstehen. *Z. Wiss. Zool.* **1858**, *9*, 439–453.
26. Hodge, K.T.; Gams, W.; Samson, R.A.; Korf, R.P.; Seifert, K.A. Lectotypification and status of *Isaria* Pers.: Fr. *Taxon* **2005**, *54*, 485–489. [CrossRef]
27. Mongkolsamrit, S.; Noisripoom, W.; Thanakitpipattana, D.; Wutikhun, T.; Spatafora, J.W.; Luangsaard, J. Disentangling cryptic species with *Isaria*-like morphs in Cordycipitaceae. *Mycologia* **2018**, *110*, 230–257. [CrossRef] [PubMed]
28. Aini, A.N.; Mongkolsamrit, S.; Wijanarka, W.; Thanakitpipattana, D.; Luangsa-ard, J.J.; Budiharjo, A. Diversity of *Akanthomyces* on moths (*Lepidoptera*) in Thailand. *MycoKeys* **2020**, *71*, 1–22. [CrossRef] [PubMed]
29. Mains, E.B. Entomogenous species of *Akanthomyces*, *Hymenostilbe* and *Insecticola* in North America. *Mycologia* **1950**, *42*, 566–589. [CrossRef]
30. Chen, W.H.; Han, Y.F.; Liang, Z.Q.; Jin, D.C. *Lecanicillium araneogenum* sp. nov., a new araneogenous fungus. *Phytotaxa* **2017**, *305*, 29–34. [CrossRef]
31. Chen, W.H.; Chang, L.; Han, Y.F.; Liang, J.D.; Tian, W.Y.; Liang, Z.Q. *Akanthomyces araenicola*, a new araneogenous species from Southwest China. *Phytotaxa* **2020**, *409*, 227–232. [CrossRef]
32. Chen, W.H.; Han, Y.F.; Liang, J.D.; Liang, Z.Q. *Akanthomyces lepidopterorum*, a new *Lecanicillium*-like species. *Phytotaxa* **2020**, *459*, 117–123. [CrossRef]
33. Chen, W.H.; Han, Y.F.; Liang, J.D.; Liang, Z.Q. *Akanthomyces neocoleopterorum*, a new *Verticillium*-like species. *Phytotaxa* **2020**, *432*, 119–124. [CrossRef]
34. Wei, D.P.; Wanasinghe, D.N.; Chaiwat, T.A.; Hyde, K.D. *Lecanicillium uredinophilum* known from rusts, also occurs on animal hosts with chitinous bodies. *Asian J. Mycol.* **2018**, *1*, 63–73. [CrossRef]
35. Meng, Y.; Wellabada Hewage Don, P.I.D.; Wang, D. A New Strain of *Lecanicillium uredinophilum* isolated from Tibetan Plateau and its insecticidal activity. *Microorganisms* **2022**, *10*, 1832. [CrossRef]
36. Romina, M.G.; Christina, S.; Katharina, S.; Claudia, C.L.L.; Andreas, L. Genetic characterization, pathogenicity and benomyl susceptibility of *Lecanicillium* fungal isolates from Argentina. *J. Appl. Entomol.* **2018**, *143*, 204–213. [CrossRef]
37. Goettel, M.S.; Inglis, G.D. Fungi: Hyphomycetes. In *Manual of Techniques in Insect Pathology*; Lacey, L., Ed.; Academic Press: London, UK, 1997; pp. 213–249.
38. Humber, R.A. Fungi: Identifcation. In *Manual of Techniques in Insect Pathology*; Lacey, L., Ed.; Academic Press: San Diego, CA, USA, 1997; pp. 153–189.
39. Scorsetti, A.C.; Humber, R.A.; García, J.J.; López Lastra, C.C. Natural occurrence of entomopathogenic fungi (Zygomycetes: Entomophthorales) of aphid (Hemiptera: Aphididae) pests of horticultural crops in Argentina. *BioControl* **2007**, *52*, 641–655. [CrossRef]
40. Landa, Z.; Osborne, L.S.; Lopez, F.; Eyal, J. A bioassay for determining pathogenicity of entomogenous fungi on whiteflies. *Biol. Control* **1994**, *4*, 341–350. [CrossRef]
41. Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. MEGA 6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **2013**, *30*, 2725–2729. [CrossRef] [PubMed]
42. Vilgalys, R.; Hester, M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. *J. Bacteriol.* **1990**, *172*, 4238–4246. [CrossRef] [PubMed]
43. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*; Innis, M.A., Gelfand, D.H., Snisky, J.J., White, T.J., Eds.; Academic Press: San Diego, CA, USA, 1990; pp. 315–322. [CrossRef]
44. Rehner, S.A.; Buckley, E. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-a sequences: Evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* **2005**, *97*, 84–98. [CrossRef] [PubMed]
45. Stiller, J.W.; Hall, D. The origin of red algae: Implications for plastid evolution. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 4520–4525. [CrossRef] [PubMed]
46. Goetsch, L.; Eckert, A.J.; Hall, B.D. The molecular systematics of Rhododendron (ericaceae): A phylogeny based upon RPB2 gene sequences. *Syst. Bot.* **2005**, *30*, 616–626. [CrossRef]
47. Thompson, J.D.; Higgins, D.G.; Gibson, T.J. ClustalW: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **1994**, *22*, 4673–4680. [CrossRef]
48. Marshall, R.K.; Lester, M.T.; Glare, T.R.; Christeller, J.T. The fungus, *Lecanicillium muscarium*, is an entomopathogen of passionvine hopper (*Scolypopa australis*). *N. Z. J. Crop Hortic. Sci.* **2003**, *31*, 1–7. [CrossRef]
49. Kouvelis, V.N.; Sialakouma, A.; Typas, M.A. Mitochondrial gene sequences alone or combined with ITS region sequences provide firm molecular criteria for the classification of *Lecanicillium* species. *Mycol. Res.* **2008**, *112*, 829–844. [CrossRef]
50. Johnston, S.R.; Boddy, L.; Weightman, A.J. Bacteria in decomposing wood and their interactions with wood-decay fungi. *FEMS Microbiol.* **2016**, *92*, fiw179. [CrossRef] [PubMed]
51. Shahriari, M.; Zibaee, A.; Khodaparast, S.A.; Fazeli-Dinan, M. Screening and virulence of the entomopathogenic fungi associated with *Chilo suppressalis* Walker. *J. Fungi* **2021**, *7*, 34. [CrossRef] [PubMed]
52. Askary, H.; Carrière, Y.; Bélanger, R.R.; Brodeur, J. Pathogenicity of the fungus *Verticillium lecanii* to aphids and powdery mildew. *Biocontrol. Sci. Technol.* **1998**, *8*, 23–32. [CrossRef]

53. Wang, D.; Deng, J.; Pei, Y.; Li, T.; Jin, Z.; Liang, L.; Wang, W.; Li, L.; Dong, X. 2017. Identification and virulence characterization of entomopathogenic fungus *Lecanicillium attenuatum* against the pea aphid *Acyrthosiphon pisum* (Hemiptera: Aphididae). *Appl. Entomol. Zool.* **2017**, *52*, 511–518. [CrossRef]
54. Kim, J.J.; Goettel, M.S.; Gillespie, D.R. Potential of *Lecanicillium* species for dual microbial control of aphids and the cucumber powdery mildew fungus, *Sphaerotheca fuliginea*. *Biol. Control.* **2007**, *40*, 327–332. [CrossRef]
55. Kim, J.J.; Goettel, M.S.; Gillespie, D.R. Evaluation of *Lecanicillium longisporum*, Vertalec for simultaneous suppression of cotton aphid, *Aphis gossypii*, and cucumber powdery mildew, *Sphaerotheca fuliginea*, on potted cucumbers. *Biol. Control.* **2008**, *45*, 404–409. [CrossRef]
56. Broumandnia, F.; Rajabpour, A.; Hamed Ghodoum Parizipour, M.; Yarahmadi, F. Morphological and molecular identification of four isolates of the entomopathogenic fungal genus *Akanthomyces* and their effects against *Bemisia tabaci* on cucumber. *Bull. Entomol. Res.* **2021**, *111*, 628–636. [CrossRef]
57. Lu, Q.; Wang, P.; Ali, A.; Sheng, Z.L. Molecular identification and virulence of four strains of entomopathogenic fungi against the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). *J. Econ. Entomol.* **2022**, *115*, 731–738. [CrossRef]
58. Bamisile, B.S.; Akutse, K.S.; Siddiqui, J.A.; Xu, Y. Model application of entomopathogenic fungi as alternatives to chemical pesticides: Prospects, challenges, and insights for next-generation sustainable agriculture. *Front. Plant Sci.* **2021**, *12*, 741804. [CrossRef]