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A Longman's beaked whale (*Indopacetus pacificus*) strands in Maui, Hawaii, with first case of morbillivirus in the central Pacific

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The Longman's beaked whale (*Indopacetus pacificus*) is one of the world's most poorly known whales. Until 1999 this species had not been identified from either a live or a dead whale and was known only from the holotype skull collected from Queensland, Australia in 1882 (Longman 1926) and one additional skull from Somalia (Azzaroli 1968). After a detailed assessment of photographs of an unidentified tropical "bottlenose whale" (Pitman *et al.* 1999), and later genetic confirmation of species identity from stranded animals (Dalebout *et al.* 2003), at sea identification of this species became possible and sighting reports are no longer uncommon in subtropical and tropical waters, especially in the Indian Ocean (Pitman *et al.* 1999, Anderson *et al.* 2006). In the Pacific, a few sighting reports suggest that Longman's beaked whales inhabit Hawaiian waters in low abundance (Shallenberger 1981, Barlow 2006, McSweeney *et al.* 2007). However, specimens remain scarce and to date this species is known from less than 10 confirmed strandings world-wide (Pitman 2009). This is the first report on a stranding and necropsy findings from a Longman's beaked whale from the Hawaiian archipelago and confirms the presence of this species in waters of the United States. This is the only case of a Longman's beaked whale stranding world-wide where the response included collection of morphometrics, computed tomography (CT) scanning, gross necropsy, histopathology, genetics, and molecular diagnostics for pathogens.

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At approximately 1430 HST on 22 March 2010 a juvenile male Longman's beaked whale stranded live at Hamoa beach, Hana, Maui (22°43'10"N, 155°59'13"W). The 371 cm whale was reportedly thrashing at the water line with blood coming from the mouth. The animal died on site at 1445 and was cooled on ice until transported by air cargo from Maui to Oahu the following day. It was assigned specimen no. KW2010005.

The gross necropsy began on 23 March in Honolulu, Hawaii. A CT scan of the head was conducted at Koolau Radiology (Queens Medical Center, Honolulu) on 23 March. Morphometric measurements and internal organ weights are provided in Tables 1 and 2. Few measurements and no internal organ weights from Longman's beaked whales are available for comparison. The total body length of three stranded adult females measured between 565 cm and 650 cm (Dalebout *et al.* 2003, Pitman 2009). In South Africa, a neonate male that stranded in 1976 measured 291 cm and a juvenile male stranded in 1992 measured 363 cm (Dalebout *et al.* 2003). The Hawaiian juvenile male had a similar total body length of 371 cm and weighed 530 kg. The ventral body surface was light gray in color while the dorsal surface was medium gray. Dark gray coloration surrounded each eye, and a thick dark vertical band extended dorsally from each eye, forming a circular band posterior to the blowhole. Another similar dark band extended laterally from the anterior insertion of each flipper, but this was not as obvious when viewed dorsally (Fig. 1a, d). There were multiple, fresh cookie cutter shark bites over the entire body with a concentration on the posterior ventral surface (Fig. 1b, c). Papillae were observed on the tongue, which may be representative of a nursing individual. Two unerupted teeth at the tips of the mandible were apparent in the CT scan. The stomach was empty. The liver had a soft and modulated lumpy texture and the spleen was emphysematous and friable.

The skeletal system was similar to previously described Longman's beaked whales with the exception of characteristics consistent with a young individual. There was trauma to the mandible and maxilla apparent both visually and from CT images. The left mandible and maxilla had open, comminuted fractures with marked displacement of bone fragments, with a straight 20 cm laceration perpendicular to the gape of the mouth and slightly caudal to the left mandible. A 3-D reconstruction of the fractures was generated from the CT images (Fig. 2a, b). General softness of the bones was apparent during necropsy, and a CT scan of lumbar vertebrae L1-L3 suggested very low bone density in this juvenile whale that may indicate incomplete ossification that could be normal for age or pathological. The splintered nature of the jaw fracture is interesting in light of the softness of long bones elsewhere, yet is consistent with the high density and degree of calcification characteristic of beaked whales in general (Currey *et al.* 2001). However, the ossification process during growth has not previously been described for whales, and a general lack of comparative data make it difficult to interpret these observations. The vertebral formula in this individual was C7 T10 L19 Cd19 = 55. The first five cervical vertebrae were fused as reported in two other museum specimens (Dalebout *et al.* 2003). The cranial pairs of seven ribs were double-headed, followed by three single-headed ribs and one floating rib. The observation of 10 pairs of ribs also agrees with the prior examination of two museum specimens and the suggestion that 10 rib pairs and five fused cervical vertebrae are diagnostic characteristics of *Indopacetus* (Dalebout *et al.* 2003). This individual had 5 metacarpals and six carpal bones arranged in two rows of three bones each. The phalangeal formula of the left flipper was I-0, II-5, III-5, IV-4, V-3 and I-O, II-5, III-5, IV-4, V-2 for the right flipper. The only other phalangeal formula report for

Table 1. External measurements for the juvenile male Longman's beaked whale, *Indopacetus pacificus,* that stranded in Maui, Hawaii, on 22 March 2010 (specimen no. KW2010005).

Morphometric parameter	Measurement (cm)
Total length (tip of rostrum to fluke notch)	370.8
Tip of rostrum to apex of melon	104.1
Tip of rostrum to gape of mouth	25.4
Tip of rostrum to center of eye	45.7
Tip of rostrum to center of blowhole	45.7
Tip of rostrum to anterior insert of pectoral fin	76.2
Tip of rostrum to anterior insert of dorsal fin	223.5
Tip of rostrum to dorsal fin tip	246.4
Tip of rostrum to umbilicus	172.7
Tip of rostrum to center of genital slit	218.4
Tip of rostrum to anus	248.9
Fluke notch to anus	121.9
Girth at axial	170.2
Girth at anterior insert of dorsal fin	182.9
Girth at anus	144.8
Dorsal fin height	17.8
Pectoral Fin anterior length	11.4
Pectoral fin max width	35.6
Fluke width	74.9
Dorsal blubber thickness	3.2
Lateral blubber thickness	3.0
Ventral blubber thickness	3.8

Table 2. Organ and tissue weights from the juvenile male Longman's beaked whale, *Indopacetus pacificus,* that stranded in Maui, Hawaii, on 22 March 2010 (specimen no. KW2010005).

	Weight (kg)	Percentage of body weight
Body weight	530.2	–
Muscle	234.4	44.2
Blubber	133.2	25.1
Bones	94.2	17.8
Brain	2.7	0.5
Heart	3.6	0.7
Lungs	11.7	2.2
Liver	7.5	1.4
Left kidney	1.5	0.3
Right kidney	1.3	0.2
Spleen	0.5	0.1
Pancreas	0.2	0.0
Intestine	3.4	0.6
Other tissues	36.2	6.8

the species comes from a mother and calf that stranded in Taiwan. The Taiwan adult phalangeal formula was the same for both flippers: I-O, II-6, III-5, IV-4, V-3. The juvenile male had a normal left flipper with a phalangeal formula of I-0, II-6, III-6, IV-4, V-3 and a polydactylous right flipper with a phalangeal formula of I-0, II-5,

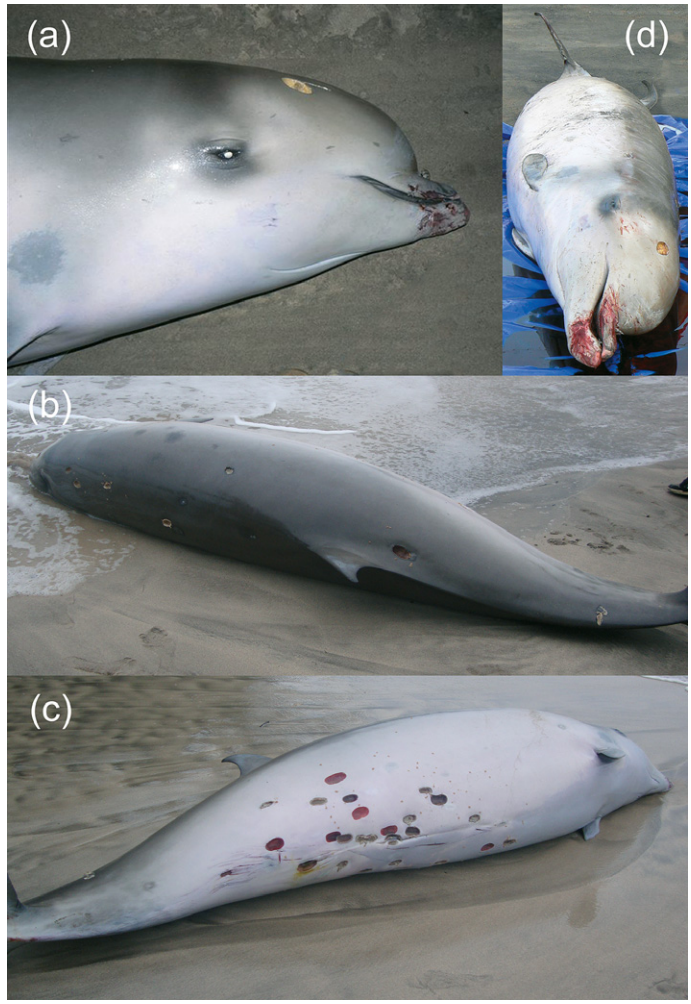

Figure 1. Indopacetus pacificus juvenile male that stranded in Hana, Maui, on 22 March 2010. (a) Right lateral head view, (b) dorsal surface with multiple, fresh cookie cutter shark bites, (c) ventral surface view with a high density of fresh cookie cutter shark bites in the caudal region of the abdomen, (d) right lateral view that illustrates the coloration pattern and the severely fractured mandible.

[5] III-5, IV-4, V-3, there being an additional smaller digit of five phalanges between digits II and III (Watson *et al.* 2008).

The species identification of the stranded Longman's beaked whale was confirmed by genetic analysis of a skin sample at the Southwest Fisheries Science Center, National Marine Fisheries Service. Standard protocols were used for DNA extraction (sodium chloride extraction, Miller *et al*. 1988), mitochondrial DNA (mtDNA) PCR and sequencing (Saiki *et al.* 1988, Sambrook *et al.* 1989, Palumbi *et al.* 2002). A 400 base pair region of the 5′ end of the hypervariable mtDNA control region was ampli-

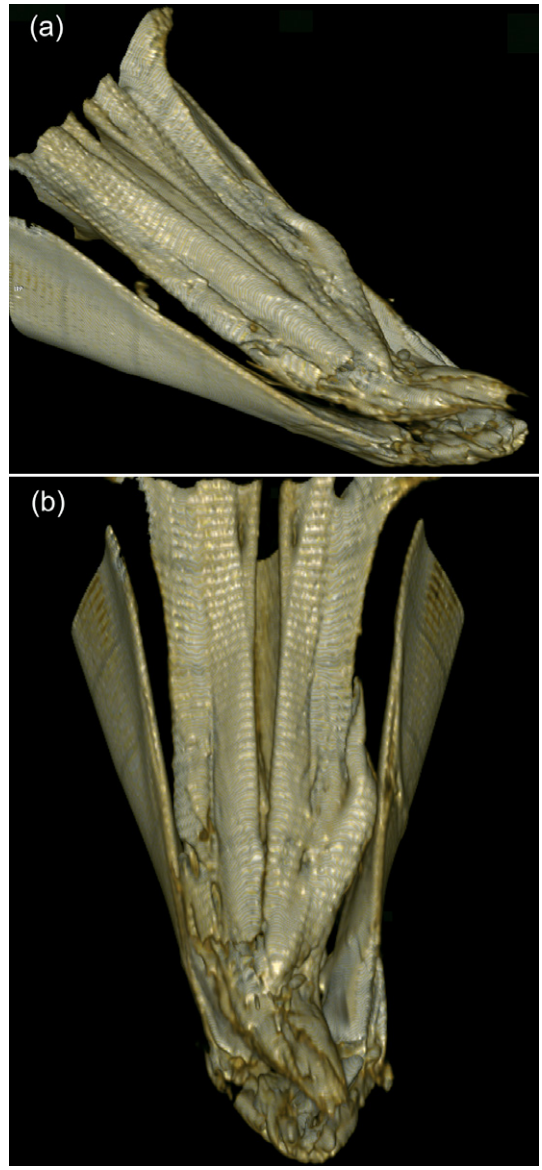

Figure 2. 3-D reconstructions of the skull of the Maui, Hawaii, *Indopacetus pacificus* were generated from the CT scan. (a) View from dorsal illustrating damage to the maxilla and mandible. The animal's left is to the left of the image and the deviation of the rostral fragments is evident. (b) Maxilla and mandibular damage view from right dorsolateral.

fied using primers TRO (5′-CCTCCCTAAGACTCAAGG-3′); developed at SWFSC and D (5′-CCTGAAGTAAGAACCAGATG-3′; Rosel *et al.* 1994). Sequencing of the PCR product in both directions was performed with the same primers as above using the ABI 3130XL Automated Sequencer (Applied Biosystems Inc., Foster City,

CA). All sequences were aligned using Sequencer v4.1 software (Gene Codes Corp., Ann Arbor, MI). The aligned sequence was submitted to DNA Surveillance (Ross *et al*. 2003) to obtain a species identification. The sequence matched 100% with the reference sequence of *Indopacetus pacificus* (IpMZUF1959).

Histopathological findings were extensive, with significant findings in the skeletal and nervous systems. Mandibular and maxillary osteonecrosis and fibrosis (early callus formation) were observed, indicating a chronic response, and it is likely that the trauma occurred several weeks prior to the stranding event. The severity of the fractures combined with the empty gastrointestinal tract noted on necropsy strongly suggest that feeding was impeded. A lymphoplasmacytic (nonsuppurative), primarily cerebral encephalitis was observed (Fig. 3). Cranial nerve VIII was unilaterally thickened (left) on CT and histopathology. Additional findings included renal lympoplasmacytic periglomerulitis, mild lymphoid depletion, pulmonary edema, thyroid gland atrophy, and bilateral hypertrophy of the adrenal cortex.

Because of the rarity of this stranding event, we conducted a number of follow-up analyses. The encephalitis was nonspecific, but the pattern was indicative of an infectious cause. Despite no previous reports of morbillivirus in Hawaiian waters, testing included the known cetacean viruses, morbillivirus and herpesvirus (Van Bressem *et al*. 1999, 2009). Tissues frozen at −80°C from the cerebrum, cerebellum, lung, spleen, thymus, and various lymph nodes (mediastinal, scapular, mesenteric, and colonic) were sent to the Athens Diagnostic Laboratory at the University of Georgia for molecular diagnostics. The morbillivirus PCR test utilized primers directed against the phosphoprotein (P) gene of cetacean morbillivirus (Nollens and Sanchez, unpublished data). All tissues tested positive for morbillivirus as indicated by visualization of a single band of the expected size. Sequencing of this P gene fragment revealed that it had 86% similarity to dolphin morbillivirus (EF451565) and 84%

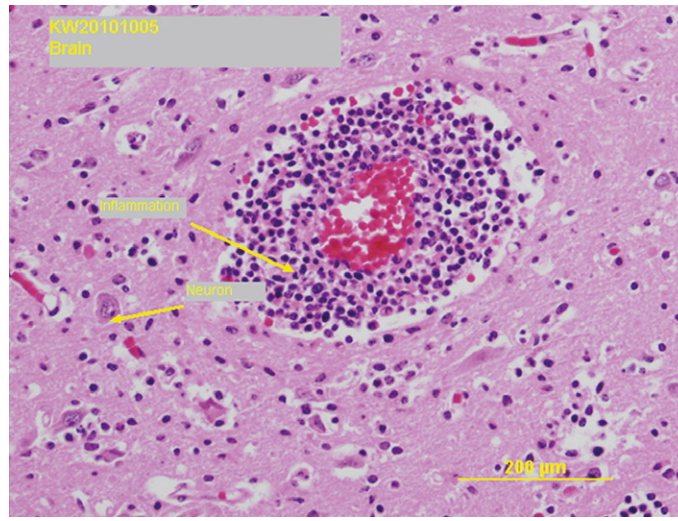

Figure 3. Cerebral vascular Virhcow-Robin spaces are expanded by abundant lymphocytes, plasma cells and macrophages. Lining endothelial cells are reactive. Within the neuropil, there are inflammatory cells and increased microglial cells.

similarity to pilot whale morbillivirus (AF200817) and the pilot whale-like cetacean morbillivirus (FJ842381). This partial P gene sequence has been deposited in GenBank under accession JX195718. Viral antigen was detected in neurons in the brain and spinal cord from this individual by immunohistochemistry using a monoclonal antibody directed against the nucleoprotein of CDV but known to cross-react with other morbilliviruses (Fig. 4). In addition, brain and mediastinal lymph node samples were sent to the University of California, Davis Marine Ecosystem Health Diagnostic and Surveillance Laboratory for herpesvirus testing. PCR to detect known and novel herpesviral DNA was performed using primary and nested consensus primers for the DNA-dependent-DNA polymerase (Dpol) gene of herpesviruses to amplify a fragment of 250 bp (VanDevanter *et al.* 1996). Brain from this individual tested positive, but no virus was detected in the mediastinal lymph node. Sequencing indicated that the herpesvirus identified in the Longman's beaked whale belongs to the alphaherpes subfamily with closest similarity to the common bottlenose dolphin (*Tursiops truncatus*) alpha herpesvirus (AY757301). Complete genetic analyses of these potentially novel strains of morbillivirus and herpesvirus are currently underway.

Morbillivirus infection in this cetacean was likely chronic stage and neurotropic. There was no evidence of viral inclusions or syncytia within the brain, lung, or lymph nodes. Prior multisystem infection resulting in bronchointerstitial pneumonia and immune suppression could have occurred or infection may have been relegated solely to the brain. Recent reports following an epizootic suggest that primarily neurotropic morbillivirus could be the chronic form that persists after the initial systemic infection (Domingo *et al.* 1995, Soto *et al.* 2011). However, in the Longman's beaked whale, positive PCR in all organs tested suggests that this may represent a different, earlier phase of infection. Encephalitis could have played a role in the stranding if neurological dysfunction occurred.

This is the first report of morbillivirus in a marine mammal from the central Pacific. Other reports of morbillivirus in the North Pacific include the detection of this virus from stranded short-beaked common dolphins (*Delphinus delphis*) in California, a

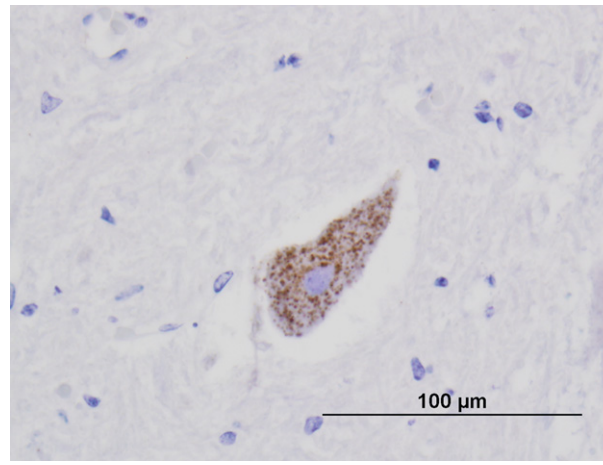

Figure 4. Morbilliviral antigen was detected by immunohistochemistry in the spinal cord neurons of the stranded Maui, Hawaii, *Indopacetus pacificus* juvenile male.

Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) from Japan and a pygmy sperm whale (*Kogia breviceps*) from Taiwan (Reidarson *et al.* 1998, Uchida *et al.* 1999, Yang *et al.* 2006). The surprising finding of morbillivirus in the Longman's beaked whale generates many questions about the history and prevalence of this disease in Hawaii and the potential impact on Hawaiian marine mammal populations. Hawaii's cetaceans may be particularly vulnerable to a morbillivirus outbreak as many of Hawaii's stocks are comprised of small, island-associated, resident populations where any reduction in population size may be devastating because of an already low number of breeding individuals (*e.g.,* Baird *et al*. 2008, Chivers *et al*. 2010, Aschettino *et al*. 2011, Carretta *et al*. 2011). Additionally, Hawaii is home to the endemic and already critically endangered Hawaiian monk seal (*Monachus schauinslandi*). Future planned work includes a genetic characterization of the identified Hawaiian morbillivirus, an investigation into the prevalence of this disease among Hawaii cetaceans and an assessment of the potential impact of this disease on Hawaiian marine mammal populations.

In summary, potentially novel virus strains were detected in the first Longman's beaked whale to strand in the United States. Genetic testing of this specimen confirmed the presence of Longman's beaked whales in Hawaiian waters. The complete skeleton is vouchered at the Natural History Museum, Smithsonian Institution (accession number: USNM 593534).

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