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Franciscana (*Pontoporia blainvillei*) found dead in Northern Patagonia, Argentina: record, description and sampling. Preliminary report

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Franciscana (*Pontoporia blainvillei*) found dead in Northern Patagonia, Argentina: record, description and sampling. Preliminary report

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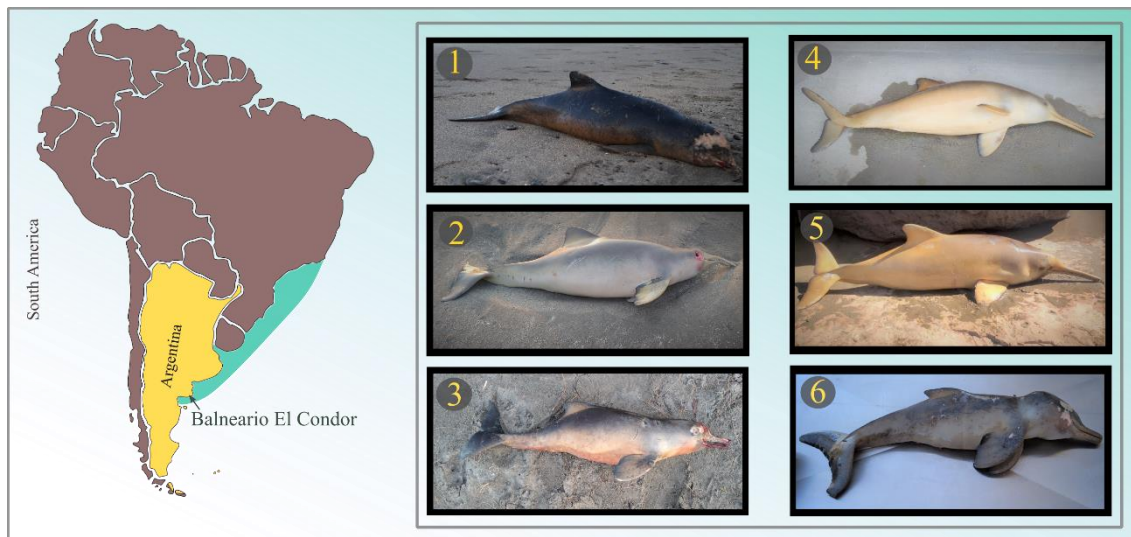
Abstract The Rio Negro estuary is considered the southernmost breeding and feeding area of the franciscana. Preliminary results of necropsies performed on six dead specimens found dead during 2020 are reported here. One calf, two juveniles/subadults and three adults were analyzed. Two of the adults died in gill nets. Macroparasites of Nematoda and Acantocephala phyla were found in the stomach inspections of four animals. Two dead specimens showed beak deviations which would represent the southernmost report of beak anomalies for the species throughout its range. Scattered granulomatous lesions compatible with mycobacteria was detected in one franciscana. Cultured samples of the whitish nodular lesions showed the development of acid-fast bacilli detection. In addition, molecular identification of *Mycobacterium tuberculosis* complex was positive. This report will be the first case of *Mycobacterium tuberculosis* complex in this cetacean. Based on the macroscopic lesions compatible with infectious diseases discovered here, it is necessary to extend the sanitary study of this population of franciscana.

Key words: monitoring, biopsy sampling, disease, gillnets, South America

The franciscana (*Pontoporia blainvillei*) is a small cetacean endemic to the Atlantic coast of South America (Zerbini *et al.*, 2017). Currently incidental captures in fishing nets represent the main threat to *P. blainvillei* (Zerbini *et al.*, 2017; Reeves *et al.*, 2012). It is listed as "vulnerable" by the IUCN Red List (Zerbini *et al.*, 2017) and also on the Red List of Mammals of Argentina (Denuncio *et al.*, 2019).

During 2020, six franciscanas were found dead and studied in Balneario El Cóndor, Río Negro Estuary, Patagonia Argentina (41°2'S, 62°48'W) (Figure 1). Preliminary results of examination performed on six dead specimens found dead during 2020 are reported here.

53 Figure 1. Distribution area of franciscana (in light blue, adapted from Zerbini *et al.*, 2017) and study area.
 54 Numbers 1 to 6 identify the different dead specimens studied.



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 57 Each specimen underwent an external examination with collection of morphometric data (Norris,
 58 1961), a gross necropsy examination (Pugliares *et al.*, 2007; Young *et al.*, 2007), collection of
 59 tissue samples for molecular and bacteriological diagnostics and photographic documentation.
 60 These tasks were performed *in situ* and within 12 hours of finding each stranding to facilitate
 61 fresh study.
 62 According to the results of Arruda Ramos *et al.* (2000) who describe that males attain sexual
 63 maturity at two years and 115cm long and females at three years and 130cm long, individuals
 64 found dead were classified into: calf, juvenile/ subadult and adult. Table 1 shows the date of
 65 death, sex, age category and total length.
 66

67 Table 1. Date of death and general description of each franciscana studied. Numbers 1 to 6 identify the
 68 different dead specimens studied.

Franciscana studied	Date	Sex	Age category	Length
1	July 27 2020	female	juvenile/ subadult	104cm
2	August 28 2020	female	adult	143cm
3	September 10 2020	female	juvenile/ subadult	85cm
4	September 25 2020	female	adult	155cm
5	December 02 2020	male	adult	123cm
6	December 02 2020	nd	calf	64cm

69 nd: no data

70
 71 None of the adult females was pregnant on or showed signs of suckling. To assess incidental
 72 mortality, net marks were searched for. Although gillnet marks were only observed on the
 73 individual identified as number 4, the individuals identified as number 2 and 4 were removed
 74 from the fishing nets, according to witness accounts (Table 2).
 75 Also, the general nutritional status of each individual and the location of the organs (to
 76 determine if there are any displaced organs) were evaluated before the organs were removed.
 77 Later, individual organs were removed, examined externally and internally for macroscopic
 78 lesions (Table 2) and sampled systematically (except in franciscana identified as number 2).
 79 Lungs, liver, kidneys, muscle tissue, blubber, intestines, gonads and mammary glands were
 80 sampled. Serum was also collected for franciscana identified as number 4. The samples were
 81 frozen for further studies.

82 Stomachs of four specimens were analyzed and only two of them contained macroparasites:
 83 Nematoda and Acantocephala (Table 2). Their identification is still in process. The presence of
 84 plastics was not detected. The intestines of each individual were frozen for further analysis.
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 86

87 Table 2. External and internal examination of each franciscana studied. Numbers 1 to 6 identify the
 88 different dead specimens studied.

Franciscana studied	1	2	3	4	5	6
Presence of net marks	no	no*	no	yes*	no	no
External morphology	normal	beak deviation	normal	normal	beak deviation	normal
General nutritional status	normal	normal	normal	normal	normal	malnutrition
Location of organs	normal	normal	normal	normal	normal	normal
Internal macroscopic lesions compatible with infectious diseases	yes	no	no	no	no	no
Stomach contents	nd	full	nd	full	empty	empty
Stomach plastics	nd	not found	not found	not found	not found	not found
Stomach macroparasites	nd	yes A	yes A	yes A	yes A and N	no

89 * Removed from fishing nets; nd: no data; A: Acantocephala; N: Nematoda

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 91 The franciscana identified with the numbers 2 and 5 had beak deviations in the skull (Figure 2).
 92 Recently Denuncio *et al.* (2016) described and quantified the beak anomalies of franciscanas
 93 from the coastal waters of Argentina reporting that 12% showed beak deviations. The natural or
 94 anthropogenic causes of these anomalies are still unknown. The dead specimens reported here
 95 would represent the southernmost reports of beak anomalies for the species in its entire range.
 96 This skull was frozen for posterior studies.
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98 Figure 2. Beak deviations in the skull of two Franciscana identified as number 2 (left) and 5 (right) of the
 99 present study.

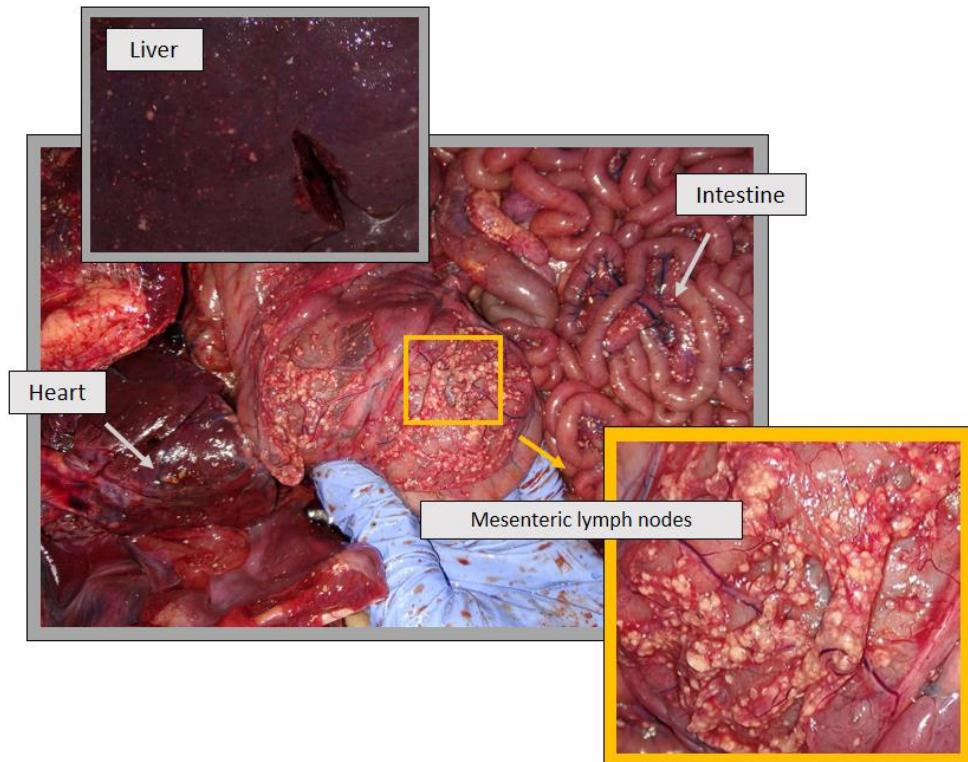


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 114 Macroscopic granulomatous lesions compatible with mycobacteria were found was detected at
 115 the internal inspection of the individual number 1 (Figure 3). The finding of macroscopic lesions,
 116 the impossibility of move the corpse to a laboratory in the context of covid-19 and the weather

117 conditions at the time of the necropsy did not allow made detailed observation of the lungs and
118 stomach. Samples with macroscopic granulomatous lesions were cultured in Ziehl-Neelsen
119 staining and after two weeks development of plenty acid-fast bacilli was observed. Also,
120 molecular identification of the isolate was made. Likewise, by end-time PCR, it was determined
121 that the species of *Mycobacterium* present is within the pathogenic *Mycobacterium tuberculosis*
122 complex .The identification of mycobacterial species and the typing of the strain is being
123 processed through the reverse hybridization technique of spoligotyping. This is the first record
124 of *Mycobacterium tuberculosis* complex in franciscana.

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Figure 3. Macroscopic granulomatous lesions compatible with mycobacteria detected in franciscana identified as number 2 of the present study.



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Despite not finding marks on all the specimens, the strongest hypothesis of the cause of death captures by gill nets. Franciscanas are accidentally captures by gill nets throughout their geographic distribution range (Ott *et al.*, 2002; Negri *et al.*, 2012). Balneario El C3ndor, municipality of Viedma (province of R3o Negro) introduced since 2013 a ban on the use of gillnets from 15th November to 15th March (Ordinance N7326-Consejo Deliberante de Viedma) and it have been implemented every year. Although the Ordinance was enacted to order nautical and fishing activity in the area, it benefits indirectly the franciscana, since it is applied during the reproductive season of the species at the R3o Negro Estuary. The discovery of six dead franciscanas, two confirmed by anthropic causes, reveals the need to review (extend the closed period) local fisheries regulations, in order to conserve the species. The first report of the *Mycobacterium tuberculosis* complex in the species shows the need to continue and expand the investigations. Know the health of this population of Franciscans also contributes to knowing the health of the ecosystem. However, the limitations to develop a complete necropsy of an animal with strong indications of lesions compatible with infectious diseases are recognized. These recommendations become even more relevant when it is noted that the R3o Negro Estuary is considered the southernmost reported breeding and feeding area (Failla *et al.*, 2012) for these vulnerable dolphins.

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