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Characterization of *Aspergillus salvadorensis* Isolated from *Caeselpinia coriaria* Seed, El Salvador.

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ABSTRACT **Original research paper**

The study focused on the identification the phenotypic and genotypic characterization of the *Aspergillus* genus using the Next-Generation Sequencing (NGS), which was sent to MACROGEN SOUTH KOREA Illumina NovaSeq 6000. Carried initial phase involved the collection of *Caesalpinia coriaria* seeds. The second phase began in 2006 with the initial macroscopic and microscopic characterization and isolation of *Aspergillus.* The gDNA was extracted, yielding a maximum concentration of 12.297 ng/µl (with a volume of 30 µl and a total amount of 0.369 ng), and a DNA Integrity Number (DIN) of 6.4. The maximum sample intensity was observed for 15000 bp during the Tape Station gDNA Screen quality control. qPCR analysis showed a qDNA fragment of 624 bp at a concentration of 103.24 nM (41.87 ng/µl). For the sequencing process, a total of 11,705,895,990 bp and 77,522,490 total reads were obtained using the TruSeq Nano DNA library. The nucleotide content was 49.7% GC and 50.3% AT. Quality metrics were excellent, with Q20 at 95.1% and Q30 at 88.3%, confirming high data quality. After quality and adapter trimming, and contaminant elimination, the raw data value was refined from 38,761,245 N to 30,961,740. Krona taxonomy and Heatmaps confirmed the genus as *Aspergillus*. In conclusion. The Aspergillus sp was successfully identified showing a variety of species, which aligns with previous studies in 2006 where it was named *Aspergillus salvadorensis*. The ITS and BenA sequences are not identical known species, which validates their identification as the new taxon *Aspergillus salvadorensis*.

Keywords: *Aspergillus salvadorensis*, *Caeselpinia coriaria*, ITS, BenA.

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Introduction

A research project focused on the Nacascol seed in El Salvador, which requires the presence of an *Aspergillus sp* fungus to be pulverized and used for dyeing pottery (yielding a characteristic black clay) and tanning leather. The fungus facilitates the extraction of tannins (for brown color) and contributes to the intense black color when combined with clay's iron. *Aspergillus* species are widespread saprophytes found in organic material, exhibiting high temperature adaptability (typically 5∘C to 60∘C). It is a diverse genus comprising 260 to 837 species. Fungal pigmentation, including melanins, is crucial for protection against temperature and radiation. The research project, which began in 2006 with phenotyping and culminated in 2024 with Next-Generation Sequencing (NGS) Illumina NovaSeq X Plus in South Korea, aims to extract, identify, and characterize the native *Aspergillus* species circulating in El Salvador through phenotypic and genotypic analysis.

Material and methods

Tools Sequencing accuracy was assessed per cycle, with each cycle representing the incorporation of a single base (A, T, C, or G), detected by fluorescent signals. Cycle-by-cycle sequencing ensured high precision. To identify genes associated with enzymes, proteins, and secondary metabolites. The study for the characterization of the Aspergillus fungus was conducted in two methodical phases:

[1]**GenBank ID: PRJNA1306032. Mycobank MB 860456**. *Aspergillus uessalvadorensis is clarified* and will be called *Aspergillus salvadorensis*.

Phenotypic Characterization

The Phenotypic Characterization began with the collection of five batches of Nacascol seeds (*Caesalpinia coriaria*) from the northern zone, followed by their purification and selection. Inoculant were prepared in Sabouraud culture medium, and growth was observed after 48 hours under an optical microscope at 40x and 100x. This phase included taking electron microphotographs at CENSALUD and performing a preliminary PCR in Mexico.

Genotypic Characterization

Genotypic Characterization focused on advanced molecular analysis. Three samples were sent to MACROGEN INC laboratories in South Korea for the extraction, analysis, and identification of various forms of DNA, including gDNA, qDNA, dsDNA, cDNA, and seqDNA, with the goal of determining the species. Laboratory procedures included preparing a spore suspension from the culture, re-seeding it, and counting it with a Neubauer chamber to ensure adequate concentration. A microculture was also performed in a sterile wet chamber at CENSALUD, where the sample was incubated for 7 days, and characteristic structures were observed with Lactophenol Blue under a 100x objective. [10,11,12]Nacascol seeds were collected and stored at room temperature.

Morphological Characterization

For Morphological Characterization, fungal samples were cultured in Sabouraud and incubated at 37∘C for 72 hours and then at room temperature, with structures observed using the same stain under an optical microscope. Isolation and Purification were achieved by scraping the material from the seed and depositing it on Sabouraud Dextrose Agar (ASD) until pure isolates were obtained, which were then inoculated into liquid medium (PW) and incubated before drying the biomass at 42∘C. Regarding DNA Methods, both the Sanger sequencing method and, primarily, the Illumina platform were used. Illumina is characterized by amplifying DNA fragments using bridge PCR to generate clusters and detecting bases with fluorescent markers, allowing fragments to be sequenced from both ends. gDNA Extraction aimed to release intracellular DNA by breaking the fungal cell wall, concentrating and purifying the target molecules without degradation, through chemical, enzymatic, or mechanical methods. Finally, the qPCR (Quantitative DNA) technique was used for quality control and quantification of the fragmented DNA. DNA quality control at MACROGEN was performed using the QuantiFluor dsDNA System and a microplate reader, which more accurately quantifies double-stranded DNA. It was noted that the *Aspergillus* genus has a variable genome size (approximately 27 to 39 Mb) with an average of 9,000 to 14,000 genes approx. to 99,980,000 pb. Quality per Cycle refers to evaluating the quality of sequencing reads in each cycle, where an incorporated base is detected and recorded using an optical signal.

Results

Taxonomic Description

Etymology

The specific epithet *salvadorensis* refers to El Salvador, the country where the species was first isolated. Diagnosis is *Aspergillus salvadorensis* is differentiated from closely related species by a unique combination of morphological and molecular characters. Material examined (Holotype). Isolation from Nacascol seeds from the northern area of Morazán El Salvador, collected in February 2006, deposited in the culture collection of the University of El Salvador (UES-1) as a type strain. Reference sequences deposited in GenBank under BioProjects PRJNA1306032 and PRJNA1303219. Habitat and distribution Isolated from plant matter in seeds and soil. El Salvador. So far, it has only been reported in this region. Taxonomic notes *Aspergillus salvadorensis*belong to the *nigri* section of the genus *Aspergillus*, where it forms a distinct and well-supported monophyletic clade. Consistent differences in the ITS, β-tubulin, calmodulin, and rpb2 sequences support its recognition as an independent species. The combination of molecular, phylogenetic and morphological evidence justifies its designation as a new species. In addition, it is a producer of black dye from fungus. Phylogenetically, it forms a well-supported monophyletic lineage within the genus *Aspergillus*, separated from related species in multilocus analyses (ITS, BenA). Morphologically, it presents distinctive characteristics in the coloration of the colonies, the morphology of the conidiophores and the ornamentation of the conidia.

Description

Microculture Procedure

The initial stage involved isolation and phenotypic observation. The brownish black Nacascol seeds were collected and stored at room temperature. Instead of being cleaned, the scraped material from the seeds was directly inoculated onto Sabouraud Dextrose Agar (ASD) plates for isolation, and this purification process was repeated until pure fungal isolates were obtained. Taxonomic features were then identified using the microscope and online databases. For the molecular analysis, the fungal biomass was cultivated in PW liquid medium at 37∘C, dried at 42∘C, and stored. The main goal of genomic DNA (gDNA) extraction was to release the intracellular DNA by breaking the fungal cell wall (through chemical, enzymatic, or mechanical methods) and purifying it by removing contaminants without degrading the nucleic acid. The qPCR (quantitative polymerase chain reaction) technique was used to measure DNA concentration via fluorometry (using the QuantiFluor dsDNA System), which provides greater accuracy than spectrometry. Once the DNA met quality control requirements, it was fragmented and prepared for sequencing. General methods like Sanger sequencing were mentioned, and more specifically, the

Illumina NovaSeq 6000 platform. The latter technology involves DNA fragment amplification from mycelia into clusters via bridge PCR and base detection using fluorescent markers. This allows sequencing from both ends and the analysis of various DNA types (gDNA, qDNA, cDNA, etc.). The reliability of the results is ensured by evaluating the quality of the reads at every cycle of the sequencing process. Sequencing accuracy was evaluated at each cycle. The reliability of the sequencing results was guaranteed by a meticulous base detection process. In every sequencing cycle, a single base (A, T, C, or G) was added, and this incorporation was detected through fluorescent signals, ensuring precise, step-by-step sequencing. To identify the genes related to enzymes, proteins, and secondary metabolites, MACROGEN utilized several specialized databases. These included Egg-NOG, which categorizes orthologous groups (COGs); Meta-Cyc, which provides detailed information on metabolic pathways, enzymes, and reactions; UniRef, which clusters protein sequences; and KEGG, which focuses on annotating gene functions. These tools allowed for the classification of the genes and metabolic pathways specific to *Aspergillus salvadorensis*, enhancing the understanding of its biological capabilities. Extraction of the black dye (Melanin) The extraction of melanin, the black pigment produced by the *Aspergillus* fungus under laboratory conditions, is a methodical procedure due to the pigment's inherent insolubility. The process begins with the controlled cultivation of *Aspergillus* on Sabouraud Agar medium, which supplies essential nutrients like carbon and nitrogen sources. The culture is grown at room temperature or slightly higher (approximately 25∘C to 30∘C) with high humidity. Submitting the fungus to environmental stressors, such as pH changes or UV radiation, stimulates melanin production.[55]

Phenotypic [5,8,51]

NACASCOL whose scientific name is *Caeselpinia co*riaria, of the family *Caesalpnieaceae*, of the genus *Caesalpinia*, a leguminous plant with a stem from 3 to 11 meters high, with leaves in pairs pinnae 5 to 10 cm long, each with more than 10 leaflets 4 to 8 mm long and 2 mm wide, The seeds are about 3 to 4 cm long, apex rounded, brown seeds of black appearance, from which grows a fungus whose resemblance is to a possible *Aspergillus* sp. The *Caeselpinia coriaria* tree contains tannins, triterpenes, glycosides, and flavonoids. The fungus reproduces exclusively in the seeds of the Nacascol tree, from which it is prepared for the extraction and preparation of the dye and then cultivated in vitro for the production of the dye by Salvadoran artisans. The fungus found in the seeds is described as follows: Sterygmas. The head of the conidia is black, smooth conidiophore 1 to 4 µm long with moderate conidia or internal spores of 1 to 3 microns, brown to black. It is a hyaline, saprophytic filamentous fungus, belonging to the phylum Ascomycota.

Macroscopic characteristics

When grown in special Saboraud medium, they produce a black coloration with production of pigment in the tube in the posterior region of the tubes within 7 days. In the cultivation of Saboraud agar in plate and tube: the colony its mycelium is white with columnar appearance and then turns black with a size approx 30-40 mm. The size can vary significantly depending on the culture medium and incubation temperature used in the study. The texture of the colognes, they look like cotton or velvet, there are no sclerotes. The reverse of the colony is black or pigmented black depending on the oxidizing agent, low height of the mycelium, appearance of the colony is dusty black. It is a filamentous fungus. [14,31,43] The fungus, a saprophytic, filamentous hyaline organism belonging to the phylum Ascomycota, exhibits distinct colonial and structural features: Culture Growth: When grown on Sabouraud medium, the colony initially displays white, columnar mycelium that later turns black. The texture is cotton-like or velvety, and no sclerotia are observed. In culture tubes, the fungus produces a visible black pigment at the bottom within seven days. The reverse side of the colony appears black or pigmented, with a dusty black, low mycelium appearance. Microscopic features: The conidiophore of Aspergillus has three distinct parts: a terminal globose vesicle with a uniseriate phialide without metal, a tubular stipe, and the foot cell that joins the conidiophore to the mycelium. Conidiophores are smooth and rough and clump together to form a compact, hyaline or pigmented mass and are 3 to 5 µm long and 15 to 20 µm in diameter. Hundreds of spores or conidia are observed inside. The spore is spherical and irregular, aseptate, ameroporous, colored and dark of the columnar pheospore type, large, with a diameter of 1 to 3 µm. The vesicle is globose with 52-66 µm in diameter with a stipe length of 100-300 µm, a stipe width of 2-2 µm, irregular ornamentation and produces phialide around it. The phialide are monoseriate, the primary branches are 30 µm long, short and 8 microns long, from which the conidia sprout, which are globose and rough 4 to 5 µm in diameter, brown or brown to black. The diameter of the colony is 9-10 µm, the conidia are columnar and have a length of 3-5 µm and a width of 1.96-2 µm. Heads of smooth conidia with a round and irregular wall, arranged in a column; smooth, pronounced, thin-walled, brown to black stipes; no columella is observed; there are abundant conidia that detach from the head; It has a row of phialides. The sporangium is a simple globose peridial structure, the sterygma is black. Mature brown conidia are spherical with abundant spiny projections throughout the periphery; and the central ones are few, forming spicules in number greater than ten. The conidia have a spherical appearance in the form of a mallet with spicules, from the ends emerge long filaments arranged in the form of linear chains that come out of the protuberances. The entire structure forms a solid shell, It is characterized by not dyeing with dyes. Immature asexual spores are spherical, thin-walled, colorless, large in size, which then fill in to form internal brown masses. The conidiophore is long, smooth,

uniseriate, with abundant spores. In general, the spore is elongated and irregular; Hyphae are not septate with continuous mycelium. The head is not radiated, it is columnar, it is uniserial, from which the conidia are detached, it grows at room temperature of 37°C.Immature asexual conidia/spores are irregular with a thin, colorless wall of large size, which are then filled in forming brown inner masses, the appearance of the colony is powdery black. The mycelium is short or flattened and thin and black as it ages. This species of Aspergillus found, it is suggested that given its morphological characterization it is another found in El Salvador. When comparing both the *niger* and the discovered species, the vesicle of the *niger* species is irradiated with round conidia and the other discovered is in the form of an elongated mallet without irradiation. At 100x it can be seen that the niger conidia are round with thick walls and a smooth biserial, Metula and radiated center [46]. The one of the new species has spicules coming out of the armor. Both conidia can be observed, in which it is highlighted that the conidia of the new species are not stained with cotton blue lactophenol and those of niger are [51].*Aspergillus* has asexual reproduction because it has conidia formation, at first the mycelium is white and then after weeks at room temperature it changes to black in 7 days, with progressive increase in brown sporulation. These grow at the ends of the phialide. Due to the presence of pigments, it looks black in color. The fungus of the genus *Aspergillus spp* has the particularity of being mycotoxin producers. Mycotoxins are secondary metabolites produced and secreted by the fungus during the degradation process of organic matter, as a defense mechanism against other microorganisms. [43-14,17]. The hyphae continue their way in a conidiophore that at its terminal end ends in a globose conidial head from which the phialide emerge and the spores or conidia detach to return to their cycle. Each hypha, at its end, forms globose conidial heads that can produce more than 500,000 conidia. [31]. These conidia at 100x have a rough appearance like pectorals, thick wall with brown spicules projecting outward, filled and whole that are not colored with cotton blue lactophenol. From the base of the hyphae, each conidia is pushed into the conidiophore to the end until it reaches the vesicle where it accumulates, exerting a mechanical effect of pressure outwards from where it emerges from the phialides to the outside, this is constant over and over again. Hyphae are filamentous cylindrical structures that form the body of multicellular fungi. They are made up of a row of elongated cells wrapped by a chitinous cell wall. The cells that make it up may or may not be separated from each other by a transverse cell wall called septa. These conidia are considered infectious and constitute the starting point for the development of the mycelium of the fungus. In summary, it belongs to the Kingdom Fungi, Filo Ascomycota, Genus *Aspergillus*, Species *salvadorensis*. It is an active metabolite. [9,11, 34,35,36,40,41,47,48-50,51].

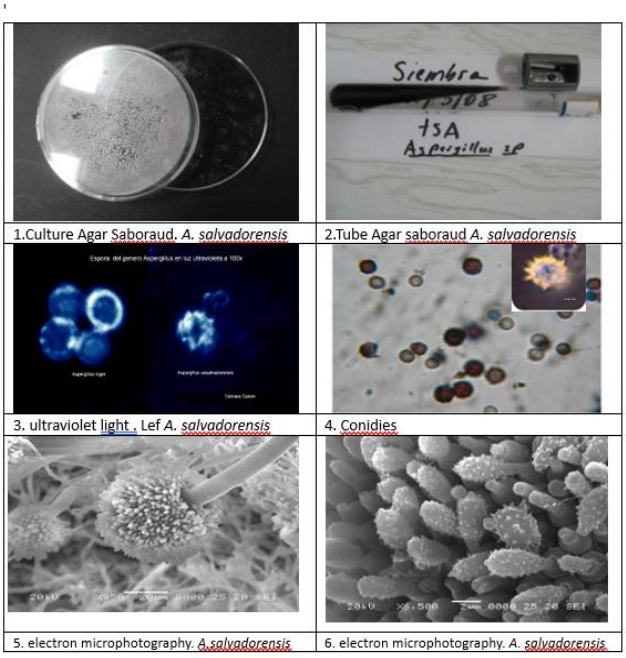

Figure 1. *Aspergillus salvadorensis.* Phenotypic characterization.
1. Culture Agar saboraud
2. Culture tuve Saboraud
3. Ultravioleta light
4. Aspergillus conidial heads 100x
5. Electron microphotography 2550x
6. Electron microphotography 10000x

Figure 1 Fungal Morphology and Adaptation to Heat. It was grown on saboraud agar in which the black colonies are observed on plate and back of the tube in photos 1 and 2, in photo 3 it is observed that in ultraviolet light qualitatively there is a difference between the *niger and salvadorensis species*, in the first there is a halo around and in the second there is the presence of spicules it is not dyed with cotton blue lactophenol, other species fungus do, In photo 4 there are conidia at 100x that reflect spicules and compact armor, in 5 and 6 it is an electronic microphotograph in which a conidiophore and vesicle plus conidia in the form of a cactus are observed at 2550x and 10,000x . [4]

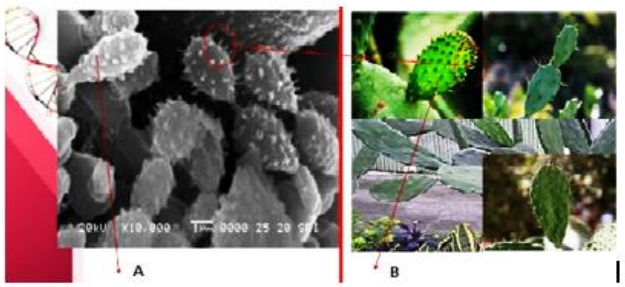

Figure 2. Comparison of species with natural cactus plant.

En la fig 2. The Aspergillus species studied shows an adaptation to extreme heat, a trait visually compared to a cactus plant. The fungus's spiculated conidia (spores covered in fine, spine-like projections) are believed to provide heat resistance and help retain moisture, allowing the fungus to survive temperatures above 60−100∘C. This heat adaptation

is significant because while high humidity typically stops the growth of toxin-producing fungi like those that create aflatoxins, this particular *Aspergillus* thrives under heat. Microscopy confirmed rapid and prolific sporulation, with conidia emerging from the uniseriate phialides of the vesicular column within two weeks of culture. Extremely fast sporulation was recorded millions of spores were observed in ten visual fields within an hour. The fungal body consists of a rough, hyaline conidiophore with a globose head and hyphae that elongate and accumulate filaments. Racket-shaped or in a bottle conidia were also observed. Spore Characteristics and Biological Structure The simple biological cycle of *Aspergillus* involves the formation of spores for reproduction, which germinate into hyphae, the fungus's invasive form. The high rate of sporulation and high environmental concentration are attributed to the spores being found in angiospores. The small size of the conidia, varying between 0.2 and 3.5 microns, is particularly noteworthy as it allows them to be easily inhaled into the pulmonary alveoli, which measure only 0.2 to 0.5 mm. The spores' resistance to high temperatures comes from their hard outer shell, and some spores contain melanin for added heat protection. This resilience enables them to travel long distances and colonize new areas, maturing rapidly in less than five days. Fungal cells are protected by a rigid wall composed of about 80% carbohydrates, including chitosan, chitin, peptidoglycans, and mannans—but unlike plants, they mostly lack cellulose. This wall also contains proteins, carbohydrateslipids, and minerals. Proteins are used for nitrogen and growth, carbohydrates and lipids for energy In the lab, chitin is often softened with potassium hydroxide for morphological analysis. Comparison to Related Species and Genomics A comparison of the studied species (*A. salvadorensis*) with A. niger revealed clear differences in sporeproducing structures. *A. niger* has a round, radiate head that is biseriate (having two rows of phialides), while *A. salvadorensis* has an elongated columnar, mallet-shaped head that is uniseriate (one row of phialides) and lacks a vesicle. Globally, the Aspergillus genus is vast, with Rapper and Fennell reporting about 900 known species.

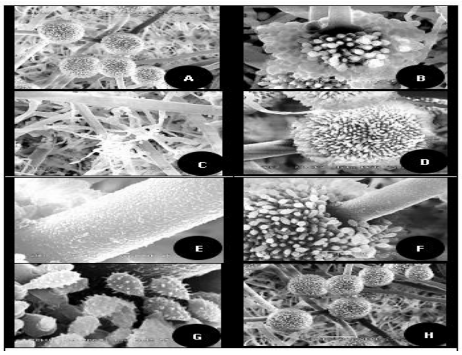

Figure 3. Aspergillus salvadorensis Scanning electron microphotography. Courtesy of CENSALUD by Dr. Vianney de Abrego. A.Morphology Aspergillus, B. vesicle, C: Zapata, elongation of hyphae with filament and branching D. Head of conidia, E. rough codiophore, F. globose and fialide vesicle, G. conidia, H. head of columnar. 1000x y 100,000x

Only 12 species are commonly associated with human diseases. Generally, *Aspergillus* species have a genome size ranging from 30 to 40 megabases (Mb), which is equivalent to 30 to 40 million base pairs (bp), *A. salvadorensis* has 11,705,895,990 bp.[1,2,3,4,5,6,7,8,9,18,19,20,12,49]

In Fig 3 you can see electron microphotographs of *Aspergillus salvadorensis* where the conidiophore can be seen in a,e, f, the vesicle in b,c,d, and the conidia in f,g at 1000x and 100,000x.

qPCR technique

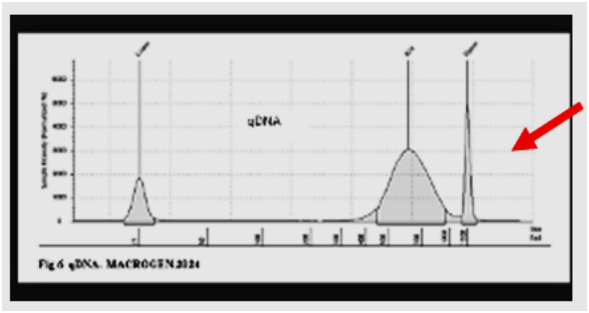

Figure 4. qDNA. Extraction *Aspergillus salvadorensis* MACROGEN INC. 2024

Figure 4. Molecular Quantification and DNA Library Preparation. DNA Quantification and Quality (qDNA). Analysis of the genomic DNA (gDNA) sample from El Salvador, conducted using LightCycle qPCR, yielded a concentration of 103.24 nM and 41.87 ng/µl for a 624 bp fragment. The TruSeq Nano DNA (350_META) library was utilized. These values indicate that the DNA had been prepared and analyzed to meet high standards of purity and quality, a prerequisite for molecular biology applications like sequencing. In general, concentrations above 10 ng/µl are considered ideal for sequencing, as they ensure sufficient genetic material for accurate readings. Low concentrations, conversely, may require reextraction or additional concentration. The number of base pairs (bp) needed to sequence a fungus depends on the study's objective; millions of base pairs are usually required for complete genomic coverage, given that fungal genomes range from a few megabases (Mb) to over 100 Mb. Complementary DNA (cDNA) Library For the subsequent phase, a total of 33 library kits were used for cDNA. This technique is critical for creating a complementary DNA (cDNA) copy from the organism's messenger RNA (mRNA) via reverse transcription. The resulting cDNA is then used to construct the sequencing library. The construction of the cDNA library involves assessing its quality and representativeness. Visualizing the fragments of the extracted double-stranded DNA is vital: short fragments may indicate RNA degradation, while unusually long fragments suggest issues with fragmentation during preparation. In the final process, adaptors are added to the ends of the cDNA fragments so they can bind to the flow cell surface of the NovaSeq platform Illumina (5 to 10 nM concentration) and be amplified during sequencing. [37,21,22]

dsDNA

Raw Data Statistics

The total number of bases, reads, GC (%), Q20 (%), and Q30 (%) are calculated for the 1 sample(s).
For example, in Aspergillus sample, 77,522,490 reads are produced, and total read bases are 11.7 Gbp.
The GC content (%) is 49.7% and Q30 is 88.3%.

* Raw Data

Sample ID	Total bases (bp)	Total reads	GC (%)	AT (%)	Q20 (%)	Q30 (%)
Aspergillus	11,705,895,990	77,522,490	49.7	50.3	95.1	88.3

- Sample ID : Sample name.
- Total Bases (bp) : Total number of bases sequenced.
- Total Reads : Total number of reads. For illumina paired-end sequencing, this value refers to the sum of read1 and read2.
- GC (%) : Ratio of GC content.
- AT (%) : Ratio of AT content.
- Q20 (%) : Ratio of bases that have phred quality score of over 20.
- Q30 (%) : Ratio of bases that have phred quality score of over 30.

Table 1. Raw data.Macrogen. 2025

In Table 1. Analysis of Sequencing Data and Quality Control. Double-Stranded DNA and Total Bases. A high concentration of double-stranded DNA (dsDNA) was confirmed, which is necessary for accurate sequencing, with concentrations above 10 ng/µl being ideal. During the sequencing process, this dsDNA is denatured into single strands for reading the nucleotide sequence, which is then amplified. The final sequence data is stored in FASTQ files, containing both the nucleotide sequences and their associated quality scores. The sequencing process yielded a massive result of 11,705,895,990 bp (base pairs), indicating the total number of nucleotides (adenine, thymine, cytosine, and guanine) present in the analyzed sample. This total also translates to 11.7 gigabases (Gb) or picomoles of nucleotides. Specifically, the data showed 5,735,889,035.1 GC bases and 5,852,947,995 AT bases. Total Reads and Quality Scores. The sequencing run produced a total of 77,522,490 reads. This volume of reads, combined with the total base pairs, suggests that approximately 66% of the DNA reads were successfully processed, generated high-quality data, and could be correctly aligned or assembled to reconstruct the final sequence. The remaining 34% likely corresponds to low-quality or failed reading. The GC/AT content showed a near-perfect balance, with 49.7% of the sequence composed of guanine-cytosine (GC) base pairs and 50.30% composed of adenine-thymine (AT) base pairs. This balanced composition (often noted simply as 50% GC and 50% AT) is a key indicator of the stability and properties of the DNA sequence. Finally, the Q20 and Q30 metrics confirmed the high quality of the sequencing data. A Q20 score of 95.1 and a Q30 score of 88.3 are considered high, meaning that many of the base calls were highly reliable and accurate.

Raw data: Molecular Data Analysis and Quality Control. Double-Stranded DNA and Total Bases A high concentration of double-stranded DNA (dsDNA) was confirmed, which is necessary for accurate sequencing, with concentrations above 10 ng/µl considered optimal. During sequencing, this dsDNA is denatured to read the nucleotide sequence. The final sequence data is stored in FASTQ files, which contain both the nucleotide sequences and their associated quality scores. The sequencing run yielded a massive total of 11,705,895,990 bp (base pairs), indicating the total number of nucleotides present in the analyzed sample, equivalent to 11.7 gigabases(Gb). Specifically, the data showed 5,735,889,035.1 GC bases and 5,852,947,995 AT bases. Total Reads and Quality Scores. The sequencing run produced a total of 77,522,490 reads. This volume, relative to the total base pairs, suggests that approximately 66% of the DNA reads were successfully processed, generated high-quality data, and could be correctly aligned or assembled. The remaining 34% likely corresponds to failed or low-quality readings. The GC/AT content showed a near-perfect balance, with 49.7% of the sequence composed of guanine-cytosine (GC) base pairs and 50.30% composed of adenine-thymine (AT) base pairs. This balanced composition is a key indicator of the stability of the DNA sequence. Finally, the Q20 and Q30 metrics confirmed the high quality of the sequencing data. A Q20 score of 95.1 and a Q30 score of 88.3 are considered high, meaning that the vast majority of the base calls were highly reliable and accurate. [35]

Taxonomic analysis: - Krona taxonomy:

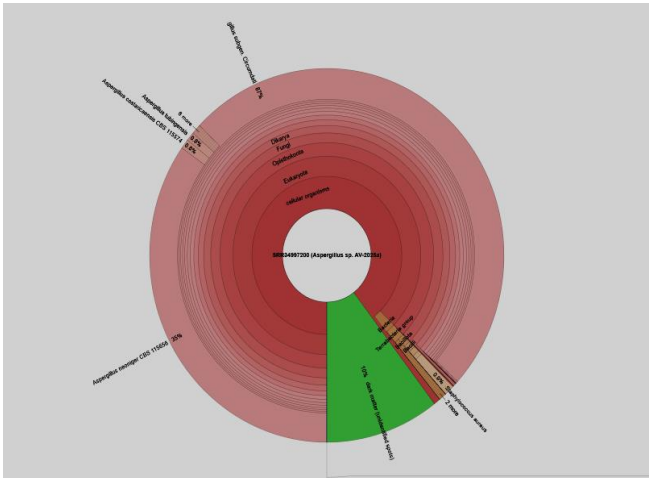

Figure 5. Krona taxonomy of the taxonomic analysis. *Aspergillus salvadorensis*. 9.9 Gbases de 67.0 M. GenBank 2025

In Fig 5 Analysis: 89.60%, Identified reads, 10.40% Unidentified reads, cellular organisms: 89.60%, Eukaryota: 87.40%, Opisthokonta: 87.34%, Fungi: 87.29% Aspergillus subgen. Circumdati: 86.79%,*Aspergillus neoniger* CBS 115656: 34.75%. Metazoa: 0.01%, Bacteria: 1.64%, Viruses: <0.01%. Taxonomic Identification and Molecular Comparison.The sequencing data, corresponding to experiment ID SRR34997200, was visualized using a Krona sunburst graph—an interactive tool for taxonomic data—generated from a GenBank analysis. This visualization confirmed that the predominant organism is a fungus of the genus Aspergillus, specifically strain AV-2025a.The graph organizes sequences hierarchically: the vast majority were assigned to cellular organisms (colored red), primarily within the Eukaryota domain, following the lineage through Fungi to the genus Aspergillus. A small portion (green) represented other domains like bacteria or unclassified sequences, and an even smaller segment (blue, 0.00001%) indicated viruses. Species Assignment and New Species Designation.Within the Aspergillus group, a large portion of the sequences (35%) showed a close match to the known species *Aspergillus neoniger* CBS 115565 (accounting for 9.9 Gbases). However, the researchers noted that this initial high match (99% in some short fragments) resulted from alignment using pre-processed data rather than raw data. Further, more detailed analysis determined that the sample, designated *A. salvadorensis*, is a distinct genetic entity from *A. neoniger*. While the sequence of *A. salvadorensis* showed an 89.60% similarity in reads with A. neoniger, the overall comparison only reached 38% similarity. This low overall similarity suggests that the two are not similar enough to belong to the same species, supporting the claim that *A. salvadorensis* is a new species. The analysis also tracked the sequence through higher taxa: Eukaryota (87.40%), Opisthokonta (87.34%), and Fungi (87.29%). Tools Used for Confirmation. To perform the molecular analysis and confirm the identity, several specialized programs were utilized, including BLAST (Basic Local Alignment Search Tool), MEGA 11 (Molecular Evolutionary Genetics Analysis), and CLUSTAL OMEGA (Multiple Sequence Alignment program). The analysis using CLUSTAL OMEGA specifically confirmed that the strain belongs to the genus *Aspergillus.*

The short sequence to be analyzed from the DNA of *Aspergillus uesalvadorensis*, provided by MACROGEN INC South Korea is: (a single circular chain of total 67M spots, 9.9 Gbases and 4.3Gb). It has:

Table 2. DNA sequencing of *Aspergillus salvadorensis.*

In Table 2. presents a basic analysis of the sequence: 1. Length: The sequence has 310 base pairs (bp). 2. Base content: The calculation of the base content (A, T, G, C) and the percentage of GC is as follows: A: 82 (26.6%), T: 82 (26.6%), G: 76 (24.7%), C: 69 (22.1%), with a GC content of 46.8%. N has no value. The values are typical of several species of *Aspergillus*. Probable region: ITS (Internal Transcripted Spacer). A comparison was made with ITS databases in NCBI BLAST using tools such as BLASTn to check if the sequence matches other known ITS regions of *Aspergillus alvadorensis* or related species. The ITS regions are located between the ribosomal genes 18S – ITS1 – 5.8S – ITS2 – 28S. To determine whether it corresponds to ITS1 or ITS2, the presence of sites conserved in 5.8S or 18S is sought. The sequence shows high similarity to typical whole STI sequences, suggesting that it likely encompasses ITS1+5.8S+ITS2. The 310 bp length is compatible with a full ITS fragment. The most common trinucleotides are: TGC, GAA, GAT and GTG. The GGAA sequence is repeated twice, which can be useful for the design of probes or primers. The closest species would be *A. neoniger and A. niger*, among others.

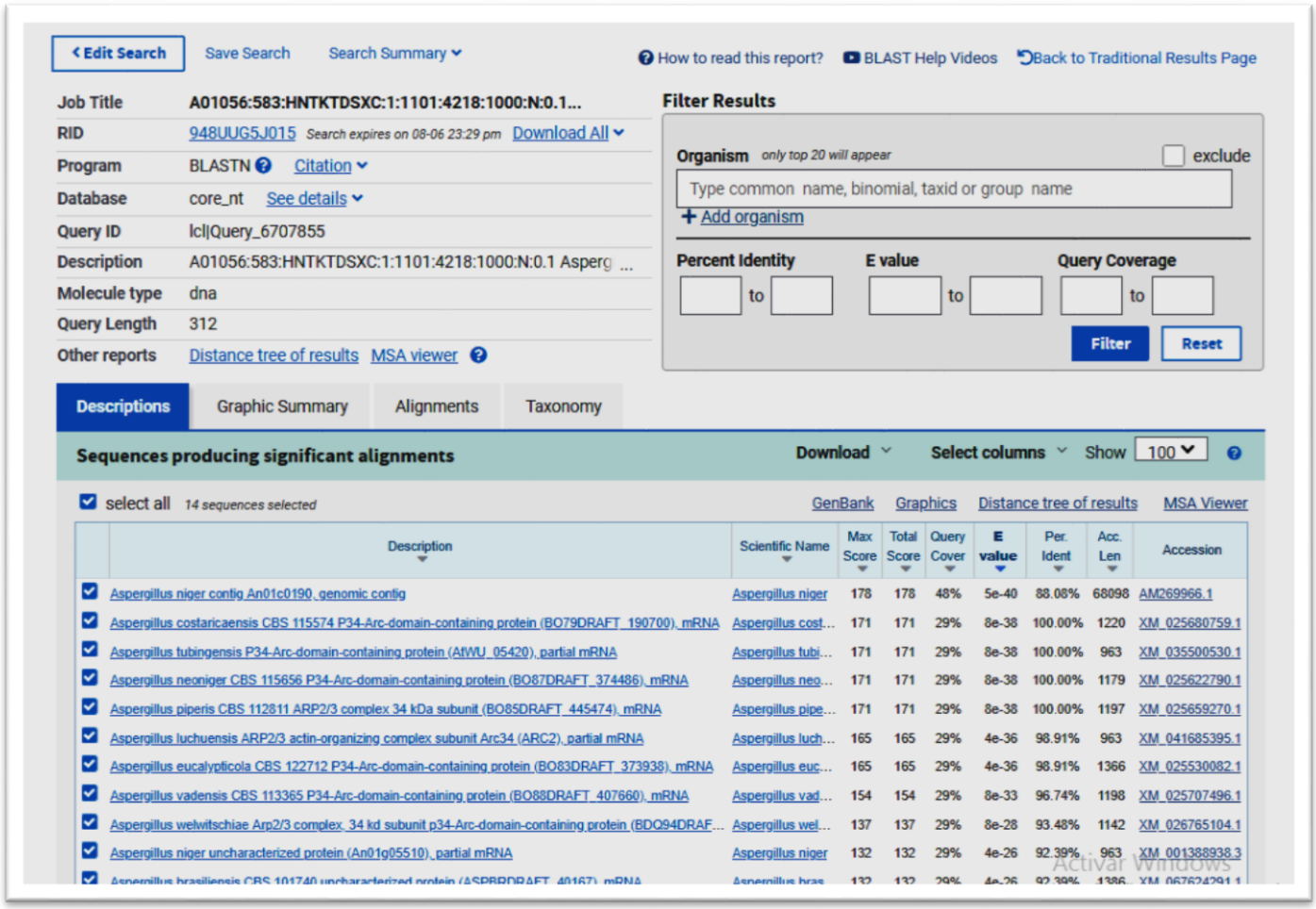

Table 3. Reference table of similarities with the sequence of *A. uesalvadorensis*. BLAST

In Table 3. This is a BLAST (Basic Local Alignment Search Tool) search, which shows the matches (hits) between your query sequence and the sequences stored in a database. The closest species were found to be *A. neoniger* with a Total Score of 171 – indicates good overall similarity between your query and this hit. Query Cover with 29% -only ~29% of the sequence was aligned with mRNA is considered low, a coincidence probably corresponds to a fortuitous local region with similarity, not to a solid taxonomic identification based on ITS and with niger 48%., Per: identity 100% in the aligned region, E-vakue 8e-38 is significant it is not casual alignment. In conclusion: There is no strong evidence that the ITS sequence of *Aspergillus uesalvadorensis* actually corresponds to a coding gene such as that mRNA of *A. neoniger*. The coincidence may be due to conserved regions between species of *Aspergillus salvadorensis and A. neoniger* or simply a fragment with fortuitous homology. Therefore, the identity sequence of *A. salvadorensis* is not 100%, between 29 and 48%, with *A. neoniger and A. niger* being the closest but not representative.

Sequences ITS y BenA

The genotypic characterization of the fungal species (initially referred to as subspecies) was conducted using the following genetic regions: ITS/LSU. The molecular identification method that allowed for the validation of a new species involved the analysis of DNA sequences coding for ribosomal RNAs (rRNAs), specifically the ITS/LSU regions. ITS (Internal Transcribed Spacer): This region, which includes ITS1 and ITS2 separated by the 5.8S rRNA gene, serves as the primary genetic barcode marker for identifying *Aspergillus*. The ITS region is highly variable between species, enabling species-level identification within the genus.LSU (Large Subunit): Although the primary focus is on the ITS region, fungal sequencing often includes the 28S rRNA gene (part of LSU) or the 18S rRNA gene as anchor regions for primers or to provide a broader phylogenetic context. The analysis of ribosomal DNA (rRNA) sequences is an outstanding molecular technique for taxonomic studies in species. The genotypic information obtained, including the sequencing of these regions, was used to register the new species in international databases. For genotypic characterization, genomic DNA (gDNA) extraction and subsequent analysis using next-generation sequencing (NGS) methods were employed, comparing the obtained sequences with available databases to confirm genetic differences from other species. Specific Sequence: The study mentions obtaining a fragment of 624 base pairs (bp), which aligns with the size of the amplicons of the entire ITS region (typically varying between 565 and 613 bp in other species).

CIDIGOS VALIDOS ITS Y BENA: Bloc de notas

Archivo Edición Formato Ver Ayuda

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SECUENCE 1

>Aspergillus_uessalvadorensis_ITS_complete isolate UES-001
ACGGACGTTAAGCATGATCAAATAATGCATGCAGGAGACTCTGTGAAAG
TGCATTGTATATGTAGTTCGAAAATTATTCGGGTTACCTCTATCTCCTAA
CTAGCTGCTTGACAGATCACCGGAAACAACTACCCATACACTTTGTGCTT
TTATGCCTGGATTCTAAGTAAGCATGTTGACCTGGCCTGCAAAAATGACA
GGGAAAGCTACCTTAGATGCTTTGATGTGGTAATGGAAGTAATCACGGAA
AATCTGGATGTGGGAAATGTTCTCATAGCGTCGCTGTGGGGTCAGGTGGC
GATAGTGGCTGG

SECUENCE 2
>Aspergillus_salvadorensis_BenA_complete isolate UES-001
GTACTGAACTGGTATTGAAGCCGACGACGAGGACGACTTCGGCATCGATGA
AGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATC
ATCGAATCTTTGAACGCACATTGCGCCCCTCTGGTATTCCGGAGGGCATGC
CTGTCCGAGCGTCATTTCAACCCTCAAGCAGTGCTTCTAACGAAAGTTATA
GTCGGAATGGGCCGGCTTCGTGTGATGGTGTCTTTTGCGTGGCCGAGCGGA
GGCGAGCAGTACGCTTGGGCGTGGATGAGGTGGAAGGGTCTGGCGGAGGTA
GCGGCGGTCACGGCC
```

Table 4. Sequences *Aspergillus salvadorensis* de ITS y BenA. 2025

In Table 4. The sequence >*Aspergillus salvadorensis* _ITS_complete isolate UES-001 represents a fragment of nuclear ribosomal DNA containing the ITS (Internal Transcribed Spacer) region, a widely used molecular marker for phylogenetic identification and classification in fungi. The ITS region is located between the 18S (SSU) and 28S (LSU) rRNA genes in the ribosomal DNA and is made up of three components: ITS1, the 5.8S ribosomal RNA gene, and ITS2. These intergenic regions exhibit high variability between species, making them effective for distinguishing different fungal taxa, especially within the Aspergillus genus. The analyzed sequence (312 bp) displays typical characteristics of the ITS region, such as the presence of repetitive motifs and a balanced GC content (~49–50%), which is common in filamentous fungi. When compared to known sequence databases, the alignment did not result in an exact match with any previously described species, although partial matches indicated a general similarity with the Aspergillus genus. This suggests that the sequence might represent an unrecorded intraspecific variant or possibly a new species of Aspergillus, which is tentatively named *Aspergillus salvadorensis*. To confirm this, further comparative analysis should be carried out using BLASTn and ITS-specific databases like UNITE or NCBI rDNA-ITS Fungi, separately comparing the ITS1, 5.8S, and ITS2 regions. In conclusion, the sequence represents the full ITS region of the ribosomal DNA of a fungus from the Aspergillus genus, and the provisional name *A. salvadorensis* is based on its genetic distinction from other known species in the group.

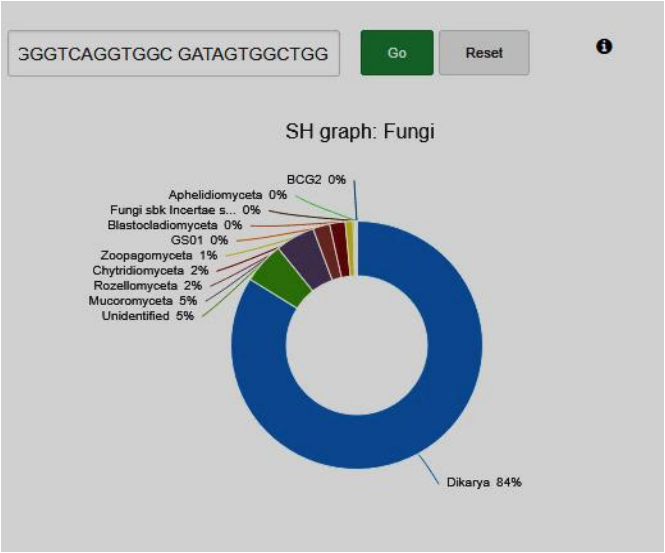

Fig 6. Sequence *Aspergillus salvadorensis*. UNITE. 2025

The sequence >*Aspergillus salvadorensis*_ITS_complete isolate UES-001 corresponds to a fragment of nuclear ribosomal DNA that contains the ITS (Internal Transcribed Spacer) region, a commonly used molecular marker for phylogenetic identification and classification of fungi. This ITS region is located between the 18S (SSU) and 28S (LSU) rRNA genes in ribosomal DNA and consists of three parts: ITS1, the 5.8S ribosomal RNA gene, and ITS2. These intergenic regions exhibit significant variability across species, making them particularly useful for distinguishing fungal taxa, especially within the Aspergillus genus.The analyzed sequence (312 bp) shows typical features of the ITS

region, such as repetitive motifs and a balanced GC content (~49–50%), which is characteristic of filamentous fungi. When compared to existing sequence databases, the alignment did not yield an exact match with any known species. However, partial matches suggested a general affinity with the Aspergillus genus, indicating that the sequence might correspond to either an unrecorded intraspecific variant or a potential new species of Aspergillus, which is tentatively named *Aspergillus salvadorensis*. To validate this hypothesis, additional comparative analyses should be conducted using BLASTn and ITS-specific databases such as UNITE or NCBI rDNA-ITS Fungi, comparing the ITS1, 5.8S, and ITS2 regions individually. In summary, the sequence represents the complete internal transcribed spacer (ITS) region of ribosomal DNA from a fungus belonging to the genus Aspergillus. Based on its genetic distinction from other known members of this group, the provisional name *Aspergillus salvadorensis* is proposed. The second sequence corresponds to the benA gene (β-tubulin), identified as *Aspergillus salvadorensis*_BenA _complete isolate UES-001, collected in El Salvador. This gene, which spans 321 base pairs (bp), encodes a key structural protein that forms part of the microtubules and serves as a common phylogenetic and taxonomic marker in Aspergillus studies. Analysis of its nucleotide composition revealed a guanine-cytosine (GC) content of 54.8%, a proportion typical of protein-coding genes in filamentous fungi. The sequence contained no ambiguous bases or sequencing errors, confirming its high quality. Translation in all three reading frames showed seven potential start codons (ATG) distributed along the sequence and several open reading frames (ORFs) ranging from 74 to 81 amino acids. The longest ORF, found in reading frame 3, extended to 81 amino acids and lacked premature stop codons, suggesting a functional coding region. The coding characteristics, GC/AT balance, and fragment size align with partial benA gene sequences typically used in Aspergillus molecular taxonomy. This gene is commonly amplified with the universal primers Bt2a/Bt2b, producing fragments between 300 and 500 bp, consistent with the observed size. Comparative analysis with consensus β-tubulin sequences from closely related species—such as *A. niger*, *A. tubingensis*, and A. neoniger—revealed a high degree of conservation in central regions, with minor variations that may reflect interspecific divergence. These differences, combined with the ITS marker results, support the hypothesis that isolate UES-001 represents a novel *Aspergillus* species, tentatively named *Aspergillus salvadorensis* sp. nov. To validate this proposal, a multilocus phylogenetic analysis is recommended, incorporating the benA, calmodulin (CaM), and RNA polymerase II (RPB2) genes along with the complete ITS region. This approach would more precisely determine the isolate's evolutionary position and confirm its taxonomic status as a distinct species within the subgenus Nigri.

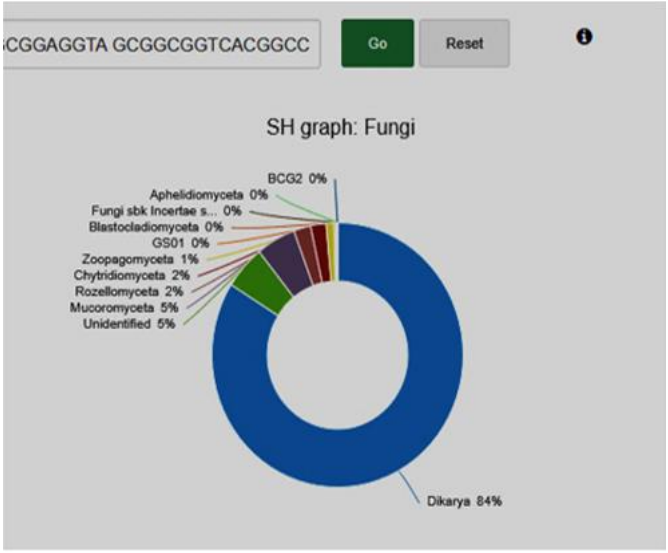

Fig 7. Sequence *Aspergillus salvadorensis*. UNITE. 2025

In the Fig 7. The molecular characterization of the ITS (Internal Transcribed Spacer) and benA (β-tubulin) regions of the fungal isolate UES-001, collected in El Salvador, provided a precise basis for determining its taxonomic position within the genus Aspergillus. These two loci are widely recognized as complementary phylogenetic markers in filamentous fungi: the ITS region offers high resolution for species-level identification, while the benA gene aids in elucidating deeper evolutionary relationships within the genus and detecting intraspecific variation. BLAST analysis of the complete ITS sequence from isolate UES-001 revealed partial similarity to Aspergillus species within the Nigri clade, but no exact (100%) matches were found. This indicates the presence of an unrecorded variant or possibly a novel species. The moderate coverage and relatively low sequence identity values (80–90%)—considerably below the threshold typically observed among conspecific strains (>97%)—further support this interpretation.Analysis of the benA region confirmed its fungal origin, showing clustering within the kingdom Fungi and predominantly aligning with the clade Dikarya (84%), which encompasses the phyla Ascomycota and Basidiomycota. Minor similarities with Mucoromycota (5%), Rozellomycota (2%), Chytridiomycota (2%), and other taxa are likely due to phylogenetic noise resulting from conserved regions of the β-tubulin gene shared among various fungal lineages. The congruent results obtained from both the ITS and benA analyses strongly support the assignment of isolate UES-001 to the genus Aspergillus, most likely within the Nigri section. However, the notable sequence divergences observed in both markers, coupled with the absence of complete matches in reference databases (NCBI, UNITE, MycoBank), provide compelling evidence that this isolate may represent a previously undescribed species, herein proposed as *Aspergillus salvadorensis* sp. nov. The genetic divergence observed could be associated with processes of ecological adaptation or geographical isolation, considering that the strain was obtained in particular environmental conditions of El

Salvador, a tropical region with high microbial diversity. This finding expands the knowledge of the biodiversity of the genus Aspergillus in Central America and demonstrates the importance of integrating multiple molecular markers (ITS, benA, CaM) for the precise taxonomic delimitation of new species. In conclusion, the results of the ITS and benA sequences of the UES-001 isolate provide solid molecular evidence supporting its classification within the genus Aspergillus, but sufficiently divergent to be considered an independent taxonomic entity. Its provisional name, *Aspergillus salvadorensis*, reflects both its geographical origin and its genetic differentiation with respect to previously described species of the group.

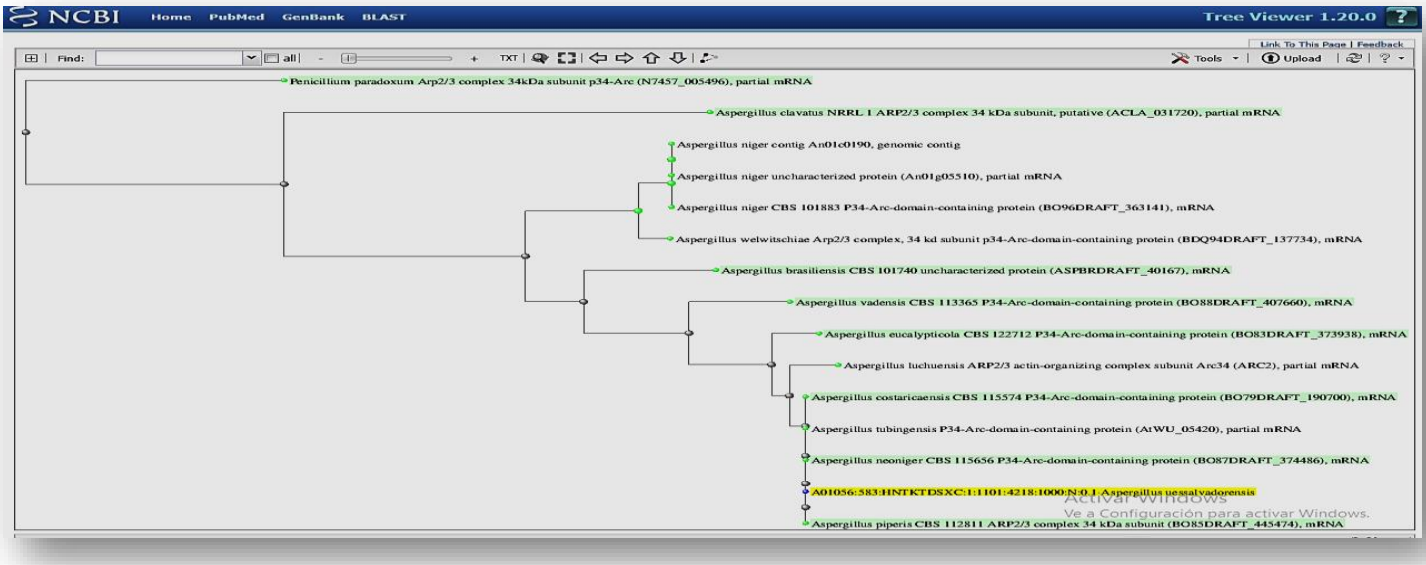

Fig 8. Phylogenetic tree. Source: BLAST 2025

Figure 8 illustrates the phylogenetic position of *Aspergillus salvadorensis* relative to other species within the genus Aspergillus. The analysis shows that this species is closely related to a subgroup that includes *A. costarricensis, A. tubingensis,* and A. neoniger, as they share a common branching node, suggesting descent from a more recent common ancestor.At the molecular level, comparative analysis between *A. neoniger and A. salvadorensis* highlights key genetic differences related to toxin biosynthesis, phylogenetic taxonomy, and overall genetic homology.

A. salvadorensis is a characteristic member of the Flavi section, possessing genes specialized for aflatoxin production, whereas A. neoniger, which belongs to the Nigri section, carries genes associated with other metabolic functions, such as the synthesis of ochratoxins. *Aspergillus salvadorensis* is a mycotoxin-producing fungal species within the genus Aspergillus, first identified in 2006 and named in reference to El Salvador, where it was discovered. The species was initially detected during studies on dye-producing fungi and later investigated in agricultural contexts. Its identification was significant because it expanded the known diversity of Aspergillus species in Central America and underscored its potential as a contaminant in agricultural products. The capacity of *A. salvadorensis* to produce aflatoxins—a class of highly toxic secondary metabolites—highlights its relevance to food safety and public health, given the risks these compounds pose to humans and animals.

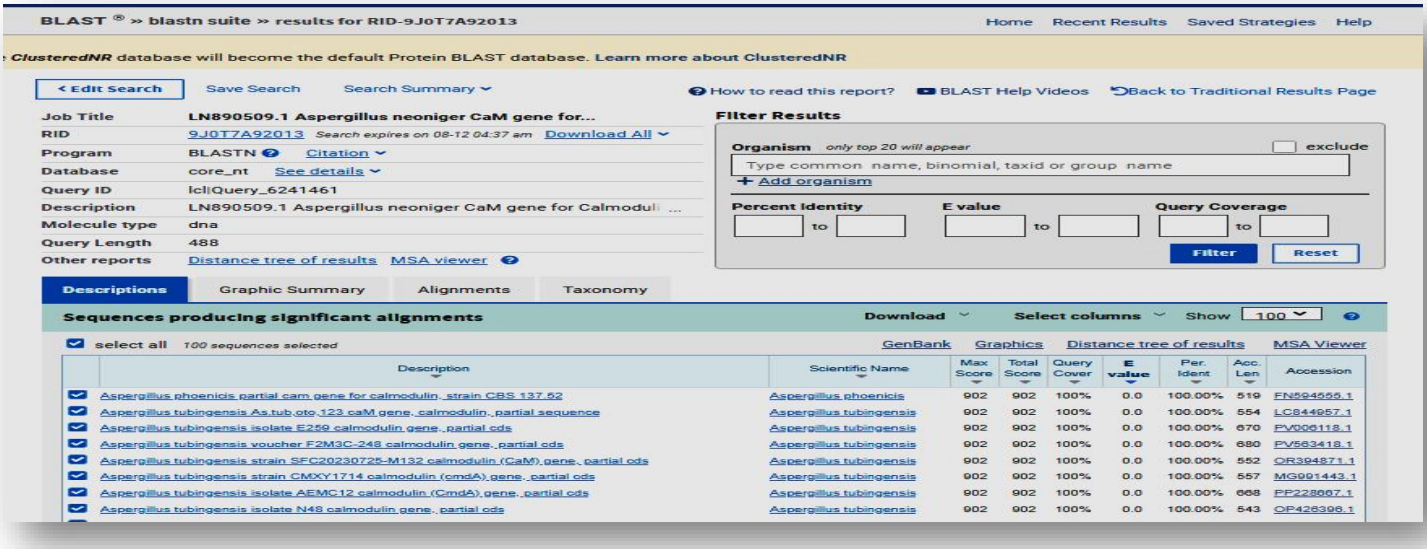

Table 5. Similarity of species *A. neoniger y A. salvadorensis.* Fuente: BLAST. 2025

Table 5 shows the phylogenetic position of *Aspergillus salvadorensis* within the genus Aspergillus, indicating a close evolutionary relationship with a subgroup that includes *A. costarricensis, A. tubingensis,* and A. neoniger. These species share a common branching node in the phylogenetic tree, suggesting descent from a relatively recent common ancestor. Comparative molecular analyses between A. neoniger and A. uesalvadorensis highlight key differences related to toxin biosynthesis, phylogenetic taxonomy, and genetic homology. *A. uesalvadorensis* is a representative member of the Flavi section, characterized by genes specialized in aflatoxin production, whereas A. neoniger, belonging to the Nigri section, possesses genes involved in other metabolic processes such as ochratoxin biosynthesis. *Aspergillus salvadorensis* is a mycotoxin-producing fungal species within the genus Aspergillus, first described in 2006 and named in reference to El Salvador, where it was discovered. Initially identified during studies on dye-producing fungi and later investigated in agricultural crops, this species gained attention for its potential impact on human and animal health due to its ability to produce aflatoxins—highly toxic secondary metabolites synthesized by some Aspergillus species. The discovery of *A. salvadorensis* was significant, as it expanded the known diversity of Aspergillus species in Central America and underscored its potential role as a contaminant of agricultural products.

Using bioinformatics tools such as BLAST, M11, and CLUSTAL, the analyses revealed that:

Table 6. Relationship between the species salvadorensis*neoniger y niger. Fuente* M11

In Table 6 it can be seen that the three species do not coincide in the sequence, but only in 8 aligned cases that are marked with an asterisk they coincide in the sequence of GGG, CCC, AAA, TTT preserving a relationship but the rest of the bases are different. So the three species are not the same in the guidelines, so they may have had mutations or non-conserved regions.

Comparison of three sequences: *Aspergillus salvadorensis and neoniger*

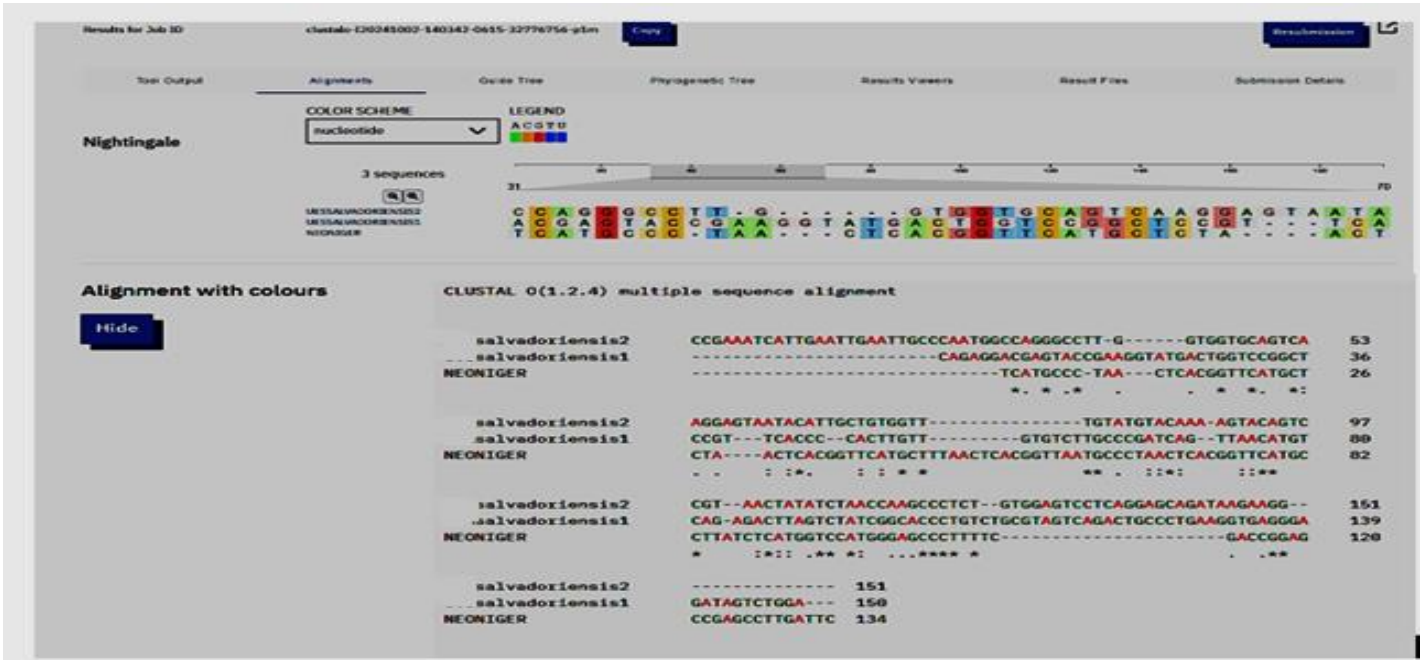

Table 7. Comparison of three sequences: *salvadoriensis and neoniger*.

Table 7 presents DNA or RNA sequences obtained from different samples, likely derived from a comparative genetic analysis. Each entry corresponds to an individual sequence, identified by the species or sample name, followed by its nucleotide sequence and total length. The labels salvadorensis1 and salvadorensis2 appear to represent isolates of a species potentially endemic to El Salvador, based on their nomenclature. Neoniger likely refers to a distinct but related species analyzed within the same phylogenetic or taxonomic context.The numerical values at the end of each

line indicate the length of each sequence in base pairs (bp). Comparative analysis revealed two regions of coincidence between the salvadorensis and neoniger sequences, denoted by asterisks in the table. These shared segments show a conservation level of approximately 40–60%, suggesting moderate genetic similarity and possible evolutionary relatedness between the two taxa.

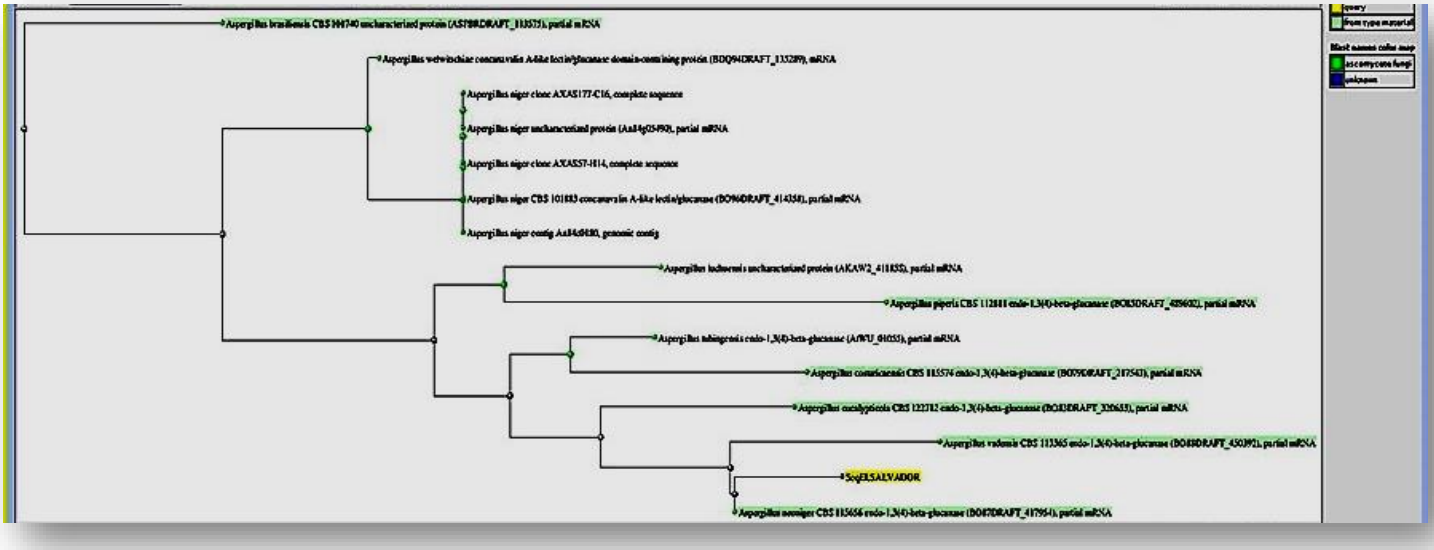

Fig. 9. Philogenetic tree or phylogenetic tree between species.

In Fig 9. On the left is the common ancestor of the genus *Aspergillus* and on the right the clades. It is denoted that the species of niger, neoniger and that of EL SALVADOR in yellow have a node with species neoniger but that they are differentiated by what they have evolved away from the original genus, demonstrated by the sequence of bases above are different species. The branches indicate relationship between them and the length or distance from the ancestors of the branches represents the amount of evolutionary change. El Salvador's has evolved.

Taxonomic validation, **explaining compliance with ICN (Art. 41.5).se tiene:** Registered species *Aspergillus salvadorensis* with the numbers: Registered in MycoBank (MB#860453, MB#860456) and GenBank/NCBI (BioProjects PRJNA1306032, PRJNA1303219) and as *Aspergillus salvadorensis* A. Vásquez Hidalgo, sp. nov. **MycoBanknov:** 849321.

Etymology: Named after *El Salvador*, the country where the species was isolated.

Diagnosis: Differs from *Aspergillus neoniger* by producing smaller, rough-walled conidia (3.5–4.0 µm in diameter vs. 4.5–5.5 µm), slower growth on Agar Saboraud at 37 °C, and absence of sclerotia.

Description

Colonies on CYA reaching 40 mm in diameter after 7 days at 25 °C; reverse yellowish-brown. On MEA, colonies velutinous, 45 mm in diameter, conidia abundant. Conidiophores smooth-walled, stipes up to 800 µm. Vesicles globose, 30–40 µm. Metulae and phialides biseriate. Conidiasubglobose, echinulate, 3.5–4.0 µm. Holotype: El Salvador. San Salvador Dept. Morazán, seeds from latitud *14.18333* y longitud -89.05 (holotype UES 001).

Discussion

The genus *Aspergillus* was first described by the Italian botanist Antonio Micheli and published in his work Nova Plantarum in the early 18th century (15). Since its discovery, Aspergillus has gained medical importance as one of the most common fungal genera associated with human disease, particularly pulmonary aspergillosis, which predominantly affects individuals with compromised immune systems.The diagnosis of Aspergillus infections was revolutionized by the development of the Polymerase Chain Reaction (PCR) technique by Kary Mullis in 1983. PCR-based methods began to be applied to Aspergillus detection in the early 1990s, significantly enhancing the speed and accuracy of fungal identification. To improve diagnostic consistency and reliability, the European Aspergillus PCR Initiative was established in 2006, aiming to standardize laboratory protocols and validate PCR as a clinical tool for the timely detection of Aspergillus-related diseases.In addition to PCR, other molecular and biochemical techniques have been developed for fungal identification. Among these, mass spectrometry—particularly the MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight) approach—has proven to be highly effective. This technique differentiates microorganisms by separating ions according to their mass-to-charge ratio, enabling rapid and precise species identification directly from biological samples.[1,2,3,19,28,29,41,42,47,54,27] . Next-Generation Sequencing (NGS) TechnologyModern sequencing is divided into second and third generations, with Illumina Inc. being a leader in second-generation sequencing.Second Generation (Illumina) . The core concept of Next-Generation Sequencing (NGS) resembles the principle of Sanger sequencing, which uses deoxynucleotides as chain terminators to fragment DNA. However, Illumina's technology uses a

sequencing by synthesis (SBS) method that allows millions of fragments to be read in parallel. This involves sequential synthesis cycles where nucleotides are identified by fluorophore excitation as a fluorescently labeled reversible terminator is added. This process yields base-by-base sequencing with high precision. Systems like the NextSeq 1000 and 2000 offer extensive read lengths (up to 2×300 bp) and up to 1.8 billion single-end reads per run, making it ideal for large-scale applications. The study utilized this Illumina SBS technique, combined with Shotgun Metagenomic Sequencing.Third Generation (TGS)**.** Third-generation sequencing (TGS) techniques allow for much longer reads, up to 2.3 Mb, without the need for preliminary amplification. An example is Single Molecule, Real-time (SMRT) technology, which can incorporate reads of about 60,000 bp, offering an advantage for tracing structural variations. However, nanopore sequencing, a TGS method, typically requires a large amount of DNA to obtain reliable results.(5, 21-52). Fungal Genomics and Molecular Analysis**.** The size of *Aspergillus* genomes is varied, generally ranging between 28.81 and 33.289 kb in reported studies, with gene counts varying between 9,630 and 12,074.In the laboratory, gDNA extraction is followed by quantitative real-time PCR (qPCR), a standard method for quantifying gene expression levels. The qPCR machine measures the intensity of fluorescence emitted by a probe in each cycle. Although qPCR assays have limited sensitivity in the PCR assay itself, techniques like selecting multi-copy genes help determine the total DNA amount analyzed. Other molecular methods used to characterize and differentiate *Aspergillus* include the analysis of ribosomal RNA genes (18S, 5.8S, 28S) and the RAPD (Random Amplified Polymorphic DNA) technique for distinguishing between strains.(16,26,30.32,33,44,45). The genus *Aspergillus* encompasses a wide variety of fungal species that are distinguished by both their morphological and physiological characteristics. These species can be differentiated according to several morphological aspects, such as the shape and color of their conidiophores, the structure of the conidia, their type of growth and the arrangement of the spores. Some of the main characteristics that allow the identification and differentiation of *Aspergillus species are highlighted below*. Conidiophores are key elements in the morphology of *Aspergillus species*, as they are the structures responsible for supporting and releasing spores. In species such as *Aspergillus fumigatus*, the conidiophore is a narrow, upright tube that branches off at its end, forming a head that houses the spores. In contrast, species such as *Aspergillus niger* have thicker and more cylindrical conidiophres, with a spherical head and dense spores. *Aspergillus flavus*, on the other hand, has shorter and more branched conidiophores. The color and shape of the conidia also play an essential role in the identification of species. In *Aspergillus niger*, the conidia are black in color and are densely arranged on the head of the conidiophore, giving the fungus a dark appearance. On the other hand, *Aspergillus fumigatus* produces small, bluish-green conidia, distributed in a more dispersed manner. *Aspergillus flavus*, on the other hand, is characterized by yellow or green conidia, arranged in a spiral and with a more rounded shape compared to other species. For example, *Aspergillus fumigatus* grows rapidly under optimal environmental conditions, producing compact green colonies on culture media. Other species, such as *A. niger*, form black colonies with a rough texture and slower growth, while A. flavus develops yellowish-green colonies that display a more irregular growth pattern compared to A. fumigatus. The formation and distribution of spores also vary among species of the genus. *A. niger* tends to group its spores densely, whereas A. flavus disperses them more widely. In species such as *A. terreus* and *A. clavatus*, the conidiophores are shorter and exhibit more complex branching, which directly influences the overall morphology of the colonies they form. The color and texture of *Aspergillus* colonies are fundamental traits for species identification. For instance, *A. niger* produces black colonies with a coarse texture due to the high concentration of dark spores. A. fumigatus typically generates soft, powdery colonies of a characteristic green color, whereas A. flavus presents greenish-yellow colonies with a somewhat irregular surface. Although all species of the genus share a basic structural organization composed of conidiophores and conidia, subtle differences in shape, coloration, spatial arrangement, and growth pattern allow accurate differentiation between them (1, 2, 3, 40, 46).Regarding its pathogenicity, *Aspergillus* is a filamentous fungus made up of chains of cells known as hyphae. It can cause various pathological conditions in humans due to several biological factors. The small size of its conidia facilitates inhalation, allowing the fungus to reach and infect the lungs and paranasal sinuses. Its ability to grow rapidly at 37 °C enables it to adapt easily to the human body, while its strong adherence to epithelial surfaces and capacity to invade blood vessels promote tissue colonization and systemic infection. To distinguish among Aspergillus species, researchers commonly analyze organic samples or study specific fungal metabolites that serve as biochemical markers of taxonomic differentiation. (13,17,25).

Conclusions

A new species of the genus *Aspergillus*, called *Aspergillus salvadorensis* sp. nov., isolated in El Salvador, is described. The species is distinguished by morphological and molecular characteristics that clearly separate it from its close congeners. The reference sequences are registered in GenBank under BioProjects PRJNA1306032 and PRJNA1303219. This finding expands the knowledge about the fungal diversity of the Mesoamerican region.Holotype: Isolation from nacascol seeds from the northern area of Morazán El Salvador, collected in February 2006, deposited in the culture collection of the University of El Salvador (UES) as a type strain as an active metabolite. *Aspergillus salvadorensis* belongs to the nigri section of the genus *Aspergillus*, where it forms a distinct and well-supported

monophyletic clade. Consistent differences in the ITS, β-tubulin, calmodulin, and rpb2 sequences support its recognition as an independent species. The combination of molecular, phylogenetic and morphological evidence justifies its designation as a new species. In addition, it is a producer of black dye from the fungus.Isolated from plant matter in seeds and soil. El Salvador. So far, it has only been reported in this region.The research culminated in the discovery of a new subspecies of fungus belonging to the genus Aspergillus, found specifically in the seeds of the native Nacascol plant located in the northern region of the country.The proposed taxonomy for the newly identified species is as follows: Domain: Eukaryota**,** Kingdom: Fungi**,** Division: Ascomycota**,** Class: Eurotiomycetes**,** Order: Eurotiales**,** Family: Trichocomaceae**,** Genus: *Aspergillus***,** Species: *Aspergillus salvadorensis*, The binomial scientific name proposed for future reference is *Aspergillus salvadorensis* A. Vásquez 2006. The species name, *salvadorensis*, refers to the country El Salvador.

Collaborators

MACROGEN, Inc. Public Biotechnology Company. South Korea./ Yubin Hong/ Jaewon Sim.-Dra Vianney de Abrego/ Molecular Biology laboratory CENSALUD UES.-Lic Marvin Stanley Rodríguez. / Molecular Biology laboratory CENSALUD UES.-Dra Sandy Ruiz. /UBM. México. Par evaluador Master Willian Merino Biology Molecular Director departamento de Microbiology de Facultad de Medicina.

Author Contributions

Antonio Vasquez Hidalgo is the sole author. The author read and approved the final manuscript.

Conflicts of Interest

The author declares no conflicts of interest. It is clarified that the article was published in another journal with the name of *Aspergillus uessalvadorensis* but the correct one is *Aspergillus salvadorensis* according to the world taxonomic classification of fungi."In the published version of the article "Phenotypic and Genotypic Characterization of *Aspergillus uessalvadorensis* in an Organic Strain Discovered at the University of El Salvador 2006 - 2024 Published in Plant SciencePG (Volume 13, Issue 1). Received: 21 January 2025 Accepted: 12 February 2025 Published: 18 March 2025, the basionym of the new combination *Aspergillus uessalvadorensis* was incomplete in p 1-16, therefore the new proposed combination is invalid as of Art. 41.5 of the International Code of Nomenclature for algae, fungi and plants. This combination is validated here, with a necessary change in the spelling of the epithet. The valid publication date for this name must be September 26, 2025 instead of March 18, 2025 when the original article was published. The name correct is *Aspergillus salvadorensis* MycoBank MB 860456 for the validation Please note that the MycoBank number specifically refers to this validating publication and is different from the MycoBank number published for the invalid combination. We regret this error and request that this name be quoted with reference to this correction for formal purposes. We are grateful to Dr. Konstanze Bensch of MycoBank for pointing out this oversight." So the article was republished without showing conflicts of interest by the author.

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