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Additional Record of the Indo-Pacific Burrowing Goby *Trypauchen vagina* (Bloch & Schneider, 1801) in the South of the Mediterranean Sea off Port Said Coast, Egypt

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ABSTRACT

The Mediterranean Sea is considered a novel habitat that comprises extensive diversity of non-indigenous marine species. Here, we present an additional –but comprehensive– description of the newly recorded burrowing goby *Trypauchen vagina* on the southern coast of the Mediterranean Sea. In total, 53 individuals had been recorded as a bycatch collected using the beach seine netting method in 2021 on Port Said coast. Morphometric and meristic characters were studied in 12 samples, and species identity was further confirmed by COI barcoding technique that revealed high similarity with *T. vagina* inhabiting the Indo-pacific region. Given that the current record represents the largest recorded bycatch size of *T. vagina* in the Mediterranean Sea, therefore, our study broadens the knowledge on the occurrence and abundance of that species off the southern coast. In this context, we highlighted the potential challenges encountered by the Mediterranean Sea environment facing the growing diversity of non-indigenous marine organisms.

INTRODUCTION

One of the world's biodiverse regions is thought to be the Mediterranean Basin. Its unique climate circumstances and geological history have resulted in physiographical complexity, which has led to a remarkable diversity of marine creatures. Although it makes up less than 1% of the world's water surface, the Mediterranean Sea is home to 7% of all macroscopic marine species, between 25% and 30% of which are endemic. Furthermore, the majority of the previously cited biodiversity statistics only take into consideration the most well-known and well-studied groups of creatures though they only represent a small portion of the overall number of species found in the Mediterranean. Notably, an estimate of 17,000 species has been recorded in the maritime environment alone (**Coll et al., 2010**).

With over than 2000 species, family Gobiidae (generally known as gobies) is one of the largest families of fishes that can be found worldwide in marine, brackish and freshwater habitats (**Van Tassell, 2001**). *Trypauchen vagina* is an amphidromous fish,





commonly found in tropical and Indo-Pacific region and can live on soft bottoms with a wide range of salinity levels (**Çiftçi & Ayas, 2018; Riede, 2004**).

According to many literatures, *T. vagina* was recorded in many regions around the world. In the Pacific, it was recorded in many regions off the coasts of Indonesia (Bleeker, 1860; Kottelat & Whitten, 1996), China (Herre, 1927), the Philippines (Herre, 1953), Caledonia (Kulbicki *et al.*, 1993), Vietnam (Dinh, 2018; Dinh, 2017; Rainboth, 1996) and Taiwan (Chen & Fang, 1999). In the Indian Ocean, *T. vagina* was recorded in India (Hora, 1924) and South Africa (Kottelat & Whitten, 1996), and additionally it was identified at high latitudes in the Arabian Gulf (Al-Daraji *et al.*, 2017; Yeganeh *et al.*, 2015). Similarly, the individuals of this species are known between the Pacific and Indian waters of Thailand (Fowler, 1935; Smith, 1945) and Singapore (Larson & Lim, 2005). Recently, *T. vagina* was reported in the Atlantic Ocean in Brazil, a habitat outside its natural Indo-Pacific region (Trevisan *et al.*, 2022).

T. vagina has also been recorded in the Mediterranean Sea. However, the occurrence of this species was largely limited to the northeast and eastern parts of the Mediterranean basin (Akamca *et al.*, 2011; Salameh *et al.*, 2010; Yaglioglu *et al.*, 2013). On the southern coast, Nour *et al.* (2021) recorded this species for the first time and introduced a brief description on only four specimens collected from the Egyptian coast off Alexandria. In the current study, an additional record of *T. vagina* was presented, supported with a morphometric, meristic and genetic description for individuals of this species collected from Port Said coast at the southern coast of the Mediterranean Sea, Egypt.

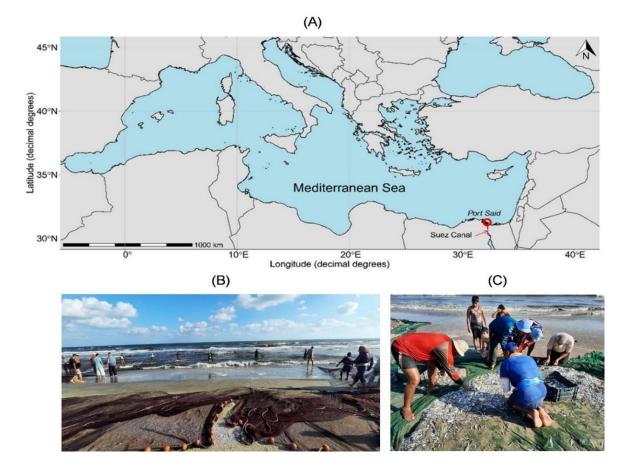
MATERIALS AND METHODS

1. Study site

In the present study, individuals of *T. vagina* were recorded in a catch collected from Port Said coast; a site located on the Egyptian coast of the Mediterranean Sea at the entrance of the Suez Canal (Fig. 1). A weekly fish examination was performed from December 2018 to December 2021. During this period, the presence of *T. vagina* was only recorded in the 13^{th} of March 2021. The catch was caught using a beach seine netting method from a depth ranging from 1- 10m. It is worthy to mention that, the beach seine fishing method is one of the most common fishing methods on Port Said coast and usually used to catch some economic fish species all over the year. For a purpose outside the scope of the current study, all the catch was examined to determine catch composition. The *T. vagina* individuals (n=53) were counted in the catch and subsamples were selected for morphological/meristic (n= 12) and molecular analyses (n= 2).

2. Morphometric and meristic measurements

A total of 12 samples were examined; 23 morphometric as well as 7 meristic characters were recorded for each fish sample. All the measured characters were determined following the description of **Yeganeh** *et al.* (2015), **Ciftci and Ayas** (2018)



and **Ergüden** *et al.* (2018). Lengths illustrated in Fig. (2) are measured to the nearest 0.01cm using a digital caliper.

Fig. 1. Sampling site of *T. vagina* from the Mediterranean Sea, Egypt. (A) Location of Port Said coast; (B) Beach sein fishing method, and (C) Fishermen sort the catch on the coast of Port Said.

3. COI barcoding

Total DNA was extracted from the muscles of the freezed samples (n= 2) using DNeasy Blood and Tissue Extraction Kit (Qiagen) following the manufacturing instructions. Both integrity and quantity of DNA were assessed using 1.5% agarose gel electrophoresis and NanoDrop (ND-1000), respectively. Afterwards, COI from each sample was amplified via polymerase chain reaction (PCR), using FishF1 [5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3'] and FishR1 [5'TAG ACT TCT GGG TGG CCA AAG AAT CA-3'] primer set (**Ward** *et al.*, **2005**). Each PCR reaction was consisted of 12.5µL of 2x PCR Master Mix (GeneDireX), 1µL of each primer (20 pmol), 2µL DNA template (20 ng/µL) and was completed to a final volume of 25µL with 8.5µL deionized water. PCR samples were subjected to the following thermal conditions: initial denaturation at 94°C for 4min, followed by 30 cycles of 94°C for 45sec, 53°C for 45sec, 72°C for 90 sec and a final extension at 72°C for 7min. COI products were then purified

using QIAquick PCR Purification Kit (QIAGEN) and sequenced using 3500 Genetic Analyzer (Applied Biosystems) at the Biotechnology Research Center in the Suez Canal University.

4. Phylogenetic analysis

Sequences of COI were used to investigate the phylogenetic relationship between *T. vagina* recorded in the present study and that found in the Mediterranean and Indo-Pacific region. To achieve this step, raw sequences obtained by the current study were checked and edited, when required, and finally uploaded to the NCBI GenBank (accession numbers MZ412464.1 and MZ412465.1). Sequences obtained by the current study and those downloaded from BOLD system were then aligned using Clustal W. Phylogenetic tree was constructed based on the maximum likelihood method (ML) of 1000 bootstrap value under the HKY+G substitution model (**Hasegawa** *et al.*, **1985**). *Tridentiger bifasciatus* COI sequences were used to root the tree. The phylogenetic analysis and illustration were principally performed using phangorn, msa, and ggtree R packages (**Team, 2020**).

RESULTS AND DISCUSSION

In the present study, a total of 53 individuals of *T. vagina* have been identified from the examined catch. To the best of our knowledge, this number of individuals represents the largest bycatch size recorded for *T. vagina* (total weight= 480g); suggesting the expansion of this species in the southeast part of the Mediterranean Sea. *T. vagina* has been recently identified from some regions in the Mediterranean Sea (Akamca *et al.*, 2011; Nour *et al.*, 2021; Salameh *et al.*, 2010; Yaglioglu *et al.*, 2013). However, the available data indicate a very limited abundance and restricted occurrence of this fish species in the southern coast (Nour *et al.*, 2021).

In addition, 23 morphological as well as seven meristic characters were measured for *T. vagina* individuals (n= 12) and summarized as in table 1. All individuals generally have an elongate and reddish-pink body with all fins present and well-defined. Our morphometric analysis showed that the total length (TL) ranged from 9.37cm to 18.91cm where the mean±SD length was 14.51 ± 2.46 cm. On the other hand, the standard length (SL) of the body was between 7.98cm and 16.55cm (SL= 12.63 ± 2.25 cm), a length that typically 87.08% of the TL. The dorsal fin (DFL= 9.93 ± 1.84 cm) composed of two parts connected by a membrane and contained 52 -59 fin rays with no fin spines. The anal fin was also long, averaged 54.65% of the TL (AFL= 7.93 ± 1.60 cm), and is supported by no less than 45 fin rays. Both dorsal and anal fins are directly connected to a caudal fin that extends to 2.02 ± 0.26 cm and supported by 21-24 caudal vertebrae (Fig. 2). The pectoral and pelvic fins, on the other hand, are tiny and have average lengths of 0.75 ± 0.17 cm and 0.83 ± 0.21 cm, respectively. The pelvic fins, however, are notably joined to form a funnel that supported by many fin rays. The head is small and represents 13.15% of the TL.

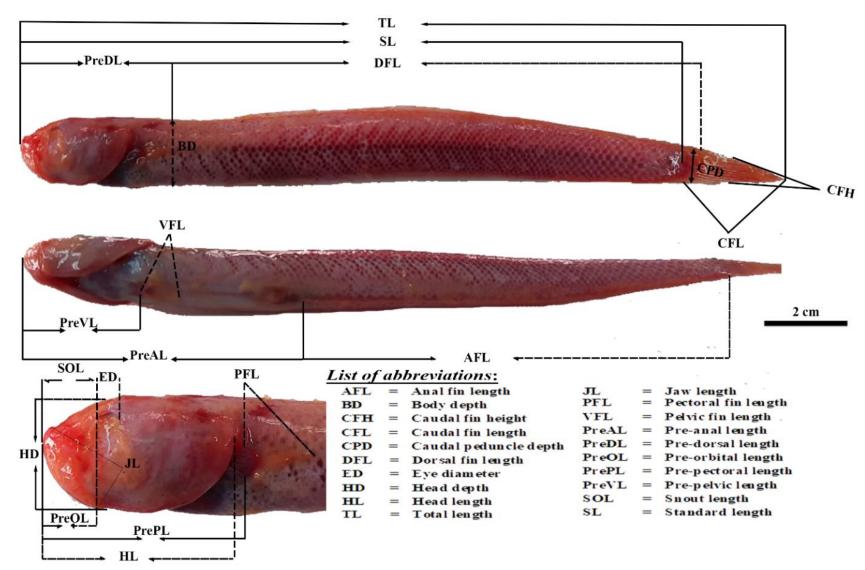


Fig. 2. Standard morphometric length-measurements of the body of *T. vagina* caught from the Mediterranean Sea, Egypt.

In the mouth, teeth are canine-like and visible on both jaws. Eyes are covered by a membrane-like skin that makes the fish looks blind. Additional examination also revealed that the gills are covered by opercula and have oval pouch-like cavities at the dorsal margin. Except the head, body is covered by 69-87 cycloid scales along the lateral line. All body measurements, however, were comparable to that recorded in the Mediterranean Sea and that identified from the Indo-Pacific (Akamca *et al.*, 2011; Çiftçi & Ayas, 2018; Nour *et al.*, 2021; Yeganeh *et al.*, 2015).

Table 1. Morphometric and meristic characters of *T. vagina* collected from the Egyptian coast of the Mediterranean Sea.

Character	Unit	Minimum	Maximum	Mean	SD
Anal fin length		4.67	10.51	7.93	1.60
Body depth		1.03	1.95	1.41	0.24
Caudal fin height		0.39	0.85	0.67	0.13
Caudal fin length		1.5	2.48	2.02	0.26
Caudal peduncle depth		0.48	0.93	0.71	0.12
Dorsal fin length		5.84	13.08	9.93	1.84
Eye diameter		0.14	0.26	0.18	0.03
Head depth		0.91	1.98	1.42	0.31
Head length		1.39	2.44	1.91	0.29
Head width		0.62	1.1	0.87	0.15
Interorbital width		0.19	0.39	0.31	0.06
Jaw length	Centimeter	0.51	0.86	0.70	0.11
Nape width		0.56	1.12	0.83	0.14
Pectoral fin length		0.42	1.08	0.75	0.17
Pelvic fin length		0.5	1.2	0.83	0.21
Pre-anal length		2.89	5.75	4.39	0.73
Pre-dorsal length		1.88	3.14	2.46	0.38
Pre-orbital length		0.33	0.65	0.47	0.11
Pre-pelvic length		1.17	2.25	1.86	0.32
Pre-pectoral length		1.51	2.69	2.11	0.32
Snout length		0.27	0.54	0.43	0.09
Standard length		7.98	16.55	12.63	2.25
Total length		9.37	18.91	14.51	2.46
Dorsal fin rays		52	59	0.73	2.53
Anal fin rays		45	50	0.45	1.56
Pectoral fin rays		16	18	0.18	0.62
Caudal vertebrae	Count	21	24	0.29	1.00
Precaudal vertebrae		9	10	0.08	0.29
Gill rakers		6	13	0.71	2.45
Lateral line scales		69	87	1.94	6.71
Total body weight	Gram	3.46	23.73	11.05	5.34

Phylogenetic analysis showed that the examined samples (n= 2) are highly related to *T. vagina* inhabiting the Indo-pacific region. Also, based on COI sequences, there were two main haplotypes, and the two samples collected from the Egyptian coast were belonging to one of those haplotypes (Fig. 3).

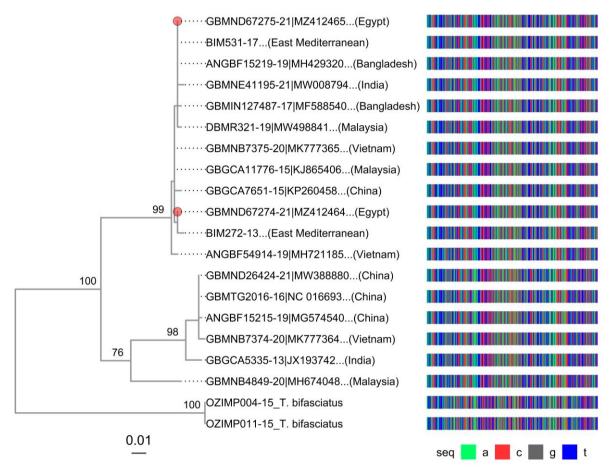


Fig. 3. Phylogenetic relationship between *T. vagina* recorded in the present study (denoted by Red circles) and those retrieved from BOLD system. Phylogenetic tree (left) was constructed based on ML method of COI sequence alignment (right). Only bootstrap values above 75% are shown.

One scenario that may explain the presence of this non-indigenous fish species in the Mediterranean Sea is the Lessepsian migration (Akamca *et al.*, 2011), proposed that this species may have migrated from the Red Sea to the Mediterranean throughout the Suez Canal. However, there is no evidence for the presence of this fish species in the Red Sea so far despite the intensive studies carried there. Also, until now, there is no record for this species in the Suez Canal is a primitive assumption, particularly with the lack of the relevant data. Alternatively, adult *T. vagina*, or its larvae, may be transported from the Indo-Pacific to the Mediterranean Sea through the ballast water of ships (Çiftçi & Ayas, 2018; Golani, 2004; Goren *et al.*, 2009; Trevisan *et al.*, 2022).

CONCLUSION

Despite further molecular studies are required, our results provide basic information on the genetic identity of the burrowing goby *T. vagina* inhabiting the Egyptian coast of the Mediterranean Sea. Also, in the light of the current results, the need to address the potential factors responsible for expanding diversity of non-indigenous ichthyofaunal community in the Mediterranean region may have high priority particularly with the recent-accelerated global environmental changes. Finally, we add an additional record of *T. vagina* and highlight the potential expansion and adaptation of this species in the Mediterranean Sea.

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