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New Data on the Systematics of Comb-fin Squids *Chtenopteryx* spp. (Cephalopoda: Chtenopterygidae) from the Canary Islands

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Alejandro Escánez, Álvaro Roura, Rodrigo Riera, Ángel Francisco González, and Ángel Guerra (2018) The systematics of the comb-fin squid species is problematic and poorly resolved. In total, 53 specimens of comb-fin squids (*Chtenopteryx* spp.) were caught at depths ranging from 30 to 800 m off the Canary Islands (NE Atlantic Ocean). Mantle lengths of the individuals ranged from 18 to 43 mm and the sample included immature, mature male and mature female specimens. Two species of comb-fin squids, *Chtenopteryx canariensis* and *C. sicula*, were identified by combining traditional morphological characters with a molecular analysis of a fragment of the cytochrome *c* oxidase subunit I (COI) gene. Intra- and interspecific genetic distances and maximum likelihood tree analyses based on COI sequences available from GenBank suggest the existence of at least four species, two from the Pacific and two from the Atlantic Ocean. Our data expand the current geographic range of *C. canariensis* from the NE to NW Atlantic. In the GenBank database, several sequences of comb-fin squid in different species-specific clades have been attributed only to *C. sicula*, indicating the possible existence of cryptic species and the need to re-analyse these data.

Key words: *Chtenopteryx sicula*, *Chtenopteryx canariensis*, Cytochrome *c* oxidase subunit I (COI), Systematics, Central east Atlantic.

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BACKGROUND

The genus *Chtenopteryx* Appellöf, 1890 is composed of a group of small, muscular mesopelagic squids within the monogeneric family Chtenopterygidae Grimpe 1922. Comb-fin squids are distributed in tropical and subtropical waters worldwide at depths from sea surface to 1,000 m, showing a gradual ontogenic descent into deeper waters with increasing dorsal mantle length (ML) (Shea and Vecchione 2010). Generally, large specimens of chtenopterygiids with ML up to 100 mm are scarce in mid-water trawl samples, while small specimens are common in plankton nets (Vecchione et al. 2001). Unfortunately, most of the descriptions of *Chteropteryx* species are based on damaged specimens collected from stomach contents of predators such as fishes and cetaceans, or small poorly preserved specimens (Young and Vecchione 2016).

The diagnostic characters that differentiate *Chtenopteryx* species are currently limited to the following: (i) the maximum number of sucker series on arms and tentacular clubs, (ii) the presence/absence and size of visceral photophores, (iii) the presence/absence of eyeball photogenic patches and (iv) mantle width relative to ML (Roper and Jereb 2010). Nesis (1987), Guerra (1992) and Roper and Jereb (2010) indicated that mature males develop a large, dorsally directed photophore in the posterior mantle cavity. Although Young and Vecchione (2010) indicated that males of some *Chtenopteryx* species possess a large photophore that lies within the shell sac, they did not consider this light organ as a diagnostic character within the genus. According to Young and Vecchione (2010), only *Chtenopteryx sicula* Vérany 1851, *C. sepioloides* Rancurel 1970 and *C. canariensis* Salcedo-Vargas & Guerrero-Kommritz, 2000 are currently considered to be valid species. In contrast, Jereb and Roper (2010) considered that *C. sicula* and *C. sepioloides* should be recognized. *Chtenopteryx chuni* Pfeffer, 1912 and *C. canariensis* have an undetermined status due to their small size and the low number of specimens studied. Several inappropriately described species like *C. neuroptera* Jatta, 1896; *C. fimbriatus* Appellöf, 1890 and *C. cyprinoides* Joubin, 1894 currently have been synonymized to *C. sicula* (Young and Vecchione 2016).

Chtenopteryx sicula is the type species of the genus. However, a detailed description of this squid has not been published to the best of our knowledge. Joubin (1900), Pfeffer (1912) and Naef (1923) re-described the species, but always based on poor material or very few and small

individuals. Young and Vecchione (2016) examined two specimens from the Mediterranean Sea that do have the visceral photophore, which contrasts with the description by Naef (1923). Therefore, concerning photophores, *C. sicula* should have a large photophore on the ventral surface of the eyeball and a visceral photophore; however, sometimes these are not discernible as they are in other descriptions, e.g. lacking (Naef 1923; Jereb and Roper 2010), supposedly present (Nesis 1987), present (Guerra 1992), lacking or present (Young and Vecchione 2016). As a result, the identity of *C. sicula* remains questionable and its presence outside the Mediterranean is considered unresolved (Young and Vecchione 2016).

Chtenopteryx sepioloides was described based on four specimens found in the stomachs of lancetfish (*Alepisaurus pherox*) captured in the Pacific Ocean (Rancurel 1970). *Chtenopteryx chuni* was described by Pfeffer (1912) based on a single young specimen with 7 mm ML, from the outer edge of the Benguela Current in the South Atlantic Ocean and is currently considered as taxon *inquirendum.* The original description of *C. canariensis* by Salcedo-Vargas and Guerrero-Kommritz, 2000 was based on four specimens - 2 mature males (63 and 65 mm ML), 1 immature female (55 mm ML) and 1 juvenile (12 mm ML) - collected from deep trawls (1,000 m) in the eastern Atlantic Ocean. The holotype (mature male) was captured south of the Canary Islands (26°20'N, 19°21'W). The description of this species differed from the remaining species of the genus in its lack of photophores on both eyes and viscera (Salcedo-Vargas and Guerrero-Kommritz 2000).

The aim of this paper is to undertake a detailed morphological and molecular analysis of the two morphologically different comb-fin squid species recently caught off the Canary Islands and provide some additional diagnostic characters for these uncommon and poorly described mesopelagic squid species.

MATERIALS AND METHODS

Two specimens of *Chtenopteryx canariensis* and fifty-one *C. sicula* identified according with Guerra (1992) were caught between 30 and 800 m depth, with an open mid-water trawl of 300 m^2 mouth area and 45 m length. The mesh size was 80 cm near the opening, decreasing to 1 cm in the last 10 m of the cod end. The hauls were performed over the SW continental slopes off El Hierro, La Palma and Tenerife (Canary Islands, NE Atlantic Ocean) (Fig. 1), during the mesopelagic survey CETOBAPH carried out in April 2012 (4-19th), on board R/V “Cornide de Saavedra”. Data from hauls are summarized in table 1. On board, the specimens were identified to the species level following Nesis (1987), Guerra (1992), Roper and Jereb (2010) and Young and

Vecchione (2010), their ML was measured to the nearest mm and they were sexed. The specimens were fixed in 70% ethanol and stored in the collection of the Department of Animal Biology of the University of La Laguna (Tenerife, Canary Islands, Spain). In addition, three Mediterranean specimens from the Biological Collection of Marine Institute of Barcelona (ICM-CSIC), classified as *C. sicula* reference numbers (ICMC 13050202; ICMC 13050201; ICMC 594/1991) were used for morphological comparative study. These specimens were preserved in formaldehyde solution, inappropriate for molecular analysis.

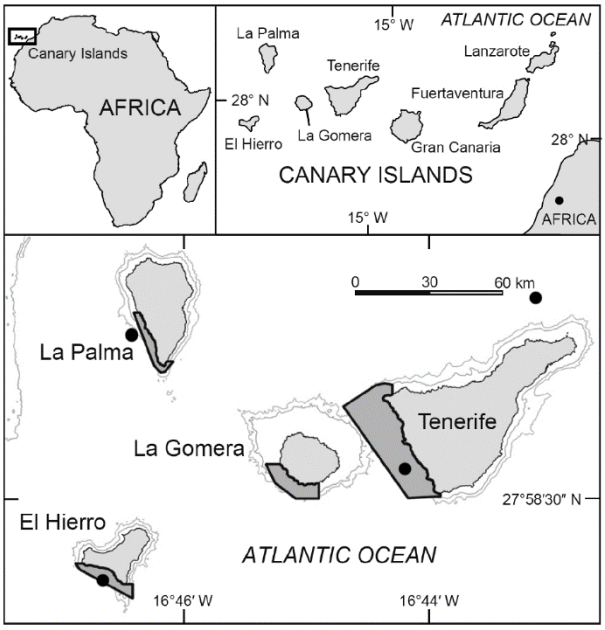

Fig. 1. Map of the sampling areas. Black dots indicate fishing areas. Grey zones correspond to marine protected areas.

Table 1. Data of hauls in which the *Chtenopteryx* specimens were captured; Min. Depth: minimum depth of trawl expressed in metres; Max. Depth: maximum depth of trawl expressed in metres; Av. Depth: average depth of trawl expressed in metres

Haul code	Date (m/d/y)	Latitude (N)	Longitude (W)	Time	Min. Depth	Max. Depth	Av. Depth
EH-01	04/05/12	27º 38.860'	18º 02.450'	23:30	30	190	130,5
EH-08	04/08/12	27º 39.300'	18º 04.113'	14:04	603	800	684
TF-05	04/16/12	28º 04.500'	16º 49.300'	1:44	349	531	472
TF-01	04/14/12	28º 05.681'	16º 50.305'	22:40	97	208	150
TF-02	04/15/12	28º 04.670'	16º 49.440'	1:20	360	486	453
TF-06	04/16/12	28º 04.216'	16º 49.063'	4:30	89	199	149
TF-04	04/15/12	28º 05.300'	16º 50.000'	22:54	526	670	586
TF-09	04/17/12	28º 05.000'	16º 49.900'	16:25	710	900	833
LP-12	04/14/12	28º 35.400'	18º 00.200'	4:12	40	206	130
OC-01	04/19/12	28º 49.100'	16º 00.500'	23:20	50	150	105

DNA amplification, sequencing and analysis

Small pieces of mantle tissue of one *C. canariensis* and three *C. sicula* were sampled and stored in 70% ethanol at 4°C. Genomic DNA was isolated using Macherey-Nagel NucleoSpin® tissue kit following the manufacturer's recommended protocols. The barcoding region of the mitochondrial COI (650 base pairs) gene was amplified using the forward primer HCO 2198 and reverse primer LCO1490 (Folmer et al. 1994). The polymerase chain reactions (PCRs) were carried out in a total volume of 25 µl containing 100 ng of genomic DNA, 10 µM of each primer, 2.5 µl of 10 x buffer, 0.5 µl of dNTPs and 5 U/µl of Taq DNA polymerase from *Thermus Aquaticus* BM, recombinant, Roche. PCR cycling parameters were: denaturation at 94°C for 2 min, followed by 39 cycles of 94°C for 15 s, annealing at 48°C for 30 s and extension at 72°C for 45 s, and a final extension at 72°C for 7 min. PCR products were purified using Illustra ExoStar 1-Step© following the manufacturer's recommended protocols, with minor modifications in the incubation temperature and enzyme concentration. We added 4 µl of reactive Illustra ExoStar 1-Step© and incubated the mix for 15 min at 37°C. In order to inactivate the reaction, we incubated the mix for 20 min at 80°C. Samples with DNA concentrations of 20 ng/ml were sequenced by Secugen® (Madrid).

Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990) was used to find the closest homologous sequences available in GenBank online database (Benson et al. 2011). The search was conducted in the nucleotide collection database (nr/nt), optimizing for highly similar sequences (megablast). Eight COI homologous sequences from GenBank were downloaded, and one more sequence was selected as an out-group (Table 2). The program CLUSTAL-W was used to compare and align all sequences. Phylogenetic relationships between the taxa analyzed were inferred by maximum likelihood (MaxL) topologies. Strength of support for internal nodes of ML construction was measured using 1,000 bootstrap replicates under GTR + I + G evolutive model. Inter and intragroup genetic distances were calculated using the Tamura-Nei model and 1,000 bootstrap replicates (Tamura and Nei 1993). All procedures were conducted in MEGA7 software (Kumar et al. 2016). The measurements for ML, mantle width, weight, sex and maturity of all specimens analysed for each species are shown in table 3.

Table 2. GenBank accession numbers for taxa used in this study. Asterisk (*) indicates unpublished data. Accs. Number: accession number reference in GenBank

Accs. number	Species	Author	Location
AY293705	*C. sicula*	Nishiguchi et al. 2004	South Pacific, Japan
AY557526	*C. sicula*	Lindgren 2004	Pacific Ocean, Hawaii

HQ386019	*C. sicula*	Elliger et al. 2010*	Pacific Ocean
HQ386018	*C. sicula*	Elliger et al. 2010*	Pacific Ocean
GU145076	*C. sicula*	Bucklin et al. 2009*	Atlantic Ocean
AF000033	*C. sicula*	Carlini and Graves 1999	Pacific Ocean
EU735388	*Chtenopteryx* spp.	Lindgren et. al 2004	North Atlantic
EU735369	*C. sicula*	Lindgren et al. 2004	North Atlantic
B1_0F74M	*C. sicula*	Herein	North Atlantic, Canary Islands
G4_0F74L	*C. sicula*	Herein	North Atlantic, Canary Islands
E4_0F74L	*C. sicula*	Herein	North Atlantic, Canary Islands
H4_OF74K	*C. canariensis*	Herein	North Atlantic, Canary Islands
GU145064	*Pterygioteuthis giardi*	Jennings 2009*	Northeast Atlantic

Table 3. Biometric data of *Chtenopteryx* spp. caught in the Canary Islands. Reference number (RN); dorsal mantle length (ML, mm); mantle width (MW, mm), weight in grams (W); Sex (S): In: indeterminate; M: male, F: female; Maturity stage (MI): I: immature, II: maturating, III: mature; -: no data; D: damaged

Species	RN	ML	MW	W	S	MI	Species	RF	ML	MW	W	S	MI
C. sicula	EH1-1	21	-	0.92	-	-	*C. sicula*	EH1-32	24	13	1.24	In	-
C. sicula	EH1-10	29	17	1.70	M	III	*C. sicula*	EH1-33	20	-	0.83	In	-
C. sicula	EH1-11	27	17	1.18	M	II	*C. sicula*	EH1-34	26	13	1.13	M	II
C. sicula	EH1-12	27	15	1.28	M	II	*C. sicula*	EH1-35	27	-	-	-	D
C. sicula	EH1-13	29	19	1.39	M	III	*C. sicula*	EH1-36	18	-	-	-	D
C. sicula	EH1-14	24	15	1.25	In	-	*C. sicula*	EH1-4	-	-	0.26	In	-
C. sicula	EH1-15	28	14	1.97	F	II	*C. sicula*	EH1-5	-	-	0.62	In	-
C. sicula	EH1-16	30	-	2.25	M	II	*C. sicula*	EH1-6	-	-	0.51	In	-
C. sicula	EH1-17	19	-	0.86	In	-	*C. sicula*	EH1-7	23	14	0.87	M	II
C. sicula	EH1-18	34	-	3.51	F	III	*C. sicula*	EH1-8	24	16	1.30	M	II
C. sicula	EH1-19	24	-	1.01	M	I	*C. sicula*	EH1-9	27	17	1.40	M	II
C. sicula	EH1-2	20	-	0.61	In	-	*C. sicula*	EH8-1	20	-	1.14	In	-
C. sicula	EH1-20	25	13	1.24	In	-	*C. sicula*	EH8-2	19	-	0.76	In	-
C. sicula	EH1-21	22	-	0.95	In	-	*C. sicula*	EH8-3	-	-	0.68	In	-
C. sicula	EH1-22	22	11	0.99	In	-	*C. sicula*	LP12-1	33	-	1.80	F	I
C. sicula	EH1-23	25	-	1.27	In	-	*C. sicula*	TF2-1	25	-	1.32	In	-
C. sicula	EH1-24	30	14	1.95	F	D	*C. sicula*	TF2-2	27	-	1.73	In	-
C. sicula	EH1-25	24	-	1.26	M	II	*C. sicula*	TF2-3	23	-	1.03	In	-
C. sicula	EH1-26	24	-	0.92	In	-	*C. sicula*	TF2-4	-	-	0.20	In	-
C. sicula	EH1-27	24	10	1.14	In	-	*C. sicula*	TF4-1	25	-	0.84	M	I
C. sicula	EH1-28	23	-	1.03	In	-	*C. sicula*	TF5-1	33	-	3.00	F	III
C. sicula	EH1-29	22	-	0.99	F	I	*C. sicula*	TF6-1	30	-	2.10	In	-
C. sicula	EH1-3	19	-	0.55	In	-	*C. sicula*	TF6-2	26	-	1.37	In	-
C. sicula	EH1-30	21	-	0.76	In	-	*C. sicula*	TF6-3	23	-	1.29	In	-
C. sicula	EH1-31	23	-	1.29	M	II	*C. sicula*	TF6-4	29	-	1.85	In	-
C. canariensis	TF-9	43	19	13.80	F	III	*C. canariensis*	OC-1	32	13	2.80	M	III
C. canariensis	TF-1	27	-	2.40	In	-							

RESULTS

SYSTEMATICS

Phylum Mollusca Linnaeus, 1758

Class Cephalopoda Cuvier, 1797

Subclass Coleoidea Bather, 1888

Superorder Decapodiformes Leach, 1817

Family Chtenopterygidae Grimpe, 1922

Genus *Chtenopteryx* Appellöf, 1890

***Chtenopteryx canariensis* Salcedo-Vargas & Guerrero-Kommritz, 2000**

(Fig. 2A, C)

Material examined: 2 specimens, Spain: Tenerife, Canary archipelago; TF10411512 South of Tenerife and OC1041412 north of Tenerife.

Description: The two *C. canariensis* specimens had an ML of 27 and 43 mm, with a weight of 2.4 and 13.8 g. *Chtenopteryx canariensis* is characterized by a wide mantle flattened from above and a fin almost equal to the ML, composed of muscular soft rays. Very similar externally to its congeners. Arm pairs I-II-III with 2 series of suckers proximally, 8 series of suckers in the central part of arm and 8 to 10 suckers distally. Arms IV with 2 series of zig-zag shaped suckers. Tentacular clubs with 6 series of suckers in the proximal part, 16-18 sucker series in the central part of clubs, and 6-8 series of suckers in the distal part. Visceral photophore lacking. Elongated photophore on the ventral aspect of the eyeball present. Mantle width corresponds to 47 and 50% of the ML. Figure 2A shows the ventral view of 43 mm ML female. The ink sac lacks a photophore, as illustrated in figure 2C. The ventral eyeball photogenic tissue is shown in figure 3A. The diagnostic characters of the two studied specimens agree with those ones analysed by Salcedo-Vargas and Guerrero-Kommritz (2000).

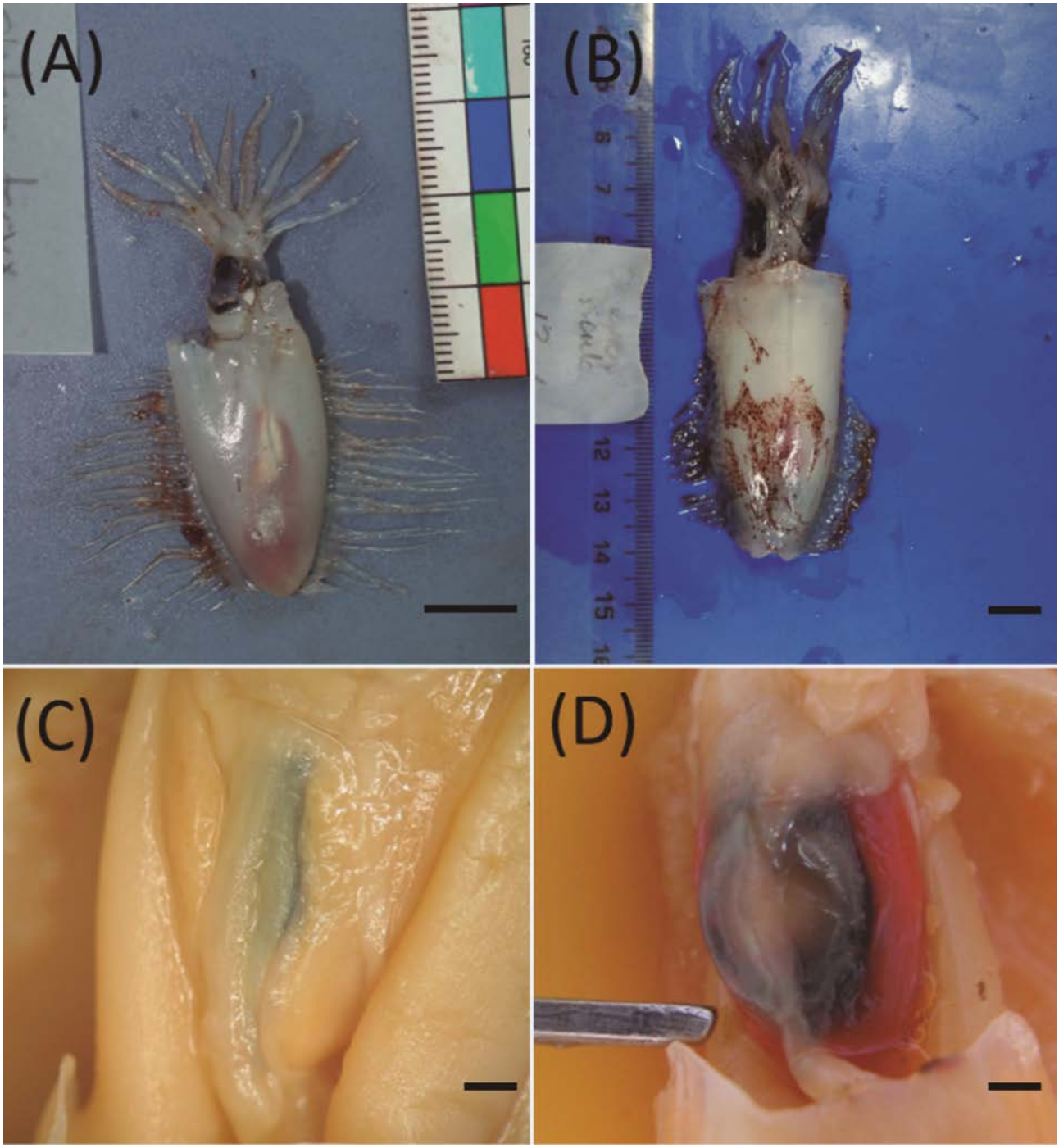

Fig. 2. (A) *Chtenopteryx canariensis*, 43 mm ML female specimen captured in Canary Islands, ventral view. (B) *Chtenopteryx sicula*, 34 mm ML mature female captured in Canary Islands. (C) Ink sack of *C. canariensis* without visceral photophore. (D) Visceral photophore on ink sack of *C. sicula*.

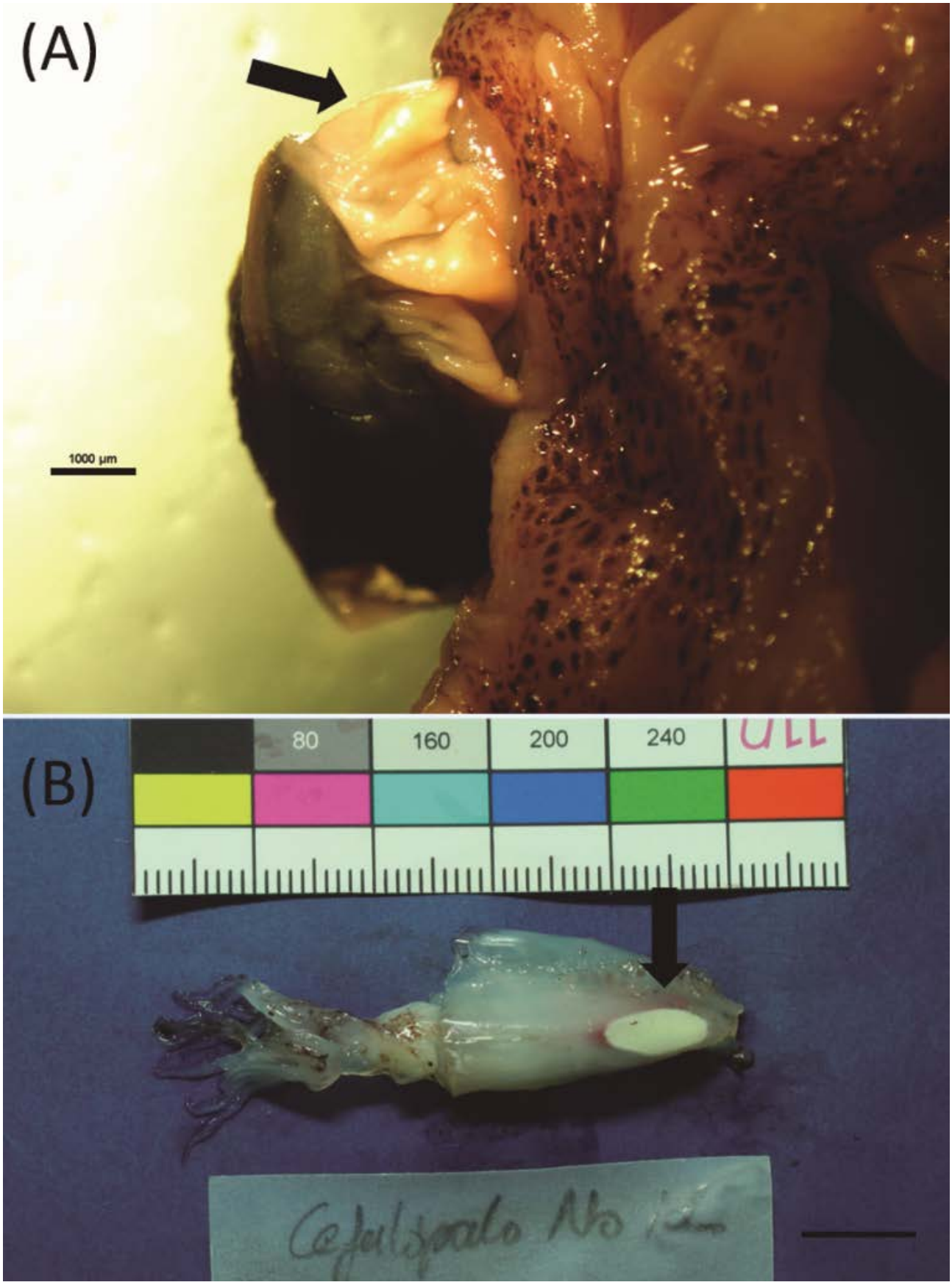

Fig. 3. Ventral eyeball photogenic tissue in *C. canariensis*. (B) Dorsal light organ on mantle of mature male of *C. sicula*.

***Chtenopteryx sicula* (Vérany, 1851)**

(Figs. 2B, 2D, 4)

Synonyms: Calliteuthis neuroptera Jatta, 1896; *Chtenopteryx fimbriatus* Appellöf, 1890; *Ctenopteryx cyprinoides* Joubin, 1894.

Material examined: Fifty-one specimens of *C. sicula* were captured around El Hierro, La Palma and Tenerife (Spain, NE Atlantic Ocean) (see Table 1 and 3 for details).

Description: The diagnostic characters of the specimens caught in the Canary Islands agree with those described by Guerra (1992). A dorsal light organ is present in the posterior part of the mantle in mature males (Fig. 3B). The studied specimens have a clear visceral photophore on the ink sac dorsal to the intestine in immature and mature males and females (Fig. 2D). Buccal membrane with two series of suckers. Suckers on arms I-II and III have the suckers in two transversal series along the arms and four series distally (Fig. 4A, B), while sucker on arms IV are positioned in two zigzag transversal series (Fig. 3C), whilst the tentacular clubs present 8-14 series of suckers.

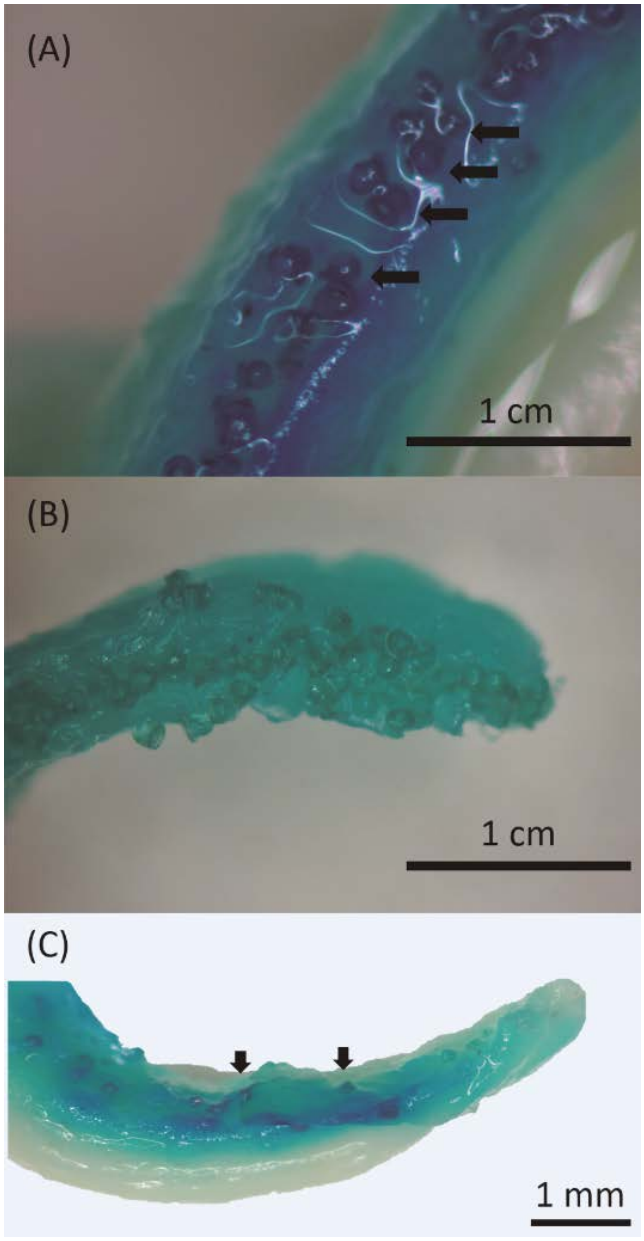

Fig. 4. (A) Detail of the two suckers series on proximal part of arm I in *C. sicula*. (B) Detail of the four suckers series on distal par of arm III, in *C. sicula*. (C) Arm IV of *C. sicula*, with two zig-zag transversal series of suckers. Black arrows point to suckers.

Genetic analysis

Of the 582 bp aligned for COI sequences, 95 were variable and 78 were parsimony-informative sites. Mean nucleotide composition was 29.8% (A), 35.6% (T), 19.8% (C) and 14.8% (G). The twelve partial COI sequences of *Chtenopteryx* species analysed in this study clustered in four distinct groups in the MaxL phylogenetic tree (Fig. 5). A well supported group with bootstrap values of 99 (BP = 99) was identified as *C. sicula* (Atlantic Ocean). A second group was identified as *C. sicula* (Pacific Ocean) (BP = 100). A third clade was identified as *C. canariensis* group (BP =

99), and finally a clade formed by a unique sequence was identified as *Chtenopteryx* sp. (West Pacific) (Fig. 5).

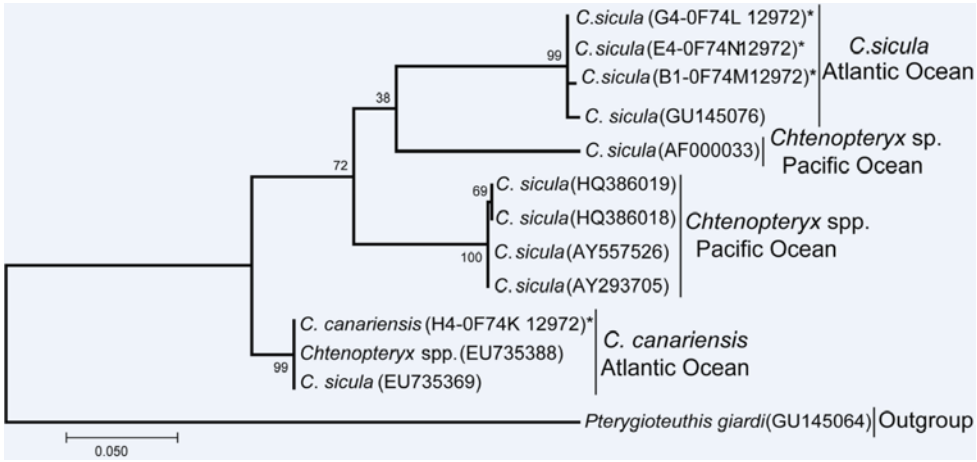

Fig. 5. Maximum-likelihood phylogenetic tree of the genus *Chtenopteryx* based on COI sequences. Bootstrap values are shown in the nodes. Accession numbers correspond to table 2. *: samples collected in the Canary Islands.

The partial COI sequence obtained for *C. canariensis* was clustered with two COI sequences classified as *C. sicula* (EU735369: caught in the Bear Seamount, NW Atlantic) and *Chtenopteryx* sp. (EU735388: collected north of the Azores archipelago, eastern Atlantic). The specimens belonging to this clade shared the same haplotype and therefore intragroup genetic divergence was TN = 0. The three *C. sicula* partial COI sequences obtained in this study were grouped together with a *C. sicula* (GU145076) collected in the SW Atlantic Ocean. The intragroup genetic distance for this clade was TN = 0.004 ± 0.002. Finally, the clade formed by the Genbank sequences HQ386019; HQ386018; AY557526; AY293705, classified as *C. sicula* and collected from the Pacific Ocean, showed an intragroup genetic distance of TN = 0.001 ± 0.001.

The pairwise intergroup genetic distances between the four clusters formed in the MaxL phylogenetic tree ranged from 9.8% to 12.4% (Table 4). The more divergent clades corresponded to those including *C. sicula* and *C. canariensis*, while the lowest divergence was obtained between *C. sicula* from the Pacific Ocean and *C. canariensis*.

DISCUSSION

Two species of comb-fin squids coexist in the Canary Islands, *C. canariensis* and another congener with a visceral photophore that contrasts with the original description of *C. sicula*, which

lacks a visceral photophore. Morphological and genetic evidence supports the presence of two different *Chtenopteryx* species in the oceanic waters surrounding the Canary Islands (NE Atlantic): *C. canariensis* and *C. sicula*. In addition, genetic analyses using COI sequences of the Chtenopterygidae available on GenBank showed the existence of four different clades within this group, likely to correspond to four different species. Our DNA-barcode results showed intragroup distances ranging from 0-0.4% and intergroup distances ranging from 9.8-12.4%, suggesting the existence of up to four *Chtenopteryx* species within the data set analyzed in this study.

Few *Chtenopteryx* specimens from the Mediterranean have been caught and described in detail morphologically or molecularly since the original description made by Naef (1923). Two recent Mediterranean specimens described by Young and Vecchione (2016), as well as the three Mediterranean specimens examined here, clearly showed a visceral photophore on the ink sac and morphometric/meristics measures that matched with the original description made by Naef. These Mediterranean specimens agree with our description of *C. sicula* from the Atlantic Ocean. Accordingly, we consider that the original description by Naef (1923) was based on an incomplete specimen, and *C. sicula* have a visceral photophore and, thus, it is reported in the Mediterranean and the Atlantic Ocean. However, a thorough revision of Mediterranean specimens is needed to clarify the real diversity of *Chtenoteryx* species, including morphological and molecular analyses. It is also necessary to establish a neotype for *C. sicula* due to the loss of the original type of Naef 1923 deposited in the Museum National d'Histoire Naturelle of Nice (France).

At least two morphologically-close chtenopterygid species have been confused in the Atlantic Ocean. The two species, *Chtenopteryx canariensis* and *C. sicula*, can be distinguished by their morphologies, phylogenetic tree topologies and genetic distances. Although *C. canariensis* can be distinguished from its Atlantic congener species by the absence of a ventral visceral photophore (see supplementary material), it has been misclassified in previous studies. The sequence EU735369, classified as *C. sicula* in GenBank, was obtained from an incomplete individual (only mantle) caught in the Bear Seamount (NW Atlantic). The second sequence, EU735388, classified at the genus level (*Chtenopteryx* sp.), was captured in the Mid-Atlantic Ridge close to the Azores archipelago, during the Mar-Eco expedition (Vecchione et al. 2010). Both sequences, showed a 100% sequence similarity with our *C. canariensis* partial COI sequence. The geographic origin of the sequences EU735369 (Bear Seamount, NW Atlantic) and EU735388 (Azores islands) considered by us as *C. canariensis*, in addition to three more specimens of *C. canariensis* deposited in the collection of the National Museum of Natural History (Washington D.C., United States) collected from South Africa, Sierra Leone and the north Atlantic Ocean, reference numbers USNM 730696, USNM 730697, USNM 730700, respectively, expand the current geographic range of this species to the North, NW and South Atlantic waters. Unexpectedly, the interspecific distance

obtained seems to show a closer relationship between *C. canariensis* and *C. sicula* from the Pacific Ocean than between *C. canariensis* and *C. sicula* cohabiting in the Atlantic (Table 4).

Regarding to the morphology of the studied specimens of *C. canariensis*, we found one discrepancy between our specimens and the original description. This is related to the light organ on the eyeball, described by Salcedo-Vargas and Guerrero-Kommritz (2000) as a "golden iridescent tissue". The presence of an elongated photophore on the ventral side of the eyeball was always considered a diagnostic character of the genus *Chtenopteryx* (*e.g.* Appellöf 1890; Nesis 1987; Guerra 1992). In the studied specimens, the eyeball photophore covers the whole ventral side of the eye and should be considered a true light organ. However, this fragile tissue may be easily damaged or lost in poorly preserved specimens, which may have occurred to the four specimens examined by Salcedo-Vargas and Guerrero-Kommritz (2000).

The presence of several sequences of *C. sicula* within three distinct clades (Fig. 4), with genetic distances between and 11.5% and 11.7% (Table 4), apart from evidencing misclassification of the species present on Genbank, points out to the probable existence of cryptic species in this genus and, most likely, the end of the circumglobal distribution of *C. sicula* in tropical and subtropical waters (Jereb and Roper 2010). The analysis of COI sequences proved to be useful as a molecular marker for identifying cephalopods at the species level, including distinguishing between closely related species (cryptic species) (*e.g.* Allcock et al. 2010; Undheim et al. 2010; Dai et al. 2012; Braid et al. 2014; Katugin et al. 2015; Sales et al. 2017). Intraspecific genetic distances for 46 cephalopods species ranged from 0 to 6.8%, while interspecific distances detected ranged from 3.31 to 22.1% (Dai et al. 2012; Gebhardt and Knebelsberger 2015). Considering these ranges, and assuming that *C. sicula* present in the Atlantic/Mediterranean is a valid species, the results suggest the presence of a least two other species that have been wrongly assigned to *C. sicula* in the Pacific Ocean. One *Chtenopteryx* species (HQ386019; HQ386018; AY557526; AY293705) is widely distributed from Japan to Hawaii and another *Chtenopteryx* species (AF000033) from Japan. We consider that one of these "Pacific clades" will correspond to *C. sepioloides*, a valid species from the Pacific Ocean, morphologically very similar to *C. sicula.* The remaining clade would correspond to a sibling species that needs to be described. Accordingly, the Pacific chtenopterygids should be subject to further genetic and detailed morphological analyses in order to enhance the systematics of this family.

CONCLUSIONS

In conclusions, our results suggest that only *Chtenopteryx sicula* and *C. canariensis* inhabit in the Atlantic Ocean and both species are distinguishable at morphological and molecular levels. The status of *C. canariensis*, considered in Jereb and Roper (2010) as undetermined, should be treated as such valid species. Two more *Chtenopteryx* species have been detect in the Pacific Ocean, suggesting the existence of cryptic species in "*C. sicula*", but further morphological and molecular analysis must be carried out in Pacific specimens to confirm this. Morphological species characterized in the past, such as *C. sepioloides* and *C. chuni*, should be accessed using recent molecular systematics methods, as well new specimens of *Chtenopteryx* caught in the Mediterranean and different regions of the world. This effort is needed to advance into the systematics, taxonomy and phylogeography of these poorly-studied mesopelagic squids.

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Supplementary Material

Identification key to *Chtenopteryx* spp. from the Atlantic Ocean. (download)