

Systemic infection of *Beauveria bassiana* in a stranded endangered green turtle *Chelonia mydas*: A case report

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Abstract

The green turtle (*Chelonia mydas*) is the most frequently encountered marine turtle species in Taiwan. This study describes the postmortem findings of a juvenile green turtle with injuries to the right foreflipper that was rescued off the Taiwan coast and died after 3 months in rehabilitation. Necropsy revealed large, yellow, multifocal caseous lesions involving the right foreflipper, spleen, liver, kidneys, and lungs. Fungal infection of *Beauveria bassiana* was confirmed by culture, morphological and molecular characterization, phylogenetic analysis, and histopathological examination. This report provides evidence that *B. bassiana* is associated with a systemic fungal infection which can be fatal to green turtles. Fungal infections are considered important emerging diseases and threats to the conservation of sea turtles. Additional research into the prevalence of infection, geographic distribution, and pathogenicity of *B. bassiana* should be conducted to better understand the role of *B. bassiana* in sea turtle health and to inform conservation efforts in Taiwan.

Keywords: *Chelonia mydas*, fungal hyphae, fungal infection, histopathology, internal transcribed spacer (ITS) gene

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Introduction

Green turtles (*C. mydas*) are widely distributed throughout subtropical and tropical oceans; however, they face numerous threats, most of which are associated with humans, including boat propellers (Work *et al.*, 2015), fishing nets (Cheng and Chen, 1997), marine debris (Wabnitz and Nichols, 2010), chemical pollution (Ng *et al.*, 2018), habitat degradation (Wang and Li, 2008), and infectious diseases (Sarmiento-Ramírez *et al.*, 2014; Arpini *et al.*, 2019; Tsai *et al.*, 2019; Li and Chang, 2020; Li *et al.*, 2023). While information related to fungal disease in marine turtles is presently limited, a few studies have investigated the emergence of fungal disease in sea turtle populations and reported that fungal disease appears to be linked to egg mortality (Sarmiento-Ramírez *et al.*, 2010; Sarmiento-Ramírez *et al.*, 2014; Smyth *et al.*, 2019). Recent work has further defined sea turtle egg fusariosis, caused by pathogenic members of the *Fusarium solani* species complex, as a newly emerging fungal disease linked to egg mortality and hatch failure in nests of several globally distributed sea turtle species (Gleason *et al.*, 2020). Susceptibility to fungal infections has been described in hawksbill turtles (*Eretmochelys imbricata*) (Posthaus *et al.*, 1997), green turtles (Jacobson *et al.*, 1979), and loggerhead sea turtles (*Caretta caretta*) (Arpini *et al.*, 2019). A recent study has documented mycotic dermatitis in captive leatherback neonates and decreased hatching success in leatherback nests associated with *Fusarium* spp. (Kuschke *et al.*, 2023). Mycotic infections in stranded sea turtles are increasingly recognized as critical yet underdiagnosed causes of morbidity and mortality, with pneumonia being the primary clinical presentation. However, antemortem detection poses substantial diagnostic challenges and frequently fails when using standard sampling methods. A comprehensive case series of 20 stranded sea turtles revealed that fungal infections were detected antemortem in only 30% (6/20) of cases, with 90% (18/20) of the affected turtles ultimately dying despite intensive care. Notably, multiple antemortem cultures from deep tissue or lavage sites yielded no fungal growth, despite postmortem histological confirmation of systemic mycoses. The predominantly isolated genera included *Purpureocillium*, *Beauveria*, and *Fusarium*, underscoring the clinical threat of entomopathogenic fungi to debilitated sea turtles (Mastrostefano *et al.*, 2024). Similarly, a recent case of fatal pneumonia caused by *Beauveria bassiana* in a stranded Kemp's ridley sea turtle (*Lepidochelys kempii*) in Portugal demonstrated that the fungal infection was only confirmed postmortem after 11 days of intensive care that included antibiotic but not antifungal therapy, emphasizing the critical need for early mycological diagnostics in sea turtle rehabilitation (Marques *et al.*, 2025). Furthermore, some fungi have been recognized as pathogenic for reptiles, for example, *Purpureocillium lilacinum*, *Metarhizium anisopliae*, and *Beauveria bassiana* (Schmidt, 2015). However, *B. bassiana* infections in green sea turtles have only rarely been reported worldwide (Mastrostefano *et al.*, 2024; Marques *et al.*, 2025). Given that green turtles are widely distributed throughout

the East Asia region (Ng and Matsuzawa, 2020), this is, to our knowledge, the first description of systemic *B. bassiana* infection in a stranded green turtle from this region.

Case Description

This case study reports on a stranded juvenile green turtle (curved carapace length, CCL = 42.5 cm; body weight, BW = 7.2 kg; body condition index, BCI = 1.08) (Fig. 1) with injuries to the right foreflipper, presumably due to entanglement with fishing gear. The animal was brought to the sea turtle rehabilitation facility at the National Museum of Marine Biology and Aquarium, Taiwan, where it underwent antibiotic therapy for approximately 3 months. As the primary and most extensive therapeutic regimen implemented during this period, empirical antimicrobial therapy was initiated with a combination of enrofloxacin (20 mg/kg IM) and amikacin (5 mg/kg IM) every 48 to 72 hours for approximately 6 weeks, supported by susceptibility testing. Over the following 1.5 months, the regimen intermittently consisted of amikacin (5 mg/kg IM q72h) and ceftiofur (3 mg/kg IM q24h) for approximately 2 weeks, followed by amikacin (5 mg/kg IM q72h) and ceftiofur (10 mg/kg SC q24h) for nearly 2 weeks. Nevertheless, during clinical management the wound to the right foreflipper progressed to pronounced swelling with yellow caseous debris, and the turtle showed no clinical improvement, ultimately dying during the hospitalization period. Necropsy was immediately performed, and tissue specimens were saved for histological examination. The most notable macroscopic postmortem observation revealed large, yellow, multifocal caseous lesions which covered the right foreflipper (Fig. 2A), spleen (Fig. 2B), liver (Fig. 2C), kidneys (Fig. 2D), and lungs (Fig. 2E). Yellow fluid was also present in the peritoneal cavity (Fig. 2F). Tissue samples obtained during necropsy were fixed by immersion in 10% formalin and sent to National Pingtung University of Science and Technology, Pingtung, Taiwan for standard histopathological sectioning (hematoxylin and eosin; H&E). Histological analysis revealed fibroblastic and necrotic cells, together with macrophage infiltration in multiple organs. Fungal hyphae were demonstrated by periodic acid-Schiff (PAS) staining in the coelomic wall, spleen, liver, kidney, lung, and intestines (Fig. 3). To further elucidate the effects of the fungal infection, ascitic fluid samples were incubated in Sabouraud dextrose agar (SDA; DifcoTM, USA) at 25°C for 5 days to obtain a fungal culture (Arpini *et al.*, 2019). Throughout this period, the SDA was checked regularly for evidence of conidia germination and hyphae growth. Fungal colonies (Fig. 4; main image) collected from the SDA were subsequently prepared and mounted on wet slides. The morphologic features of fungal fruiting structures were identified via direct microscopic examination (Fig. 4; inset image). Isolated hyphae underwent DNA extraction and DNA sequencing for species identification using the PCR protocol described by Devi *et al.* (2013). Briefly, DNA fragments were amplified using the primers ITS1 (5'-TCCGT AGGTG

AACCT GCGG -3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), whereupon PCR amplification products were analyzed via gel electrophoresis. PCR products were also sent to Genomics, Taiwan, for DNA sequencing, and sequencing results were compared with strains in the GenBank database. The DNA sequences obtained in this study were identified as *Beauveria bassiana* (DNA identity: 100 %). The *B. bassiana* DNA sequence obtained in the present study was deposited in

GenBank [accession number: MT107137.1]. The Clustal W module in MEGA4 software was used for sequence alignment, and the neighbor-joining method was used to construct a phylogenetic tree via bootstrapping using 1000 resampling replicates. Phylogenetic analysis confirmed that the homologous DNA sequences of *B. bassiana* obtained in the current study shared significant similarity with sequences reported in previous studies (Fig. 5).



Figure 1 Green sea turtle (*Chelonia mydas*) rescued off the coast of Taiwan.

Discussion

B. bassiana infections in green sea turtles have only rarely been reported worldwide (Marques *et al.*, 2025), and, to our knowledge, this is the first description of systemic *B. bassiana* infection in a stranded green turtle from East Asia. Here, we also provide the first evidence of *B. bassiana* infection in a green turtle from Taiwan, through the culture technique, morphologic features of the fungus, molecular characterization, phylogenetic analysis, and histopathological examination. Previous studies reported that *B. bassiana* can be used as a biological insecticide (Bidochka *et al.*, 1990; Quesada-Moraga and Vey, 2004; Xu *et al.*, 2009). The virulence of *B. bassiana* can be attributed to the secretion of secondary metabolites, such as beauvericin, bassianolide, bassianin and bassiacridin, which have been shown to possess cytotoxic properties (Quesada-Moraga *et al.*, 2004; Zimmerman, 2007; Xu *et al.*, 2009; Valero-Jiménez *et al.*, 2016; Al Khoury *et al.*, 2019). The relatively limited amount of research on *B. bassiana* infection in vertebrates includes studies on Pond sliders (*Trachemys scripta*) (González Cabo *et al.*, 1995), American alligators (*Alligator mississippiensis*) (Fromtling *et al.*, 1979) and Galapagos tortoises (*Testudo elephantopus*) (Georg *et al.*, 1963). In the present case, the caseous material was also found in the lungs, as documented in a previous study on fungal infection in captive loggerhead sea turtle hatchlings (Arpini *et al.*, 2019). One previous study reported on loggerhead sea turtles co-infected with *B. bassiana* and *Purpureocillium lilacinum* (Schumacher *et al.*, 2014). In that research, the

infected loggerhead sea turtles (*C. caretta*) displayed abnormal buoyancy and decreased appetite, which was observed in green sea turtle in the current study as well. Furthermore, our histopathologic results, in which we observed organ damage and the infiltration of white blood cells with fungal hyphae in infected organs, were also similar to findings reported in other animal studies (Georg, 1963; González Cabo *et al.*, 1995). However, the source of *B. bassiana* infection in the current study remains unclear due to the ubiquity of these fungal cells in soil (González Cabo *et al.*, 1995; Schumacher *et al.*, 2014). Mycotic infections in reptiles are generally associated with opportunistic pathogens. In wild animals, these infections are often linked to a range of predisposing stressors, including concurrent bacterial infections, parasitic infestations, ingestion of foreign material, exposure to environmental pollutants, traumatic injuries, and entanglement in fishing gear (Nardoni and Mancianti, 2023). Mastrostefano *et al.* (2024) also postulated that factors such as aspiration of seawater, exposure to unusually cold water, and trauma may predispose sea turtles to opportunistic infections. In the present case, the turtle had injuries to the right foreflipper, presumably resulting from entanglement in fishing gear, which may have acted as a predisposing factor for secondary fungal infection. The detection of *B. bassiana* in green turtles is an important finding. Recently, fatal pneumonia associated with *B. bassiana* has also been reported in a stranded Kemp's ridley sea turtle (*L. kempii*) on the Portuguese coast, where histopathology, culture, and molecular identification confirmed

infection by this entomopathogenic fungus (Marques *et al.*, 2025). In that case, the turtle died after 11 days of intensive care that included antibiotic but not antifungal therapy, as the fungal infection was only confirmed postmortem. Antifungal susceptibility testing in that case revealed susceptibility to voriconazole and itraconazole but resistance to fluconazole and amphotericin B, highlighting that empirical antifungal therapy without susceptibility testing may fail in refractory pulmonary mycoses (Marques *et al.*, 2025). However, antifungal susceptibility testing was not performed for our case. Similarly, a recent multi-species case series of 20 stranded sea turtles with histologically confirmed mycotic infections reported that fungal infection was detected antemortem in only 6 cases (30%), with the majority diagnosed postmortem (Mastrostefano *et al.*, 2024). Importantly, seven antemortem fungal cultures from nonsuperficial sites yielded no fungal growth despite postmortem confirmation of fungal infection in the same organ system, emphasizing the diagnostic challenges of mycoses in debilitated sea turtles

(Mastrostefano *et al.*, 2024). The study also demonstrated that fluconazole resistance is common among fungi isolated from sea turtles, and recommended itraconazole, voriconazole, or terbinafine as more appropriate treatment options based on susceptibility testing (Mastrostefano *et al.*, 2024).

Mycobacteriosis is a well-known disease that has been reported in a wide range of reptile species. Goldberg *et al.* (2023) described a juvenile green turtle infected with *Mycobacterium gordonae*. Postmortem examination revealed granulomatous pneumonia, hydropericardium, and small granulomas in the myocardium, liver, thyroid, spleen, and brain, as well as caseous exudate in the meninges of the brain. Hence, it is important for clinicians to consider mycobacterial infection among the differential diagnoses for our turtle case. However, in our case, the diagnosis of *B. bassiana* infection was further supported by culture, the morphologic features of the fungus, molecular characterization, phylogenetic analysis, and histopathological examination.

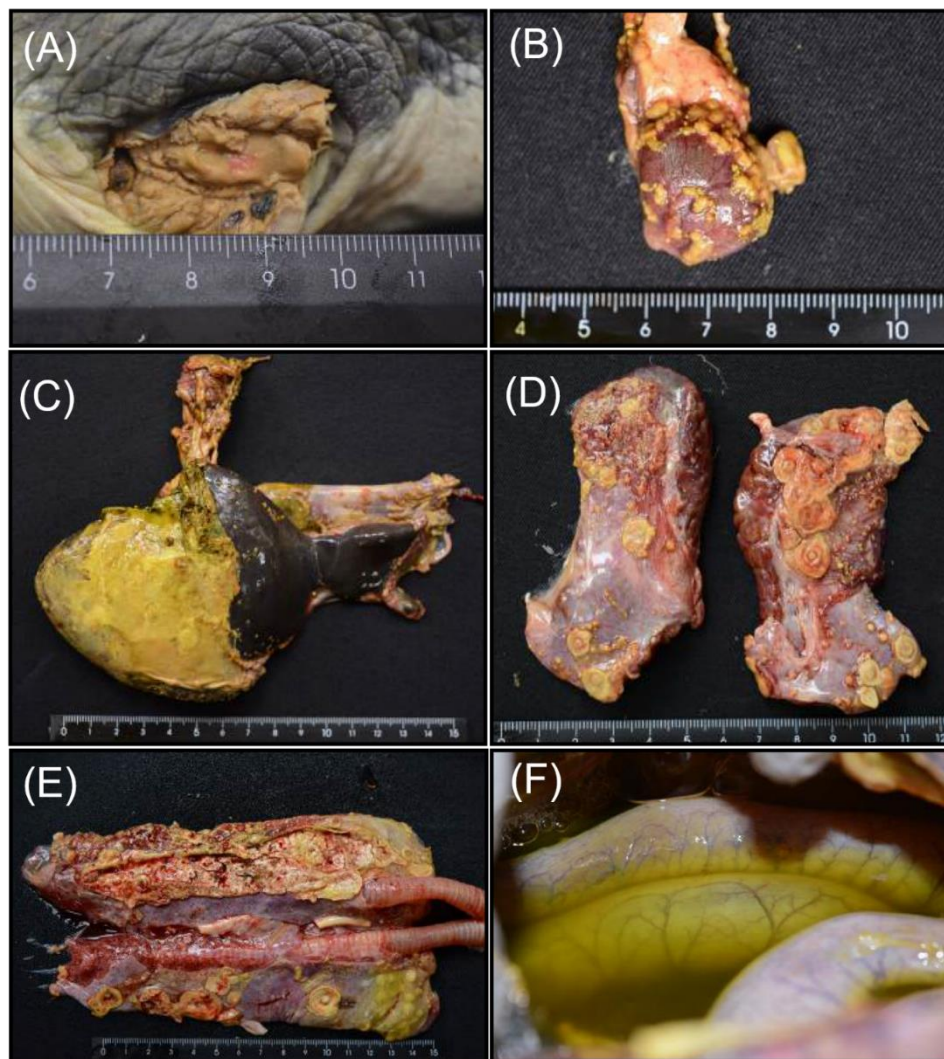


Figure 2 Gross findings in the green turtle (*Chelonia mydas*). Caseous material in the (A) fore flipper and multiple organs: (B) spleen, (C) liver, (D) kidneys, and (E) lung. (F) Yellow fluid is present in the peritoneal cavity.

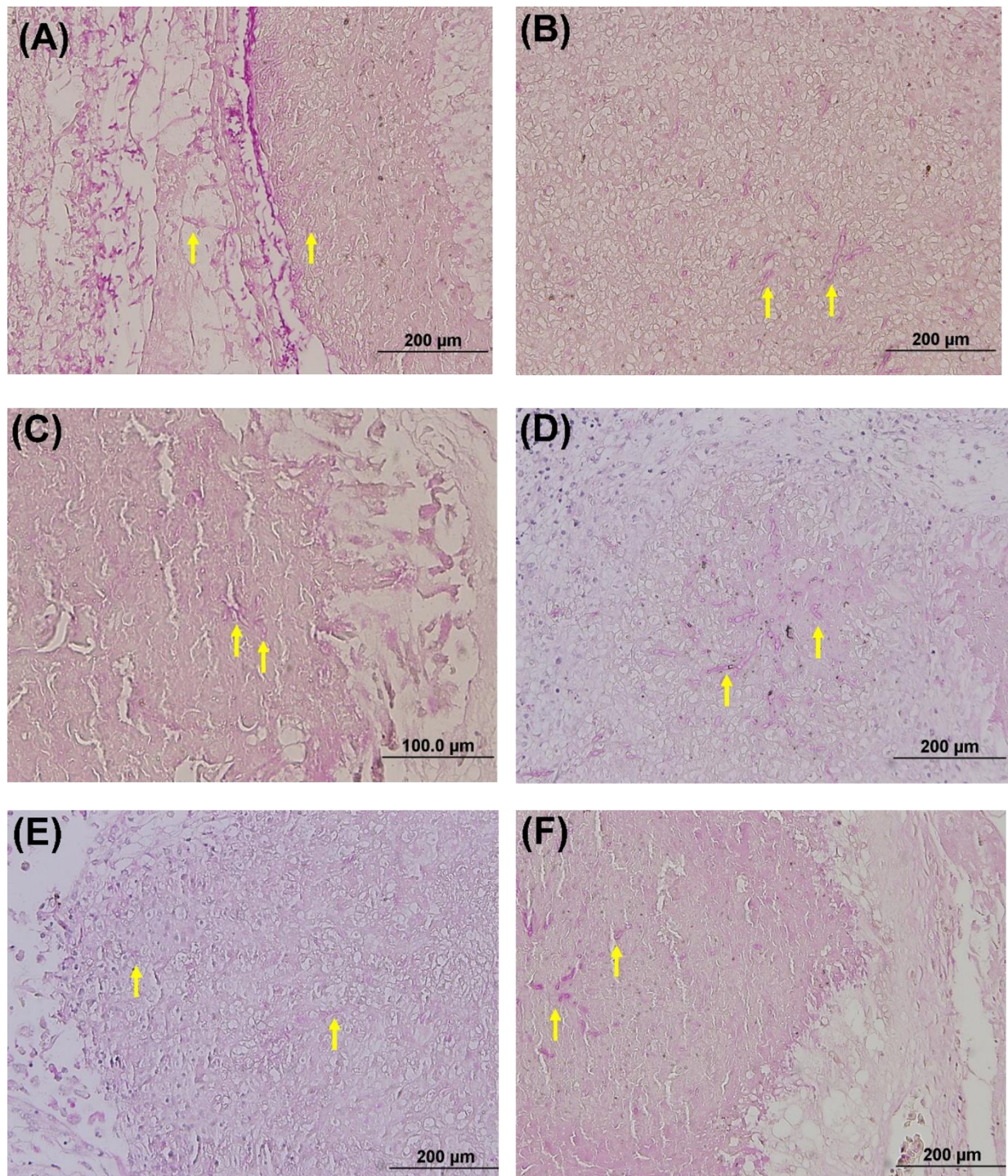


Figure 3 Fungal hyphae (arrows) were observed associated with the (A) coelomic wall, (B) spleen, (C) liver, (D) kidney, (E) lung, and (F) intestines (Periodic Acid-Schiff stain). Histopathologically, multifocal, chronic, severe fibrinous and granulomatous serositis involving the lungs, liver, spleen, kidney, coelomic wall, and intestinal serosa, as well as severe fibrinous, multifocal granulomatous pleuritis, are shown.

The fact that *B. bassiana* is a common soil saprophyte makes it very likely that other animals could be infected through the ingestion or inhalation of spores (Fromtling *et al.*, 1979). Thus, *B. bassiana* should be considered when performing differential diagnosis in sea turtles, particularly when these animals do not respond to antibiotic therapy. More aggressive methods of diagnosis, such as lung biopsy, may be

indicated for suspected cases of fungal infection, as antemortem culture of tracheal lavage and bronchoscopic lavage has been shown to lack sensitivity for diagnosing fungal pneumonia in sea turtles (Mastrostefano *et al.*, 2024). At present, there is a shortage of reports on *B. bassiana* infection in sea turtles. Indeed, this is the first report of *B. bassiana* infection in a green turtle in Taiwan.

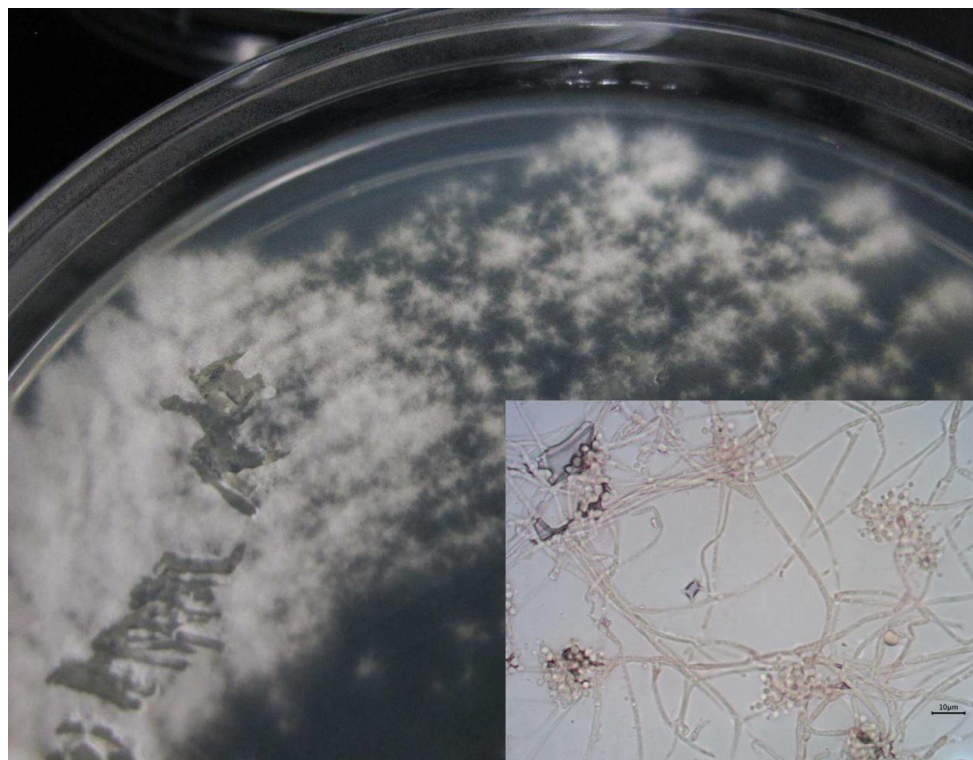


Figure 4 *Beauveria bassiana* isolated from the ascitic fluid of the green turtle (*Chelonia mydas*), showing characteristic colony morphology on Sabouraud dextrose agar (SDA) (main image) and microscopic features at 1000× (iodine stain; inset image).

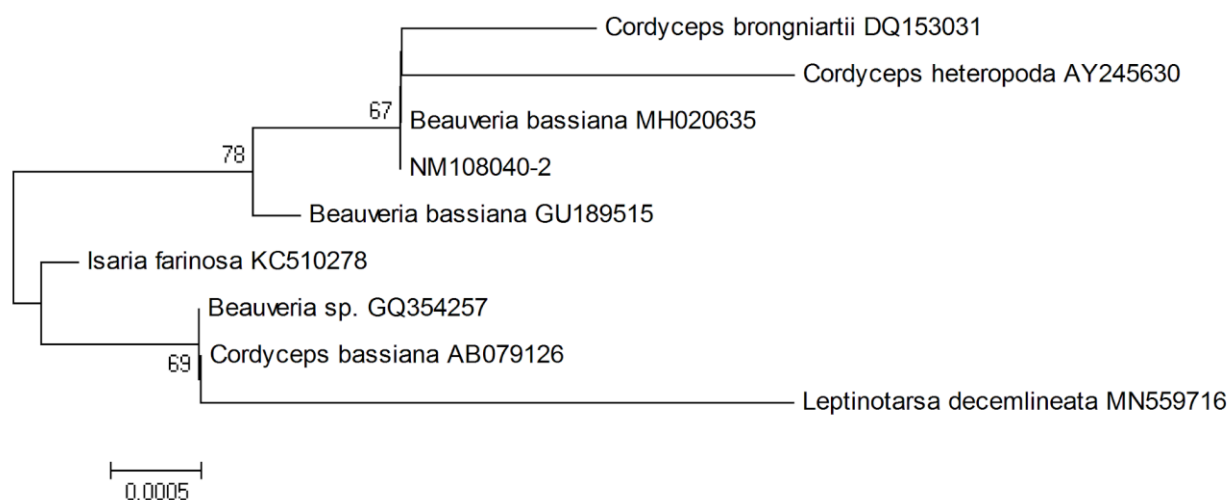


Figure 5 Phylogenetic tree based on internal transcribed spacer (ITS) gene sequence in NM108040-2 isolate and its 564 bp sequence from green turtle (*Chelonia mydas*). The phylogenetic tree was constructed by using the neighbour-joining method. Bars indicate genetic distance. Numbers at each node indicate percent bootstrap values. Scale represents 0.0005 nucleotide substitutions per position.

In conclusion, fungal infections are considered important emerging diseases and threats to the conservation of endangered sea turtles (Sarmiento-Ramírez *et al.*, 2014; Arpini *et al.*, 2019; Smyth *et al.*, 2019; Marques *et al.*, 2025). Further research on the prevalence of infection, geographic distribution, and pathogenicity of *B. bassiana* in sea turtles is needed to advance efforts to preserve endangered sea turtles. Early mycological diagnostics and antifungal susceptibility testing should be incorporated into sea turtle rehabilitation protocols to improve treatment outcomes.

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draft, Writing – review & editing. **I-Chun Chen:** Data curation, Investigation, Visualization, Writing – original draft. **Tsung-Hsien Li:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of artificial intelligence use: During the preparation of this work, the author(s) used Perplexity for language editing and grammar checking. The authors take full responsibility for the integrity, accuracy, and originality of the work.

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