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Hazeus ingressus sp. nov. a new goby species (Perciformes: Gobiidae) and a new invasion in the Mediterranean Sea

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Abstract

A new species of gobiid, *Hazeus ingressus* sp. nov. (Teleostei: Gobiidae) is described from the Levantine coast of Turkey. The species probably originates from the Red Sea and represents the 11th alien gobiid species in the Mediterranean Sea. The new species is distinguished from its Indo-Pacific congeners by a combination of the following characters: no dark blotch on the first dorsal fin; caudal fin coloration; scales in lateral series 25-28 (modally 27); second dorsal fin rays I,8; anal fin rays I,8-9; predorsal scales ctenoid 7; short, stout gill rakers 2+8. This finding suggests that the Lessepsian invasion continues with the inclusion of known species as well as undescribed species.

Keywords: Gobiidae; *Hazeus*; gobiid fish; alien species; Lessepsian; Levantine Sea.
zoobank code: urn:lsid:zoobank.org:pub:07D66563-D207-4CA6-A640-1B5136C4F851

Introduction

Gobiids are one of the most diverse groups of fishes, consisting of more than 2000 described species (Eschmeyer *et al.*, 2017). The family Gobiidae forms 10% of the total fish biodiversity of the Red Sea (Golani & Bogorodsky, 2010) and is also the largest fish family in the north-eastern Atlantic, Mediterranean Sea and the Black Sea with 104 species (Engin *et al.*, 2016; Akel, 2017; Akel & Samir, 2017; Engin & Innal, 2017; Engin & Seyhan, 2017; Kovačić *et al.*, 2017; Kovačić *et al.*, 2018). However, the small body size and morphological similarities of many of these species frequently leads to misidentification, even today.

Gobies are an important component of the reef fauna of the Red Sea based on abundance, species richness and degree of habitat specialization (Bogorodsky *et al.*, 2010). Their species richness, usually benthic lifestyles and their function in the food web linking small invertebrates to fishes at the coastal zones also makes gobies important within the Mediterranean (Kovačić, 2001; Ahnelt & Dorda, 2004; Engin & Seyhan, 2009, 2010; Engin *et al.*, 2016).

The Red Sea is a hotspot with a high level of endemism (12.9%) of marine organisms, with the endemism level along the Red Sea coasts being higher than all other hotspots recorded in the Indian Ocean, the Arabian Gulf and Southern Oman (DiBattista *et al.*, 2016a, b). However, knowledge of the gobiid fauna in the Red Sea is still in progress and there is a lack of information about the distribution and habitat preference of many species. Through the last decade, few studies have been published on gobiid species in the Red Sea (Kovačić *et al.*, 2014a, b, 2016; Kovačić & Bogorodsky, 2014; Greenfield *et al.,* 2014; Gill *et al.,* 2014; Hoese *et al.*, 2015; Bogorodsky *et al.,* 2016; Delventhal & Mooi, 2013; Delventhal *et al.,* 2016). More importantly, not only gobiids but also there are some Lessepsian migrant fishes described as new species in the Mediterranean Sea. (e.g. DiBattista *et al.,* 2012; Russell *et al.*, 2015, Fricke *et al*., 2017).

The genus *Hazeus* Jordan and Snyder, 1901 has a wide distribution range in the Indo-Pacific region, from the western Pacific (Japan, Taiwan, Philippines and Indonesia) to the northern coast of the Red Sea. However, knowledge of the genus is limited, as specimens in collections are few and its relatives (e.g. *Oplopomus, Oplopomops*) are often confused due to their rigid and pungent first spine of dorsal fins and none have been reviewed. Due to this study, the distribution of *Hazeus* would be extended to the Mediterranean Sea.

The genus was first described as monotypic, with the type species *Hazeus otakii* Jordan & Snyder, 1901. Later, *Oplopomops elati* Goren, 1984 and *Opua maculipinna* Randall & Goren, 1993, were moved into *Hazeus* without comprehensive redescription or revision (Anderson *et al.*, 1998; Eschmeyer *et al*., 2017). The samples recently collected from the eastern Mediterranean Sea differ from all the native Gobiidae species according to their morphology, sensory papillae and scale patterns. *Hazeus ingressus* sp. nov. is distinct from its congeners based on morphological characters. The present study describes a new species of *Hazeus,* revealing a newly established population of an alien fish in the Mediterranean Sea.

Material and Methods

Sample Collection

Specimens were collected with hand nets at 5-20 m depth by SCUBA diving in Inlice during November 2014 (36°43’44” N/ 28°58’10” E) and in Aliosman Bay in June 2015 (36°44’05” N/ 28°55’37” E), in the province of Fethiye, Turkey. Additionally, many individuals were observed in Kilye Bay in July 2016 (36°41’59” N/ 28°52’45” E), during underwater surveys. A total of 11 specimens were collected although hundreds were observed in the areas mentioned (Fig. 1). Before the specimens were fixed in 4% formalin solution for morphological study, pectoral fin clips were fixed in 96% ethanol for genetic analysis. The collected specimens were deposited in the Fish Collection Centre of Izmir Katip Celebi University (IKC.PIS.1170-1175) and the Queensland Museum, Brisbane (QM I.40709).

Morphological Analysis

Specimen measurements were taken under a stereomicroscope using a digital caliper point to point (accuracy of 0.1 mm) and reversibly stained with 1% toluidine blue before the morphological analysis. Morphometric methods follow Goren, 1984; Randall & Goren (1993); Miller (1986) and Larson & Wright (2003) except for osteological information.

Meristic abbreviations: A, anal fin; C, caudal fin; D1, first dorsal fin; D2, second dorsal fin; P, pectoral fin; V, pelvic fins; LL, scales in lateral series; TRB, transverse scale counts, taken by counting the number of scale rows from the anal fin origin diagonally upward and back toward the second dorsal fin base. LL is counted from the pectoral axilla along the lateral midline, including the scales at the hypural crease; in D2 and A counts the last bifid ray is counted as one. Body depth (BDA) was measured at the anal fin origin; caudal peduncle length (CPL) measured the end of A base to the hypural crease; head length (HL) was measured from snout to midline opposite the upper origin of the opercle; snout length measured from tip of upper jaw to anterior margin of eye. Head width was measured between the upper origin of opercle, head depth measured from the upper edge of eyes. The terminology of the LL system follows Miller (1986) based on Sanzo (1911). An asterisk in the description indicates the counts of the holotype of *Hazeus ingressus* sp. nov.

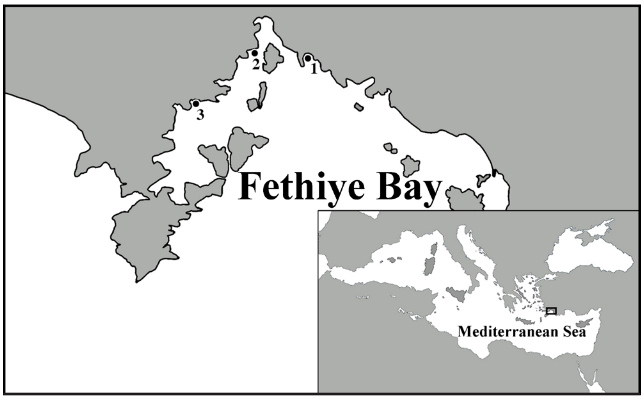

Fig. 1: Sampling location of *Hazeus ingressus* sp. nov.

Genetic analysis

Total genomic DNA was isolated from the fin clips using the PureLink Genomic DNA mini kit (Invitrogen). Approximately 655 bp were amplified from the 5′ region of the COI gene using primers described in Ward *et al.* (2005) by polymerase chain reaction (PCR) using the 5x FIREPol® Master Mix (Solis Biodyne; https://www.sbd.ee/) according to the manufacturer’s instructions.

The thermal regime consisted of an initial step of 2 min at 95°C followed by 35 cycles of 0.5 min at 94°C, 0.5 min at 54°C, and 1 min at 72°C, followed in turn by 10 min at 72°C and then held at 4.0 °C. PCR products were checked by 2% agarose gel.

Nucleotide sequences were aligned using ClustalW (Thompson *et al*., 1997) implemented in MEGA 6.0 (Tamura *et al*., 2013) and edited with BioEdit (Hall, 1999). Consensus sequences of *Hazeus* species were compared with other sequences producing similar alignments in databases using BLASTN 2.6.1 (Zhang *et al*., 2000). Aligned sequences were also compared with existing and public data on Atlanto-Mediterranean and several morphologically similar Pacific gobies (*Oplopomus oplopomus* was the only “pungent-spined goby” with publicly available genetic data) then submitted to GenBank with the accession numbers KY867537 - KY867546.

The methodology of Tamura & Nei (1993) performed better than the other nucleotide substitution models but Kimura two Parameter (K2p) distance model (Kimura, 1980), used for standard DNA barcoding analysis, was also used in the genetic analysis. Due to this, nucleotide composition, nucleotide pair frequencies, sequence divergence values within and between species and Neighbour-Joining (Saitou & Nei, 1987) topology were calculated using Kimura two Parameter (K2p) distance model with “pair-wise deletion” option for the treatment of gaps and missing data, to retrain all sites initially, excluding them as necessary using MEGA 6.0 software. The character-based ML analysis also tested. The bootstrap tests (Felsenstein, 1985) of 1000 replicates were performed to verify the robustness of the tree.

Results

Generic identification. The generic identification has been represented based on the original descriptions of three valid *Hazeus* species (Jordan & Snyder, 1901; Goren, 1984; Randall & Goren, 1993) and Larson & Murdy (2001).

The genus *Hazeus* is characterized by having mostly ctenoid scales on the entire body and the first spines of the first and the second dorsal fin are stiff and pungent, unlike the remaining flexible spines and rays; there is no spine on the preopercular margin, and the sensory papillae rows on head are in *a* reduced and disorganised transverse pattern. In addition, squamation on the opercle is a conspicuous character for discrimination of closely related *Opua* and *Oplopomops* species.

Hazeus ingressus sp. nov.

Type material

Holotype (Fig. 2): female, 32.2+ 5.1 mm (IKC. PIS.1170), Turkey, Aliosman Bay/ Fethiye 36°44'05" N/ 28°55'37" E, 15 m depth June 2015, collector, Semih Engin.

Paratypes: 5 males and 5 females. Female 26.0 + 6.5 mm, male 23.0 + 6.1 mm, male 25.0 + 6.1 mm, male 21.5 + 6.0 mm (QM I.40709); female 26.1 + 5.0 mm (IKC. PIS.1171), female 25.1 + 5.1 mm (IKC.PIS.1172), male 25 + 4.01 mm (IKC.PIS.1173), female 25.9 + 4.4 mm (IKC.PIS.1174), female 25.1 + 4.6 mm (IKC.PIS.1175), male 25.7 + 4.5 mm (IKC.PIS.1176). All samples were collected from Inlice Bay/Fethiye 36°09'31" N/ 29°37'53", 37 m depth August. Collector, Semih Engin.

Diagnosis. (1) no black spot/blotch at rear of first dorsal fin; (2) second dorsal fin rays I,8; anal fin rays I,8-9; pectoral fin rays 17-18; (3) scales in lateral series 25-28; (4) predorsal scales ctenoid 6-7; (5) short, stout gill rakers 2+8; (6) suborbital rows of sensory papillae with rows *a* and *c* in reduced and disorganised transverse pattern.

Description

General morphology. Counts and proportions are given in Table 1. Body more cylindrical anteriorly, compressed posteriorly; head moderately rounded; wider than deep. First fin element in each dorsal fin a stiff and pungent spine. Eyes dorsolateral, top forming part of the dorsal profile with narrow interorbital space. Anterior nostril short and tubular, close to upper lip; posterior nostril pore-like and close to mid-level of orbit. Mouth moderately oblique; angle of jaws ending below mid eye. Gill opening reaching to under mid-opercle or nearly to preopercular rear margin. Gill rakers 2+8; short and stout. Tongue rounded. Teeth in two rows in both jaws; very small, sharp and evenly sized. Sexual dimorphism not observed.

Fig. 2: *Hazeus ingressus* sp. nov. (A) live coloration of holotype (IKC.PIS.1170), female, 32.2+ 5.1 mm (B) preserved holotype, (IKC.PIS.1170), female, 32.2+ 5.1 mm (Photographs by Semih Engin).

Table 1. Standard length (SL) and proportional measurements (%) with meristic characters of *Hazeus ingressus* sp. nov. Values, except holotype, are range and in parentheses with mean ± S.D. and mode for meristic characters.

		Hazeus *ingressus* **sp. nov.**	
n	**Holotype**	10	
SL (mm)	32.2	21.5-26.1 (24.8 ± 1.5)	
HL (mm)	9.2	6.7-8.2 (7.4 ± 0.5)	
% of SL			
Head length	28.6	28.0-31.5 (29.7 ± 1.2)	
Body depth	17.3	15.87- 20.00 (17.2 ±1.3)	
Caudal peduncle length	21.6	20.2-24.2 (22.3 ± 1.2)	
Caudal peduncle depth	9.6	8.3-11.5 (10.3 ± 1)	
Pectoral fin length	20.1	22.8-27.9 (25.2 ± 1.6)	
Pelvic fin length	21.4	20.6-27.9 (24.6 ± 2.0)	
Caudal fin length	19.5	20.1-27.9 (23.4 ± 2.6)	
Third dorsal fin spine	12.6	12.7-17.3 (12.9 ± 4.8)	
Depressed dorsal fin	18.5	16.3-20.0 (17.7 ± 1.2)	
% of HL			
Head depth	65.7	59.0-72.00 (66.5 ± 4.2)	
Head width	60.8	62.7-74.6 (68.2± 3.9)	
Eye width	26.9	31.3-35.9 (32.7 ± 1.3)	
Interorbital length	6.2	5.1-8.4 (6.6 ± 1.0)	
Snout length	18.3	16.0-26.9 (23.2 ± 4.0)	
Jaw length	37.9	34.2-44.9 (37.7 ± 3.3)	
Meristic characters	**Holotype**	**Paratypes**	**mode**
First dorsal fin spines	VI	VI	VI
Second dorsal fin rays	I+8	I+8	I+8
Anal fin rays	I+8	I+8-9	I+8
Caudal segmented fin rays	17	16-17	17
Pectoral fin rays	17	17-18	17
Scales in lateral series	27	25-28	27
Transverse scales backward	7	7-8	7
Transverse scales forward	8	8-9	8

Fins. First dorsal VI*, first spine thickened and pungent; second dorsal I,8*, spine thickened and pungent; anal I,8-9* (modally I,9), pectoral rays 17*-18 (modally 17), segmented caudal rays usually 16-17* (modally 17); branched caudal rays 12-13* (modally 13).

Fin-bases and lengths in proportion to standard body length are given in Table 1. First fin spine in both dorsal fins thickened and sharp. First dorsal fin spine lengths almost equal but third spine slightly the longest. Narrow interdorsal space without membrane between D1 and D2. Last ray of D2 ends at vertical almost equal to end of anal fin. A originates at vertical between D2 second or third ray. Caudal fin rounded. Pelvic disc anterior membrane without lateral lobes. Pelvic fin rounded and reaching to anal fin, covering urogenital papilla in adult males.

Squamation. Body with large ctenoid scales including predorsal area (seven rows of ctenoid scales), cheeks (two or three rows of scales on the cheek; upper two rows ctenoid, lower row cycloid), opercles (five rows of ctenoid scales) and breast (six rows of ctenoid scales). Embedded cycloid scales in prepectoral area. Scales in lateral series 25-28 (modally 27), scales in transverse series backward 7-8.

Lateral line system (Fig. 3). Figure 3 represents a composite of the sensory papillae pattern of specimens of *Hazeus ingressus* including the holotype. Head with anterior oculoscapular canal with pores σ, λ, κ, ω, α, β, ρ; posterior oculoscapular canal with pores $\rho_{1,}$ ρ_2 and preopercular canal with pores ε, δ, γ. Anterior oculoscapular head canal ends in front of the anterior edge of the eye with paired σ pores (Fig 3b). Fine dark melanophores inside the σ pores.

Preorbital rows of sensory papillae: row *r* (6-7) split into two sections between the σ pores. Row s^1 (2) under pore σ, and lower horizontal row s^3 above the upper lip.

Lateral series *c* in four parts: superior c^2 variable, clustered as pair between anterior nostril and posterior nostril or not visible; middle transverse c^1 (2-3) close to anterior nostril; superior c_1 transverse (3) and inferior c_2 (3-4) longitudinal above lips.

Suborbital rows of sensory papillae (Fig 3a): row *a* with transverse paired or triple proliferations but ending with single papilla. Nine transverse well organized suborbital rows of sensory papillae. *c*1, *c2*, *c3*, *c4*, c5, *c6*, *c*9 rows paired, *c*7 tripled, *c8* with single papillae.

Longitudinal row *b* (20-21) beginning below anterior edge of the pupil and extending to preoperculum.

Longitudinal row *d* (13-14) continuous. Row *d* beginning below the transverse row *c2* and ending anterior to *c7.*

Preopercular-mandibular rows of sensory papillae (Fig. 3c): Row *e* discontinuous and in two parts. superior e^1 (16-17) and e^2 (20-22). Row *i* divided into anterior i^1 (10-12), and posterior sections i^2 (14-15). Row *f* with 6-7 papillae arranged in longitudinal line.

Oculoscopular rows of sensory papillae: longitudinal row x^1 (23-24) continuous. Transverse row *z* (4-5) located between the pore ρ and β. Row *q* (4) longitudinal, between pore ρ and ρ^1; row *u* longitudinal (4-7) between ρ^1 and ρ^2 and may be continuous after ρ^2 with 2-3 papillae in some specimens. Row *y* not visible. Transverse rows *trp* and *q* not visible.

Transverse axillary rows as^1 (6), as^2 (6-7), as^3 (9) and as^4 (7) present, longitudinal rows la^1 (8-9) present as variably scattered papillae pattern between and above upper parts of rows.

Opercular rows of sensory papillae: Transverse row *ot* (16-17), longitudinal row *os* (13-14) and *oi* (9-10).

Anterior dorsal rows of sensory papillae: anterior transverse row *n* proliferated (10-11) and behind pore ω. Transverse rows *o* and *h* absent. Row *g* and row *m* also absent.

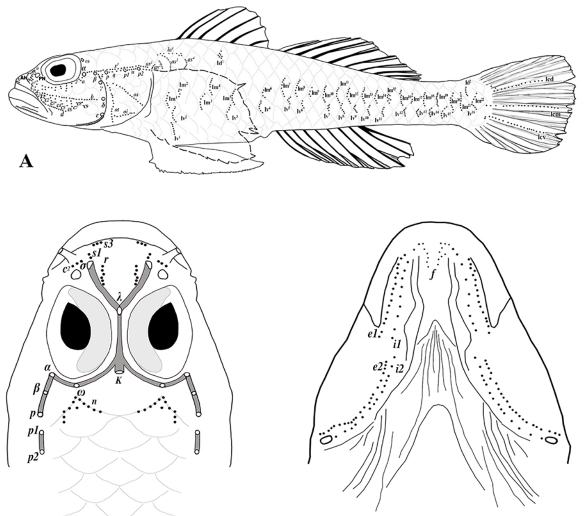

Fig. 3: Sensory papilla pattern and sensory head canals and pores in *Hazeus ingressus* sp. nov. (A) Head, trunk and fin papilla pattern, (B) Anterior oculoscapular head canals, (C) Preopercular-mandibular rows of sensory papillae.

Trunk (Fig. 3a): Three series of longitudinal and transverse series of *Id, Im, Iv*. Dorsal series of Id^1 row below the end of first dorsal fin and Id^2 row close to origin of caudal peduncle. Sixteen or seventeen ventral series of *lv*. All *Iv* rows transverse beginning at origin of pectoral fin and extending to caudal peduncle. *Iv* rows located between and below lower parts of *Im* rows. Median lateral series *Im* in 21-22 regularly spaced transverse rows posterior to origin of anal fin base; last *lm* row V shaped.

Caudal fin: Three longitudinal rows of *Icd, Idm, Icv*. Longitudinal rows on interradial membranes; anteriormost neuromasts largest, gradually decreasing in size posteriorly. Transverse row *Ict* absent.

Coloration in life. (Fig. 2a). Translucent whitish-grey head and body with many irregular rows of dark brown spots on nape and body, those on dorsum usually larger. Underside of head and body whitish; lower belly silvery white. Distinct pattern of five blackish paired blotches along lateral midline, beginning behind pectoral fin to base of caudal fin; posteriormost blotch joining black blotch on mid-base of caudal fin itself. Diffuse whitish longitudinal stripe above lateral midline. Several small-interconnected dark brown blotches above pectoral fin base and two small dark brown blotches on upper fin base. A few scattered bright blue-green spots along lateral midline. Branchiostegal membrane whitish with pearly iridescence. Preopercle dusted with small melanophores and several irregular yellow-brown blotches. Both dorsal fins transparent, tinged with very pale brown submarginal band; first dorsal fin with margin bright bluish to whitish, with 1-2 rows of small brown spots on lower part of fin spines; second dorsal fin with 2-3 rows of brown spots on fin rays. Pectoral fins transparent with yellowish-green rays. Pelvic fins transparent, speckled with whitish to yellowish white. Caudal fin transparent, crossed by 4-5 brown, convexly curved vertical bands.

Coloration of preserved specimens (Fig. 2b): Trunk white to grayish white, with irregular rows of small dark brown to yellowish blotches and spots. Five dark, relatively large dark brown paired blotches along lateral midline. Branchiostegal membrane, isthmus and chest white to whitish. Mid-base of caudal fin with dark brown blotch (posteriormost of lateral series), joined by similar blotch on mid-part of fin. Dorsal fins translucent with indistinct pale brownish margin and several irregular brownish blotches on lower part of fins; second dorsal fin with two rows of dark brown spots on rays in female. Anal fin dusky grey with dark brown to blackish margin. Pectoral and pelvic fins translucent. Caudal fin translucent with 3-4 broad convexly curved brown to blackish vertical bands.

Etymology. The specific name is derived from the Latin word which means enter, step or go into, with regard to the species' entering the Mediterranean.

Ecology. Hazeus ingressus sp. nov. has been observed in vegetated (the Lessepsian seagrass *Halophila stipulacea* (Forsskål) Ascherson, 1867) soft bottom and sloping habitats in the Fethiye region, on the south-western coasts of Turkey. It was also observed that the species more often prefers areas where the water movement is limited, such as in sheltered bays. *Hazeus ingressus* populations were observed at 5-20 m depth but were more abundant at 7-15 m depth. In this depth range on soft bottom, approximately 1-3 individuals per m^2, were observed which means that *H. ingressus* is one of the dominant fish species in the area. The dominancy value of this new species indicates that a successful invasion process is in the region. In addition, *Gobius niger* Linnaeus, 1758 and three Lessepsian migrant fishes *Vanderhorstia mertensi* Klausewitz, 1974, *Oxyurichthys petersii* (Klunzinger, 1871) and *Torquigener flavimaculosus* Hardy & Randall, 1983 were observed as dominant syntopic species in the area.

Remarks. The taxonomy of *Hazeus* and its possible relatives *Oplopomops, Oplopomus, Opua* and *Echinogobius,* all possessing sharp pungent spines in the dorsal fins, is unresolved (and see Iwata *et al.*, 1998). Most species within this group are poorly known, with few specimens in collections and they are probably often misidentified. We acknowledge that the generic assignments used here are provisional.

Since there is a scarcity of knowledge about the detailed lateral line system of *Hazeus* species, detailed comparison of free neuromast pattern and lateral line system among the species could not be done. Although *H. ingressus* is similar to its close relatives in most respects, a number of differences are notable. In this part of the study, the comparisons between the three valid *Hazeus* species and *H. ingressus* are based on the original descriptions of these species with additional studies (Jordan & Snyder, 1901; Goren, 1984; Masuda *et al*., 1984; Randall & Goren, 1993).

Hazeus elati and *H. otakii* all share with *Hazeus ingressus* variably proliferated papilla rows *a* and *c* (immediately below the eye and along mid-cheek). However, the second dorsal fin counts differ in *H. ingressus* (I,8) versus *H. maculipinna* (I,10) and *H. elati* (I,9-10), as does predorsal scale count (7 in *H. ingressus* versus 9 in *H. maculipinna* and *H. elati*).

The three previously described species all possess distinct black blotches on the first dorsal fin: *H. elati* and *H. maculipinna* have an oval black blotch at the rear of the fin (absent in female *H. elati*), and *H. otakii* has a black elongated blotch between the first and fifth spines of the first dorsal fin. *Hazeus ingressus* differs from these by having no black blotch on the dorsal fin and also the cheek and opercle are fully scaled (Fig. 5).

Additionally, *H. otakii* has large cycloid scales on the nape versus ctenoid scales in *H. ingressus*. The gill rakers are rather long and not very slender in *H. otakii* while they are short and stout in *H. ingressus*. The caudal fin has small black blotches arranged in wavy lines and a conspicuous V-shaped black line on its outmost part in *H. otakii* versus the caudal fin translucent with 3-4 broad convexly curved brown to blackish vertical bands in *H. ingressus*. The posteriormost large tooth on each side of the lower jaw is a little larger than the others and curved backward in *H. otakii*, versus the teeth being smaller in the posterior part of the lower jaw.

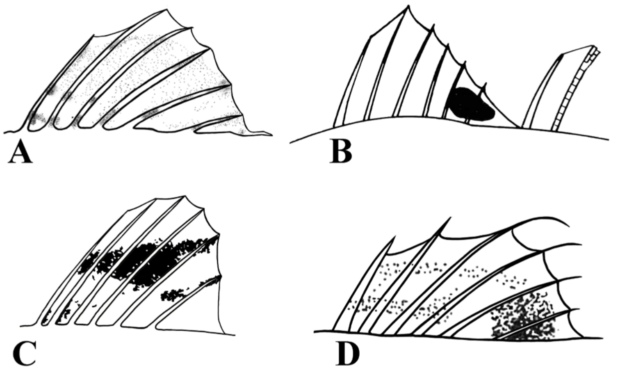

Fig. 5: First dorsal fins of *Hazues* species. a) *Hazeus ingressus* sp. nov., b) *Hazeus elati*, c) *Hazeus otakii*, d) *Hazeus maculipinna.*

Its geographically closest species is *H. elati*, described from the Gulf of Aqaba, Red Sea, from which it differs by having a lateral scale count of 25-27 (versus 23-26 in *H. elati*), in lacking any distinct black spot in the first dorsal fin in either sex (versus black blotch at the rear of the fin in males) and living in shallower habitats at 5-20 m depth (versus 27-80 m depth in *H. elati*).

Genetic Analysis. DNA barcodes obtained from ten *H. ingressus* specimens were compared with publicly available barcodes for 21 different gobiid species including Atlanto-Mediterrenean and Pacific gobies. Both standard distance model of DNA Barcoding (K2p) and Tamura & Nei distance model resulted in similar topologies. The intraspecific K2p distance of *Hazeus ingressus* specimens ranged between 0-0.09% with mean 0.04% ± 0.01% SD and it clustered with Pacific Ocean gobies such as *Oplopomus oplopomus* (Valenciennes, 1837); *Cryptocentrus leptocephalus* Bleeker, 1876; *Istigobius ornatus* (Rüppell, 1830); *Istigobius campbelli* (Jordan & Snyder, 1901); *Istigobius rigilius* (Herre, 1953) and *Istigobius decoratus* (Herre, 1927). The closest neighbours of *Hazeus ingressus* were determined as *C. leptocephalus* and *O. oplopomus* with 22.63% and 23.71% K2p distances respectively (22.70% and 22.78% Tamura & Nei distances respectively). The interspecific distances ranged between 3.0 - 37.76% (mean 24.48% ± 7.8% SD). The minimum interspecific genetic distance was observed between *I. decoratus* and *I. rigilius* while the maximum observed between *I. campbelli* and *Pomatoschistus marmoratus* (Risso, 1810). In addition, the character-based ML analysis also represented a similar topology with NJ analysis and *Hazeus ingressus* specimens clustered with Pacific Ocean gobies. Although there is a lack of publicly available data of the closest relatives of *Hazeus,* all analyzed sequences showed a well-supported monophyletic clade and basically shows that the pungent-spined gobies are

Indo-Pacific in origin and quite unrelated to the European taxa (Fig. 4).

Discussion

The genus *Hazeus* has an Indo-Pacific distribution but so far there has been no record of any *Hazeus* species in the Mediterranean Sea. The discovery of *Hazeus ingressus* from the northern Levantine coast and the presence of *Hazeus elati* in the northern Red Sea (Goren, 1984) could be indicative of other undiscovered species of this genus in the Red Sea. Recently, the Indo-Pacific species *Diplogrammus randalli* Fricke, 1983 was reported from the Mediterranean Sea at the same location as *H. ingressus*, also far from the Suez Canal. These possible introduction pathways for such small and benthic fishes were discussed by Seyhan *et al*. (2017), who concluded that direct migration via the recently enlarged canal was likely. The morphological similarities, cryptic coloration, habitat preferences and insufficient ichthyological surveys may result in misidentification and these species being overlooked in traditional ichthyofauna research (Engin *et al*., 2014; 2016).

Hazeus ingressus also greatly resembles a specimen from Maumere Bay, Flores, Indonesia, held at NTM (Fig. 6), but as the specimen was collected from over black volcanic sand, all its colour patterns are intensified. This may represent another undescribed species as some ray

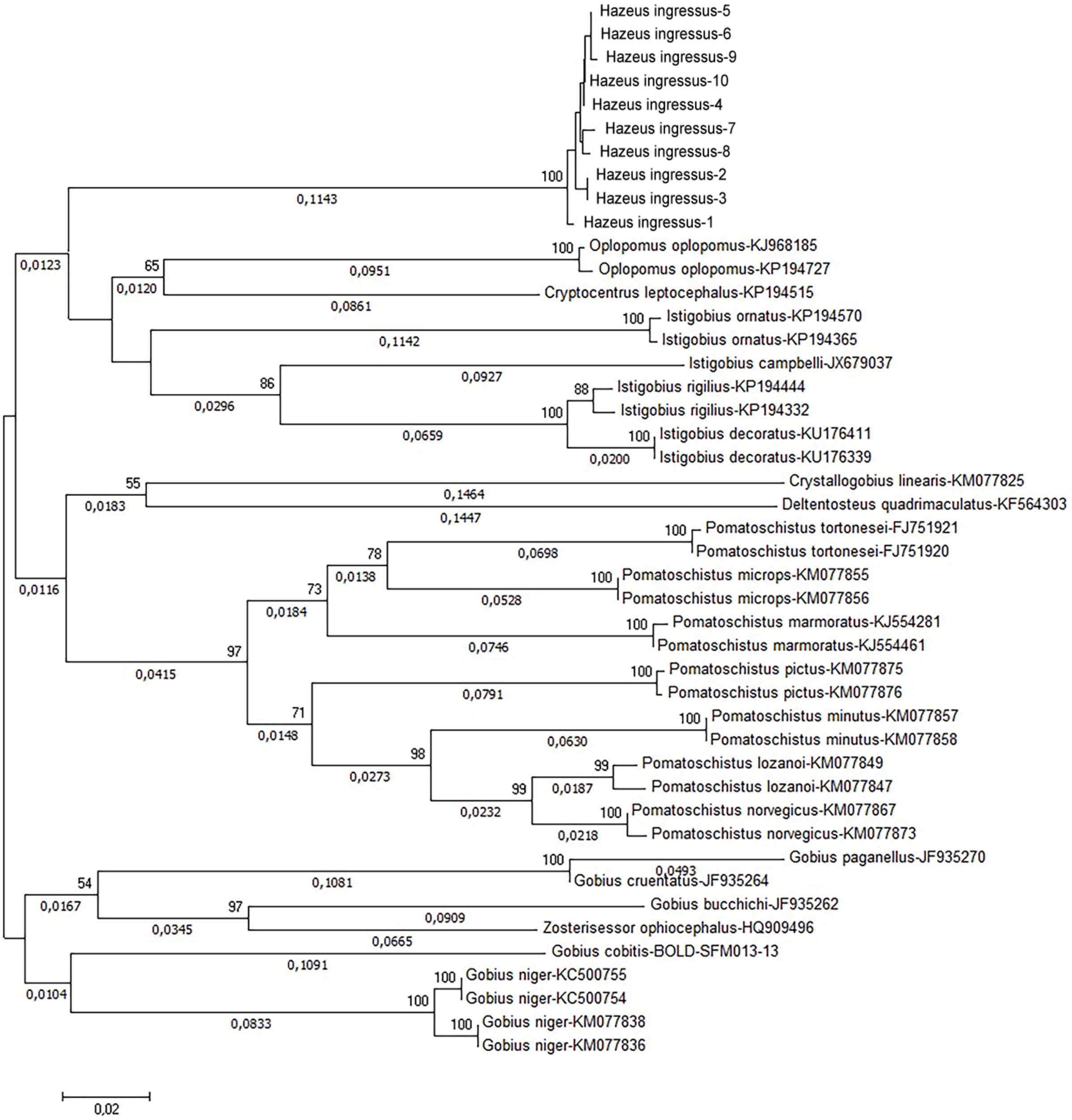

Fig. 4: Tree based on neighbour joining (NJ) analysis of Atlantic and Indo-Pacific gobiid species including *Hazeus ingressus* sp. nov. Numbers next to branches indicates bootstrap values >50 for 1000 replicates.

Fig. 6: A specimen resembling *Hazeus* sp. from Maumere Bay, Flores, Indonesia, held at NTM (Photograph by Michael Hammer).

counts differ. The fish is illustrated here in the hope that workers might recognise more specimens in collections and contribute to an understanding of this poorly known group.

The DNA barcoding technique has been used to contribute to molecular databases and an understanding of the basic position of *Hazeus* species within Atlanto-Mediterranean and Pacific species. Molecular studies on genus *Hazeus* and its possible relatives (*Oplopomops, Oplopomus, Opua* and *Echinogobius*) are very limited and inadequate. However, the barcode sequences discriminated all examined *Hazeus ingressus* specimens from Atlanto-Mediterranean species and they clustered with Pacific gobiids.

The increase of Lessepsian invasion in the Mediterranean is expected, by the recent enlargement of the Suez Canal (Fricke *et al.,* 2015). The most important and usually ignored anthropogenic factor, which creates gaps in the marine ecosystems and eases the introduction of non-indigenous species into the Levantine Sea, is the reduction or disappearance of native predators and competitors (sharks, groupers, carangids, tunas, sparids, etc.) due to overfishing (Engin *et al*., 2016a,b). As a result of all these negative processes, it is thought that the both the number of native gobiid species is decreasing and their distribution is being restricted throughout the Northern Levantine while the number of non-indigenous species is increasing.

We expected to observe small native *Pomatoschistus* species because of suitable available habitats but instead, only the more resistant and larger native *Gobius* species were observed during our fieldwork. Besides, the abundance of the non-indigenous gobiid fishes *Oxyurichthys petersii* (Klunzinger, 1871), *Vanderhorstia mertensi* Klausewitz, 1974 and *H. ingressus* in the area seem to support the theory that the distributions of native species are being restricted by alien species. The other important finding during the underwater surveys is the high abundance of the Lessepsian pufferfish *Torquigener flavimaculosus* in the area. In addition to all this, the potential threat of acquiring feral populations of lionfish *Pterois miles* (Bennett, 1828) is present, which would affect native and small gobiids including those yet undiscovered (Engin & Seyhan, 2017; Tornabene & Baldwin, 2017). A total of 11 gobiid species [*Coryogalops ochetica* (Norman, 1927), *Cryptocentrus caeruleopunctatus* (Rüppell, 1830), *Silhouetta aegyptia* (Chabanaud, 1933), *Tridentiger trigonocephalus* (Gill, 1859), *Trypauchen vagina* (Bloch & Schneider, 1801), *Oxyurichthys petersi* (Klunzinger, 1871), *Vanderhorstia mertensi* Klausewitz, 1974, *Favonigobius melanobranchus* (Fowler, 1934), *Aulopareia unicolor* (Valenciennes, 1837), *Bathygobius cyclopterus* (Cuvier & Valenciennes 1837)] including *Hazeus ingressus* sp. nov. are known to be of Indo-Pacific or Red Sea origin within the 107 known Lessepsian migrant fish species (Kovačić & Golani, 2007; Goren *et al*., 2009; Rothman & Goren, 2015; Engin *et al*., 2016; Seyhan *et al*., 2017; Fricke *et al*., 2017; Akel, 2017; Akel & Samir, 2017; Bariche & Fricke, 2018).

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