

First case of the pigo-ischio-cephalopago deformation in a neonate of a gallube shark (*Squalus acanthias*- Linnaeus -1758) captured in Peruíbe, south coast of São Paulo, in the Cananea -Iguape-Peruíbe environmental protection area - APACIP and Jureia-Itatins Mosaic Conservation Units -SP

Primeiro caso de deformação Pigo-isquio-cefalópago em neonato de tubarão galhudo (*Squalus acanthias*- Linnaeus -1758) capturado em Peruíbe, litoral sul de São Paulo, na área de proteção ambiental Cananea -Iguape-Peruíbe – APACIP e Unidades de Conservação do Mosaico Jureia-Itatins-SP

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ABSTRACT

Connatural alterations can be deformations or malformations discovered in the embryonic development phase of the animal, which can disguise structures or roles of the systems. Congenital disorders can range from small changes, slight changes, serious adulterations and also genetic inconsistencies. There are no apparent defined reasons, and these changes may be caused by environmental and genetic factors. Pigopagus twins are joined

at the back, have a coccyx, the gastrointestinal tract may have a single or separate rectum, a single bladder in some cases and obligatorily separate spinal cord and always with sharing of the pelvic bones. The ischiopagus are united by the ventral region from the navel to the pelvis, two sacrum and two pubic symphysis, in addition to a single gastrointestinal tract and a varied number of limbs. Reporting and discussing the morphological and anatomical deformities presented in a species of *Squalus acanthias*, recorded by LOPES in 2020, is a big step to better understand the anatomy and physiology of animals, which are considered the top of the food chain in the oceans and are in serious trouble. risk of extinction.

Keywords: Siamese twins, diprosopia, two heads, squalus acanthias, deformations.

RESUMO

Alterações conaturais podem ser deformações ou más-formações descobertas na fase de desenvolvimento embrionário do animal, podendo disfarçar estruturas ou papéis dos sistemas. Os distúrbios congênitos podem ocorrer desde pequenas mudanças, alterações leves, adulterações sérias e também incoerências genéticas. Não existem motivos aparentes definidos, podendo estas alterações serem causados por fatores ambientais e genéticos. Os gêmeos Pigopagus são unidos pelo dorso, apresentam um coccix, o trato gastrointestinal pode apresentar reto único ou separado, bexiga única em alguns casos e obrigatoriamente medula espinhal separada e sempre com compartilhamento dos ossos pélvicos. Os isquiópagos apresentam-se unidos pela região ventral do umbigo à pelve, dois sacros e duas sínfises púbicas além de tratos gastrointestinal único e número de membros variados. Relatar e discutir as deformidades morfológicas e anatômicas apresentadas em uma espécie de *Squalus acanthias*, registrada por LOPES em 2020, é um grande passo para entender melhor a anatomia e fisiologia de animais, que são considerados o topo da cadeia alimentar nos oceanos e correm um sério risco de extinção.

Palavras chaves: gêmeos siameses, diprosopia, duas cabeças, squalus acanthias, deformações.

1 INTRODUCTION

In elasmobranchs, duplication anomalies such as dicephaly (or bicephaly) (Lopes et al., 2020) and diprosopia, which are related to duplication in the head region, have already been recorded. In the first case, there is total separation of the duplicated structures, joining in the distal region of the skull or the beginning of the spine, thus the animal is born with two heads, brain, and the beginning of the spine (not necessarily), a result of the error in embryogenesis where there is duplication of neural plates that result in two partially modified neural crests or, depending on the case, totally duplicated, thus forming double facial structures (Sans-Coma et al., 2017).

When diprosopia or incomplete duplication occurs, the skull remains fused and there is duplication of the face or structures contained therein (eyes, nose) of the

individual. In these cases, the nomenclature "Siamese" may or may not be employed (Carles et al., 1995).

Genetic mutations have been confirmed in elasmobranch species, and cases of albinism have been reported (Botarro, et.al., 2005; Saidi et.al., 2006); mutations in pectoral fins, head, anophthalmia, presence of only one clasper, and cases of dicephaly (Gopalan, 1971; Masatoshi et al., 1981; Lamilla et al., 1995; Heupel et al., 1999; Mancini et al., 2006).

Concerning Siamese characteristics and classification, considerable difficulty has been encountered in interpreting the variations in nomenclature and designation of the different types of union, as well as the identification of individual twins and their anatomical relationship to each other (Spencer, 1996),

Pygopagus are united by the back, have a coccyx, the gastrointestinal tract may have a single or separate rectum, a single bladder in some cases, and separate spinal cord, and always with shared pelvic bones (Denardin et al., 2013). Ischiopagus are joined by the ventral region of the umbilicus to the pelvis, two sacrums, and two symphysis pupicus in addition to a single gastrointestinal tract and number of legs varying from 2 to 4. (Denardin et. al., 2013).

Cephalopod twinning, an extremely rare type of conjoined twins, is characterized by the anterior union of the upper half of the body, with two faces each on the opposite side of a conjoined head (Kuroda et. al., 2002). Thoracopagus refers to Siamese who has large junctions at the thorax and upper abdomen levels up to the umbilical scar, an anomaly that is difficult to surgically separate compatible with life because it most often involves shared hearts (Mei-Hwan Wu et al., 1992). The spinal form of Siamese twins joined by an ectopic or accessory limb attached to the back of both, whether or not involving spinal union, and whether or not the second individual is whole is called Rachipagus (Naveis et al., 2015). Another variation described is the union of twins by the ventral region, which may or may not have a union of the trunk, liver, terminal ileum, and colon or even unite at the level of the diverticulum with separation of the rectum, but necessarily with independent hearts called Omphalopagus (Denardin et. al., 2013).

Parapagos are also joined by ventrolateral fusion and may be dividing from the lower abdomen to the pelvis, but obligatorily shared pubic symphysis and urinary tract.

This paper aims to report and discuss the morphological and anatomical deformities presented in a species of *Squalus acanthias*, recorded by LOPES et al., (2020), which is considered the first record in the world literature. The species is classified as

vulnerable in the list of endangered species, according to the IUCN Red List of Threatened Species.

2 MATERIAL AND METHODS

The specimen with anomaly was caught in a seabass shrimp trawl by artisanal fishermen from the city of Peruíbe, São Paulo, Brazil at the geographic coordinates 24°22'38.78 "S and 47°01'12.15 "W (Figure 1). The specimen was donated to IBIMM (Institute of Marine Biology and Environment) in July 2013. The institution, which aims to work with elasmobranch conservation in the region usually receives donations from fishermen.

The animal was fixed in 4% formalin, then placed in a 70% alcohol solution. Currently, it is deposited in the scientific collection of the Museu do Mar, under IBIMM number 005/2013 and number 019/2021, Bioceua-IBIMM Ethics Committee.

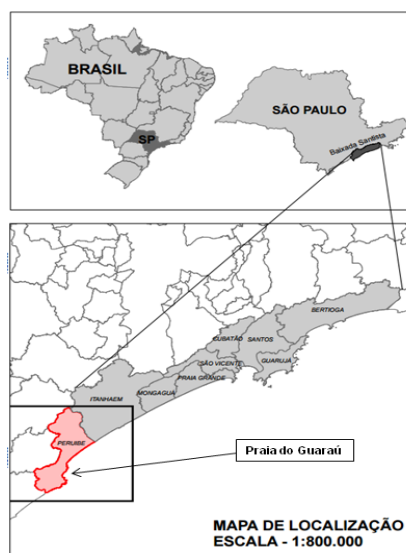


Figure 1 – Location of the *Squalus acanthias* capture area, Peruíbe, SP, Brazil. Photo: (available in the site - SIMA- Secretary of Infrastructure and Environment-SP-2021). Photo: (author).

IDENTIFICATION

The specimen was identified through morphological and morphometric characteristics, with emphasis on the dermal denticles and dentition, according to (Lopes, et al., 2020). Several photographs were taken using a cell phone camera with a resolution of 1,500 pixels, for a better definition of the images. Pictures were taken of all the structural parts of the animal.

EXTERNAL ANATOMY

In anatomy the general morphological characteristics can be observed according to (Compagno, 1984).

INTERNAL ANATOMY

Following the methodology of the Atlas of Shark Anatomy Photography - The Glossary of Morphology of *Squalus acanthias*, (Gans 1964), a longitudinal cut was made in the ventral region of *Squalus acanthias* up to the oral cavity to the cloacal cavity, using blunt-ended scissors, to avoid perforation and cuts of the external organs. Photographs were then taken for further photo-identification of the structures and organs of the animal.

COMPUTED TOMOGRAPHY

For the compaction of the internal structures, a computed tomography scan was performed in a Toshiba tomograph at the Animal Care Veterinary Hospital, where the specimen was positioned with the ventral part facing down. The program K-sas diagnostics was used to read the images.

3 RESULTS

IDENTIFICATION

The specimen was identified as a *Squalus acanthias* (Linnaeus, 1758), male weighing 800g, total lengths of 23.5 cm (right specimen) and 22.8 cm (left specimen) seen in figure (2). Complete biometrics are shown in Table 1.



Figure 2 - Dogfish shark, *Squalus acanthias* found on the coast of Peruibe-SP. (Photo: author).

Table 1. Morphometric relationship of *Squalus achantias* captured in Peruíbe, SP, Brazil.

Measures	Right	Left
(TOT) TOTAL LENGTH (CM)	23.5	22.8
(FOR) LENGTH FROM NOSE TO TAIL BIFURCATION	20.0	20.1
(PRC) PRE-CAUDAL LENGTH	18.6	18.2
(PD2) LENGTH OF THE PRE-SECOND DORSAL	14.9	14.6
(PD1) LENGTH OF THE FIRST DORSAL PRE	7.9	7.6
(HDL) HEAD LENGTH	4.8	4.8
(PG1) PRE-GILL LENGTH	4.0	3.7
(PSP) PRE SPICULAR LENGTH	2.6	2.5
(POB) PRE-ORBITAL LENGTH	1.3	1.2
(PP1) PRE-CHEST LENGTH	4.9	4.7
(IDS) INTERDORSAL SPACE	4.4	4.3
(DCS) CAUDAL DORSAL SPACE	1.8	1.5
(PRN) SNOUT LENGTH	1.2	1.0
EYE LENGTH (POR) PREORAL LENGTH	2.3	2.2
EYE LENGTH (EYL)	1.3	0.9
EYE HEIGHT (EYE)	0.6	0.5
INTERGILL LENGTH (ING)	0.6	0.5
HEIGHT OF FIRST GILL SLIT (GS1)	0.6	0.5
EARLY PECTOR MARGIN RIGHT (PIAR)	2.7	2.6
PECTORAL BACK MARGIN LEFT (PIAL)	2.5	2.4
RIGHT PECTORAL BASE (PIBR)	1.4	1.3
LEFT PECTORAL BASE (PIBL)	1.2	1.4
RIGHT INTERNAL PECTORAL MARGIN (PIIR)	1.5	1.2
LEFT INTERNAL PECTORAL MARGIN (PIIL)	1.0	1.4
RIGHT POSTERIOR PECTORAL MARGIN (PIPR)	1.6	1.7
LEFT PECTORAL POSTERIOR (PIPL)	1.8	1.9
RIGHT PECTORAL HEIGHT (PIHR)	1.9	1.6
LEFT PECTORAL HEIGHT (PIHL)	2.3	1.5
FIRST DORSAL LENGTH (DIL)	2.9	2.7
FIRST ANTERIOR DORSAL MARGIN (D1A)	2.6	2.3
FIRST DORSAL BASE (DIB)	2.1	1.8
FIRST DORSAL HEIGHT (D1H)	1.5	1.1
FIRST BACK MARGIN DORSAL (DIP)	1.4	0.9
SECOND DORSAL LENGTH (D2L)	3.1	2.7
SECOND ANTERIOR MARGIN DORSAL (D2A)	2.1	1.8
SECOND BACK FOOT (D2B)	1.7	1.5
SECOND DORSAL HEIGHT (D2H)	0.7	0.7
SECOND DORSAL POSTERIOR EDGE (D2P)	1.2	1.1
PELVIC LENGTH (P2L)	2.2	2.0
ANTERIOR PELVIC MARGIN (PZA)	1.0	1.0
PELVIC BASE (P2B)	0.7	0.6
PELVIC HEIGHT (P2H)	1.1	1.1
PELVIC INTERNAL MARGIN LENGTH (P2I)	0.6	0.5
LENGTH OF POSTERIOR PELVIC MARGIN (P2P)	0.7	0.6
HEAD HEIGHT (HDH)	2.1	1.9
TRUNK HEIGHT (TRH)	1.8	1.8
MOUTH LENGTH (MOL)	1.5	1.4
MOW = WIDTH OF MOUTH	1.8	1.7
RIGHT UPPER LIP LINE LENGTH (ULAR)	0.7	0.4
LEFT UPPER LIP LINE LENGTH (ULAL)	0.6	0.4
LOWER RIGHT LABIAL GROOVE LENGTH (LLAR)	0.3	0.4
LOWER LABIAL FURROW LENGTH LEFT (LLAL)	0.4	0.5
NOSTRIL WIDTH RIGHT (NOWR)	0.3	0.3
NOSTRIL WIDTH LEFT (NOWL)	0.4	0.4
INTERNARIAL SPACE RIGHT RIGHT (INWR)	1.1	1.1

EXTERNAL MORPHOLOGY

Externally the animal showed lateral fusion of two complete embryos. Regarding head duplication, they are separated until the 5th-gill slit.

It had two pairs of gill slits, two pairs of dorsal fins with spines at the origin, and two pairs of pectoral fins, which are joined at the base, being divided at the anterior and posterior margin.

One pelvic fin, with a pair of claspers, one caudal fin, with two upper lobes and one lower lobe.

Two pairs of lateral lines, run from the origin of the first dorsal at the junction site ending at the end of the tail (Figure 3A and 3B).

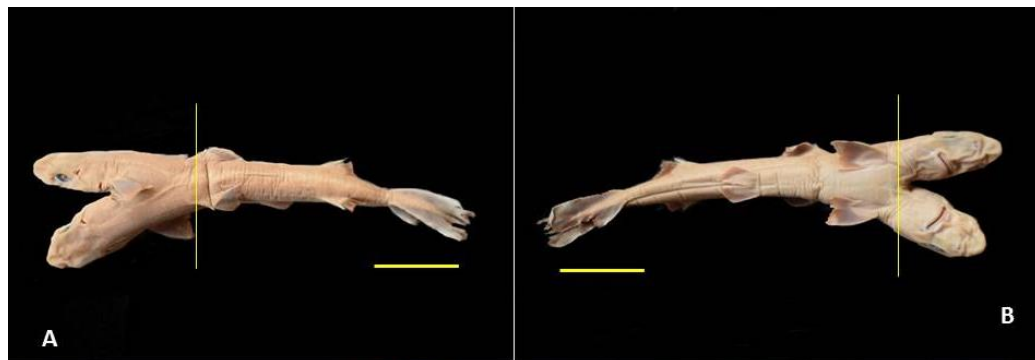


Figure 3 - Dorsal and ventral view of lateral fusion of *Squalus achantias* embryos, with separation up to 5th gill slit, presence of pelvic fin with 02 upper and 01 lower lobes. Bar=5 cm. (Photo: author).

INTERNAL MORPHOLOGY

In the analysis of the morphology of the animals in question, we found 02 completely separate chondochraniums, two oral cavities followed by two esophagi, two hearts, two stomachs, one more developed than the other, two livers two pairs of kidneys, and two hearts. One intestine is attached to the stomach, one rectal gland, one pair of male gonads, and one cloacal opening (Figure 4).

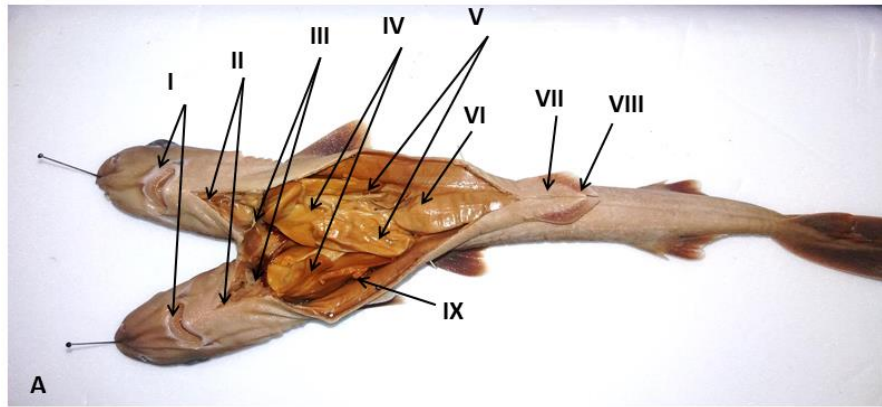


Figure 4. internal morphology: (I)-oral cavity; (II)-esophagus; (III)-heart;(IV)-liver; (V)-stomach; (VI)- intestine; (VII) cloaca; (VIII)-Clasper,male gonads (IX)-kidneys, of *Squalus acanthias*, captured in Perufe, SP, Brazil. (Photo: author).

COMPUTED TOMOGRAPHY

It was possible to evaluate the internal structures by means of computerized tomography and to prove the separation of the vertebral columns in the entire anatomical structure of the animal according to figure (5).

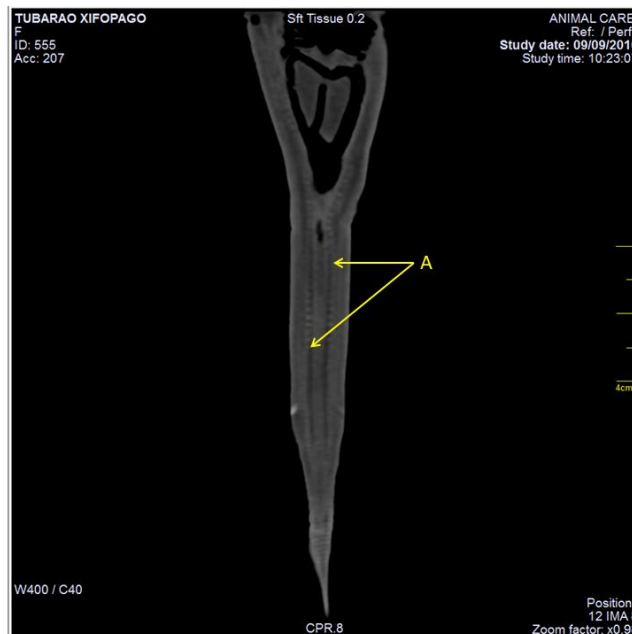


Figure 5. internal anatomy by CT scan, with presence of the two vertebral columns in *Squalus acanthias*.

In figure (6) we have sagittal sections of the internal structures, with presentation of the chondocranium and presence of the pelvic nares.

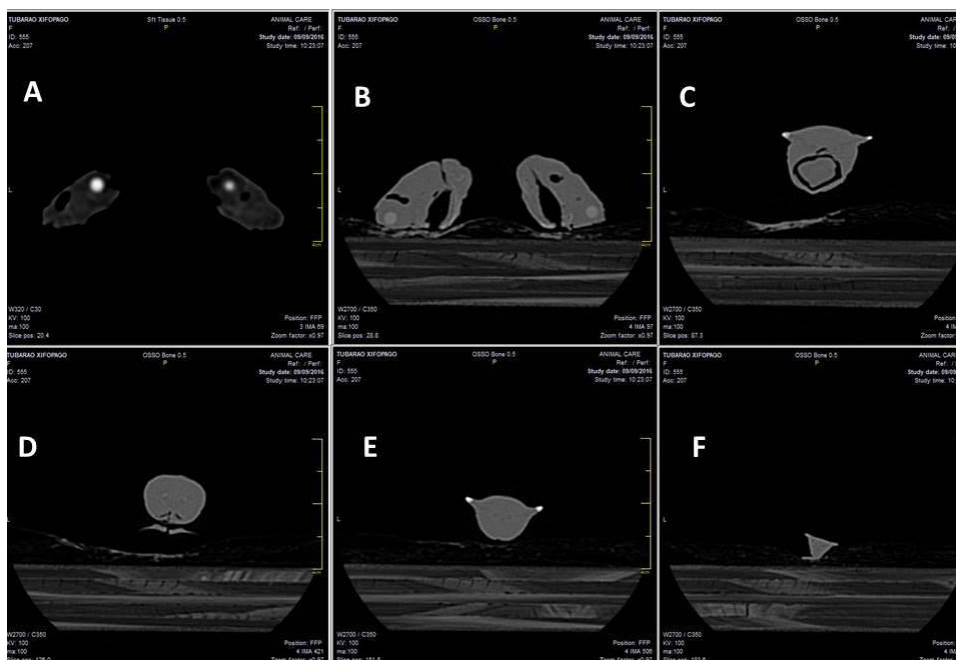


Figure 6 - Image of the internal anatomy through CT scan, based on the spine, in sagittal sections of *Squalus achantias*, with the following demonstrations: A) end of the chondrocranium; B) pectoral fin height; C) first dorsal fin height; D) pelvic fin height; E) second dorsal fin height; and F) caudal fin height. Photo: (author).

4 DISCUSSION

There are several causes referred to as natural anthropogenic agents implicated in elasmobranch abnormalities, such as parasite infection, mechanical injuries, physiological diseases, poor nutrition, congenital abnormalities and environmental conditions, but these assumptions cannot always be conclusive in all cases of abnormalities. Parasitic infestation seems to be excluded as a direct agent in anatomical anomalies in neonate elasmobranchs. (Rosa et al., 1996; Mancini et al., 2006; Delpiani, et al., 2011; Dulvy et al., 2014).

However, potential causes of anomalies probably occur during embryonic development and unfavorable environmental conditions such as chemical pollutants, may also be considered as they exert influence on embryo development. (Mancini, et al., 2006; Lamarca, et al., 2014).

Anomalies in neonates of elasmobranchs are rare and their causes are still unknown. What is supposed to give rise to these genetic defects or malformations related to organ duplication can have several causes: pollution, tumors, overfishing, hypoxia, malnutrition, environmental changes, zygotic division (Costa et al., 2014, Delpiani et al. 2011, Mancini et al., 2006).

Comparing the external and internal morphology of our specimen with other cases of anomaly reports, it is evident that it cannot be included in any of the alterations such

as: xipophagy or bicephaly described by Mancini et al, (2006) Goto et al.,(1981) Ehemann et al.(2016), Diprosopus, Dicephaly, described by Bosincaano(1934), Risso(1827) Lozano Cabo(1945) Muller & Henle 1839) and Tetrophthalmos, described by Lamarca et al.(2017).

There are two contradictory theories to explain the origins of Siamese twins. The traditional theory is fission, in which the fertilized egg partially splits the twins, representing the late separation of the embryonic mass. The second theory is fusion, in which a fertilized egg separates completely, but the stem cells (which have trophies for similar cells) fuse the stem cells of the other embryo (T. Abossolo et. al., 1994; R. Spencer, 2000; R. Spencer, 2000).

The specimen under study does not fit the term dicencephalic or bicephalic cited by Bosincaano (1934). The term "pigo" is used when completely independent spinal columns are recognized in the specimen. However, when it presents double internal organs and fusion of the intestine (colon) with double size in the pelvic region it is considered "ischiopagus" (Denardim et al., 2013). Cephalopagous twinning, an extremely rare type of conjoined twins, is characterized by anterior union of the upper half of the body, with two faces each on the opposite side of a conjoined head. After analysis of the external and internal anatomy of *Squalus acanthias*, it was shown that the best definition would be the pygmy-ischiium-cephalopagus.

5 CONCLUSION

This is probably the first record of Siamese twins with pygmy-ischiophagus deformity in a *Squalus acanthias* neonate, as no record has been found so far in the world literature.

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