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Morphology, biology and molecular characterizations of *Opisthoteuthis calypso* (Cephalopoda: Octopoda) from the Sardinian Channel (central western Mediterranean)

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Several aspects of the biology of Opisthoteuthis calypso *were studied based on 38 individuals (23 males, 14 females and 1 indeterminate) retrieved from bottom trawls fished at 871 to 1420 m depth in the Sardinian Channel (central western Mediterranean). Data on full maturity of females are presented for the first time for this species. Information on the distribution, size, structure and fecundity for both sexes is provided, and the biological characteristics of the Sardinian samples are compared to those of other Mediterranean and Atlantic samples. Morphological variability and the peculiar structure of the shell and beak are reported and discussed. Molecular DNA sequences were obtained and compared with sequences available for the genus* Opisthoteuthis.

Keywords: morphology, biology, genetics, *Opisthoteuthis calypso*, Mediterranean Sea

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INTRODUCTION

Recent investigations of deep sea habitats have improved our knowledge of the behaviour, morphology and reproductive biology of deepwater cephalopods (e.g. Villanueva, 1992b, 2000; Villanueva *et al.*, 1997; Boyle & Daly, 2000; Cuccu *et al.*, 2007). These include deep-sea octopuses belonging to the suborder Cirrata (=Cirroctopoda *sensu* Young, 1989), commonly known as cirrate octopods, some of the largest invertebrate organisms in the bathyal and abyssal megafauna, found to depths of over 7000 m. They are gelatinous in consistency, with neutral buoyancy and soft, watery flesh, particularly susceptible to damage during capture (Collins & Villanueva, 2006).

Due to their relative rarity, the scarcity of collected specimens, and the often very poor condition of specimens available for identification and comparison, the systematics of this group has long been confused and controversial (e.g. Villanueva *et al.*, 2002; Collins & Villanueva, 2006). The development of new technologies, along with the extension of commercial fishery into deeper waters, allowed the capture of considerably more material, which is sometimes in excellent condition (especially specimens caught by submersibles), and stimulated renewed interest for this group in the last two decades. Much of recent research focused on taxonomy, distribution and comparative aspects (e.g. Villanueva *et al.*, 2002; Collins, 2003, 2005; Piertney *et al.*, 2003; Collins & Villanueva, 2006), and video recordings made by ROVs and manned submersibles provided important information on the behaviour of these octopods in their natural environment (e.g. Vecchione & Roper, 1991; Vecchione & Young, 1997; Villanueva *et al.*, 1997).

Recent results based on molecular studies (Piertney *et al.*, 2003) suggested a division of the suborder into four families, including the Opistoteuthidae, considered by some authors to be monotypic, with the sole genus *Opisthoteuthis* (see Collins & Villanueva, 2006 for a review). At present, the genus contains 19 species broadly distributed in the world's oceans; six species are recognized in the Atlantic Ocean. Among these, *Opisthoteuthis calypso*, distributed in the eastern Atlantic from Ireland to South Africa, is the only species recorded also in the Mediterranean Sea (Villanueva *et al.*, 2002).

Information on the biology of *O. calypso* in Mediterranean waters is still limited to data on a few specimens from the Balearic Sea (Quetglas *et al.*, 2000) the Catalan Sea (Morales, 1959, 1962; Villanueva, 1992b) and Italian waters (Orsi-Relini *et al.*, 2001; Cuccu *et al.*, 2006; Sartor & Belcari, in press). This paper aims to improve our present knowledge on this species, by providing information on the morphology, biology and genetics of specimens from the Sardinian Channel (central western Mediterranean).

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MATERIALS AND METHODS

The material of this study was collected during scientific trawl surveys carried out in the Sardinian Channel from 2003–2007, using a bottom otter trawl net with a 20 mm cod-end stretched mesh size, at depths ranging between 700 and 1600 m (Figure 1). Each specimen was separately sealed in a plastic bag as soon as arriving on board and rapidly deep-frozen (−20°C). Once in the laboratory, specimens were weighed before removal from their bags, so the total wet weight recorded (TW, measured to the nearest 0.01 g) included the water lost from the tissues during freezing; according to Boyle & Daly (2000) this water normally equals about 15% of the weight before freezing. Specimens were then classified and sexed following Villanueva *et al.* (2002). The dorsal mantle length (ML) was measured as in other octopods, to the nearest mm (Roper & Voss, 1983). Sucker counts and measurements (to the nearest 0.1 mm) were made starting at the mouth, using a dissecting microscope provided with a graduate scale. The gill lamellae counts refer to the total number of lamellae on each gill (i.e. left/right). The mandibles and the shell were removed and photographed. For each mandible, upper crest (UC), upper hood (UH), lower crest (LC) and lower hood (LH) measurements were taken (Clarke, 1986). Each measurement was related to mantle length and total weight by linear regression, after logarithmic transformation. Specimens were considered sexually mature based on the presence of spermatophores in the seminal vesicle and/or penis of males and eggs in the oviducts and/or oviducal gland of females (Villanueva *et al.*, 2002). Specimens were considered 'maturing' when gamete maturation was well underway but no eggs/spermatophores were present in the proximal oviducts/seminal vesicle (Rosa *et al.*, 2008).

Moreover, spent specimens were observed; in these specimens the gonads were flaccid but a few gametes were still present inside (i.e. a few eggs within the oviduct and/or oviducal gland and a few spermatophores in the penis).

After fixation in 5% formalin, ovarian oocytes and spermatophores were counted and measured (to 0.01 mm).

Total fecundity in females was determined by adding the number of eggs found in the ovary, oviduct and oviducal gland to the follicular sheaths found in the ovary (Boyle & Daly, 2000).

Thirty-one stomachs were examined for a contents analysis; prey remains were identified to the lowest possible taxonomic level. The occurrence index (OCI) and the frequency of occurrence (f%) of prey items were computed combining data from both sexes (Cortez *et al.*, 1995).

A sample of 10 specimens (6 males and 4 females) with different lateral wing tip morphologies was selected for the molecular analysis. Small pieces of mantle tissue were sampled and stored in absolute ethanol at −20°C. Genomic DNA was extracted with a salting-out method (Miller *et al.*, 1988) and analysed through PCR amplification and direct DNA sequencing. The PCR was used to selectively amplify the 16S ribosomal gene (16S rDNA), using the primers described by Palumbi *et al.* (1991). Amplification products were purified with magnetic beads (ChargeSwitch® PCR CleanUp Kit, Invitrogen) and directly sequenced using the same PCR primers. Sequences were aligned in CLUSTAL-W implemented by the MEGA v4 (Tamura *et al.*, 2007), using default parameters, and adjusted by eye. Divergence values between sequences were determined following the Kimura 2-parameter distance model (K2P; Kimura, 1980) with MEGA4; positions containing alignment gaps and missing data were totally eliminated only in pair-wise sequence comparisons (pair-wise deletion option). A neighbour-joining tree of the K2P distances was generated in MEGA4 and linearized assuming equal evolutionary rates in all lineages (Takezaki *et al.*, 2004).

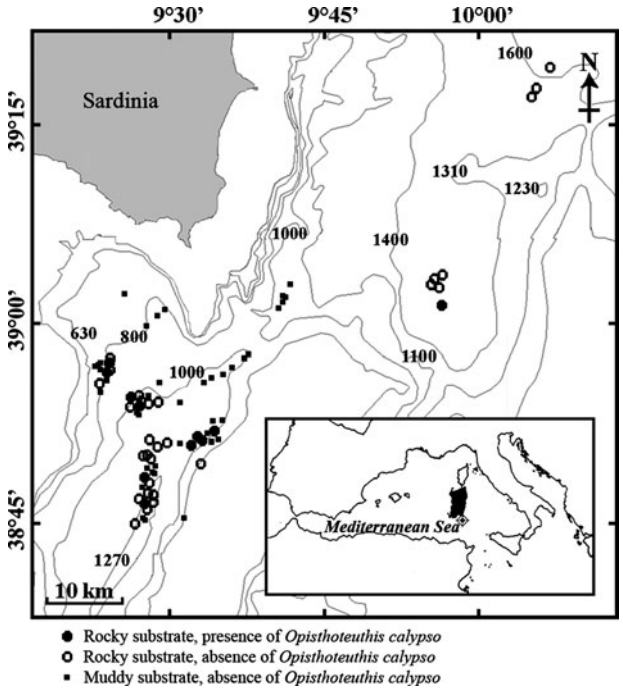

Fig. 1. Map of investigated area.

RESULTS

The sampled population

A total of 38 specimens of *Opisthoteuthis calypso* (23 males, 14 females and 1 unsexed) were collected between 871 and 1420 m of depth, in 11 hauls carried out on hard substrates. Both females (ML: 11–42 mm; TW: 18.10–141.46 g) and males (ML: 17–50 mm; TW: 20.02–243.06 g) were caught at all seasons of the year. Maturing and mature females were present only in January; maturing and mature males were caught in January, May and September. The minimum mantle length at maturity was 26 and 23 mm for females and males, respectively. One female (ML: 27 mm; TW: 24.75 g) caught in January and one male (ML: 50 mm; TW: 243.06 g) caught in May were spent. Additional data on the sample are reported in Table 1.

Morphological data

ARMS AND SUCKERS

Arm length was inconsistent, ranging between 39.3 and 87.1 mm in females and between 36.8 and 113.6 mm in males. Average arm sucker numbers ranged between 41 and 51 in females and between 42 and 54 in males. As to sucker diameters, several sectors were identified on the arms, respective sequences

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Table 1. *Opisthoteuthis calypso* from the Sardinian Channel: details of depth, frequency of occurrence, month of capture, mantle length (ML) total weight (TW), sex and percentage of different maturity stages.

Depth (m)	700–800	801–900	901–1000	1001–1100	1101–1200	1201–1300	1401–1500	1501–1600
Occurrence hauls	0/7	2/11	4/14	3/16	0/22	1/3	1/4	0/3
Monitored months*	1, 5, 6, 11	1, 5, 6, 9, 11	**1, 5, 6, 7**, 9, 11	1, 3, 4, **5**, 6, 7, **9**, 11	1, 3, 4, 5, 6, 7, 9, 11	**1**, 11	3, **7**, 9, 11	3, 9, 11
Females		N = 2	N = 10			N = 2		
		ML: 31–37 (33.9 ± 3.9)	ML: 11–41 (28.0 ± 9.2)			ML: 22–42 (32.1 ± 13.9)		
		TW: 86.19–112.98 (99.59 ± 18.94)	TW: 18.10–132.69 (77.69 ± 40.83)			TW: 49.19–141.46 (95.33 ± 65.24)		
		Maturing: 50%	Immature: 30%			Immature: 50%		
		Mature: 50%	Mature: 60%			Mature: 50%		
			Spent: 10%					
Males		N = 2	N = 17	N = 3		N = 1		
		ML: 33–35 (34.2 ± 1.1)	ML: 17–49 (36.0 ± 7.5)	ML: 23–34 (27.0 ± 0.6)		ML: 31		
		TW: 123.00–138.00 (130.84 ± 11.09)	TW: 20.02–343.06 (158.6 ± 70.32)	TW: 25.43–143.54 (75.61 ± 61.02)		TW: 103.83		
		Mature: (100%)	Maturing: 11.8%	Mature: 100%		Immature: 100%		
			Mature: 82.3%					
			Spent: 5.9%					
Un-sexed							ML: 9 TW: 9.17	

*from January = 1 to November = 11; the months of catch are in bold. ML and TW are expressed in mm and in g respectively.

starting at the mouth: four sectors (A–B–C–D) in females and six sectors (A–B–B_1–C–C_1–D) in males (Table 2). Proximal enlarged suckers (PES) and distal enlarged suckers (DES) were present in sector B_1 and sector C_1, respectively, on all arms of mature males. In the single immature male examined, only proximal enlarged suckers were observed. Females lacked both proximal and distal enlarged suckers. Except for suckers in sector A, where sucker diameters were equal in both sexes, suckers were always larger in males. Generally, the maximum distal enlarged sucker diameter exceeded that of the proximal enlarged sucker.

The position of enlarged proximal and distal suckers varied from individual to individual and within different arms (I–IV) in the same specimen (Table 3).

The first cirrus was located between the second and the third sucker (46%) or between the first and the second sucker (31%); variability in the position of this first cirrus (between the second and the sixth suckers) on different arms of the same specimen was observed in 23% of the sample. The longest cirrus measured 5.00 mm in length.

GILLS

Gills with 7/7 and 6/7 lamellae were observed in 74% and 11.1% of the sample, respectively. Gills with 5/5, 6/6, 7/6 and 7/5 lamellae were also present, each representing 3.7% of the sample.

MANDIBLES

The morphology of the upper and lower beak is shown in Figure 2A, B. The relationship between the growth of the crest and hood of each mandible, and the size (ML) and

Table 2. *Opisthoteuthis calypso* from the Sardinian Channel: sucker arrangement along the arm sectors (A, B, B1, C, C1). B1 proximal enlarged suckers, C1 distal enlarged suckers.

Arm sector	Arm suckers			
	Females		Males	
	Number (mean)	Size (mm)	Number (mean)	Size (mm)
A	3–4 (3)	0.80	2–5 (3)	0.80
B	0–1 (1)	1.00	0–2 (1)	1.30
B_1			4–8 (6)	2.60
C	15–29 (24)	1.1 0	9–29 (12)	1.45
C_1			0–5 (3)	2.74
D	10–25 (20)	<1.10	14–29 (23)	<1.45

Table 3. *Opisthoteuthis calypso* from the Sardinian Channel: position and number of the enlarged suckers by arm (I–IV) in mature males (N = 19). PES, proximal enlarged sucker; DES, distal enlarged sucker.

	First sucker	Last sucker	No. suckers mean	Range of position of largest suckers (mean)
PES-I	4–5	8–11	6	6–7 (7)
PES-II	4–5	8–11	6	5–8 (6)
PES-III	5–6	8–11	6	6–8 (7)
PES-IV	4–6	8–11	6	5–8 (7)
DES-I	21–25	23–29	3	23–28 (25)
DES-II	21–25	23–28	3	22–26 (24)
DES-III	20–25	23–27	3	22–25 (24)
DES-IV	20–26	23–28	3	22–27 (24)

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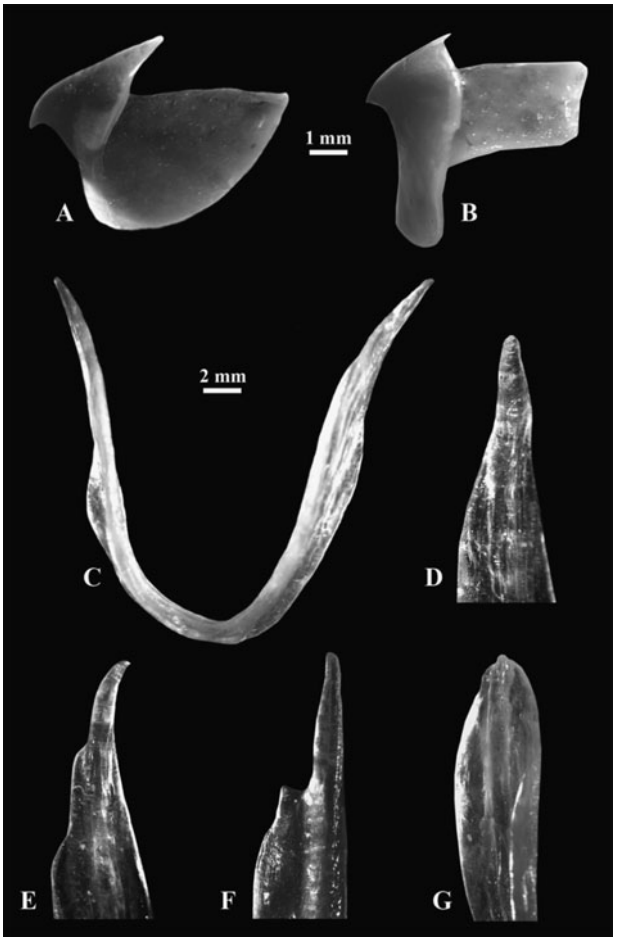

Fig. 2. *Opisthoteuthis calypso* from the Sardinian Channel: A, upper beak; B, lower beak; C, shell with standard; (D) and different degrees of differentiation of the shape of the lateral wings (E, F, G).

mass (TW) of the specimens (sex mixed) are reported in Table 4. The slopes of the regressions indicate the allometric nature of beak growth.

SHELL

Shell morphology is shown in Figure 2C–G. The typical morphology of lateral wing tips (Figure 2D) was observed in about 64.5% of the specimens; variations in these structures were also observed, both at the left (29.1%) and right (6.4%) tip, as shown in Figure 2 E, F & G (percentage of occurrence: 54.5, 36.4 and 9.1, respectively).

Molecular analysis

A total of 485 bp of nucleotide sequence of 16S rDNA were determined. Two haplotypes were obtained; the most common was shared by 9 specimens (GenBank Accession Number FJ403541) and differed from the second one by a single transition (GenBank Accession Number FJ403542).

The relationships among the 16S sequences obtained from the Sardinian *O. calypso* specimens and those present in the NCBI database for the genus *Opisthoteuthis* are shown in the tree given in Figure 3.

The Sardinian specimens had sequences well differentiated from the sequences of the other *Opisthoteuthis* species available; they clustered together and formed a well supported clade with the sequence AJ315374 that, according to the supplementary information reported by Piertney *et al.* (2003), is to be attributed to a specimen of *O. calypso* from the south-eastern Atlantic Ocean.

However, the 16S sequences of the Atlantic and the Mediterranean specimens were not identical, differing by 5 indels and 4 substitutions (all being transitions).

Reproductive data

The reproductive system of a mature female is shown in Figure 4A. The ovary is round and transparent, with ovarian oocytes of different developmental stages and sizes (0.10–6.76 mm) clearly visible; oocyte number, size and relative percentage of occurrence are reported in Table 5. The largest fraction (70.26%) was constituted of small oocytes (0.51 ± 0.31 mm); only 1.46% of the eggs measured between 6.12 and 6.76 mm.

The length of the oviduct ranged between 17.00 and 19.11 mm (mean 17.70 ± 1.10 mm) in the proximal tract and between 10.30 and 15.41 mm (mean 12.66 ± 1.90 mm) in the distal tract.

The maximum length of the oviducal gland varied between 6.70 and 11.40 mm with a mean value of 9.10 ± 2.00 mm. Both proximal and distal tracts were brown in fresh specimens (Figure 4A).

On average, a single egg (6.18–7.50 mm) was present in each, the proximal oviduct, the oviducal gland and the distal oviduct. The eggs inside the proximal and distal oviduct were smooth and encapsulated, respectively (Figure 4B, D).

Total fecundity of eight mature ovaries was 344 ± 83.78 including a mean of 26 ± 4 follicular sheaths.

One maturing ovary had 341 eggs; of these, 85% were small oocytes (<0.20 mm) and the rest ranged between 0.80 and 6.00 mm.

Table 4. *Opisthoteuthis calypso* from the Sardinian Channel: linear regression equations and statistics of beak measurements. UC, upper crest length; UH, upper hood length; LC, lower crest length; LH, lower hood length.

Regression equations	No.	Regression coefficient r	Standard error	r^2	F	*P*
Log UC = 0.533339 + 0.465081 log ML	32	0.8735	0.438	0.763	96.56	<0.000
Log UC = 0.471786 + 0.180774 log TW	31	0.9220	0.353	0.850	164.45	<0.000
Log UH = 0.366759 + 0.425793 log ML	34	0.8493	0.044	0.721	82.853	<0.000
Log UH = 0.312454 + 0.164021 log TW	33	0.9125	0.341	0.833	154.32	<0.000
Log LC = 0.460055 + 0.435909 log ML	33	0.8634	0.043	0.746	90.79	<0.000
Log LC = 0.407253 + 0.164884 log TW	32	0.8956	0.385	0.802	121.58	<0.000
Log LH = 0.138806 + 0.492487 log ML	33	0.8507	0.051	0.724	81.20	<0.000
Log LH = 0.078243 + 0.187108 log TW	32	0.8868	0.046	0.787	110.50	<0.000

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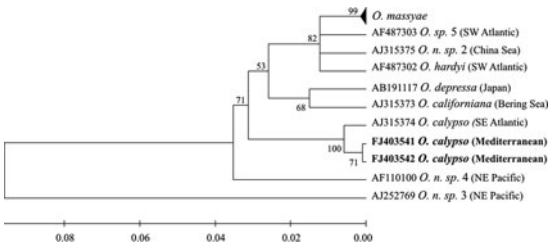

Fig. 3. *Opisthoteuthis* species comparison: neighbour-joining tree based on Kimura distances of 16S rDNA sequences; the alphanumeric code refers to the NCBI accession number; sequences of *O. calypso* from the Sardinian Channel are in bold. Only bootstrap support values >50 are indicated next to each node. The clade containing 11 sequences of *O. massyae* (AF299265; AF487297–301; AJ315371–72; AJ414702; AY545103; AY616970) was condensed in order to minimize the size of the figure. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

The reproductive system of a mature male is shown in Figure 4E. The testis is large and white in colour with a few small brown spots on the external epithelium; the accessory glands are white and rounded, with accessory gland 2 largest; spermatophores were oval in shape (Figure 4F) and located in the seminal vesicle and penis. In nineteen mature males spermatophore numbers varied between 16 and 59; spermatophore sizes ranged between 1.00 and 2.91 mm (average 1.59 ± 0.31 mm).

Diet

Twenty-one of the stomachs examined (i.e. 68%) contained between 1 and 9 prey items (average 3 ± 2); of these stomachs 7 belonged to females and 14 to males at different maturity stages.

Polychaeta and Crustacea were the most abundant prey; they were present, respectively in 95.24% and 76.19% of the stomachs analysed. Polychaeta and Crustacea made up 40% and 36.37% of the total stomach contents, respectively (Table 6). Sand was present in all stomachs.

DISCUSSION

The presence of *Opisthoteuthis calypso* in the Mediterranean Sea was confirmed for the Spanish waters and in Italian waters (i.e. Orsi-Relini *et al.*, 2001; Villanueva *et al.*, 2002; Cuccu *et al.*, 2006; Sartor & Belcari in press); however, samples examined were small in numbers of specimens and, consequently, the biological information provided fragmentary.

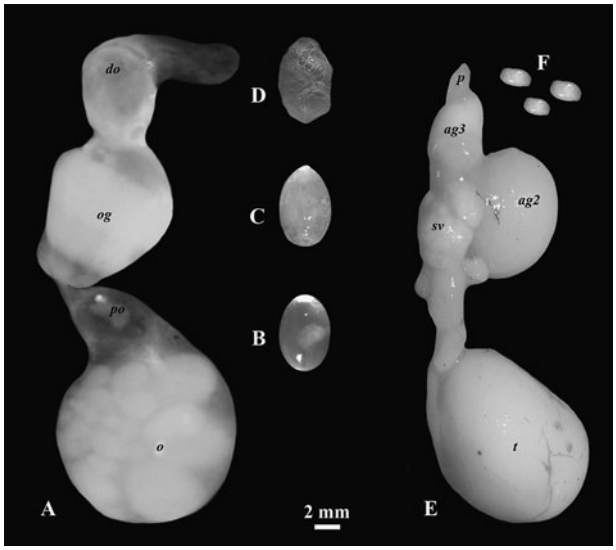

Fig. 4. *Opisthoteuthis calypso* from the Sardinian Channel; fresh material: (A) female reproductive system; o, ovary, po, proximal oviduct; og, oviducal gland; do, distal oviduct; (B) egg from proximal oviduct; (C) egg from oviducal gland; (D) egg from distal oviduct; (E) male reproductive system; t, testis, sv, seminal vesicle complex; ag2, accessory gland 2; ag3 = accessory gland 3; p, penis; (F) spermatophores from seminal vesicles and penis.

The sample analysed in this paper made it possible to analyse several aspects of the biology and ecology of the species. Also, the good condition of the specimens examined permitted detailed observations on external and internal morphology.

Our data confirmed that *O. calypso* is well represented in the Sardinian Channel at depths below 800 m, both sexes sharing the same areas throughout the year. Catch depth data are in agreement with previous results obtained for the same area that indicated the absence of the species in shallower waters (Cuccu *et al.*, 2003; Cuccu unpublished data).

Our results clearly indicate that in the Sardinian Channel *O. calypso* finds ecological environments suitable for feeding and reproduction only in the deeper bathyal. Stomach contents confirm that the species feeds on both epibenthic and suprabenthic deep species such as deep sea sponges (*Hyalonema* sp.) and the carol bobtail squid (*Neorossia caroli*). Also, the high percentage of mature and maturing specimens found in the sampled area indicate that this represents a suitable habitat for spawning events; the presence of rocky substrates for egg attachment could be an important requisite (Drazen *et al.*, 2003) as recently found in the same area for *Neorossia caroli* egg-clutches (Cuccu *et al.*, 2007).

Table 5. *Opisthoteuthis calypso* from the Sardinian Channel: number, % and size of the oocytes observed in the reproductive system of eight mature females.

	Eggs	Number		Length (mm)	
		Range	Mean ± SD	Range	Mean ± SD
In ovary	Small (70,26%)	132–372	241 ± 87	0.10–2.00	0.51 ± 0.31
	Medium (20,70%)	58–95	71 ± 16	2.01–5.94	3.19 ± 1.05
	Large (1,46%)	2–10	5 ± 4	6.12–6.76	6.40 ± 0.19
	Follicular sheaths (7,58%)	20–30	26 ± 4		
Inside	Proximal oviduct	0–2	1	6.18–7.07	6.53 ± 0.36
	Oviducal gland	0–1	1	6.46–7.20	6.90 ± 0.30
	Oviduct distal	1–2	1	6.50–7.50	7.10 ± 0.50

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Table 6. *Opisthoteuthis calypso* from the Sardinian Channel: frequency of occurrence (f%) and occurrence index (OCI) of food categories in the stomach contents.

Food category	(f%)	(OCI%)	Family	Species
Crustacea Amphipoda	23.81	14.55		
Crustacea Cumacea	9.52	3.64	Diastylidae	
Crustacea Ostracoda	14.29	7.27	Gammaridea	
Crustacea unidentified	28.57	10.91		
Foraminifera	9.52	5.45	Globigerinidae	*Globigerina* sp.
Mollusca Bivalvia	9.52	3.64	Limopsidae	*Limopsis* sp.
Mollusca Cephalopoda	4.76	3.64	Sepiolidae	*Neorossia caroli*
Nemertea	4.76	1.82		
Polychaeta	95.24	40.00	Sigalionidae	
Tunicata	9.52	3.64	Thaliacea	*Pyrosoma atlanticum*
Porifera	14.29	5.45	Hyalonematidae	*Hyalonema* sp.

Interestingly, the depth-ranges reported for the species in the Mediterranean Sea confirm its deeper location in this sea with respect to the Atlantic Namibian and Guinea waters. Even in the Portuguese waters, where the deepest reported bathymetric limits of the catches are 960 m, the upper limit was 553 m (Rosa *et al.*, 2008).

The size of the Sardinian specimens was definitively smaller than that reported for the Atlantic Sea sample (Villanueva *et al.*, 2002; Rosa *et al.*, 2008), both in length and weight; consistently, full maturity was reached at a smaller size in both sexes. Villanueva *et al.* (2002) stated that considerable growth took place after the onset of sexual maturity; however, spent specimens examined in the present work did not exceed 250 g of total weight, far from the maximum weight of 5400 g and 1600 g reported for males and females, respectively, from Namibian waters. Additional reports of the species from other Mediterranean areas also refer to small and medium size specimens (i.e. from the Northern Tyrrhenian Sea, the Ligurian Sea and Spanish waters as reported in Villanueva *et al.*, 2002). Apart from body size, reproductive features observed in the Sardinian sample agree with what is known for the species (e.g. Villanueva, 1992a; Villanueva *et al.*, 2002).

The general morphology of the reproductive system as well as the size and shape of the eggs and spermatophores conformed to what was previously observed, even if spermatophore maximum length observed in our sample was a little larger than that reported in the literature (Villanueva *et al.*, 2002; Collins & Villanueva, 2006). All reproductive features observed indicate a continuous spawning strategy, as already suggested for this species (Villanueva, 1992a; Villanueva *et al.*, 2002; Collins & Villanueva, 2006).

Results of the fecundity analysis showed lower fecundity values in both sexes in comparison with values previously reported (Villanueva *et al.*, 2002). In particular, fecundity observed in the fully mature female examined (344 eggs), was conspicuously lower than the mean value reported for the species in the Atlantic (648 eggs), probably due to the smaller size of the Mediterranean specimens. This is the first fully mature specimen recorded in the Mediterranean, and the fecundity value is unlikely to be underestimated in relation to the continuous spawning, being similar to that of the maturing female close to spawning observed in the present sample, and to maturing females from the Tyrrhenian Sea (Sartor & Belcari, in press).

Proximal oviduct and oviducal gland lengths observed in the present sample emphasize the fact that only very few eggs fit inside these structures, and confirm that only one or two eggs can be released at a time.

The presence of two fields of enlarged suckers in mature males, as indicated by Villanueva *et al.* (2002) for the species is here confirmed, but the arrangement of both, suckers and cirri were variable between specimens and also between the different arms of the same specimens; also, all suckers analysed, including enlarged suckers, were smaller than those reported by Villanueva *et al.* (2002).

As already observed (Villanueva *et al.*, 2002) sexual dimorphism affects only the proximal suckers in immature males, confirming that in this sex sucker enlargement in the distal field is closely linked to the achievement of full maturity.

Our analysis of the internal anatomy revealed the presence of different numbers of lamellae in the left and right gills in the same specimen, a condition never reported before in *O. calypso* but previously observed in *O. agassizii* (Villanueva *et al.*, 2002).

Also, the morphology of the mandibles and the degree of variation in the shape of the lateral shell wing tips were more similar to what was described and illustrated for *O. agassizii* than of *O. calypso* (Villanueva *et al.*, 2002).

These observations raise new questions and highlight the need for further morphological studies.

Despite the great intra-species morphological variability observed, our molecular analyses confirmed that all specimens belonged to the same species, *O. calypso*. However, while the Sardinian specimens clearly clustered with the south-eastern Atlantic specimen in our neighbour-joining tree based on the 16s rDNA sequences, our results revealed differences between the molecular sequences of *O. calypso* from the Mediterranean and those of the south-eastern Atlantic specimen.

In addition to the different size distribution observed, these findings clearly suggest the need for an extensive molecular taxonomic analysis encompassing all the species of the genus *Opisthoteuthis* (some of which are still undescribed), in order to support and confirm the morphological reclassification still underway and to clarify the differences between Atlantic and Mediterranean *O. calypso*.

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