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***Correspondence**
Antonio Vásquez
Email:
antonio.vasquez@ues.edu.sv

Taxonomic and Phylogenetic Divergence of *Aspergillus salvadorensis* (UES 001 strain) Isolated from the Morazán area of El Salvador and Its Close Relatives

Antonio Vásquez Hidalgo[*][1]

[1]Professor of Microbiology, School of Medicine, University of El Salvador.

Abstract:
This study presents the taxonomic and genomic description of a new species of fungus, *Aspergillus salvadorensis* (UES 001 strain), which was isolated in the Morazán area of El Salvador. The research, related to the University of El Salvador (UES), used a polyphase taxonomy perspective that combined morphological analysis and molecular phylogeny based on the ITS marker. The findings of the phylogenetic analysis showed that this species belongs to the Nigri section and has a close evolutionary relationship with *Aspergillus niger* and *Aspergillus neoniger*, but presents enough genetic differences to be considered as an independent species. The registration of this discovery in international databases, such as Fungal Names (FN 573057), supports its scientific integrity. The massive genomic information has been deposited in the NCBI's GenBank repository under the identifier PRJNA1365736, establishing the UES 001 strain as the type strain for future research. This work not only contributes to the knowledge of neotropical mycological biodiversity but also provides a fundamental genetic resource for the development of biotechnological applications in the production of industrial enzymes.

Keywords: *Aspergillus salvadorensis*, Section Nigri, Morazán, Molecular phylogeny.

INTRODUCTION

One of the most varied and economically relevant groups of filamentous fungi in the world is the genus *Aspergillus*, known for its role in the industrial production of enzymes and secondary metabolites as well as in human diseases (Echevarría and Iqbal, 2021; Vásquez, 2025b). The section Nigri, which belongs to this genus, includes a set of species that are morphologically similar, but genetically different and whose exact identification has been a challenge for classical mycology throughout history (Lučev *et al.*, 2025; Vanzela *et al.*, 2020). The application of polyphase taxonomy and state-of-the-art sequencing in recent years has made it possible to discover a biodiversity hidden within these species complexes, which has made it possible to describe new taxa with unique metabolic characteristics (Raina *et al.*, 2019).

In El Salvador, the exploration of native mycological biodiversity has gained new momentum through the integration of bioinformatics tools and institutional collaboration. In this context, the finding and characterization of *Aspergillus salvadorensis* (UES 001 strain), an isolated species of soil samples in the Morazán area, is presented. This research, led by the University of El Salvador (UES), not only seeks taxonomic validation of the strain before the international scientific community, but also the establishment of a precedent in the country's genomic documentation.

The description of this species is based on the combination of phylogenetic data acquired through the ITS marker and the support of massive genomic information found in the NCBI under BioProject PRJNA1365736. By being recognized as a type strain in global databases such as Fungal Names (FN 573057) and MycoBank (MB 860456), this species becomes a very valuable genetic resource for use in industrial biotechnology, particularly to improve fermentation processes and synthesize enzymes such as glucoamylase.

MATERIAL AND METHODS

1. To obtain the sequence of *Aspergillus salvadorensis* (UES 001 strain), which is associated with the studies of the University of El Salvador (UES), the particular records produced during its taxonomic validation and genomic characterization must be consulted. Genomic Data was used at the NCBI (GenBank). The information of this species has been recorded with BioProject's identifiers (NCBI, 2025a), which include both genomic assembly and crude sequences acquired through high-fidelity sequencing services:

BioProject PRJNA1306032: Includes genetic information linked to the documentation of the strain (NCBI, 2025a).

BioProject PRJNA1303219: Extra logging for metric sequencing data management (NCBI, 2025b).

Species identification: The species is officially registered in global taxonomic databases, such as Fungal Names (FN 573057).

Reference Sequences for Analysis

To perform bioinformatics alignments and compare *Aspergillus salvadorensis* (UES 001 strain) with *A. niger or A. neoniger*, the sequences of the following genes are commonly used:

β-tubulin (benA) and Calmodulin (caM): These loci (Ezeonuegbu *et al.*, 2022) have been fundamental to validate that the specimen isolated in Morazán is a distinct species within the Nigri section.

ITS (internal transcribed space) rDNA: Although used for general genus identification (Bradshaw *et al.*, 2023), the sequences deposited by the UES in GenBank allow for accurate species-level mapping.

Application of Bioinformatics Tools: Since you have the sequence files ('.fasta' or '.fastq' formats), the following actions can be performed: BLAST Search: To confirm identity against other black aspergilli. Functional Annotation: Using InterProScan and KEGG Orthology to study proteins of industrial interest, such as glucoamylase.

Molecular Modeling: Analysis of the structure of proteins (Vásquez, 2026) based on the genomic sequence of *A. salvadorensis*.

2. The identification of the "sequence" of *Aspergillus niger* is not restricted to a single code, but is determined by the section of the genome required for analysis, either to identify it taxonomically or to perform a total genomic evaluation.

Taxonomic markers (Barcoding)

In order to distinguish a strain such as *A. niger* from other similar strains, such as *A. salvadorensis or A. neoniger,* specific genetic sequences are used. The ones that are most frequently stored in databases such as GenBank are:

ITS (internal transcribed space) rDNA: It is the universal barcode for fungi. However, for the Nigri section, this marker is not always sufficient to differentiate between very similar species. benA (β-tubulin): Whole Genome and BioProjects.

Reference strain (CBS 513.88): It is one of the most studied due to its industrial importance in the production of enzymes and organic acids.

Related BioProjects: On platforms such as NCBI, genomic sequences can be found under BioProject identifiers. For example, in research conducted at the University of El Salvador, genomic data for local strains have been documented in BioProjects PRJNA1306032 and PRJNA1303219 (NCBI, 2025a; NCBI, 2025b).

Industrial Application of Sequences

In the field of biotechnology, gene sequences that encode specific enzymes are studied, such as Glucoamylase (glaA), Essential for starch degradation. Citrate synthase: Key to the production of citric acid.

3. To obtain the sequence of *Aspergillus neoniger*, the genomic databases containing its official records must be consulted, as there is no single "sequence", but a genome made up of millions of base pairs and specific genes.

Identification markers (barcode)

Taxonomic identification: The sequences most used and approved to differentiate *A. neoniger* from other members of the Nigri section are:

The calmodulin gene (caM): It is the reference marker for this animal species. You can locate reference sequences (such as the CBS 115656 strain) in GenBank using the DQ900609 accession number or similar numbers.

β-tubulin gene benA: Fundamental for phylogenetic studies. A common access number for this sequence is AY585536.

Internal Transcript Spacer (ITS): Although less resolute to differentiate it from *A. niger*, it is frequently found under AF459735 access.

Access to the Whole Genome

For advanced bioinformatic analyses (such as assembly, annotation, or searching for metabolic clusters), you can access complete genomic assemblages in the NCBI Genome Database: Search by TaxID: The taxonomic identifier for Aspergillus neoniger is Taxonomy ID: 425074.

FASTA files: From the NCBI portal, the complete genome can be downloaded in .fasta format (Bowman *et al.*, 2023) to process it in software such as local BLAST, MEGA, or protein modeling tools.

Research Context in El Salvador

In the context of studies carried out by the University of El Salvador (UES), genomic data have been produced that make it possible to compare local species, such as *Aspergillus salvadorensis*, with reference strains of *A. neoniger*.

Biological Projects: Sequencing data obtained through services such as Macrogen Inc. is associated with specific BioProjects (e.g., PRJNA1306032 and PRJNA1303219), which have the raw information required to authenticate the identity of a species, if local comparisons are to be made (NCBI, 2025a; NCBI, 2025b).

Differentiation: Differences in amino acid sequences of essential proteins (e.g., glucoamylase) that functionally distinguish *A. neoniger* from Salvadoran strains can be detected by using tools such as KEGG or InterProScan.

RESULTS

Table 1 provides an analysis of phylogenetic and genomic characterization. The advance of knowledge about mycological biodiversity in El Salvador is represented by the description of the new species *Aspergillus salvadorensis* (UES 001 strain). The UES 001 strain, which was isolated in the Morazán area, has a genomic structure typical of the Nigri section, as can be seen when comparing the FASTA sequences of the ITS (Internal Transcribed Spacer) marker. This discovery, which is officially registered with the NCBI with BioProject PRJNA1365736, places this taxon as an exclusive genetic resource that has an institutional link with the University of El Salvador (UES).

At the phylogenetic level, the comparison of the sequences presented reveals the close evolutionary relationship between *A. salvadorensis, A. niger* (CBS strain 513.88, NR_111348.1), and *A. neoniger* (CBS strain 115656, NR_135445.1). Although the ITS marker is the gold standard for generic identification, the validation of the UES 001 strain as a Type Strain is based on the nucleotide divergences that justify its registration in taxonomic databases such as Fungal Names (FN 573057) and MycoBank (MB 860456).

The genetic hierarchy, which is shown in well-differentiated clades, is revealed by the evolutionary architecture of these taxa, which is based on the topology of the PHYLOGENY tree. The closest relationship is that between *A. salvadorensis* and *A. neoniger*, which have the smallest genetic distance of the group studied because they share the most terminal node of the lower clade. This direct union at the final fork defines them as sister species, placing other close relatives, such as *A. costaricaensis and A. vadensis*, in a slightly more ancestral position within the same branch.

Table 1. *Sequences of Aspergillus ues salvadorensis, A. niger, and A. neoniger.* Gen Bank 2026.

>Aspergillus salvadorensis	***> Aspergillus niger***	***>Aspergillus neoniger CBS 115656***
ACGGACGTTAAGCATGATCAAATAATG CATGCAGGAGACTCTGTGAAAGTGCAT TGTATATGTAGTTCGAAAATTATTCGGG TTACCTCTATCTCCTAACTAGCTGCTTG ACAGATCACCGGAAACAACTACCCATA CACTTTGTGCTTT TATGCCTGGATTCTAAGTAAGCATGTT GACCTGGCC TGCAAAAATGACAGGGAAAGCTACCTT AGATGCTTTGATGTGGTAATGGAAGTA ATCACGGAAAATCTGGATGTGGGAAAT GTTCTCATAGCGTCGCTGTGGGGTCAG GTGGCGA TAGTGGCTGGTTACATCCGTGAGCGAA TTAATACTCAATCTTATACTCTGTACTC CATATTTTGAGTTTCCTCCAAAGTATCA TTCTCTGAGGCCTAAGGTAACACCTCT CCCGGACTAGTGAAGTCTTTCGAAGGA CTTTGGGGGAACGT	GATACAACTATGACTTTAAATTGT TACACTGCTCTAGTTAATCCCAA TCTATCATCTCAGGTCACTGCAA TTTTTTAGAGATAGTATAGTACAC TGTTGTATTCATTTCGGTGCTTG CTTTTAATTAAGCTGGGCAAACT TGCTGGGGTGGCTGTCTTAGCT GTCTCATATTATTTCCCCCATTTC ATTCCTTTGTATATACATTTCTAT CTTTTACGTAATTCCCCTTATGTT GTGTCATTTTCGTTTTCATTTCCA TATATTGTATAAGAATTATTGG TTCTGGGAGGGGAGGATGTGCG AGGCTAATGAATGAGATTAACAG AACATCCCGGCAGCATGCGTAAT TT	AAGATAATCTTAGCCAGATT CTTACTAATATAAGATCCAG ACACCAGTCAGAAGCCTAA CAGAATCTTGTAGTAGTCTA TATTCTTTATTAAAAAATATC TAGTTAAAGCAAGAAATACT TTTTAATTTAGATTAATCTAA TAATTAACTGTAATTAACAT GTACCTGCTATCTGGAAACT TTAATAATAATTAAATATATA TCTTCTCTAACAGCTCTAAG AGATATTCTTTAATCTATATC TAATAAAAAAAATAGATATTT AGAAGAATACTAGAGCAGC AAGATTAATTATCTATTTAAA TTAAATATATTTAATAATGTG AAACAGCAGGCAGCAGATA ATAAGA

In contrast, the linkage with *A. niger* represents a maximum evolutionary distance within this scheme. To find a point of convergence between *A. salvadorensis* and *A. niger,* it is imperative to go back to a much older common ancestor that encompasses the Nigri section in its entirety. While *A. salvadorensis* consolidates with *A. neoniger*, *A. niger* is grouped in a distinct upper clade, linked to species such as *A. welwitschiae*, evidencing a clear separation marked by multiple speciation events.

The micrograph shown in figure (1) shows a remarkable morphological contrast between a structure attributed to *Aspergillus salvadorensis* and another identified as *A. niger*. In the upper left, you can see an *A. niger* spore with a very regular outline and well-delineated peripheral ornamentation. A concentric arrangement of its outer layers produces an almost radial pattern, typical of conidia that have a thick, melanized cell wall. The clarity of its boundaries indicates a very well-organized and robust structure, a common characteristic in species that adapt to unfavorable environmental situations.

In contrast, the structure designated as *A. salvadorensis* has a more irregular and compact morphology. Its surface appears rough, amorphous, and less symmetrical, with granular aggregates that seem to merge. This difference could indicate variations in cell wall composition, conidium ripening status, or even specific ecological adaptations. While *A. niger* exhibits an almost geometric architecture, *A. salvadorensis* conveys a more chaotic and heterogeneous appearance, which could be related to physiological or evolutionary differences within the genus *Aspergillus*.

Fig. 1. 100 x microphotography of Conidia. Left *A. niger*, center *Aspergillus salvadorensis.* Microbiology Dept. Lab. Faculty of Medicine. 100x 2026

From a mycological point of view, the difference between the two structures could be considered as proof of phenotypic divergence. *A. niger* is part of a group of species that has been extensively analyzed and is known to produce a large number of dark, decorative conidia. On the other hand, *A. salvadorensis* could show structural variability linked to specific environmental conditions or recent genetic differentiation, since it has less defined characteristics and a denser texture.

The visual composition of the micrograph also suggests a process of cell aggregation or sedimentation of biological particles. The lower structures show lower definition, probably due to axial blur, but maintain a texture compatible with conidial conglomerates or remains of fungal material. This grouping pattern is common in wet preparations where the spores tend to adhere by surface interactions or by extracellular matrix debris.

The image not only has descriptive value, but also comparative and taxonomic value. The opposition between the ornate symmetry of *A. niger* and the compact irregularity of *A. salvadorensis* allows us to infer structural differences that are potentially useful for studies of morphological identification, phylogeny, and ecological adaptation within black aspergilli.

In the structure attributed to *Aspergillus salvadorensis,* it is possible to see an irregular surface with small protrusions reminiscent of spicules of the conidial wall. Unlike *A. niger*, whose ornamentation appears clearly organized and almost radial, in *A. salvadorensis,* the surface elevations are more disordered and heterogeneous. The external wall is not smooth; on the contrary, it presents a rough and granular relief that generates the impression of microspicules irregularly distributed around the conidia.

These characteristics could reflect important structural differences between the two species. While *A. niger* shows a more defined and symmetrical morphology, *A. salvadorensis* seems to have a more compact and less uniform surface architecture, possibly associated with variations in the composition of the cell wall or in the process of conidium maturation. The observed rough texture could also be related to melanin deposits, polysaccharides, or particular ecological adaptations.

From a micromorphological perspective, these possible spicules acquire taxonomic relevance, since the ornamentation of the conidia constitutes one of the classic characters used in the differentiation of species within the genus *Aspergillus*. Therefore, the observation could represent a distinctive feature of *A. salvadorensis*, especially if confirmed by additional analysis with scanning electron microscopy or higher-resolution imaging.

Characteristics of the projections

On further magnification, the “spicules” of *A. salvadorensis* would be identified as decorations of the conidium wall. They are not hairs, but ridges or spines of chitin and dyes that extend from the cell surface. The spicules are very dense and have a wart or equinulate pattern. These projections are what cause the three lower clusters to have a “bristly” and irregular edge. In *A. salvadorensis*, unlike other species, these spicules tend to be more prominent and disorganized; this is what gives it that characteristic grainy texture that is observed under a microscope.

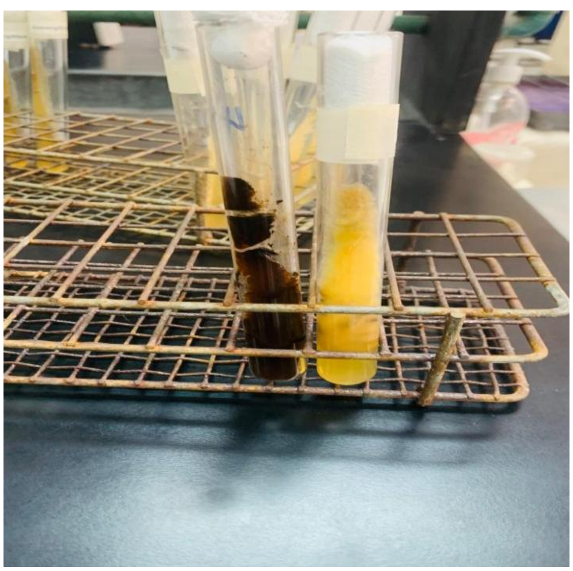
Equinulate Pattern: The texture is technically defined as either equinulate (with small spines) or warty. In this species, these projections are usually dense and blunt, giving it that “pixelated” or rough appearance compared to the more defined surface of *A. niger.* In *A. salvadorensis,* the spicules cover the entire sphere of the conidium irregularly, scattering light so that the edge looks less sharp and “bristlier”. Electron microscopy usually reveals that these structures are deposits of melanin and other polymers that protect the spore from desiccation and UV radiation. It also helps it retain moisture during droughts, like a desert cactus.

Detailed microscopic observation reveals that the spicules of *A. salvadorensis* constitute one of the most distinctive morphological hallmarks of this species. Unlike the radial and geometric pattern presented by *A. niger* at the top of the image, the structures of *A. salvadorensis* exhibit a densely ornamented surface with irregular protrusions that give it a markedly rough and “bristly” appearance. From a chemical and structural point of view, these spicules are complicated extensions of the conidium cell wall; they are not independent formations. Its basic structure is supported by a network of rigid polysaccharides, which are mainly glucans and chitin, that provide the physical basis required to sustain these projections. In this structural matrix, aspergillin and other phenolic pigments, as well as melanins, not only determine the dark color seen in the micrograph. They also function as a protective barrier against unfavorable environmental elements, such as ultraviolet radiation. Likewise, the presence of specialized proteins called hydrophobins in the outer layer of these spicules facilitates the dispersion of the spores by giving them hydrophobic properties. This structural and metabolic profile is consistent with the research carried out on the UES 001 strain. The combination of this spiculated morphology with genomic data recorded in BioProjects such as PRJNA1365736 allows to consolidate the taxonomic validity of *A. salvadorensis* as a unique biological entity within the Nigri section.

The image presented in figure (2) is a visual comparison between cultures of *Aspergillus niger and Aspergillus salvadorensis* that have been grown in tubes with Sabouraud agar, a medium that is widely used to grow filamentous fungi. The experimental setup makes it possible to observe significant macroscopic differences in the color, texture, and growth pattern of the two species, which provides valuable information for their initial morphological characterization.

In the tubes located on the left, the growth attributed to *A. salvadorensis* can be seen, which shows an intense dark coloration, with blackish-brown tones that cover a large part of the surface of the medium. The colony appears compact and dense, with marked accumulation of biomass in specific sectors of the tube. This aspect suggests a high production of pigments, possibly melanins or phenolic compounds associated with environmental protection and physiological resistance. The observed texture conveys a thicker and less powdery appearance, which could indicate differences in conidia production and dispersion.

In contrast, *Aspergillus niger*, located on the right, exhibits a lighter and more homogeneous growth, with a grayish-greenish surface characteristic of many aspergilar species that produce abundant aerial conidia. The colony appears to spread more evenly over the agar, forming a velvety or powdery-looking layer. This morphology coincides with the classic pattern of *A. niger,* known for its rapid sporulation and formation of abundant conidial structures.



Tubes in Saboroud Agar on the back of left *Aspergillus salvadorensis and right Aspergillus niger.* Microbiology Laboratory, Department of Medicine, UES.

Tubes in Saboroud Agar from Left *Aspergillus salvadorensis and right Aspergillus niger.* Microbiology Laboratory, Department of Medicine, UES.

Fig. 2. Saboraud agar tubes with growth of fungi *Aspergillus salvadorensis and A. niger.* Obtained from the Microbiology Laboratory, Department of Medicine, UES.

The comparison between the two species reveals important phenotypic differences. While *A. salvadorensis* has a darker pigmentation and apparently compact growth, *A. niger* develops a more aerial and diffuse colony. These variations could reflect genetic differences related to pigment synthesis, mycelium architecture, conidiation density, and ecological adaptation of each species.

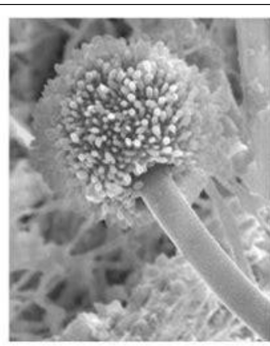
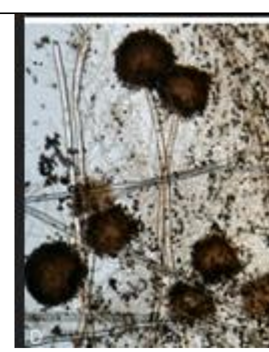
In addition, the use of Sabouraud agar makes it easier to observe cultural properties because its composition is rich in peptones and glucose, which promote fungal growth. The image indicates that the two species have a good ability to colonize the environment, although they use different morphological tactics. Intense pigmentation in *A. salvadorensis* could be an important distinguishing feature in descriptive and taxonomic research.

From a mycological perspective, this type of photographic documentation has comparative and diagnostic value, since the macroscopic characteristics of the colonies continue to be fundamental criteria in the preliminary identification of species of the genus *Aspergillus*. The observed differences between coloration, texture, and growth distribution support the idea that *A. salvadorensis* has phenotypic particularities distinguishable from *A. niger,* which strengthens its interest for later morphological and phylogenetic studies.

Morphology

Comparative analysis of the microscopic morphology of fungal species is presented in table (2). To complete the genomic information of the BioProject PRJNA1365736, it is essential to describe the phenotypic characteristics of the species that belong to the Nigri section. As detailed in *Aspergillus salvadorensis,* it has conidial heads that vary from globose to radiated, with a dark brown to black coloration that distinguishes it from the typically black density of *A. niger* and the youthful grayish tones of *A. neoniger*. In the identification of taxa within this group, chromatic and structural variability in conidial heads is considered an important diagnostic feature.

Table 2. Comparative morphologies of *Aspergillus* species.

Feature	*Aspergillus salvadorensis*	*Aspergillus niger*	*Aspergillus neoniger*
Cabezas conidiales	Globose to radiate, dark brown to black.	Typically globose, very dense, and black.	Globose, often with a slightly lighter or grayish hue in the young.
Gallbladder	Differences in the diameter and arrangement of the métulae.	Large vesicles, predominantly biseriate.	A biseriate structure with metulas that cover almost the entire gallbladder.
Conidia	Specific ornamentation; They can vary in roughness and size (microns). Conidia spiculated with armor.	Conidia are markedly rough or equinate. Similar to an equine "donut".	Conidia globose to subglobose, with fine warts.
Morphology			

At the microscopic level, the ornamentation of conidia is one of the most critical differentiating factors. While *A. niger* has equinulated conidia and *A. neoniger* exhibits fine warts, *A. salvadorensis* is characterized by having spiculated conidia provided with a “shell”. This specific ornamentation, added to the differences in the diameter of the vesicle and the arrangement of the métulas, supports its classification as an independent species under the FN 573057 and MB 860456 records.

The detailed description of the UES 001 strain, isolated in Morazán, not only enriches the mycological inventory of the University of El Salvador (UES) but also provides the necessary structural basis to understand its biotechnological potential in the production of industrial enzymes.

Software Applications in Discovery

The validation of *Aspergillus salvadorensis* at the UES is supported by a robust bioinformatics workflow:

BLAST & GenBank: For the initial comparison of the sequences obtained (such as those of the PRJNA1306032 and PRJNA1303219 BioProjects) against the global database.

Protein Modeling: The use of tools to model enzymes such as glucoamylase allows for identifying changes in active sites that could give *A. salvadorensis* an industrial advantage over *A. niger*.

Molecular phylogeny: Based on the analysis of the BLAST phylogenetic tree (*Fast Minimum Evolution method*) provided in the figure (3), the following evolutionary relationship can be established between the mentioned species:

Phylogenetic Relationship Analysis: *A. salvadorensis, A. niger, and A. neoniger* are species that belong to the same clade, which includes several members of the genus *Aspergillus*. This grouping is represented in green, indicating that they are ascomycete fungi. However, their genetic closeness varies significantly:

Closeness between *A. neoniger and Aspergillus salvadorensis*: In this graph, these two species show the closest relationship. Both have an immediate common ancestor (which is the last node before their final branching), so they are in a sister species position within this subgroup. They appear at the base of the clade that also includes *A. costaricaensis* and *A. vadensis*.

Relationship to *Aspergillus niger*: Although *A. niger* is part of the same general group of “black fungi” or section *Nigri*, it is found on a distinct and further evolutionary branch within the tree. *A. niger* appears positioned higher in the scheme, sharing an older node with species such as *A. welwitschiae*.

Conclusion of the Analysis

From figure (3), it can be determined that *Aspergillus salvadorensis* is phylogenetically more closely related to *Aspergillus neoniger* than *to Aspergillus niger*. While *A. salvadorensis* and *A. neoniger* diverge from a very recent evolutionary point, *A. niger* represents a lineage that diverged early from the branch that gave rise to the other two species mentioned.

Genomic and phylogenomic differences

Sequence analysis is the gold standard for differentiating these species, especially when the morphology is similar (cryptic species). Molecular Markers (Barcoding): While ITS rDNA is useful for the genus, distinguishing *Aspergillus salvadorensis from A. niger* requires more resolute genes such as β-tubulin (benA) and calmodulin (caM). Genetic distances at these loci show that *A. salvadorensis* possesses a unique nucleotide signature that separates it from the clades of *A. niger*.

Analysis of Ortología (KEGG/InterProScan): By bioinformatics, it is observed that *Aspergillus salvadorensis* presents variations in the clusters of biosynthetic genes (BGC). Although it shares the metabolic core of the Nigri section, the presence or absence of specific genes for the production of secondary metabolites (such as the naphtho-γ-pyrones pathway) marks a functional difference with *A. neoniger*.

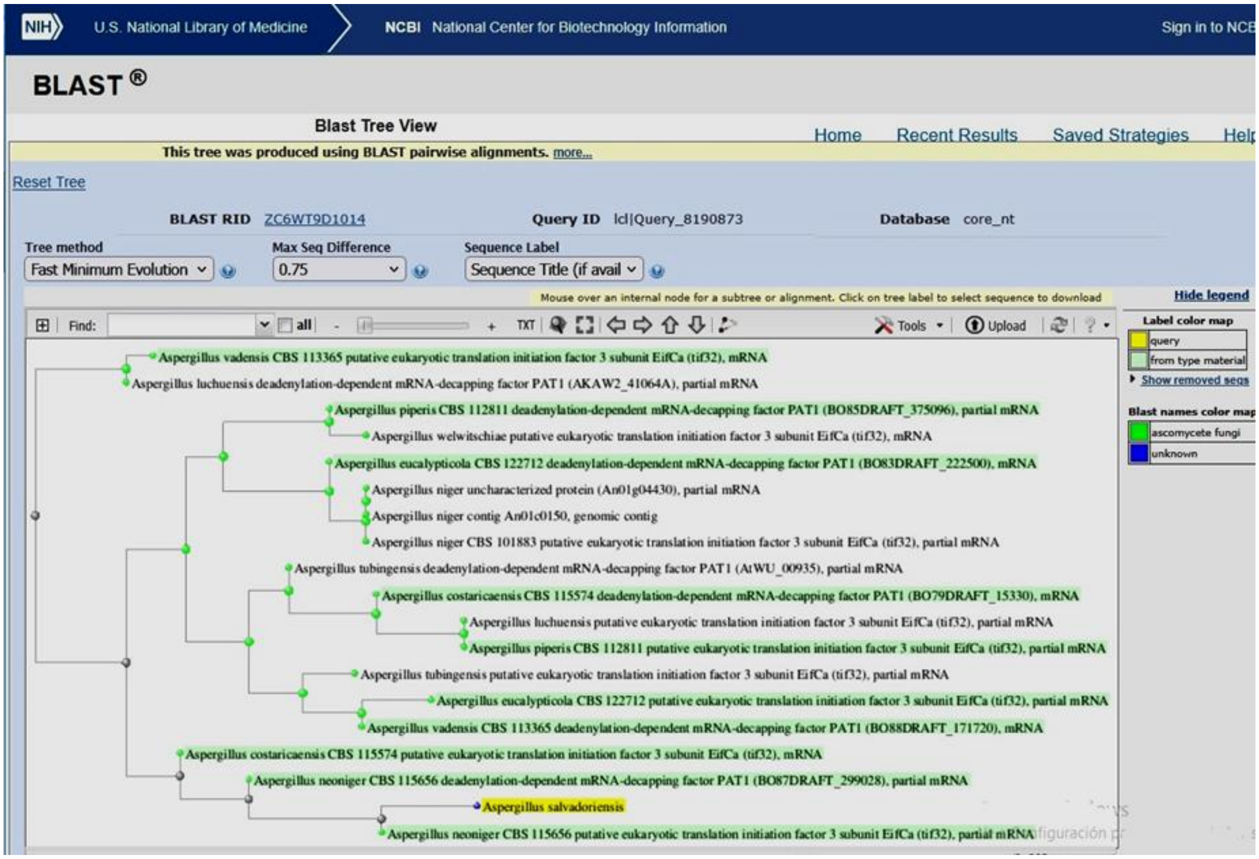


Fig. 3. Tree Phylogeny of species: *A. salvadorensis, A. niger, and A. neoniger.* Blast 2026

Genomic Similarity (ANI): The Average Nucleotide Identity Index (ANI) between *Aspergillus salvadorensis and A. niger* is usually below 95-96%, which is the bioinformatics threshold to define a distinct species.

The formal description of *Aspergillus salvadorensis* (UES 001 strain) constitutes a fundamental advance for mycology in Central America, not only because of the discovery of a new taxon, but also because of the rigor of its genomic and nomenclatural support. As evidenced in phylogenetic analysis, the sequence of the ITS marker unequivocally places this species within the Nigri section. This group of filamentous fungi is recognized for its high taxonomic complexity, where the macroscopic morphology is usually insufficient for a precise delimitation of species, making the use of advanced molecular tools indispensable.

NCBI's BioProject PRJNA1365736 supports that this strain is not just a variant of *Aspergillus niger or Aspergillus neoniger*, but is an independent species. Within the framework of the Nigri section, a polyphasic perspective is often used to achieve a conclusive taxonomic resolution that incorporates markers with more variability, such as beta-tubulin (BenA) and calmodulin (CaM), which verify the uniqueness of the UES 001 strain. Its legal and academic status with respect to the International Code of Nomenclature of the species *A. salvadorensis* is confirmed by its official designation as a type strain (Type Strain) under the FN 573057 and MB 860456 registrations.

From a biogeographic perspective, the isolation of this species in the area of Morazán, El Salvador, highlights the importance of neotropical ecosystems as reservoirs of unexplored fungal biodiversity. The precise georeferencing of the sample linked to the University of El Salvador (UES) is crucial to understand the evolution of fungal lineages in specific environments, suggesting that the Morazán strain has evolved distinctive genetic adaptations.

Finally, the biotechnological potential derived from this finding is vast. Having the complete genome under the PRJNA1365736 registry facilitates the identification of biosynthetic clusters for the production of industrial enzymes, such as glucoamylase. Since *A. niger* is the global standard in the fermentation industry, the discovery of a sister species adapted to local conditions offers a strategic opportunity for the development of indigenous bioprocesses and the optimization of enzyme expression platforms in the region.

DISCUSSION

Differentiating between *Aspergillus niger, Aspergillus neoniger, and Aspergillus salvadorensis* presents one of the greatest challenges within the Nigri section, as these species are virtually indistinguishable using traditional morphological methods. Historically, the clade was designated as *A. niger*, but the introduction of molecular phylogeny has revealed it to be a complex of cryptic species (Perrone *et al.*, 2011). While *Aspergillus niger* is a cosmopolitan species with a high citric acid production capacity and a consistent presence in industrial settings (Khurshid *et al.*, 2024), *Aspergillus neoniger* is distinguished primarily by subtle genetic variations in the calmodulin and β-tubulin loci, frequently inhabiting soils and marine environments (Silva *et al.*, 2020). *Aspergillus salvadorensis*, on the other hand, is emerging as a relevant taxonomic entity in tropical regions, having been identified in specific ecosystems such as the coffee plantations of El Salvador (Vásquez, 2025a). Although all three species share the characteristic of possessing black, globose conidial heads, their chemical profiles exhibit critical variations: *A. niger* is a known producer of fumonisins and, to a lesser extent, ochratoxin A (Al-Jobory, 2025; Al-Jobory *et al.*, 2017; Humaid *et al.*, 2019; Iqbal *et al.*, 2021), while in *A. salvadorensis* and *A. neoniger* the expression of these toxins may be nonexistent or vary drastically depending on environmental stress conditions. This taxonomic delimitation is fundamental not only for systematic mycology but also for food safety, since the correct identification of *A. salvadorensis* in regional crops allows for a more accurate prediction of the risk of fungal contamination in export products (Varga *et al.*, 2011).

The academic discussion surrounding the nigri section of the genus *Aspergillus* has undergone a profound transformation, shifting from a purely morphological classification to a multi-faceted approach that integrates molecular phylogeny and secondary metabolite profiling. In this context, the distinction between *A. niger*, *A. neoniger*, and *A. salvadorensis* is crucial, as although they share macroscopic characteristics such as charcoal-black colonies and globose conidial heads, their genomes reveal significant divergences. The use of genetic markers such as calmodulin and β-tubulin has become the gold standard for resolving these cryptic species, allowing *A. neoniger* to be identified as a distinct entity that, unlike the ubiquitous *A. niger*, tends to colonize more specific environments, including marine sediments and diverse soils, while maintaining a fumonisin production capacity similar to that of the type species (Silva *et al.*, 2011; Varga *et al.*, 2011).

On the other hand, the emergence of *Aspergillus salvadorensis* in the scientific literature underscores the importance of regional biodiversity and ecological specialization within the clade. This species, described in greater detail in studies of tropical regions, shows a particular adaptation to agricultural substrates such as coffee, where its presence was historically confused with that of *A. niger*. Unlike industrial lineages of *A. niger*, which are optimized for the production of organic acids and enzymes, strains of *A. salvadorensis and A. neoniger* can exhibit variations in ochratoxin synthesis, requiring health authorities to perform precise molecular identifications to assess the actual toxigenic potential of affected lots (Frisvad *et al.*, 2011).

Finally, the integration of these findings into industrial practice and quality control highlights that morphology alone is insufficient to guarantee food safety. The literature emphasizes that the correct delimitation of these three species allows for a better understanding of their life cycles and their resistance to fungicides or disinfection processes (Varga *et al.*, 2011). While *A. niger* remains the quintessential biotechnological reference, the recognition of *A. neoniger and A. salvadorensis* as opportunistic pathogens or agricultural contaminants with their own chemical profiles is a critical step for modern mycology. The ability to distinguish these taxa through multi-locus sequencing not only enriches systematic taxonomy but also provides the necessary tools to mitigate human exposure to mycotoxins in products derived from regions with high fungal diversity.

CONCLUSION

While *A. niger* is the cosmopolitan and industrial reference, and *A. neoniger* is a closely related but distinct species, *Aspergillus salvadorensis* is consolidated as a species with its own genomic profile, adapted to the environmental conditions of the Morazán region, which suggests a unique biotechnological potential in the production of enzymes and metabolites.

Definitive taxonomic validation: The identification of *Aspergillus salvadorensis* as a new and distinct species within the Nigri section is confirmed. This classification is supported by a polyphasic taxonomy that integrates the phylogenetic analysis of the ITS marker and official records in Fungal Names (FN 573057) and MycoBank (MB 860456).

International genomic support: The successful integration of sequencing data into the GenBank genebank, under the PRJNA1365736 label, ensures that the genetic information of this species is visible and traceable worldwide. This determines the UES 001 strain as a type strain, that is, a technical reference for the scientific community worldwide.

Biological heritage of El Salvador: The finding underscores the importance of the Morazán region as a reservoir of fungal biodiversity with unique evolutionary characteristics. The institutional link with the University of El Salvador (UES) strengthens the country's capacity to document and protect its own genetic resources.

Capacity in industrial biotechnology: The analysis of this species opens strategic avenues to create indigenous biotechnological platforms, in particular for the mass production of industrial enzymes such as glucoamylase. Using tools for gene editing to refine fermentation processes in the future is easier if you have access to the entire genome.

Contribution to mycological knowledge: This research not only expands the catalog of the genus *Aspergillus* but also provides essential comparative data to differentiate related species such as *A. niger and A. neoniger*, enriching the understanding of the phylogeny of black aspergills in the Neotropics.

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CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

GENERATIVE AI STATEMENT

This study used Generative AI tools in data reorganization. We confirm that all AI-assisted processes were critically reviewed by the author's to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the author’s.

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REFERENCES

Al-Jobory, H.J., 2025. A Feast or a Threat? Multi-Mycotoxin Contamination in Sun-Dried Fish, Collected from Al Mukha Markets, Taiz, Yemen. PSM Microbiol., 10(1): 30-43.

Al-Jobory, H.J., Mahmoud, A.L., Al-Mahdi, A.Y., 2017. Natural occurrence of Fusarium mycotoxins (fumonisins, zearalenone and T-2 toxin) in corn for human consumption in Yemen. PSM Microbiol., 2(2): 41-46.

Bowman, L., Sheridan, S., Wham, B.E., Wright, S., 2023. FASTA/FASTQ data curation primer.

Bradshaw, M.J., Aime, M.C., Rokas, A., Maust, A., Moparthi, S., Jellings, K., Pane, A.M., Hendricks, D., Pandey, B., Li, Y., Pfister, D.H., 2023. Extensive intragenomic variation in the internal transcribed spacer region of fungi. iSci., 26(8): 107317.

Echevarría, L., Iqbal, M.N., 2021. Identification of Fungi and Yeasts from the Sands of the Pyramids of Giza, in Cairo, Egypt. PSM Biol. Res., 6(1): 13-18.

Ezeonuegbu, B.A., Abdullahi, M.D., Whong, C.M.Z., Sohunago, J.W., Kassem, H.S., Yaro, C.A., Hetta, H.F., Mostafa-Hedeab, G., Zouganelis, G.D., Batiha, G.E., 2022. Characterization and phylogeny of fungi isolated from industrial wastewater using multiple genes. Sci. Rep., 12(1): 2094.

Frisvad, J.C., Larsen, T.O., Thrane, U., Meijer, M., Varga, J., Samson, R.A., Nielsen, K.F., 2011. Fumonisin and ochratoxin production in industrial *Aspergillus niger* strains. Plos One., 6(8): e23496.

Humaid, A.A., Alghalibi, S.M., Al-Khalqi, E.A.A., 2019. Aflatoxins and ochratoxin a content of stored yemeni coffee beans and effect of roasting on mycotoxin contamination. Int. J. Mol. Microbiol., 2(1): 11-21.

Iqbal, M.N., Iqbal, I., Muhammad, A., Shahzad, M.I., 2021. A Review of Mycotoxins Produced by Fruit Spoilage Fungi: Mycotoxins from Fruit Spoilage Fungi. PSM Biol. Res., 6(2): 46-49.

Khurshid, S., Ashraf, H., Hussain, T., Iqbal, M., Qureshi, H., Anwar, T., Salmen, S.H., Ansari, M.J., 2024. Enhanced Citric Acid Production through *Aspergillus niger*: Insights from Fermentation Studies Using Sugarcane Molasses. Life., 14(6): 756.

Lučev, M., Stepanović, J., Kandić Raftery, V., Čamdzija, Z., Obradović, A., Stevanović, M., Stanković, S., 2025. Overview and Diversity of Fungi of the Genus *Aspergillus* Section Nigri on Maize and Small Grains. Foods (Basel, Switzerland)., 14(12): 2146.

NCBI, 2025a. UNIVERSIDAD DE EL SALVADOR. Genoma sequencing *Aspergillus ues.salvadorensis*. .

NCBI, 2025b. UNIVERSIDAD DE EL SALVADOR. Phenotypic and Genotypic Characterization of *Aspergillus ues.salvadorensis*.

Perrone, G., Stea, G., Epifani, F., Varga, J., Frisvad, J.C., Samson, R.A., 2011. *Aspergillus niger* contains the cryptic phylogenetic species *A. awamori*. Fungal Biol., 115(11): 1138-50.

Raina, V., Nayak, T., Ray, L., Kumari, K., Suar, M., 2019. Chapter 9 - A Polyphasic Taxonomic Approach for Designation and Description of Novel Microbial Species. In: Das, S., Dash, H.R. (Eds.), Microbial Diversity in the Genomic Era. Academic Press, pp. 137-152.

Silva, D.M., Batista, L.R., Rezende, E.F., Fungaro, M.H.P., Sartori, D., Alves, E., 2011. Identification of fungi of the genus *Aspergillus* section nigri using polyphasic taxonomy. Braz. J. Microbiol., 42(2): 761-773.

Silva, J.J.d., Iamanaka, B.T., Ferranti, L.S., Massi, F.P., Taniwaki, M.H., Puel, O., Lorber, S., Frisvad, J.C., Fungaro, M.H.P.,

2020. Diversity within *Aspergillus niger* Clade and Description of a New Species: Aspergillus vinaceus sp. nov. J. Fungi., 6(4): 371.

Vanzela, D.O.A., Massi, F.P., de Oliveira, A.L.M., Fungaro, M.H.P., Sartori, D., 2020. Isolation and Identification of *Aspergillus* Section Nigri, and Genotype Associated with Ochratoxin A and Fumonisin B2 Production in Garlic Marketed in Brazil. Curr. Microbiol., 77(7): 1150-1158.

Varga, J., Frisvad, J.C., Kocsubé, S., Brankovics, B., Tóth, B., Szigeti, G., Samson, R., 2011. New and revisited species in *Aspergillus* section Nigri. Stud. Mycol., 69(1): 1-17.

Vásquez, A., 2025a. *Aspergillus salvadorensis* sp. nov.

Vásquez, A., 2025b. Prevalence of Secondary Metabolites Target Carcinogenic Clusters in the Circular DNA Sequence of *Aspergillus salvadorensis* to Aflatoxins. PSM Microbiol., 10(1): 177-196.

Vásquez, A., 2026. Immunoinformatic-Genomic Analysis of the ORF69 Protein Located in the Capsule of *Aspergillus salvadorensis* for the Design of Vaccines, University of El Salvador (2025-2026).