

Report on the Final 2024 Collaborative Beaked Whale Expedition off Baja California, Mexico

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INTRODUCTION

Beaked whales (Family Ziphiidae; Order Cetacea) are the least well-described group of marine mammals because they conduct very long deep dives, spend little time on the surface where they display cryptic behavior, and are difficult to identify due to the similarity between species. Beaked whales are also known to be susceptible to high-energy sound sources in the ocean (e.g. seismic research, sonars used by military activities). Previous research conducted off the western coast of Baja California, Mexico recorded vocalizations of two unidentified and possibly new species of beaked whales of the genus *Mesoplodon*; these echolocation pulses have been called the BW43 and BWB pulses based on their peak frequency and location where they were recorded, respectively (Baumann-Pickering et al. 2013; Barlow et al. 2021). During the initial effort in 2020, photographs were also obtained of one group of three unidentified animals (Barlow et al. 2021; Henderson et al. 2021, 2022, 2024), but the water sample collected for environmental DNA (eDNA) did not produce any genetic results. The goal of the current effort was to identify which species of beaked whales occur off Baja California using a combination of photo-identification and genetic sampling, and link them to their vocalizations via simultaneous acoustic recordings in order to better understand the status of these poorly known species.

Given the difficulties in finding these beaked whales in the previous expeditions using platforms of opportunity, this expedition was conducted in collaboration with Oregon State University to improve the chances of success. The Oregon State University research vessel *R/V Pacific Storm* allowed for the use of long-range binoculars (called "big eyes") that increased the probability of finding beaked whales; the research vessel also supported the use of a towed hydrophone array to detect the acoustic presence of beaked whales in real time. Due to the morphological similarity between species of beaked whales of the genus *Mesoplodon*, it was also important to collect dermal tissue (via biopsies and eDNA) from individuals that were sighted to confirm the identification at the species level with genetic analysis. Some species of beaked whales can be identified based on diagnostic characteristics, mainly on the position of the pair of teeth in the jaw in male individuals, but some species look very similar in photographs and need to be further distinguished with genetics. Therefore, the collection of biopsies was key to the success of this project. This type of collection, together with the simultaneous recording of the vocalizations of the beaked whales sighted, allowed the identification at the species level of the beaked whales previously sighted and recorded in Mexican waters.

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OBJECTIVES

The objectives of this effort were to 1) record vocalizations, collect photographs, video recordings, and genetic samples of beaked whales of the genus *Mesoplodon* in the waters of the Pacific Ocean coast off the Baja California Peninsula, Mexico; and 2) to use these data to determine the status and identify at the species level the beaked whale species of the *Mesoplodon* genus found in this region.

METHODS

Visual Monitoring and Photo-Identification

During the expedition, a rotation of five visual observers monitored from two platforms onboard the *R/V Pacific Storm*; these included a crow's nest 10.7 m above the water level where handheld binoculars were used, and a large platform above the wheelhouse 6.7 m above the water line where two 25 x 150 mm big-eye binoculars were positioned. Sighting and general event data were recorded in SeaScribe software (Bureau of Ocean Energy Management). Once a group of beaked whales were sighted, the observers took photographs of all individuals for identification of species and photo-identification of individuals. This photo-identification technique is a way of "marking" animals for further study, based on individual recognition of an animal using permanent characteristics or long-lasting marks on the skin (for example, color patterns, scars caused by sharks, etc.), or by using the shape of a specific body part such as the head, dorsal fin, etc. Images were recorded with digital cameras in JPG format and were edited using software to achieve the best possible image quality to facilitate species-level identification of the cetaceans and beaked whales observed. A beaked whale photo-identification catalogue was created with all the information related to the sighting. The catalogue also allowed us to differentiate how many individuals there were in the beaked whale groups. This catalogue was also compared against the catalog from the original sighting in 2020 to determine if the same species and individuals were resighted (Henderson et al. 2021; Barlow et al. 2021).

Acoustic Monitoring

Multiple instruments were used to record the sounds produced by the beaked whales to detect their vocalizations both in real time and autonomously. The first was a hydrophone array made up of three HTI-96-min (High-Tech, Inc.; Long Beach, MS) hydrophone elements in an oil-filled tube, towed 150 m behind the vessel at a mean depth of 16 m. The array was connected by a cable to a data acquisition system (NI-USB-6356, National Instruments; Austin, TX), and from there the data were recorded to a hard drive and monitored in real time. This system was monitored during all daylight hours by a rotation of three expert acousticians, allowing us to detect beaked whales in real time and provide information to the visual team. In addition to the towed array, Drifting Acoustic Pole Buoy Recorders (DASPRs) were deployed at each beaked whale sighting location, as well as various locations throughout the survey to be recovered later. The DASBRs were equipped with Soundtrap loggers (Ocean Instruments, NZ; www.oceaninstruments.co.nz) of the ST300 and ST600HF models, which were tied to a rope with one end at a depth of 10 meters below the surface; the other end of the rope was attached to a pole and large buoy so the system could be placed in the water and set adrift. The Soundtrap sound logger is designed for general purpose stand-alone acoustic monitoring with a recording frequency range of 100 to 200,000 Hz. The buoys also had a satellite device (Spot tracker; www.findmespot.com) that allowed us to track the location of the buoy for later recovery. These DASPRs were deployed on a time scale of hours

to days, and provided a secondary mechanism to record beaked whale vocalizations separate from the vessel.

Genetic Sampling

Once a group of beaked whales were photographed, attempts were made to either collect water samples from the dive “footprint” of the group for eDNA or to take a biopsy sample directly from an animal. The collection of biopsies from the cetaceans consisted of collecting skin and fat was done using a modified arrow with a metal “sampler” tip measuring 2 to 3.5 cm long by 8 mm in diameter, previously sterilized with alcohol, propelled by a 25 kg/force crossbow. Once the samples were taken, with the help of previously sterilized forceps, the sample was removed from the metal tip and preserved in a 96% molecular grade alcohol solution and refrigerated in a freezer on the ship. In the Genetic Traceability Laboratory of the Fish Farming Biotechnology Unit of the Autonomous University of Baja California located in the City of Ensenada, BC, DNA was extracted from approximately 10 mg of epidermal tissue using the rapid salt extraction protocol (Aljanabi & Martinez, 1997). To assign the species, ~500 bp mtDNA fragments from the control region (primers Dlp1.5-L and Dlp5-H) and the cytochrome b gene (primers GLUDG-L and CB2-H) were independently PCR amplified and sequenced following the methods described in Dalebout et al. (2004). Sequences were reviewed and edited by eye in ChromasPro software v2 (Technelysium Pty Ltd, Australia). The edited mtDNA sequences were assembled, uploaded, and compared to sequences available in the GenBank database (June 2024) using NCBI's BLAST online software. The water samples taken for eDNA underwent a filtering, storage, and filtration process to obtain diverse genetic material. The filters were used later in the laboratory for the development and improvement of techniques for extracting and identifying cetacean species from small amounts of genetic material left by the animals in the water.

STUDY AREA

Navigations were carried out following proposed transects (Figure 1) of up to 100 km to search for beaked whales. These transects were demarcated into two areas: a main area where the greatest search effort was planned and a potential area further south where efforts could be made if weather conditions were not safe to navigate in the main area. In addition, it is important to note that beaked whales of the genus *Mesoplodon* are no longer than 6 meters and their blow is not readily visible. Therefore, very calm sea conditions (little wind and swell, i.e. Beaufort sea state 0 or 1) are needed to increase the probability of sighting them. Therefore, the survey had to be adaptable to local weather conditions and move where calm seas might be available. The primary study area was delimited along the western coast of the Baja California Peninsula in front of the municipalities of Ensenada and San Quintín, Baja California and the municipalities of Mulegé and Comondú, Baja California Sur. This area is delimited by the northwest vertex of the potential study area with coordinates 32° 20.068'N and 118° 34.006'W and by the southwest vertex with coordinates 23° 38.653'N and 118° 11.487'W.

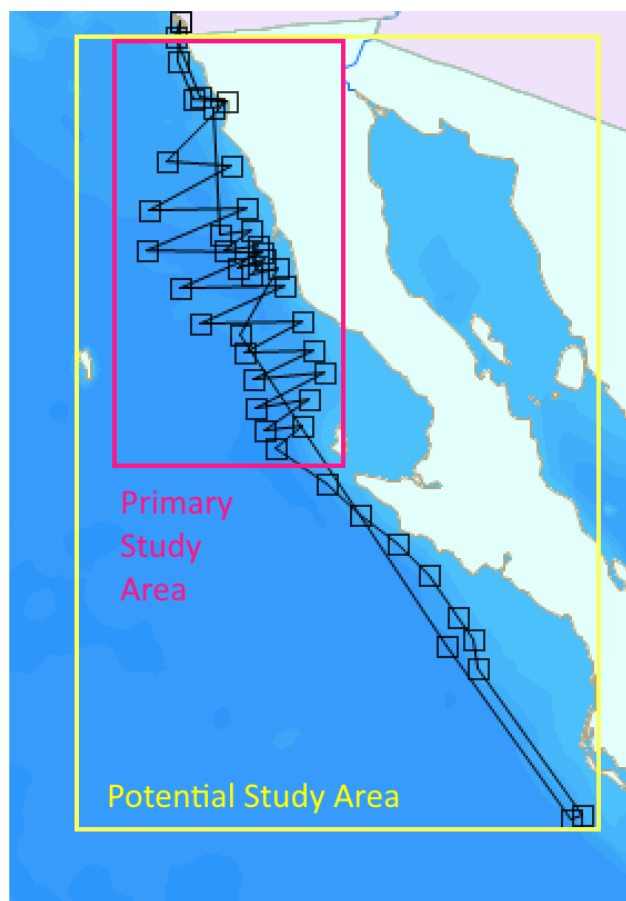


Figure 1. Map showing the study area. A primary study area is demarcated in red where the main search effort for beaked whales was planned. A potential study area in yellow is also demarcated that could be utilized in case the weather conditions in the primary area are unsafe for navigation or not suitable for beaked whale study.

RESULTS

The R/V *Pacific Storm* departed from the port of San Diego, California, USA, heading to Mexican waters on May 31, 2024, and returned to port on June 20, 2024. The search effort targeted seamounts and other seafloor features where the bathymetric relief might be suitable beaked whale habitat. The sighting and detection locations of the ginkgo-toothed beaked whales (*Mesoplodon ginkgodens*) are visualized in Figure 2 and are described in greater detail below.

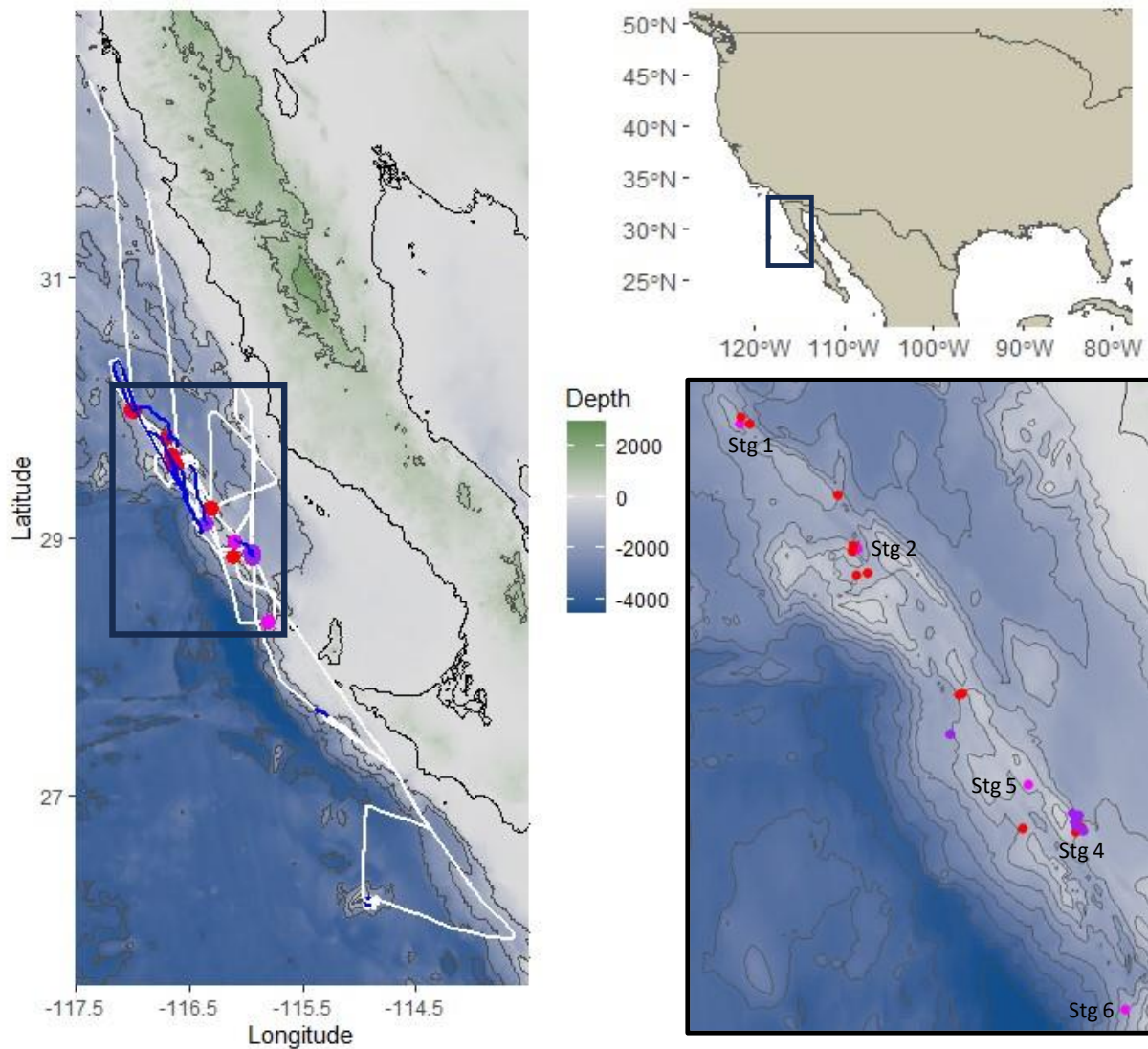


Figure 2: Left plot indicates the area of the survey, with the survey trackline in white, DASPR tracks in blue, sightings of BW43 whales in magenta, and acoustic detections of BW43 from towed array in red and from DASPRs in purple. Top right map is a map of North America north of 20° indicating where the survey took place; bottom right map is zoomed into the area of the survey outlined on the lefthand map, with a close-up of the locations of ginkgo-toothed beaked whale sightings and acoustic detections. Map bathymetry data retrieved from www.gebco.net/data_and_products/gridded_bathymetry_data/.

Sightings and Photo-identification

There were six beaked whale sightings (Table 1), one of which was a goose-beaked whale (*Ziphius cavirostris*), while the remaining five were of an unidentified *Mesoplodon* species that were later determined to be ginkgo-toothed beaked whales through photographs, genetic, and/or acoustic analyses. In addition to these beaked whales, there were also sightings of multiple species of baleen

whales, pinnipeds, and odontocetes, including several encounters of sperm whales (*Physeter macrocephalus*) that may have impacted our abilities to detect beaked whales (Table 2).

Table 1: Sightings observed on the research cruise in June 2024. A * indicates recordings of BW43 echolocation pulses were made in the immediate area of the sighting.

Sighting	Date	Time (PDT)	Species Common Name	Count	Latitude	Longitude
1	6/1/2024	16:57	ginkgo-toothed beaked whale*	1	29.977	-117.011
2	6/5/2024	12:32	ginkgo-toothed beaked whale*	5	29.633	-116.639
3	6/6/2024	12:35	goose-beaked whale	2	29.483	-116.443
4	6/10/2024	9:02	ginkgo-toothed beaked whale	2 (3?)	28.358	-115.798
5	6/10/2024	9:15	ginkgo-toothed beaked whale	2	28.881	-115.954
6	6/18/2024	9:59	ginkgo-toothed beaked whale*	2	27.072	-115.954

Table 2: Other marine mammal species observed during the expedition.

Species Name	Latin Name	# Observations	Total Count
Blue Whale	<i>Balaenoptera musculus</i>	2	2
Unidentified Baleen Whale	--	1	1
Unidentified Balaenopterid Whale	--	4	4
Common Dolphin	<i>Delphinus sp.</i>	13	719
Unidentified Beaked Whale	--	1	1
Unidentified fur seal	--	23	36
Guadalupe Fur Seal	<i>Arctocephalus townsendi</i>	6	17
Northern Elephant Seal	<i>Mirounga angustirostris</i>	7	7
Pacific White-sided Dolphin	<i>Lagenorhynchus obliquidens</i>	2	80
Killer Whale	<i>Orcinus orca</i>	1	6
Sperm Whale	<i>Physeter macrocephalus</i>	30	121
Unidentified Otariid	--	6	74
Risso's Dolphin	<i>Grampus griseus</i>	1	12
Bottlenose Dolphin	<i>Tursiops truncatus</i>	1	25
Humpback Whale	<i>Megaptera novaeangliae</i>	2	2
Bryde's whale	<i>Balaenoptera edonii</i>	1	2
Unidentified Dolphin	--	1	3

The sightings of ginkgo-toothed beaked whales represent the first identified sightings of this species of beaked whale at sea. The adult male was photographed in the group of five beaked whales (Figure 3); the head of the male clearly demonstrates the location of the tooth in the lower jaw, as well as the color of the tip of the face to be observed, as well as the curvature and shape of the jaw (Figure 4). From these photographs, identification at the species level could be inferred when coupled with the genetic analysis.



Figure 3: The heavily scarred back of the adult *M. gingkodens*; the large white spots are cookie cutter shark bite wounds. The numerous fine white lines are tooth rake marks from the small teeth of other males; the marks are all single lines because the teeth do not project high enough above the rostrum to leave parallel tracks. Photo by C. Hayslip.

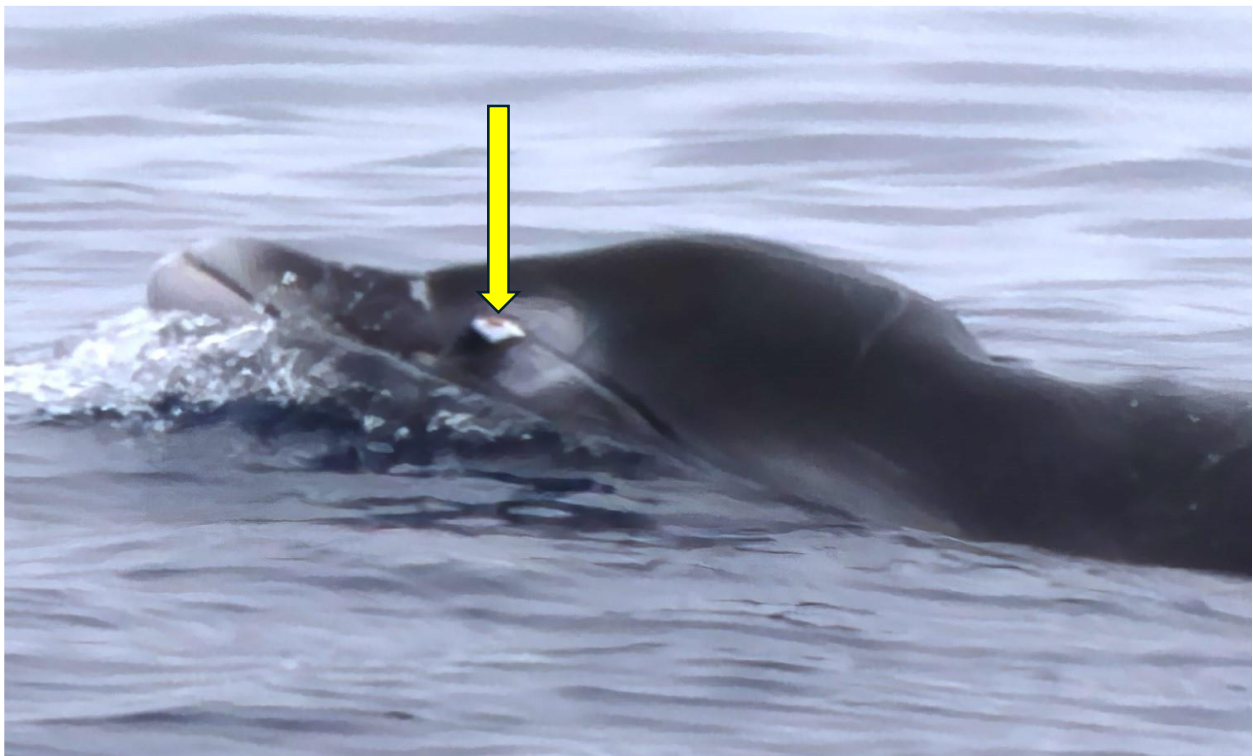


Figure 2: The head of the adult male *M. gingkodens*. Visible is the relatively short, white-tipped beak, and tiny, erupted tooth (orange spot; arrow) surrounded by white scar tissue. The mouthline curves up to a peak, with the tooth at the apex, then angles back and down toward the eye in a relatively straight line. Behind the tooth, on the melon and on lower jaw, are pale gray “bruise” marks (see text), possibly from forceful contact with other males. Photo by C. Hayslip.

Acoustic Detections

The towed array was in the water for most of the survey, only coming out of the water to deploy or recover the pole buoys or the small boat. DASPRs were deployed 18 times; eight were deployed at the location of beaked whale sightings, while 10 were deployed along the trackline and left to drift (Table 3). Between the towed array and the DASPRs, there were 19 acoustic detections of BW43 echolocation pulses, 2 mixed BW43/BWB acoustic detections, and three detections of unidentified beaked whales (Table 4). Not included in the table are 45 acoustic detections of goose-beaked whales, and several detections last multiple days of sperm whales. BW43 detection events lasted from 0.05 to 28.83 minutes and had a mean acoustic encounter duration of 4.72 minutes. Several of the sightings and acoustic detections were related; some sightings were triggered by an acoustic detection, and some acoustic detections followed a sighting. These detections occurred on 7 separate days and corresponded with ginkgo-toothed beaked whale sightings 1, 2, 3, and 5 (Table 1). Acoustic detections on June 8 and 12 were not sighted, and the June 10 sighting was not detected on the towed hydrophone array. These associations are indicated in the following tables.

Table 3: DASPR deployment information. Drifts marked with an * were deployed in the area of a visual sighting.

Drift #	Start Date-Time (PDT)	Retrieve Date-Time (PDT)	Dur (day)	Recorder Type	Sample Rate kHz	Duty Cycle On/Off Min
1	6/2/2024 8:08	6/4/2024 10:00	2.1	ST300	576	2/0
2	6/2/2024 15:56	6/4/2024 6:30	1.6	ST300	576	2/0
3	6/5/2024 6:35	6/7/2024 17:36	2.5	ST300	576	2/0
4*	6/5/2024 20:20	6/8/2024 15:35	2.8	ST300	576	2/0
5*	6/5/2024 20:36	6/8/2024 15:55	2.8	ST300HF	576	2/0
6*	6/5/2024 21:03	6/8/2024 14:34	2.7	ST300HF	576	2/0
7	6/9/2024 8:03	6/10/2024 7:00	1.0	ST300	576	2/0
8*	6/10/2024 7:38	6/10/2024 16:03	0.4	ST300	576	2/0
9*	6/10/2024 8:26	6/10/2024 15:49	0.3	ST300HF	576	2/0
10*	6/10/2024 11:28	6/10/2024 16:58	0.2	ST300	576	2/0
11	6/12/2024 7:42	6/12/2024 17:20	0.4	ST300	576	2/0
12	6/12/2024 10:40	6/12/2024 17:05	0.3	ST600HF	384	2/0
13	6/13/2024 8:20	6/13/2024 18:40	0.4	ST600HF	384	2/0
14	6/15/2024 13:02	6/15/2024 18:57	0.2	ST300	576	2/0
15	6/15/2024 14:46	6/15/2024 19:43	0.2	ST300HF	576	2/0
16*	6/18/2024 10:51	6/18/2024 18:10	0.3	ST300	576	2/0
17*	6/18/2024 12:26	6/18/2024 18:00	0.2	ST300HF	576	2/0
18	6/19/2024 9:45	6/20/2024 4:17	0.8	ST600HF	384	2/0

Table 4: Beaked whale acoustic detections on the towed array and DASPRs with at least three pulses. Each row represents an acoustic encounter, which could be from one or more beaked whales in a group echolocating together. BW43 are ginkgo-toothed beaked whales, BWB are the beaked whales detected off Baja California, Mexico in 2020 with clicks similar to BW43, and unid BW are unidentified beaked whale clicks, likely *Mesoplodon*. Detections associated with a sighting are indicated with an *.

Species	Recorder	Start Time (PDT)	Duration (min)	Latitude	Longitude
BW43*	Towed Array	6/1/24 13:14	2.01	29.976	-116.979
BW43*	Towed Array	6/1/24 16:25	28.83	29.993	-117.005
BW43	Towed Array	6/2/24 12:28	2.63	29.641	-116.653
BW43	DASPR 2	6/4/24 3:43	14.82	29.121	-116.348
BW43*	Towed Array	6/5/24 13:42	0.05	29.628	-116.657
BW43*	Towed Array	6/5/24 16:58	5.30	29.562	-116.641
BW43	Towed Array	6/6/24 4:11	0.25	29.568	-116.608
BW43	Towed Array	6/7/24 20:13	0.08	29.781	-116.700
Unid BW	Towed Array	6/8/24 3:43	5.42	29.958	-116.936
BW43	Towed Array	6/8/24 19:12	4.62	28.863	-116.118
Unid BW	Towed Array	6/8/24 23:59	8.53	29.042	-115.964
BW43/BWB	DASPR 7	6/9/24 20:59	0.89	28.904	-115.962
BW43	DASPR 8	6/10/24 1:06	11.72	28.879	-115.957
BW43	DASPR 12	6/12/24 1:11	0.36	28.893	-115.944
BW43/BWB	DASPR 11	6/12/24 8:08	17.30	28.898	-115.943
BW43	Towed Array	6/12/24 8:12	4.00	28.873	-115.944
BW43	Towed Array	6/12/24 14:25	1.63	28.866	-115.935
Unid BW	DASPR 11	6/12/24 14:25	0.25	28.867	-115.946
BW43	DASPR 12	6/12/24 15:12	0.21	28.869	-115.954
BW43	DASPR 11	6/12/24 15:14	23.01	28.855	-115.930
BW43	Towed Array	6/12/24 15:19	6.63	28.853	-115.954
BW43*	Towed Array	6/18/24 10:03	29.02	28.875	-115.935
BW43	Towed Array	6/19/24 0:12	0.05	29.233	-116.308
BW43	Towed Array	6/19/24 0:14	5.22	29.230	-116.321

All BW43 clicks from the towed array detections were processed in PAMpal to extract click features and statistics. The clicks had a mean peak frequency of 42.3 kHz (SD = 11.1 kHz), a 10 dB down lower endpoint value of 35.8 kHz (SD = 10.96 kHz), a mean ICI of 0.25 ms (SD = 0.16 ms), and a duration of 141.0 μ s (SD = 274.0 μ s). These values largely match those described from previous recordings of BW43 pulses (Baumann-Pickering et al. 2013, 2014; Fregosi et al. 2020), although pulse duration here is shorter, possibly due to more off-axis clicks being recorded. There were also lower peaks at higher frequencies and occasionally at 23 kHz as well (Figure 3).

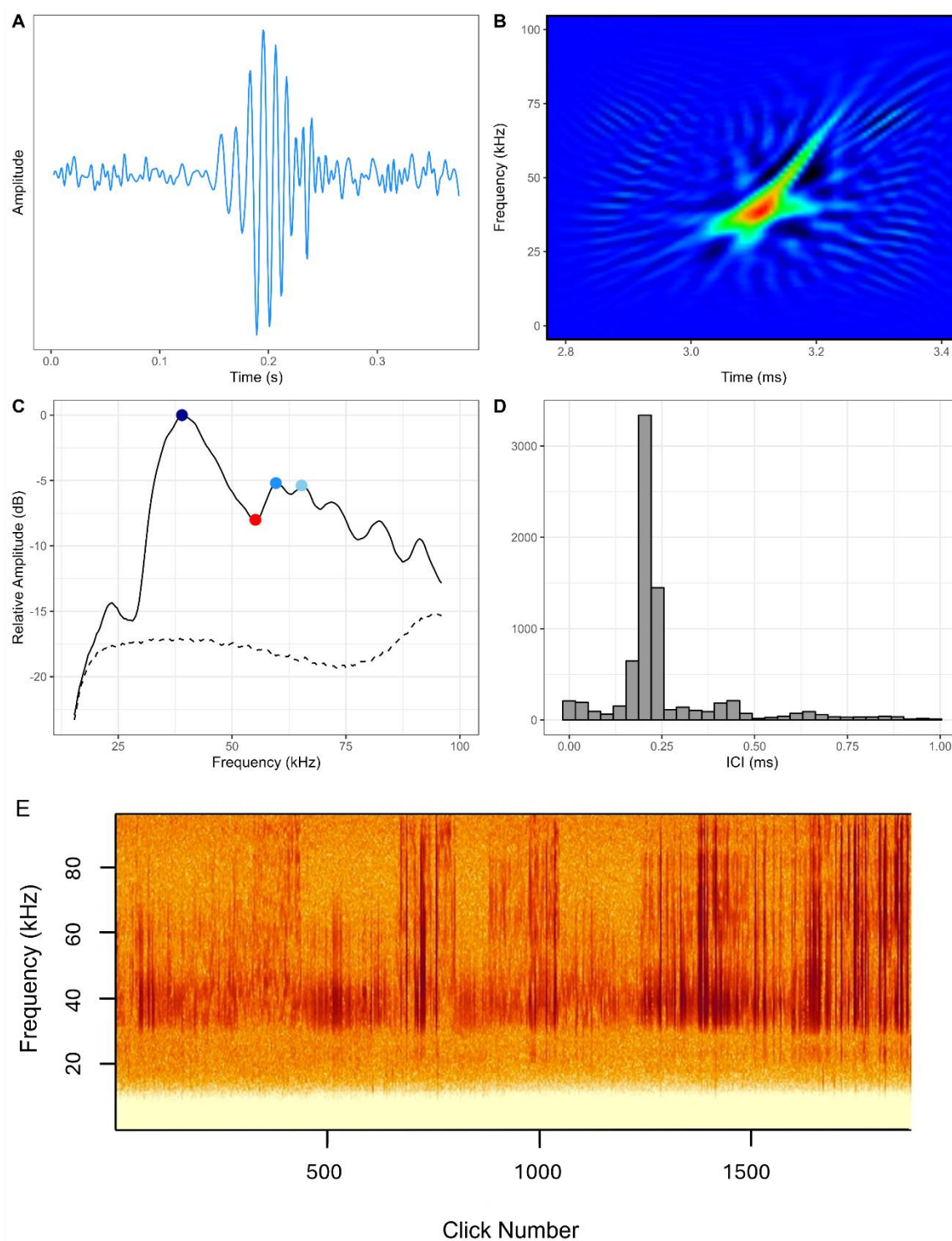


Figure 3: (A) Amplitude of a BW43 click train over 1 sec; (B) Wigner-Ville plot and associated timeseries of a BW43 click; (C) Relative amplitude vs time, with markers at secondary peak frequencies; and (D) Histogram of inter-pulse intervals taken from the concatenated click spectrogram (E).

Genetic Identification

During sighting #4 of the ginkgo-toothed beaked whales, a biopsy was collected from one animal. The dart tip was only able to obtain a very small piece of skin, but it was enough for the sample to be processed in the laboratory for analysis. Water samples for eDNA were collected during sightings #2, 3, and 4. From the edited sequences of the mtDNA control region (427 bp) and cytochrome b (416 bp), the species was identified as the ginkgo-toothed beaked whale. The BLAST search yielded close matches with the published records MH019963, from strandings in Korea (100% coverage, 99.77% identity), and NC_027593, from a stranding in Taiwan (98% coverage, 98.54% identity). Therefore, what had been inferred through the analysis of the beaked whale photographs could be confirmed with genetic analysis. The processed biopsy sample is stored in the office of the Directorate Baja California Peninsula and North Pacific Region of the National Commission of Protected Natural Areas (CONANP) located in the city of Ensenada, Baja California. The processed cytology samples are stored at the genetics lab at Oregon State University.

DISCUSSION

These data represent the first at-sea confirmed sighting of ginkgo-toothed beaked whales, verified through genetic analysis and photo-identification. This species was also linked to the BW43 echolocation pulse that had previously been hypothesized to belong to Perrin's beaked whale (*Mesoplodon perrini*; Baumann-Pickering et al. 2013). While the individual animals from the original 2020 sighting were not seen during this expedition, the features from those animals closely resemble the external features and color patterns observed during this current effort (Barlow et al. 2021; Henderson et al. 2021). In addition, although at the time it was determined that the BWB echolocation pulse was distinct from the BW43 echolocation pulse due to the presence of a secondary peak at 26 kHz (Barlow et al. 2021), there were two instances in the current effort where both pulse types were present in the same bout of clicks. Therefore, it may be that the BWB and BW43 beaked whales are the same species, and ginkgo-toothed beaked whales were observed in 2020 as well. This will be explored in greater detail in a follow-on acoustic analysis.

A review of the distribution of ginkgo-toothed beaked whales is currently being conducted (Henderson et al, in prep), as this was previously considered a tropically distributed species. However, BW43 echolocation pulses have been recorded along the Baja California Peninsula, Mexico in this and other efforts (Henderson et al. 2021, 2022, 2024; Keating et al. 2018; Simonis et al. 2020), as well as in southern California (Baumann-Pickering et al. 2013, 2014). The confirmation that these pulses are produced by ginkgo-toothed beaked whales therefore extends the known range of that species considerably.

These results also lead to two further unresolved mysteries. First, since Perrin's beaked whales do not produce the BW43 echolocation pulse, their echolocation pulse remains to be identified. This species has only been visually identified from five strandings in California (Dalebout et al., 2002) and has never been seen alive at sea. Extensive acoustic monitoring has been conducted in southern California, and the only remaining echolocation pulse yet to be linked to a species is BW40 (Baumann-Pickering et al. 2013, 2014). Therefore, it is likely that Perrin's beaked whale produces the BW40 echolocation pulse, but this needs to be confirmed. Second, Baumann-Pickering et al. (2013, 2014) had hypothesized that the ginkgo-

toothed beaked whale produces the BWC echolocation pulse, based on that pulse's tropical distribution (McCullough et al. 2024). Now that we know that is not the case, the beaked whale species needs to be identified that produces that pulse, which is the only beaked whale pulse to be produced only at night. Once these mysteries are solved, all known beaked whales in the North Pacific will have been linked to their echolocation pulses, allowing researchers to better understand the occurrence and distribution of these species throughout this ocean basin, as well as their specific habitat use and seasonal and diel patterns through acoustic monitoring.

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