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MORPHOLOGICAL AND MOLECULAR ANALYSIS OF CEPHALOPODS PREY RETRIEVED FROM THE DIGESTIVE TRACT OF AN EXCEPTIONALLY FRESH CUVIER'S BEAKED WHALE (*ZIPHIUS CAVIROSTRIS*) STRANDED IN GRAN CANARIA (CANARY ISLANDS)

*ANALISI MORFOLOGICA E MOLECOLARE DI CEFALOPODI ESTRATTI DAL TRATTO DIGERENTE DI ESEMPLARE DI ZIFIO (*ZIPHIUS CAVIROSTRIS*) ECCEZIONALMENTE FRESCO SPIAGGIATOSI IN GRAN CANARIA (ISOLE CANARIE)*

Abstract - *Cuvier's beaked whale (*Ziphius cavirostris*) remains one of the least understood cetaceans, with limited information on its behavior, diet, and ecological role. Understanding its trophic ecology is essential to expand knowledge of deep-diving cetaceans and improve conservation strategies. This study examines the cephalopod preys consumed by a individual shortly before its death, in the Canary Islands, combining morphological and molecular methods to identify species from beaks and undigested tissues. The whale was exceptionally well preserved, and necropsy confirmed recent foraging activity. Cephalopod beaks cross-referenced with molecular data strengthened species identification and revealed dietary preferences consistent with literature, with Cranchidae and Octopoteuthidae species as main prey. Some unusual items were also detected, including specimens of undescribed or genetically uncharacterized species. Since such well-preserved specimens are extremely rare and considering the difficulty of studying deep-diving cetaceans, this case study provides valuable insights into the feeding ecology of *Z. cavirostris *and a natural sampler for deep-sea cephalopods' species.*

Key-words*:* Ziphius cavirostris*, Canary Archipelago, cephalopods, cetacean, species identification*

Introduction – Among cetaceans the family Ziphiidae includes species highly adapted to deep diving. Within ziphiids, the Cuvier's beaked whale (*Ziphius cavirostris* G. Cuvier, 1823) is the species with the widest geographic distribution, occurring in all oceans, from temperate to subpolar waters (Arcangeli*,* 2015). Its trophic ecology, based primarily on meso- and bathypelagic cephalopods captured through suction feeding (Heyning*,* 1996), makes it both a key predator of the deep ocean and an excellent biological sampler of abyssal communities. In particular, several studies show that most preyed species belong to families such as Histioteuthis, Octopoteuthis, Ancistroteuthis, Gonatidae and Cranchiidae. Fish and crustaceans occur only occasionally and represent a minor component of the Cuvier's beaked whale's diet (Blanco*,* 2000). This study employs a combination of molecular and morphological analyses over cephalopods' remains found in the stomach and intestinal content of a Cuvier's beaked whale. Using this integrative approach, we examined the taxonomical composition of cephalopods consumed by a single individual to identify the prey species foraged in the deep central Atlantic region surrounding the Canary Islands archipelago.

Materials and Methods - On 9 December 2022, a sexually mature male of *Z. cavirostris* (560 cm long) was found floating off Taurito beach (Gran Canaria, Canary Islands). The carcass was recovered and transported to the port. The necropsy was then conducted at the University of Las Palmas de Gran Canaria by the Istituto Universitario de Sanidad Animal (IUSA) and the Society for the Study of the Cetacean in the Canary Archipelago (SECAC), following ACCOBAMS protocols for cetacean post-mortem investigations. As the specimen was fresh, samples of the contents of both the stomach and the intestine were collected. A total of 63 prey cephalopods' items were identified and catalogued based on the presence of muscular tissue and beaks. Among them, 38 possessed both upper and lower beak, 16 only the upper portion and 9 none of the above. Despite only 38 individuals possessed lower beaks (the key structure for reliable morphological identification), in some cases, diagnostic features of the upper beak alone provided sufficient characters for confident taxonomic assignment. Cephalopod beaks were classified morphologically through diagnostic features and morphometrics (Clarke, 1986). Muscle tissue associated to beaks (n=54) or isolated by any hard structure (n=9) was subjected to DNA extraction (Qiagen DNeasy Kit), PCR amplification of a portion of the mitochondrial COI gene (Folmer *et al.,* 1994) followed by Sanger sequencing. Sequences were quality-checked, aligned, compared with GenBank and BOLD databases, and curated to identify the prey taxa.

Results - The integration of morphological and molecular analyses revealed a diverse assemblage of deep-sea cephalopods consumed by the stranded specimen (Tab. 1). The 54 complete and partial cephalopods beaks were morphologically assigned to 6 families: Architeuthidae, Chiroteuthidae, Cranchiidae, Gonatidae, Mastigoteuthidae and Octopoteuthidae. The most abundant taxa were *Octopoteuthis sicula* (Rüppell, 1844) (n = 19) and *Teuthowenia megalops* (Prosch, 1849) (n = 10), followed by *Taonius pavo* (Lesueur, 1821) (n = 7). Less frequent but still noteworthy were *Liocranchia* sp. (n = 1), *Chiroteuthis veranii* (A. Férussac, 1834) (n = 1), *Gonatus* sp. (n = 1), and *Mastigoteuthis* sp. (n = 2). A single large beak was attributed to *Architeuthis* sp., though species-level resolution was not possible based solely on morphology. A total of 21 specimens could not be assigned even at the family level: 9 due to the absence of beaks and the remaining 12 due to complications encountered during the identification process. Conversely, the genetic analysis produced DNA amplifications for all but one of the 63 tissue samples. However, only 50 yielded high-quality COI sequences suitable for taxonomic assignments. BLAST searches and phylogenetic reconstruction confirmed 5 of the 6 families identified through morphology and resolved 8 species, partially overlapping with morphological identifications. Among Octopoteuthidae, molecular data confirmed the dominance of *O. sicula* (n = 21) and revealed the presence of *O. megaptera* (A. E. Verrill, 1885) (n = 1), not distinguishable through morphology. One sequence exhibited only 92.45% similarity to known Octopoteuthidae and instead clustered close to *Lepidoteuthis grimaldii (*Joubin, 1895*)*, suggesting the possible presence of either a previously undescribed lineage, or the first genetic information regarding an already described species. The Cranchiidae family was strongly represented in both approaches, although molecular analyses expanded morphologic taxonomic resolution by confirming *Taonius pavo* (n = 8) and detecting *Liocranchia reinhardtii* (Steenstrup, 1856) (n = 1). *Teuthowenia megalops*, however, could not be confidently verified by DNA data, reflecting either limitations in available reference sequences or potential misassignments in existing databases. For Mastigoteuthidae, the two morphologically identified specimens were successfully resolved at species level through sequencing: *Mastigoteuthis agassizii* (Verrill, 1881) and *M. magna* (Joubin, 1913).

A single specimen, assigned morphologically to Gonatidae, was genetically analyzed but did not cluster with any recognized species of the latter family; it showed closer affinity to a sequence originally attributed to *Gonatus steenstrupi* (Kristensen, 1981), which subsequent molecular analysis revealed to be misidentified. This discrepancy may reflect misclassification within public databases or unresolved cryptic diversity. Finally, the giant squid beak (morphologically not fully resolved) was unequivocally annotated, with a 100% identity match, to reference sequences of the *Architeuthis dux* (Steenstrup, 1857).

Table 1: table resuming and comparing the data obtained from the two identification analysis methods. *Tabella che riprende e confronta i risultati ottenuti dai due approcci di identificazione.*

	Morphological identification		**Molecular identification**		
Family	**Species**	**Total number**	**Species**	**Total number**	**Concordance/Notes**
Architeuthidae	*Architeuthis sp.* (n=1)	1	*Architeuthis dux* (n=1)	1	Molecular analysis revealed a higher taxonomic resolution power
Chiroteuthidae	*Chiroteuthis veranii* (n=1)	1	*Chiroteuthis veranii* (n=1)	1	Full agreement
Cranchiidae	*Teuthowenia megalops* (n=10); *Taonius pavo* (n=7); *Liocranchia* sp. (n=1)	18	*Liocranchia reinhardtii* (n=1); *Taonius pavo* (n=8); *Cranchiidae* sp. (n=12)	21	Molecular analysis revealed a higher taxonomic resolution power
Gonatidae	*Gonatus* sp. (n=1).	1	None	0	Uncertainties in the molecular identification due to lacking/ incorrect data
Mastigoteuthidae	*Mastigoteuthis* sp. (n=2)	2	*M. agassizii* (n=1); *M. magna* (n=1)	2	Molecular analysis revealed a higher taxonomic resolution power
Octopoteuthidae	*Octopoteuthis sicula* (n=19)	19	*Octopoteuthis sicula* (n=21); *Octopoteuthis megaptera* (n=1); *Octopoteuthis* sp. (n=3)	25	Molecular analysis revealed a higher taxonomic resolution power
Unidentified	21 specimens	21	13 specimens	13	n.a.

Conclusions - This study demonstrates the value of combining morphological and molecular approaches for a reliable identification of individuals, in this case pelagic cephalopod prey of Cuvier's beaked whales in the Canary Islands. The two methods yielded complementary insights: morphology provided fine species-level resolution when beaks were intact, whereas DNA barcoding proved essential taxonomic information for degraded material and/or partial beak apparatus and for verifying or refining morphological identifications. The fact that some of the species retrieved from the stomach contents of this deep-diving cetacean may not have been previously characterized, either morphologically (through beak descriptions) or molecularly (via reference sequences on GenBank or other DNA databases), presents an inherent limitation while simultaneously underscoring the substantial knowledge gap that persists with regard to abyssal species.

Direct comparison between the two methodologies revealed 6 mismatches, showing taxonomic assignments disagreement. These results reflected both the strengths and limitations of each technique: morphological examination of beaks enables taxonomic identification when soft tissues are absent, though it relies on known species; molecular analysis provides finer taxonomic resolution and greater reliability, since it can be performed even without hard material (fundamental for morphologic analysis), but can be hindered by missing reference sequences for undescribed or unsequenced taxa, and occasionally by misidentified references. Notable discrepancies, such as specimens assigned differently to *Octopoteuthis* and *Taonius*, highlight the continuing need to expand and curate genetic databases to reduce taxonomic uncertainty. The detection of rarely documented species, including *Architeuthis dux* and *Liocranchia reinhardtii*, illustrates the role of *Z. cavirostris* as an effective biological sampler of otherwise inaccessible bathypelagic biodiversity. Despite the challenges of tissue degradation, taxonomic uncertainties and sequence divergence, this work provides new genetic information that strengthens public reference databases and improves future species identification. Overall, the integration of molecular and morphological tools significantly enhances our understanding of the trophic ecology of *Z. cavirostris* and contributes to the broader characterization of deep-sea cephalopod diversity, underscoring the importance of continued development of genetic resources for marine biodiversity research.

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