Latin American Journal of Aquatic Mammals

ISSN: 2236-1057 | Vol. 19 No. 1, April 2024

# Oral cleft in an Amazonian manatee (*Trichechus inunguis*) (Mammalia, Sirenia)

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The Amazonian manatee, *Trichechus inunguis*, is the only aquatic mammal with an herbivorous diet living in freshwater (Amaral et al., 2023). The species was heavily hunted for its meat, fat, and hide since the Brazilian Colonial times and although protected in all countries where it occurs, it is still hunted for subsistence purposes and sometimes sold at illegal markets (Amaral et al., 2023).

Since 1976, Amazonian manatees have been examined alive or necropsied as part of a long-term program of rescue and rehabilitation of orphan manatee calves, whose mothers were victims of illegal hunting and accidental catch in fishing nets in the Brazilian Amazon region. Rescued manatees presented several types of diseases (d'Affonseca Neto et al., 2002) although, to our knowledge, there is no record of a congenital anomaly of the face, known as an oral cleft, in Amazonian manatees.

**Keywords:**
Birth defect, cleft lips, congenital anomaly, facial malformation.

**ARTICLE INFO**

**Manuscript type:** Article

**Article History**
Received: 21 August 2023
Received in revised form: 19 November 2023
Accepted: 19 November 2023
Available online: 23 February 2024

**Handling Editor:** Nataly Castelblanco-Martínez

**Citation:**
da Silva, V. M. F., Rosas, F. C. W., d'Affonseca Neto, J. A., Feldberg, E., Franco-de-Sá, J. F. O., Lazzarini, S. M., & Amaral, R. S. (2024). Oral cleft in an Amazonian manatee (*Trichechus inunguis*) (Mammalia, Sirenia). *Latin American Journal of Aquatic Mammals, 19*(1), 120-124. https://doi.org/10.5597/lajam00320

This anomaly is a common birth defect in humans, affecting approximately four out of 10,000 newborns (Salari et al., 2022). It is characterized by openings in or discontinuity of the structures of the lips and/or palate and can occur in different locations and to a variable extent in the face (Montagnoli et al., 2005). While many syndromes involving cleft lips, affecting or not the cleft palate, are recognized in humans, the majority of oral clefts fall into the category of 'non-syndromic oral clefts' and their etiology remains poorly understood. However, the general agreement is that oral clefts are of multifactorial origin, with both genetic and environmental factors playing a role (Schutte & Murray, 1999; Alade et al., 2022).

Syndromic cases of oral clefts in humans can be subdivided into chromosomal anomalies, Mendelian syndromes, teratogens, and uncategorized syndromes (Schutte & Murray, 1999; Alade et al., 2022). Genetic factors are related to gene and chromosome alterations, with specific depletion or duplication, where the predisposition of the fissure depends on the integration of polygenic factors associated with environmental conditions. In humans, the depletion and duplication of portions of the chromosomal arms, including the X-chromosome, have been associated with clefts (Schutte & Murray, 1999). The most common environmental factors associated with that anomaly are viral infections, nutritional disorders, and contamination by pesticides, herbicides, heavy metals, and petrochemical products (Axelsson & Molin, 1988; Datubo-Brown & Kejeh, 1989). In non-human animals, oral cleft has already been recorded in all domestic mammals and some wild mammals, including aquatic mammals, and as in humans, this alteration has multifactorial origins (Gogan & Jessup, 1985; Suzuki et al. 1992; Goldschmidt et al., 2010; Krief et al., 2015; Uzal et al., 2015; Lewis et al., 2016).

Congenital malformations are rarely described for all Sirenian species; therefore, punctual records are very important to record their occurrence and highlight potential causes. In this case study, we report for the first time the occurrence of oral cleft in an Amazonian manatee calf, describing the clinical evaluation and discussing the potential causes.

In June 2004, a female Amazonian manatee calf, measuring 113 cm long and weighing 24.2 kg, was found swimming alone near the confluence of the Amazon and Negro rivers, near the city of Manaus (Amazonas, Brazil), in the Catalão area (03°9'33.8" S; 59°54'44.2" W). By the condition of the skin, flexibility and fold marks of the tail and the soft umbilical scar, it was evident that this animal was a neonate manatee, no more than two weeks old. Despite the presence of a facial anomaly, the general physical condition, reflexes and behavior of the animal were considered normal and it was transported to the Aquatic Mammals Laboratory (LMA) of the National Institute of Amazonian Research (INPA) in Manaus, Brazil, where she was fed with an artificial milk formula. Two years later, in June 2006, this manatee was weaned at 147 cm in length and 70 kg in body mass. The female died in December 2007 due to gastrointestinal problems.

In order to investigate the extent and potential causes of the anomaly in this calf, three different approaches were taken: 1 – clinical exams of the palate, 2 – a series of radiographic images of the head and face to examine the physical effects and extent of the fissure in the bones of the rostrum, and 3 – a cytogenetic diagnosis to identify a possible karyotype anomaly. A clinical exam of the palate was performed by opening the mouth of the animal and exploring manually the upper parts of the mouth and its cavity. Radiographic images of the head and rostrum were taken in a dorsoventral (D-V), and latero-lateral oblique (L-Lo) positions. The genetic basis of this anomaly was studied using three different methods of karyotype analysis: the standard Giemsa staining to confirm the number of pairs of chromosomes of the species, nucleolus organizer regions NOR-banding to highlight the organizational region of the nucleolus (Howell & Black, 1980), and G-banding to verify the existence of chromosomal rearrangements (Seabright, 1971).

Externally, this female calf presented a deep labial fissure dividing the oral disc up to the nasal cavity, and affecting the nostril's valve of the right nasal orifice (Fig. 1). The clinical exam showed a sectioning of the right side of the oral disk and the widening of the right nostril, with an accentuated deviation of the left nostril to the right. It did not reveal any apparent palate cleft or changes in the upper horny palate (Figs 1A, 1B). The prehensile function of the upper perioral bristles was not totally affected, and the animal was able to take solid food and handle the nipple of the milk bottle. During the bottle feeding, no reflux was observed. Some difficulty in controlling the right nostril's valve was observed in the early stage of its life but improved with age. Despite this morphological alteration, the animal grew at a similar rate to other orphaned calves raised with an artificial diet at INPA and did not show any pathological alteration during its life related to a possible evolution of the oral cleft.

The D-V radiograph revealed a non-fused palatine fissure in the maxillary region, represented by a radiolucent line with a moderate lateral deviation, and an area of approximately 1-1.5 cm with a radiopaque reduction in the right pre-maxillary bone and soft tissue areas. The region of the mandibular symphysis presented a small lateral deviation, with angles and deviation probably accentuated by a small lateral rotation of the animal's head (Fig. 2A). The L-Lo radiograph also revealed an area of radiolucency in the pre-maxillary region (not shown). These results revealed a probable partial agenesis of the pre-maxillary bone. These findings were later confirmed by the skull evaluation (Fig. 2B). Fig. 2C shows a normal calf Amazonian manatee skull, with

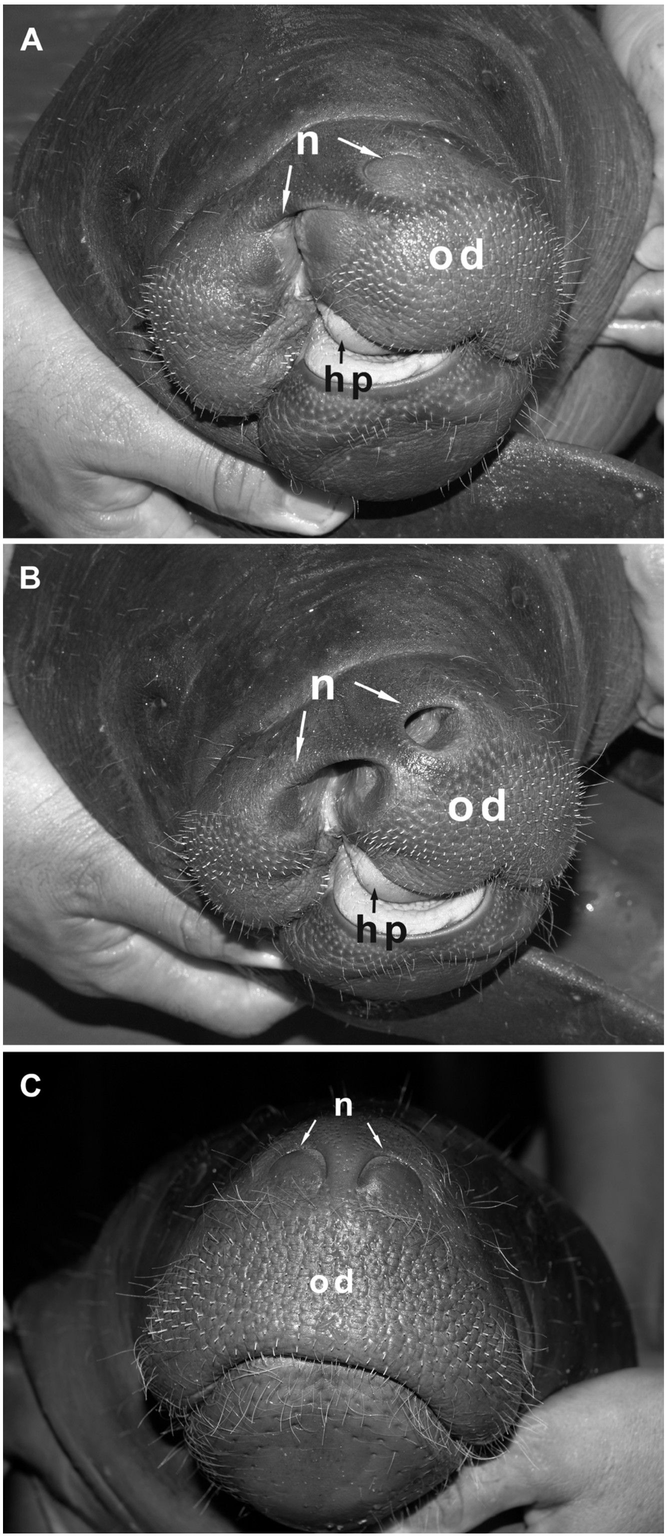


**Figure 1.** Frontal photograph of the rostrum of the 113 cm long female Amazonian manatee calf (*Trichechus inunguis*), showing the external appearance of the right-sided unilateral cleft anomaly, affecting the oral disk (od), widening of the right nostril (n), and with an accentuated deviation of the left nostril to the right, with a closed (A) and an open (B) nostrils' valve. hp: hard palate. C. Photograph of a normal rostrum of an Amazonian manatee calf with closed nostrils (n), showing a normal rostrum and complete oral disk (od).

the complete maxillary and pre-maxillary bones, for comparison. The results of the karyotype analysis showed that this animal had a normal number of chromosomes (2n = 56; FN = 82, Fig. 3), as described by Assis et al. (1988) without alterations in the chromosome structure or number.

Based on the physical appearance and age of the calf at the time of rescue, the oral cleft observed was consistent with a congenital anomaly. The occurrence of some congenital anomalies in sirenians has been reported (Watson & Bonde, 1986; Varela-Lasheras et al., 2011; Carvalho et al., 2019; Weisbrod et al., 2021), including a photograph of a possible oral cleft in an African manatee calf (https://www.instagram.com/p/BKbXRAmAMsp); however, this is the first report of a clinical evaluation of an Amazonian manatee with oral cleft.

The karyotype analysis indicated that in this case, this anomaly did not represent a chromosomal syndrome. However, other genetic disorders related to oral cleft were not evaluated and cannot be discarded (Kohli & Kohli, 2012; Alade et al., 2022). Similarly, viral infections or nutritional disorders during the mother's pregnancy in this case are unknown and difficult to identify during the calf evaluation. Among the environmental factors, the exposition to teratogenic agents is the most plausible cause for oral cleft observed in this case.

Contaminant-induced pathologic effects and associated diseases in aquatic mammals are not well known, although high levels of several contaminants have been documented. Exposure to endocrine-disrupting chemicals during gestation, lactation, or adulthood is known to alter reproductive function and development in laboratory and domestic animals, humans, and free-ranging wildlife species (O'Shea et al., 1999).

In the last decades, the development of the Amazon region has expanded dramatically, with new areas of industrial plants, including oil and natural gas prospecting, transport and refineries (Laurance et al., 2004; Lessmann et al., 2016; Albert et al., 2023), with eventual oil spills and leaking in the rivers. The effects of these activities in the aquatic environment and on the aquatic mammals in the Amazon region are not yet known, although studies on the effects of contamination of aquatic mammals and their habitats by different agents are abundant elsewhere (O'Shea et al., 1999).

Amazonian manatees feed mainly on aquatic macrophytes abundant in the white waters of the Amazon River basin such as *Eichhornia* spp, *Pistia* sp, and *Salvinia* spp, among others (Best, 1981; Guterres-Pazin et al., 2014; Crema et al., 2019). These aquatic plants are well known for their ability to absorb and accumulate heavy metals such as mercury, and other pollutants derived from oil and other petrochemicals (Lopes et al., 2009; Kimura et al., 2017). The Catalão area, where this calf was rescued, is close to an oil refinery and other petrochemical-related activities. Thus, the chances of consumption of contaminated macrophytes by manatees in that area are high. Therefore, reproductively active female manatees could be vulnerable to physiological disturbances in early pregnancy, which could induce fetal malformations such as the oral cleft described here. The absence of other animals with the same pathology may be due to the limited number of animals in our sample from this area.

This was the first occurrence of oral cleft among the 341 Amazonian manatees examined by us since 1976. Although the cause of the congenital abnormality in this Amazonian manatee is unknown, this report brings light to further investigations of the effects of teratogenic agents on reproduction and embryo/fetal development in Amazonian manatees and other aquatic mammals in the region.

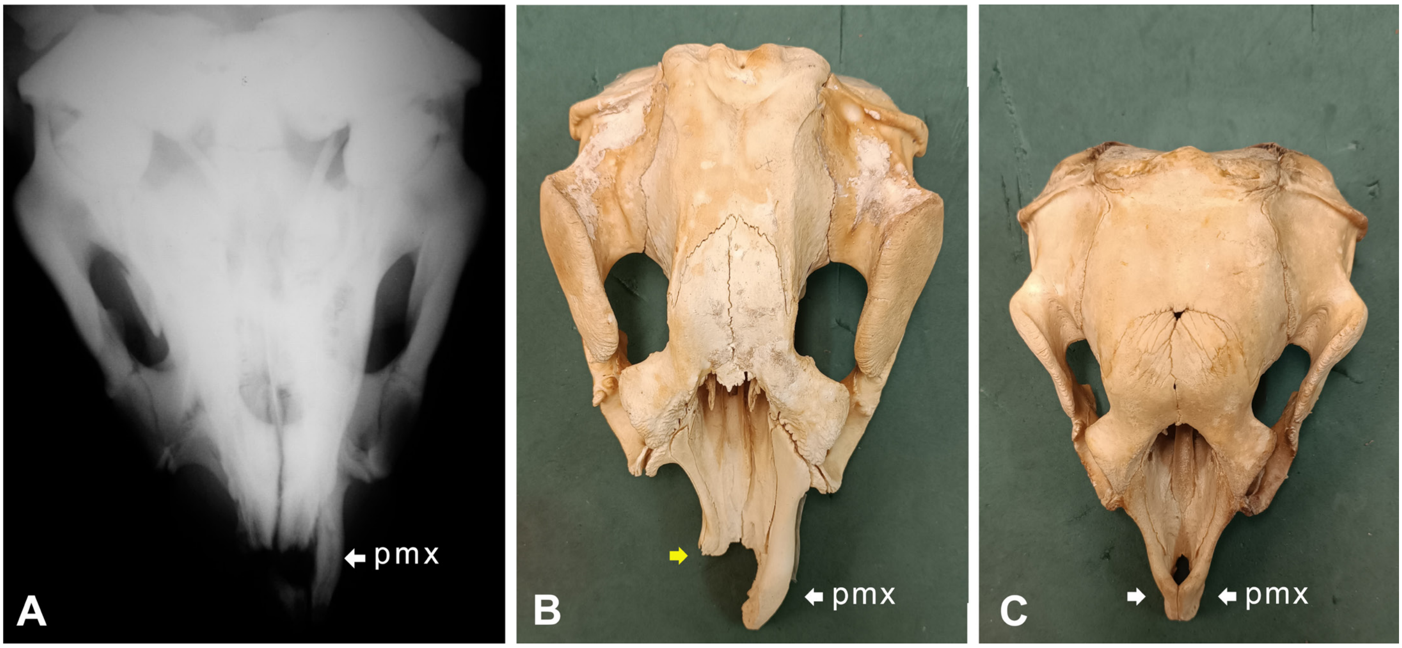


**Figure 2.** A. Dorso-ventral radiograph of the head of a live female Amazonian manatee calf (*Trichechus inunguis*) measuring 113 cm long with oral cleft anomaly. Note the presence of the left pre-maxillary bone (pmx) and the radiopaque reduction in the right pre-maxillary bone and soft tissue areas, suggesting the partial agenesis of the bone. B. Photograph of the skull of the female Amazonian manatee showing the partial agenesis of the right pre-maxillary bone (yellow arrow). C. Photograph of a normal skull of an Amazonian manatee calf (C) showing the complete maxillary bone (white arrows).

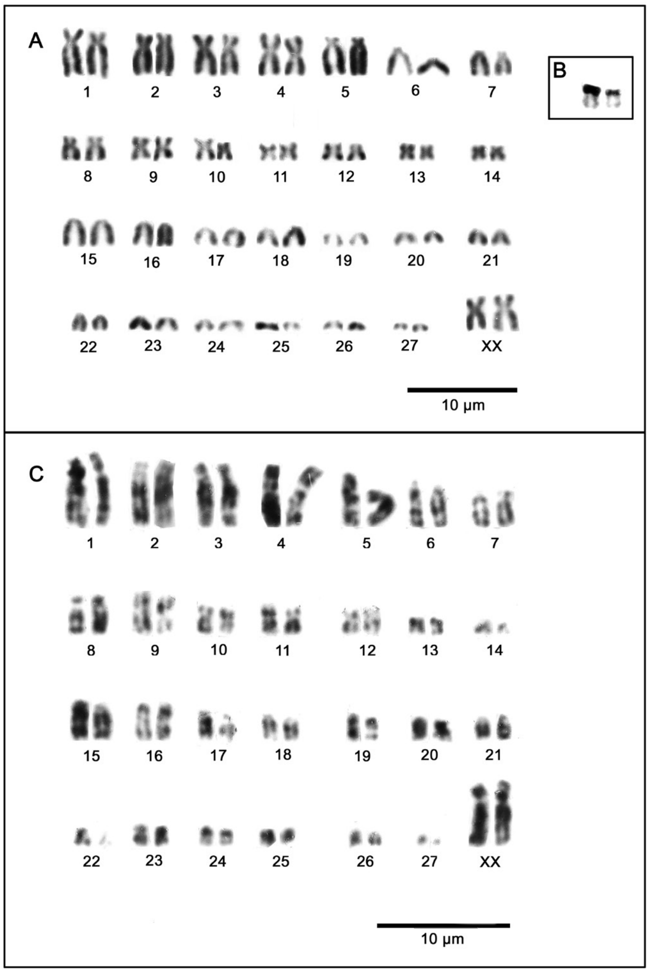


**Figure 3.** Karyotype of a live female Amazonian manatee calf (*Trichechus inunguis*) with oral cleft anomaly: A. Giemsa standard staining; B. Nucleolar pair visualized with silver nitrate; C. G-banding (Bar = 10μm).

## Acknowledgments

We thank Érika Yumie Hiraishi from Pires Veterinary Clinic in Manaus, for her technical support during the radiographic procedures, as well as Marcelo, Daniel, Nazaré, Jeová, Raimundo, Nildon, and Raimundo Manoel for their dedication and care during the rescue and rehabilitation of the manatee calves. We also thank Daryl Domning and Tony Martin for their constructive comments and suggestions on the first draft. Kesä K. Lehti (in memoriam) kindly revised the earlier English version of the manuscript. The Amazonian manatee Project (Projeto Peixe-boi) was supported by grants from PPG-7/FINEP, Ministry of Science and Technology of Brazil (MCT), INPA, CNPq, Petrobras Ambiental and through the Associação Amigos do Peixe-boi (AMPA) and its partners.

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