Arch Biol Sci. 2025;77(2):185-195 https://doi.org/10.2298/ABS250410015S

Diversity of fungi isolated from the carapace of the European pond turtle (*Emys orbicularis*, L. 1758) in South Banat, Serbia

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Received: April 10, 2025; **Revised**: May 6, 2025; **Accepted**: May 16, 2025; **Published online**: May 21, 2025

Abstract: This study aimed to isolate and identify a fungal community on the carapace of the European pond turtle (*Emys orbicularis*) and to discuss their potential role as constituents of the turtle mycobiome. The study was conducted during the summer of 2020 within the special nature reserve Deliblato Sands (Deliblatska Peščara), situated in northern Serbia. We used a direct microscopy technique along with culture-based methods and molecular barcoding of the internal transcribed spacer (ITS) region. A plethora of fungal spores were documented as constituents of the biofilm present on the carapace surfaces. A total of 12 fungal isolates from the *E. orbicularis* carapace were identified, belonging to *Alternaria*, *Aureobasidium*, *Cladosporium*, *Curvularia*, *Didymella*, *Microsphaeropsis*, *Pseudopithomyces*, *Naganishia*, *Rhodotorula*, and *Mucor* genera. Ecological analyses showed that the majority of documented fungal species originated from terrestrial and aquatic environments, and many could be regarded as potential chelonian pathogens. The study presents pioneering research on fungal dwellers of *E. orbicularis* in Serbia.

Keywords: Chelonia; fungi; turtle carapace; pathogens

INTRODUCTION

The ventral and dorsal surfaces of turtles (order Testudines) are protected by their shells, which enclose all of their internal organs and, in certain situations, even their heads, extremities, and tails. These shells are primarily derived from dermal and endochondral bones. The carapace, or dorsal portion of a turtle's shell, is long and curved, with a brown to dark brown exterior that is narrower at the front and wider at the back [1]. The chelonian shell helps turtles survive in both water and land [2]. Freshwater turtles are exposed to terrestrial or aquatic microbiota in such a way that bacteria, algae, and fungi can rapidly colonize the carapace and sometimes, in the case of lesions on the protective corneous layer of the epidermis, invade the inner tissues [3].

Research addressing fungal interactions with vertebrates was prompted by the discovery of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* Longcore, Pessier & D.K. Nichols, a pathogen that has devastated frog populations worldwide and caused the extinction of several anuran species [4]. Recent years have seen the identification of onygenalean fungi, specifically those within the genera *Nannizziopsis*, *Ophidiomyces*, and *Paranannizziopsis*, as primary pathogens affecting reptiles. These fungi have posed significant challenges to *in situ* conservation efforts [5]. Fungal infections of chelonians are considered to be rare. However, in recent years, newly emerging fungal pathogens have begun to affect freshwater and sea turtles, as well as their eggs, leading to clinical outcomes that had not been previously documented. These pathogens have the potential to significantly reduce turtle populations on a global scale [2]. A newly described onygenalean fungus named *Emydomyces testavorans* A.N. Mill. & D.B. Woodburn is linked to freshwater environments and is the causative agent of shell disease of turtle and terrapin species [5,6]. Furthermore, *Aphanoascella galapagosensis* Deanna A. Sutton, Y. Marín, E.H. Thomps. & Guarro, an onygenalean fungus was for the first time isolated and described from Galapagos tortoise *Chelonoidis niger* Quoy & Gaimar), which showed symptoms of carapace

keratitis [7]. Also, recently discovered sea turtle egg fusariosis (STEF), a disease associated with increased egg mortality of endangered sea turtles worldwide, is caused by two closely related *Fusarium* species, *F. keratoplasticum* Geiser, O'Donnell, D.P.G. Short & Ning Zhang and *F. falciforme* (Carrión) Summerb. & Schroer [8]. Hatchling Florida soft-shell turtles (*Apalone ferox* Schneider) have been reported to succumb to mucormycosis, which affects both skin and shell [9].

Emys orbicularis Linnaeus is the European pond turtle from the order Testudines, the family Emydidae. *E. orbicularis* is an omnivorous and long-living freshwater species which is endemic to the Western Palearctic, with habitats documented throughout East and Central Europe and in areas around the Mediterranean Sea [10]. *E. orbicularis* is classified as "near threatened" on the IUCN Red List (IUCN 2020) because of direct human disturbance, habitat degradation, and aquatic environment pollution, especially with pesticides, poaching, illegal online pet trafficking, and competition from invasive species, such as *Trachemys* sp. [11,12]. Also, eggs and juveniles of *E. orbicularis* are more vulnerable to predators, including mammalian and avian species, due to the disrupted nesting habitat close to waterbodies [13]. *Spirhapalum polesianum* Ejsmont, L. (class Trematoda, order Diplostomida, family Spirorchiidae), the blood fluke parasite, is also regarded as a threatening factor for European pond turtle populations [14]. Guz et al. [15] showed that the population of free-living European pond turtles in eastern Poland was highly colonized by Gram-negative facultative anaerobic bacteria from the genus *Aeromonas*, which are causative agents of different types of diseases in many cold-blooded animals. The hemoprotozoan *Haemogregarina stepanowi* Danilewsky (phylum Apicomplexa, class Conoidasida, order Eucoccidiorida) was found on poached *E. orbicularis* in poor health conditions held in the quarantine section at Belgrade Zoo [16]. To our knowledge, there is no literature data regarding fungal pathogens of *E. orbicularis* or any other biotic interactions between fungi and the European pond turtle. Considering the complex role of fungi in various ecosystems and their diverse interactions with other organisms, ranging from neutralism and commensalism to parasitism, predation, and symbiosis, the diversity of fungi on chelonian carapaces is often overlooked in studies. The importance of these organisms has yet to be fully explored.

The main hypothesis of this study was that the carapace of *E. orbicularis* harbors diverse fungal communities originating from the surrounding aquatic and terrestrial environments. The main goal of this study was to use a direct optical microscopy technique along with culture-based methods and molecular barcoding to isolate and identify a presumably broad spectrum of fungi dwelling on the investigated European pond turtle carapace, to discuss their potential role as constituents of the turtle mycobiome, and to emphasize the potential threat to this turtle species.

MATERIALS AND METHODS

Ethics statement

This research does not require special permission, since no animal has been hurt or killed during the study. Nonetheless, this study is in accordance with the permission of the Ministry of Environmental Protection of the Republic of Serbia for conducting research on protected amphibian and reptile species in localities in Serbia (Permission No: 353-01-189/2020-04).

Field sampling

The study took place in June 2020 at the Deliblato Sands Special Nature Reserve in northern Serbia, the largest European continental sand area. Deliblato Sands is among the most important centers of biodiversity not only in Serbia but in Europe as well, and as an internationally significant bird habitat (IBA), it is included in the Ramsar List of Wetlands of International Importance [17]. Two waterbodies within the Deliblato Sands were chosen as study sites: Kraljevac Lake (KL) and the village of Dubovac, situated on the banks of the Danube (Du). (Supplementary Figure S1).

KL is a protected area, a special nature reserve situated in southern Banat on the western edge of Deliblato Sands, near Deliblato village [18]. It is a marsh-swamp type of ecosystem that was formed by the partitioning of local watercourses and is characterized by a specific combination of aquatic and terrestrial habitats. There are floating islands of marsh vegetation in the lake itself, and it is surrounded by wet valley meadows, as well as degraded steppe elements with significant

anthropogenic areas. The village of Dubovac (Du) is located on the southern edges of Deliblato Sands on the bank of the Danube, 16 km from the locality (KL). Marsh-swamp ecosystems, where sampling was carried out, are located in Deliblato Sands and are under the direct influence of the Danube River. This area along the Danube is surrounded by pastures and fragments of forest ecosystems [18].

A total of 11 *Emys orbicularis* specimens were captured at KL, while 4 specimens were captured at the Du sampling site. No turtle was hurt, and after the sampling, they were released to their original habitat.

Sampling of turtle carapace microbiota for direct microscopy analyses

Adhesive tape was used for sampling *E. orbicularis* microbiota and the detection of fungal structures by gently adhering the tape to selected areas on the turtle carapace. Samples were taken from the top and sides of the carapaces in triplicate for each individual. After removing the adhesive tape, the collected material was used for the preparation of microscopic slides. For better visualization of fungal structures during optical microscopy (Zeiss Axio Imager M.1 microscope, equipped with the AxioVision Release 4.6 software, Carl Zeiss AG, Oberkochen, Germany), a standard mycological dye, Lactophenol Cotton Blue (LCB), was used. Photographs were taken of the observed fungal structures, and existing literature was consulted for their accurate description.

Fungal isolation and identification

The carapace of each captured *E. orbicularis* specimen was sampled with a sterile cotton-tipped swab brushed all over the shell surface for at least half a minute. Samples were also taken in triplicate for each individual. In laboratory conditions, the swabs were inoculated on malt extract agar (MEA) (LAB M Ltd., Heywood, UK) and incubated at 25±2°C in a thermostat (UE 500 Memmert).

After seven days of incubation, primary isolates were examined, and morphologically different colonies were re-inoculated on MEA media. Isolated fungi were inspected using a stereomicroscope (Stemi DV4, Carl Zeiss) and light microscope (Zeiss Axio Imager M.1) and identified according to morphological criteria using the identification keys of Watanabe [19] and Samson et al. [20]. Approximately 50 mg of marginal mycelia was harvested for DNA isolation with the DNAeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany). The internal transcribed spacer (ITS) region of extracted fungal DNA was amplified by using ITS1/ITS4 primers [21]. PCR amplification of ITS region was performed as follows: initial denaturation (95°C, 4 min), followed by 35 amplification cycles (95°C, 30 s; 52°C, 1 min; 72°C, 1 min) with a final extension (72°C, 7 min). Amplification reactions were conducted in a Mastercycler personal model (Eppendorf, Hamburg, Germany) in 25 µl of the reaction mixture (5 ng DNA, 1 × PCR buffer (20 mM Tris/HCl pH 8.4, 50 mM KCl), 1 µM of each primer, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP, and 1 unit of Taq polymerase). The amplified DNA fragments were fractionated in 1% agarose gels consisting of 0.5 × TBE buffer, stained with Midori Green dye, and visualized by UV illumination [22]. The obtained PCR products were then shipped for purification and sequencing to Macrogene (Netherlands). The resulting sequences were then compared with other related sequences deposited to the National Center for Biotechnology Information (NCBI) using the BLAST program (BLAST + 2.7.1 of the NCBI). Fungal DNA sequences obtained in this research were then deposited in the GenBank database of NCBI. Sequence alignment was performed using the CLUSTALW algorithm in MEGAX software [23]. A phylogenetic tree was constructed based on the alignment and comparison of DNA sequences, using neighbor-joining phylogeny of 1,000 bootstrap replicas. By applying the model test within the MEGAX program, a Kimura 2-parameter model was determined as the best for estimating genetic distances between samples. The ITS sequence of the reference strain *Batrachochytrium dendrobatidis* JEL 197 (NR 119535.1) was used as the outgroup.

Ecological function assessment

All fungi detected on the *E. orbicularis* carapace were assigned to the species level according to the NCBI database, and isolates were sorted using the FUNGuild v1.0 tool (Guilds_v1.1.pyscript, database: http://stbates.org/funguild_db.php, viewed on August 7th, 2024). All fungal species were classified based on their trophic mode into ecological categories: pathotroph (P),

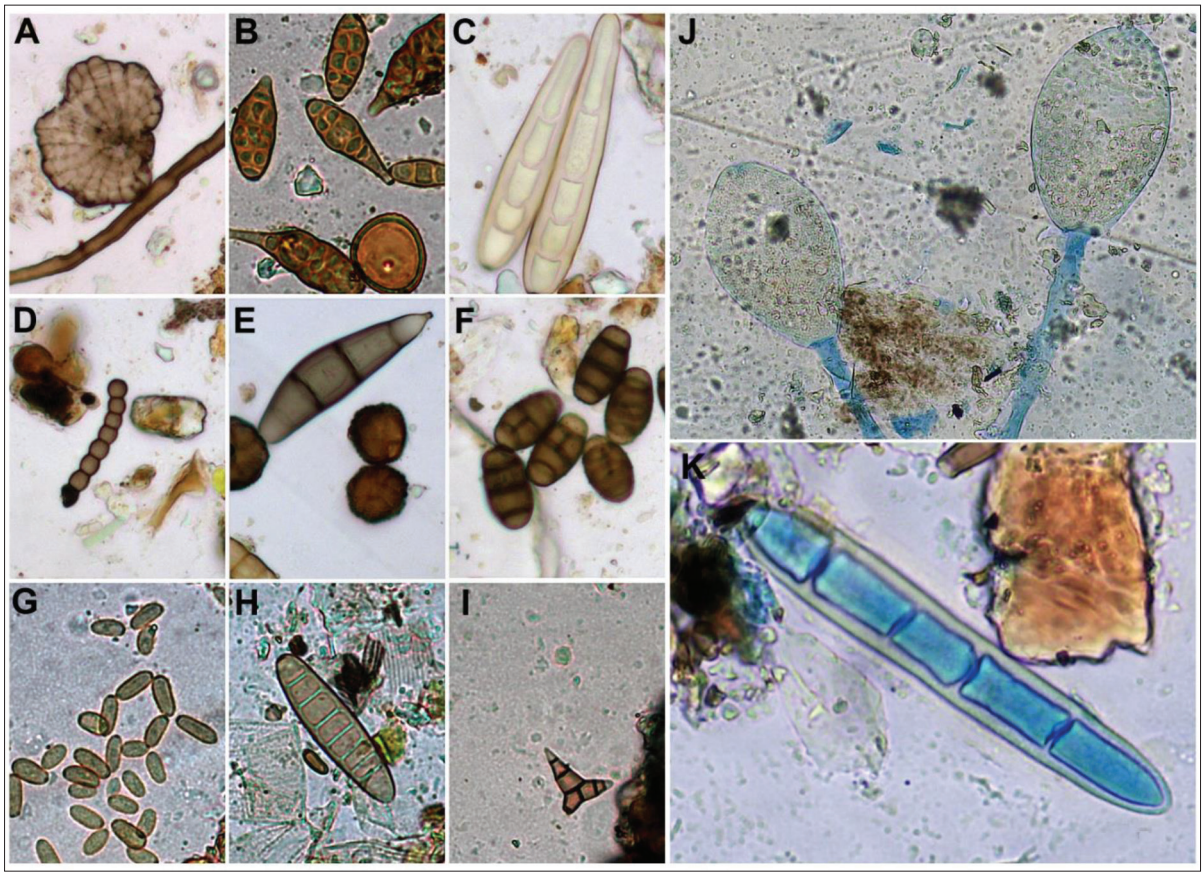

Fig. 1. Fungal and fungus-like organism propagules documented on analyzed samples of *Emys orbicularis* carapaces via the adhesive tape method: **A** – melanized *Dictyosporium*-like dictyospores; **B** – melanized *Alternaria*-like dictyospores; **C** – melanized *Exserohilum*-like fragmospores; **D** – melanized amerospores in chains; **E** – melanized *Curvularia*-like fragmospores and *Epicoccum*-like dictyospores; **F** – melanized *Canalisporium*-like dictyospores; **G** – melanized *Cladosporium*-like amerospores in mass; **H** – melanized *Bipolaris*-like fragmospore; **I** – melanized *Diplocladiella*-like staurospore; **J** – sporangium of zoosporic microorganism (probably Oomycetes); **K** – melanized *Trichophyton*-like fragmospore

saprotroph (S), and symbiotroph (Sy), as well as into the corresponding ecological guilds: animal pathogen (ap), endophyte (en), epiphyte (ep), plant pathogen (pp), plant saprotroph (ps), and wood saprotroph (ws). Additional data regarding ecological categories of fungal isolates were found in the literature [20,24,25]. A review paper by Calabon et al. [26] was used for the comparison of fungi detected on *E. orbicularis* carapaces with previous findings of micromycetes in various freshwater habitats.

RESULTS

Optical microscopy analyses of samples taken directly from the *Emys orbicularis* carapace via adhesive tape revealed the presence of several fungal propagules, primarily melanized spores, which morphologically could be classified into dematiaceous amerospores, fragmospores (resembling *Bipolaris*, *Brachysporium*, *Curvularia*, *Trichophyton*, *Pseudospiropes*, *Pleiochaeta*, *Corynespora*, *Drechslera*, *Taeniolella*), dictyospores (resembling *Alternaria*, *Canalisporium*, *Epicoccum*, *Pseudopithomyces*, *Monodictys*, *Berkleasmium*), didymospores (resembling *Pseudobotrytis*, *Trichocladium*, *Triadelphia*), and staurospores (resembling *Diplocladiella*). Sporangia of zoosporic microorganisms (chytrids and oomycetes) were also detected in adhesive tape samples but with less frequency. No notable difference in the fungal composition of the turtles' carapaces from both sampling sites was detected in adhesive samples. The most representative detected fungal structures are shown in Fig. 1.

Using culture-based isolation methods combined with morphological identification and ITS barcoding, a total of 12 fungal isolates from the *Emys orbicularis*

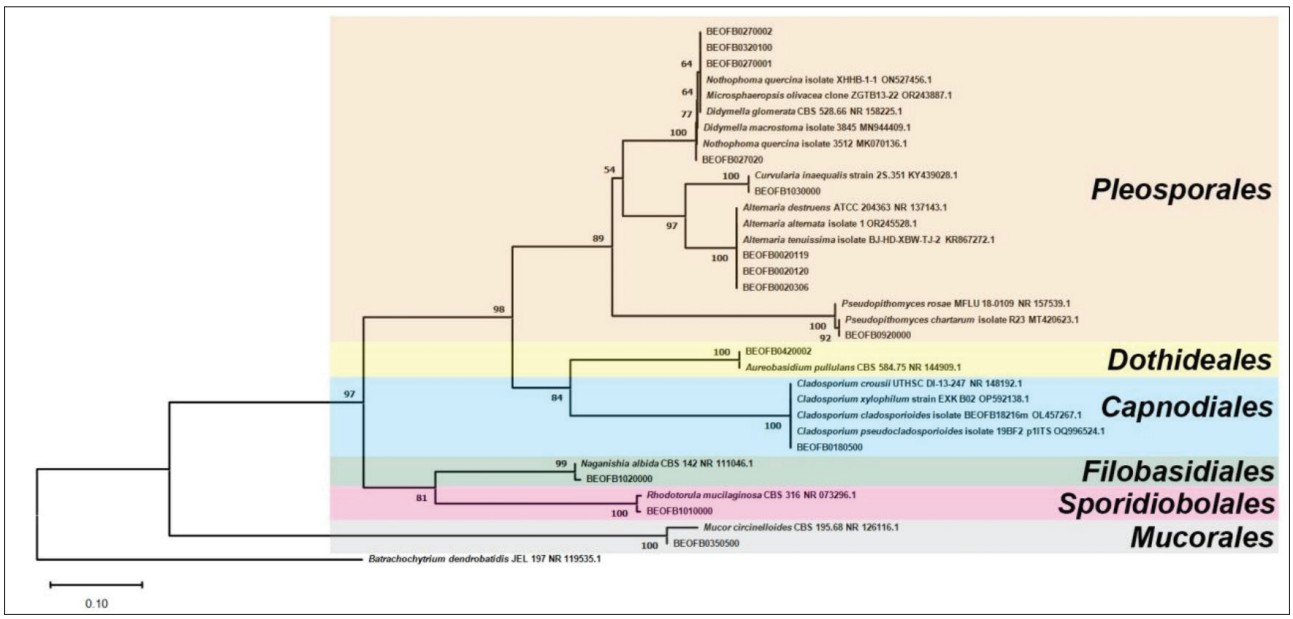

Fig. 2. The phylogenetic relationship between the fungi isolated from *Emys orbicularis* carapace based on the ITS region (neighbor-joining cladogram).

carapace were successfully identified at the species level. Fungal isolates documented in this research were exclusively assigned to Ascomycota (75%), Basidiomycota (16.6%), and Mucoromycota (8.33%). Species of the genera *Alternaria*, *Aureobasidium*, *Cladosporium*, *Curvularia*, *Didymella*, *Microsphaeropsis*, and *Pseudopithomyces* formed a well-supported clade within Dothideomycetes/Ascomycota with a bootstrap value bs of 98. Basidiomycetous yeasts from the genera *Naganishia* and *Rhodotorula* also clustered together (bs=81). Finally, *Mucor* species clustered together in a separate Mucoromycota clade (bs=100) (Fig. 2).

The dominant genera were *Alternaria* and *Didymella* (each comprising 20% of total mycobiota), while other genera were detected only once (6.67%). The majority of isolated fungi were detected in the locality KL (78.57%), while only *A. alternata*, and *D. glomerata* were detected at both sampling sites (14.29%). *Alternaria tenuissima*, *Curvularia inaequalis*, and *Didymella macrostoma* were detected only in Du, while others were reported in KL. Examination of primary isolates grown on MEA showed a high abundance of dematiaceous micromycetes at both sampling sites, while yeast isolates were less frequent.

All fungal isolates, along with assigned NCBI accession numbers, trophic mode, and ecological guilds, are listed in Table 1. Ecological analyses revealed a notable presence of potential animal pathogens (66.67%) and fungal species already reported in freshwater habitats (42%).

DISCUSSION

Fungal propagules – primarily various types of spores – were identified in samples collected using the adhesive tape method, along with cultivable fungi isolated from the carapaces of captured *Emys orbicularis* specimens. These findings indicated a moderate diversity of micromycetes and also reflected the composition and quality of the mycobiome present on substrates with which the turtles frequently come into contact. The habitat of the European pond turtles (wetlands with surrounding vegetation) is the main source of fungi detected on turtle shells, so it could be concluded that carapace fungi originated from both aquatic and terrestrial environments. In chelonians, the shell represents a significant adaptive feature that enables these reptiles to thrive in both aquatic and terrestrial environments. However, the presence of fungi on the shell may indicate a potential health risk [2]. Ascomycetes described in freshwater habitats encompass three different ecological adaptations and, as such, are divided into aquatic

Table 1. Fungi identified on the *Emys orbicularis* carapace in South Banat with associated trophic mode and ecological guild

NCBI Blast	Isolate Number	Accession number	Isolation Locality	Trophic mode	Ecological guild	Freshwater checklist according to Calabon et al. [26]
Alternaria alternata	BEOFB0020119	PQ637631	KL, Du	P, S, Sy	ap, en, pp, ws	Water (river)
	BEOFB0020120	PQ637646				
Alternaria tenuissima	BEOFB0020306	PQ644086	Du	P, Sy	en, pp	-
Aureobasidium pullulans	BEOFB0420002	PQ637629	KL	P, Sy	ap, en, ep, pp	Water (lake, pond), glacial meltwater river
Cladosporium pseudocladosporioides	BEOFB0180500	PQ637630	Du	P, S	en, ep, pp, ps	-
Curvularia inaequalis	BEOFB1030000	PQ637670	Du	P	pp	-
Didymella glomerata	BEOFB0270001	PQ637633	KL, Du	P, S	pp, ws	-
	BEOFB0270002	PQ637661				
Didymella macrostoma	BEOFB0270201	PQ637711	Du	S	-	-
Mucor circinelloides	BEOFB0350500	PQ637659	Kl	P	ap, pp	-
Microsphaeropsis olivacea	BEOFB0320100	PQ637663	Kl	P, Sy	ap, ep	Submerged decaying wood (stream)
Naganishia albida	BEOFB1020000	PQ637667	Kl	P, S, Sy	ap, us, en	Water (lake, pond), rock pools, surfaces of freshwater turtles *Podocnemis expansa*, *P. unifilis*, polluted water (river)
Pseudopithomyces chartarum	BEOFB092000	PQ637632	Kl	P	ap	-
Rhodotorula mucilaginosa	BEOFB1010000	PQ637662	Kl	P	ap	Glacial meltwater (lake, river), tropical freshwaters, phytotelmata of bromeliad tanks, rock holes, surfaces of freshwater turtles *Podocnemis expansa*, groundwater, mud (river, canal), water (lake, wetland, river), acidic water (lake, river), sediment (lake)

*** KL** –Kraljevac Lake, **Du** – Dubovac, **P** – pathotroph, **S** – saprotroph, **Sy** – symbiotroph, **ap** – animal pathogen, **en** – endophyte, **ep** – epiphyte, **pp** – plant pathogen, **ps** – plant saprotroph, **ws** – wood saprotroph

hyphomycetes (Ingoldian fungi), aeroaquatic hyphomycetes, and submerged-aquatic hyphomycetes [27]. Fungal spores detected on the adhesive tape samples, primarily dematiaceous amerospores, fragmospores, dictyospores, didymospores, and staurospores, are often found in submerged-aquatic hyphomycetes. The asexual spores of these fungi are not specifically adapted for aquatic environments. Consequently, these species primarily colonize decaying herbaceous material and wooden debris [28]. The ecological behavior of *E. orbicularis* includes a preference for waterbodies with soft, muddy bottoms and rich submerged vegetation, bringing them into frequent contact with aquatic and submerged-aquatic fungi. Analyses of the adhesive tape samples revealed an abundant presence of melanized spores, which are the main feature of dematiaceous fungi, causative agents of chromomycosis and phaeohyphomycosis in vertebrates [29]. Phaeohyphomycosis caused by the dematiaceous fungus *Exophiala jeanselmei* (Langeron) McGinnis & A.A. Padhye is reported for *Terrapene carolina carolina* L. (Eastern box turtle) in the USA [30]. Additionally, fungus-like organisms were detected on the adhesive tape samples, including zoosporangia oomycetes from *E. orbicularis* captured at the locality KL. This result is also very significant since oomycetes are recognized as facultative pathogens of a variety of aquatic animals [31]. Also, turtles could be reservoirs of oomycetes pathogenic for other aquatic organisms. Blackwell et al. [32] reported that oomycetes of the genus *Lagenidium*, which parasitize

cladophoraceous algae (*Basicladia* sp.), have been found on the dorsal surface of the carapace of freshwater turtles collected in Alabama and Mississippi. Apart from water, other sources of fungi on *E. orbicularis* carapaces are the soil and vegetation in terrestrial environments, as well as some airborne deposits. Many of the detected fungal propagules, particularly melanized spores, likely originate from the terrestrial environment and vegetation with which the pond turtles are in close contact. Ecological analyses also revealed a high abundance of fungi classified into various ecological guilds, including endophytes, epiphytes, plant pathogens, plant saprotrophs, and wood saprotrophs.

Even though many fungal species may be overlooked when culture-based methods are used, the isolation of fungi colonizing turtle shells serves as an important first step toward understanding the carapace mycobiota and gaining better insight into the ecological roles of these microeukaryotes. Furthermore, the detection of fungal taxa on the adhesive tape samples is also very important because many of them are hard to isolate in axenic cultures. Mycological analyses based on culture-based methods of chelonians, including their eggs and nests, are scarce and have mostly focused on the sea turtles *Chelonia mydas* L. (green sea turtle), *Caretta caretta* L. (loggerhead sea turtle), *Lepidochelys kempii* Garman (Kemp's ridley sea turtle), and *Dermochelys coriacea* Vandelli (leatherback sea turtle). According to the IUCN database, all these sea turtles are threatened, especially *L. kempii*, which is the rarest of all sea turtles listed as Critically Endangered (CR) [33]. To name a few studies, Candan [34] isolated 23 different cultures belonging to 5 distinct fungal genera from *C. mydas* eggshells from nests on Sugözü Beaches (Türkiye). Phillott and Parmenter [35] reported that soil fungi *Fusarium solani* (Mart.) Sacc. and *Pseudallescheria boydii* (Shear) McGinnis, A.A. Padhye & Ajello are frequent colonizers of the exterior of unhatched eggs in sea turtle nests of *C. mydas* and in *C. caretta* in eastern Australia. Sarmiento-Ramırez et al. [36] reported the presence of 46 fungal isolates from *C. mydas* eggshells exhibiting unusually colored spots collected from different Ascension Island beaches. In these studies, the dominant fungal isolate identified on sampled eggshells was *Fusarium keratoplasticum* Geiser, O'Donnell, D.P.G. Short & Ning Zhang, while fungi from the genera *Pseudallescheria*, *Scedosporium*, *Aspergillus*, *Phoma*, *Alternaria*, and *Gymnascella* were found with lower isolation frequency. Mastrostefano et al. [37] reported that members of the genera *Purpureocillium*, *Beauveria*, and *Fusarium* are among the most frequently isolated fungi from the lungs of sea turtles *L. kempii*, *C. mydas*, and *D. coriacea* in the New England Aquarium (Boston, MA, USA). It is also worth mentioning that many advanced scientific studies characterizing microbial communities employ metabarcoding analyses, a technique that could also be applied to studying the microbiota of turtles. With this in mind, using Illumina MiSeq sequencing with bioinformatics analysis, Filek et al. [38] detected diverse communities of endozoic cloacal fungi in *C. caretta* in the Adriatic Sea. Terrestrial chelonians are seldom subjected to mycological analyses. However, the results of this research correspond with other findings. The occurrence of yeasts (*Candida* spp., *Cryptococcus* spp., *Geotrichum candidum* Link and *Rhodotorula* sp.) and molds (*Absidia*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Drechslera*, *Fusarium*, *Mucor*, *Penicillium*, *Stemphylium*, and *Ulocladium* species) has been frequently observed in tortoises but seldom in turtles [2]. Flamant et al. [39] reported in an extensive study carried out in France the dominance of dematiaceous fungi, with *Alternaria*, *Cladosporium*, and *Aureobasidium* identified as the most prevalent genera on *Testudo* spp. The presence of these fungi on tortoise or turtle shells must not be ignored because they are potential causative agents of chromomycosis and phaeohyphomycosis in vertebrates. The presence of *Mucor circinelloides* on the carapace of a turtle from KL should not be overlooked, as several members of the order *Mucorales* have been reported to cause shell mycosis in several turtle species [40]. *M. circinelloides* is commonly associated with opportunistic infections, which may lead to mucormycosis in immunocompromised animals, including humans. Lozano et al. [41] sequenced the whole genome of *Mucor circinelloides* and identified genes encoding four virulence factors: permease, iron receptors, ADP-ribosylation factors, and GTPase. Both basidiomycetous yeasts found on *E. orbicularis* carapaces from specimens captured in KL, *Naganishia albida* (Saito) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout, and *Rhodotorula mucilaginosa* (A. Jörg.) F.C. Harrison, have also been isolated from the plastron and mouth cavities from *Podocnemis expansa* Schweigger (Arrau turtle) and *P. unifilis* Troschel (yellow-spotted Amazon River turtle) specimens captured in the Javaes River

in Araguaia National Park in Brazil [42]. *N. albida* (formerly *Cryptococcus albidus* (Saito) C.E. Skinner) is considered a saprobe yeast and is a rarely reported human pathogen causing skin infections. However, occurrences of human *N. albida* infections have increased during the past few years [25]. Therefore, the turtle shell could be regarded as a potential reservoir of human pathogens. Endozoospory, an interesting biotic interaction between turtles and fungi, has been reported by Jones et al. [43]. To investigate the role of *Terrapene carolina carolina* in fungal spore dispersal, Jones et al. [43] isolated 23 different fungal species from fecal samples of turtles collected in Clark, Clay, Coles, Moultrie, and Shelby counties in Illinois. *Rhodotorula* species have also been reported as emerging opportunistic human pathogens, particularly affecting immunocompromised patients. Numerous cases of fungemia caused by *Rhodotorula* species have been linked to serious infections such as endocarditis, peritonitis, meningitis, and endophthalmitis [44]. For *Rhodotorula mucilaginosa*, the yeast detected on *Emys orbicularis* carapaces in this study, it has been shown that *T. carolina carolina* could be a dispersal vector through the incidental ingestion of fungal spores. Additionally, *R. mucilaginosa*, along with *A. pullulans*, which was also isolated in our work, was documented as a potential pathogen of snakes – *Coronella austriaca* Laurenti, and *Vipera aspis* L. [45]. Both terrestrial and aquatic fungi have been reported to effectively colonize lesions in chelonians, leading to superficial and systemic mycoses – for example, following injuries caused by fish hook ingestion or carapace damage. Furthermore, as poikilotherms, chelonians are prone to adverse environmental factors. Fungal infections can be triggered by poor cellular immunity, as well as by adverse environmental conditions such as overcrowding, poor water quality, drops in body temperature, or concurrent infections. However, the primary causes of these diseases often remain unidentified, especially in wild animals, because diagnoses are typically based on observations of dying individuals or carcasses without access to detailed medical histories [2].

Studying the fungal diversity on turtle carapaces enhances our understanding of the ecological interactions between turtles and microorganisms in their environment. Since some fungi are opportunistic pathogens, knowing which species are present can help identify risks to turtle health, especially in stressed or immunocompromised individuals. Shifts in fungal diversity may signal health issues before symptoms appear, allowing for proactive intervention. Fungal isolates from *Emys orbicularis* carapaces should be considered not only as potential pathogens for chelonians but also as valuable candidates for testing the antifungal efficacy of various synthetic and natural compounds aimed at mitigating fungal infections in reptiles [37].

CONCLUSIONS

This study is a pioneering effort to describe fungal communities present on chelonian carapaces in Serbia. A total of 12 culturable fungal species were identified along with a plethora of fungal propagules. The surrounding environment, including marsh vegetation, water, and soil, is the potential source of pathogenic mycobiota. Among the isolated mycobiota, ecological analyses revealed a significant presence of potential animal pathogens as well as fungal species previously reported in freshwater habitats. Further research is needed to clarify the exact biological roles and potential interactions between these fungi and chelonians.

Funding: The authors are grateful to the Ministry of Science, Technological Development and Innovation of the Republic of Serbia for the financial support according to the contract with the registration numbers (451- 03- 66/2024- 03/200178 and 451-03- 65/2024- 03/200178).

Author contributions: All listed authors have contributed sufficiently to the work be included as co-authors. Conceptualization: MS and IK; investigation: MS, IK, and ŽS; data processing: MS, MLjG and ŽS; writing of the original draft: MS and ŽS; revision and editing: MLjG and IK

Conflict of interest disclosure: The authors declare no conflict of interest.

Data availability: The data sets generated during the current study are available from https://doi.org/10.5281/zenodo.15188827.

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SUPPLEMENTARY MATERIAL

Supplementary Fig. S1. Map of investigated localities in South Banat (Serbia). KL – Kraljevac Lake, Du – Dubovac village. Map sources: https://www.alo.rs/data/images/2015-11-19/21475_profimedia-0088349174-1000x0_orig.jpg and https://www.google.com/maps [Cited 2025 Feb 13]