

First confirmed case of neonatal fraternal twin humpback whales in the Mexican Pacific Distinct Population Segment

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Life-history strategies of mammals vary widely, with reproductive traits evolving to maximize fitness (Bronson, 1985). A strong negative correlation exists between offspring number and birth weight (Enzmann & Crozier, 1935; Clapham, 1996). Consequently, all cetaceans are considered uniparous, typically giving birth to a single calf (Slijper, 1949; 1966; Clapham, 1996). In smaller cetaceans, rare cases of multiple fetus pregnancies have been documented in the wild (González et al., 1999; Davison et al., 2016) and in captivity (Gray & Conklin, 1974; Osborn et al., 2012), but successful twin births have not been documented (Osborn et al., 2012). In large whales, confirmed evidence of twins is limited to multiple fetuses recovered from harvested females (Slijper, 1949; Kimura, 1957; Chittleborough, 1958; Best et al., 1984; Drinkwater & Branch, 2022) and unsuccessful births of conjoined animals (Tamburin et al., 2017).

Knowledge of multiple pregnancies in large whales largely derives from industrial whaling records. Using International Whaling Commission data, Drinkwater & Branch (2022) reported that 0.87% (2,197 out of 252,651) of pregnancies involved multiple fetuses in baleen whales. In humpback whales (*Megaptera novaeangliae*), the reported frequency is lower, at approximately 0.57%, although twin fetuses have been documented in several studies (Slijper, 1949; Kimura, 1957; Chittleborough, 1958; Drinkwater & Branch, 2022).

Multiple fetuses in pregnant large whales were most frequent in females of greater length and age (Kimura, 1957), which suggests that most twins are produced by physically well-developed mothers that have attained a greater length than the average for their age. Males predominated among fetuses and size asymmetry was often observed between twin fetuses (Slijper, 1949; Kimura, 1957). In uniparous mammals, multiple pregnancies can be classified as either monozygotic (identical twins), which arise from the division of a single fertilized egg and produce genetically identical embryos that share nearly all polymorphic alleles, or multizygotic (fraternal twins), which result from the fertilization of two or more separate eggs (Endres & Wilkins, 2005). In the latter case, offspring share on average 50% of their alleles, a level of genetic similarity comparable to that observed among non-twin siblings (Sahu & Prasuna, 2016; Beck et al., 2021). In a global analysis of whaling-derived datasets, Drinkwater & Branch (2022) reported that the proportion of monozygotic twin fetuses was consistently lower than that of dizygotic twins across mysticete species (4–34%), with the notable exception of humpback whales, in which monozygotic twins represented a higher proportion (57%).

Despite increasing documentation of multiple fetuses, there remains no confirmed evidence of cetaceans successfully birthing twin calves (Drinkwater & Branch, 2022). Rare reports include

ARTICLE INFO

Manuscript type: Note

Article History

Received: 18 January 2026

Received in revised form: 07 April 2026

Accepted: 08 April 2026

Available online: 30 April 2026

Responsible Editor: Jorge Urbán

Citation:

Frisch-Jordán, A., Enríquez-Paredes, L. M., Llamas-González, M., Victoria-Cota, N. L., Flores-Márquez, F., Morales-Vázquez, J. E., Torres-Campos, E., Reyes-Núñez, C., Ransome, N., & Ortega-Ortiz, C. D. (2026). First confirmed case of neonatal fraternal twin humpback whales in the Mexican Pacific Distinct Population Segment. *Latin American Journal of Aquatic Mammals*, 21(1), 27-35. <https://doi.org/10.5597/lajam000364>

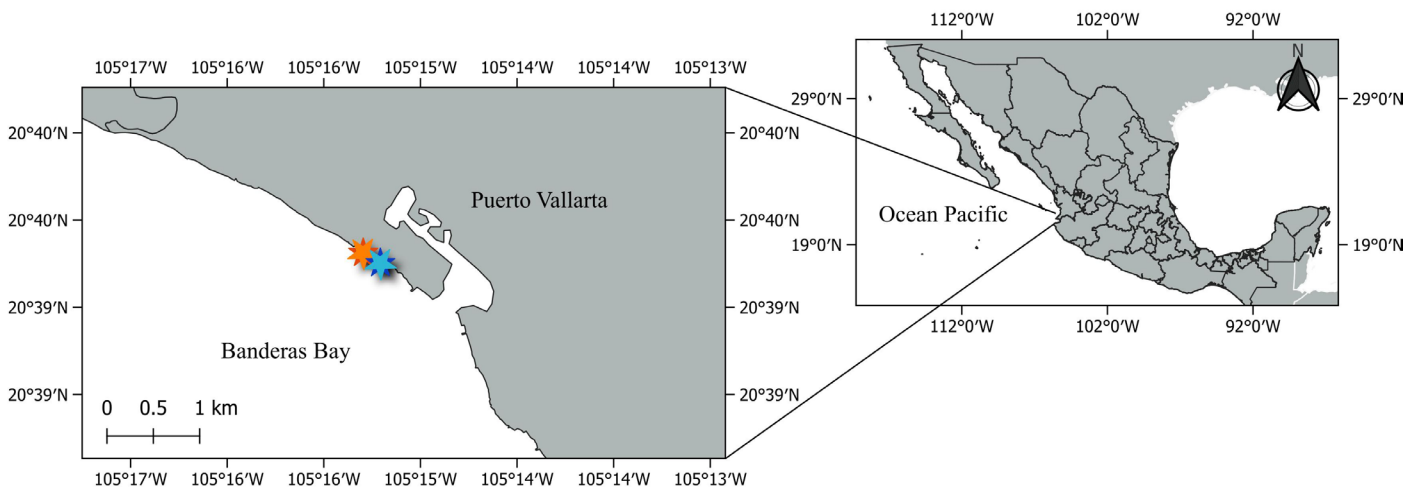


Figure 1. Location of the simultaneous strandings of two humpback whale calves (Calf 1: orange star, Calf 2: blue star) on 17 January, 2021, in Marina Vallarta, Puerto Vallarta, Jalisco, Mexico.

stillbirths of conjoined animals, including one humpback whale (Zemsky & Budylenko, 1970; Kompanje, 2005; Kompanje et al., 2017; Tamburin et al., 2017), as well as cases of premature twin fetuses (Tiongson et al., 2025). In captive cetaceans, a single case of a full-term twin pregnancy has been reported in a beluga whale, in which only one calf survived (Osborn et al., 2012). Genetic evidence of twin calves born to the same female in the wild has remained elusive.

Cetaceans exhibit the fastest fetal growth rates among mammals (Frazer & Huggett, 1959; van Aswegen et al., 2025a). This represents an extreme energetic demand on pregnant females (Lockyer, 1981; van Aswegen et al., 2025b). Modeling studies suggest that while twin survival declines sharply with fetal growth, humpback whales may be capable of carrying twins to full term, albeit with high mortality risk (Drinkwater & Branch, 2022).

Because most knowledge of whale multiple pregnancies derives from historical whaling, strandings remain the main source of evidence in modern populations, including studies documenting vessel strikes and fishing-gear interactions affecting humpback whales in the Mexican Pacific (Ransome et al., 2021; Lazcano-Pacheco et al., 2022; Frisch-Jordán & López-Arzate, 2023).

Here, we report the first confirmed documentation of a live twin birth in a large whale species, based on the stranding of two full term, neonate humpback whale calves in January 2021, in Puerto Vallarta, Jalisco, Mexico.

Humpback whales have a cosmopolitan distribution and migrate between high latitude feeding areas and low latitude breeding areas (Dawbin, 1966), with 14 distinct population segments (DPSs) recognized in (sub-) tropical breeding regions worldwide (Bettridge et al., 2015; NOAA, 2016). Banderas Bay is located at the center of the mainland Mexico breeding area, of the Mexico DPS (Calambokidis et al., 2008; Bettridge et al., 2015), with sexually mature adults of both sexes assembling each winter to breed, and females arriving after approximately 11 months gestation (Chittleborough, 1958) to give birth (Ransome et al., 2022).

At approximately 06:00h on 17 January, 2021, the Banderas Bay Stranding Network received a report of a live humpback whale calf stranded on Marina Vallarta beach, in Puerto Vallarta, Banderas Bay, Mexico (Fig. 1). Upon arrival of stranding network

personnel at the site at ~07:00h, it was determined that there were two calves stranded on the same beach approximately 250 m apart (Fig. 1). According to local surveillance personnel, the calves likely stranded around 01:30h that morning. The calf initially reported alive (Calf 1) had already died, and the second calf (Calf 2) was also found dead. Following the guidelines of the stranding protocol (DOF, 2014), the species was identified, an external examination was performed to look for wounds and/or scars, to determine the sex of the individuals, and photographs and videos were taken. Additionally, body measurements were made, and skin tissue and baleen samples were collected from both carcasses. Both calves were located in the wave-breaking zone, oriented parallel to the waves, and their position varied with wave action. Calf 1 stranded further north on the beach (Fig. 1) and had a total curved length of 4.87 m, while Calf 2 measured 3.78 m (Table 1). The weight of both calves was estimated using the WhaleScale application v4.0.1 (Harms et al., 2024). Both calves presented folds typical of newborns. Calf 1 presented fresh attached remnants of a bleeding umbilical cord measuring 10.5 cm in length, as well as an open umbilicus with no signs of cicatrization. Calf 2 presented an open and bleeding umbilicus with 1 cm of remaining umbilical cord. No external parasites, such as cyamids or barnacles, were observed on either calf

Table 1. Geographic position and general body measurements of humpback whale calves stranded on 17 January, 2021, in Marina Vallarta, Puerto Vallarta, Jalisco, Mexico. The weight of both calves was estimated using the WhaleScale application.

Location and corporal measurements	Calf 1	Calf 2
Latitude	20°39'34" N	20°39'31" N
Longitude	105°15'18" W	105°15'11" W
Decomposition Condition (fresh)	1	1
Body Condition	4/5	2/5
Total curve length	4.87 m	3.78 m
Dorsal fin width	0.56 m	0.41 m
Dorsal fin height	0.18 m	0.083 m
Pectoral fin length	1.33 m	1.0 m
Eye aperture length	0.05 m	0.053 m
Baleen plates length	6.50 cm	5 cm
Estimated weight	1,758 kg	832 kg



Figure 2. A) Rostrum of Calf 1, B) ventral region section of Calf 1, C) ventral view of Calf 2, and D) necropsy on Calf 2.

(Fig. 2). Based on the size, physical condition, and state of each animal, both were identified as neonates within a few hours of birth. Furthermore, both calves were male, as determined by observation of the protruding penis (Glockner, 1983) and later genetic confirmation.

Both stranded neonate calves presented a small amount of straw-colored fecal discharge, pale mucous membranes, complete tongue, short and soft baleen plates (Table 1) and vibrissae in each tubercle in the anterior part of the rostrum. No apparent trauma or malformations were observed in either carcass. Neither exhibited any macroscopic lesions indicative of death from trauma, severe physical injury, entanglement, chronic disease, or widespread systemic damage. These findings suggest that death was likely due to natural causes. Calf 2 was in poor body condition for its species (2/5), showed no signs of dehydration, and displayed conjunctival hyperemia in the eyes. Due to the area's popularity as a tourist beach and the typical high number of daily visitors, along with tidal conditions and personnel availability, only Calf 2 was necropsied (Fig. 2). Subsequently, both carcasses were towed by boat to the center of the bay to minimize impacts on the tourist area and allow natural decomposition.

The fecal and blood samples were analyzed at a human clinical laboratory that frequently analyzes marine mammal samples. Heart and stomach tissue samples were collected and sent for examination by a pathologist specialized in marine mammals. The necropsy of Calf 2 revealed that its lungs were active and inflated. One lung was filled with fluid and the other with air, and water was also present in the stomach, indicating that this

calf was born alive. Blood chemistry results for Calf 2 showed deviations from published reference values for humpback whale neonates (Holyoake et al., 2012), including low levels of glucose, BUN, total protein, albumin, and phosphorus, while creatinine and bilirubin levels were elevated (Table 2). This biochemical profile is suggestive of metabolic stress and possible multi-organ compromise, likely resulting from systemic metabolic stress or a shock state. The combination of elevated creatinine, low BUN, and low protein and albumin levels suggests impaired renal filtration and hepatic synthetic function, potentially accompanied by protein loss through renal excretion or malnutrition. Elevated bilirubin further supports hepatic dysfunction, possibly associated with hemolysis or biliary obstruction. Collectively, these findings indicate acute multi-organ failure, primarily affecting the kidneys and liver. Additionally, low phosphorus may have contributed to neuromuscular or respiratory compromise, which, although possibly artifactual due to delayed processing, could also reflect metabolic exhaustion. Reference ranges for AST and ALT were unavailable, but these values are not considered clinically relevant in this context (Groch et al., 2018).

Coprological analysis revealed a significant number of inflammatory cells in the sample, indicative of acute intestinal irritation likely triggered by stress. An acidic pH (6) was recorded, which may also be associated with this severe inflammatory response. Low counts of polymorphonuclear leukocytes (0–1 per field) and red blood cells (0–5 per field) were observed, suggesting mild gastrointestinal mucosal damage, most likely stress-related. Gram-negative and Gram-positive bacilli, as well as

Table 2. Clinical analyses results of humpback whale Calf 2 which stranded on 17 January, 2021 in Puerto Vallarta, Mexico, and comparison with results from two stranded neonate humpback whales from Western Australia (Holyoake et al., 2012).

Clinical analyses	Calf 2 Banderas Bay, Mexico	Peaceful Bay, Australia (Holyoake et al., 2012)	Quinns Rock, Australia (Holyoake et al., 2012)
Glucose (mg/dL)	18	66.78	
Blood urea nitrogen (mg/dL)	23	62.72	82.88
Creatinine (mg/dL)	2.9	1.469	1.41
ALT (U/L)	32	101	108
AST (U/L)	159		
ALP (U/L)	677		
Amylase (U/L)	30		
Total bilirubin (mg/dL)	1.1	0.0584	0.03504
Total protein (g/L)	2.2	43.1	72.5
Albumin (g/L)	1.3	22.2	38.1
Globulin (g/dL)	0.9		
Phosphorus (mg/dL)	4.7	9.77	36.46

Gram-negative coccobacilli, were detected; however, these bacteria are considered non-clinically relevant (Sandle, 2014). Protozoa were present in low numbers and without species identification, likely representing environmental contamination rather than true parasitic infection, as their presence was minimal. Samples of stomach and heart tissue were submitted for histopathological processing and analysis. According to the pathology report, the stomach tissue showed gastric mucosa erosion, congestion, hemorrhage, and inflammatory cell infiltration. These changes are consistent with a state of shock preceding death, suggesting the animal was in a terminal (agonal) state. There were no relevant pathological changes in the heart tissue found.

Based on the combined histopathological, clinical, and biochemical findings, the cause of death in Calf 2 is consistent with drowning, as the only significant lesions were observed in the pulmonary tissue (pulmonary edema). These findings were accompanied by multiple systemic alterations—including hepatic and renal dysfunction—compatible with terminal shock and metabolic exhaustion. The absence of trauma, congenital anomalies, or signs of infectious disease further supports the conclusion that the death was not due to pre-existing pathological conditions but rather resulted from acute physiological collapse during the agonic state.

To investigate the parental relationship of the stranded calves, skin tissue samples from both individuals were preserved in 96% molecular grade ethanol and kept at -20°C until processed. In addition, tissue samples from three other stranded calves collected during a different season (2024) were included as reference samples to provide baseline information for relatedness estimations. These additional samples were not part of the focal event and were exclusively used to improve the performance and calibration of relatedness estimators, but were not included in the biological interpretation of the focal relationship. DNA extractions were carried out from 15 mg of tissue using the DNeasy Blood and Tissue Kit (Qiagen Ltd.), following the manufacturer's instructions. Extracted DNA was quantified with a Qubit 3.0 fluorometer using a dsDNA HS Assay Kit (Life Technologies), diluted to 20 ng/ μL in ultrapure water, and stored at -20°C until use. DNA was extracted from two tissue samples per individual (pseudo-replicates). Gender

identification and maternal lineage analysis were performed concurrently with multilocus genotyping to confirm that both tissue samples corresponded to a single stranded specimen. Gender identification was performed by PCR amplification of the ZFX and ZFY genes according to the protocol described by Bérubé & Palsbøll (1996). For maternal lineage assignment, an approximately 800-base pair section of the mtDNA control region was amplified using Dlp-1.5 and Dlp-8G primers (Garrigue et al., 2004) and sequenced with BigDye v3.1 on an ABI 3730xl (Applied Biosystems). Sequences were reviewed and manually edited in the software ChromasPro v2.1.10.1 (Technelysium Pty Ltd) to define haplotypes and compare them with those reported for the species in the North Pacific Ocean (Baker et al., 2013), thus assigning each specimen to its corresponding maternal lineage.

For multilocus genotyping, 34 cetacean microsatellite markers were amplified in eight multiplex PCR reactions (Supplementary Material 1) using the Qiagen™ Multiplex Kit Plus (Qiagen Inc.), 0.25 μM of each primer, and 15 ng of DNA. The overall quality of the PCR amplification products was first assessed by electrophoresis in 2% agarose gel with 0.5X TBE and GelRed® 3X dye (Biotium, Inc.), at 125 volts for 35 minutes, and visualized under UV light at 260 nm. Duplicates and negative PCR controls were included as quality controls (see Morin et al., 2010). Amplicons were separated and detected by capillary gel electrophoresis on an ABI 3730 Genetic Analyzer (Applied Biosystems Inc.) including GeneScan 600 LIZ as size standard (Applied Biosystems Inc.). Fragment analysis was performed with GeneMarker® ver. 3.0 (Soft Genetics LLC). Genotyping error rate was assessed using both DNA extraction and PCR duplicates, with four multilocus genotype readings per specimen. Complete multilocus genotypes (allele sizes for each locus and individual, including replicate consistency), as well as pairwise relatedness estimates for all individuals, are provided in Supplementary Material 1. MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004) was employed to test for the presence of null alleles, large allele dropout, and scoring errors, using 95% confidence intervals in Monte Carlo simulations. Kinship (r_{xy}) was estimated using COANCESTRY 1.0.1.2 (Wang, 2011), which incorporates five minimum likelihood (Lynch, 1988; Queller & Goodnight, 1989; Li et al., 1993; Ritland, 1996; Lynch & Ritland, 1999; Wang, 2002) and two maximum likelihood kinship estimators (Milligan, 2003; Wang, 2007). Although the best-performing kinship estimator depends mainly on the number of genetic markers and the levels of variation detected (Wang, 2011), COANCESTRY incorporates Monte Carlo simulations to test the performance of each estimator in real kinship relationships. After the simulation, the best-performing estimators were selected based on the correlation between simulated and observed values, using the full dataset (including reference individuals) to improve estimator calibration. Because all individuals in a population share ancestry to varying degrees (Guan & Levy, 2024), inbreeding was incorporated into the inference of pairwise kinship estimates.

The genetic data showed that there was no evidence of cross-contamination or sample mislabeling; gender and maternal lineages matched with the stranding data records for tissue samples and amplification duplicates. All specimens were genotyped at least for 33 loci, and no evidence of null alleles or genotyping errors was found when scoring replicates (see Supplementary Material 1). Queller & Goodnight's (1989) moment

estimator (Q&G) and DYADML likelihood estimator (Milligan, 2003) are widely used for estimating pairwise relatedness in population and molecular ecology (Blouin, 2003). Both estimators showed high accuracy in predicting the outcomes of 100 simulated pairs representing true parent–offspring, full-sibling, half-sibling, first-cousin, and unrelated relationships ($R^2 = 0.999$ and $P < 0.05$ for Q&G; $R^2 = 0.990$ and $P < 0.05$ for DYADML). Relatedness between the stranded calves was estimated as Q&G = 0.5017 and DYADML = 0.4010 (Table 3). Mitochondrial control region sequencing demonstrated that both calves shared the same maternal haplotype (F2), consistent with either a mother–offspring or full-sibling relationship, whereas nuclear genetic data supported two possible relationships: parent–offspring or full siblings (fraternal twins). Because first-degree relationships (parent–offspring and full siblings) are genetically indistinguishable based solely on relatedness coefficients, inferring kinship categories requires the integration of genetic, developmental, and ecological evidence.

This stranding event represents the first molecularly confirmed report of a live birth of fraternal twin humpback whale calves, and potentially the first confirmed twin birth in any large whale species. Although both calves were deceased when the stranding was attended, at least one had been alive prior to our arrival, making this case only the second documented instance of a cetacean twin pregnancy reaching full term (Osborn et al., 2012). Multiple lines of evidence support the interpretation that the stranded calves were fraternal twins. Both were neonates with active bleeding from the umbilical tissue and no signs of healing (Fig. 2). They were found in close proximity and displayed size asymmetry consistent with historical observations of twin development in large whales (Slijper, 1949; Kimura, 1957). Furthermore, both calves were male, consistent with male-biased sex ratios reported for multiple pregnancies (Kimura, 1957; Ichihara, 1962).

Genetic analyses supported these findings: both calves shared the same mitochondrial haplotype (F2), which is common in the North Pacific (Baker et al., 2013) and prevalent in the Mexican Central Pacific (Llamas-González et al., 2024), and exhibited a high proportion of shared alleles (~50%), consistent with a first-degree relationship (*i.e.*, full siblings). Under these conditions, multilocus genotypes provide sufficient resolution to effectively exclude alternative kinship scenarios arising from independent reproductive events. This level of multilocus allele sharing aligns with expectations for close kin and is highly unlikely to arise among independent offspring from different reproductive events, given the number and variability of loci analyzed. Taken together, the genetic and morphological evidence consistently supports a full-sibling relationship between the two individuals. Given that first-degree relationships (parent–offspring and full siblings) are genetically indistinguishable based solely on relatedness coefficients, the inference of kinship category was refined by integrating genetic results with developmental and ecological context. In this case, the shared mitochondrial haplotype, comparable body size, neonatal condition, and close spatial association strongly support the inference that both individuals originated from the same reproductive event, consistent with fraternal twins.

These findings align with survival models based on whaling fetal length data that suggest sperm whales (*Physeter macrocephalus*) and humpback whales are the only two large whale species that

Table 3. Microsatellite-based multilocus relatedness estimates for the two humpback whale calves stranded on 17 January, 2021 at Marina Vallarta, Puerto Vallarta, Jalisco, Mexico. Simulated kinship values are shaded in grey.

Individuals	r QG	r QG I.C. 95%	r Dyad ML	r Dyad ML I.C. 95%
Calf 1 vs Calf 2	0.5017	(0.0172, 0.6201)	0.4010	(0.2512, 0.7154)
Parent - Offspring	0.5118	(0.4967, 0.5268)	0.5559	(0.5481, 0.5636)
Full-sibs	2.9	1.469	1.41	
Fraternal twins	0.4891	(0.4689, 0.5093)	0.5187	(0.4983, 0.5392)
Half-sibs	0.2545	(0.2315, 0.2774)	0.2849	(0.2631, 0.3074)
First cousins	0.1220	(0.0947, 0.1492)	0.1727	(0.1503, 0.1951)
Unrelated	-0.0014	(-0.0191, 0.0296)	0.0700	(0.0057, 0.0842)

could potentially carry twins to full term (Drinkwater & Branch, 2022), despite the substantial energetic demands of late gestation (van Aswegen et al., 2025b). In this case, twin fetuses were large, with one having a greater body length (4.87 m) and the other slightly smaller (3.78 m) than the 4.1 m average for males at birth found in a recent drone videogrammetry study from the nearby North Pacific breeding area of the Hawai'i DPS (van Aswegen et al., 2025b). Gestational survival is constrained in multiple pregnancies of whales due to mother's uterine capacity, maternal body condition, resource availability, and environmental variability (Ichihara, 1962; Argente et al., 2006; Drinkwater & Branch, 2022).

Behavioral observations are suggestive of a maternal relationship. During the stranding, an adult female, identified as 3BB162 ("Guerrera", Fig. 3), was observed exhibiting agitated behavior near the calves, consistent with maternal distress. The whale was observed for approximately seven hours, swimming back and forth in 1.4-3.7 m deep water within a 500 m area in front of the beach where the calves had stranded. Based on the observations of Slijper (1949) and Kimura (1957), most twin births in baleen whales are thought to occur in physically well-developed females that have reached a body length greater than the average for their age. Female 3BB162 is a good candidate for such a reproductive capacity, as she was first identified as an adult in 2004 in Banderas Bay Humpback Whale Photoidentification Project/FIBB Catalog (<https://ecobac.org/en/research/>), making her more than 20 years old at the time of the 2021 stranding event. She was first documented with a calf in 2010, when she was disentangled by the Mexican disentanglement network (Red de Asistencia a Ballenas Enmalladas; Frisch-Jordán & López-Arzate, 2023), and her life history indicates at least four pregnancies (Supplementary Material 2; FIBB Catalog/ECOBAC, unpub. data). Subsequent observations of "Guerrera" in a courtship group the next day (18 January, 2021), and then with a calf in March 2022 and subsequently in December 2022 (Supplementary Material 2; FIBB Catalog/ECOBAC, unpub. data) further corroborate her reproductive capacity and provide additional ecological context to the event. Studies into the female reproductive organs of Australian harvested humpback whales in the 1950s found evidence of ovulation after calf loss during/around parturition

(Chittleborough, 1958). While there is no genetic evidence that the female 3BB162 sighted offshore from the stranding was the mother of the twin calves, the abnormal behavior observed is noteworthy and suggests a maternal relationship to the neonates.

Moreover, on 21 March of the same year, 2021, sport fishermen notified one of the authors (CDOO) of the observation of an adult humpback whale pushing a dead calf to the surface off the coast of Colima, Mexico (19°05'00" N, 104°28'00" W), south of Puerto Vallarta. For several minutes, they observed how the adult whale moved the calf from side to side, as if shaking it, and emitting agitated breaths. The calf was very small, about the size of a newborn; it is unknown whether it had just been born or if it had already been born and subsequently died. Minutes later, other adult whales were observed nearby. After witnessing the event, the sport fishing boat had to move away from the area, and the outcome of the event was unknown. There were no reports of the calf being stranded in the following days. This event stands out to show similar behavior, of an adult whale, assuming it is the mother, displaying activities of behavior similar to that of a 'mourning' or 'concern' for the calf. It also stands out because it occurred in the same winter season in which the twin calves were found stranded in Puerto Vallarta, Jalisco.

Epimeletic behaviour typically involves stationing near the carcass or actively carrying it on the dorsum or melon in odontocetes (Bearzi & Reggente, 2018). Thus, there is little information available on this behavior in mysticetes (Bearzi et al.,

2018), and the observations reported in this document represent the only records in Mexican waters.

While the two neonates did not survive the birthing event and only one was necropsied, this case study provides new insight into why large whales are so rarely documented with twin calves. Calf 2 was concluded to have drowned, and it seems likely its fraternal twin may have succumbed to the same fate. Therefore, confirming that even if twin fetuses survive to full term, complications from prolonged birthing in an aquatic environment may result in drowning.

Considering that fewer than one in 500 multiple pregnancies result in live births (Drinkwater & Branch, 2022), the successful development of apparently healthy fraternal twins represents a rare and noteworthy event. These observations contribute new information on humpback whale reproductive biology and demography. There is a significant lack of published data regarding clinical laboratory reference ranges for humpback whales and other large cetaceans. This scarcity of baseline information poses a major challenge for the interpretation of diagnostic findings in stranded or diseased individuals. Many aspects of this topic remain poorly understood, but the present study provides valuable preliminary data that can serve as a reference for future health assessments in the species. Expanding long-term monitoring, genetic sampling, behavioral observations, and enhancing clinical evaluations will be critical to improving understanding of multiple pregnancies in cetaceans and their ecological implications, ultimately supporting conservation and management efforts in cetacean populations.

Acknowledgments

The response to the double stranding was carried out under the authorization of PROFEPA Jalisco and the research and collection permit Number SGPA/DGVS/06738/20. We want to thank the local staff from PROFEPA Jalisco, David Tapia, and Jonathan Tirado Ortiz. We thank the staff of Hotel Melía Marina Vallarta and Condominio Shangrilá for their facilities and support. We also appreciate the support provided by the 12th Naval Zone of Puerto Vallarta (at that time the 8th Naval Zone) and the staff from Civil Protection and Firefighters of Puerto Vallarta. To the members of the Marine Mammal Stranding Network of Bahía de Banderas, GRIMMA, and volunteers Eder Soriano, Florencia Flores, Gaia Soriano, and Frank McCann. We thank M.V.Z. MSc David Espinosa Avilés from Consultoría Patología Veterinaria for conducting the histopathologic analysis. Advice and comments from Erin John Gless were also greatly appreciated. We also greatly appreciate the ongoing support of Fundación Ecológica Biomar, Stanley Ekstrom Foundation, Karel Beets, and Ecotours Vallarta team for ECOBAC's research projects. Finally, thanks also go to Oscar Enciso, a sport fisherman from Colima, for the description of the event of the mother interacting with the dead calf in March 2021.

References

Argente, M. J., Santacreu, M. A., Climent, A., & Blasco, A. (2006). Influence of available uterine space per fetus on

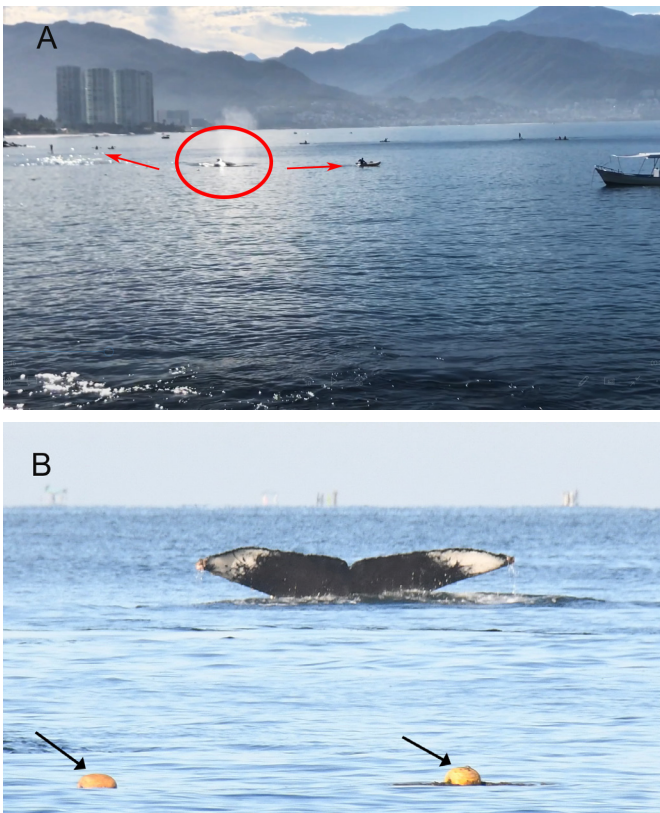


Figure 3. A) Position of the whale 3BB162 "Guerrera" (highlighted with a red circle) and red arrows indicating the locations of people in kayaks. B) Photoidentification showing the whale deep diving in 1.4-3.7 m of water near the swimming-buoy line (black arrows) in front of the stranding beach of the two twin calves.

- fetal development and prenatal survival in rabbits selected for uterine capacity. *Livestock Science*, 102(1–2), 83–91. <https://doi.org/10.1016/j.livprodsci.2005.11.022>
- Baker, C. S., Steel, D., Calambokidis, J., Falcone, E., Gonzalez-Peral, U., Barlow, J., Burdin, A. M., Clapham, P. J., Ford, J. K. B., Gabriele, C. M., Mattila, D., Rojas-Bracho, L., Straley, J. M., Taylor, B. L., Urbán, J., Wade, P. R., Weller, D., Witteveen, B. H., & Yamaguchi, M. (2013). Strong maternal fidelity and natal philopatry shape genetic structure in North Pacific humpback whales. *Marine Ecology Progress Series*, 494, 291–306. <https://doi.org/10.3354/meps10508>
- Bearzi, G., Kerem, D., Furey, N. B., Pitman, R. L., Rendell, L., & Reeves, R. R. (2018). Whale and dolphin behavioural responses to dead conspecifics. *Zoology*, 128, 1–15. <https://doi.org/10.1016/j.zool.2018.05.003>
- Bearzi, G., & Reggente, M. A. (2018). Epimeletic behavior. In B. Würsig, J. G. M. Thewissen, & Kit M. Kovacs (Eds.), *Encyclopedia of marine mammals* (pp. 337–338). Academic Press. <https://doi.org/10.1016/B978-0-12-804327-1.00121-7>
- Beck, J. J., Bruins, S., Mbarek, H., Davies, G. E., & Boomsma, D. I. (2021). Biology and genetics of dizygotic and monozygotic twinning. In A. Khalil, L. Lewi, & E. Lopriore (Eds.), *Twin and higher-order pregnancies* (pp. 31–50). Springer. https://doi.org/10.1007/978-3-030-47652-6_3
- Bérubé, M., & Palsbøll, P. (1996). Identification of sex in cetaceans by multiplexing with three ZFX and ZFY specific primers. *Molecular Ecology*, 5(2), 283–287. <https://doi.org/10.1046/j.1365-294X.1996.00072.x>
- Best, P. B., Canham, P., & McLeod, N. (1984). Patterns of reproduction in sperm whales, *Physeter macrocephalus*. *Report of the International Whaling Commission, Special Issue*, 6, 51–79.
- Bettridge, S. O. M., Baker, C. S., Barlow, J., Clapham, P. J., Ford, M., Gouveia, D., Mattila, D. K., Pace III, R. M., Rosel, P. E., Silber, G. K., & Wade, P. R. (2015). Status review of the humpback whale (*Megaptera novaeangliae*) under the Endangered Species Act. *NOAA Technical Memorandum NMFS-SWFSC-540*. Department of Commerce.
- Blouin, M. S. (2003). DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. *Trends in Ecology & Evolution*, 18(10), 503–511. [https://doi.org/10.1016/S0169-5347\(03\)00225-8](https://doi.org/10.1016/S0169-5347(03)00225-8)
- Bronson, F. H. (1985). Mammalian reproduction: an ecological perspective. *Biology of Reproduction*, 32(1), 1–26. <https://doi.org/10.1095/biolreprod32.1.1>
- Calambokidis, J., Falcone, E. A., Quinn, T. J., Burdin, A. M., Clapham, P. J., Ford, J. K., & Wade, P. R. (2008). SPLASH: Structure of populations, levels of abundance and status of humpback whales in the North Pacific. *Final Report for Contract AB133F-03-RP-00078*.
- Chittleborough, R. G. (1958). The breeding cycle of the female humpback whale, *Megaptera nodosa* (Bonaterre). *Marine and Freshwater Research*, 9(1), 1–18. <https://doi.org/10.1071/MF9580001>
- Clapham, P. J. (1996). The social and reproductive biology of humpback whales: an ecological perspective. *Mammal Review*, 26(1), 27–49.
- Davison, N. J., Doeschate, M. T., Dagleish, M. P., Reid, F. L., Reid, R. J., Foster, G., Brownlow, A., & Barley, J. (2016). Twin foetuses in an Atlantic white-sided dolphin (*Lagenorhynchus acutus*) stranded on the coast of Scotland, United Kingdom. *Journal of the Marine Biological Association of the United Kingdom*, 96(4), 841–844. <https://doi.org/10.1017/S0025315415002246>
- Dawbin, W. H. (1966). The seasonal migratory cycle of humpback whales. In M. Carwardine (Ed.), *Whales, dolphins and porpoises* (2nd ed., pp. 145–170). University of California Press.
- Diario Oficial de la Federación (DOF) (2014). *Protocolo de Atención a Varamientos de Mamíferos Marinos*. https://dof.gob.mx/nota_detalle.php?codigo=5348898&fecha=17/06/2014#gsc.tab=0
- Drinkwater, R. W., & Branch, T. A. (2022). Estimating proportions of identical twins and twin survival rates in cetaceans using fetal data. *Marine Mammal Science*, 38(4), 1398–1408. <https://doi.org/10.1111/mms.12929>
- Endres, L., & Wilkins, I. (2005). Epidemiology and biology of multiple gestations. *Clinics in Perinatology*, 32(2), 301–314. <https://doi.org/10.1016/j.clp.2005.04.002>
- Enzmann, E. V., & Crozier, W. J. (1935). Relation between birth weight and litter size in multiparous mammals. *Journal of General Physiology*, 18(6), 791. <https://doi.org/10.1085/jgp.18.6.791>
- Frazer, J. F. D., & Huggett, A. S. G. (1959). The growth rate of foetal whales. *Journal of Physiology*, 146(3), 21–22.
- Frisch-Jordán, A., & López-Arzate, D. (2023). Large whale entanglements in México, a 25-year review from 1996 to 2021. *Marine Mammal Science*, 40(3), e13106. <https://doi.org/10.1111/mms.13106>
- Garrigue, C., Dodemont, R., Steel, D., & Baker, C. S. (2004). Organismal and ‘gametic’ capture-recapture using microsatellite genotyping confirm low abundance and reproductive autonomy of humpback whales on the wintering grounds of New Caledonia. *Marine Ecology Progress Series*, 274, 251–262. <https://doi.org/10.3354/meps274251>
- Glockner, D. A. (1983). Determining the sex of humpback whales (*Megaptera novaeangliae*) in their natural environment. In R. Payne (Ed.), *Communication and Behavior of Whales* (pp. 447–464). Westview Press.
- González, A. F., López, A., & Benavente, P. (1999). A multiple gestation in a *Delphinus delphis* stranded on the north-western Spanish coast. *Journal of the Marine Biological Association of the United Kingdom*, 79(6), 1147–1148. <https://doi.org/10.1017/S0025315499001502>
- Gray, K. N., & Conklin, R. H. (1974). Multiple births and cardiac anomalies in the bottle-nosed dolphin. *Journal of Wildlife Diseases*, 10(2), 155–157. <https://doi.org/10.7589/0090-3558-10.2.155>
- Groch, K. R., Diaz-Delgado, J., Marcondes, M. C., Colosio, A. C., Santos-Neto, E. B., Carvalho, V. L., Boos, G. S., Meirelles, A. C. O., Ramos, H. G. C., Guimarães, J. P., Borges, J. C. G., Vergara-Parente, J. E., St. Leger, J. A., Fernandez, A. & Catão-Dias, J. L. (2018). Pathology and causes of death in stranded humpback whales (*Megaptera novaeangliae*) from Brazil. *PLoS One*, 13(5), e0194872. <https://doi.org/10.1177/0300985820954550>
- Guan, Y., & Levy, D. (2024). Estimation of inbreeding and kinship coefficients via latent identity-by-descent states. *Bioinformatics*, 40(2), btae082. <https://doi.org/10.1093/bioinformatics/btae082>

- Harms, C., Finkel, S., Wischusen, K., & Burdett-Hart, L. (2024). *WhaleScale* v4.0.1 application. College of Charleston.
- Holyoake, C., Stephens, N., & Coughran, D. (2012). Collection of baseline data on humpback whale (*Megaptera novaeangliae*) health and causes of mortality for long-term monitoring in Western Australia C. Murdoch University, Report for the Western Australian Marine Science Institution.
- Ichihara, T. (1962). Prenatal dead fetus of baleen whales. *Scientific Reports of the Whales Research Institute*, 16, 47–60.
- Kimura, S. (1957). The twinning in southern fin whales. *Scientific Reports of the Whales Research Institute*, 12, 103–125.
- Kompanje, E. J. O. (2005). A case of symmetrical conjoined twins in a bottlenose dolphin *Tursiops truncatus* (Mammalia, Cetacea). *Deinsea*, 11(1), 147–150.
- Kompanje, E. J. O., Camphuysen, C. J., & Leopold, M. F. (2017). The first case of conjoined twin harbour porpoises *Phocoena phocoena* (Mammalia, Cetacea). *Deinsea*, 17, 1–5.
- Lazcano-Pacheco, C., Onofre-Díaz, M. A., Meza-Yañez, R., Llamas-González, M., Bautista-Moreno, L. M., Olivos-Ortiz, A., & Ortega-Ortiz, C. D. (2022). Cetacean mortality related to ship traffic in the Mexican Central Pacific. *Aquatic Mammals*, 48(6). <https://doi.org/10.1578/AM.48.6.2022.737>
- Li, C. C., Weeks, D. E., & Chakravarti, A. (1993). Similarity of DNA fingerprints due to chance and relatedness. *Human Heredity*, 43, 45–52. <https://doi.org/10.1159/000154113>
- Llamas-González, M., Ortega-Ortiz, C. D., Enríquez-Paredes, L. M., González-Peral, Ú. A., Bautista-Guerrero, E., & Martien, K. K. (2024). Genetic data reveal wintering ground affiliation of humpback whales from the Mexican Central Pacific. *Marine Mammal Science*, 40(1), 54–72. <https://doi.org/10.1111/mms.13053>
- Lockyer, C. H. (1981). Growth and energy budgets of large baleen whales from the southern hemisphere. *Mammals in the Seas*, *FAO Fisheries Series*, III(5), 379–487.
- Lynch, M. (1988). Estimation of relatedness by DNA fingerprinting. *Molecular Biology and Evolution*, 5, 584–599. <https://doi.org/10.1093/oxfordjournals.molbev.a040518>
- Lynch, M., & Ritland, K. (1999). Estimation of pairwise relatedness with molecular markers. *Genetics*, 152, 1753–1766. <https://doi.org/10.1093/genetics/152.4.1753>
- Milligan, B. G. (2003). Maximum-likelihood estimation of relatedness. *Genetics*, 163, 1153–1167. <https://doi.org/10.1093/genetics/163.3.1153>
- Morin, P. A., Martien, K. K., Archer, F. I., Cipriano, F., Steel, D., Jackson, J., & Taylor, B. (2010). Applied conservation genetics and the need for quality control and reporting of genetic data used in fisheries and wildlife management. *Journal of Heredity*, 101(1), 1–10. <https://doi.org/10.1093/jhered/esp107>
- NOAA - National Oceanic and Atmospheric Administration (2016). Endangered and threatened species; identification of 14 distinct population segments of the humpback whale (*Megaptera novaeangliae*) and revision of species-wide listing. *Federal Register*, 81, 62260–62320.
- Osborn, S., Dalton, L., Dold, C., & Robeck, T. (2012). Management of twin pregnancy and perinatal concerns in a beluga (*Delphinapterus leucas*). *Journal of Zoo and Wildlife Medicine*, 193–196. <https://doi.org/10.1638/2011-0161.1>
- Queller, D. C., & Goodnight, K. F. (1989). Estimating relatedness using molecular markers. *Evolution*, 43, 258–275. <https://doi.org/10.1111/j.1558-5646.1989.tb04226.x>
- Ransome, N., Loneragan, N. R., Medrano-González, L., Félix, F., & Smith, J. N. (2021). Vessel strikes of large whales in the eastern tropical Pacific: a case study of regional underreporting. *Frontiers in Marine Science*, 8, 675245. <https://doi.org/10.3389/fmars.2021.675245>
- Ransome, N., Bejder, L., Jenner, M., Penfold, G., Brosig, V. J., Kitson, C., Skjothaug, R., Neilson, E., Loneragan, N. R., & Smith, J. N. (2022). Observations of parturition in humpback whales (*Megaptera novaeangliae*) and occurrence of escorting and competitive behavior around birthing females. *Marine Mammal Science*, 38(2), 408–32. <https://doi.org/10.1111/mms.12864>
- Ritland, K. (1996). Estimators for pairwise relatedness and inbreeding coefficients. *Genetics Research*, 67, 175–186. <https://doi.org/10.1017/S0016672300033620>
- Sahu, M., & Prasuna, J. G. (2016). Twin studies: A unique epidemiological tool. *Indian Journal of Community Medicine*, 41(3), 177–182. <https://doi.org/10.4103/0970-0218.183593>
- Sandle, T. (2014). Biochemical and modern identification techniques: Enterobacteriaceae, Coliforms, and *Escherichia coli*. In C. A. Batt & M. L. Tortorello (Eds.), *Encyclopedia of Food Microbiology* (2nd ed., pp. 232–237). Academic Press. <https://doi.org/10.1016/B978-0-12-384730-0.00037-9>
- Slijper, E. J. (1949). On some phenomena concerning pregnancy and parturition of the Cetacea. *Bijdragen tot de Dierkunde*, 28(1), 416–448.
- Slijper, E. J. (1966). Functional morphology of the reproductive system in Cetacea. In K. S. Norris (Ed.), *Whales, dolphins, and porpoises* (pp. 277–319). American Institute of Biological Science.
- Tamburin, E., Carone, E., López, I. G., & Magaña, F. G. (2017). First report of gray whale (*Eschrichtius robustus*, Lilljeborg, 1861) conjoined twin calves in the Eastern Pacific Ocean. *Turkish Journal of Zoology*, 41(5), 951–954. <https://doi.org/10.3906/zoo-1609-50>
- Tiongson, A. J. C., Uzzurum, J. A., Divinagracia, D. G., & Acebes, J. M. (2025). First record of twin fetuses in a stranded Cuvier's beaked whale or goose-beaked whale (*Ziphius cavirostris*). *Current Zoology*, 71(2), 263–266. <https://doi.org/10.1093/cz/zoae030>
- Van Aswegen, M., Szabo, A., Currie, J. J., Stack, S. H., Straley, J., Neilson, J., Gabriele, C., Cates, K., Baker, C. S., Steel, D., Pack, A. A., West, K. L., Hofmann, N. J., Toonen, R. T., Loockerman, C., Sullivan, F. A., Cheeseman, T., Sharpe, F., & Bejder, L. (2025a). Age-specific body length, mass, and energetic cost of growth in humpback whales. *Marine Ecology Progress Series*, 770, 171–194. <https://doi.org/10.3354/meps14937>
- Van Aswegen, M., Szabo, A., Currie, J. J., Stack, S. H., West, K. L., Hofmann, N., Christiansen, F., & Bejder, L. (2025b). Energetic cost of gestation and prenatal growth in humpback whales. *The Journal of Physiology*, 603(2), 529–550. <https://doi.org/10.1113/JP287304>
- Van Oosterhout, C., Hutchinson, W. F., Wills, D. P., & Shipley, P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, 4(3), 535–538. <https://doi.org/10.1111/j.1471-8286.2004.00684.x>

- Wang, J. (2002). An estimator for pairwise relatedness using molecular markers. *Genetics*, 160, 1203–1215. <https://doi.org/10.1093/genetics/160.3.1203>
- Wang, J. (2007). Triadic IBD coefficients and applications to estimating pairwise relatedness. *Genetics Research*, 89, 135–153. <https://doi.org/10.1017/S0016672307008798>
- Wang, J. (2011). COANCESTRY: A program for simulating, estimating and analyzing relatedness and inbreeding coefficients. *Molecular Ecology Resources*, 11, 141–145. <https://doi.org/10.1111/j.1755-0998.2010.02885.x>
- Zemsky, V. A., & Budylenko, G. A. (1970). Siamese twins of humpback whale. *Whales of the Southern Hemisphere. Trudy Atlant NIRO*, 29, 225–230.

Supplementary material

Supplementary Material 1- Microsatellite markers, genetic data summaries, and relatedness estimates for the humpback whale calves.

Supplementary Material 2- Photograph registers in the FIBB-ECOBAC Catalog of the mature female humpback whale 3BB162, nicknamed “Guerrera”. G1=single, G2=pair, CG=courtship group, MC=mother and calf, MCE= mother, calf and escort.