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Fungal Community Associated with Loggerhead Sea Turtle Caretta caretta eggshells and Nest sand from Sirte, Libya

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المجتمع الفطري المرتبط بقشور البيض وتربة العش للسلحفاة البحرية ضخمة الرأس *Caretta caretta* من سرت، ليبيا

Article history

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الملخص:

باستخدام تقنيات تعتمد على زراعة الفطريات في المعمل، تكشف هذه الدراسة النقاب عن التنوع الفطري المرتبط بأعشاش السلاحف البحرية ضخمة الرأس (*Caretta caretta*) بشاطئ الثلاثين، غربي سرت، ليبيا. من خلال عزل الفطريات وتحديدها من قشور البيض الفاقس وعينات رمل الأعشاش التي تم جمعها خلال موسم التعشيش لعام 2023م، لتساهم في موضوع لم يدرس سابقا بليبيا. كشفت تحليلاتنا عن مجتمع فطري غني يضم عشرة أنواع، بما في ذلك فطر الفيوزاريوم *Fusarium* (تم تحديد نوعي *F.* solani و *F. oxysporium*)، وفطر الاسبراجيلس (*Aspergillus niger* و *Aspergillus* sp.)، والكلادوسبوريوم *Cladosporium* sp ، والبينيسليوم *Penicillium* sp واللترناريا *Alternaria* sp ، والستاكيبوترس *Stachybotrys* sp والتي كانت معزولة بشكل متكرر من كل من قشر البيض ورمل العش. ومن المثير للاهتمام ، تم العثور على فطريات *Trichoderma* sp و *Chaetomium* sp. حصريا في عينات رمال العش، مما يشير إلى تفضيلات متخصصة محتملة داخل الموائل الدقيقة للعش. تتوافق هذه النتائج مع الملاحظات في مناطق أخرى، مما يشير إلى احتمال وجود هذه الأصناف في كل مكان عبر شواطئ التعشيش. إن وجود أنواع *Fusarium* و *Aspergillus* ، وهي مسببات أمراض معروفة لأنواع مختلفة من السلاحف البحرية ، يستدعي مزيدا من التحقيق بسبب تأثيرها المحتمل على صحة البيض وتأثيره على فقس البيض. يمكن للدراسات المستقبلية التي تستخدم الأساليب الكمية لتقييم وفرة الفطريات والمراقبة في الموقع لأنماط نمو الفطريات طوال فترة الحضانة أن توفر رؤى أعمق لهذه التفاعلات. بالإضافة إلى ذلك، فإن التحقيق في عوامل البيئة الدقيقة للعش مثل درجة الحرارة والرطوبة جنبا إلى جنب مع جهود عزل الفطريات سيساعد في استكشاف الارتباطات بين الظروف البيئية للعش وتكوين المجتمع الفطري. من خلال توضيح هذه العلاقات، يمكننا تطوير استراتيجيات الحفظ المستهدفة لتحسين نجاح الحضانة وفقس السلاحف ضخمة الرأس بشكل أفضل في شمال إفريقيا.

الكلمات المفتاحية: التنوع الفطري، السلاحف البحرية ضخمة الرأس، البيئات الدقيقة للأعشاش، فطريات الفوسياريوم، استراتيجيات الحفظ.

A B S T R A C T:

Employing culture-dependent techniques, this study unveils the fungal diversity associated with Loggerhead Sea turtle (*Caretta caretta*) nests on Al-Thalateen beach, Libya, the first to explore this aspect in the country. By isolating and identifying fungi from eggshells and nest sand samples collected during the 2023 nesting season, we contribute valuable insights into a previously understudied topic. Our analyses revealed a rich fungal community encompassing ten genera, including *Fusarium* (identified as *F. solani* and *F. oxysporium*), *Aspergillus* (*Aspergillus niger* and *Aspergillus* sp.), *Alternaria* sp., *Cladosporium* sp., *Penicillium* sp., and *Stachybotrys* sp., all were frequently isolated from both eggshells and nest sand. Interestingly, *Trichoderma* sp. and *Chaetomium* sp. were found exclusively in nest sand samples, hinting at potential niche preferences within the nest microhabitat. These findings align with observations in other regions, suggesting a possible ubiquity of these taxa across nesting beaches. The presence of Fusarium and Aspergillus species, known pathogens in various sea turtle species, warrants further investigation due to their potential impact on egg health. Future studies employing quantitative methods to assess fungal abundance and in situ monitoring of fungal growth patterns throughout the incubation period could provide deeper insights into these interactions. Additionally, investigating nest microenvironment factors like temperature and humidity alongside fungal isolation efforts would aid in exploring correlations between the nest's ecological conditions and fungal community composition. By elucidating these relationships, we can inform the development of targeted conservation strategies to improve Loggerhead turtle nest success in North Africa.

Keywords: Fungal Diversity, Loggerhead Sea Turtles, Nest Microhabitats, Fusarium Fungi, Conservation Strategies

Introduction:

Marine turtles face a multitude of threats throughout their life cycle, including habitat degradation, climate change, and bycatch in fisheries (Wallace et al., 2022). Loggerhead turtles (*Caretta caretta*) are a critical component of marine ecosystems in the Mediterranean Sea, and populations in North Africa, particularly Libya (SPA/RAC-UNEP/MAP, 2021). Egg mortality is a major bottleneck hindering loggerhead turtle population recovery (Philott, 2001). While predation and environmental factors undeniably contribute to egg loss, emerging research suggests fungal pathogens pose a growing threat to sea turtle egg health in various regions (Tiwari et al., 2014). Fungi are ubiquitous in the environment, and their presence within nests is not inherently detrimental. Specific fungal species can act as opportunistic pathogens, compromising egg development and viability (Güçlü et al., 2010). A new recognized fungal disease called Sea Turtle Egg Fusariosis (STEF) is spreading globally (Gleason et al., 2020). This disease infects eggs in sea turtle nests, leading to death for the developing embryos. It's caused by several fungal species within the Fusarium genus, especially those in the Fusarium Solani Species Complex (Nardoni and Mancianti, 2023).

Understanding the factors influencing loggerhead turtle nest success is particularly important in North Africa, where knowledge gaps persist. Recent studies in Libya have highlighted threats such as predation and beach erosion at nesting areas (SPA/RAC-UNEP/MAP., 2021), but research on the specific microbial communities within Libyan loggerhead turtle nests are not available.

Previous studies in the Mediterranean have documented the presence of potentially pathogenic fungi associated with loggerhead turtle nests, including Aspergillus and Fusarium species (Güçlü et al., 2010, Candan, 2018, Risoli et al., 2023). Some studies included other forms of microbiota from the eggs and nest sand of Sea turtles (Vecchioni et al., 2022).

In Libya, a comprehensive understanding of the fungal communities within Libyan nests and their potential impact on egg health is lacking. Given the vulnerability of the Loggerhead Sea turtle population, a thorough investigation of potential threats, including threats within the incubated nest, is crucial for setting up effective conservation strategies

(SPA/RAC-UNEP/MAP, 2021). The present study investigates the mycobiota, or fungal communities, associated with loggerhead turtle nests on the nesting beaches of Al-Thalateen, Libya, one of the major nesting sites along the Libyan coast, and ranked second in terms of nesting density (SPA/RAC-UNEP/MAP, 2021). Our findings contribute to the knowledge base on fungal communities within loggerhead turtle nests in the Mediterranean region, with a specific focus on a previously understudied population in North Africa. By elucidating the composition of the fungal community, we aim to gain insights into potential risks posed by fungal pathogens to loggerhead turtle egg health at Al-thalateen beach. This knowledge can inform future management practices aimed at improving nest success and population recovery efforts (Filek et al., 2024).

Materials and Methods:

Sample Collection and Processing: Fungal isolates were collected from eggshells and nest sand samples collected during the 2023 nesting season, after the end of the incubation period and hatchling emergence. Sand eggshell samples were collected using a randomized sampling design from the 5 Km long Al-Thalateen nesting site (31°13'38.24"N, 16°17'16.84"E). To minimize potential contamination, sterile collection techniques were employed throughout the process. Samples were transported to the laboratory in sterile containers and air-dried at room temperature under aseptic conditions.

For sand samples, a one-gram aliquot was subjected to serial dilution using a sterile diluent, typically phosphate-buffered saline (PBS). One millilitre of aliquots 10^-2 and 10^-3 dilutions was then plated onto Petri dishes containing potato dextrose agar (PDA) media using an aseptic technique. Eggshells collected from unhatched eggs were directly similarly plated onto PDA media. All plated samples were incubated at room temperature for a period ranging from 7 to 14 days, to allow for fungal growth.

Fungal Isolation: Following incubation, plates were examined for the presence of fungal colonies. Colonies exhibiting distinct morphologies, such as variations in colour, texture, and growth patterns, were marked for further analysis. A single representative subculture from each morphotype was isolated using sterile techniques to ensure pure cultures for identification.

Fungal Identification: Isolated fungal cultures were maintained on fresh PDA media and subjected to microscopic examination for species-level identification, Standard identification keys were used (Summerell et al., 2003; Raghukumar, 2017). Utilizing a light microscope, fungal structures were examined at various magnifications. At 10x magnification (low power), the overall morphology of the vegetative mycelium was assessed, including characteristics like colony pigmentation, colony texture, and the presence or absence of septa within the hyphae. Higher magnification objectives, typically 40x and 100x, were employed to visualize and identify diagnostic reproductive structures, such as conidiophores, conidia, ascospores, or basidiospores, which are crucial for definitive identification at the species level.

Results:

Fungal isolation from eggshells and nest sand of loggerhead turtles (*Caretta caretta*) nesting at Al-Thalateen beach, Libya, revealed a diverse fungal community (Figure 1). Eight fungal genera and ten species were identified, including *Fusarium*, *Aspergillus*, *Trichoderma*, *Alternaria*, *Stachybotrys*, *Chaetomium*, *Cladosporium*, and *Penicillium* (Table 1).

Table 1. Fungal isolation from eggshells and nest sand of loggerhead turtles nesting at Al-Thalateen beach, Libya.

Fungal Genus	Eggshells	Nest Sand
Fusarium solani	+	+
Fusarium oxysporium	+	+
Aspergillus niger	+	+
Aspergillus sp.	+	+
Alternaria sp.	+	+
Cladosporium sp.	+	+
Penicillium sp.	+	+
Stachybotrys sp.	+	+
Chaetomium sp.	-	+
Trichoderma sp.	-	+

Note: ‘+’ indicates the presence of the fungus, ‘-‘ means absence.

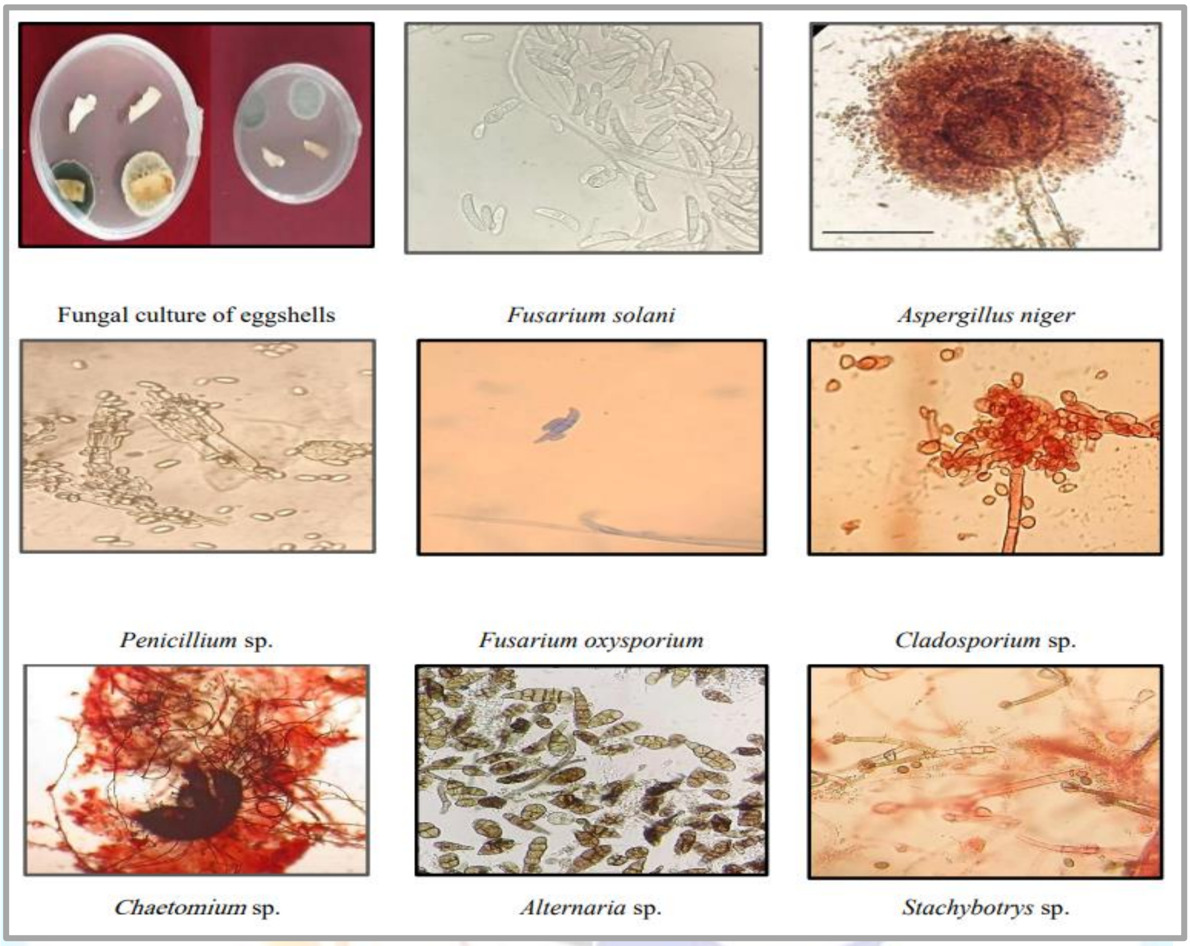

Figure 1. Microscopic images of isolated fungal species, under lens x40.

The most frequently isolated fungi were *Fusarium* (identified as *Fusarium solani* and *Fusarium oxysporium*), followed by Aspergillus (*Aspergillus niger* and *Aspergillus* sp.), *Alternaria* sp., *Cladosporium* sp., and *Penicillium* sp. and *Stachybotrys* sp. Additionally, *Trichoderma* sp. and *Chaetomium* sp. were isolated exclusively from nest sand samples.

The presence of these fungi, particularly *Fusarium* and Aspergillus species, known pathogens associated with egg mortality in sea turtles (Güçlü et al., 2010; Tiwari et al., 2014; Santos et al., 2015), and for Fusarium species, Sea Turtle Egg Fusariosis (STEF) can spread and cause catastrophic consequences on incubated clutches, leading to mass egg mortality in some areas (Gleason et al., 2020).

Discussion:

Studies on fungal communities in marine animal species are limited in Libya, recently Al-Masri et al., (2023) identified fungal species from the skin and gills of the White

Seabream *Diplodus sargus*. The current study sheds light on the fungal community associated with Loggerhead Sea turtle (*Caretta caretta*) nests at Al-Thalateen Sirte Beach, Libya. By isolating and identifying fungi from eggshell and nest sand samples collected during the 2023 nesting season, we contribute valuable insights to the understudied topic of fungal diversity in North African loggerhead turtle nests and expand the knowledge base on fungal communities within Mediterranean loggerhead nests in general.

Our culture-dependent approach revealed a diverse nesting environment mycobiota, encompassing eight fungal genera (Table 1). The most frequently isolated fungi – *Fusarium* (including *F. solani* and *F. oxysporium*), *Aspergillus* (*A. niger* and *Aspergillus* sp.), *Alternaria* sp., *Cladosporium* sp., and Penicillium sp. – align with findings from previous studies on loggerhead nests in other countries, like Turkyie (Güçlü et al., 2010); Indian Ocean (Tiwari et al., 2014) and Brasil (Santos et al., 2015). This suggests a potential omnipresence of these taxa across nesting beaches globally. Interestingly, *Trichoderma* sp. and *Chaetomium* sp. were isolated exclusively from nest sand samples, hinting at possible niche preferences within the nest microhabitat, which in agreement with similar studies in Turkey (Güçlü et al., 2010) and Oman (Elshafie et al., 2007). Studies on leatherback and green turtle nests revealed a diverse array of fungi, with *Aspergillus*, *Cladosporium*, and *Curvularia* prevalent in leatherback nests (Patino-Martinez et al., 2014) while *Aspergillus variecolor*, *Aspergillus quadrilinieatus*, and *Rhizopus oryzae* are common in green turtle nests (Elshafie et al., 2007). Additionally, *F. oxysporum, F. solani, and Pseudallescheria boydii* are isolated from failed eggs of various sea turtle species (Güçlü et al., 2010; Tiwari et al., 2014; Santos et al., 2015).

The presence of *Fusarium* and *Aspergillus* species in both eggshells and sand require closer attention. These genera are known to include pathogens associated with egg mortality in various sea turtle species, including Loggerheads (Güçlü et al., 2010; Tiwari et al., 2014; Santos et al., 2015). Studies have identified various fungal species like *Fusarium oxysporum* and *Aspergillus* spp. colonizing turtle eggs, potentially leading to mortality and developmental issues (Elshafie et al., 2007). Additionally, in situ monitoring of fungal growth patterns throughout the incubation period would provide valuable insights into potential interactions between fungi and developing embryos (Tiwari et al., 2014).

The nest microenvironment, encompassing factors like temperature, humidity, and organic matter content, significantly influences fungal growth patterns within nests (Güçlü et al., 2010). The ideal temperature range for fungal growth is typically between 24°C to 28°C, with optimal relative humidity levels ranging from 60% to 75% (Zhan et al., 2021). Measuring these parameters alongside fungal isolation efforts would allow for exploration of potential correlations between the nest microenvironment and fungal community composition (Santos et al., 2015). Understanding these relationships could inform management strategies aimed at optimizing nest conditions to mitigate the negative impacts of fungal colonization on egg health (Tiwari et al., 2014).

The composition of the nest microenvironment, including factors such as temperature, humidity, and organic matter content, can influence fungal growth. The ideal temperature range for fungal growth is typically between 24°C to 28°C, with optimal relative humidity levels ranging from 60% to 75% (Zhan et al., 2021). Measuring these parameters alongside fungal isolation would allow for the exploration of potential correlations between the nest microenvironment and fungal community composition. Understanding these relationships could inform management strategies to optimize nest conditions and potentially mitigate negative fungal impacts.

Our study employed a culture-dependent approach for fungal isolation, which may not capture the entire fungal diversity within the nesting environment. Future studies could incorporate molecular techniques, as advocated by Candan et al. (2018) in their work on Green Turtle fungal communities. This would allow for a more comprehensive understanding of the fungal community composition, potentially revealing unculturable but ecologically relevant species. Additionally, investigating the abundance of each fungal genus through quantitative methods would provide a more nuanced understanding of the fungal populations within eggshells and nest sand. Studies on loggerhead turtle nests showed a correlation between fungal presence and hatching success, for example, fungal presence on unhatched eggs of both loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) turtles can lead to complete egg mass mortality, resulting in zero hatching success (Phillott & Parmenter, 2001, Tiwari et al,., 2014), which highlight the importance of understanding fungal populations in such ecosystems (Güçlü et al., 2010).

To gain a deeper understanding of the fungal community within the nest environment, further studies employing quantitative methods to assess fungal abundance for each genus would be valuable. This would allow for comparisons between fungal populations in eggshells and nest sand, and potentially elucidate variations across nest sites or incubation stages. Additionally, employing molecular identification techniques could provide a more precise understanding of fungal species diversity within each genus.

In conclusion, this study provides the first preliminary data on the fungal community associated with Loggerhead Sea turtle nests from Libya. The identification of a diverse fungal assemblage, including potentially pathogenic genera, underscores the need for further research on the interplay between fungi and egg health in this understudied population. By elucidating these interactions and the role of the nest microenvironment, we can contribute to the development of informed conservation strategies to improve Loggerhead turtle nest success in North Africa.

References:

Al-Masri, T. A., Benzeglam, S. A. B., & Hamza, A. (2023). Mycobiota of the White Seabream Diplodus sargus (Linnaeus, 1758) from Northwestern Libya. *Faculty of Education Tripoli Journal*, *1*(18).

Canadn, E. D. (2018). Molecular identification of fungal isolates and hatching success of green turtle (Chelonia mydas) nests. Archives of microbiology, 200, 911-919.

Elshafie, A., Al-Bahry, S. N., AlKindi, A. Y., Ba-Omar, T., & Mahmoud, I. (2007). Mycoflora and aflatoxins in soil, eggshells, and failed eggs of Chelonia mydas at Ras Al-Jinz, Oman. Chelonian Conservation and Biology, 6(2), 267-270.

Filek, K., Vuković, B.B., Žižek, M., Kanjer, L., Trotta, A., Di Bello, A., Corrente, M. and Bosak, S., (2024). Loggerhead Sea Turtles as Hosts of Diverse Bacterial and Fungal Communities. Microbial ecology, 87(1):1-16.

Gleason, F. H., Allerstorfer, M., & Lilje, O. (2020). Newly emerging diseases of marine turtles, especially sea turtle egg fusariosis (SEFT), caused by species in the *Fusarium solani* complex (FSSC). Mycology, 11(3), 184-194.

Güçlü, Ö., Bıyık, H., & Şahiner, A. (2010). Mycoflora identified from loggerhead turtle (*Caretta caretta*) eggshells and nest sand at Fethiye beach, Turkey. African Journal of Microbiology Research, 4(5), 408-413.

Nardoni, S., and Mancianti, F. (2023). Mycotic diseases in chelonians. Journal of Fungi, 9(5), 518.

Patino-Martinez, J., Marco, A., Quiñones, L., Abella, E., Abad, R. M., & Diéguez-Uribeondo, J. (2012). How do hatcheries influence embryonic development of sea turtle eggs? Experimental analysis and isolation of microorganisms in leatherback turtle eggs. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology, 317(1), 47-54.

Phillott, A. D., & Parmenter, C. J. (2001). The distribution of failed eggs and the appearance of fungi in artificial nests of green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) sea turtles. Australian Journal of Zoology, 49(6), 713-718.

Raghukumar, S. (2017). Fungi in coastal and oceanic marine ecosystems (Vol. 378). New York, NY, USA:: Springer

Risoli, S., Sarrocco, S., Terracciano, G., Papetti, L., Baroncelli, R., & Nali, C. (2023). Isolation and characterization of Fusarium spp. From unhatched eggs of *Caretta caretta* in Tuscany (Italy). Fungal Biology, 127(10-11), 1321-1327.

Santos, R. C., Pereira, R. G., & Barreto, A. S. (2015). Mycobiota associated with loggerhead turtle (*Caretta caretta*) nests in southeastern Brazil. Marine Environmental Research, 101, 114-119.

SPA/RAC-UNEP/MAP (2021). Marine Turtle Research and Conservation in Libya: A contribution to safeguarding Mediterranean Biodiversity. By Abdulmaula Hamza. Ed. SPA/RAC, Tunis: pages 77.

Summerell, B. A., Salleh, B., & Leslie, J. F. (2003). A utilitarian approach to *Fusarium* identification. Plant disease, 87(2), 117-128.

Tiwari, S., Bhadauria, V., & Sanyal, A. (2014). Fungal flora associated with eggs of Indian Ocean loggerhead sea turtle (*Caretta caretta*) and their potential role in embryonic mortality. Mycoscience, 55(1), 71-76.

Vecchioni L, Pace A, Sucato A, Berlinghieri F, Cambera I, Visconti G, Hochscheid S., Arculeo M., and R. Alduina (2022) Unveiling the egg microbiota of the loggerhead sea turtle *Caretta caretta* in nesting beaches of the Mediterranean Sea. PLoS ONE 17(5): e0268345. https://doi.org/10.1371/journal.pone.0268345

Zhan, Z., Xu, M., Li, Y., & Dong, M. (2021). The Relationship between Fungal Growth Rate and Temperature and Humidity. International Journal of Engineering and Management Research, 11(3), 78.