Research Article

Molecular Identification of Mushroom Flies by Utilizing 28S rRNA

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Abstract | On Al-Qadisiyah farms, mushroom flies can cause several damages by reducing growth of mushroom body. Recently, mushroom flies have significantly reduced mushroom production in Iraq. moreover, larvae and adults of these pest cannot be identified due to their lack of morphological and genetical information. Thus, Iraqi farmers are unable to identify which pest is the primary cause of the mushroom damage in their farms. In this study, mushroom flies (adults) were collected from damage mushroom farms at Al-Qadisiyah governorate in Iraq, and subsequently determined by using 28S ribosomal RNA. Divergences of the 28S ribosomal RNA sequences among the species discriminated the clusters clearly, and the mushroom flies were identified as *Scatopse notata* and *Coboldia fuscipes*.

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Introduction

Mushroom is a fungus that belongs to the family of Basidiomycetes, and it is saprophytic, which decomposes food waste, wheat straw, and wooden plant to absorb the nutrients (Khorsheed and Ahmed, 2023; Sabri *et al*., 2020). Since days of yore, mushroom has been utilized by humans as an antimicrobial activity, food, antibacterial, antiviral, and other medicinal purposes (Hassan and Al-Qiassi, 2022; Chechan *et al*., 2020). Mushroom also is grown for purifying polluted soils from crude oil and heavy metals (Owaid *et al*., 2015). In Iraq, edible mushrooms grow naturally in several places, with the Kurdistan Region-Iraq recognized as the primary area for mushroom cultivation and production (Owaid *et al*., 2014, 2018; Nadir *et al*., 2020; Muslat *et al*., 2020). Nonetheless, mushroom production remains inferior to worldwide levels due to its vulnerability to several diseases and pests, which affect both the amount and quality of output (Navarro *et al*., 2021).

Mushroom flies are the primary pest of oyster mushrooms. These insects inflict significant harm to mushrooms as the larvae of the mushroom fly consume the fruiting body and mycelium of the oyster mushroom. Additionally, mushroom fly adults carry many pathogens, including mites, mold spores, and nematodes, on mushrooms (Bae *et al*., 2001; Rijal *et al*., 2021; Navarro *et al*., 2021). Different

types of mushroom flies cause significant damage on mushroom development and productivity. The predominant species in mushroom cultivation and production areas globally, including Iraq, are the sciarid flies (Diptera: Sciaridae), the phorid fly (Diptera: Phoridae), and the cecid flies (Diptera: Cecidomyiidae) (Nguyen *et al*., 2023; Duarte *et al*., 2020; Erler and Polat, 2015).

The mushroom cultivation and production at Al-Qadisiyah governorate and other regions in Iraq can be infested by different types of peat, creating serious threats to the mushroom cultivation process. Therefore, this study becomes essential to identify different pests by using molecular techniques to get the optimum yield of mushroom in different Iraqi farms.

Materials and Methods

Mushroom fly samples collection

Adult mushroom flies were collected utilizing an aspirator at several mushroom farms in Al-Qadisiyah governorate, Iraq (Figure 1). Samples collected in the mushroom field were transferred to the laboratory and kept into the plastic container of 1000 g capacity. Moreover, samples stored in 70% ethanol were utilized for molecular analysis.

DNA extraction, amplification, sequencing, and phylogenetic tree

Whole-body DNA was extracted from individual adult specimens with the G-spin DNA extraction kit. The ribosomal RNA gene (28s) was expanded by using the Polymerase Chain Reaction (PCR) employing primers: 28s forward (5'- GAG AGT TMA ASA GATCGT GAA AC- 3') and reverse (5'- TGC GAR GAGACC AGC TAC TA - 3'). The PCR Master Mix 2X (iNtRON, Korea) was employed, consisting of an over-all reaction volume of 25 µL, which comprised i-Taq DNA Polymerase at 5 U/mL, DNTPs at 2.5 mM, a 10X reaction buffer at 1X, 1.5 µL of DNA, 16.5 µL of distilled water, and 1 µL of each primer (10 picomoles/µL).

PCR was conducted on a Multi Gene Opti Max Gradient Thermal Cycler (Labnet). The thermal cycling protocol comprised a premier denaturation at 94 °C for 1 minute, followed by 5 cycles of 94 °C for 45 seconds, 58 °C for 1 minute and 45 seconds, and 72 °C for 45 seconds, concluding with an additional 35 cycles and a last expansion at 72 °C for 7 minutes. The PCR produces were divorced via 2% agarose gel electrophoresis and seen under ultraviolet light (302 nm) following staining with Red Stain. They were subsequently refined and bidirectionally sequenced utilizing the BigDye Terminator v3.1 Cycle Sequencing kit from Macrogen Inc. (Korea) on an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA).

The sequence was analyzed in nucleotide files using NCBI's Basic Local Alignment Search Tool Bio ID for sample identification and subsequently submitted to GenBank (ID). Samples were acquired from the NCBI nucleotide database (www.ncbi.nlm.gov/nucleotide) and incorporated into multiple alignments utilizing the Bio ID software (Tamura *et al*., 2011). The evolutionary history was inferred using the Neighbor-Joining method. The Jukes-Cantor model was utilized to calculate evolutionary distances for determining phylogenetic relationships, as executed in the Gene 6 software.

Figure 1: *Sampling location of Scatopse notata and Coboldia fuscipes in Al-Qadisiyah, Iraq.*

Results and Discussion

The results demonstrate that high-quality sequences of the gene samples (Ribosomal RNA 28s) from the fly were acquired from Microgen. A homology search was conducted using the BioEdit application with the Basic Local Alignment Search Tool (BLAST),

attainable by the National Center for Biotechnology Information (NCBI) at (http://www.ncbi.nlm.nih.gov). Our samples corresponded to two species of flies from the Scatopsidae family (Diptera), namely Scatopse notata and Coboldia fuscipes. We selected one specimen from each species for prioritized application and subsequently registered it with NCBI after successful validation. Utilizing the universal time reversible model and the maximum likelihood technique, we inferred the evolutionary history based on Nei and Kumar (2000). The tree demonstrating the most significant heuristic search was autonomously produced utilizing the Neighbor-Joining and BioNJ algorithms on a matrix of pairwise distances. The distances were computed utilizing the Maximum Composite Likelihood (MCL) method. The topology exhibiting of the highest log probability value was then carefully chosen. The central nodes of the tree signify the percentage of places that include at least one unambiguous base in at least one sequence for each descendant clade. This research includes five nucleotide sequences (Figure 2). The completed dataset included 131 sites. The sequences of both samples were identified. One of the sample sequences (PP756476) displayed a dominant haplotype. The sequence exhibited 98% similarity to the publicly available *C. fuscipes* sequences KC177651 from the USA and KJ136764 from the Czech Republic, whereas the other sequence (PP756633) shown 97% similarity to the publicly accessible *Scatopse notata* sequences at KJ136763.1 from the Czech Republic. Evolutionary studies were carried out utilizing the Mega X, which explained by Kumar *et al.* (2018). Our results correspond with those of Nguyen *et al.* (2023), who identified the DNA barcode of *Coblodio fuscipes* in Australian fungi. The findings align with a comparable work aimed at discovering Scatopse notata that infects fungi through the examination of 12S and 16S ribosomal RNA genes (Basbagci, 2012; Ševčík *et al.*, 2014; Zhang *et al.*, 2016; Alameri and Alhasan, 2020). The Mushroom flies, *Coblodio fuscipes*, and *Scatopse notate*, are the principal nuisance flies in mushroom farms, owing to their significant reproductive capacity in these settings, which diminishes mushroom yield. The proliferation of *Coblodio fuscipes* and *Scatopse notate* flies in the mushroom farms in Al-Qadisiyah Governorate is attributable to the utilization of plant waste and organic fertilizer as substrates for mushroom cultivation without prior sterilization.

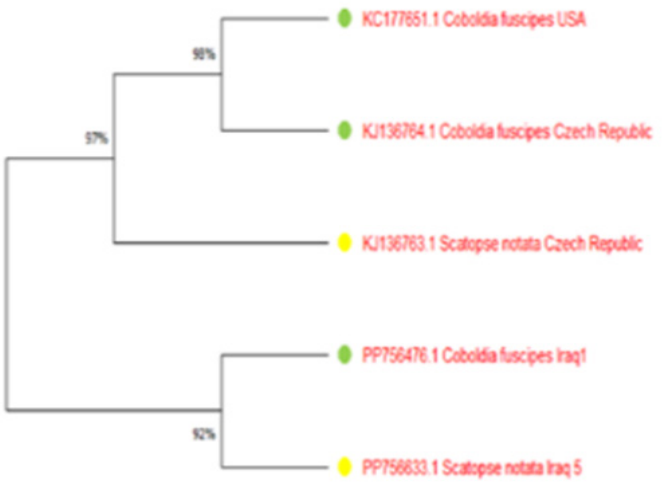

Figure 2: *A maximum likelihood phylogenetic tree utilizing the ribosomal RNA gene (28s) of Coboldia fuscipes and Scatopse notata serves as the outgroup.*

Conclusions and Recommendations

Reporting the incidence infection of mushroom flies (*Scatopse notata* and *Coboldia fuscipes*) in Al-Qadisiyah, Iraq is the first, based on our knowledge. These flies can be a threat to mushroom production in Iraq, especially when disseminating. Besides can mushroom flies transport different germs such as mites, mold spores, and nematodes on mushroom. Moreover, construction of the 28S ribosomal RNA gene database can be a useful tool for the lab of applied entomology, especially for mycophagous dipteran pests at the adult stage.

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Novelty Statement

The research and experimental work on the subject title is original and new in the field of entomology science in Iraq.

Author's Contribution

Dalal Tareq Al-Ameri: Investigation, methodology, data curation, writing – original draft, and funding acquisition
Ali Sabah Alhasan: Writing – review and editing

Conflict of interest

The authors have declared no conflict of interest.

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