

Rapid Communication

The yellowstripe barracuda *Sphyraena chrysotaenia* (Kluzinger, 1884) in Crete (GSA 23, eastern Mediterranean): first genetically verified records and highlighted issues on the lessepsian barracudas nomenclature ambiguities

Sotiris Kiparissis^{1,2,*}, Dimitris Tsaparis³, Panagiota Peristeraki^{1,4}, Amalia Giannakaki¹, Giannis Kosoglou¹, Manolis Metaxakis¹ and George Tserpes¹

¹Hellenic Centre for Marine Research, Institute of Marine Biological Resources and Inland Waters (IMBRIW), 71003, Heraklion, Greece

²Hellenic Agricultural Organization-DEMETER, Fisheries Research Institute, 64700 Nea Peramos, Kavala, Greece

³Hellenic Centre for Marine Research, Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Heraklion, Greece

⁴University of Crete, Biology Department, Stavrakia, Heraklion, Greece

Author e-mails: skipariss@inale.gr (SK), tsaparis@hcmr.gr (DT), notap@hcmr.gr (PP), amalg28@gmail.com (AG), kosoglou@gmail.com (GK), m.metaxakis@hcmr.gr (MM), gtserpes@hcmr.gr (GT)

*Corresponding author

Citation: Kiparissis S, Tsaparis D, Peristeraki P, Giannakaki A, Kosoglou G, Metaxakis M, Tserpes G (2020) The yellowstripe barracuda *Sphyraena chrysotaenia* (Kluzinger, 1884) in Crete (GSA 23, eastern Mediterranean): first genetically verified records and highlighted issues on the lessepsian barracudas nomenclature ambiguities. *BioInvasions Records* 9(4): 814–826, <https://doi.org/10.3391/bir.2020.9.4.15>

Received: 10 January 2020

Accepted: 15 May 2020

Published: 23 September 2020

Handling editor: Michel Bariche

Thematic editor: Andrew David

Copyright: © Kiparissis et al.

This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International - CC BY 4.0).

OPEN ACCESS

Abstract

On April 17, 2018, four specimens of the lessepsian barracuda *Sphyraena chrysotaenia* were captured in the Gulf of Heraklion in Crete. The specimens were genetically identified and their full morphological description is provided. The close morphological similarities of the lessepsian barracudas, as well as multiple nomenclature changes through the years have resulted in an extended confusion regarding their accurate identification and taxonomy. The detailed morphological descriptions provided in this work coupled with DNA barcoding of the specimens, intends to provide verified data that will hopefully aid future specialized research aiming to alleviate this problem. To date, this is the first genetically confirmed record of this particular barracuda in Crete.

Key words: invasive species, DNA barcoding, Sphyraenidae, morphometry

Introduction

Barracudas (Family: Sphyraenidae) comprise a cosmopolitan group of pelagic ferocious predatory fish that live solitarily or in large shoals, depending on the species (de Sylva 1973). All species of this group are very similar in morphology and biology and with life histories remarkably alike, probably all around their distribution (Williams 1959; de Sylva 1973). In many places of their distribution they are exploited commercially and the smaller members of the genus may occasionally constitute a noteworthy proportion of the catch (Smith 1956; Williams 1959). In certain areas of the Caribbean they appear to be highly toxic (Smith 1956; de Sylva 1973; Rose 1983). Certain solitary species appear dangerous to man because of their aggressiveness, with several attacks to humans and fatalities reported throughout the tropics (Smith 1956; de Sylva 1963; Rose 1983).

The taxonomy of this group is largely under consideration with varying numbers of genera and different number of species described in the past.

Nowadays, all barracuda species are grouped into a single genus (*Sphyraena*), even though a more elaborate division of the family into six genera has been suggested in the past (Smith 1956). However, the eventual congruity of including all barracudas into a single genus seems to be the only point of agreement between taxonomists and great confusion and disagreement exists when it comes to species identifications and descriptions. Over 69 to 74 nominal barracuda species have been described to date (de Sylva 1973; Eschmeyer and Fong 2019 respectively), of which, a much smaller number is considered valid (20 species by Schultz (1953), de Sylva (1973), de Sylva and Williams (1986) and Doiuchi and Nakabo (2005), probably less than 20 species by Smith (1956), 21 by Nelson (2006) and 27 by Eschmeyer and Fong (2019)), yet, with their validity still under consideration. At the same time, potential new species are suggested (e.g., Pastore 2009). Many of these species have been synonymized with other species over the past years, depending on the criteria used each time as taxonomic characters, eventually resulting in a rather confusing taxonomical and nomenclature status.

In the Mediterranean, the species of the genus *Sphyraena* did not evade the general rule of taxonomic confusion, particularly the lessepsian ones. Seven nominal species have been reported in the Mediterranean to date, of which two are considered synonyms. Most likely, in the basin today there are four *Sphyraena* species. Two of them (*S. sphyraena* (Linnaeus, 1758) and *S. viridensis* (Cuvier, 1829)) are indigenous whereas the other two (*S. chrysotaenia* (Kluzinger, 1884) and *S. flavicauda* (Rüppel, 1838)) are lessepsian migrants (de Sylva and Williams 1986; Golani et al. 2006). The latter two species relatively recently have been synonymized to *S. pinguis* and *S. obtusata* respectively, by Doiuchi and Nakabo (2005). A fifth new species (*Sphyraena intermedia*) has also been described by Pastore (2009), which however has not been reported again since its first description. Like the rest of the barracuda species, the close morphological similarity among the barracudas present in the Mediterranean, their common habitat and their potential coexistence, has resulted in taxonomic confusions in earlier studies (Corsini and Economidis 1999; Kalogirou et al. 2012). The problem has been exacerbated for the two lessepsian barracudas by their nomenclature ambiguity. For example, *S. chrysotaenia*, which was originally recorded in the basin in 1931 misidentified as *Belone acus* (Spicer 1931), was in the following years regarded either as a synonym to *S. obtusata* (e.g., Williams 1959; Ben-Tuvia 1966, 1986; Allam et al. 2004), or as a misidentified younger stage of *S. flavicauda* (De Sylva 1973), or as a synonym to *S. pinguis* (e.g., Doiuchi and Nakabo 2005). To date, still there has not been one generally accepted nomenclature for the lessepsian barracudas. The two latest nomenclature suggestions of Doiuchi and Nakabo (2005) (*S. chrysotaenia* a synonym of *S. pinguis* and *S. flavicauda* a synonym of *S. obtusata*) have been adopted by some authors however the former ones (*S. chrysotaenia*

and *S. flavicauda*) are the most frequently used in the majority of the latest works. These works are based either exclusively on the examination of key morphological criteria (e.g., Golani et al. 2006; Rim et al. 2007; Galil 2007; Halim and Rizkalla 2011) or on both morphological and genetic data (e.g., Karahan et al. 2017). Even though the majority of the authors agrees on the presence of two lessepsian barracudas in the basin (regardless the name they choose, i.e. *S. chrysotaenia* vs *S. pinguis* and *S. flavicauda* vs *S. obtusata*), the recent works of Santini et al. (2015), Shirak et al. (2016) and Karna et al. (2018), who discriminated the aforementioned nominal species as separate ones, only add to the general bemusement regarding the lessepsian barracudas, resulting in a rather obscure picture of their presence in the basin and with the information on their distribution possibly misleading (Golani et al. 2006). This situation calls for a more rigorous approach on the matter of their identification where useful information can be extracted only from meticulous descriptions of captured, genetically described and deposited specimens.

In the present study, detailed information for four *Sphyraena* specimens caught in Crete and genetically verified as *S. chrysotaenia*, is presented. Crete is the second largest island in the Eastern Mediterranean, with unique geomorphological and oceanographic characteristics (Psarra et al. 1996; Danovaro et al. 1996; Chronis et al. 1996; Lykousis et al. 2002) and a hotspot area for alien species in the basin, functioning as an intermediate stopover in the lessepsian migratory route (Peristeraki et al. 2007, 2017). The captured *S. chrysotaenia* specimens are thoroughly described, and they provide undisputable proof for the presence of the particular species in Crete. All four vouchers have been deposited in the Natural History Museum of Crete (NHMC) accredited with the collection numbers: NHMC80.1.705.1, NHMC80.1.705.2, NHMC80.1.705.3 and NHMC80.1.705.4

In a recent study, the presence of *S. chrysotaenia* was reported in Crete (Bilecenoglu et al. 2013), however this reference comes from visual *in situ* observations with no captured specimen descriptions and certainly no genetic validation. Visually, *in situ* detections in certain occasions constitute a valuable sampling technique since they can provide a wide range of data regarding the organisms observed. However, its value is limited when it comes to identifying very similar congeneric species whose legitimate identification requires captured specimens scrupulously examined in the lab. The close similarity of the lessepsian barracudas has quite often resulted in being confused (Smith 1956) and also in disputable nomenclature. So, it is the authors' opinion that meaningful information that actually contributes to the knowledge on the presence and distribution of these fishes in the Mediterranean can be extracted only from elaborately examined and genetically verified captured specimens. In similar cases, visual records should probably be confined to the taxonomic level that can be indisputably determined.



Figure 1. One of the four specimens of *Sphyraena chrysotaenia* caught in North Crete.

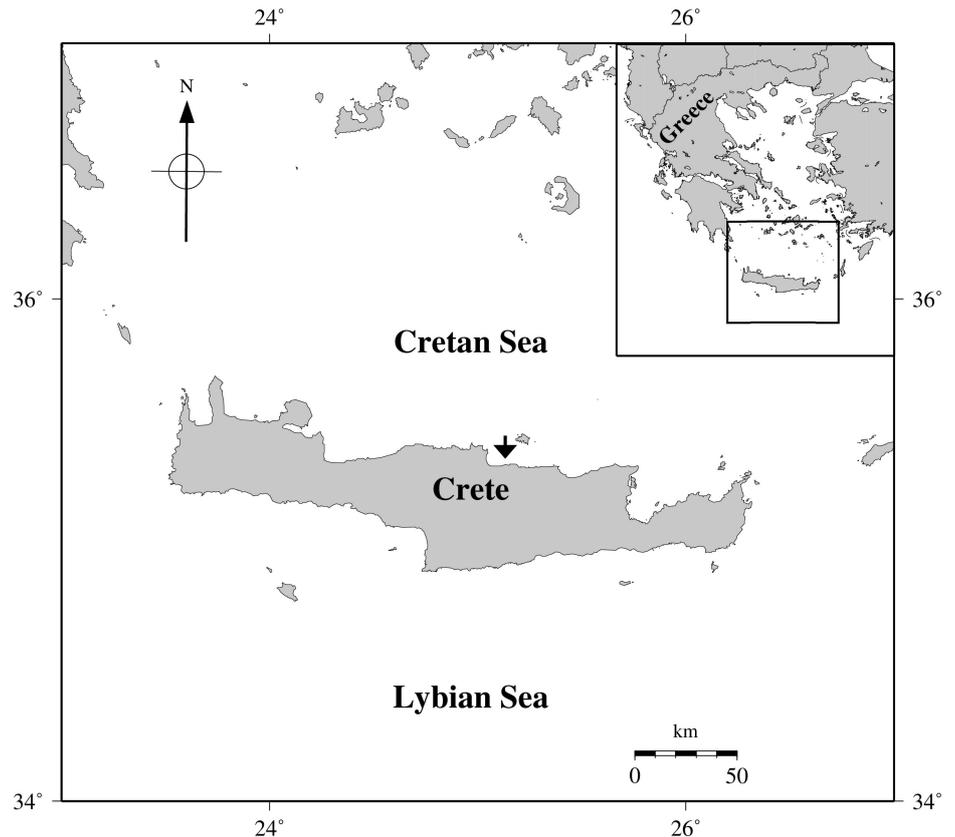


Figure 2. Collection site in Crete of the four specimens of *S. chrysotaenia* described in this study.

Materials and methods

Specimen collection

The four specimens of the lessepsian barracuda *S. chrysotaenia* (Figure 1) were captured on April 17 2018 by the commercial fishing vessel “STEFANOS III” in the Gulf of Heraklion, North Crete (Figure 2). They were captured during a purse-seine fishery expedition targeting *Boops boops*, at a depth of 49.3 m. The specimens were transferred to the laboratory of the Institute of Marine Biological Resources and Inland Waters (IMBRIW) of the Hellenic Center for Marine Research (HCMR) in Crete, where they were identified to the species level, following the species description provided by Golani et al. (2002). The nomenclature adopted in this work is the one most frequently used for this species by the majority of

Table 1. Morphology description of the four specimens of *S. chrysotaenia* caught in the Gulf of Heraklion, North Crete. All specimens had two gillrakers on the first gill arc. Length measurements are presented in millimeters (mm).

Meristics	Specimen 1	Specimen 2	Specimen 3	Specimen 4
First Dorsal (D ₁)	V	V	V	V
Second Dorsal (D ₂)	I + 9	I + 9	I + 9	I + 10
Pelvic (V)	I + 5	I + 5	I + 5	I + 5
Pectoral (P)	15	14	13	14
Anal (A)	II + 9	I + 9	II + 9	II + 9
Morphometry				
Total Length	220	222	225	213
Fork Length	203	204	208	197
Standard length	196	196	202	187
Head Length	61	60	62	57
Head Length at the end of the gill slit	23	23	25.5	23
Head Depth at the middle of orbit	19	18	19	18
Head Width at gill slit	18.5	18	18.5	18.5
Head Width at orbits	15	15	15.5	14
Eye Diameter (horizontal)	12.5	12	11	11.5
Eye Diameter (vertical)	12	12	11.5	10
Inter-Orbital	10.5	8.5	10	8
Pre-Orbital Length (Snout length)	29	29	29.5	26
Post-Orbital Length	177	182	184	175
Body Width at dorsal fin origin	19	20	20.5	17
Body Depth (maximum)	28	27	28	25
Body Depth (minimum)	13.5	13.5	13.5	12.5
Pre Dorsal Length	82	83	85	76
Dorsal Fin Base Length	23	19	17	22
Dorsal Fin Length	27	26	28	25
Second Dorsal Fin Base Length	18.5	21	18	22.5
Second Dorsal Fin Length	24	22	26	24
Pre Pectoral Length	62	62.5	64	58
Pectoral Fin Base Length	7.5	6.5	7.5	7.5
Pectoral Fin Length	22	23.5	25.5	24.5
Pre Pelvic Length	77	73	78.5	70
Pelvic (Ventral) Fin Base	5	4	4.5	4
Pelvic (Ventral) Fin Length	22	23	23	23.5
Pre-Anal Length	138	134	139.5	128.5
Anal Fin Base Length	20	19	20	17
Anal Fin Length	25.5	24	26	21.5
Caudal Peduncle Depth	15.5	14	14.5	14.5
Caudal Fin Height (vertically extended)	32	36.5	41	35

the authors in recent works (*S. chrysotaenia* over *S. pinguis*), as well as in official sites (e.g., CIESM, EASIN, ELNAIS). By adopting the specific nomenclature, we do not oppose the alternative suggestion; we rather stay on the conservative side until one generally accepted nomenclature is eventually adopted.

Morphological – Biological data collection

The morphological and biological data of all four specimens were recorded. Morphological data included a number of standard meristic counts and morphometry measurements (Table 1), while biological data included weight measurements (total and eviscerated), sex and maturity (Table 2). The maturity staging was done according to the MEDITS maturity scale protocol (MEDITS Working Group 2017: Instruction Manual). Lastly,

Table 2. Biological parameters of the four specimens of *S. chrysotaenia* caught in the Gulf of Heraklion, N. Crete. All weights are presented in grams (g).

Biological Parameter	Specimen 1	Specimen 2	Specimen 3	Specimen 4
Total Weight	55.9	51.6	58.8	48.3
Eviscerated Weight	52.5	49.2	56.5	46.4
Sex	male	female	female	female
Maturity Stage	developing	developing	developing	developing
Gonad Weight	0.6	0.8	1.1	0.7

tissue samples were extracted for genetic analysis in order to verify the taxonomy of the specimens.

DNA barcoding

Total genomic DNA was extracted from muscle tissue using a standard salt extraction protocol (Miller et al. 1988). DNA amplification of the COI gene was achieved with Polymerase Chain Reaction (PCR) using the primers FishF2 and FishR2 (Ward et al. 2005). The 12.5 µL PCR mix included 50–70 ng of template DNA, 1x Taq buffer, 0.2 µM of each primer, 0.2 mM dNTP mix, 1 U of Taq polymerase, 1.5 mM MgCl₂ and RNase-free pure water. The PCR cycling protocol 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 by a final extension step at 72 °C for 10 min. After purification with ethanol/sodium acetate precipitation, PCR products were sequenced with the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems Inc.) using an ABI 3730 capillary sequencer (Applied Biosystems) and following the manufacturer's instructions. Individual sequences were edited with MEGA 6.06 (Tamura et al. 2013) and were re-checked by visual inspection of raw fluorogram data. Amino acid translations were examined to ensure the absence of stop codons. The retrieved COI haplotypes were submitted to GenBank via Blastn (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and to the BOLD Identification Engine (<http://www.boldsystems.org>) in order to search for the nearest matches at species level in public libraries of sequences. In BOLD Identification Engine we used the Public Record Barcode Database (2,002,083 Sequences/131,781 Species/45,356 Interim Species) which includes all published COI records from BOLD and GenBank with a minimum sequence length of 500 bp. This library is a collection of records from the published projects section of BOLD. Genetic distances between haplotypes (p-distances) were also estimated with MEGA 6.06.

Results

Description of S. chrysotaenia specimens

The examined specimens had elongated bodies with large eyes and two dorsal fins well separated. The head was large with pointed snout with the lower jaw slightly protruded and without a fleshy knob on its tip. The posterior end of the maxillary bones did not reach the anterior edge of the

orbit. Large canine-like teeth, located on the jaws and on the palatine, vomer toothless. The first gill arch with two prominent gill rakers, the first on the junction of the upper and the lower part of the arch and the second raker on the lower part of the arch. The posterior tip of the opercular sharply pointed dorsally at the level of the pectoral fin base in specimens 1, 3, 4 and obtuse in specimen 2. Preopercular with a protrude membrane flap posteroventrally in all specimens.

The origin of the second dorsal fin was located just in front of the anal fin origin. The pectoral fin tips of all specimens extend slightly over the origin of the first dorsal fin. The pelvic fin origin was located well before the origin of the first dorsal and below the pectoral fin, about half way its length. Lateral line almost straight, scales small and cycloid. Color: grey-slightly yellowish on the back and white silvery on the belly. The caudal fin was forked, yellow in color, with whitish areas dorsally and ventrally in the posterior edges of the fin. The second dorsal fin was yellow and the pectoral fins yellowish. However, no obvious stripes were observed (frozen specimens).

The morphometry (meristics and measurements) of each specimen is presented in Table 1 while the biological characters examined are presented in Table 2. All four specimens had five spines on the first dorsal, which is a common taxonomic character of all Sphyraenidae (e.g. Abe 1974; Ben-Tuvia 1986). In those specimens with two spines on the anal fin, the first spine was very short and the second spine always shorter than the rays of the fin. The first two rays in the pectoral fin and the first ray on the dorsal were unbranched.

In the published literature on the lessepsian barracudas, there is a number of contradicting descriptions regarding certain taxonomic characters. A synoptic review of these characters for all four nominal lessepsian species is provided in Table 3 firstly for comparison reasons and secondly for establishing the ranges of the morphology characteristics used in the literature for each nominal species. The above comparisons highlight the existing problem in the descriptions of these species. The features for which there was an overall agreement (thus not included in Table 3) were the number of gill rakers on the first gill arc (2), the number of spines on the first dorsal (5), the number of spines (1) and rays (5) on the pelvic fin and the origin of the pelvic fin which was located before the origin of the first dorsal and below the pectoral, most frequently described as originating at about half the length of the pectoral fin.

DNA barcoding

A segment of COI gene 625 bp long was successfully sequenced in three specimens (DNA extraction failed in Specimen 4), resulting in two unique haplotypes (SC1 and SC2) which differ by only one nucleotide site (p-distance: 0.2%). Both haplotypes matched with sequences already retrieved from

Table 3. Agreements and disagreements in the literature regarding the described taxonomic characters for the four nominal lessepsian barracudas. The morphology descriptions are presented according to the nomenclature used in the published papers. Also, the meristics are presented the same way they were in the published works. Latin letters, represent the number of spines, Arabic numerals represent the number of soft rays. The small case letter “i”, according to the authors, denotes soft unbranched rays. D₁ = 1st dorsal, D₂ = 2nd dorsal, P = pectoral, V = pelvic, A = anal and C = caudal fins. According to the latest synonymization (Doiuchi and Nakabo 2005) *S. chrysotaenia* = *S. pinguis* and *S. flavicauda* = *S. obtusata*.

Taxonomic characters	<i>Sphyaena chrysotaenia</i>	<i>Sphyaena pinguis</i>	<i>Sphyaena flavicauda</i>	<i>Sphyaena obtusata</i>
D ₂	I+9 ^{[3][5][12][13][14]} / I+i8 ^{[4][10]} / 9-10 ^[17]	I+9 ^{[8][16]}	I+9 ^{[3][17][18]} / I+i8-9 ^[4] / I+i8 ^[10]	19(?) ^[2] / I+9 ^{[16][19][20]} / I+9-10 ^[21]
P	2, 12 ^[3] / ii 11-13 ^{[4][10]} / 13-14 ^[5] / 12-13 ^[13] / 15 ^[14] / 13 ^[17]	13-14 ^[8] / 11-13 ^[16]	2, 10, 1 ^[3] / ii 11 ^{[4][10]} / 14 ^[17] / 13 ^[18]	14 ^{[2][20]} / 11 ^[16] / 13 ^[19] / 11-13 ^[21]
A	II+ 8 ^{[3][13][14]} / II + i8 ^{[4][10]} / II+8-9 ^[5] / I+9 ^{[12][17]}	II+8-9 ^[8] / II+9 ^[16]	II+8-9 ^[3] / II+i7-8 ^[4] / II+i8 ^[10] / II+9 ^{[18][17]}	II+9 ^{[2][16][19][20][21]}
C	IV+15+IV ^[14]	17 ^[8]	16-17 ^[18]	17 ^[20]
P tip vs D ₁ origin	P tip reaches or passes D ₁ origin ^{[1][3][10][11][13][14][17]} to 1/6 of P length ^{[4][5]}	Did not reach D ₁ origin in half cases, it did in the rest ^[16] / it reaches ^{[8][20]} or does not reach ^[8] D ₁ origin	P tip does not reach D ₁ origin ^{[3][4][6][10][11][17][18][15][20]} or just reaches it ^{[3][4][10][11]}	P tip reaches or passes D ₁ origin ^{[2][16][19][20][15]} / P tip does not reach or just reaching D ₁ origin ^[16]
Operculum	Distinct skinny flap at angle of preoperculum ^{[3][5][10]} opercle with single membranous flap ^[10]	Posterior tip of operculum: sharply pointed ^{[16][20]} / a thin membranous point just above P base ^[8]		Lower posterior angle of preoperculum with produced membranous flap ^[4] / Posterior tip of operculum: obtuse ^[16]
Dorsal ray spines	2 nd D ₁ spine the longest ^[3] / 1 st D ₁ spine < 2 nd D ₁ spine or 1 st D ₁ spine ≥ 2 nd D ₁ spine ^[4]	1 st D ₁ spine > 1 st D ₂ ray ^{[16]*}	1 st D ₁ spine the longest ^[3] / 1 st D ₁ spine < 2 nd D ₁ spine or 1 st D ₁ spine ≥ 2 nd D ₁ spine ^[4]	1 st D ₁ spine < 1 st D ₂ ray ^[16]
Stripe position	Single brown-green stripe from snout through center of eye and above P base ^{[4][10]}	1 stripe ^[16] / 2 stripes in fresh specimens, lower stripe from snout through eye and above P base ^[8]	Single brown-green stripe from snout through eye and through base of P ^{[4][10]} / Two brown or brownish-yellow stripes ^[15]	Broad bands on the body dorso laterally ^[5] / no distinct stripes ^[15] / 2 stripes, the lower running through eye and upper part of P base ^[16] / 2 yellow-brown stripes ^[21] /
LL scales	85-87 ^[3] / 85-96 ^{[4][10]} usually 85-90 ^[4] / 86-87 ^[5] / 74-85 ^[12] / 82-86 ^[17]	80-95 ^[8]	80-90 ^{[4][10]} usually 84-88 ^[4] / 80-88 ^[3] / 85-90 ^[18] / 72-90 ^[17] / 84-91 ^[15]	83-86 ^[2] / 80-95 (usually 88-90) ^[8] / Less than 100, usually 80-90 ^{[7][9]} / 78-85 ^{[16][21]} / 90 ^[19]
C coloration	Yellow with dark margins ^{[4][5]} / yellow with trailing edge and upper edge black ^[10] / yellowish ^[14] / yellow ^[17]	Yellow with dark margin, especially in the fork ^[8]	Yellow with dark margin ^{[3][11]} / yellow-green, upper edge dusky ^[4] / dusky yellow ^[10] / yellow with upper, lower and posterior margins black ^[18]	Yellow ^{[2][20]} / yellow, entire margin dusky ^[16]
Maxillary vs orbit	Maxilla before eye ^{[5][10]} about width of pupil ^{[3][4]}	Maxilla before eye about width of pupil ^[8]	Maxilla before eye about width of pupil ^{[3][4]} / Maxilla reaching below anterior nostril ^[15]	Maxillary reaching nostrils ^[2] / Maxilla reaching to anterior margin of eye ^[9] / Maxilla not reaching anterior edge of eye ^[15] / Maxilla reaching or extending beyond anterior nostril ^[21]

*: Variation in this feature – a few times in *S. pinguis* 1st D₁ spine < 1st D₂ ray.

[1]: Schultz 1953, [2]: Ben-Tuvia 1953, [3]: Smith 1956, [4]: Williams 1959, [5]: Dutt and Rao 1967, [6]: de Sylva 1973, [7]: Abe 1974, [8]: Au 1979, [9]: Rose 1983, [10]: de Sylva and Williams 1986, [11]: Golani 1992, [12]: Gucu et al. 1994, [13]: Corsini and Economidis 1999, [14]: Pallaoro and Dulcic 2001, [15]: Senou 2001, [16]: Doiuchi and Nakabo 2005, [17]: Golani et al. 2006, [18]: Corsini et al. 2005, [19]: Iglésias and Frotté 2015, [20]: Karna et al. 2018, [21]: Kimura et al. 2009.

S. chrysotaenia specimens that have been collected in the Mediterranean coasts of Israel, Turkey and Lebanon (Table 4). These sequences are included in BOLD BIN AAD0400 which consists of *S. chrysotaenia* specimens collected

Table 4. Similarity of COI sequences from three specimens with available barcodes of *S. chrysotaenia* in public repositories.

Species	Specimen no	Haplotype	Genbank Accession Number	BOLD top hit (similarity)	BOLD BIN ID <i>Species within BIN</i>	Genbank closest match
<i>Sphyraena chrysotaenia</i>	1	SC1	MT348376	BIM300-13 (100%)	BOLD:AAD0400 <i>S. chrysotaenia</i> <i>S. obtusata</i>	-KY176643.1 (100%) <i>S. chrysotaenia</i> , Turkey, Gulf of Iskenderun
	3	SC1	MT348378	<i>S. chrysotaenia</i> Israel, Nitzanim (Med. Sea)		-KR861562.1 (100%) <i>S. chrysotaenia</i> , Lebanon
	2	SC2	MT348377	BIM016-13 (100%) <i>S. chrysotaenia</i> Israel, Nitzanim (Med. Sea)		-KY176643.1 (99%) <i>S. chrysotaenia</i> , Turkey: Gulf of Iskenderun -KR861562.1 (99%) <i>S. chrysotaenia</i> , Lebanon

not only in the Mediterranean Sea but also in the native range of the species (Indo-Pacific).

Discussion

If we consider that all species identifications and the morphological descriptions provided for them by the different authors are accurate, then, after summing up all the information provided for each nominal species, (presented in Table 3), the meristic formulas for the four nominal lessepsian sphyraenids develop as follows:

For *S. chrysotaenia*: D1: V, D2: I + 9–10, P: 11–15, V: I + 5, A: II + 8–9, C: IV + 15 + IV, LL scales: 74–96, for *S. pinguis*: D1: V, D2: I + 9, P: 11–14, V: I + 5, A: II + 8–9, C: 17, LL scales: 80–95, for *S. flavicauda*: D1: V, D2: I + 9, P: 13–14, V: I + 5, A: II + 8–9, C: 16–17, LL scales: 72–91, for *S. obtusata*: D1: V, D2: I + 9–10, P: 11–14, V: I + 5, A: II + 9, C: 17, LL scales: 80–95.

The above formulas show considerable overlap on the meristics for these nominal species, thus minimizing their value as species diagnostic characters. As we can see in Table 3, there are also important discrepancies in the formulas presented by different authors, even for the same species. Other descriptive characters that have been used for distinguishing these species, such as the relation of the pectoral fin tips in respect to the dorsal origin, also show considerable contradictions even within each species, thus almost cancelling out their usefulness. Lastly, other characters that have been commonly used when describing the species, such as the length of the dorsal spines in relation to the dorsal ray lengths and the scale count along the lateral line, present significant variability in the published literature, rendering them as weak taxonomic characters.

These contradictory descriptions could partly be explained by the successive nomenclature changes that took place for these species, which have resulted to either identifying the same species under different names, or describing different species under the same species name. The outcome of all this confusion is revealed in Table 3, where the four nominal species are presented with overlapping (and even contradictory) morphological characteristics.

Other possible reasons for these inconsistencies could be either the morphological variability within each species, which may be greater than the one considered so far, and/or the existence of various allometric changes with age and/or size. The above would have resulted in classifying specimens of the same species but of different ontogenetic stage to different nominal species (e.g. Smith 1956; de Sylva 1973). The existence of such allometric changes in the barracudas has been suggested by Smith (1956) and Williams (1959), but it has not been confirmed for all *Sphyraena* species. If this is the case, the detection of these varying characters and their exclusion from the list of the species' diagnostic characters will hopefully minimize the confusion in these species descriptions. The problem with the systematics of the Sphyraenidae and the lack of comprehensive reviews for this family has been stressed many times over in the past (e.g. de Sylva 1973; Au 1979; Rose 1983; Doiuchi and Nakabo 2005), emphasizing the need for a world-wide revision of this family (e.g. Dutt and Rao 1967). This group of fishes certainly requires a closer and more elaborate study of their morphology, in order to discover more consistent and adequate species-specific characters, coupled with specimens' genetic descriptions. This may eventually clarify the number of the existing barracuda species as well as the nomenclature status of the lessepsian ones.

Even though the specimens described in this work constitute the first verified and registered specimens of *S. chrysotaenia* in Crete, another 67 specimens of the group of the lessepsian barracudas were recorded during on-board sampling expeditions around the Island from 2015 to 2018. Those specimens were captured by professional fishermen using a variety of fishing gears (trammel nets, gill nets, beach seines, bottom trawls and purse seines), deployed both in coastal and offshore areas in depths ranging from 6 to 277 m. The lessepsian *Sphyraena* species are easily distinguished from the two native ones since they exhibit conspicuous morphological differences (e.g., the origin of the ventral fins *vs* the origin of the dorsal fin). However, their identification to species level requires more meticulous examination, most safely done in the lab. Thus, these on-board identifications were preferably restricted to the genus level.

The present work verifies the presence of *S. chrysotaenia* in Crete. As for the second lessepsian sphyraenid (*S. flavicauda*), there are no records in the island yet, however this could be due to the great difficulty in distinguishing these two species. These species share common ecological habits, often dwelling in the same areas. *Sphyraena flavicauda* in particular has been reported in Rhodes 14 years ago, dwelling along with the other three barracudas found in the Mediterranean (Corsini et al. 2005). Since Crete constitutes a stop-over in the migratory route of the majority of the lessepsian migrants, it is only natural to suspect this species' presence in the Island as well, especially if we consider the relatively long presence of *S. flavicauda* in the basin and its pelagic mode of life which favors its

spread (Corsini et al. 2005). Otherwise, *S. flavicauda* must have followed a very unusual migratory route very different from the one followed by the other lessepsian migrants. The issue of the number of lessepsian barracudas in Crete can be resolved only with persistent and attentive morphological and genetic examinations of as many as possible new *Sphyraena* specimens captured in the Island.

Acknowledgements

The authors wish to express their gratitude to Mr. Nikolaos Meris, captain of the fishing Vessel “STEFANOS III” for providing the specimens and to Dr. Nikolaos Lampadariou for providing the map. We would also like to thank the two anonymous reviewers for their detailed comments and suggestions that helped improve the manuscript. The authors received no specific funding for this work. Declaration of interest: none. All research pertaining to this article did not require any research permits.

References

- Abe T (1974) Sphyraenidae. In: Fisher W, Whitehead PJP (eds), FAO Species Identification Sheets for Fishery Purposes. Eastern Indian Ocean (Fishing Area 57) & Western Central Atlantic (Fishing Area 71), Volume 4: Bony Fishes: Families from S (in part) to Z. FAO, Rome, 10 pp
- Allam SM, Faltas SN, Ragheb E (2004) Reproductive biology of *Sphyraena* species in the Egyptian Mediterranean waters off Alexandria. *Egyptian Journal of Aquatic Research* 30(B): 255–270
- Au K-C (1979) Systematic study on the barracudas (Pisces: Sphyraenidae) from a northern sector of the South China Sea. *Journal of Natural History* 13: 619–647, <https://doi.org/10.1080/00222937900770471>
- Ben-Tuvia A (1953) Mediterranean fishes of Israel. The Sea Fisheries Research Station, Caesarea, Israel, Bulletin no 8, 40 pp, <https://doi.org/10.2307/1441133>
- Ben-Tuvia A (1966) Red Sea fishes recently found in the Mediterranean. *Copeia* 2: 254–275, <https://doi.org/10.2307/1441133>
- Ben-Tuvia A (1986) Sphyraenidae. In: Whitehead PJP, Buchot M-L, Hureau J-C, Nielsen J, Tortonese E (eds), Fishes of the North-eastern Atlantic and the Mediterranean. Unesco, Paris, Volume III, pp 1015–1473
- Bilecenoglu M, Alfaya J, Azzurro E, Baldaconi R, Boyaci R, Circosta V, Compagno L, Coppola F, Deidun A, Durgham H, Durucan F, Ergüden D, Fernández-Álvarez F, Gianguzza P, Giglio G, Gökoğlu M, Gürlek M, Ikhtiyar S, Kabasakal H, Karachle P, Katsanevakis S, Koutsogiannopoulos S, Lanfranco E, Micarelli P, Özvarol Y, Pena-Rivas L, Poursanidis D, Saliba J, Sperone E, Tibullo D, Tiralongo F, Triperi S, Turan C, Vella P, Yokeş M, Zava B (2013) New Mediterranean marine biodiversity records (December 2013). *Mediterranean Marine Science* 14: 463–480, <https://doi.org/10.12681/mms.676>
- Chronis G, Stavrakakis S, Danovaro R, Tselepedes A, Wassmann P, Duineveld G, Della Croce N, Karageorgis A, Zivanovic S, Ypma JE, Dell’Anno A, Marralle D, Martorano D, Kabouri G, Pagou K (1996) Downward fluxes. In: Tselepidis A, Papadopoulou KN, Polychronaki T (eds), CINCS: Pelagic-benthic coupling in the oligotrophic Cretan Sea. MAST-II, Mediterranean targeted project, Final Report. Heraklion, Greece, pp 82–112
- CIESM (2020) The Mediterranean Science Commission, CIESM Atlas of Exotic Fishes in the Mediterranean Sea. <http://www.ciesm.org/atlas/appendix1.html> (accessed 13 April 2020)
- Corsini M, Economidis PS (1999) Distribution extension of two lessepsian migrants found in the marine area of the island of Rhodes (Aegean Sea, Greece). *Cybium* 23: 195–199
- Corsini M, Margies P, Kondilatos G, Economidis PS (2005) Lessepsian migration of fishes to the Aegean Sea: First record of *Tylerius spinosissimus* (Tetraodontidae) from the Mediterranean, and six more fish records from Rhodes. *Cybium* 29: 347–354
- Danovaro R, Della Croce N, Dell’Anno A, Marralle A, Pusceddu A (1996) Seasonal and spatial distribution of pico-, nano-, and microparticulate matter and bacterioplankton in the Eastern Mediterranean Sea. In: Tselepidis A, Papadopoulou KN, Polychronaki T (eds), CINCS: Pelagic-benthic coupling in the oligotrophic Cretan Sea. MAST-II, Mediterranean targeted project, Final Report. Heraklion, Greece, pp 69–81
- de Sylva DP (1963) Systematics and life history of the great barracuda, *Sphyraena barracuda* (Walbaum). Studies in Tropical Oceanography 1, University of Miami Press, 179 pp, https://scholarlyrepository.miami.edu/trop_ocean/1

- de Sylva DP (1973) Barracudas (Pisces: Sphyraenidae) of the Indian Ocean and adjacent seas - A preliminary review of their systematics and ecology. *Journal of the Marine Biological Association of India* 15: 74–94
- de Sylva DP, Williams F (1986) Sphyraenidae. In: Smith MM, Heemstra PC (eds), *Smith's Sea Fishes*. Smith Institute of Ichthyology, Grahamstown, South Africa, pp 721–726
- Doiuchi R, Nakabo T (2005) The *Sphyraena obtusata* group (Perciformes: Sphyraenidae) with a description of a new species from southern Japan. *Ichthyological Research* 52: 132–151, <https://doi.org/10.1007/s10228-004-0263-1>
- Dutt S, Rao BVS (1967) The Sphyraenidae of the Indian coasts - I. *Sphyranella chrysotaenia* (Klunzinger), 1844 and *Sphyraena genie* Klunzinger, 1870. *Proceedings of the Indian Academy of Sciences - Section B* 65: 238–248, <https://doi.org/10.1007/BF03052548>
- EASIN (2020) European Alien Species Information Network, Joint Research Centre (EASIN). <http://alien.jrc.ec.europa.eu/SpeciesMapper> (accessed 13 April 2020)
- ELNAIS (2020) Ellenic Network on Aquatic Invasive Species (ELNAIS). <https://elnais.hcmr.gr/?s=Sphyraena> (accessed 13 April 2020)
- Eschmeyer WN, Fong JD (2019) Eschmeyer's catalog of fishes. <http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp#Sphyraenidae> (accessed 21 May 2019)
- Galil BS (2007) Seeing Red: Alien species along the Mediterranean coast of Israel. *Aquatic Invasions* 2: 281–312, <https://doi.org/10.3391/ai.2007.2.4.2>
- Golani D (1992) *Rhabdosargus haffara* (Forsskål, 1775) and *Sphyraena flavicauda* Rüppell, 1833—new Red Sea immigrants in the Mediterranean. *Journal of Fish Biology* 40: 139–140, <https://doi.org/10.1111/j.1095-8649.1992.tb02561.x>
- Golani D, Orsi-Relini L, Massutí E, Quignard JP (2002) CIESM Atlas of exotic species in the Mediterranean. Volume 1: Fishes. Briand F (ed), CIESM, Monaco, 254 pp
- Golani G, Öztürk B, Başusta N (2006) The Fishes of the Eastern Mediterranean. Turkish Marine Research Foundation, Istanbul, Turkey, 260 pp
- Gucu AC, Bingel F, Avsar D, Uysal N (1994) Distribution and occurrence of Red Sea fish at the Turkish Mediterranean coast—northern Cilician basin. *Acta Adriatica* 34: 103–113
- Halim Y, Rizkalla S (2011) Aliens in Egyptian Mediterranean waters. A check-list of Erythrean fish with new records. *Mediterranean Marine Science* 12: 479–490, <https://doi.org/10.12681/mms.46>
- Iglésias SP, Frotté L (2015) Alien marine fishes in Cyprus: update and new records. *Aquatic Invasions* 10: 425–438, <https://doi.org/10.3391/ai.2015.10.4.06>
- Kalogirou S, Mittermayer F, Pihl L, Wennhage H (2012) Feeding ecology of indigenous and non-indigenous fish species within the family Sphyraenidae. *Journal of Fish Biology* 80: 2528–2548, <https://doi.org/10.1111/j.1095-8649.2012.03306.x>
- Karahan A, Douek J, Paz G, Stern N, Kideys AE, Shaish L, Goren M, Rinkevich B (2017) Employing DNA barcoding as taxonomy and conservation tools for fish species censuses at the southeastern Mediterranean, a hot-spot area for biological invasion. *Journal for Nature Conservation* 36: 1–9, <https://doi.org/10.1016/j.jnc.2017.01.004>
- Karna SK, Manna RK, Mukherjee M, Suresh VR (2018) Occurrence of obtuse barracuda *Sphyraena obtusata* Cuvier, 1829 (Actinopterygii: Perciformes: Sphyraenidae) from Chilika lagoon, Odisha coast of India. *Indian Journal of Geo Marine Sciences* 47(12): 2549–2551
- Kimura S, Satapoomin U, Matsuura K (2009) Fishes of the Andaman Sea: West Coast of Southern Thailand. National Museum of Nature and Science, Tokyo, 346 pp
- Lykousis V, Chronis G, Tselepidis A, Price NB, Theocharis A, Sikou-Frangou I, Van Wambeke F, Danovaro R, Stavrakakis S, Duineveld G, Georgopoulos D, Ignatiades L, Souvermezoglou A, Voutsinou-Taliadouri F (2002) Major outputs of the recent multidisciplinary biogeochemical researches undertaken in the Aegean Sea. *Journal of Marine Systems* 33–34: 313–334, [https://doi.org/10.1016/S0924-7963\(02\)00064-7](https://doi.org/10.1016/S0924-7963(02)00064-7)
- MEDITS Working Group (2017) Codes of sexual maturity for Fish, Crustaceans and Cephalopods, In: International Bottom Trawl Survey in the Mediterranean, Instruction Manual, version 9, 106 pp, ANNEX VIII
- Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* 16: 1215, <https://doi.org/10.1093/nar/16.3.1215>
- Nelson JS (2006) *Fishes of the World*, 4th edn. Hoboken, New Jersey, 601 pp
- Pallaoro A, Dulčić J (2001) First record of the *Sphyraena chrysotaenia* (Klunzinger, 1884) (Pisces, Sphyraenidae) from the Adriatic Sea. *Journal of Fish Biology* 59: 179–182, <https://doi.org/10.1111/j.1095-8649.2001.tb02349.x>
- Pastore MA (2009) *Sphyraena intermedia* sp. nov. (Pisces: Sphyraenidae): a potential new species of barracuda identified from the central Mediterranean Sea. *Journal of the Marine Biological Association of the United Kingdom* 89: 1299–1303, <https://doi.org/10.1017/S0025315409000575>
- Peristeraki P, Lazarakis G, Skarvelis K, Georgiadis M, Tserpes G (2007) Additional records on the occurrence of alien fish species in the eastern Mediterranean Sea. *Mediterranean Marine Science* 7: 61–67, <https://doi.org/10.12681/mms.170>

- Peristeraki P, Tserpes G, Lampadariou N, Stergiou KI (2017) Comparing demersal megafaunal species diversity along the depth gradient within the South Aegean and Cretan Seas (Eastern Mediterranean). *PLoS ONE* 12: e0184241, <https://doi.org/10.1371/journal.pone.0184241>
- Psarra S, Tselepidis A, Ignatiades L, Dafnomili E (1996) Primary production estimates in the Cretan Sea. In: Tselepidis A, Papadopoulou KN, Polychronaki T (eds), CINCS: Pelagic-benthic coupling in the oligotrophic Cretan Sea. MAST-II, Mediterranean targeted project, Final Report. Heraklion, Greece, pp 46–56
- Rim Z-K, Ennejjar S, Bradai M-N, Ghorbel M, Bouain A (2007) Age and growth of the lessepsian migrant *Sphyraena chrysaenia* Klunzinger, 1884 from the Gulf of Gabes (Eastern Mediterranean). *Reviews in Fisheries Science* 15: 169–181, <https://doi.org/10.1080/10641260701484259>
- Rose JM (1983) Sphyraenidae, In: Fischer W, Bianchi G, (eds), FAO Species Identification Sheets, Fishing Area 51: Western Indian Ocean, Volume 4. FAO, Rome, 10 pp
- Santini F, Carnevale G, Sorenson L (2015) First timetree of Sphyraenidae (Percomorpha) reveals a Middle Eocene crown age and an Oligo-Miocene radiation of barracudas. *Italian Journal of Zoology* 82: 133–142, <https://doi.org/10.1080/11250003.2014.962630>
- Schultz LP (1953) Order Percomorphida, Suborder Sphyraenina, Family Sphyraenidae: Barracudas. In: Schultz LP, Herald ES, Lachner A, Welander AD, Woods LP (eds), Fishes of the Marshall and Marianas Islands. Smithsonian Institution, United States National Museum, Bulletin 202, pp 279–286
- Senou H (2001) Sphyraenidae. In: Carpenter KE, Niem VH (eds), The Living Marine Resources of the Central Pacific. FAO Species Identification Guide for Fishery Purposes, FAO, Rome, Vol 6, pp 3685–3693
- Shirak A, Dor L, Seroussi E, Ron M, Hulata G, Golani D (2016) DNA barcoding of fish species from the Mediterranean coast of Israel. *Mediterranean Marine Science* 17: 459–466, <https://doi.org/10.12681/mms.1384>
- Smith JLB (1956) The fishes of the family Sphyraenidae in the Western Indian Ocean. *Ichthyological Bulletin* 3: 37–46
- Spicer IJ (1931) Fisheries. In: Report of the Department of Agriculture and Forests for the years 1927-30. Printing Office, Russian Building, Jerusalem, pp 159–160
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729, <https://doi.org/10.1093/molbev/mst197>
- Ward RD, Zemplak TS, Innes B, Last PR, Hebert PDN (2005) DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London. Series B Biological Sciences* 360: 1847–1857, <https://doi.org/10.1098/rstb.2005.1716>
- Williams F (1959) The barracudas (Genus *Sphyraena*) in British East African waters. *Annals and Magazine of Natural History* 2: 92–128, <https://doi.org/10.1080/00222935908651031>