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Morphological Comparison of the Long-snouted Seahorse (*Hippocampus guttulatus* Cuvier, 1829) Populations in the Black Sea and the Aegean Sea

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Abstract: Seahorses are popular species due to their unique morphology, but they are under global threat. The plasticity of seahorses' morphology and phenotype causes confusion in species identification, leading to taxonomic uncertainties that make effective conservation and management activities difficult. In this study, 80 (46♀; 34♂) specimens belonging to long-snouted seahorse populations distributed in the Black and Aegean Seas were collected from fishermen and analyzed. As a result of the analyses, it was determined that there was a statistically significant difference between the populations in terms of total lengths, eye diameters, dorsal fin lengths, and body height values ($p<0.05$), and in the SIMPER analysis, based on metric measurements and meristic counts, it was calculated that the total difference between the populations was 3.13%. To refine and improve conservation actions for seahorses, more studies, including information about seahorses' taxonomy, biology and ecology are necessary.

Keywords: Aegean Sea, Black Sea, *Hippocampus*, long-snouted seahorse.

Karadeniz ve Ege Denizi'ndeki Uzun Burunlu Denizatı *(Hippocampus guttulatus* Cuvier, 1829) Popülasyonlarının Morfolojik Olarak Karşılaştırması

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Öz: Denizatları, benzersiz morfolojileri nedeniyle popüler olan ancak küresel tehdit altındaki türlerdir. Denizatlarının morfolojik ve fenotipik esnekliği, tür tanımlamasında karışıklığa yol açmakta, etkili koruma ve yönetim faaliyetlerini zorlaştıran taksonomik belirsizliklere neden olmaktadır. Bu çalışmada, Karadeniz ve Ege Denizi'nde dağılım gösteren uzun burunlu denizatı popülasyonlarına ait toplam 80 (46♀; 34♂) örnek balıkçılardan toplanarak analiz edilmiştir. Analizler sonucunda popülasyonlar arasında total boy, göz çapları, dorsal yüzgeç uzunlukları ve vücut yükseklikleri değerleri açısından istatistiksel olarak anlamlı bir fark olduğu tespit edilmiş ($p<0.05$), metrik ölçümler ve meristik sayımlara dayalı SIMPER analizinde ise popülasyonlar arasındaki toplam farkın %3,13 olduğu hesaplanmıştır. Denizatlarının koruma eylemlerini iyileştirmek ve geliştirmek için taksonomileri, biyolojileri ve ekolojileri hakkında bilgi içeren daha fazla çalışmaya ihtiyaç vardır.

Anahtar kelimeler: Ege Denizi, *Hippocampus*, Karadeniz, uzun burunlu denizatı.

INTRODUCTION

Seahorses are charismatic organisms of marine life and have an unusual life history. However, these sensitive fishes are under the threat of anthropogenic activities such as coastal habitat degradation, accidental capture, and illegal catch and trade (Lourie et al., 2016; Correia et al., 2018; Pierri et al., 2021). There are 48 valid species of seahorses in the genus *Hippocampus* (Koning and Hoeksema, 2021) that live in shallow-water seagrass beds, mangroves, and coral reef ecosystems worldwide (Foster and Vincent, 2004; Cohen et al., 2017).

The first seahorse species was described as *Syngnathus hippocampus* by Linnaeus in 1758 because of

the poor descriptions, insufficient morphological variations among the species and camouflage abilities characterized by using a number of different names (Lourie al., 1999). And even at the beginning of these confusions, the taxonomic instability ongoing about Linnaeus' seahorse is short or long-snouted (Vasileva, 2007; Kuiter, 2009; Lourie et al., 2016; Woodall et al., 2018). Currently, seahorses in the Mediterranean are represented by two native species: the *Hippocampus hippocampus* (Linnaeus, 1758), *Hippocampus guttulatus* (Cuvier, 1829) and one Lessepsian migrant, *Hippocampus fuscus* Rüppel, 1838 (Golani & Fine, 2002). The presence of these three species has been documented on the coasts of Turkey, and numerous studies have been conducted on their distribution, biology, and ecology (Gokoglu et al., 2004; Gurkan and Taskavak, 2007; Basusta et al., 2014; Bilecenoglu et al., 2014; Kasapoglu and Duzgunes, 2014; Uzer et al., 2019; Gurkan, 2020; Taylan et al., 2020; Erguden et al., 2023).

Uncertainties and disagreements in seahorse taxonomy make it difficult to determine the existence of species, their ecology, and to carry out effective conservation and management activities (Perry et al., 2005; Lavergne et al., 2010; Woodall et al., 2018). For conservation actions, all *Hippocampus* species were incorporated into Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in 2004, however The Red List from the International Union for Conservation of Nature (IUCN) categorized *H. guttulatus* as "Data Deficient" and *H. hippocampus* as "Near Threatened" for the Mediterranean region.

In this study, specimens of the long-snouted seahorse, which shows intraspecific variability in morphology, from the Black Sea and the Aegean Sea were examined, and the obtained data were presented.

MATERIAL AND METHOD

Long-snouted seahorse specimens were collected from the Turkish coast of the Black Sea (eastern: Rize; western: Kırklareli) and the Aegean Sea (İzmir). No seahorse was caught specifically for this work, and all the specimens were obtained as by-catch from small-scale fishermen.

The examination of the specimen was carried out by utilizing the studies of Lourie et al., (2004). Metric characteristics were measured with a digital calliper to the nearest 0.1 mm. Head-related measurements were transformed as a percentage of head length (HL%), whereas characteristics related to the body and fins were transformed as a percentage of total length (TL%).

Abbreviations: TL= total length; HL= head length; SnL= snout length; PO= postorbital length; Sd1= snout depth from the anterior edge of the eye; Sd2= depth from the tip of the snout; Ed= eye diameter; Io= interorbital distance; CorL= coronet length; DfL= dorsal fin length; Prp-L= prepectoral length; Bd-Pdf= body depth from the posterior part of the dorsal fin; Bw-Pdf= body width from the posterior part of dorsal fin; Bd-Adf= body depth from the anterior part of the dorsal fin; Bw-Adf= body width from the anterior part of dorsal fin; Bd-Poc= body depth from the post opercle. Meristic characters: Br= the number of rings in the body; Tr= the number of rings in the tail; Dfr= dorsal fin rays; Pfr= pectoral fin rays.

Body proportions based on morphometric measurements and meristic counts were analyzed by One-way ANOVA and posthoc Tukey test (T). The SIMPER test was also used to determine which character was responsible for the possible population differences (Primer V7).

Figure 1. Sampling locations.

RESULTS AND DISCUSSION

A total of 80 long-snouted seahorse individuals obtained from the Black Sea (BS) (21♀; 19♂) and the Aegean Sea (AS) (25♀; 15♂) were examined. The Black Sea population samples length (TL) ranged from 6.5-9.3 cm (7.9±0.7cm), dorsal fin rays (Dfr) 18–20 (mainly 19), pectoral fin rays (Pfr) 15-18 (mainly 16), body rings (Br) 11 and tail rings (Tr) 33-37 (37). The Aegean Sea population TL ranged 5.7-12.0 cm (8.6±0.9cm), Dfr 18-19 (18), Pfr 15-16 (16), Br 11 and Tr 34-38 (37). The ratio of HL/SnL varies from 2.41-2.65 (2.5±0.14) for the BS and 2.54-2.89 (2.7±0.21) for AS with no statistically significant difference between populations ($p>0.05$).

The morphometric evaluations showed a statistically significant difference in the characters that eye diameter (Ed), dorsal fin length (DfL) and body depth (Bd-Adf) between populations. In accordance with Woodall et

al., (2018), the total length of the Black Sea population was found significantly smaller than the Aegean population ($p<0.05$). The mean length of the BS population in this study was similar to Basusta et al., (2014) (5.78- 9 cm) and Kasapoglu and Duzgunes, (2014) which obtained 8.31 cm. In AS, Gurkan et al., (2007); Gurkan and Taskavak, (2007) (13.3cm) and in BS, Uzer et al., 2019 (females: 11.8 cm, males: 12.2 cm) obtained larger specimens from this study. Details of morphometric proportions are listed in Table 1. Almost all of the meristic characteristics of short and long-snouted sea horses distributed in the Mediterranean and Black Sea basins overlap and cannot provide healthy data for separation. The meristic counts obtained in this study are presented in Table 2.

Table1. Proportional measurements of long-snouted seahorse specimens. Details of the abbreviations were explained in the material and methods section.

%		Min.	Max.	Mean	Std. dev.	Sig.
HL/TL	Black Sea	16.38	21.80	18.39	1.63	0.639
	Aegean Sea	14.92	23.24	18.75	2.61	
SnL/HL	Black Sea	44.62	63.20	54.84	4.91	0.792
	Aegean Sea	44.11	61.99	55.40	6.69	
Ed/HL	Black Sea	16.64	29.00	21.86	2.77	0.047*
	Aegean Sea	15.44	27.08	19.54	3.45	
Sd1/SnL	Black Sea	30.33	42.87	34.91	3.07	0.326
	Aegean Sea	27.15	42.08	33.59	4.29	
Sd2/SnL	Black Sea	23.76	33.73	27.77	2.63	0.961
	Aegean Sea	25.36	33.01	27.73	2.24	
SnL/TL	Black Sea	9.22	10.90	10.02	0.43	0.352
	Aegean Sea	8.13	11.44	10.27	1.02	
PO/HL	Black Sea	24.78	46.92	19.70	3.61	0.339
	Aegean Sea	13.84	41.04	20.67	5.93	
DfL/TL	Black Sea	9.11	11.34	10.55	0.74	0.00*
	Aegean Sea	7.59	10.37	9.15	0.86	
Bd-Adf/TL	Black Sea	13.29	18.63	16.65	1.51	0.029*
	Aegean Sea	10.42	17.82	15.04	2.39	
Bw-Adf/TL	Black Sea	5.65	9.26	7.40	0.98	0.221
	Aegean Sea	4.61	9.89	6.78	1.77	
CorL/Bd-Poc	Black Sea	14.25	26.73	19.70	3.61	0.570
	Aegean Sea	11.85	31.48	20.67	5.93	

SIMPER analysis revealed that the dissimilarity between the populations 3.13%. The characteristics contributing to the difference were determined as Tr (13.5%); PO/HL (18.5%); Pfr (9.7%); Br (9.4%); CorL/Bd-Poc (9.1%); Bw-Adf/TL (7.9%); Ed/HL (6.7%); Bd-Adf/TL (5.5%).

In both populations, the body colour is variable brown with irregular small white spots scattered all over the body. The spines of individuals from the Black Sea were short and blunt, while the Aegean population mostly branched. The cirri presence in larger specimen was more likely on supporting findings by Curtis, (2006) and Woodall et al., (2018).

Table 2. Meristic counts of the short and long-snouted seahorses (BS: Black Sea; AS: Aegean Sea; LS: long-snouted; SS: short-snouted). Details of the abbreviations were explained in the material and methods section.

References	Species	Snout	Tr	Pfr	Dfr	HL/SnL
Lourie et al., (2004;2016)	*H.guttulatus*	LS	37-39 (35-40)	17 (16-18)	19-20 (17-20)	2.6 (2.3-2.9)
	H.hippocampus	SS	37 (35-38)	14 (13-15)	17 (16-19)	3.0 (2.8-3.4)
Vasil'Eva, (2007)	*H. hippocampus*	LS	37 (34-38)	17 (15-18)	19-20 (17-21)	2.3-2.6 (2.2-2.9)
	H. brevirostris	SS	36	14 (13-15)	16-17 (16-19)	2.8-3 (2.6-3.4)
Slastenenko, (1956)	*H. hippocampus microstephanus*	LS	34-37	16-19	18-22	-
Gurkan and Culha, (2008)	*H. guttulatus*	LS	36-40	15-18	18-21	2.5-2.75
Kasapoglu and Duzgunes, (2014)	*H. guttulatus*	LS	31-36	14-18	16-20	2.17
This study	*H. guttulatus* BS population	LS	37 (33-37)	16 (15-18)	19 (18-20)	2.5 (2.4-2.6)
	H. guttulatus AS population	LS	37 (34-38)	16 (15-16)	18 (18-19)	2.7 (2.5-2.9)

It is known that *H. guttulatus* prefers densely vegetated habitats (Curtis & Vincent, 2005; Caldwell & Vincent, 2012; Correia, 2022). In this study, it was observed that the specimens were mostly distributed in *Zostera spp.* and *Cystoseira barbata* facies in shallow coastal waters in the Black Sea and Aegean Sea.

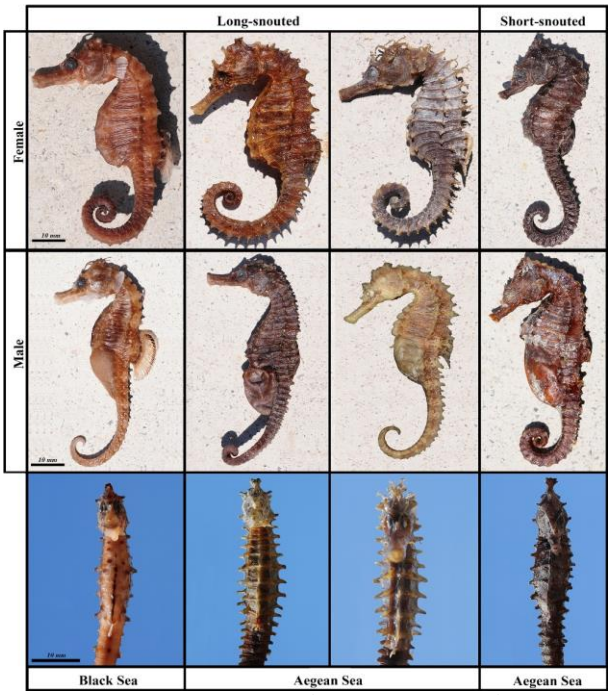

Figure 2. A general appearance of the original samples from the Black Sea and the Aegean Sea.

Although there are studies on short-snouted seahorses in the Black Sea (Dawson, 1986; Gurkan & Culha, 2008; Keskin, 2010; Basusta et al., 2014; Taylan et al., 2020; Pierri et al., 2022; Iftime, 2023), no individuals were found in the Eastern and Western Black Sea samplings. Within the scope of the study, short-snouted

seahorses were observed in the Aegean Sea samplings and it was observed that these individuals spread to deeper depths than the long-snouted seahorses. Photographs of individuals belonging to the populations obtained from the field studies are presented in Figure 2.

Seahorses are the flagship species in conservation biology (Lourie et al., 1999; Pierri et al., 2022) and have an important role as indicators of environmental quality (Gristina et al., 2015). Due to the lack of ecological data and also indicated need researches on biology. ecology. habitat. abundance and distribution of these species. many of them are categorized as "Data Deficient" in IUCN.

These specialized organisms show phenotypic plasticity. such as color variation and cirri (Curtis, 2006), caused by adaptive modifications for different habitats and environmental conditions common in syngnathids (Tortonese, 1970; Lourie et al., 2004). Examining the historical process of syngnathid taxonomy. it is clear that the identification of this family based on morphological characters has caused much taxonomic confusion. including numerous misidentifications and synonyms (Dawson, 1986; Hablutzel, 2009).

With the support of molecular analyses, studies have been carried out to clarify the phylogenetic relationships of the species belonging to the genus *Hippocampus* (Casey et al., 2004; Teske et al., 2004, 2005, 2007; Boehm et al., 2013; González et al., 2014; Silveira et al., 2014; Woodall et al., 2015). The accepted status of seahorses is that *H. algiricus* and *H. erectus* are distributed in the NE Atlantic, *H. hippocampus* and *H. guttulatus* are native, and *H. fuscus* is distributed in the Mediterranean as a lessepsian representative (Woodall et al., 2018).

Considering the communication possibilities in the past, one of the main problems was the limited communication among the researchers who talking about the seahorses using same or different nomenclatures (Lourie et al., 1999). With the help of revisions in the genus, many species have been synonymized, but the taxonomic instability continues whether the Linnaean seahorse is short or long-snouted (Vasil'eva, 2007; Maclaine, 2015). In this context, as mentioned in detail by Vasil'eva, (2007) and Mclaine, (2015), the European seahorses were characterized by using different names since the first seahorse species described as *Syngnathus hippocampus* by Linnaeus in 1758. In 2007, Ekaterina Vasil'eva suggested using the name *H. hippocampus* for the long-snouted, and *Hippocampus brevirostris* Schinz, 1822 for the short-snouted seahorse but it was not adopted as it would lead more confusions and the possibility of the long-snouted seahorse in Black Sea may have been different species (Lourie et al., 2004; Maclaine, 2015; Fricke et al., 2024), however it was not supported by genomic studies (Woodall et al., 2015; Lourie et al., 2016). It is obvious that especially the illustrations of Linnaeus's original description that he cited from Bradley, (1721) and Olearius, (1674), images of the specimens from the Fish Collection of Carl Linnaeus (1707-1778) and also M.E. Bloch's illustrations in "Naturgeschichte der ausländischen Fische" have characterized the long-snouted specimen. In addition, the distribution area, depth, and abundance data of these species the sampling opportunities at the time when the species was identified, and the images in the existing literature. In addition, the species distribution area, depth range, and the fact that it is more abundant in the shallows than the short-snouted increase the possibility that the first described seahorse was a long-snouted.

CONCLUSION

Every piece of information is valuable in clarifying the conservation status and management actions for seahorses. In this context, the present study provides insights about the populations of long-snouted seahorses distributed in the Black Sea and Aegean Sea. In the future, studies that include molecular techniques supported by detailed morphology, ecological, and distributional investigations on large scales will be effective for this group.

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