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ORIGINAL PAPER

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Biological aspects of Cuvier's beaked whale (*Ziphius cavirostris*) recorded in the Croatian part of the Adriatic Sea

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Abstract The paper describes two stranded ziphiids from Croatia: a subadult female (length 430 cm, body mass 610 kg) that was stranded in 2001 and an adult male (length 510 cm, body mass ~1,000 kg) that was stranded in 2002. Both were confirmed to be Cuvier's beaked whales (*Ziphius cavirostris* Cuvier, 1823) from analysis of mitochondrial DNA sequences and osteological features. There are no previous records of Cuvier's beaked whales from the Croatian part of the Adriatic. The external shape of the head of the female specimen appears to be significantly different from the heads of Cuvier's beaked whales from other seas. The Croatian specimen exhibited embedded pieces of gravel in the gum tissue around the tip of the lower and upper jaws, which was observed for the first time in a Cuvier's beaked whale. The presence of the female in shallow coastal waters for several weeks and her boat-positive behaviour are apparently also first records of this kind for the species. The female was found to have ingested several plastic bags which likely caused her death. These are the northernmost findings of this species in the Adriatic Sea.

Keywords DNA identification · External morphology · Osteometry · Behaviour

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Introduction

The family Ziphiidae is the most poorly known group of cetaceans (Rice 1998) and includes 21 species in six genera (Dalebout et al. 2003). Many ziphiid species are difficult to identify and differentiated from one another because the diagnostic morphological features are generally subtle and may be present only in adult specimens (Heyning 1989; Mead 1989; Gomercic et al. 2002). An alternative and unambiguous method of accurately distinguishing among species of the family Ziphiidae is provided by sequencing a portion of the mitochondrial (mt) DNA control region or cytochrome *b* and comparing it with the validated database of reference sequences that have been compiled for all 21 beaked whale species (Dalebout et al. 2003).

Before the findings described here, only one specimen of beaked whales was recorded from the Croatian part of the Adriatic Sea. At Cavtat, near Dubrovnik, an animal (length 535 cm, body mass ~2,000 kg) identified as *Hyperoodon ampullatus* was killed in 1939 (Hirtz 1940). It is to be regretted that nothing was retained of this specimen, and only photographs and a general description are available. The only other ziphiids recorded from the Mediterranean Sea are Cuvier's beaked whale and Blainville's beaked whale, *Mesoplodon densirostris* (Rice 1998). The former is seen as rare in this region (Kinzelbach 1985). This species was mentioned for the first time in the Croatian scientific literature by Brusina (1889), who identified Cuvier's beaked whale as one of the species that inhabits or accidentally strays into the Mediterranean Sea but which had yet to be recorded from the Adriatic Sea. In 1986 and

1992, the carcasses of two female Cuvier's beaked whales were found near Bari, on the southern part of Italy's Adriatic coast (Centro Studi Cetacei 1987, 1995). In 2001 and 2002, two specimens of Cuvier's beaked whale were stranded at the Croatian Adriatic coast (Gomercic et al. 2002; Gomerčić et al. 2003).

The aim of this paper is to describe these two specimens. The description includes morphological data, observation of unusual behaviour in one of the two individuals, its parasites and the possible cause of its death. The morphological findings are compared with literature data on other specimens of the species recorded in other parts of its range.

Materials and methods

The first specimen was observed alive for a period of 36 days (from 7 March to 11 April 2001) in a shallow bay (42°37′18" N, 18°12′24" E) near Srebreno, south of Dubrovnik, Croatia (Fig. 1). Around 8 A.M. on 12 April 2001, the animal was found dead, floating on the surface of the sea. The second specimen was found dead on a beach in Pupnatska luka (42°55′54" N, 17°25"; E), on the island of Korčula, Croatia, on 7 February 2002 (Fig. 1). The carcass was in advanced decomposition, and the tip of the upper jaw was missing. Both carcasses were transported to the Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Zagreb, Croatia. Necropsy and X-ray examination were performed on both specimens. Both skeletons are stored at the above-named institution. The presence of fused epiphyses of the vertebrae, humerus, radius and ulna was used as criterion for physical maturity (Moore 1968). The sex of the specimens was determined from morphological features for the first animal and through molecular sexing using the SRY method of Gilson et al. (1998) for the second. Tissue samples were collected from both animals and sent to the University of Auckland, New Zealand for DNA extraction, mitochondrial DNA sequencing and analysis to determine species identity following standard methods (Dalebout et al. 2005). Twenty-two external measurements were taken for the first animal (after Perrin 1975), while only four of them for the second animal due to its advanced decomposition. Skull measurements were taken after Omura (1972) and postcranial skeletal measurements and meristics after Perrin (1975). The morphological data were compared with those in the literature (Omura 1972; Heyning 1989). Ectoparasities and endoparasites were examined macroscopically, and the species or genus identified according to Hogans (1987) and Dailey and Brownell (1972), respectively. The behaviour of the first specimen was observed and documented (slide film and video) during 11-h total observation time from an inflatable boat (5.5 m long with an outboard engine) on 9 and 10 March. Weather conditions were good with no wind and the sea was calm. The water was clear enough to watch the animal below the surface. For the rest of this period, its presence was confirmed only by observation from land due to prohibition of sea traffic in the bay during the animal's stay.

Results

Species identification

The mtDNA control region sequences were amplified successfully from both specimens. These 'test' sequences (~435 bp) were compared to the beaked whale reference database (Dalebout et al. 2003). The two Croatian animals shared the same haplotype, which grouped strongly with reference sequences from Cuvier's beaked whale, *Ziphius cavirostris* (bootstrap score, 100%), to the exclusion of sequences from all other beaked whale species in the database. Based on these results, the specimens were identified as Cuvier's beaked whale. A sequence representing this haplotype has been deposited in Genbank (Accession No. DQ068239).

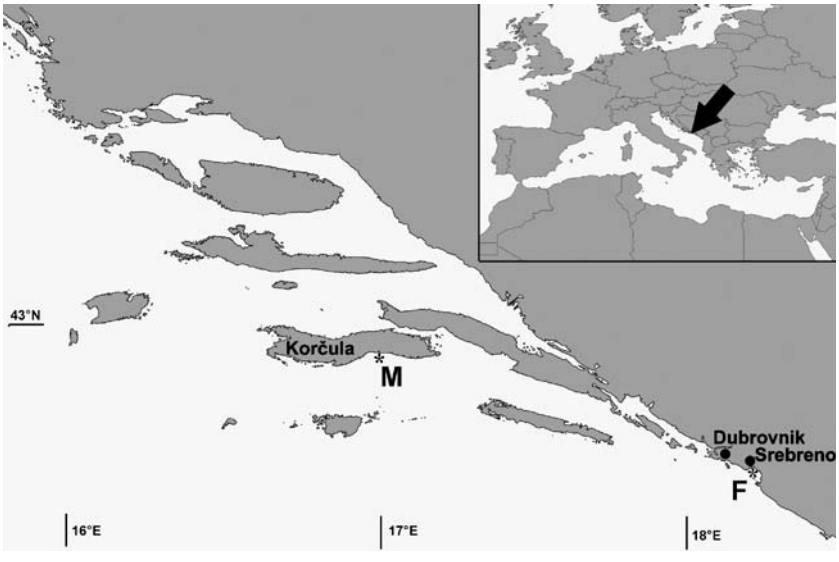

Fig. 1 Geographic locations of the two Cuvier's beaked whale findings in Croatian waters. *F* represents the female specimen and *M* the male

Morphology

The first animal was a juvenile female with uniform dark grey–brown coloration on the back and light grey coloration ventrally. The animal was 430 cm long, with a body mass of 610 kg. The skin surface was smooth, without scars except for a round, depigmented patch 5 cm in diameter below the dorsal fin on the right side of the body. The melon was distinct and sloped gently down to the short beak (Fig. 2c). The caudal edge of the tail flukes was flat, laterally curved and lacked a median notch (Fig. 2d). The blowhole was large and semi-lunar with the horns pointing anteriorly (Fig. 2a). The flippers were relatively small. The dorsal fin was small, falcate and set far back on the body (Fig. 2b). In the gum of the tip of upper jaw, several pieces of gravel or shell were embedded while others were overgrown and covered by epithelium. Pieces of overgrown gravel or shell were also found in the gum at the tip of the lower jaw. The area of gum where this material (size up to 5 mm, Fig. 3a,b) was embedded was circular with a diameter of 5–6 cm.

A round, fatty, soft organ, possibly homologous to the spermaceti organ (Heyning 1989), was found beneath the blubber of the melon in a prenarial basin. The organ was transparent, light yellow and had a gelatinous consistency at room temperature (mass 5.67 kg, volume 6–7 l).

There was neither chyle in the mesenterial lymph vessels nor any traces of food in the digestive organs. The thickness of the blubber was 3 cm on the back and abdomen. The cause of death was probably the obstruction of the opening between the fundic and first pyloric compartment of stomach by four plastic bags. Two of the bags (size of 40×15 and 30×25 cm, respectively) were shopping bags made of soft plastic. The other two bags (size of 15.5×11 and 20×14 cm, respectively) were made of more solid plastic. The latter two bags were of a type in which sweets and snacks are sold in Croatia. One of these bags must have been ingested after 10 March 2001 because the product was not sold before this date. Thus, the animal likely ingested this bag during its stay in the bay near Srebreno.

Ovaries were 4.5×2.0×1.0 (left) and 4.3×1.8×1.0 cm (right) in size, with a mass of 4.0 and 3.0 g, respectively (measured after formalin fixation), with no visible corpora from previous ovulations. The surface of both ovaries was smooth and macroscopically lobulated.

The heavily decomposed carcass of the second animal (length 510 cm, body mass approximately 1,000 kg) possessed incomplete viscera and a damaged skeleton. Molecular sexing indicated that this specimen was a male. Its teeth had fallen out due to decomposition and were missing; however, two large alveoli in the tip of the lower jaw were present.

The female possessed a single pair of unerupted teeth at the tip of the lower jaw, together with a large number of unerupted, small, vestigial teeth attached loosely in the gum of both the upper and lower jaw. The two large mandibular teeth were set in alveoli and covered with gum. They were cylindrical–conical in shape with sharp unworn tips (Fig. 3a,c). Tooth lengths were 41 (left) and 42 (right)

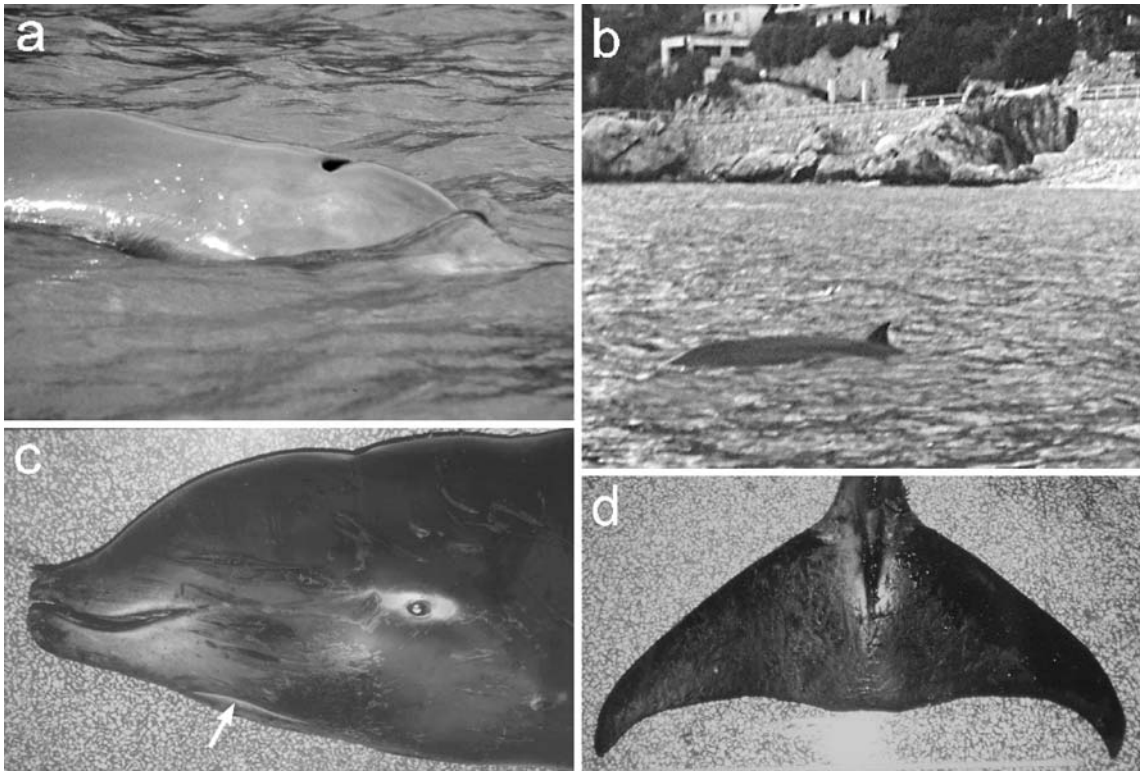

Fig. 2 Female Cuvier's beaked whale: **a** alive near research boat; **b** alive near the coast in Srebreno; **c** head of dead animal, *arrow points* at left throat groove; **d** dorsal view of tail flukes

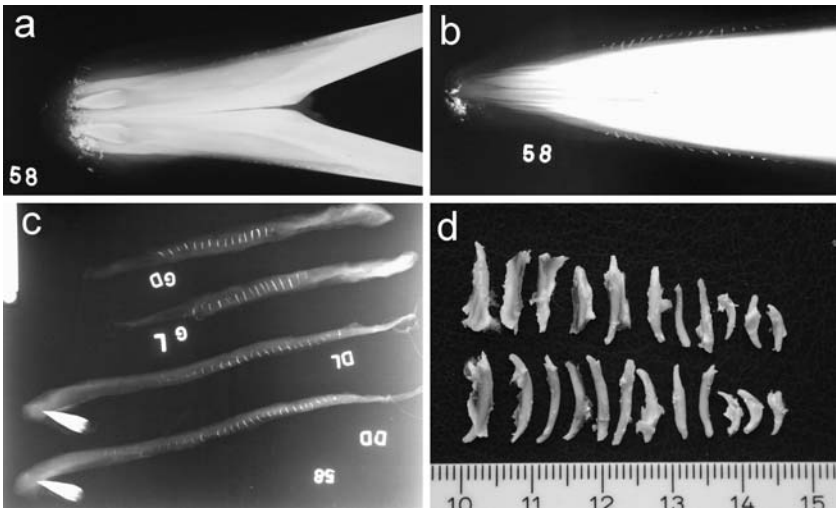

Fig. 3 Female Cuvier's beaked whale: **a** X-rays of lower jaw with small pieces of gravel around the tip; **b** X-rays of upper jaw with big stones on tip; **c** X-rays of gum, upper right (*GD*), upper left (*GL*), lower left (*DL*) and lower right (*DD*), with apical mandibular and vestigial teeth; **d** some of the vestigial teeth

mm. Both teeth were almost completely covered with cement, except for the upper 7 mm of the tips which were covered with enamel. Dentin was almost completely absent, and the pulp cavity was broad and unfilled. The tooth wall at the root was only 0.5- to 0.8-mm thick. The vestigial teeth in the gums of both upper and lower jaws (24 in the upper left, 26 in the upper right, 33 in the lower left, and 31 in the lower right) were up to 13 mm long and up to 2 mm in diameter (Fig. 3).

The epiphyses of the last nine vertebrae (39th–48th) of the female were unfused, as were the epiphyses of humerus, radius and ulna, indicating that the specimen was a juvenile. In contrast, in the male, the epiphyses of all 46 vertebrae and epiphyses of humerus, radius and ulna were completely fused, indicating that this was an adult animal. The female had five and the male six fused cervical vertebrae.

The structure of the synvertex cranii was identical in both animals, with a characteristic extremely high vertex and enlarged nasals which protruded anteriorly to overhang the external bony nares (Fig. 4c,d). Condylobasal length was 730 (female) and 840 mm (male). The greatest width of skull across the postorbital processes of the frontals was 390 mm (female). In the male, this measure could not be taken because of postmortem damage of the skull in this region.

Both specimens possessed ten pairs of ribs. The eighth and ninth left and right ribs had developed only the tuberculum costae, which was joined with vertebral transverse process.

Parasites

The female had 38 openings in its skin on the left body side and 12 on the right side, through which parasitic copepods *Pennella balaenoptera* protruded to a length of approximately 10 cm. The cephalothorax of each copepod sat inside a suppurating abscess with a diameter of 3–4 cm that penetrated deep in the blubber but did not reach the fasciae or muscles. The parasites could easily be pulled out of the abscesses. In the urinary ducts of the kidneys of the same animal, nematode conglomerates were found, probably *Crassicauda* sp. (possibly *Crassicauda crassicauda*). These parasites (length 20–30 cm) were not attached to the duct wall and could easily be removed.

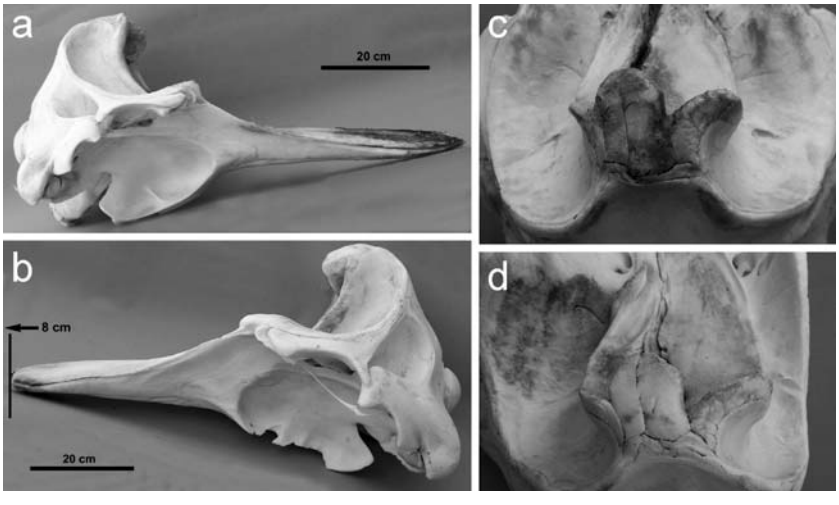

Fig. 4 Skulls of Cuvier's beaked whale: **a** juvenile female; **b** adult male; **c** synvertex of female; **d** synvertex of male

Behaviour

The female was seen daily over a period of 36 days in a shallow bay near the shore where the sea is only 2- to 3-m deep (Fig. 2a,b). Water depth in the greater part of the bay is 30–50 m. It is, at least, a further 20 km from the shore before depth increases to 200–250 m, and with at least 30–50 km distance before a water depth of 1,000 m is reached. We estimated that the animal surfaced every 10–20 min, while swimming and diving slowly (with an estimated speed of 2–5 km/h). We never observed raising of the tail flukes. The individual approached within 10 m of the shore at times and as close as 2 m from the research boat (Fig. 2a). At times, it would not be seen for a period of 1–3 h but would always return several times each day to the same bay near Srebreno.

Discussion

Species identity of female could not be determined with confidence based on its external morphology and behaviour. Due to advanced decomposition, the identity of the male specimen could also not be determined with certainty from external appearance. Analysis of mtDNA sequences together with skull osteological features, however, confirmed that both specimens represented Cuvier's beaked whales. These are the northernmost records of this species in the Adriatic Sea.

The morphological differences observed between both Croatian animals and Cuvier's beaked whales from other parts of the world suggest that there may be more geographic variation in some of these features than previously realised. Contrary to Nishiwaki (1972), Zemskij (1980), Watson (1981) and Jefferson et al. (1993), the tail flukes of our female specimen had a flat, laterally curved caudal edge without a median notch (Fig. 2d). This observation is, however, in agreement with both Leatherwood and Revees (1983) and Heyning (1989) who stated that Cuvier's beaked whale has no distinct notch between the tail flukes. In three [distances: (1) between tip of upper jaw to centre of eye, (2) between tip of upper jaw to blowhole along midline and (3) between tip of upper jaw to anterior insertion of flipper] of 16 external measurements, the female differs significantly (i.e. by more than one standard deviation) from the majority (95%) of females measured by Heyning (1989). The external shape of the head of the female (Fig. 2c) appears to be significantly different from the heads of other Cuvier's beaked whales (Heyning 1989), although the rest of the body proportions are similar.

Mead (1984) reported an ovary mass of 5.2 g for a neonate Cuvier's beaked whale. In the Croatian female, the ovaries had a mass of only 3 and 4 g, respectively. It is likely that ovaries of our juvenile animal were smaller than the ovaries in the neonate (Mead 1984) due to the atresia of large numbers of primary oocytes between birth and puberty.

Heyning (1989) examined a female Cuvier's beaked whale of 470-cm length and 1,300-kg body mass. This differs markedly from the Croatian female, which was slightly shorter (430 cm) but weighed only 610 kg. The absence of food in the digestive system, the presence of plastic bags blocking the first of the stomach openings and the overall poor physical condition indicated that this animal was starving. Comparisons with the presumably healthy female of similar size examined by Heyning (1989) suggest that our female animal had lost (or failed to gain) almost half its normal body mass.

Our observations of embedded pieces of gravel around the tip of the lower and upper jaws of the female animal are highly unusual. It is difficult to explain how this could have occurred except as a result of the animal repeatedly striking its beak against such gravel on the seafloor over an extended period of time, sufficient for some of these wounds to heal and the embedded gravel become overgrown with gum tissue. Ziphiids feed primarily on deep-water squid (Heyning and Mead 1996). They are not known to be bottom feeders, although North Atlantic bottlenose whales would take small numbers of benthic echinoderms and were observed to come to the surface with mud from the seafloor on their beaks (Benjaminsen and Christensen 1979). Necropsies of this species have not, however, revealed the presence of embedded gravel in their jaws.

The discovery that the female specimen most likely died as a direct result of ingesting plastic bags is of particular concern. Marine debris is a growing threat for cetaceans worldwide (e.g. Laist 1997). A sick or starving animal may be more likely to take up foreign material than a healthy one (Kastelein and Lavaleije 1992). That at least two of the four plastic bags found in the stomach of the female animal appeared to have been ingested during the time it spent in the shallow coastal waters could be taken as support for the hypothesis that the animal was starving.

Off the coast of Japan, Cuvier's beaked whale is most commonly found in waters over 1,000-m deep (Nishiwaki and Oguro 1972). In the Mediterranean, four pairs of Cuvier's beaked whales were sighted in the Central Tyrrhenian Sea, where water depth is 1,200–1,800 m (Marini et al. 1992). Our observation of an individual of this species in shallow coastal waters (30–50 m deep) over 30–50 km away from areas with a water depth of >1,000 m is, therefore, highly unusual.

Unlike most other ziphiids, North Atlantic bottlenose whales will often approach slow-moving or stationary vessels (Gray 1882). In contrast, Cuvier's beaked whale often avoid vessels (Heyning 1989). The boat-positive behaviour exhibited by the female animal was, therefore, also unusual. Our observation of Cuvier's beaked whale in shallow coastal waters for several weeks and its boat-

positive behaviour during this time seems to be first of this kind of behaviour in Cuvier's beaked whales. This is also the first case of death of Cuvier's beaked whale in the Mediterranean, i.e. Adriatic Sea, caused by ingestion of plastic bags and constipation of the digestive system.

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