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Molecular-Based Diagnosis, Phylogenetic and Secondary RNA Structure Analysis of Three *Aspergillus* Species Isolated from Local Mosquito, *Culex poicilipes* Collected in Erbil City

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Abstract

This study investigates fungi associations in *Culex poicilipes* mosquitoes, which have been collected from Erbil City, Kurdistan Region, Iraq. A total of 155 adult mosquitoes were analyzed, with 18% showing fungal infections. Molecular analysis using COI and LSU genes identified three *Aspergillus* species (*A. niger*, *A. inflatus*, and *A. tubingensis*). Sequences were submitted to GenBank, and all of them were recorded under the accession numbers PV085519.1, PV085520.1, and PV085521.1, respectively. Phylogenetic analysis and secondary RNA structure prediction confirmed species-level relationships and showed genetic polymorphism. The study concludes that *C. poicilipes* may act as a vector for *Aspergillus* spp., with implications for public health.

1. Introduction:

Despite extensive control efforts over centuries, mosquito-borne diseases continue to spread globally, disproportionately affecting children and adolescents, and contributing significantly to global morbidity and mortality [1]. Over a span of tens of millions of years, mosquitoes and fungi, along with viruses and bacteria, have had ample time to interact and establish a variety of connections [2]. The World Health Organization has indicated that vector-borne diseases represent over 17% of all infectious diseases, resulting in more than 700,000 deaths each year, and can be attributed to parasites, bacteria, viruses, or fungi [3].

The survival rate of the transmission rate of infections carried by mosquitoes is likewise significantly influenced by the same conditions [4]. Specifically, the insect's rate of multiplication is influenced by temperature; this in turn influences the pace of infection of the salivary secretions and, thus, the probability of successful transfer to another host [5]. In order to diagnose and track vector-borne illnesses with high sensitivity and specificity, molecular detection methods are

essential [6]. Pathogens, including Plasmodium, Borrelia, Dengue virus, and Zika virus, could be quickly and accurately detected in vectors and hosts using techniques like conventional polymerase chain reaction (PCR), real-time quantitative PCR (qPCR), and loop-mediated isothermal amplification (LAMP) [7].

A useful molecular marker for mosquito identification and detection, cytochrome C is a highly conserved mitochondrial protein that is involved in cellular respiration and the electron transport chain [8]. The gene which encodes large subunit ribosomal RNA (LSU 28rRNA) is a useful molecular marker for identifying and detecting mosquito-associated fungus species [9]. The LSU rRNA sequencing offers a high resolution of taxonomic classification because of its conserved and variable sections, which makes it a useful method for researching fungal communities linked to mosquitoes [10].

The aims of this study can be summarized as follows: various mosquitoes which were found in the country were tested for their vector competency whereby captured mosquitoes were tested for potential pathogens and their capacity to transmit pathogens. Investigation of genetic variation in mosquito overlapping pathogens via secondary RNA analysis is among additional aim of the present study. This study involves Ministry of Health and Environmental Protection and Improvement Board because the related ministry can depend on our results in order to control transmitted diseases in the study area.

2. Materials and Methods:

2.1 Description of Study Area:

Erbil City, referred to as Hawler in Kurdish and historically known as Arbela, serves as the capital and most populous urban center of the Kurdistan Region in northern Iraq. Its geographic coordinates are 36.206293 latitude and 44.008870 longitude, which translate to GPS coordinates 36^o12'22.6548" N and 44^o0'31.932" E. Materials and kits that have been utilized for the present study are summarized in Tables 1 and 2.

2.2 Sample Collection:

A total of 155 adult mosquito specimens were collected throughout the period from October 1, 2024, to May 29, 2025, at several locations in Erbil City, Kurdistan region, Iraq (Figure 1). Each mosquito was examined with a microscope, and the females were identified and separated. To detect fungi associated with mosquitoes, each sample was individually homogenized and inoculated on Sabouraud Dextrose Agar (SDA) followed by incubation at 25-28^oC [11].

2.3 Molecular study:

2.3.1 DNA Extraction:

Ethanol was extracted from parasites before DNA extraction. Ethanol was eliminated from the specimens by air-drying them [12]. Using extraction, genomic DNA was obtained from over 20 samples that were collected. With little alterations, the Beta Bayern tissue DNA preparation kit aligns with the production's instructions. Extraction protocol.

Table 1. *In Vitro* Diagnostic Research Kits were utilized in this Study.

Kits	Company	Origin
Applied Biosystem's Prism Terminator Sequencing Kit	Macrogen Molecular	Korea
The tissue DNA preparation kit from Beta Bayern	Beta Bayern GmbH .90453 Bayern	Germany
Master Mix	A/S Stenhuggervej 22 AMPLIQON	Korea

2.3.2 Genomic DNA Quantification and Qualification:

NanoDrop (ND-1000, USA)was used to achieve both the quantity and quality of DNA concentration. It was possible to get genomic DNA samples with more than 0.5µg and an A260–A320/A280–A320 ratio of more than 1.7 qualities.

2.3.3 Amplification of DNA:

Since the gene's Cytochrome Oxidase C Subunit I (COI) and LSU gene were amplified by PCR, each PCR reaction was conducted in a total amount of 50 μl of reaction mixture that contained; The Bioresearch PTC-200 Gradient thermocycler contains 2x Taq DNA Polymerase Master Mix (AMPLIQON A/S Stenhuggervej 22), ten Picomol (pmol) primers, DNase-free water, and template DNA (Table 3). In the temperature profile, step one consists of an initial denaturation at 95^oC for 5 minutes. Step two is followed by 35 cycles of denaturation at 95^oC for 40 seconds, followed by primer annealing at 55^oC for 45 seconds, an additional extension at 72^oC for a minute, and final extension at 72^oC for 5 minutes.

2.3.4 Agarose Gel Electrophoresis Preparation:

A clear solution was prepared by heating two grams of agarose powder in 1X TBE buffer in a microwave [13]. The solution was left to cool down between 37 and 45 oC by swirling the flask. After melting the agarose gel, 5–10 μl of safe dye solution was added, and everything was thoroughly mixed. After being poured into the casting tray, the melted agarose solution was allowed to harden. The combs were cautiously removed. The electrophoresis chamber was filled with the gel. Enough TBE buffer was added to cover the gel by two to three millimeters. Five microliters of extracted DNA were pipetted onto different gel wells. A DNA ladder with 100–3000 base pairs was meticulously pipetted onto a different gel well.

Table 2. Pair of Gene Primers Used in this Study.

1-The primer is used for identifying mosquitoes with PCR conditions			
Primer code	Sequence order and direction	Amplicon size [bp]	Condition of PCR
COI-F	5'GGTCAACAAATCATTAAAGATATTGG-3'	720	95^o-5 minutes, 95^o-35 seconds, 59^o-35 seconds, 72^o-1 minute, 72^o-10 minute, and 4^o ∞
COI-R	5'TAAACTTCAGGGTGACCAAAAAATCA-3'	720	95^o-5 minutes, 95^o-35 seconds, 59^o-35 seconds, 72^o-1 minute, 72^o-10 minute, and 4^o ∞
2-The primer is used for identifying fungi with PCR condition			
Primer code	Sequence order and direction	Amplicon size (bp)	Condition of PCR
LSU-F (28S rRNA)	ACCCGCTGAACTTAAGC	1200	95^o-5 minutes; 95^o-40 seconds, 56°-40 seconds, 72^o-1 minutes; 72^o-10 minutes; 4^o ∞
LSU-R (28S rRNA)	CGCCAGTTCTGCTTACC	1200	95^o-5 minutes; 95^o-40 seconds, 56^o-40 seconds, 72^o-1 minutes; 72^o-10 minutes; 4^o ∞

Table 3. Reagents used for PCR Amplification.

No.	components of PCR	Concentration	Volume [μl]
1	Master Mix	2x	25
2	Forward Primer	20 Pmol	2
3	Reverse Primer	20 Pmol	2
4	DNase-free Water	-	18
5	Template DNA	50ng/µl	3
	Total		50

2.3.5 Expression of DNA Fragments:

DNA doesn't show up on the gel because it isn't naturally colored. Therefore, a DNA-specific dye is applied to the gel before electrophoresis. After adding an intercalating dye, such as ethidium bromide, to an agarose gel, the bands' locations are identified by looking at the gel under a UV transilluminator.

2.3.6 DNA Sequencing:

The ABI Prism Terminator Sequencing Kit (Applied Biosystem) at Macrogen Molecular Company of Korea was used to sequence samples of the PCR product for the COI partial gene and the LSU gene. The Finch TV program software was used to check base calls and edit the chromatogram.

2.4 Bioinformatics and Sequence Analysis:

2.4.1 Sequence Editing:

The software programs Finch TV and Jalview (https://www.jalview.org/getdown/release/) were used to modify the chromatograms of all isolated genes and check base calls.

2.4.2 Sequence Similarity Search Tool:

The Basic Local Alignment Search Tool, which applies the sequence similarity method, was used to investigate all isolated gene sequences from fungi, bacteria, and mosquitoes (using both, BLASTN) (https://blast.ncbi.nlm.nih.gov/), this information can be accessed on the National Center for Biotechnology Information (NCBI) website at https://www.ncbi.nlm.nih.gov/ for the comparison between laboratory or query sequences (subjects) to see how similar isolated sequences are to the subject sequences that were previously stored in the gen bank.

2.4.3 Submitting of the Isolated Sequences to NCBI Databases:

Sequences with high similarity were placed in an authenticated culture collection center for reference, GenBank in NCBI in order to record all of them and receive the accession number for each sequence separately.

2.4.4 Sequence Alignment Search Tool:

Multiple sequence alignment among highly similar sequences (Subject) and isolated sequences (Query) was done, using the

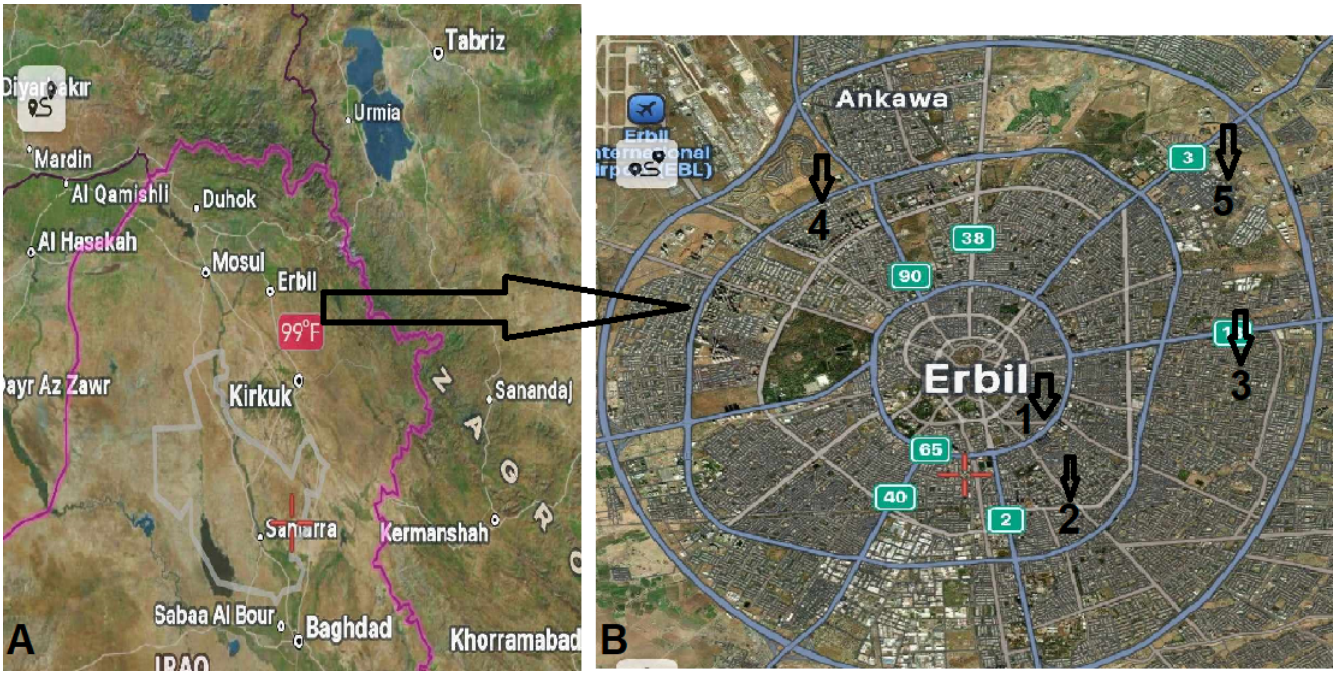

Figure 1. A-Map of Iraq. B-Map of Erbil City showing the specific quarters within Erbil City where mosquito sampling was conducted: 1- Brayaty,2- Zanko Two, 3-Zilan City, 4-Bakhtiari, and 5-Zin City. These locations were selected based on environmental variability and urban diversity.

MUSCLE program in MEGA11 software ("$C:Files11_64.exe$").

2.4.5 Phylogenetic Tree Construction and Analysis:

Multiple alignments among different similar sequences were performed in order to determine the isolated sequences' phylogenetic position. Through using the MUSCLE model in MEGA program v.11, similar sequences were chosen and retrieved from NCBI for this purpose. They were subsequently aligned with one another (adding an outgroup for each creature). The phylogenetic trees of all isolated sequences were based on the Neighbor-Joining (NJ) and the Maximum Likelihood of the Kimura 2 parameter model. Clade support was given using 100 and 1000 bootstrap replicates [14], and for the estimation of the substitution matrix, the Tamura-Nei model was used.

2.4.6 Secondary RNA Structure Prediction:

The RNA fold web server (http://rnatbi.univie.ac.atcgi-bin) was used to derive the minimal free energy prediction for the secondary structure of the separated sample sequences. Fold is a vital bioinformatics software in molecular science for the forecast of secondary RNA structure, which depends on the minimal free energy (MFE) state [15].

3. Results and Discussion:

A total of 155 adult mosquito specimens were collected from different parts of Erbil City, including Brayaty, Zanko 2, Zilan City, Bakhtiari, and Zin City. Cytochrome Oxidase C Subunit I (COI) gene analysis indicates that the collected mosquito is *Culex poicilipes*, and it had been recorded in the National Center for Biotechnology Information (NCBI) under the accession number PV085768 to find fungal infections, molecular screening was performed on the gathered samples. Twenty-eight mosquitoes (18.06%) had fungal infections in a total of 155 examined samples. These results imply that fungal interaction is rather common among mosquito populations in Erbil's urban environments. The small variation in infection distribution between quarters suggests that the frequency of mosquitoes' fungal symbionts may be influenced by local ecological or environmental factors, according to Figure 2. In the current study, two types of primers have been used for molecular analysis, including mitochondrial gene, the COI gene of 720 bp in length for the molecular analysis of mosquitoes and large subunit 28svedberg unit rR (LSU 28S rRNA) of 1200 bp in length for the identification of mosquito overlapping fungi (Figure 3 A and B).

The diversity analysis and taxonomic resolution at the species level may be constrained by the LSU region's generally lower degree of variability compared to the ITS region [16]. Nevertheless, it is accessible to phylogenetic investigation of the novel clades and sequence alignment [17]. Mosquitoes can serve as powerful vectors for the spread of fungi. Globally, zoonotic virus transmission is largely caused by mosquitoes belonging to the Culex genus [18]. In the present study, all collected samples were molecularly screened for the presence of mosquitoes' fungal symbionts

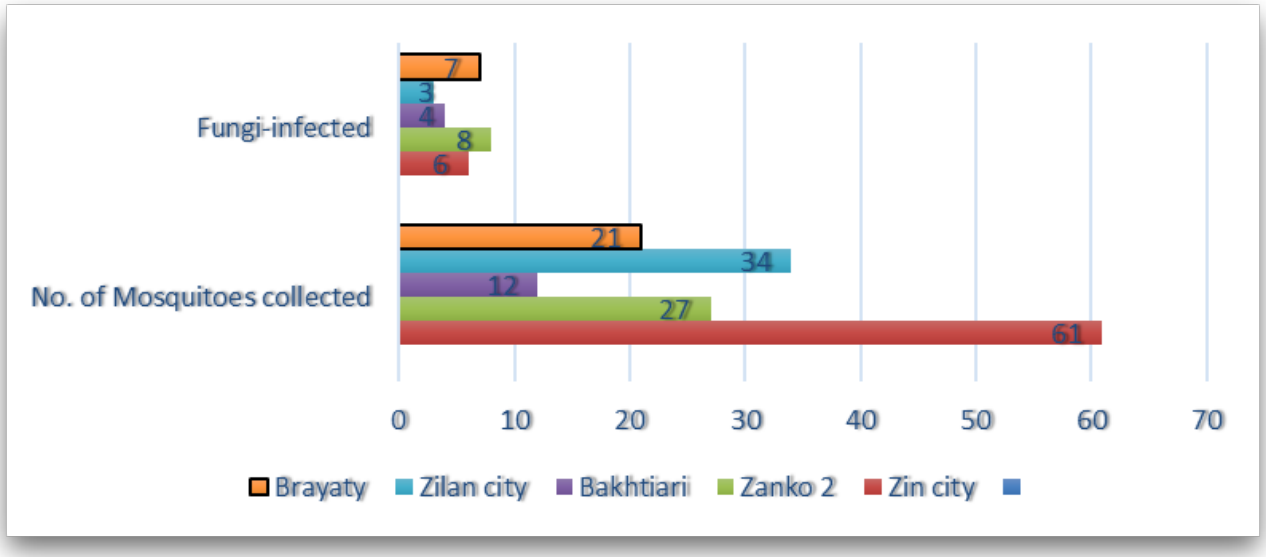

Figure 2. The histogram shows the number of mosquitoes collected from different quarters of Erbil city and the prevalence of fungal infection.

with a 100% sequence similarity. The BLASTN and sequence similarity results validate the isolation and identification of three *Aspergillus* fungal species from the collected mosquito, *Culex poicilipes*. These species include *Aspergillus* niger, *Aspergillus inflatus*, and *Aspergillus* tubingensis. Their respective accession numbers are PV085519, PV085520, and PV085521, which were submitted to GenBank. A synopsis of this isolated fungus is provided below.

3.1 *Aspergillus* niger (Ehenberg, 1822):

The filamentous fungus *Aspergillus* niger is frequently found in soil, decomposing vegetation, and interior spaces [19]. It is well known for growing quickly on a variety of culture media and producing black spores [20]. The Phylum Ascomycota, Class Eurotiomycetes, Order Eurotiales, and Family Trichocomaceae are all home to *Aspergillus* niger. On potato or Sabouraud dextrose agar at 25–28^{o}C, its colonies grow quickly, turning white at first and then black as a result of extensive sporulation [21]. It appears as septate hyphae with conidial heads made up of biseriate phialides extending from a vesicle under a microscope, although *Aspergillus* niger is not a motile organism and does not create aflatoxins, it is capable of producing ochratoxin A in specific circumstances, it is frequently utilized in industrial settings to produce enzymes and citric acid since it is catalase positive, current morphological and cultural characteristics consistent of those of *Aspergillus* niger [22].

Isolated sequences of query fungal samples were drawn from collected samples during this study and compared with the GenBank reference sequences to find out sequence similarity by using BLASTN. Results appear that the isolated sequences refer to *Aspergillus* niger with the percentage identity 100%, then it was recorded in GenBank under the name, Awaz-1 large subunit ribosomal RNA gene partial sequence and accession number of PV085519.

3.2 *Aspergillus inflatus* (Raper & Fennell, 1965):

Aspergillus inflatus is a filamentous fungus that is typically isolated from soil and decaying plant material. It belongs to Phylum: Ascomycota, Class: Eurotiomycetes, Order: Eurotiales, and Family: Trichocomaceae, Colony growth on Czapek yeast extract agar and Sabouraud Dextrose Agar at 25–28^{o}C is sluggish to moderate for this species [23]. Initially white, colonies eventually turn yellow to olive-brown, and they frequently have a velvety to granular texture. Under a microscope, *Aspergillus inflatus* creates globose conidial heads with uniseriate phialides extending from a vesicle that has been relatively inflated; it produces non-motile conidia and septate, branching hyphae [24]. The species do not create aflatoxins and is oxidase negative and catalase positive [25].

Isolated sequences of query fungal samples were drawn from collected samples during this study and compared with the GenBank reference sequences to find out sequence similarity (using BLASTN). Results appear that the isolated sequences refer to *Aspergillus* niger with the percentage identity of 100%, then it was recorded in GenBank under the name, Awaz-2 large subunit ribosomal RNA gene, partial sequence and accession number of PV085520.

3.3 *Aspergillus tubingensis* (Mosseray, 1934):

Aspergillus tubingensis is a filamentous, black-spored fungus that belongs to the *Aspergillus* section Nigri, commonly

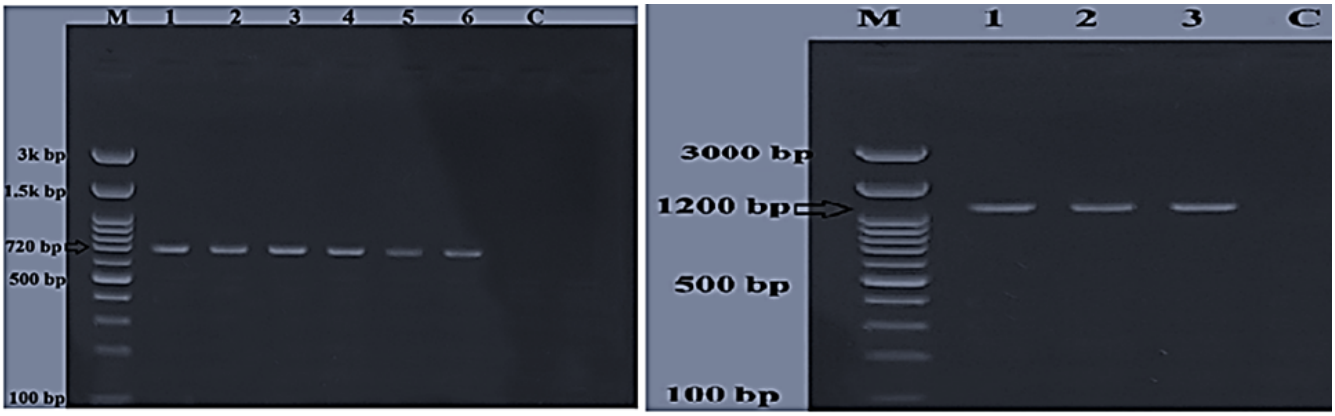

Figure 3. PCR amplification of partial COX1 gene (A) and LSU gene (B) from mosquito and fungi samples, respectively, in which the first lane is the marker ladder (3kbp-100bp) and the second to the last one are LSU gene bands with sizes 720 bp and 1200 bp, respectively. Lane C is a negative control, showing no band.

isolated from soil, food products, and indoor environments [26]. It is closely related to *Aspergillus* niger but can be differentiated based on morphological and molecular characteristics. Taxonomically, it belongs to Phylum: Ascomycota, Class: Eurotiomycetes, Order: Eurotiales, and Family: Trichocomaceae. Colonies grow quickly at 25–28^{o}C on Czapek yeast extract agar and Sabouraud Dextrose Agar [27]. Initially white, they eventually turn dark brown or black as a result of conidial production. It creates biseriate conidial heads with a smooth to slightly roughened vesicle under a microscope. Compared to *Aspergillus* niger, its conidia are rougher and smaller. *Aspergillus* tubingensis is a non-motile, catalase-positive fungus that is well-known for producing lipases and proteases [28]. The observed morphological and physiological characteristics align with Mosseray's (1934) original description.

Medical implication for these newly isolated fungal symbionts of mosquitoes is *Aspergillus* niger it is an opportunistic mold that most often acts as a saprophyte but can cause a range of human disease, particularly in predisposed patients. Clinically it has been reported as a cause of otomycosis, superficial and cutaneous infections, and less commonly invasive or necrotizing pulmonary disease in immunocompromised hosts. Because *A. niger* produces readily visible dark (black) conidia, it may be identified morphologically, but clinical relevance requires correlation with symptoms and repeated isolation or histopathologic evidence to distinguish contamination from true infection. Rare cases of invasive disease have been correlated with poor outcomes when diagnosis or appropriate antifungal therapy is delayed. Therefore, clinicians should consider *A. niger* in otologic and respiratory infections that fail routine therapy, especially in patients with local risk factors or immunosuppression [29].

Aspergillus inflatus is a less-commonly reported species within the genus that have been described taxonomically and occasionally recovered from environmental and clinical samples. Current evidence indicates *A. inflatus* to be primarily environmental but can be isolated from human specimens. However, clinical reports are sparse, and its role as a primary pathogen remains uncommon and not well characterized. From a clinical-microbiology and public-health perspective, the appearance of uncommon or "cryptic" *Aspergillus* species (including *A. inflatus*) in diagnostic laboratories highlights the importance of molecular identification (e.g., sequencing) and careful interpretation of culture results, because species-level differences can matter for epidemiology and sometimes for antifungal susceptibility. Emphasize that *A. inflatus* represents part of the expanding diversity of clinically encountered *Aspergillus* spp., it warrants surveillance and further study to define pathogenic potential and susceptibility profiles [30]. *Aspergillus* tubingensis has increasingly been recognized in clinical specimens, particularly from the respiratory tract, including patients with cystic fibrosis, chronic lung disease, or acute exacerbations.

Case reports and small series document that *A. tubingensis* can cause chronic pulmonary aspergillosis, invasive disease in immunocompromised hosts, and even rare extrapulmonary infections (for example: brain abscess). This emphasizes that cryptic species in the *A. niger* complex are clinically relevant. Importantly, the isolates within the *A. niger* complex can show variable antifungal susceptibility, so species-level identification and susceptibility testing are advisable when *A. tubingensis* is recovered from sterile sites or when infection is suspected, to guide therapy [31].

Isolated sequences of query fungal samples were drawn from collected samples during this study, and they were compared with the GenBank reference sequences to find out sequence similarity (using BLASTN). Results appear that the isolated sequences refer to *Aspergillus* niger with the percent-

age identity of 100%, then it was recorded in GenBank under the name, Awaz-2 large subunit ribosomal RNA gene, partial sequence, and accession number of PV08552.

Multiple sequence alignment was carried out across various *Aspergillus* species to ascertain the phylogenetic position of the isolated fungal sequences. Eight extremely identical sequences in all, with zero E-value and sequence identities ranging from 93.01% to 100%, were chosen. *Aspergillus* fumigatus (AF338659.1) was included as an outgroup to root the tree and provide a comparative reference. All sequences were retrieved from NCBI and aligned using the MUSCLE algorithm in MEGA software version 11($ProgramFiles11_64.exe$). Strong sequence conservation among the chosen strains was validated by the multiple alignment results. Following alignment, sequences were edited and examined using Jalview software (https://www.jalview.org/getdown/release/). To ensure statistical reliability, a phylogenetic tree was built using the Maximum Likelihood approach after alignment, using 100 bootstrap replications. The tree that was produced (Figure 4) showed distinct patterns of grouping between the reference sequences and isolates.

Isolates of *Aspergillus* niger, such as PV085519.1(Awaz-1) and MT536778.1 (Cu-17), formed a close cluster with reference strains MK273552.1 (strain ACSI) and MF187479.1 (strain CSR3) with strong bootstrap support (93–100), indicating their close genetic relationship. Similarly, isolates of *Aspergillus* infaltus, such as PV085520.1 (Awaz-2), MH871301.1, MH871300.1, MH871302.1, and MH871635.1, established a unique and well-supported clade, known as bootstrap values 97–100. Monophyly within the *Aspergillus* infaltus cluster is shown by this close grouping. Within the same major lineage, *Aspergillus* tubingiensis (PV085521.1) and *Aspergillus* luchuensis (PQ571950.1) developed distinct but closely related branches. *Aspergillus* fumigatus was used as an outgroup to further validate the tree's structure and set it apart from the ingroup species. Generally, the tree topology validated the isolated sequences' strong evolutionary ties with known *Aspergillus* species and corroborated their genetic identity.

All isolated sequences were compared in terms of the type and quantity of loops that emerged in the secondary structure of the isolated sequences' map, and the secondary structure prediction of the isolated sequences was compared with other closely related sequences under the accession number MF187479.1. The types of loops which are observed in all isolated sequences include External, Internal, Bulge, Hairpin, Helices, and Multi-branch loops are closely similar to the types of loops which are present in all previous recorded sequences. There are 119 loops in total for the query sequence and 123 loops in total for the subject.

Regarding the loop types in both sequences, the results that were mapped from earlier isolated sequences MF187479.1 were comparatively similar, as mentioned in Figure 5 and Table 4. This supports two fundamental ideas: first, that the isolated sequence is, and second, that the isolated sequence exhibits genetic diversity because of minor differences in the number of loops. This is because various studies have shown that DNA mutations that alter mRNA folding are linked to changes in gene expression. Consequently, any difference between the reference studies and the current investigation validates the genetic alterations of the locally isolated sequence.

The secondary structure of the isolated gene sequence PV085520.1 (Query) was examined and contrasted with the reference sequence MH871300.1 (Subject), as shown by the findings shown in Figure 6 and Table 5. The types and quantities of loops in both sequences were the same, totalling 105 loops each: 1 external loop, 23 internal loops, 2 bulge loops, 18 hairpin loops, 53 helices loops, and 10 multi-branched loops. A high degree of conservation between the isolated and reference sequences is suggested by this structural similarity. Furthermore, the reference sequence displayed a somewhat lower free energy of -408.60 kcal/mol, whereas the lowest free energy of the secondary structure for the query sequence was determined to be -398.60 kcal/mol. This energy difference could be a sign of small stability changes brought on by subtle nucleotide polymorphisms between the sequences. These results imply that there is little difference in the structural profile between the isolated gene sequence and the reference.

The secondary structure analysis of the isolated fungal gene sequence PV085521.1 (Query) was contrasted with the reference sequence MH871301.1 (Subject), as shown in Figure 7 and Table 6. The number of internal loops (23) and bulge loops (2 for the query and 1 for the topic) was the same in both sequences, but there were minor differences in the other loop types. There were 103 loops total in the query sequence: 20 hairpin loops, 51 helix loops, and 7 multi-branched loops. On the other hand, the reference sequence contained 107 loops in total: 19 hairpin loops, 53 helix loops, and 11 multi-branched loops. Despite being slight, these variations show some degree of structural variation among the sequences. Furthermore, the query sequence's computed minimum free energy was -355.40 kcal/mol, while the reference sequence was -374.60 kcal/mol. Subtle nucleotide variations may be the cause of the query sequence's decreased stability, as these variations might impact RNA folding and structural integrity. At a large scope, the findings show that while the isolated sequence exhibits minor structural differences that might have biological significance, it is structurally identical to the reference.

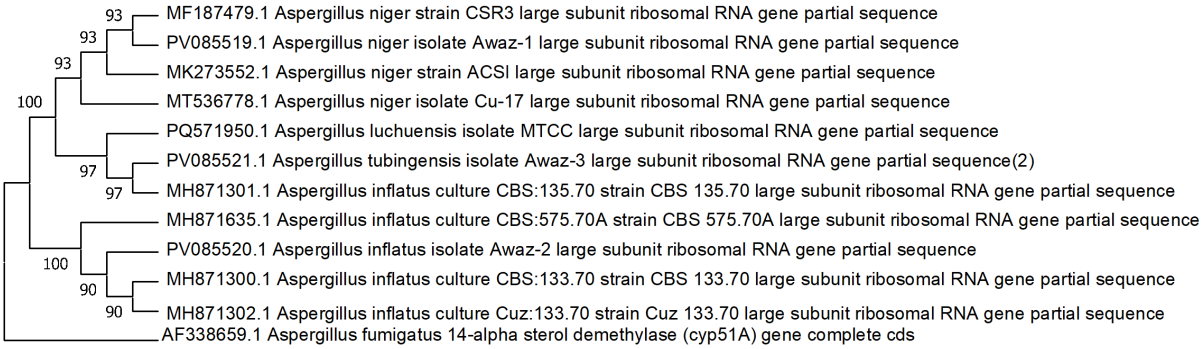

Figure 4. Represents a phylogenetic position of isolated fungal sequence among several related other fungal species and some other related sequence represented in maximum likelihood. Bar, 0.05 substitution per nucleotide position.

Table 4. Reagents used for PCR Amplification.

Sequences	External loops	Internal loops	Bulge loops	Hairpin Loops	Helices loops	Multi-branched	Total loop
PV085519.1 (Query)	0	26	4	23	59	7	119
MF187479.1 (Subject)	0	26	4	24	60	9	123

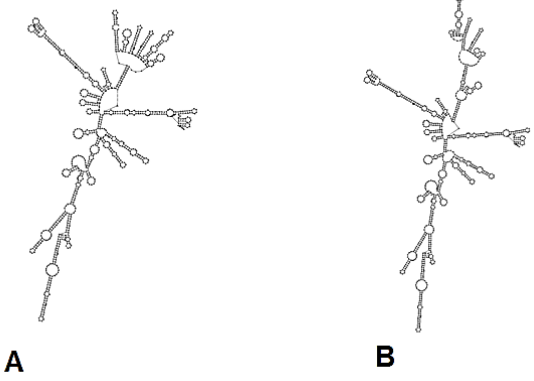

Figure 5. Schematic representation of the gene expects secondary sequence of the isolated fungi sequence, (A) PV085519.1 and the reference sequence, (B) MF187479.1.

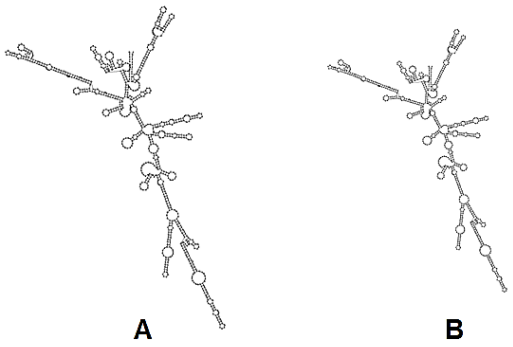

Figure 6. Schematic representation of the gene expected secondary sequence of the isolated fungi sequence, (A) PV085520.1, and the reference sequence, (B) MH871300.1.

4. Conclusions:

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This study reaches the conclusion that *Culex poicilipes* mosquitoes in Erbil carry *A. niger*, *A. inflatus*, and *A. tubingensis*, which suggest a potential role in fungal transmission. The observed genetic polymorphism in *A. niger* and *A. tubingensis* highlights the need for ongoing molecular surveillance. These findings may support the Ministry of Health in designing monitoring programs for vector-borne fungal pathogens.

Table 5. Types and Number of Loops Calculated from Secondary Structure Analysis of gene Sequence of the Isolated Sample and Previously Recorded Sequence.

Sequences	External loops	Internal loops	Bulge loops	Hairpin Loops	Helices loops	Multi-branched	Total loop
PV085520.1 (Query)	1	23	2	18	53	10	105
MH871300.1 (Subject)	1	23	2	18	53	10	105

Table 6. Types and Number of Loops Calculated from Secondary Structure Analysis of Gene Sequence of the Isolated Sample and the Previously Recorded Sequence.

Sequences	External loops	Internal loops	Bulge loops	Hairpin Loops	Helices loops	Multi-branched	Total loop
PV085521.1 (Query)	0	23	2	20	51	7	103
MH871301.1 (Subject)	0	23	1	19	53	11	107

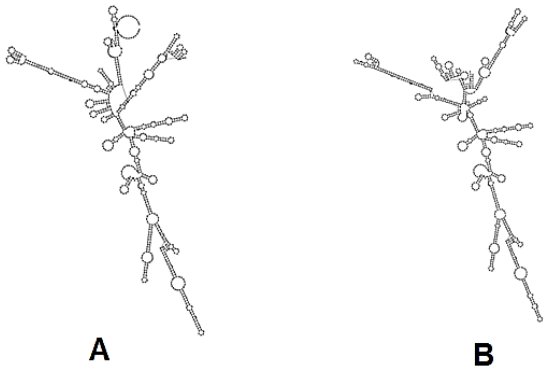

Figure 7. Schematic representation of the gene expected secondary sequence of the isolated fungi sequence, (A) PV085521.1, and the reference sequence, (B) MH871301.1.

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Data Availability Statement: All of the data supporting the findings of the presented study are available from corresponding author on request.

Declarations:

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: Our works include no human-related samples, while we conducted the procedure after obtaining the patients' verbal and informed consent before sample collection. The research protocol was approved by the local Ethical Committee at the Erbil Health and Medical Technical College, Erbil Polytechnic University.

Author Contributions: Awaz Neahmat Ahmed: data collection; data recording and analysis; writing all parts, including abstract, introduction, methodology, results, and discussion; conclusion finally references. Karwan Sallo Najm: Conceptualization; helping in molecular data analysis; reviewing and editing.

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التشخيص الجزيئي، والتحليل الوراثي التطوري، ودراسة البنية الثانوية للحمض النووي الريبي لثلاثة أنواع من فطر الرشاشيات (*Aspergillus*) المعزولة من البعوض المحلي *Culex poicilipes* المُجمّع من مدينة أربيل، إقليم كردستان، العراق

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الخلاصة

يهدف هذا البحث إلى دراسة الارتباطات الفطرية في بعوض *Culex poicilipes* الذي جُمِع من مدينة أربيل، اقليم كردستان – العراق. تم تحليل ما مجموعه 155 عينة من البعوض البالغ، وأظهرت النتائج إصابة فطرية بنسبة 18% . أُجري التحليل الجزيئي باستخدام جيني *COI* و *LSU* ، حيث تم تحديد ثلاث أنواع من جنس *Aspergillus* وهي: *A.niger* و *A.inflatus* و *A.tubingensis*. وقد أودعت التسلسلات الناتجة في قاعدة بيانات *GenBank* وسُجّلت تحت أرقام الاعتماد: *PV*085519.1 و *PV*085520.1 و *PV*085521.1 على التوالي. أكّد التحليل الوراثي (*Phylogeneticanalysis*) والتنبؤ بالبنية الثانوية للـ *RNA* على العلاقات التصنيفية على مستوى النوع، كما أظهر وجود تعددية شكلية جينية *polymorphism genetic* . وتستنتج الدراسة أنّ بعوض *C.poicilipes* قد يعمل كناقل محتمل لأنواع *Aspergillus* ، الأمر الذي يحمل دلالات مهمة على الصحة العامة.

الكلمات الدالة : البعوضة؛ الفطريات؛ التعدد الشكلي (*Polymorphism*) ؛ (*LSU*) ؛ أربيل.

التمويل: لايوجد.

بيان توفر البيانات: جميع البيانات الداعمة لنتائج الدراسة المقدمة يمكن طلبها من المؤلف المسؤول.

اقرارات:

تضارب المصالح: يقر المؤلفون أنه ليس لديهم تضارب في المصالح.

الموافقة الأخلاقية: : تشمل أعمالنا أي عينات متعلقة بالإنسان ، بينما أجرينا الإجراء بعد الحصول على الموافقة الشفهية والتحليلية للمرضى قبل جمع العينات. تمت الموافقة على بروتوكول البحث من قبل اللجنة الأخلاقية المحلية في كلية أربيل للصحة والطب التقني ، جامعة أربيل للفنون التطبيقية.

مساهمات المؤلفين: اواز نعمة أحمد: جمع البيانات؛ تسجيل البيانات وتحليلها ؛كتابة جميع الأجزاء بما في ذلك الملخص ؛ المقدمة ؛ النتائج والمناقشة ؛ المصادر. كاروان سلو نجم: صياغة المفاهيم ؛ المساعدة في تحليل البيانات الجزيئية؛ المراجعة والتنقيح.