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Lobomycosis Like Disease in Bottlenose Dolphins *Tursiops truncatus* (Artiodactyla: Delphinidae) from Costa Rica Pacific Waters

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ABSTRACT

Introduction: Lobomycosis, is a chronic mycotic disease of the skin and sub-dermal tissue caused by *Paracoccidioides ceti*, which affects dolphins worldwide. In Costa Rica, the incidence of lobomycosis Like Disease (LLD) has been documented in inshore common bottlenose dolphins (*Tursiops truncatus*) from the waters of Golfo Dulce, South Pacific.

Objective: Examine the prevalence pattern of LLD in inshore *T. truncatus* from Costa Rica Pacific waters, specifically in Golfo Dulce and Golfo de Nicoya.

Methods: We examined over 20 000 photographs of *T. truncatus*, collected during long-term research on cetaceans in 2005-2020 (n = 476 surveys), in Golfo Dulce, as well of nine sightings *T. truncatus* from the Golfo de Nicoya , obtained during 17 samplings events in 2014-2022, these records for the presence of LLD allowed to calculate the prevalence rate with a confidence interval.

Results: The prevalence rate of LLD in adult photo-identified individuals was 13.1 % (CI: 12 -14.2) in Golfo Dulce, and 100 % in Golfo de Nicoya, considering the size of the population for the period of 2022.

Conclusion: The persistence and high prevalence levels of LLD in small, localized, communities of *T. truncatus* are a cause for serious concern.

Key words: bottlenose dolphin; lobomycosis Like Disease; Golfo Dulce; Golfo de Nicoya; inshore ecotype.

RESUMEN

Enfermedad similar a la generada por *Lobomicosis* en delfines nariz de botella *Tursiops truncatus* (Artiodactyla: Delphinidae) de las aguas del Pacífico de Costa Rica

Introducción: La enfermedad similar a Lobomicosis (LLD), asociada al hongo *Paracoccidioides cetii*, es una condición dérmica frecuentemente reportada en pequeños cetáceos alrededor del mundo. En Costa Rica se ha documentado la presencia de LLD en *Tursiops truncatus* del ecotipo costero en aguas del Golfo Dulce, Pacífico Sur.

Objetivo: Examinar el patrón de prevalencia de la enfermedad en el ecotipo costero de *T. truncatus* en la costa Pacífica de Costa Rica, específicamente en Golfo Dulce y Golfo de Nicoya.

Métodos: Se examinaron cerca de 20 000 registros fotográficos de *T. truncatus* provenientes de un monitoreo de cetáceos a largo plazo entre el 2005-2020 (n = 476 muestreos) en Golfo Dulce, así como nueve encuentros de *T. truncatus* en Golfo de Nicoya derivados de 17 muestreos realizados entre 2014-2022, estos registros fueron analizados, para identificar y estimar el índice promedio de prevalencia de LLD mediante un intervalo de confianza.

Resultados: El índice promedio de prevalencia de LLD en individuos adultos marcados es de 13.1 % (CI: 12–14.2) en Golfo Dulce, y de 100 % en Golfo de Nicoya considerando el tamaño de la población para el periodo del 2022.

Conclusión: La alta incidencia de LLD y el tamaño poblacional notablemente reducido de *T. truncatus* en aguas del Golfo Dulce y Golfo de Nicoya podría ser indicativo de una baja calidad de agua y degradación ambiental, por la presión de las actividades humanas que se realizan en las zonas costeras.

Palabras clave: Delfín Nariz de Botella; enfermedad similar a Lobomicosis; Golfo Dulce; Golfo de Nicoya; Costa Pacífica; ecotipo costero.

Nomenclature: SMT1: Supplementary material Table 1; SMF1: Supplementary material Figure 1.

INTRODUCTION

Skin lesions have been described in odontocetes and mysticetes worldwide (Burdett Hart et al., 2012; Van Bressem and Van Waerebeek., 1996; Wilson et al., 1999). They may be caused by viruses, bacteria, fungi, and protozoa and appear to have been increasing in frequency during the last two decades (Daura-Jorge & Simões-Lopes, 2011; Van Bressem et al., 2009). Their occurrence and severity seem to be related to natural and anthropogenic factors such as water quality, inland water discharges, climate change, and habitat degradation (Burdett Hart et al., 2012; Daura-Jorge & Simões-Lopes, 2011; Gómez-Salazar et al., 2012; Harzen & Brunnick, 1997; Kiszka et al., 2009; Moore, 2008; Van Bressem et al., 2009; Wilson et al., 1999).

Caused by the yeast-like organism *Paracoccidioides ceti*, (Vilela, et al., 2016), lobomycosis (lacaziosis) is a chronic fungal disease of the skin and subdermal tissues that affects dolphins. It is characterized by raised, sometimes ulcerated or plaque-like, dermal lesions of grayish, whitish, or slightly pinkish color that can spread to disfigure large areas of the dorsal, dorsolateral, and pedunculated regions of the body (Bermudez et al, 2009; Bessensen et al. 2014; Daura-Jorge & Simões-Lopes, 2011; Félix, Van Bressem et al. 2019; Kiszka et al. 2009; Murdoch et al. 2010; Paniz-Mondolfi et al. 2012; Ramos et al. 2018; Rotstein et al. 2009, Van Bressem et al. 2009, Van Bressem et al. 2015).

Lobomycosis-like disease (LLD) is a condition whose clinical presentation is reminiscent of lobomycosis but lacks confirmation based on histologic and molecular diagnosis (Kiszka et al., 2009; Ramos et al., 2018; Van Bressem et al., 2009, 2015; Vilela et al., 2021). Nevertheless, there is a very good correlation between at-sea observations and corresponding photographic documentation and laboratory diagnosis (Murdoch et al. 2008; Sacristan et al. 2015; Van Bressem et al., 2007), even up to 100 %, as reported by Murdoch et al. (2008). In the common bottlenose dolphin, cases of lobomycosis appear to be significantly associated with disorders of the adaptive immune system, which may be related to chronic exposure to chemicals and biological pollutants (Reif et al., 2009).

In Costa Rica, the prevalence of LLD has been documented in inshore bottlenose dolphins in the waters of Golfo Dulce in the period 1991-1992, 2010-2011 (Bessesen et al., 2014). Here, we examine the prevalence of LLD in inshore bottlenose dolphins, *Tursiops truncatus* (Montagu, 1821)) from Costa Rican Pacific waters, update previous observations of the incidence in the Golfo Dulce bottlenose dolphin population ten years after the first observation, and report new cases of the disease in a small, localized dolphin population inhabiting Golfo de Nicoya.

Golfo Dulce is a 50 km long and 10–15 km wide tectonic embayment located in Costa Rica between 8°33'N and 83°14'W (Acevedo & Burkhart, 1998; Cubero, 1998; Oviedo et al., 2015; Rincón & Ballestero, 2015; Svendsen et al., 2006). The main freshwater inputs come from the Coto-Colorado, Tigre, Esquinas and Rincón rivers. This embayment has been divided into three sub-areas defined by topography and oceanographic conditions: the inner basin, the sill area, and the transitional oceanic area at the mouth of Golfo Dulce (Dalsgaard et al., 2003, Dalsgaard et al., 2005; Ferdelman et al., 2006; Morales et al., 2015; Oviedo et al., 2009, Oviedo et al., 2015; Quirós, 2003; Thamdrup et al., 1996).

Golfo de Nicoya is located on the Pacific coast of Costa Rica (10”N, 85°W) and is one of the largest estuaries (1 530 km²) in Central America. It extends about 80 km from its narrowest point at the mouth of the Tempisque River to its widest point (about 55 km) where it borders the open ocean (Maurer & Vargas 1984; Brenes et al., 2001; Wolf et al., 1998).

We conducted 476 photo-identification surveys in Golfo Dulce and 17 in Golfo de Nicoya, using a 7-m-long boat powered by a 115-horsepower four-stroke engine as a research platform during two seasons, wet (June - October) and dry (November - May), from 2005 to 2022. Each survey was conducted between 7:00 am and 4:00 pm on a given day. There were four observers on board the research vessel, with one observer acting as the primary photographer, usually assisted by another secondary surveyor, to photograph as many dolphins in the group as possible. Photographs were taken with an SRL digital camera (Canon 7D/70D) equipped with a 400 mm telephoto lens. In the presence of a group of dolphins, we photographed the dorsal fins of all individuals within the group, as perpendicular to the body axis as possible. Photographs were classified according to the classes described in Sanchez-Robledo et al. (2020). A quality criterion was prioritized in photographic data processing (Karczmarki et al., 2005). Therefore, only high-quality images (80 % and above on a quality scale from 1 to 100 %) were selected and used in the analysis. The photo processing was designed to eliminate any sampling bias, thereby strengthening one of the key assumptions of the capture-mark-recapture models, the likelihood of homogeneous capture of all individuals in the group. Once all LLD photographic records were organized and selected, we validated the diagnosis after consultation with an expert (Marie-Françoise Van Bressem, personal communication, 6 October, 2022). After analysis and classification of all folders, we counted the number of individuals with LLD.

We estimated an LLD prevalence rate, taking into account the individual history of encounters, which allowed us to calculate annual prevalence indices from the rainy season of 2011 to the rainy season of 2020. This was done by considering the annual presence records of non-calf individuals with photographic evidence of LLD in relation to all non-calf individuals recorded and photographed in that year. The latter resulted in a prevalence rate for Golfo Dulce, presented with a confidence interval. In the case of Golfo de Nicoya, the number of individuals with LLD in all surveys where coastal bottlenose dolphins were recorded was also equal to the number of individuals recorded and photographed per year, due to the low number of dolphins in this population. Data consisted of 590 *T. truncatus* sighting records collected during long-term cetacean research (2005 - 2020) in Golfo Dulce. In Golfo de Nicoya, the data collected consists of nine sightings of *T. truncatus* from 17 boat sampling events (2014: n = 6; 2015: n = 8, 2019: n = 1, 2022: n = 2).

In the period 2005-2020, the average percentage of dolphins identified with LLD in Golfo Dulce was 13.1 (CI: 12 – 14.2) in 105 dolphins photo-identified (Fig. 1; Supplementary Table 1). The highest prevalence estimated in 2014 was 16.2 %, whereas the lowest prevalence was estimated at 9.1 % in 2019 (SMT1), within this range most of the prevalence values varied around 12 to 14 %.

These prevalence levels are comparable with those reported for other coastal populations of *T. truncatus* from South America (5.4 % - 44.4 %), North America (12.4 %), and *Tursiops aduncus* (Ehrenberg, 1832 [1833]) from Africa (8.4 %), the percentage of individuals with skin lesions in Golfo Dulce is consistent with those reported in these locations (Kiszka et al., 2009; Moreno et al., 2008; Murdoch et al., 2008; Félix, Van Bressem et al., 2019).

The estimated prevalence of LLD in *T. truncatus* from the Golfo de Nicoya was 100 % (LLD dolphin; n = 6 from identified individuals, all of them adults; n = 6) (Fig. 1) in 2019 and 2022. Such a high prevalence rate in an inshore dolphin community is unprecedented. The highest prevalence rate reported until now was 44.4 % in a small community (n= 9) of

T. truncatus from Salinas, Ecuador (Félix, Van Bressem et al., 2019). In Golfo Dulce the majority of LLD cases were documented in males (n=5), with only two females affected by the disease and two unidentified sex individuals. Similarly, in Golfo de Nicoya three males were identified as LLD dolphins, along with two females and one unidentified sex individual. The lesions of the dorsal fin progressed over years in a dolphin TtGN005 from Golfo de Nicoya and individual TtGD003 from Golfo Dulce (Fig. 1).

There are intrinsic demographic characteristics that differ between these two populations, while the coastal bottlenose dolphin population in Golfo Dulce would be considered discrete at just over 100 individuals, with a portion of it showing important site fidelity (Oviedo, 2018), the Golfo de Nicoya population is smaller than ten individuals. In the course of our assessment, we captured the same adult individuals on each sampling occasion, suggesting not only site fidelity but also an apparent lack of immigration into this population. There are other similarly small populations of the coastal bottlenose dolphin ecotype elsewhere in Latin America, such as the Santa Elena Peninsula, Ecuador, where these dolphins were observed intermittently from 2005-2018, with a mean group size of 5.31 dolphins/group (SD = 1. 97, range 1–10) (Félix, Zavala et al., 2019), and in the Tramandai estuary in southern Brazil, where they have been observed since the early 90s, with an average of nine dolphins (including calves) commonly recorded over the years (Di Giacomo & Otts, 2016), with at least two dolphins affected by LLD (study period 1991- 2008) (Moreno et al., 2008). Félix, Zavala et al. (2019) suggested that such small dolphin populations may be a remnant of larger communities, possibly affected by external environmental stressors, likely of anthropogenic origin. In the case of Golfo de Nicoya, the high prevalence of LLD in such a small community is a cause for serious concern.

Demography and social behavior influence the incidence and transmission of the disease (Félix, Van Bressem et al., 2019). Even though the analysis of social behavior and characteristics of these populations is in progress. There are important similarities in terms of sex classes occurrence, distribution, and social behavior profiles of both populations, with that reported by Félix, Van Bressem et al (2019). In the case of Golfo de Nicoya, as mentioned above, the high prevalence could be explained primarily by the demography of such a small, localized community, where most of the individuals affected are males, basically half of the adults observed in the field, with at least a high ranked individual (TtGN001; Fig.1). Whereas, in Golfo Dulce, LLD is equally observed mostly in males (five out of nine cases). The medium to low prevalence rate (13.14 %) could be affected by the incidence of the disease in one out of two male alliances. This lower-ranked pair (TtGD015 and TtGD016; Fig.1) had a wider home range than the dominant alliance. All of this supports the notion of horizontal disease transmission and geographic spread by non-resident individuals (Félix, Van Bressem et al., 2019; Van Bressem et al., 2015).

Although differences in sample size may explain some of this variation, environmental and individual factors are also likely to play an important role. Burdett Hart et al. (2011) suggested that differences in freshwater input could influence the development and persistence of lobomycosis between *T. truncatus* communities of west and east coast estuaries in Florida; this could be the case for Golfo Dulce and Nicoya.

In Golfo Dulce and Golfo de Nicoya, the presence of polychlorinated biphenyl compounds (PCBs, DDT, DDD, DDE, dieldrin, and BHC) is associated with agriculture practices (Spongberg & Davis, 1998; Spongberg, 2004a; Spongberg, 2004b; Umaña., 1998. Along the riverbanks draining into the Golfo Dulce, a number of agricultural practices have been developed, such as cattle ranching and monocultures of African palm and banana (Umaña, 1998). The increase in these activities has introduced heavy metals (Fe 5.8 ug/g, Zn 96 ug/g, Cu 87 ug/g, Pb 6 ug/g) and organochlorine pesticides into the water column (5000 ug/g) and sediments (15.7 ug/g) (Acuña-González et

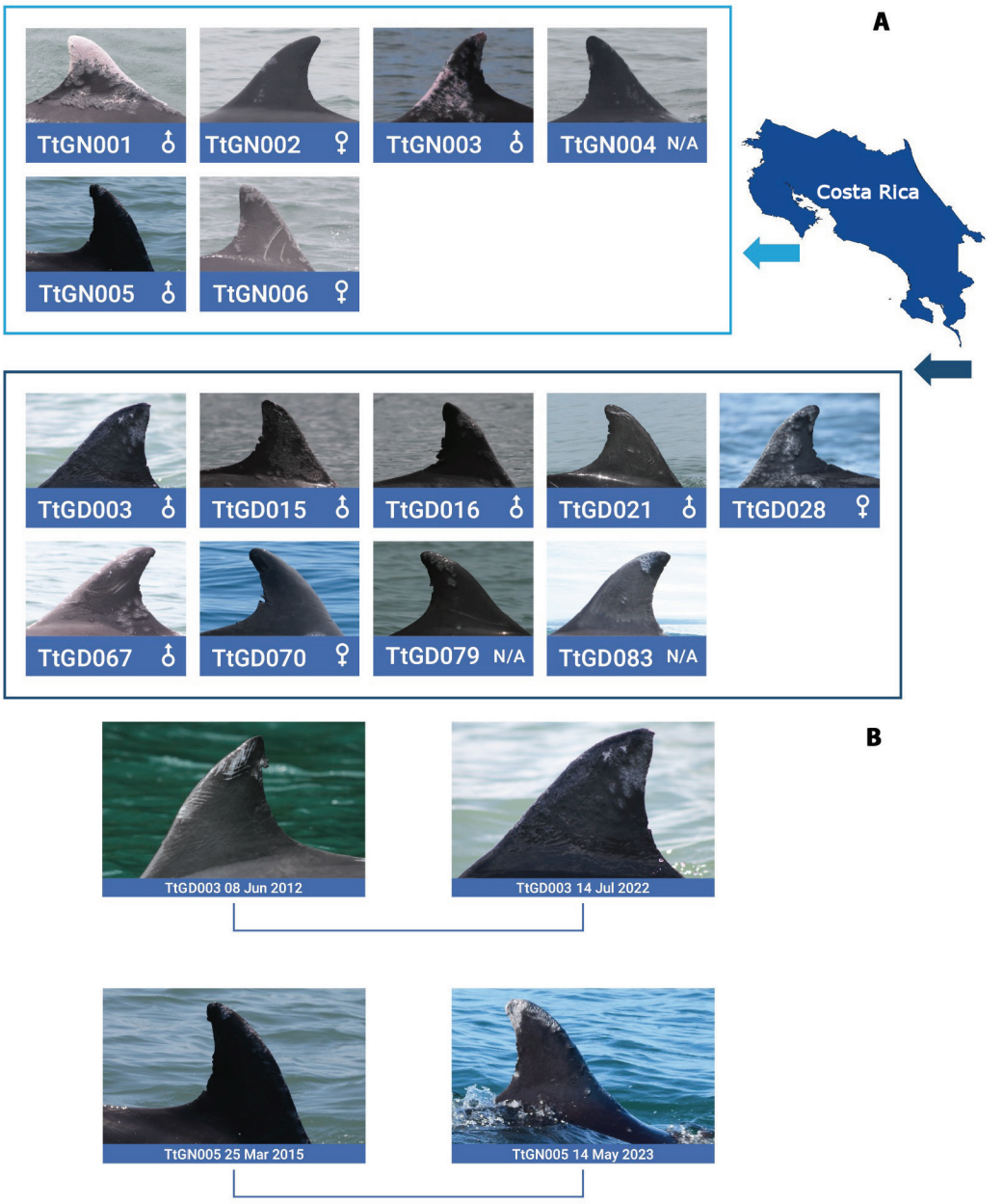

Fig. 1. A. Cases of lobomycosis-like disease in inshore bottlenose dolphins from Golfo Dulce (framed by dark blue rectangle) and Golfo de Nicoya (framed by light blue rectangle) in the Pacific waters of Costa Rica. **1.b** Lesion progression in individual TtGD003 (Golfo Dulce) and TtGN005 (Golfo de Nicoya).

al., 2004; Spongberg & Davis, 1998; Spongberg, 2004a; Spongberg, 2004b).

Many other anthropogenic activities, including agriculture, forestry, tourism, aquaculture, salt-mining, mining and industry have been carried out in the Golfo de Nicoya, making the area vulnerable to contamination by petroleum hydrocarbons, wastewater, pesticides, heavy metals and organic compounds (León Coto et al., 1998; Marín-Alpizar, 2000;

Morera-Gonzalez et al., 2019;). These can be associated with other particles and become available to biota and bioaccumulate in the lipid tissues of organisms (León Coto et al., 1998; Marín-Alpizar, 2000; Morera-Gonzalez et al., 2019).

Very low concentrations of PCBs and DDT can bioaccumulate and suppress the immune system of these animals, thereby facilitating the onset of disease (Jepson et al., 2005; Reif et al., 2009; Ross., 2002), which could be the case in the Golfo Dulce and Golfo de Nicoya bottlenose dolphin population.

The anthropogenic impacts that the coastal populations of *T. truncatus* have been exposed to may become critical and even cause a decline in the largest and smallest populations of these coastal dolphins in the country. Although there is no documented mortality caused by the disease in Golfo Dulce and Golfo de Nicoya, a high rate of disease could lead to septicemia, considering the severity and sometimes open lesions, these can be exposed to bacteria (Van Bressem et al. 2015). Additionally, the spread to key anatomical areas, such as the rostrum, could impede prey consumption and result in emaciated individuals. If we put more pressure on these areas through coastal development, such as marinas and luxury hotels, the cetacean populations and the tourism industry that depends on them would be at risk. The restricted geographic range used by these populations of *T. truncatus* and the strong dependence of these dolphins on specific environmental characteristics (structure and available resources) makes them vulnerable to habitat loss and degradation. The persistence, and high prevalence levels of LLD, in small, localized, communities of *T. truncatus* in the Pacific Ocean of Costa Rica is a cause for serious concern.

Ethical statement: the authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgments section. A signed document has been filed in the journal archives.

See supplementary material
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