Isolation and characterization of Fusarium spp. From unhatched eggs of Caretta caretta in Tuscany (Italy)

ISOLATION AND CHARACTERIZATION OF *FUSARIUM* SPP. FROM UNHATCHED EGGS OF *CARETTA CARETTA* IN TUSCANY (ITALY)

Samuele Risoli[1,2], Sabrina Sarrocco[1*], Giuliana Terracciano[3], Luana Papetti[4],Riccardo Baroncelli[5], Cristina Nali[1]

[1]Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, 56124 Pisa (Italy)

[2]University School for Advanced Studies IUSS, Palazzo del Broletto, Piazza della Vittoria 15, 27100 Pavia (Italy)

[3]Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, SS Dell' Abetone e del Brennero 4, 56123, Pisa (Italy)

[4] tartAmare, Centro Recupero Tartarughe Marine, via Bramante n. 83 Marina di Grosseto, Italy

[5] Department of Agricultural and Food Sciences (DISTAL), University of Bologna, Viale Giuseppe Fanin 42, 40127 Bologna (Italy)

*Corresponding Author: sabrina.sarrocco@unipi.it

Keywords

STEF, biodiversity conservation, sea turtle, FSSC, phylogenetic analysis

22

Abstract

Sea Turtle Egg Fusariosis (STEF) is a worldwide emergent fungal disease affecting eggs and causing embryos mortality in turtle's nests such as those of *Caretta caretta.* The disease is caused by a complex of species belonging to *Fusarium* genus, particularly those included in the Fusarium Solani Species Complex (FSSC). During the samplings carried out in summer 2020 along the Tuscany coastlines (Italy), *C. caretta* eggs showed clinical signs resembling those caused by STEF. A total of 32 fungal isolates were obtained from lesioned eggs whose molecular characterization allowing identifying as belonging to FSSC / *Neocosmospora* spp., Fusarium oxysporum Species Complex (FOSC) / *F. oxysporum* and *Fusarium nodosum*, *i.e.*, fungal *genera* and species including also well-known plant pathogens. However, sampled isolates inoculated on several plant hosts did not result in any pathogenic activity except for *F. nodosum* causing, when inoculated on wheat spikes, disease symptoms.

While FSSC / *Neocosmospora* spp. and *F. oxysporum* have previously been associated to *C. caretta* eggs displaying signs of STEF, this is the first time *F. nodosum* has been isolated from portions of eggs showing evident signs of fungal infection. This work represents the first report of *Fusarium* spp. isolated from *C. caretta* eggs showing lesions resembling those caused by STEF on Tuscan coast thus posing a significant concern to loggerhead sea turtle conservation also in this region.

Introduction

In the recent years an increase of emerging infectious diseases caused by fungi and fungal-like species has been registered in all ecosystems, thus contributing to a decline across a broad range of taxa with a consequent biodiversity loss. As example, the emerging disease Ophidiomycosis, whose causal agent is the fungus *Ophidiomyces ophidiicola*, causing local and systemic infection in affected snakes (Di Nicola et al., 2022), represents a threaten to wild snake safeguard. The white-nose syndrome (WNS), caused by the fungal pathogen *Pseudogymnoascus destructans*, caused widespread mortality in many communities of hibernating bats and decimated some species (Langwig et al., 2016). The discovery of the disease chytridiomycosis in amphibians, due to chytrid fungi belonging to the genus *Batrachochytrium*, is considered as the link between emerging infections and global amphibian declines (Fisher et al., 2020). Similarly, hatching failure in eggs linked to symptoms of fusariosis was reported in the Amazon River turtles (*Podocnemis unifilis*), highlighting important implications for turtle conservation worldwide (Carranco et al., 2022).

Fusarium (Hypocreales, Nectriaceae) genus includes approximately 300 species distributed in 23 monophyletic groups, referred to as species complexes (Summerell, 2019). These fungi cause a variety of diseases in several plant species with important damages in terms of yield and quality (Dean et al., 2012; Summerell, 2019) even if some species are now widely recognized as a major threat to animal health and biodiversity conservation worldwide (Fisher et al., 2012, 2016; Sandoval-Denis and Crous, 2018).

62 Sea Turtle Egg Fusariosis (STEF) has been marked as a worldwide emerging fungal disease,
63 associated with eggs and embryos mortality of loggerhead sea turtles (*Caretta caretta* L.) (Gleason
64 et al., 2020; Smyth et al., 2019). According to the International Union for Conservation of Nature
65 (IUCN; Ceriani et al., 2019), *C. caretta* is considered as a vulnerable species whose conservation
66 deserves continuous attention and efforts, to the point to be included under several international
67 conventions. STEF can cause significant mass mortality in the nests infected by a complex of species
68 mainly belonging to the Fusarium Solani Species Complex (FSSC; Sarmiento-Ramírez et al., 2010,
69 2014a). These fungi can infect and grow within *C. caretta* nests by, first, completely covering the egg
70 surface with a mycelial network, then destroying the shell by enzymes and organic acids that dissolve
71 organic substrates and calcium carbonate (Gleason et al., 2020; Phillott et al., 2006; Sarmiento-
72 Ramírez et al., 2010). Lesions, easily detectable by their colours on the colonized eggs, can turn into
73 necrotic portions and kill the surviving embryos. However, the aetiology and epidemiology of this
74 disease is still not completely known, while the biology and ecology of the causal agents need
75 additional investigation to reply as an example, to the question whether these pathogens are invasive
76 species or natural nest inhabitants able to cause disease under a changing environmental scenario
77 (Gleason et al., 2020; Smyth et al., 2019).
78 Despite STEF occurrence improved in the recent years at a global level (Gleason et al., 2020), in Italy
79 only few cases have been reported on *C. caretta* as in the case of Sicily (Gambino et al., 2019) and
80 Veneto (Pietroluongo et al., 2023), while any previous report in Tuscany is available. *C. caretta* is
81 the only sea turtle species nesting along the Tuscan coastline and the Tuscan archipelago, where nests
82 are becoming more numerous and widespread, as well as the number of affected nests, presumably

due to STEF. During the samplings carried out in summer 2020 in several localities along the Tuscan coast (province of Grosseto, Italy), eggs showing signs resembling those caused by STEF were found. In this work, we analysed, for the first time, eggs collected from natural nests of *C. caretta* along the Tuscan coastline, that showed visual symptoms of putative *Fusarium* infection, with the aim to (i) isolate *Fusarium* spp. present in unhatched eggs, (ii) morphologically and molecularly characterize the isolates, (iii) reconstruct the phylogenetic relationships between our isolates and those already known (animal and plant pathogens), and finally (iv) perform pathogenicity tests on plant model species known to be susceptible to infections with previously isolated pathogenic fungi.

Materials and Methods

Egg sampling, f ungal isolation and morphological observation

Nests located in three locations along the coast (Table 1) were manually excavated after the last hatchling emergence according to ISPRA guidelines (https://www.isprambiente.gov.it/it/attivita/CN-LAB/aree/area-metrologia/prodotti/linee-guida); only in the case of Rocchette location, the nest was sampled also before hatchling. In order to be sure to work in sterile conditions, and avoid external contaminations, single-use sterile gloves were used for all excavations and changed before contact with different nests. Unhatched eggs were collected and transferred in individual sterile plastic bags for successive examination. Lesioned eggs were characterized by unusual, coloured spots (reddish and grayish) of the shell compared with healthy ones and/or covered with mycelium. The content of

all contaminated eggs appeared dense and yellow apart from egg # 20106837/5, whose content appeared abnormally red.

Egg sampling was authorized by the Italian Ministry of Environment and Energy Security (authorization n. 35848, released in date 05/18/2020), according to the Council Directive 92/43/EEC of 21 May 1992.

Egg portions were firstly plated on Sabouraud Dextrose Agar (SDA, Biolife Italiana S.r.l., Milan, Italy) in order to allow the development of associated fungi. After a first mass isolation of each developing colony, pure culture, for each isolate, were obtained by single hyphal tips or, when sporulating, by single-spore cultivations on Potato Dextrose Agar (PDA, Biolife Italiana S.r.l., Milan, Italy) added with streptomycin sulphate (SIGMA-Aldrich. Milan, Italy, 300 mg L^{-1}). Plates, incubated at 24 ± 2°C, were used for morphological and molecular identification. All isolates were stored at the fungal collection of the University of Pisa, Italy (Department of Agriculture, Food, and Environment) on PDA under mineral oil at 4°C.

Table 1. List of sampling locations and corresponding nest ID number, sampling period, geographic coordinates (GCS)

Nest site (ID)	Sampling period	GCS	Eggs n°
Rocchette (2080970)	before hatching	42°46'9.75" N | 10°50'43.07" E	4
Rocchette (20106836)	after hatching	42°46'9.75" N | 10°50'43.07" E	4
Rocca a mare (20106837)	after hatching	42°46'22.04" N | 10°49'31.76" E	5
Riva del Sole (20106864)	after hatching	42°46'35.28" N | 10°47'50.08" E	5

Morphological characterisation of colonies resembling those of *Fusarium* spp. was based on the taxonomic keys of Leslie and Summerell (2007), including colony colour and morphology on top and the reverse side of the plate, colony size and texture of the mycelium growth as well as, by

121 microscopic observation (under 200X and 400X magnification, Leica Dialux 22 equipped with a
122 Leica DFC450 C camera, Leica Application Suite X –LAS X), micro- and macro-conidia shape, size,
123 number of septa, conidiogenic cells and conidiomata (if present) such as sporodochia.
124
125 **Molecular identification**
126 Fungal gDNA was extracted from all pure cultures according to Chelex 100 (Chelex® 100 sodium
127 form, MERCK SERONO S.P.A., Rome, Italy) protocol (Baroncelli et al., 2015). To identify and
128 analyse the genetic variability within *Fusarium* isolates, a region including the Translation elongation
129 factor 1 alpha (Tef1-α) gene, a useful region for fungal taxonomic and phylogenetic studies
130 (Baroncelli et al., 2015), was amplified. PCR reaction (25 µL) contained 2.0 µL of gDNA (10 ng),
131 2.5 µL of each primer (0.5 µM), 5.5 µL nuclease free H_2O and 12.5 µL GoTaq® Green Master Mix
132 (Promega Italia S.r.l, Milan, Italy). Primers Tef1-α (EF1-728F 5'-CATCGAGAAGTTCGAGAAGG-
133 3' as forward and EF1-986R 5'-TACTTGAAGGAACCCTTACC-3' as reverse) were used (Carbone
134 et al., 1999). The amplification program consisted in: 2 min denaturation (95°C); 30 cycles of
135 amplification (1 min at 94°C for denaturation, 1 min at 55°C for the annealing and 1 min at 72°C for
136 the extension); and a final extension at 72°C for 5 min. All amplifications were performed in a Q-
137 Cycler 24 (HAIN, Lifescience, Nehren, Germany). PCR products were checked by 2.0% gel
138 electrophoresis run and purified using a QIAquick PCR Purification Kit (QIAGEN, Hilden,
139 Germany). PCR products were sequenced by the Bio Molecular Research Genomics (BMR
140 Genomics, Padova, Italy). For phylogenetic analyses, reference Tef1-α sequences from the

FUSARIOID-ID database (https://www.fusarium.org) and from the FUSARIUM-ID v.3.0 (Torres-Crus et al., 2022) were retrieved and used to build a local BLAST database. Sequence produced in this work as well as Tef1-α sequences from other studies (Zhang e al., 2006; Sidique at al., 2017; García-Martín et al., 2021; Greeff-Laubscher and Jacobs, 2022) were analysed with the top-3 records retrieved from the BLAST analyses. All the sequences were aligned using MAFFT v. 7.402 (Katoh et al., 2002). FastTree v2.1.11 (Price et al., 2010) with default settings was used to perform the phylogenetic tree. Finally, each sequence was deposited in NCBI (GenBank, https://www.ncbi.nlm.nih.gov/genbank/) and an Accession number has been given.

Pathogenicity tests

In order to check the pathogenicity potential of *F. nodosum* - a species recently included within the Fusarium Head Blight Species Complex being isolated from *Triticum aestivum* in Iran (Lombard et al., 2019) and for the first time in Italy from *Triticum durum* associated with Fusarium head blight (FHB) (Felici et al., 2021) - the isolate t2 (Table 2) was tested on *T. aestivum* cv. Apogee flowering spikes, according to Vicente *et al.* (2020). In details, wheat seeds (surface sterilized with a NaClO solution - 0.6% active chlorine - for 3 min under shaking, three time washed, 10 min each, with sterile distilled water and stored in sterile distilled water at 4°C for 3 days before sowing) were sown in pots until plants reached anthesis (5 weeks). Three biological replicates, each consisting of three plants, were inoculated, by spraying, with an aqueous spore suspension of *F. nodosum* (10^5 spores mL^{-1}) made from two week old colony grown on PDA, added with Tween 80 (0.01%). Plants were covered with a white bag, previously moistened inside with water, then a black bag was placed above to favour

the infection process by the pathogen. Plants, whose spikes were inoculated with water + Tween 80 without spores, were used as negative control. After 24 h, bags were removed for plant aeration and after 1 h white bags were placed back for additional 24 h. After one week, for the following 7 days (14 dpi) plants were daily monitored for FHB symptoms in order to check the premature spike/spikelet bleaching compared to control ones.

With respect to isolates belonging to FSSC, including well known pathogens of Cucurbitaceae (Gomez et al., 2014), the isolate 3AF (Table 2) was used to inoculate *Cucumis melo* cv. Honeydew seedlings according to Gonzalez et al. (2020a, 2020b). In details, roots of melon seedlings at the 3-4 true-leaf stage, grown in pots, were immerged for 20 min in an aqueous spore suspension of the putative pathogen (10^5 spores mL^{-1}) and transplanted back in the pots. Plants whose roots were immerged for 20 min in uninoculated water were used as negative control. After one week, for the following 4 weeks, plants were daily monitored in order to check the typical symptoms of *F. solani* infection.

Finally, in order to verify if *F. oxysporum* belonged to a specific *forma specialis* or could be considered as saprotroph, artificial inoculations were performed on several model plants, according to Velarde-Felix et al. (2018). In details, tests were performed on *Capsicum annuum* (var. Cayenne and Viagra), *Cucumis melo* (var. Cantalupensis), *Cucurbita pepo* (var. Romanesco), *Eruca vesicaria* (subsp. *sativa*; var. Saetta), *Leucanthemum vulgare* (var. Bianca May Queen), *Mentha x piperita* (var. Rossa Glaciale), *Ocimum basilicum* (var. Red Rubin, Italiano and Fine Verde), *Phaseolus vulgaris* (var. Borlotto), *Pisum sativum* (var. Alderman), *Solanum lycopersicum* (var. San Marzano, Cerasiforme, Roma), *Solanum melongena* (var. Black Beauty) and *Vigna unguiculata* (var. Nano

Dolico), chosen because among the most frequent hosts for *F. oxysporum*. Three-four true-leaf stage seedlings were inoculated by immerging, for 20 min in an aqueous spore suspension (10^5 spores mL^{-1}) of the putative pathogen their roots then transplanted in plots. For this test *F. osypsorum* t7 (Table 2) was used, while plants whose roots were immerged for 20 min in uninoculated water were used as negative control. After one week, for the following 3 weeks, plants were daily monitored in order to check the typical wilt symptoms due to tracheomycosis.

All the pathogenicity tests have been performed, in triplicate, in a greenhouse with natural lighting (at plant height, the average photon flux density during measurements was around 500 µmol photons m^{-2} s^{-1}) and an average temperature of 25°C.

In case of symptomatic plants, in order to re-isolate the putative pathogen, roots and/or stem portions, as well as spikelets (for wheat) have been collected, surface sterilized for 1-3 min (depending on the host plant) in a NaClO (1% active chlorine) in 50% ethanol, washed three times in sterile water and plated on PDA added with streptomycin sulphate (SIGMA-Aldrich, Milan, Italy) at the concentration of 300 mg L^{-1}. Plates were incubated at 24 ± 2°C, under 12 h light/12 h darkness for two weeks to allow the eventual fungal colony to be developed.

Results

Fungal isolation, morphological observation and molecular identification

After the isolation to pure culture of the fungal colonies developed from the eggs, a total of 32 isolates (Table 2) were obtained and submitted, after single-spore cultures, to a first morphological

204 observation followed by a molecular identification and characterization. Morphological
205 identification, by microscopic observation, allowed to preliminary assign all the isolates to the
206 *Fusarium* genus, according to size and shape of conidia, conidiogenic cells and conidiophores
207 structures. All these strains showed a relatively fast growing on PDA, reaching a colony size of 75 ±
208 5 mm diameter after 7 days of incubation at 26 ± 1°C.
209 In order to assign a taxonomic designation to the isolates and to confirm previous results, a molecular
210 approach, consisting in the amplification and sequencing of Tef1-α regions was performed. Sequence
211 analysis allowed confirming the identity of our isolates as members of the FSSC *sensu* O'Donnell et
212 al. (2022) or *Neocosmospora* spp. *sensu* Crous et al. (2021), *F. oxysporum sensu* O'Donnell et al.
213 (2022) or FOSC *sensu* Crous et al. (2021) and *F. nodosum* (Table 2). Specifically, at the Rocchette
214 sites, a total of 14 different isolates (t7, t2, t6, 1A, 1B, 1C, 2A, 2B, 2C1, 2C2, 3A, 3B, 3C), belonging
215 to different lineages of the FSSC / *Neocosmospora* spp., FOSC and *F. nodosum* (sambucinum species
216 complex), were isolated from eggs collected before hatching (ID 2080970), while only two isolates
217 (t21, t5; FSSC / *Neocosmospora* spp.) were found in samples taken after hatching (ID 20106236). A
218 total of eight isolates were found from samples collected at the Rocca a Mare site (ID 20106837):
219 five of these were found to be FOSC (t11, t12, t14, t19, t22), while the remaining three to the FSSC
220 / *Neocosmospora* spp. (t16, t13, t18). The last nine isolates were collected from the Riva del Sole
221 sampling site (ID 20106864), and they included five isolates of the FSSC / *Neocosmospora* spp. (t3,
222 t8, t9, t10, t15) and three of FOSC (t1, t4, t17, t20). Overall, 18 strains of FSSC / *Neocosmospora*
223 spp. (56%), 12 of FOSC (38%) and two of *F. nodosum* (6%) were identified (Figure 1).

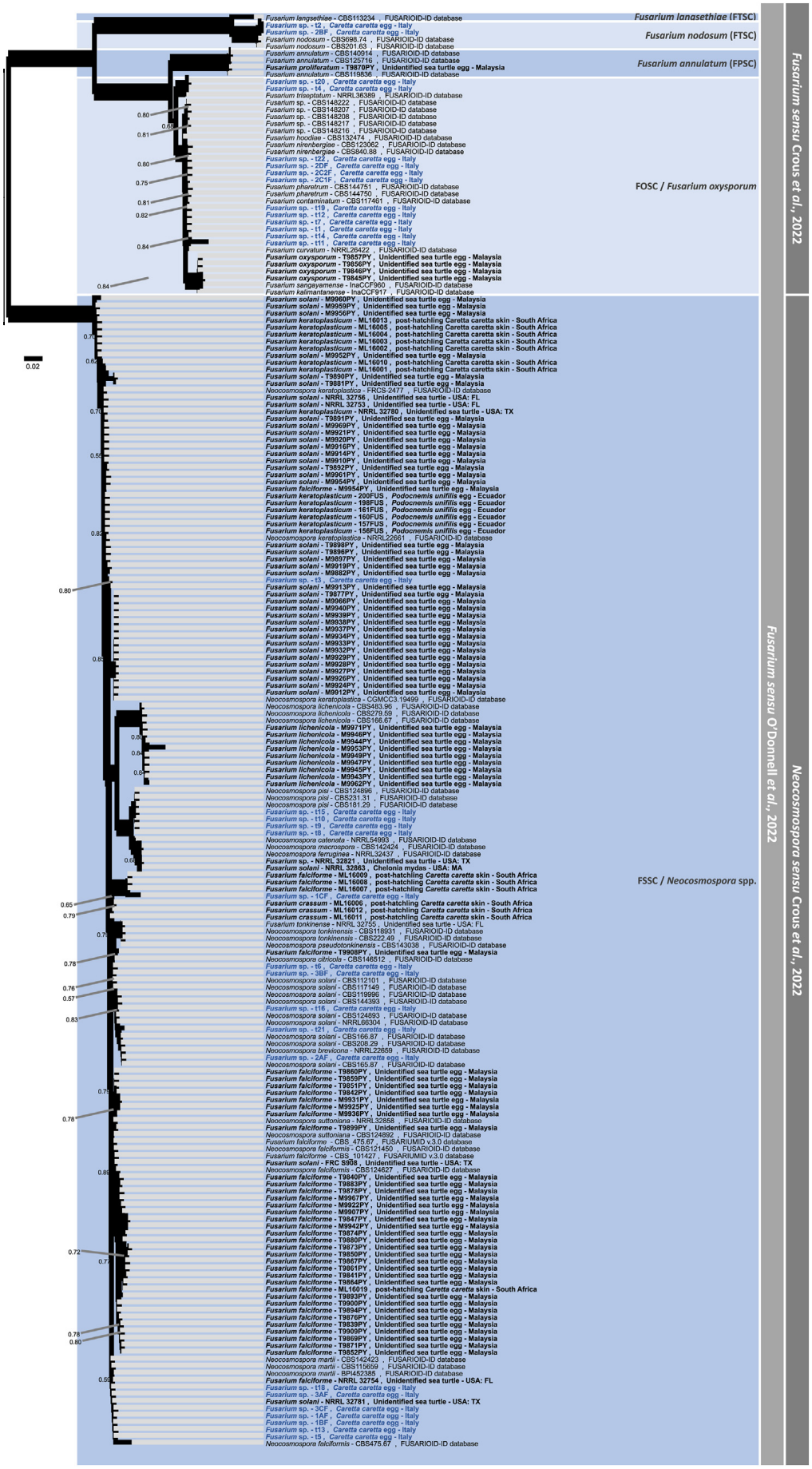
Fusarium langsethiae - CBS113234 , FUSARIOID-ID database
Fusarium sp. - t2 , Caretta caretta egg - Italy
Fusarium sp. - 2BF , Caretta caretta egg - Italy
Fusarium nodosum - CBS698.74 , FUSARIOID-ID database
Fusarium nodosum - CBS201.63 , FUSARIOID-ID database
Fusarium annulatum - CBS140914 , FUSARIOID-ID database
Fusarium annulatum - CBS125716 , FUSARIOID-ID database
Fusarium proliferatum - T9870PY , Unidentified sea turtle egg - Malaysia
Fusarium annulatum - CBS119836 , FUSARIOID-ID database
Fusarium sp. - t20 , Caretta caretta egg - Italy
Fusarium sp. - t4 , Caretta caretta egg - Italy
Fusarium triseptatum - NRRL36389 , FUSARIOID-ID database
Fusarium sp. - CBS148222 , FUSARIOID-ID database
Fusarium sp. - CBS148207 , FUSARIOID-ID database
Fusarium sp. - CBS148208 , FUSARIOID-ID database
Fusarium sp. - CBS148217 , FUSARIOID-ID database
Fusarium sp. - CBS148216 , FUSARIOID-ID database
Fusarium hoodiae - CBS132474 , FUSARIOID-ID database
Fusarium nirenbergiae - CBS123062 , FUSARIOID-ID database
Fusarium nirenbergiae - CBS840.88 , FUSARIOID-ID database
Fusarium sp. - t22 , Caretta caretta egg - Italy
Fusarium sp. - 2DF , Caretta caretta egg - Italy
Fusarium sp. - 2C2F , Caretta caretta egg - Italy
Fusarium sp. - 2C1F , Caretta caretta egg - Italy
Fusarium pharetrum - CBS144751 , FUSARIOID-ID database
Fusarium pharetrum - CBS144750 , FUSARIOID-ID database
Fusarium contaminatum - CBS117461 , FUSARIOID-ID database
Fusarium sp. - t19 , Caretta caretta egg - Italy
Fusarium sp. - t12 , Caretta caretta egg - Italy
Fusarium sp. - t7 , Caretta caretta egg - Italy
Fusarium sp. - t1 , Caretta caretta egg - Italy
Fusarium sp. - t14 , Caretta caretta egg - Italy
Fusarium sp. - t11 , Caretta caretta egg - Italy
Fusarium curvatum - NRRL26422 , FUSARIOID-ID database
Fusarium oxysporum - T9857PY , Unidentified sea turtle egg - Malaysia
Fusarium oxysporum - T9856PY , Unidentified sea turtle egg - Malaysia
Fusarium oxysporum - T9846PY , Unidentified sea turtle egg - Malaysia
Fusarium oxysporum - T9845PY , Unidentified sea turtle egg - Malaysia
Fusarium sangayamense - InaCCF360 , FUSARIOID-ID database
Fusarium kalimantanense - InaCCF917 , FUSARIOID-ID database
Fusarium solani - M9969PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9959PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9956PY , Unidentified sea turtle egg - Malaysia
Fusarium keratoplasticum - ML16013 , post-hatchling Caretta caretta skin - South Africa
Fusarium keratoplasticum - ML16005 , post-hatchling Caretta caretta skin - South Africa
Fusarium keratoplasticum - ML16004 , post-hatchling Caretta caretta skin - South Africa
Fusarium keratoplasticum - ML16003 , post-hatchling Caretta caretta skin - South Africa
Fusarium keratoplasticum - ML16002 , post-hatchling Caretta caretta skin - South Africa
Fusarium solani - M9952PY , Unidentified sea turtle egg - Malaysia
Fusarium keratoplasticum - ML16010 , post-hatchling Caretta caretta skin - South Africa
Fusarium keratoplasticum - ML16001 , post-hatchling Caretta caretta skin - South Africa
Fusarium solani - T9890PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - T9981PY , Unidentified sea turtle egg - Malaysia
Neocosmospora keratoplastica - FRCS-2477 , FUSARIOID-ID database
Fusarium solani - NRRL 32756 , Unidentified sea turtle - USA: FL
Fusarium solani - NRRL 32753 , Unidentified sea turtle - USA: FL
Fusarium keratoplasticum - NRRL 32780 , Unidentified sea turtle - USA: TX
Fusarium solani - T9891PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9969PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9921PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9920PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9916PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9914PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9910PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - T9892PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9961PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9954PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - M9954PY , Unidentified sea turtle egg - Malaysia
Fusarium keratoplasticum - 200FUS , Podocnemis unifilis egg - Ecuador
Fusarium keratoplasticum - 198FUS , Podocnemis unifilis egg - Ecuador
Fusarium keratoplasticum - 161FUS , Podocnemis unifilis egg - Ecuador
Fusarium keratoplasticum - 160FUS , Podocnemis unifilis egg - Ecuador
Fusarium keratoplasticum - 157FUS , Podocnemis unifilis egg - Ecuador
Fusarium keratoplasticum - 156FUS , Podocnemis unifilis egg - Ecuador
Neocosmospora keratoplastica - NRRL22661 , FUSARIOID-ID database
Fusarium solani - T9898PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - T9896PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9897PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9919PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9882PY , Unidentified sea turtle egg - Malaysia
Fusarium sp. - t3 , Caretta caretta egg - Italy
Fusarium solani - M9913PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - T9877PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9966PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9940PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9939PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9938PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9937PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9934PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9933PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9932PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9929PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9928PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9927PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9926PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9924PY , Unidentified sea turtle egg - Malaysia
Fusarium solani - M9912PY , Unidentified sea turtle egg - Malaysia
Neocosmospora keratoplastica - CGMCC3.19499 , FUSARIOID-ID database
Neocosmospora lichenicola - CBS483.96 , FUSARIOID-ID database
Neocosmospora lichenicola - CBS279.59 , FUSARIOID-ID database
Neocosmospora lichenicola - CBS166.67 , FUSARIOID-ID database
Fusarium lichenicola - M9971PY , Unidentified sea turtle egg - Malaysia
Fusarium lichenicola - M9946PY , Unidentified sea turtle egg - Malaysia
Fusarium lichenicola - M9944PY , Unidentified sea turtle egg - Malaysia
Fusarium lichenicola - M9953PY , Unidentified sea turtle egg - Malaysia
Fusarium lichenicola - M9949PY , Unidentified sea turtle egg - Malaysia
Fusarium lichenicola - M9947PY , Unidentified sea turtle egg - Malaysia
Fusarium lichenicola - M9945PY , Unidentified sea turtle egg - Malaysia
Fusarium lichenicola - M9943PY , Unidentified sea turtle egg - Malaysia
Fusarium lichenicola - M9962PY , Unidentified sea turtle egg - Malaysia
Neocosmospora pisi - CBS124896 , FUSARIOID-ID database
Neocosmospora pisi - CBS231.31 , FUSARIOID-ID database
Neocosmospora pisi - CBS181.29 , FUSARIOID-ID database
Fusarium sp. - t15 , Caretta caretta egg - Italy
Fusarium sp. - t10 , Caretta caretta egg - Italy
Fusarium sp. - t9 , Caretta caretta egg - Italy
Fusarium sp. - t8 , Caretta caretta egg - Italy
Neocosmospora catenata - NRRL54993 , FUSARIOID-ID database
Neocosmospora macrospora - CBS142424 , FUSARIOID-ID database
Neocosmospora ferruginea - NRRL32437 , FUSARIOID-ID database
Fusarium sp. - NRRL 32821 , Unidentified sea turtle - USA: TX
Fusarium solani - NRRL 32863 , Chelonia mydas - USA: MA
Fusarium falciforme - ML16009 , post-hatchling Caretta caretta skin - South Africa
Fusarium falciforme - ML16008 , post-hatchling Caretta caretta skin - South Africa
Fusarium falciforme - ML16007 , post-hatchling Caretta caretta skin - South Africa
Fusarium sp. - 1CF , Caretta caretta egg - Italy
Fusarium crassum - ML16006 , post-hatchling Caretta caretta skin - South Africa
Fusarium crassum - ML16012 , post-hatchling Caretta caretta skin - South Africa
Fusarium crassum - ML16011 , post-hatchling Caretta caretta skin - South Africa
Fusarium tonkinense - NRRL 32755 , Unidentified sea turtle - USA: FL
Neocosmospora tonkinensis - CBS118931 , FUSARIOID-ID database
Neocosmospora tonkinensis - CBS222.49 , FUSARIOID-ID database
Neocosmospora pseudotonkinensis - CBS143038 , FUSARIOID-ID database
Fusarium falciforme - T9908PY , Unidentified sea turtle egg - Malaysia
Neocosmospora citricola - CBS146512 , FUSARIOID-ID database
Fusarium sp. - t6 , Caretta caretta egg - Italy
Fusarium sp. - 3BF , Caretta caretta egg - Italy
Neocosmospora solani - CBS112101 , FUSARIOID-ID database
Neocosmospora solani - CBS117149 , FUSARIOID-ID database
Neocosmospora solani - CBS119996 , FUSARIOID-ID database
Neocosmospora solani - CBS144393 , FUSARIOID-ID database
Fusarium sp. - t16 , Caretta caretta egg - Italy
Neocosmospora solani - CBS124893 , FUSARIOID-ID database
Neocosmospora solani - NRRL66304 , FUSARIOID-ID database
Fusarium sp. - t21 , Caretta caretta egg - Italy
Neocosmospora solani - CBS166.87 , FUSARIOID-ID database
Neocosmospora solani - CBS208.29 , FUSARIOID-ID database
Neocosmospora brevicona - NRRL22659 , FUSARIOID-ID database
Fusarium sp. - 2AF , Caretta caretta egg - Italy
Neocosmospora solani - CBS165.87 , FUSARIOID-ID database
Fusarium falciforme - T9860PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9859PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9851PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9842PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - M9931PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - M9925PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - M9936PY , Unidentified sea turtle egg - Malaysia
Neocosmospora suttoniana - NRRL32858 , FUSARIOID-ID database
Fusarium falciforme - T9899PY , Unidentified sea turtle egg - Malaysia
Neocosmospora suttoniana - CBS124892 , FUSARIOID-ID database
Fusarium falciforme - CBS_475.67 , FUSARIUMID v.3.0 database
Neocosmospora falciformis - CBS121450 , FUSARIOID-ID database
Fusarium falciforme - CBS_101427 , FUSARIUMID v.3.0 database
Fusarium solani - FRC S908 , Unidentified sea turtle - USA: TX
Neocosmospora falciformis - CBS124627 , FUSARIOID-ID database
Fusarium falciforme - T9840PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9883PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9878PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - M9967PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - M9922PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - M9907PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9847PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - M9942PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9874PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9880PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9873PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9850PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9867PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9861PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9841PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9864PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - ML16019 , post-hatchling Caretta caretta skin - South Africa
Fusarium falciforme - T9893PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9900PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9894PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9876PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9839PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9909PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9869PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9871PY , Unidentified sea turtle egg - Malaysia
Fusarium falciforme - T9852PY , Unidentified sea turtle egg - Malaysia
Neocosmospora martii - CBS142423 , FUSARIOID-ID database
Neocosmospora martii - CBS115659 , FUSARIOID-ID database
Neocosmospora martii - BP452385 , FUSARIOID-ID database
Fusarium falciforme - NRRL 32754 , Unidentified sea turtle - USA: FL
Fusarium sp. - t18 , Caretta caretta egg - Italy
Fusarium sp. - 3AF , Caretta caretta egg - Italy
Fusarium solani - NRRL 32781 , Unidentified sea turtle - USA: TX
Fusarium sp. - 3CF , Caretta caretta egg - Italy
Fusarium sp. - 1AF , Caretta caretta egg - Italy
Fusarium sp. - 1BF , Caretta caretta egg - Italy
Fusarium sp. - t13 , Caretta caretta egg - Italy
Fusarium sp. - t5 , Caretta caretta egg - Italy
Neocosmospora falciformis - CBS475.67 , FUSARIOID-ID database
Fusarium lanasethiae (FTSC)
Fusarium nodosum (FTSC)
Fusarium annulatum (FPSC)
FOSC / Fusarium oxysporum
FSSC / Neocosmospora spp.
Fusarium sensu Crous et al., 2022
Fusarium sensu O'Donnell et al., 2022
Neocosmospora sensu Crous et al., 2022

Figure 1. Phylogenetic tree of TEF1 sequences of *Fusarium* strains isolated from *Caretta caretta*'s eggs in this study (highlighted in blue and bold), reference isolates retrieved from the FUSARIOID-ID database (https://www.fusarium.org) and from the FUSARIUM-ID v.3.0 (Torres-Crus et al., 2022) and of isolates associated with sea water and fresh water turtle (highlighted in bold) retrieved from previously published works (García-Martín et al., 2021; Greeff-Laubscher and Jacobs, 2022; Sidique at al., 2017; Zhang et al., 2006). Support values (above 50%) are shown at the nodes while the thickened nodes represent support values equal or higher than 90%.

Table 2. Identification of *Fusarium* spp. isolates, according to Tef1-α DNA sequence, collected from sea turtle eggshells and the corresponding Accession number (NCBI). Abbreviation: Egg ID, identification number of nest site and unhatched egg number (nest | egg); Sample ID, identification number of fungal isolates; FSSC, Fusarium solani species complex.

Egg ID (nest | egg)	Sample ID	Species	Accession number
2080970 | 70	t7	*FOSC / F. oxysporum*	*OQ053309*
2080970 | 70	*t2*	*Fusarium nodosum*	*OQ053304*
2080970 | 70	*t6*	*FSSC / Neocosmospora spp.*	*OQ053308*
2080970 | 1	*1AF*	*FSSC / Neocosmospora spp.*	*OQ054992*
2080970 | 1	*1BF*	*FSSC / Neocosmospora spp.*	*OQ053293*
2080970 | 1	*1CF*	*FSSC / Neocosmospora spp.*	*OQ053294*
2080970 | 2	*2AF*	*FSSC / Neocosmospora spp.*	*OQ053295*
2080970 | 2	*2BF*	*Fusarium nodosum*	*OQ053296*
2080970 | 2	*2C1F*	*FOSC / F. oxysporum*	*OQ053297*
2080970 | 2	*2C2F*	*FOSC / F. oxysporum*	*OQ053298*
2080970 | 2	*2DF*	*FOSC / F. oxysporum*	*OQ053299*
2080970 | 3	*3AF*	*FSSC / Neocosmospora spp.*	*OQ053300*
2080970 | 3	*3BF*	*FSSC / Neocosmospora spp.*	*OQ053301*
2080970 | 3	*3CF*	*FSSC / Neocosmospora spp.*	*OQ053302*
20106836 | 16	*t21*	*FSSC / Neocosmospora spp.*	*OQ053322*
20106836 | 18	*t5*	*FSSC / Neocosmospora spp.*	*OQ053307*
20106837 | 1	*t14*	*FOSC / F. oxysporum*	*OQ053316*
20106837 | 1	*t22*	*FOSC / F. oxysporum*	*OQ053323*
20106837 | 2	*t16*	*FSSC / Neocosmospora spp.*	*OQ053318*
20106837 | 2	*t19*	*FOSC / F. oxysporum*	*OQ053320*
20106837 | 3	*t18*	*FSSC / Neocosmospora spp.*	*OQ053319*
20106837 | 4	*t11*	*FOSC / F. oxysporum*	*OQ053313*
20106837 | 5	*t12*	*FOSC / F. oxysporum*	*OQ053314*
20106837 | 5	*t13*	*FSSC / Neocosmospora spp.*	*OQ053315*
20106864 | 18	*t10*	*FSSC / Neocosmospora spp.*	*OQ053312*
20106864 | 19	*t1*	*FOSC / F. oxysporum*	*OQ053303*
20106864 | 92	*t8*	*FSSC / Neocosmospora spp.*	*OQ053310*
20106864 | 92	*t9*	*FSSC / Neocosmospora spp.*	*OQ053311*
20106864 | 93	*t15*	*FSSC / Neocosmospora spp.*	*OQ053317*
20106864 | 93	*t3*	*FSSC / Neocosmospora spp.*	*OQ053305*
20106864 | 93	*t4*	*FOSC / F. oxysporum*	*OQ053306*
20106864 | 104	*t20*	*FOSC / F. oxysporum*	*OQ053321*

236

237 **Pathogenicity tests**

238 All inoculated plants were constantly monitored for the specific symptoms caused by each of the
239 potential pathogens used for pathogenicity tests.

240 After two weeks from inoculation, wheat spikes started to show a premature bleaching, firstly on
241 single spikelets and after spread all over the spike, thus confirming the pathogenicity of *F. nodosum*.
242 This last was confirmed also by plating spikelets portions on PDA + streptomycin: colony
243 morphologically similar to that of *F. nosodum* were observed.

244 With respect to all the other artificial inoculations, any of the plants inoculated with the isolate
245 belonging to the FSSC or with FOSC showed the typical symptoms of the corresponding disease,
246 thus not allowing us to confirm the pathogenicity of the isolates belonging to the FSSC /
247 *Neocosmospora* spp. and, in the case of FOSC / *F. oxysporum,* to assign our isolate to one of the
248 specific *formae speciales* of the hosts here tested.

249

250 **Discussion**

251 In the last decades, there has been an increasing number of reports concerning sea turtles (*Caretta*
252 *caretta*) infected by Ascomycota fungi belonging to *Fusarium* genus, with, among the first,
253 Wynecken and collaborators who described natural and artificial nests of *C. caretta* containing
254 diseased eggs failing in hatching (Wynecken et al., 1988). Several other reports at global level have
255 followed, all indicating *Fusarium* spp. as constantly associated with hatching failure loggerhead sea
256 turtle eggs as well as mass mortalities in nests (Sarmiento-Ramirez et al., 2010; Garcia-Hartman et

257 el. 2016). Due to the incidence of *Fusarium* spp. associated with the affected eggs/turtles, the apparent
258 disease is generally indicated as STEF. As listed by Gleason et al. (2020), Australia is the main
259 country in terms of STEF occurrence, while North and South America, Asia and Europe have only
260 few reports. With respect to Italy, very few studies are available such as that by Gambino and co-
261 authors where microbial diversity associated with turtle nests from the coastlines of Sicily and the
262 evaluation of the presence of *Fusarium* spp. have been investigated, providing key information on
263 potential pathogens that may affect hatching success (Gambino et al., 2019). More recently, two
264 northernmost worldwide loggerhead sea turtle nests were monitored along the Northern Adriatic
265 coastline (Veneto, Italy), where STEF deeply compromised the hatching success (Pietrolungo et al.,
266 2023). In this contest, the present work represents the first report of STEF on Tuscan coast. Although
267 Tuscany does not represent a primary nesting area of *C. caretta* in Italy and in the Mediterranean
268 area, the record of the disease on this coastline, in line with what recorded across the globe, confirms
269 that STEF may represent a major risk for the conservation of the loggerhead sea turtles also in this
270 region, especially considering how natural systems can be affected by the present climate change
271 perspective (Gleason et al., 2020; São Miguel et al., 2022).
272 Global climate change played an important role on the occurrence of emerging pathogens in new
273 environments and geographic areas (Fisher et al., 2012; Groner et al., 2016), thus resulting in an
274 increase of environmental stress conditions and, consequently, of fungal infection diseases also in the
275 marine ecosystems (Gleason et al., 2020), whose biodiversity is continuously threatened (Lafferty,
276 2009; Fisher et al., 2012, 2016). Climatic factors, alone and in combination with anthropogenic ones,
277 are considered as stressors that exasperated the impact of diseases since climate changes, such as

278 warming temperatures or changes in precipitation, may alter marine disease dynamics (Harvell et al.
279 2009). In addition to be perceived by living organisms as a source of stress, even if not demonstrated
280 yet specifically for sea turtles, these climatic factors can alter host-pathogen interactions in terms of
281 either alteration of pathogens' virulence that by compromising host's immune systems, as described
282 for several other marine species (Burge et al., 2014).
283 Members of the FSSC / *Neocosmospora* spp., such as *F. keratoplasticum*, *F. falciforme*, and *F.*
284 *oxysporum* were already associated with *C. caretta* eggs showing symptoms of fungal infection
285 (Candan et al., 2021; Greeff-Laubscher and Jacobs, 2022; Sarmiento-Ramírez et al., 2010, 2014a,
286 2014b). This is in line with the results here presented, where the two main fungal species isolated
287 from the three Tuscany sampling locations were identified as members of the FSSC / *Neocosmospora*
288 spp. and FOSC / *F. oxysporum*, both before and after eggs hatching. These two species are globally
289 recognized as among the most important plant pathogenic fungi, causing severe diseases on several
290 cultivated and spontaneous plants, thus representing a significant threat to human food supply and
291 agricultural biosecurity (O'Donnell et al., 2022). Despite the Fusarium solani species complex
292 (FSSC) has been considered as responsible for hatching failure in sea turtles worldwide, in 2021
293 (Carranco et al., 2022) it has also been associated with high hatching failure in eggs symptomatic of
294 fusariosis in *Podocnemis unifilis*, the yellow-spotted Amazon River turtle, thus highlighting its wide
295 dissemination among the Testudines not limited to sea environments.
296 Several *Fusarium* species can synthesize dangerous specialized secondary metabolites, defined
297 mycotoxins, that can cause adverse effects on human and animal health (D'Mello et al., 1999; Perrone
298 et al., 2020; Sarrocco et al., 2019, Pisuttu et al., 2023). It is the case of *F. nodosum* that, as far as we

299 know, has been here isolated for the first time from affected *C. caretta* eggs. *F. nodosum* is a
300 mycotoxigenic plant-pathogenic fungus, belonging to the complex of *Fusarium* species causing FHB
301 of wheat (Fusarium Head Blight Species Complex), recently reported for the first time in Italy on
302 *Triticum durum* (Felici et al., 2021). As already discussed by Gleason et al. (2020), the presence of
303 mycotoxigenic fungi on eggshell could represent a threaten for the animal since mycotoxins may
304 diffuse into embryo's tissues thus affecting its development and causing its death (Gleason et al.,
305 2020).
306 Tests here made on plants, even if performed on a limited number of fungal isolates, resulted in a lack
307 of pathogenicity of the strains included in the FSSC and of FOSC / *F. oxysporum* when inoculated
308 on some of the main plant hosts for these pathogen. The lack of pathogenicity of FOSC / *F. oxysporum*
309 here isolated is in favour of the hypothesis these isolates are included within a different *forma*
310 *specialis* than those here supposed or belong to saprotrophic populations occurring on sea turtle
311 affected eggs. The ability to infect and cause a premature blenching of wheat spikes, instead,
312 confirmed the plant pathogenic potentiality of the *F. nodosum* isolate and reinforced the trend of the
313 transmission of emerging pathogens into new environment (Groner at al., 2016).Results here reported
314 confirm a global occurrence of the problem and the need for further studies concerning the biology
315 and ecology of the pathogenic agents, as well as the aetiology and epidemiology of the disease.
316 Since fungal pathogens and associated diseases are spreading in marine environment (Pang et al.,
317 2021), it is critical to identify the biological and ecological components that could contribute to
318 disease epidemic outbreak and severity. In addition to the isolation and identification of the potential
319 causal agents of the diseases, as done in the present and several other works, other investigation are

needed, including histopathological examination. A standardize diagnostic approach appears to be essential to harmonize results in order to tackle an emergent infectious disease, as suggested by Di Nicola et al., (2022). Even if specifically referred to *O. ophidiicola* detection and infection in snakes, this paper should be considered as a guideline to be followed during investigation concerning emergent diseases potentially affecting biodiversity, in order to better discriminate among the presence of the pathogen, apparent disease and confirmed disease.

Further studies focused on pathogen phylo-biogeography, mechanisms of dispersion and colonization of coastal habitats, and environmental and physiological parameters for infection are needed. For these reasons, isolation and characterization of fungal pathogens will help us to reveal their biology and epidemiology and will allow a better management of disease and to better understand the current and future impact of STEF on sea turtles' conservation worldwide.

Acknowledgements

Authors are grateful to "Associazione tartAmare" (Grosseto, Italy) for active nests monitoring and for providing affected eggs.

References

Baroncelli, R., Sarrocco, S., Zapparata, A., Tavarini, S., Angelini, L.G. Vannacci, G., 2015. Characterization and epidemiology of *Colletotrichum acutatum* sensu lato (*C. chrysanthemi*) causing *Carthamus tinctorius* anthracnose. Plant. Pathol. 64 (2), 375–384.

341 Burge, C.A., Eakin, C.M., Friedman, C.S., Froelich, B., et al, 2014. Climate Change Influences on
342 Marine Infectious Diseases: Implications for Management and Society. Ann.l Rev. Marine Sc.
343 6:1, 249-277.
344 Candan, A.Y., Katılmış, Y., Ergin, Ç., 2021. First report of *Fusarium* species occurrence in
345 loggerhead sea turtle (*Caretta caretta*) nests and hatchling success in Iztuzu Beach, Turkey.
346 Biologia 76 (2), 565-573.
347 Carbone, I., Kohn, L.M. 1999. A method for designing primer sets for speciation studies in
348 filamentous ascomycetes. Mycologia. 91:553–556.
349 Carranco, A.S., Gillingham, M.A., Wilhelm, K., de Lourdes Torres M., Sommer, S., Romo, D., 2022.
350 Transcending sea turtles: first report of hatching failure in eggs of an Amazonian freshwater
351 turtle with symptoms of the fungal emerging disease fusariosis. Transbound. Emerg. Dis. 69 (5),
352 e3282-e3288.
353 Carvalho, A.D.M., Souza, L.K.H., Ataídes, F.S., Péres Junior A.K., 2019. Eggs of tegu lizard,
354 *Salvator merianae* (Duméril & Bilbron, 1839) (Squamata; Teiidae) damaged by fungal
355 infections. Braz. J. Biol. 80, 112-114.
356 Ceriani, S.A., Casale, P., Brost, M., Leone E.H., Witherington, B.E., 2019. Conservation implications
357 of sea turtle nesting trends: elusive recovery of a globally important loggerhead population.
358 Ecosphere 10 (11), e02936.
359 Crous, P.W., Lombard, L., Sandoval-Denis, M., *et al*., 2021. *Fusarium*: more than a node or a foot-
360 shaped basal cell. Stud. Mycol. 98, 100116.
361 D'Mello, J.P.F., Placinta, C.M., Macdonald, A.M.C., 1999. *Fusarium* mycotoxins: a review of global

362 implications for animal health, welfare and productivity. Anim. Feed Sci. Technol. 80 (3–4),
363 183-205.
364 Dean, R., van Kan, J.A.L., Pretorius, Z.A., Hammond-Kosack, K.E., Di Pietro, A., Spanu, P.D., Rudd,
365 J.J., Dickman, M., Kahmann, R., Ellis, J., Foster, G., 2012. The Top 10 fungal pathogens in
366 molecular plant pathology. Mol. Plant Pathol. 13 (4), 414-430.
367 Di Nicola, M.R., Coppari, L., Notomista, T. *et al.*, 2022. *Ophidiomyces ophidiicola* detection and
368 infection: a global review on a potential threat to the world's snake populations. Eur. J. Wildl.
369 Res. 68 (64).
370 Felici, L., Francesconi, S., Balestra, G.M., 2021. First report of *Fusarium nodosum* (L. Lombard &
371 Crous) on durum wheat (*Triticum turgidum* subsp. durum) in Italy. J. Plant Pathol. 103 (4), 1101.
372 Fisher, M.C., Garner, T.W.J., 2020. Chytrid fungi and global amphibian declines. Nat. Rev.
373 Microbiol. 18:332–343.
374 Fisher, M.C., Gow, N.A., Gurr, S.J., 2016. Tackling emerging fungal threats to animal health, food
375 security and ecosystem resilience. Philos. Trans. R. Soc. Lond., B, Biol. Sc. 371 (1709),
376 20160332.
377 Fisher, M.C., Henk, D.A., Briggs, C.J., Brownstein, J.S., Madoff, L.C., McCraw, S.L., Gurr, S.J.,
378 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature 484 (7393), 186-
379 194.
380 Gambino, D., Persichetti, M.F., Gentile, A., Arculeo, M., Visconti, G., Currò, V., Caracappa, G.,
381 Crucitti, D., Piazza, A., Mancianti, F., Nardoni, S., Vicari, D., Caracappa, S., 2020. First data on
382 microflora of loggerhead sea turtle (*Caretta caretta*) nests from the coastlines of Sicily. Biol.

383 open 9 (1), bio045252.
384 Garcia-Hartmann, M., Hennequin, C., Catteau, S. Béatini, C., Blanc, V., 2017. Cas groupés
385 d'infection à *Fusarium solani* chez de jeunes tortues marines *Caretta caretta* nées en captivité.
386 J. Mycol. Méd. 27 (1), 113-118.
387 García-Martín, J.M., Sarmiento-Ramírez, J.M., Diéguez-Uribeondo, J. , 2021. Beyond Sea Turtles:
388 *Fusarium keratoplasticum* in Eggshells of *Podocnemis unifilis*, a Threatened Amazonian
389 Freshwater Turtle. J. Fungi. 7, 742. Gleason, F.H., Allerstorfer, M., Lilje, O., 2020. Newly
390 emerging diseases of marine turtles, especially sea turtle egg fusariosis (STEF), caused by
391 species in the Fusarium solani complex (FSSC). Mycology 11 (3), 184-194.
392 Gómez, J., Serrano, Y., Pérez, A., Porcel, E., Gómez, R., Aguilar, M.I., 2014. *Fusarium solani* f. sp.
393 *cucurbitae*, affecting melon in Almería Province, Spain. Australas. Plant Dis. Notes 9, 136.
394 González, V., Armijos, E., Garcés-Claver, A., 2020. Fungal endophytes as biocontrol agents against
395 the main soil-borne diseases of melon and watermelon in Spain. Agronomy 10, 820.
396 Greeff-Laubscher, M.R., Jacobs, K., 2022. *Fusarium* species isolated from post-hatchling loggerhead
397 sea turtles (*Caretta caretta*) in South Africa. Sci. Rep. 12 (1), 1–13.
398 Groner, M.L., Maynard, J., Breyta, R., Carnegie, R.B., Dobson, A., Friedman, C.S., Froelich, B.,
399 Garren, M., Gulland, F.M.D., Heron, S.F., Noble, R.T., Revie, C.W., Shields, J.D.,
400 Vanderstichel, R., Weil, E., Wyllie-Echeverria, S., Harvell, C.D., 2016. Managing marine
401 disease emergencies in an era of rapid change. Philos. Trans. R. Soc. Lond., B., Biol. Sc. 371
402 (1689), 20150364.
403 Harvell, D., Altizer, S., Cattadori, I.M., Harrington, L. and Weil, E., 2009, Climate change and

404 wildlife diseases: When does the host matter the most? Ecology 90: 912-920.
405 Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple
406 sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30 (14), 3059-3066.
407 Lafferty, K.D., 2008. The ecology of climate change and infectious diseases. Ecology 90 (4), 888-
408 900.
409 Langwig K.E., Frick, W.F., Hoyt, J.R., Parise, K.L., Drees, K.P., Kunz, T.H., Foster, J.T., Kilpatrick.
410 A.M., 2016 Drivers of variation in species impacts for a multi-host fungal disease of bats. Phil.
411 Trans. R. Soc. B371: 20150456.
412 Leslie, J.F., Summerell, B.A., 2008. The Fusarium laboratory manual. *John Wiley & Sons*.
413 Lombard, L., Van Doorn, R., Crous, P.W., 2019. Neotypification of *Fusarium chlamydosporum* - a
414 reappraisal of a clinically important species complex. Fungal Syst. Evol. 4, 183-200.
415 Moreira, P.L., Barata, M., 2005. Egg mortality and early embryo hatching caused by fungal infection
416 of Iberian rock lizard (*Lacerta monticola*) clutches. Herpetol. J. 15 (4), 265-272.
417 O'Donnell, K., Whitaker, B.K., Laraba, I., Proctor, R.H., Brown, D.W., Broders, K., Kim, H.,
418 McCormick, S.P., Busman, M., Aoki, T., Torres-Crus, T.J., Geiser, D.M., 2022. DNA sequence-
419 based identification of *Fusarium*: a work in progress. Plant Dis. 5 (10), PDIS-09.
420 Pang, K., Hassett, B.T., Shaumi, A., Guo, S., Sakayaroj, J., Chiang, M.W., Yang, C., Jones, E.B.G.,
421 2021. Pathogenic fungi of marine animals: A taxonomic perspective. Fungal Biol. Rev. 38:92-
422 106.
423 Perrone, G., Ferrara, M., Medina, A., Pascale, M., Magan, N., 2020. Toxigenic fungi and mycotoxins
424 in a climate change scenario: ecology, genomics, distribution, prediction and prevention of the

risk. Microorganisms 8 (10), 1496.

Pietroluongo, G., Centelleghe, C., Sciancalepore, G. *et al.*, 2023. Environmental and pathological factors affecting the hatching success of the two northernmost loggerhead sea turtle (*Caretta caretta*) nests. Sci. Rep. 13:2938.

Pisuttu, C., Risoli, S, Moncini, L., Nali, N., Pellegrini, E., Sarrocco, S., 2023. Sustainable strategies to counteract mycotoxins contamination and cowpea weevil in chickpea seeds during post-harvest. Toxins 15 (1), 61.

Phillott, A.D., Parmenter, C.J., McKillup, S.C., 2006. Calcium depletion of eggshell after fungal invasion of sea turtle eggs. Chelonian Conserv. Biol. 5 (1), 146-149.

Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2–approximately maximum-likelihood trees for large alignments. PLoS ONE 5 (3), e9490.

Sandoval-Denis, M., Crous, P.W., 2018. Removing chaos from confusion: assigning names to common human and animal pathogens in *Neocosmospora*. Persoonia 41, 109–129.

São Miguel, R.A.M., Anastácio, R., Pereira, M.J., 2022. Sea turtle nesting: what is known and what are the challenges under a changing climate scenario. Open J. Ecol. 12, 1-35.

Sarmiento-Ramírez, J.M., Abella, E., Martín, M.P., Tellería, M.T., López-Jurado, L.F., Marco, A., Diéguez-Uribeondo, J., 2010. *Fusarium solani* is responsible for mass mortalities in nests of loggerhead sea turtle, *Caretta caretta*, in Boavista, Cape Verde. FEMS Microbiol. Lett. 312 (2), 192-200.

Sarmiento-Ramírez, J.M., Abella-Pérez, E., Phillott, A.D., Sim, J., van West, P., Martin, M.P., Marco, A., Diéguez-Uribeondo, J., 2014a. Global distribution of two fungal pathogens threatening

446 endangered sea turtles. PLoS ONE 9 (1), e85853.
447 Sarmiento-Ramírez, J.M., van der Voort, M., Raaijmakers, J.M., Diéguez-Uribeondo, J., 2014b.
448 Unravelling the microbiome of eggs of the endangered sea turtle *Eretmochelys imbricata*
449 identifies bacteria with activity against the emerging pathogen *Fusarium falciforme*. PLoS ONE
450 9 (4), e95206.
451 Sarrocco, S., Mauro, A., Battilani, P., 2019. Use of competitive filamentous fungi as an alternative
452 approach for mycotoxin risk reduction in staple cereals: state of art and future perspectives.
453 Toxins 11 (12), 701.
454 Short, D.P.G., O'Donnell, K., Zhang, N. Juba, J.H., Geiser, D.M., 2011. Widespread occurrence of
455 diverse human pathogenic types of the fungus *Fusarium* detected in plumbing drains. J. Clin.
456 Microbiol. 49 (12), 4264-4272.
457 Sidique, S.N.M., Azuddin, N.F., Joseph, J.. 2017. First report of *Fusarium* species at nesting sites of
458 endangered sea turtles in Terengganu and Melaka, Malaysia. Malaysian Appl Biol. 46 (3), 195–
459 205.
460 Smyth, C.W., Sarmiento-Ramírez, J.M., Short, D.P.G., Diéguez-Uribeondo, J., O'Donnel, K., Geiser,
461 D.M., 2019. Unraveling the ecology and epidemiology of an emerging fungal disease, sea turtle
462 egg fusariosis (STEF). PLoS Pathog. 15 (5), e1007682.
463 Summerell, B.A., 2019. Resolving *Fusarium*: current status of the genus. Annu. Rev. Phytopathol.
464 57, 323-339.
465 Torres-Cruz, T.J., Whitaker, B.K., Proctor, R.H., Broders, K., Laraba, I., Kim, H.S., Brown, D.W.,
466 O'Donnell, K. et al., 2022. FUSARIUM-ID v.3.0: An Updated, Downloadable Resource for

467 *Fusarium* Species Identification. Plant Dis. 106(6), 1610-1616.Velarde-Félix, S., Garzón-
468 Tiznado, J.A., Hernández-Verdugo, S., López-Orona, C.A., Retes-Manjarrez, J.E., 2018.
469 Occurrence of *Fusarium oxysporum* causing wilt on pepper in Mexico. Can. J. Plant. Pathol. 40
470 (2), 238-247.
471 Vicente, I., Baroncelli, R., Morán-Diez, M.E., Bernardi, R., Puntoni, G., Hermosa, R., Monte, E.,
472 Vannacci, G., Sarrocco, S., 2020. Combined comparative genomics and molecular biology
473 approaches provide insights into the terpene synthases inventory in *Trichoderma.*
474 Microorganisms 8 (10), 1603.
475 Wyneken, J., Burke, T.J., Salmon, M., Pedersen, D.K., 1988. Egg failure in natural and relocated sea
476 turtle nests. J. Herpetol. 22 (1), 88-96.
477 Zhang, N., O'Donnell, K., Sutton, D.A., Ameena Nalim, F., Summerbell, R.C., Padhye, A.A., Geiser,
478 D.M., 2006. Members of the *Fusarium solani* species complex that cause infections in both
479 humans and plants are common in the environment. J. Clin. Microbiol. 44 (6), 2185-2190.

Highlights

- Fungal infectious diseases have dramatically increased in marine ecosystems
- STEF represent one of the main threats to *Caretta caretta* in Mediterranean region
- A total of 32 strains of *Fusarium* strains were identified
- Phylogenetic relationships of *Fusarium* isolates have been reconstructed
- For the first time strains of *F. nodosum* were isolated from infected eggs

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Riccardo Baroncelli
Cristina Nali
Samuele Risoli
Sabrina Sarrocco
Giuliana Terracciano
Luana Papetti