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The spread of the non-indigenous species *Bregmaceros nectabanus* Whitley, 1941 (Osteichthyes: Bregmacerotidae) in the eastern Mediterranean

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Abstract

This study reports the first record and some features of two individuals of the Lessepsian migrant species smallscale codlet, *Bregmaceros nectabanus,* found in the stomachs of two individuals of European hake, *Merluccius merluccius,* sampled in the northern Ionian Sea (eastern Mediterranean) during the MEDITS International Bottom Trawl Survey. The two European hake were collected on muddy bottoms in September 2021 at 63 m depth (39.34950 ºN; 16.52350 ºE) and at 240 m depth (39.07156 ºN; 17.12626 ºE). Their morphological traits together with otolith and scale characteristics allowed the identification of the two specimens found in the stomachs as *B. nectabanus.* The analysis of the stomach contents for the larger individual is also reported. This finding underlines the utility of stomach content analysis as an indirect method to study the biodiversity of deep-sea ecosystems, including the spread of non-indigenous species as indicators of global warming.

Keywords: first record; non-indigenous species; Lessepsian migration; stomach content; Mediterranean Sea.

Introduction

Climate change, anthropogenic activity and "non-indigenous migration" have changed the faunistic composition of the Mediterranean Sea during the last few decades (Albano *et al*., 2021; Lipej *et al*., 2022; Palermino *et al*., 2022).

While "non-indigenous migration" is responsible for the arrival of species through the Strait of Gibraltar (Turan *et al*., 2011) or the Suez Canal (Goren & Galil, 2006; Albano *et al*., 2021) mainly through transport in ballast waters (Dogrammatzi & Karachle, 2015), climate change has caused northward spreading of southern thermophilous species, a process known as meridionalisation (Boero *et al*., 2008).

Of the 165 fish species from the Red Sea and the Atlantic Ocean that have been recorded in the Mediterranean (Golani *et al*., 2021; Palermino *et al*., 2022) most are Lessepsian migrants, and this is probably also the case for *Bregmaceros nectabanus* (Harold & Golani, 2016), even if the possibility of a shipping-related introduction cannot be excluded.

The smallscale codlet *Bregmaceros nectabanus* Whitley, 1941 belongs to the monogeneric and circumtropical family Bregmacerotidae, that to date includes 14 valid species (Fricke *et al*., 2020), native to the Indo-West Pacific and the Western Indian Ocean, including the Red Sea, mainly distributed down to a depth of 350 m (Kulbicki *et al*., 1994; Harold & Golani, 2016). The smallscale codlet is the only member of this family known in the Mediterranean Sea (Dulčić *et al*., 2020) and its distribution is poorly known (Vrdoljak *et al*., 2021).

Bregmaceros nectabanus has been previously described in Egyptian, Turkish, Greek, Syrian and Israeli waters (Yılmaz *et al*., 2004; Filiz *et al*., 2007; Aydin & Akyol, 2013; Dogrammatzi & Karachle, 2015; Rizkalla & Akel, 2015; Harold & Golani, 2016; Ketsilis-Rinis & Dimitriou, 2018) and more recently it has been reported in the Adriatic Sea (Dulčić *et al*., 2020; Palermino *et al*., 2022). In particular, concerning the Ionian Sea, one individual was collected in the Patraikos Gulf and five individuals in the Kerkyraikos Gulf during the DCF (Data Collection Framework) activities in 2016 (Ketsilis-Rinis & Dimitriou, 2018). Until 2016, all Mediterranean reports of this species were incorrectly identified as *Bregmaceros atlanticus* (Goode & Been, 1886).

Non-indigenous species have a low population density so they are not frequently sampled, especially by traditional gears, but they can be found within the stomach contents of commercial fish species. In fact, in the Mediterranean Sea, three specimens of *B. nectabanus* have been found in the stomachs of other fish: two specimens

in *Saurida lessepsianus* in Turkey (Yilmaz *et al*., 2004) and one specimen in *Trachurus trachurus* in the Aegean Sea (Filiz *et al*., 2007). These finds highlight the importance of stomach content analysis in the study of biodiversity of deep-sea ecosystems.

The aim of this study is to report the first record of *Bregmaceros nectabanus* in the northern Ionian Sea (eastern Mediterranean), also focusing on the utility of stomach content analysis as an indirect method to study the biodiversity of marine ecosystems.

Materials and Methods

During a study on the diet composition of the European hake *Merluccius merluccius* in the northern Ionian Sea, performed in the framework of the MEDiterranean International Bottom Trawl Survey (MEDITS) (Bertrand *et al*., 2002) carried out on muddy bottoms from Otranto to Capo Passero (Geographical Sub-Area 19, GSA 19), two specimens of the smallscale codlet *Bregmaceros nectabanus* were found from two stomachs. The first one was found in an individual of *M. merluccius* sampled on 6/09/2021 at a depth of 63 m (San Giacomo: 39.34950 ºN, 16.52350 ºE), the second one in an individual collected on 8/09/2021 at a depth of 240 m (Crotone: 39.07156 ºN, 17.12626 ºE). For each specimen of *M. merluccius* sampled, the total length (TL to the nearest 1 mm) and total weight (TW to the nearest 0.1 g) were recorded. Information on sex and stage of maturity of the gonads was recorded as well. The stomachs were removed, stored in 70% ethanol solution and kept frozen (-20 °C) until the time of analysis. The food items in the stomach contents were identified using a stereomicroscope LEICA M165C to the lowest possible taxonomic level and classified through manuals and dichotomous keys. The smallscale codlets were identified using the diagnostic characters described by D'Ancona & Cavitano (1965) and Harold & Golani (2016), supported by otolith recognition (Smale *et al*., 1995). As far as possible, the lengths were taken with a calliper and using a millimetric ichthyometric table with an accuracy of 1 mm; the weight was recorded using a digital balance with an accuracy of 0.1 g. According to digestion degree of the samples all possible morphometric measurements and meristic counts were recorded.

The sagittal otoliths of *B. nectabanus* were removed from both individuals, cleaned, immersed in glycerine and observed with a stereomicroscope LEICA M165C under reflected light against a dark background.

The stomach of the larger smallscale codlet individual was dissected and stored in 70% ethanol solution. The food items in the stomach contents were identified using a stereomicroscope to the lowest possible taxonomic level. Some scales were removed and photographed.

Finally, the two specimens of *B. nectabanus* were fixed in 70% ethanol solution and stored in the fish collection of the Ecology Laboratory of the University of Bari Aldo Moro (Italy).

Results

The first individual of European hake was a recruit with sex not identified (TL 100 mm, TW 6.43 g). Its stomach content also contained, in addition to the *B. nectabanus* specimen, one decapod cephalopod and one cumacean crustacean. The degree digestion of the gut content was medium/low. The second individual was a

Table 1. Morphometric measurements and meristic counts of the two specimens of *B. nectabanus.*

	Individuals	
Morphometric characters (mm)	1	2
Total length	-	40
Standard length	61	36
Head length	11	6
Pre-pectoral length	11.8	-
Max body height	-	7.2
Min body height	-	2.5
Eye diameter	4	1.6
Post-orbital length	6	5
Pre-orbital length	2	2
Pelvic fin ray length	34 – 25 – 17 – 15 (4 rays damaged)	-
Pectoral fin ray length	8	-
Caudal fin length	-	3.8 (the longest)
Meristic characters (count)		
Pectoral fin soft rays	16	-
Pelvic fin soft rays	5 small, 3 elongated	-
Caudal fin rays (total)	-	26

1= individual number one; 2= individual number two.

Fig. 1: Specimen of small-scale codlet *B. nectabanus* found in the stomach contents of *M. merluccius*, sampled in the northern Ionian Sea.

virgin developing female (TL 315 mm, TW 230 g); the stomach contents, in addition to *B. nectabanus,* contained one specimen of *Alpheus glaber* and one of *Sardina pilchardus*. These stomach contents were also in medium/low degree digestion.

The morphometric measurements and meristic counts of the two specimens of *B. nectabanus* are shown in Table 1.

The morphological analysis allowed the two specimens to be identified as *B. nectabanus,* according to the following traits: body elongated, slightly compressed in its ventral part; pelvic fin jugular with elongated rays, small head with a blunt snout and large eyes, a long ray on top of the head, body with dense pigmentation along the dorsum, and distally fimbriate opercular spine (Fig. 1).

The identification was confirmed by the characteristics of the otoliths, that were found intact in both specimens: their ventral margin was flat; the *pseudocolliculum* was elongated, level with, and almost joined to the *ostium* and *cauda*; the *crista superior* was relatively short (Fig. 2). The otoliths of the largest individual (SL = 61 mm) showed the presence of 5 opaque growth rings, while that of the smallest specimen (SL = 36 mm) showed 3 transparent growth rings.

The scales, taken from middle of the body, were small, cycloid and showed dense concentric *circuli* around the *focus* on exposed parts and straight and truncated at the anterior border on covered parts (Fig. 3).

The analysis of the stomach contents of the two *B. nectabanus* revealed the presence of the following prey items: 1 euphausiacean, 1 mysidacean, 1 amphipod, 2 unidentified crustaceans and 1 teleost.

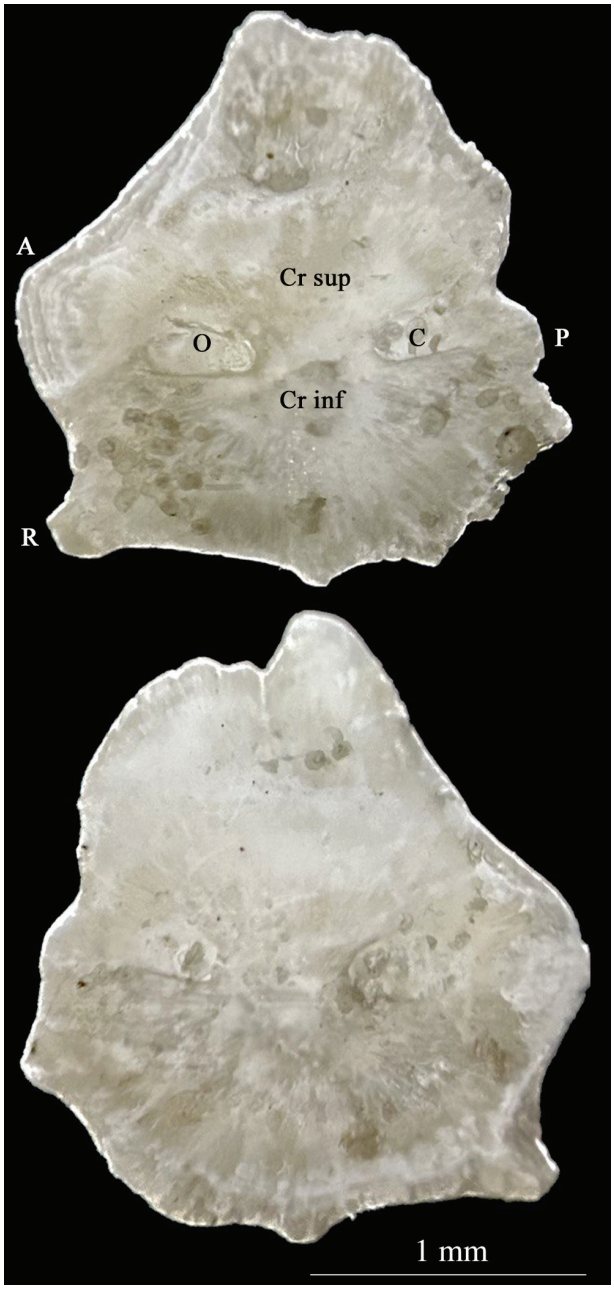

Fig. 2: *B. nectabanus* (SL = 61 mm) otoliths.
*O=ostium, C=cauda, Cr sup=crista superior, Cr inf=crista inferior, R=rostrum, A=antirostrum, P=posterior.

Discussion

This work presents the first record and some features of two individuals of *B. nectabanus* from the northern Ionian Sea, collected in the stomachs of two individuals of European hake. Previously *B. nectabanus* had been found in stomachs of other teleost fishes, such as *Trachurus trachurus* (Filiz *et al*., 2007; Othman & Galiya, 2019) sampled in the Aegean Sea and lessepsian migrant *Saurida lessepsianus* sampled along the coasts of Antalya, Turkey (Yilmaz *et al*., 2004).

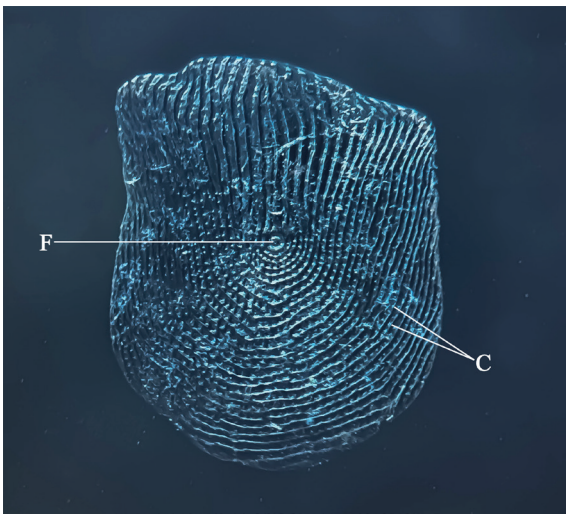

Fig. 3: *B. nectabanus* cycloid scale. *F=focus, C=circuli.

The first finding of *B. nectabanus* in the Mediterranean Sea was incorrectly reported as *B. atlanticus* by D'Ancona & Cavinato (1965).

According to Harold & Golani (2016) all the findings of *Bregmaceros atlanticus* from the Mediterranean Sea have been misidentifications of the smallscale codlet *Bregmaceros nectabanus*. This misidentification was due to the very similar morphological characteristics of the two species; *B. nectabanus* can be distinguished by some peculiarities, such as the almost non-pigmented abdomen, a thin dorsolateral longitudinal stripe under the second dorsal fin, and a distally fimbriate opercular spine (Harold & Golani, 2016).

Most of the previous records in the Mediterranean Sea come from the western coasts, such as Turkey (Yilmatz *et al*., 2004; Filiz *et al*., 2007; Turan *et al*., 2011; Aydin & Akyol, 2013; Özgül & Akyol, 2017), but recent studies show a progressive expansion of the distribution of *B. nectabanus* along the eastern coasts of the Ionian Sea along the Greek coasts (Ketsilis-Rinis & Dimitrou, 2018), and in the northern Adriatic Sea (Dulčić *et al*., 2020; Palermino *et al*., 2022). Its distribution suggests that *B. nectabanus* most probably entered the Mediterranean Sea through the Suez Canal (Harold & Golani, 2016), due to climate change and anthropogenic activity. Up to now, records of this Lessepsian migrant have been reported from the central and eastern Mediterranean Sea. The present records confirm its presence in the eastern Mediterranean.

As suggested by Palermino *et al*. (2022) and Dulčić *et al*. (2020) the spread of *B. nectabanus* towards the Adriatic Sea led researchers to suppose that the species first reached the Albanian coast carried by the Levantine Intermediate Water (LIW) and successively the Italian coast of southern Adriatic through the Adriatic Deep Water (AdDW) gyre (Santinelli, 2015). This could be the same mechanism that transported the species to the northern Ionian Sea. Although the effect of water mass movements in the transport of *Bregmaceros* larvae has been shown (Grabe *et al*., 1992), the finding of these two medium-large specimens could also be due to an active diffusion of adult individuals probably in search of a feeding area (Palermino *et al*., 2022). It should also be pointed out that the study area presents several commercial and tourist harbors, therefore, the possibility of a shipping-related introduction of *B. nectabanus* to the northern Ionian Sea cannot be excluded. In fact, Özgül & Akyol (2017) reported that *B. nectabanus* was introduced in the eastern Mediterranean via ship ballast waters in terms of it occurring near large ship harbors, and Dulčić *et al*. (2020) also noted that a shipping-related introduction of *B. nectabanus* to the Adriatic Sea was possible in relation to the area of Bari commercial harbor. Vrdoljak *et al.* (2021) supported both hypotheses (i.e., Lessepsian migration and ballast water) since two specimens have been found near the cargo port of Ploče, Croatia (Akyol, 2022). There are no substantiated records of *B. nectabanus* from the Atlantic, thus excluding it as a possible source(Harold & Golani, 2016).

As regards morphometric and meristic data, the present results agree with those reported for individuals of similar size from the Greek Ionian Sea and the Adriatic Sea (Ketsilis-Rinis & Dimitriou, 2018).

The morphology of the scales is comparable with that observed in other species of the genus *Bregmaceros* (Ho *et al*., 2020; Torii *et al*., 2003). The otolith morphology also agrees with the information provided by Smale *et al.* (1995).

As regards stomach contents of *B. nectabanus*, also from the samples found in Greek Ionian Sea, the diet was mainly composed of small crustaceans (Ketsilis-Rinis & Dimitriou, 2018).

Stomach content analysis provides a contribution to biodiversity knowledge especially in the deep-sea (Capezzuto *et al*., 2019; 2022) avoiding the issues related to traditional sampling gears, such as mesh net size, as suggested by Ali (2018) about the low catchability of *B. nectabanus*.

As previously mentioned, *B. nectabanus* has been found in the stomachs of other teleost fish, therefore the present study represents not only a new record of the species in the northern Ionian Sea but could also suggest its inclusion in the Mediterranean marine food web.

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