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The third record of black-spotted porcupinefish *Diodon hystrix* Linnaeus, 1758 in the Mediterranean Sea

Short title: *Diodon hystrix* in Cyprus and Mediterranean

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Introduction

Marine ecosystems are becoming increasingly altered worldwide as a result of human activities intensification and increasing synergistic pressures including climate change, habitat destruction, over-exploitation, and biological invasions (Crain, Kroeker, & Halpern, 2008; Halpern et al., 2015). The semi-enclosed Mediterranean Sea is at the forefront of ecosystem alterations, facing unprecedented pressures, and being characterized as a basin "under siege" (Coll et al., 2010). The spread of non-indigenous species (NIS), and ongoing shift of Mediterranean coastal species assemblages cause an increasing impact over time, resulting in changes in trophic flows and interactions between native species and NIS (Corrales, 2019). A large number of pufferfish species (Diodontidae and Tetraodontidae) have invaded or expanded their ranges in the Mediterranean Sea (Table 1). Monitoring of changes is critical towards effective adoption of management measures.

Here, we report the first confirmed record of the spot-fin porcupinefish *Diodon hystrix* Linnaeus, 1758 from the eastern Mediterranean, and the third from the entire region, after its report by a spearfisher. The spot-fin porcupinefish is a circumtropical species, widely distributed in the Atlantic, Indian and Pacific Oceans but rarely reported from the Mediterranean with only two confirmed records; the first from the Gulf of Taranto, Italy (Torchio 1963) and the second from the Balearic islands, Spain (Ordines et al. 2018) (Figure 1).

Materials and Methods

On February 4, 2017, a spot-fin porcupinefish was found drifting in the Akrotiri Peninsula (Limassol, Cyprus) (32° 56.355'E, 34° 33.592'N) at 40m depth by a spearfisher (Figure 1, A). The specimen was in relatively good physical conditions indicating a recent death (Figure 1, B). The fisher reported his finding and donated the specimen for further examination. Visual and genetic studies were conducted. DNA was extracted and the mitochondrial barcode gene CO1 (Cytochrome oxidase 1) was sequenced following published protocols (Bariche et al., 2015). Phylogenetic reconstructions were performed based on the Neighbor-Joining method generated in R (RCoreTeam, 2016) with the use of the ape package (Paradis, Claude, & Strimmer, 2004). Genetic distances were based on the Kimura 2 parameter method. The maximum likelihood (ML) method was also used as a second phylogenetic reconstruction approach, as implemented in GARLI (Zwickl, 2006). To estimate support for the nodes, 1000 bootstrap replicates were performed and we retained only the values supporting the nodes accounting for more than 50% of the bootstrap replicates.

Results

Morphological analysis

Morphological characteristics of the specimen were consistent with characters described in Leis (2016) for *Diodon hystrix*. Its general colour was tan to brown with small dark spots along the body that extended to cover most of its fins and a wide and blunt head. Its meristic characters were also consistent with that species, with 23 pectoral-fin soft rays, 16 anal-fin soft rays, 14 dorsal fin rays and no pelvic fins. Dorsal and anal fins were rounded. Morphometric measurements are presented in Table 2.

Genetic analysis

The PCR amplification and sequencing of the cytochrome oxydase 1 resulted in a 658 bp fragment (GenBank accession number MN498287). A BLAST comparison of this sequence with available sequences in GenBank placed it in a cluster with 16 sequences, all identified as *Diodon hystrix*. Six of those sequences were identical to the one obtained for our sample. These six sequences belonged to samples collected worldwide, from the Caribbean, and both Pacific and Atlantic Oceans.

Phylogenetic analyses were performed by comparing our sequence to *Diodon hystrix* sequences extracted from GenBank, using three sequences from the sister species *D. liturosus*, and one sequence from *D. nichteremus* as outgroups following Santini et al. (2013). Maximum likelihood and Neighbor-Joining methods resulted in identical tree topologies, therefore only the NJ tree is shown here (Figure 2). As indicated above from the BLAST results, our sequence clustered with *Diodon hystrix* samples, and was very well separated from outgroup sequences (bootstrap support was 100% and 88% with NJ and ML methods, respectively).

Discussion

The Eastern Mediterranean is the most invaded marine area of the world (Edelist et al., 2013) but the number of recorded NIS for Cyprus is substantially lower than for neighbouring countries, mainly due to the lack of targeted field studies (Crocetta et al., 2015). In the past years, however, citizen-scientists have substantially contributed to the detection and monitoring of a relatively large number of NIS in Cyprus waters (Giovos et al., 2019; Kleitou et al., 2019; Kousteni et al., 2019); and they continue to prove essential for monitoring the drastic changes that the Mediterranean Sea is facing.
Visual and genetic results unambiguously identify the specimen reported here as a spot-fin porcupinefish, *Diodon hystrix.* All three Mediterranean records for this species were found far from each other and do not suggest a range expansion from the eastern Atlantic. Based on our genetic results, it is difficult to determine if the Cyprus sample is an aquarium release or a Lessepsian immigrant, because this species is found worldwide and shows little genetic differentiation at the COI marker level. It is plausible that a careless aquarium hobbyist released the fish after it outgrew the aquarium, as it is a relativaley common practice (Semmens et al., 2004). Further work, sampling and observations are therefore necessary to conclusively elucidate the introduction pathway.

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Table 1. Pufferfish species (Diodontidae and Tetraodontidae) reported in the Mediterranean to date.

Family	Scientific name	Origin	Establishment success
Diodontidae	*Diodon hystrix* Linnaeus, 1758	Alien or Range expanding	Casual
Diodontidae	*Cyclichthys spilostylus* (Leis & Randall, 1982)	Alien	Casual
Diodontidae	*Chilomycterus spinosus mauretanicus* (Le Danois 1954)	Range expanding	Single record
Tetraodontidae	*Lagocephalus guentheri* Miranda Ribeiro, 1915	Alien	Established
Tetraodontidae	*Lagocephalus sceleratus* (Gmelin, 1789)	Alien	Established
Tetraodontidae	*Lagocephalus suezensis* Clark & Gohar, 1953	Alien	Established
Tetraodontidae	*Torquigener flavimaculosus* Hardy & Randall, 1983	Alien	Established
Tetraodontidae	*Tylerius spinosissimus* (Regan, 1908)	Alien	Established
Tetraodontidae	*Lagocephalus lagocephalus* (Linnaeus, 1758)	Native	Established
Tetraodontidae	*Ephippion guttifer* (Bennett, 1831)	Range expanding	Casual
Tetraodontidae	*Sphoeroides marmoratus* (Lowe, 1838)	Range expanding	Casual
Tetraodontidae	*Sphoeroides pachygaster* (Müller & Troschel, 1848)	Range expanding	Established
Tetraodontidae	*Sphoeroides spengleri* (Bloch, 1785)	Range expanding	Single record

Table 1. Morphometric measurements collected from the caught Diodon hystrix specimen

Morphometric measurements	**Absolute value (mm)**	**% TL**
Standard Length	60.77	-
Head Length	8.61	0.14
Head Width	16.87	1.96
Head Depth	8.98	0.53
Eye diameter	1.27	0.14
Body depth	12.56	9.89
Postorbital length	8.18	0.65
Pectoral fin height	5.02	0.61
Pre-pectoral length	6.35	1.26
Pectoral fin base length	3.5	0.55
Dorsal fin base length	4.91	1.40
Dorsal fin height	8.23	1.68
Pre-anal length	24.96	3.03
Anal fin base length	2.89	0.12

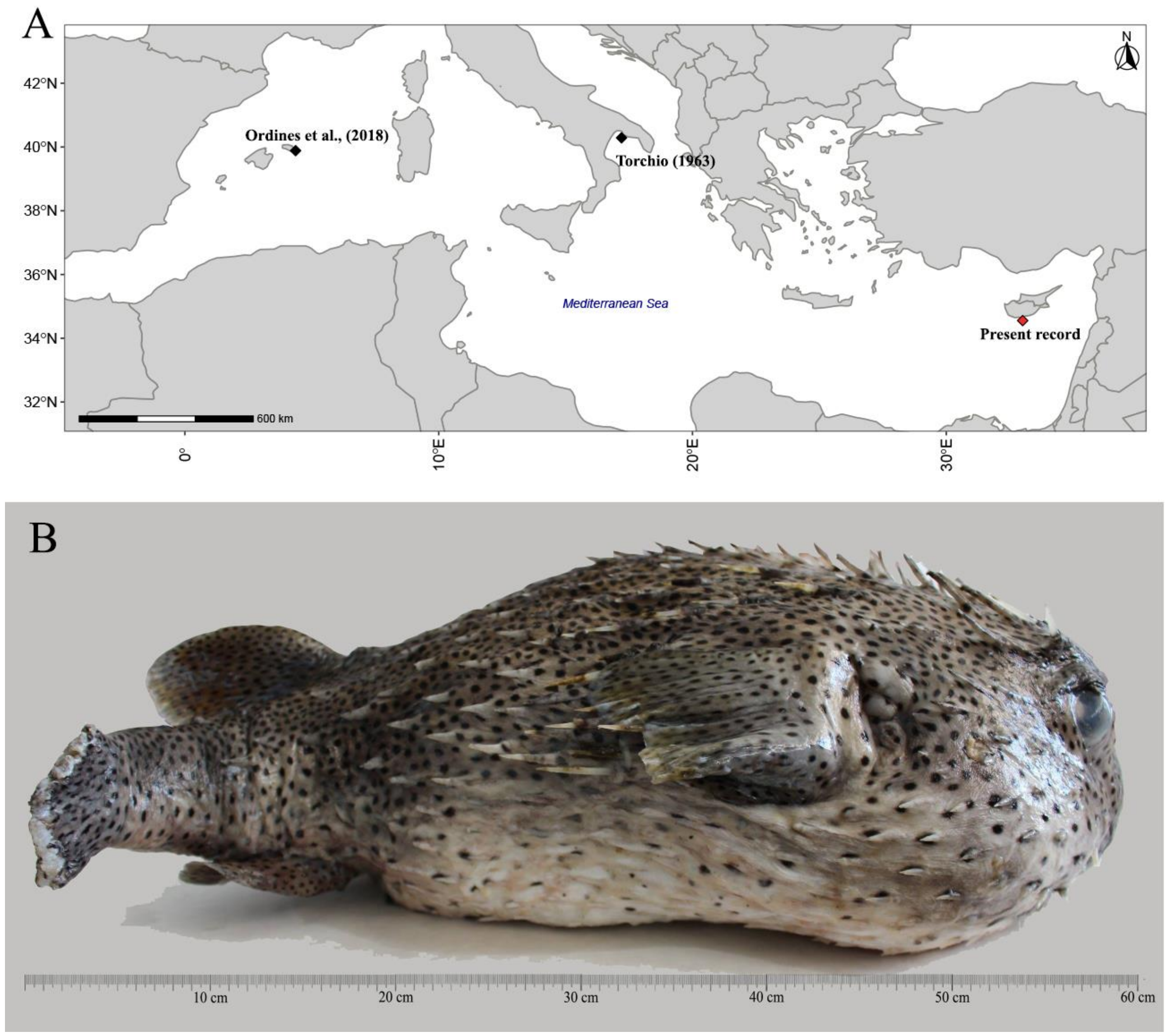

Figure 1. (A) Map with the confirmed records of *Diodon hystrix* in the Mediterranean, (B) Caught specimen reported in this study.

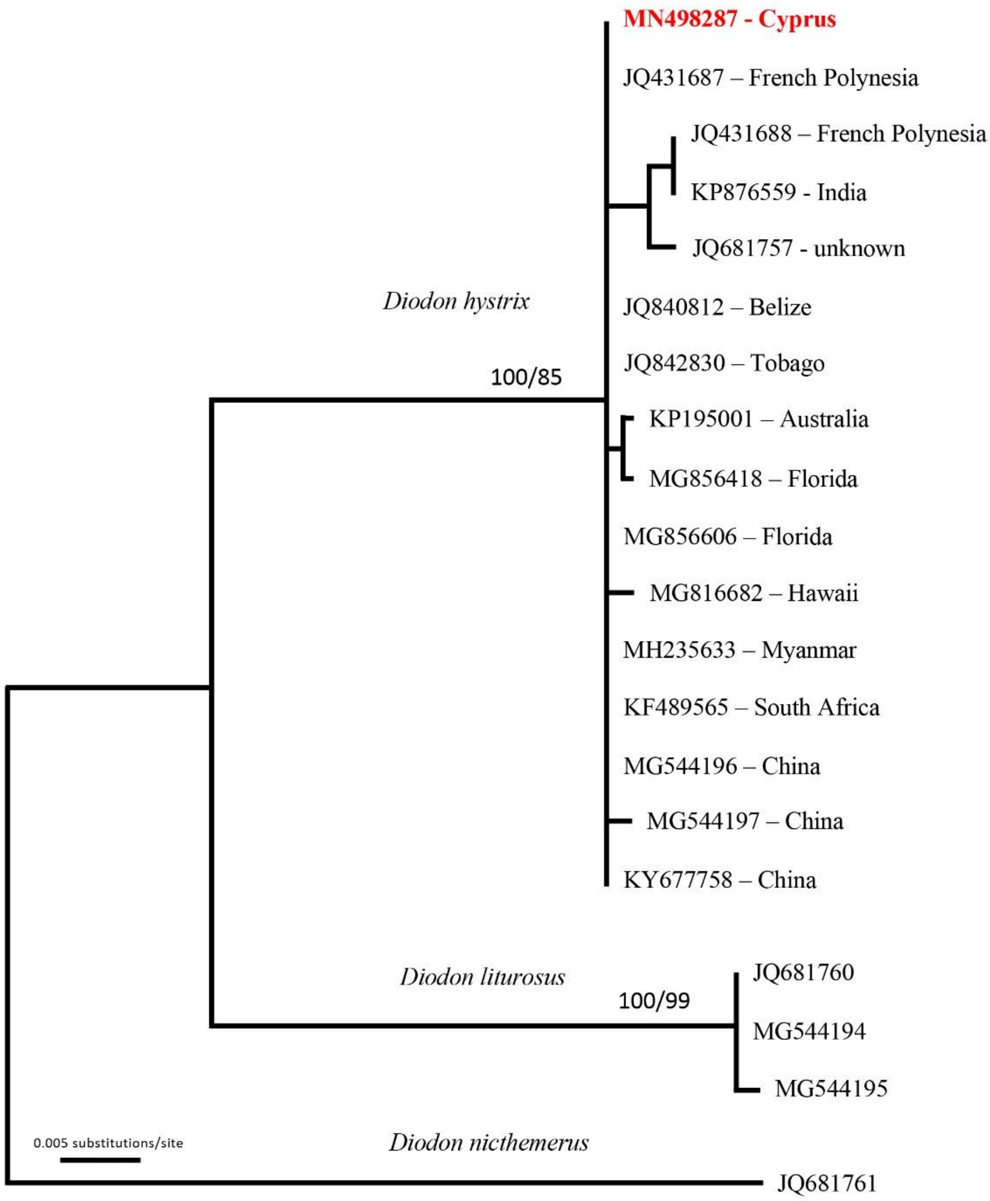

Figure 2. Phylogenetic reconstruction of *Variola* groupers based on the cytochrome oxidase marker. Tree topology is based on the Neighbour-Joining, NJ, method (identical to Maximum Likelihood, ML, topology), numbers on nodes are bootstrap values derived from 1000 replicates (only numbers above 50% are shown). Firs number is for NJ, second number for ML. Mediterranean sample is from Cyprus and is in red. All other sequences are from GenBank and are in black. Their sample origin is indicated after their accession number.