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UNWANTED GUEST CONTINUES ITS NORTHERN JOURNEY IN THE AEGEAN SEA: *PTEROIS MILES* (BENNETT, 1828)

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DOI: 10.2478/trser-2022-0020

KEYWORDS: lionfish, cytochrome oxidase I, indo-pacific sp., marine bioinvasion.

ABSTRACT

Pterois miles (Bennett, 1828), one of the most destructive marine invaders, was reported from the Mediterranean in 1991. It has invaded most parts of the Mediterranean from 2013, when it was reported for the second time, until today. On 7th October 2020, a single specimen of *P. miles* was collected by the bottom trawl from the Edremit Bay (Northern Aegean Sea). COI barcode region was used for the molecular identification of the specimen. DNA barcode data suggest that the specimen used in the present study is *P. miles* with a 99.69% probability. The current study is presented by providing the northernmost occurrence of the *P. miles* from the Aegean Sea with molecular confirmation.

ZUSAMMENFASSUNG: Unerwünschte Gäste setzen ihre Ausbreitung im Ägäischen Meer fort: *Pterois miles* (Bennett, 1828).

Pterois miles (Bennett, 1828), eine der destruktivsten Meeres-Eindringlinge, wurde aus dem Mittelmeer 1991 angegeben. Ab 2013, als sie zum zweitenmal gemeldet wurde, hat sie sich bis heute im größten Teil des Mittelmeeres invasiv ausgebreitet. Am 7 Oktober 2020 wurde ein einziges Exemplar von *Pterois miles* aus dem Bodenschleppnetz im Bereich der Endremit Bucht (Nördliches Ägäisches Meer) gesammelt. Der COI Strichcode der Region wurde zur molekularen Identifikation der Art verwendet. Die Daten des DNA Strichcodes belegen, dass es sich bei der in vorliegender Studie untersuchten Art mit einer Wahrscheinlichkeit von 99,69% um *Pterois miles* handelt. Die vorliegende Studie soll das durch molekulare Untersuchung bestätigte, nördlichste Vorkommen von *Pterois miles* aus dem Ägäischen Meer unterstützen.

REZUMAT: Oaspetele nedorit îşi continuă călătoria în nordul Mării Egee: *Pterois miles* (Bennett, 1828).

Pterois miles (Bennett, 1828), unul dintre cei mai distructivi invadatori marini, a fost raportat în Marea Mediterană în 1991. A invadat cele mai multe părţi al Mediteranei până astăzi, din 2013, când a fost raportat pentru a doua oară. În 7 Octombrie 2020, un singur specimen de *P. miles* a fost colectat cu un traul de fund din Golful Edremit (nordul Mării Egee). Codul de bare COI a fost utilizat pentru identificarea moleculară a specimenului respectiv. Datele codului de bare ADN sugerează faptul că specimenul utilizat în studiul de faţă este *P. miles* cu o probabilitate de 99.69%. Studiul actual este prezentat ca oferind date despre ocurenţa cea mai nordică din Marea Egee a lui *P. miles*, cu confirmare moleculară.

INTRODUCTION

Endemic and endangered taxa of the Mediterranean Sea have faced the invasion of the Indo-Pacific species since the opening of the Suez Canal in 1869. This invasion called Lessepsian migration is ever-growing due to climate change, constructional changes in the Suez Canal, and other anthropogenic activities. Lessepsian migration is effective throughout the Mediterranean, however, non-native species diversity still displays sharp differences between western and eastern basins of the Mediterranean Sea except for several fish species. (Coll et al., 2010) The marine ichthyofauna of the Turkish coasts currently consists of 530 species, including four classes: Actinopterygii (463 species), Elasmobranchii (65 species), Cephalaspidomorphi (one species), and Holocephali (one species). Turkish Mediterranean (Levantine coast) has 453 spp. and the Aegean Sea 453 spp., with the highest diversity, followed by Sea of Marmara (257 spp.) and Black Sea (151 spp.). Turkish coasts are hotspots of marine bio-invasion due to mainly its proximity to the Suez Canal, Mediterranean currents, and intensive maritime shipping. Considering the origin of the distributed fishes along the Turkish coasts, a total of 90 (16.9% of total) species are non-native (35 alien, 29 established, and 26 invasive), originating from Indo-Pacific (79 spp.), Atlantic (seven spp.), and cosmopolitan (four spp.) species. (Karataş et al., 2021)

The genus *Pterois* Oken, 1817, belonging to Scorpaenidae, is mainly called lionfish or firefish. It is characterized by venomous dorsal, anal and pelvic fin spines (Allen and Erdmann, 2008). *P. volitans* and *P. miles* are a recent, significant, and dangerous invasive species in the Mediterranean Sea as well as in the west Atlantic and the Caribbean Sea. *P. miles* (Bennett, 1828), reported by Golani and Sonin (1992) as first *Pterois* species in the Mediterranean area, has been expanded along Israel, Lebanon, Cyprus, Türkiye, and Greece (Dailianis et al., 2016).

We aim to present the current status, northernmost occurrence, and genetic information of the worst invader fish, *Pterois miles*, in the Aegean Sea.

MATERIAL AND METHODS

Sampling and morphological identification

On October 7th 2020, a specimen of *P. miles* (Fig. 1) was caught by commercial trawler, from Edremit Bay (39.4730°N', 26.6230°E') at a depth of approximately 70 m.



Figure 1: A view of fresh specimen of the *Pterois miles*.

The morphometric characters of this specimen were measured with a digital caliper (to the nearest 0.1 mm), while meristic details and counts were examined using a stereo zoom microscope. Morphological and taxonomic descriptions of the collected specimen were followed according to Golani and Sonin (1992).

DNA extraction, PCR and sequencing

Genomic DNA was isolated from a muscle tissue of the specimen using EurX GeneMATRIX Tissue and Bacterial DNA Kit according to manufacturer's protocol. DNA barcode region of cytochrome c oxidase subunit I (COI) was amplified using the primers of FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC) and FishR1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA) (Ward et al., 2005). DNA amplifications were performed in 50 µl volumes, each containing: 5 µl of 10X Taq Buffer with KCl (100 mM Tris-HCl, 500 mM KCl, pH 8.8), 5 µl of MgCl2 (25 mM), 1 µl of dNTPs (10 mM), 1 µl of each primer (10 pM/µl), 2 U of Taq polymerase (5 U/µl), and 3 µl of DNA (50 ng/µl). PCR amplifications were performed in Eppendorf Mastercycler® with the following cycling conditions (Keskin and Atar, 2013): preliminary denaturation at 95°C for five minutes followed by 40 cycles consisting of denaturation at 95°C for 45 seconds, primer annealing at 57°C for 45 sec., primer extension at 72°C for one minute and final extension step at 72°C for five minutes. Sanger sequencing was performed by BM Labosis (Ankara, Türkiye) using ABI 3730XL DNA Analyzer (Applied Biosystems, USA).

Data analyses

Reference sequences of the species gathered from GenBank were reported in the below table 1.

Table 1: List of GenBank COI sequences of the species used in the molecular analysis of *Pterois miles.*

Species	Country	Location	GenBank Acc. No.	Reference
Pterois miles	India	Lakshadweep	OK602740	Unpublished
Pterois miles	Yemen	Unspecified	MH331851	Unpublished
Pterois miles	Israel	Eilat	MF124020	Kimmerling et al., 2018
Pterois miles	South Africa	Vetch's Pier	GU805078	Unpublished
Pterois miles	Unspecified	Unspecified	FJ584026	Steinke et al., 2009
Pterois volitans	Unspecified	Unspecified	FJ584044	Steinke et al., 2009
Dendrochirus brachypterus	South Africa	Indian Ocean	JN312280	Unpublished
Scorpaena scrofa	Malta	Mediterranean Sea	KJ709885	Landi et al., 2014
Scorpaena porcus	Türkiye	Antalya Bay	KC501412	Keskin and Atar, 2013

Molecular data analyses of COI gene region were conducted from a specimen of *P. miles*. Nucleotide sequences were aligned using the ClustalW algorithm in MEGA X (Kumar et al., 2018). DNA sequence data were submitted to GenBank and the accession number OM128427 was assigned. *Pterois volitans* (FJ584044), *Dendrochirus brachypterus* (JN312289), *Scorpaena porcus* (KC501412), and *Scorpaena scrofa* (KJ709885) haplotypes were used as outgroup taxa in phylogenetic analysis. A phylogenetic tree was generated in MEGA X (Kumar et al., 2018) using neighbor-joining (NJ) algorithm (Saitou and Nei, 1987) of genetic p-distances with 1,000 bootstrap replications in pair wise deletion mode. Genetic p-distances have been remarked to perform better for species identification than the corrected substitution models (e.g., Kimura 2-parameter, Tamura-Nei model) (Srivathsan and Meier, 2012). Genetic distances between species were calculated in MEGA X (Kumar et al., 2018) using the p-distance model with 1,000 bootstrap replications.

RESULTS AND DISCUSSION

The examined specimen (family Scorpaenidae Risso, 1827, *Pterois* Oken, 1817, *Pterois miles* (Bennett, 1828) (Fig. 1)) has a 224 mm TL (174 mm SL) and 177.42 g in weight. Moderately compressed body with alternating wide dark brown and narrow white and reddish dorsal bands, pectoral and pelvic fins covered with black, red, and white bands. Dark spots on soft rays of dorsal, anal, and caudal fins. Several prominent white spots on pelvic fins. The morphometric measurements and meristic counts of the specimen (Fig. 1) are described as follows: body depth, 2.7 in SL. head length (HL), 3.4 in SL; snout length, 2.5 in HL, eye diameter, 3.8 in HL, and the interorbital width, 4.2 in HL; dorsal fin XIII + 10, anal fin III + 6, pectoral fin 14, pelvic fin I, 5.

Figure 2 shows phylogenetic relationships based on the COI barcode region of a specimen of *P. miles*, and its closest congeneric and same species, showing the monophyly of all of them, with good statistical support.

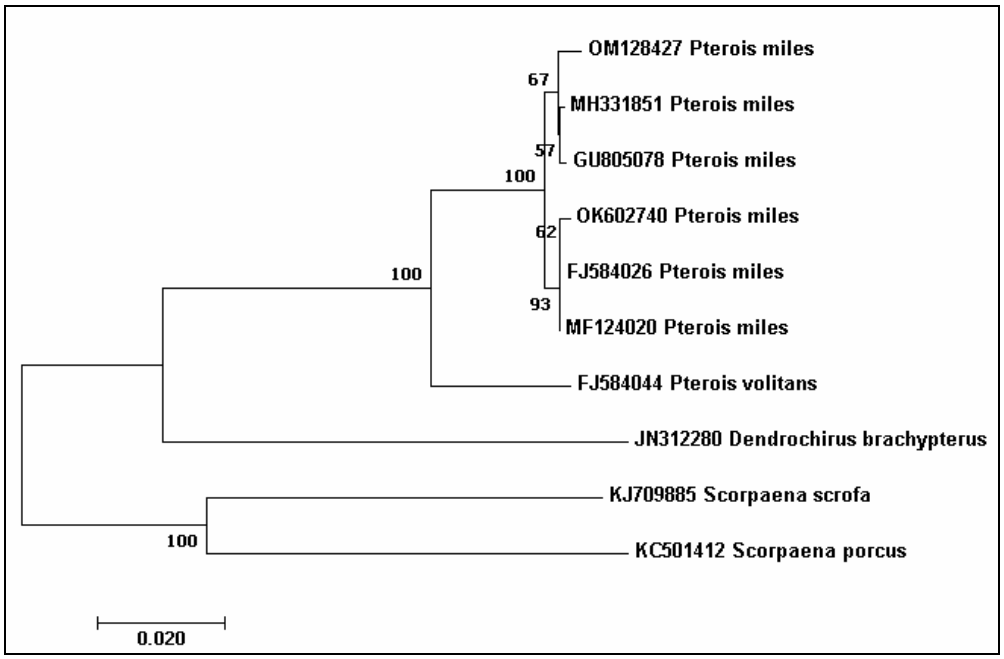

Figure 2: Tree based on the neighbor-joining (NJ) analysis of pairwise genetic p-distances. Bootstrap values for 1,000 replicates are shown above branches on the tree; scale: 2% p-distance.

COI barcode of the specimen exhibit an identical nucleotide sequence and form a cluster that is similar from its closest sequences, *P. miles* (GU805078 and MH331851), by 0.5% and 0.6%, respectively (Tab. 2). On the other hand, *P. miles* and *P. volitans* were determined as genetically distinct from each other (4.6%; Tab. 2). Additionally, according to the nucleotide data, the specimen belongs to the family Scorpaenidae, with a 100% probability of placement after a BLAST search in the GenBank database, which also matches *P. miles*, with a 99.69% probability.

Table 2: Genetic distances (p-distances) based on mitochondrial COI barcode regions of the species used in this study.

	GenBank Acc. No.	Species	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.	OM128427	*Pterois miles*	***									
2.	MH331851	*Pterois miles*	0.006	***								
3.	GU805078	*Pterois miles*	0.005	0.008	***							
4.	OK602740	*Pterois miles*	0.008	0.002	0.005	***						
5.	MF124020	*Pterois miles*	0.009	0.008	0.002	0.006	***					
6.	FJ584026	*Pterois miles*	0.008	0.002	0.005	0.000	0.006	***				
7.	FJ584044	*Pterois volitans*	0.046	0.043	0.043	0.042	0.042	0.042	***			
8.	JN312289	*Dendrochirus brachypterus*	0.138	0.137	0.135	0.135	0.133	0.135	0.138	***		
9.	KJ709885	*Scorpaena scrofa*	0.179	0.175	0.176	0.176	0.174	0.176	0.177	0.182	***	
10.	KC501412	*Scorpaena porcus*	0.180	0.179	0.180	0.179	0.181	0.179	0.176	0.192	0.127	***

Pterois miles, is one of the worst known marine invaders, is distributed naturally in the Indian Ocean and the Red Sea. It prefers coastal waters in muddy habitats (Froese and Pauly, 2022).

The first Mediterranean-based record of *P. miles* was given from Israeli coast in 1991 (Golani and Sonin, 1992). Azzurro et al. (2016) considered its first record in the Mediterranean as a failed introduction and declared: “Considering the conspicuous appearance of *P. miles*, and its relative ease in recognition, the lack of observations until 2012 is unlikely “a detection lag” and the 1991 record (Golani and Sonin, 1992) can be considered as evidence of a failed introduction”. Even if its first introduction was considered to fail, *P. miles* has invaded throughout the Mediterranean and its adjacent seas today. The chronology of the lionfish journey, which includes the period of all observation, showed a continuous progressive expansion of the lionfish westward and northward from the coast of the Levant (Fig. 1 and Tab. 3).

Table 3: Confirmed Mediterranean records of *Pterois miles* (Oruç et al. 2022).

No	Location	Coordinates	Length Range (TL, mm)	Habitat	Depth (m)	Observation Method	Reference
1.	Herzliya, Israel	–	328	–	35	Trawl	Golani and Sonin (1992)
2.	Al Minie, Lebanon	34.29N, 35.54E	209	Coralligenous	30	Trammel net	Bariche et al. (2013)
3.	İskenderun, Türkiye	36.17N, 35.46E	276	Rocky bottom	25	–	Turan et al. (2014)
4.	Rhodes, Greece	36.38N, 28.24E	–	Rocky bottom	7	Diving	Crocetta et al. (2015)
5.	Rhodes, Greece	35.91N, 27.85E	–	Shipwreck	–	Diving	Crocetta et al. (2015)
6.	Rhodes, Greece	36.45N, 28.21E	–	Rocky bottom	2	Diving	Crocetta et al. (2015)
7.	Ormidia, Cyprus	–	170	–	10	Gill net	Iglésias and Frotte (2015)
8.	Karpas, Cyprus	–	373	Rocky bottom	40	Gill net	Oray et al. (2015)
9.	Dalyan, Türkiye	–	–	Sandy bottom	11	Diving	Turan and Öztürk (2015)
10.	Datça, Türkiye	36.69N, 27.68E	–	–	10	Gill net	Bilge et al. (2016)
11.	NE Crete, Greece	35.20N, 26.30E	250	Rocky bottom	33	Gill net	Dailianis et al. (2016)
12.	SE Crete, Greece	35.01N, 25.96E	100	Rocky bottom	12-37	–	Dailianis et al. (2016)
13.	Karpathos Island, Greece	35.55N, 27.20E	100	Rocky bottom	17	Diving	Mytilineou et al. (2016)
14.	Karpathos Island, Greece	35.50N, 27.22E	200	Rocky bottom	16	Diving	Mytilineou et al. (2016)
15.	Mersin, Türkiye	36.08N, 33.40E	250	–	100-110	Trawl	Yağlıoğlu and Ayas (2016)
16.	Vendicari coast, Italy	36.49N, 15.06E	250	Sandy bottom	100-110	Diving	Azzurro et al. (2017)
17.	Kemer, Türkiye	–	85-293	Rocky bottom	10-15	Spearfishing	Özgür Özbek et al. (2017)
18.	Didim-Aydın, Türkiye	37.20N, 27.14E	–	Rocky bottom	18	Diving	Yapıcı (2018)
19.	Bodrum and Teos, Türkiye	–	100	Rocky bottom	10	Diving	Ulman et al. (2020)
20.	Kokar Bay, Türkiye	38.13N, 26.61E	144	Rocky bottom	15	Spearfishing	Özgül (2020)
21.	Kefalonia, Greece	38.16N, 20.40E	225	–	12-15	Gillnets-trammel nets	Vavasis et al. (2020)
22.	Vis Island, Croatia	48.00N, 16.00E	–	Rocky bottom	15	Diving	Dragičević et al. (2021)
23.	İzmir Bay, Türkiye	38.65N, 26.52E	309	Sandy bottom	36	Diving	Oruç et al. (2022)
24.	Edremit Bay, Türkiye	39.47N, 26.62E	224	Sandy bottom	70	Trawl	This study

Therefore are for both the Atlantic and the Mediterranean invasion processes of *P. miles* have clear evidence of how to be a successful globally marine invader. However, Kimball et al. (2004) claimed that the invasion of the lionfish in the Atlantic Ocean is dependent on temperature since their experimental studies have suggested the lionfish stops feeding below 16.1°C. In contrast, Özgür Özbek et al. (2017) noted that the *P. miles* continued feeding in low winter temperature (14.9°C) in the eastern Mediterranean. As well as in the present study, reported existences of *P. miles* from the northern Aegean sectors that has low winter inshore temperature are clearly evidence that widespread colonisation of the Mediterranean and Aegean region realized by *P. miles* seems not to be temperature dependent. Poursanidis et al. (2020) modeled the potential spread of *P. miles* in the Mediterranean Sea and declared that local adaptation and propagule spread are the main vectors to accelerate its future invasion. Nevertheless, they stated possibilities of a further expansion of the climatic niche of *P. miles* in the Mediterranean Sea seem to be likely. The occurrence of *P. miles* in the recent study may be evidence of its accelerating northern expansion in the Mediterranean and an indication of an attempt of a possible settlement in northern sectors of the Aegean Sea.

CONCLUSIONS

Inter-specific nucleotide divergences and molecular cladograms are powerful tools to determine the species (Hillis et al., 1997). The NJ analysis of the DNA barcode data concluded in congruent tree constructed with high bootstrap values. In the molecular identification of *P. miles* using COI barcode, this have proven to characterize the same species as such in traditional taxonomy. According to the NJ approach and genetic p-distances values, the specimen used in this study was *P. miles* and it was found as genetically distinct from *P. volitans*. Previous studies also found a clear distinction between these species in the phylogenetic analyses (Kochzius et al., 2003; Freshwater et al., 2009; Turan et al., 2020). Future studies performed by using other molecular markers might be enhanced to define adjunct species-specific determination between *P. miles* and *P. volitans* to simplify taxonomic identifications.

ACKNOWLEDGEMENTS

This study was supported by the project numbered 2017/SÜF/005, Ege University, Scientific Research Projects Coordination Unit. The authors would like to thank to captains Hasan Biran and Uğur Ali Biran from the F/V “Dülger”.

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