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Research Article

First record of bicephalism in the blacktip shark *Carcharhinus limbatus* (Elasmobranchii: Carcharhinidae) in the southern Gulf of Mexico

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ABSTRACT. The first case of bicephalia in a male embryo of the blacktip shark *Carcharhinus limbatus* in the southern Gulf of Mexico is described. The embryo was found and removed from a pregnant female caught off the coast of Tabasco, Mexico. The specimen had two heads, two dorsal fins, two spine columns conjoined in the pelvic region, two esophagus, and a single stomach.

Keywords: Chondrichthyes; malformation; abnormal embryo; bicephalia; blacktip shark

INTRODUCTION

The blacktip shark *Carcharhinus limbatus* (Muller & Henle, 1839) is a cosmopolitan species in tropical and subtropical waters. It ranges along the eastern coast of the Atlantic Ocean from the northeastern USA (New England) (including the Gulf of Mexico and the Caribbean Sea) to the southeast of Brazil (Compagno 1984).

On the Mexican coast of the Gulf of Mexico, *C. limbatus* is a more appreciated species by the artisanal coastal shark fishery (Tovar-Ávila 1995, Castillo-Géniz et al. 1998). In the southern Gulf of Mexico, the species ranks seventh in importance by the number of individuals captured (Pérez-Jiménez & Méndez-Loeza 2015). Due to migratory movements along the coast, the catches of the artisanal fleets are made up of subadult and adult individuals (Pérez-Jiménez & Méndez-Loeza 2015).

Bicephalia is a case of conjoined twins in which two animals are always derived from a single fertilized ovum; the animals are conjoined to varying degrees, presenting two separate heads on one body (Witschi 1952, Spenser 2000, Bodenson 2001). The phenomenon is well documented in elasmobranchs (Table 1), with about 14 species reported with a bicephalic embryo. This article reports the first case of bicephalia in the blacktip shark *C. limbatus*.

MATERIALS AND METHODS

A pregnant female blacktip shark *C. limbatus* of 222 cm total length (TL) with 10 embryos was captured in coastal waters 27.4 km off Frontera, Tabasco in the southeastern Gulf of Mexico (18°51'0''N, 92°44'24''W, Fig. 1) and landing in fishery camp of La Barra Frontera (18°35'9''N, 92°41'5''W) on September 12, 2022. The catch was made at a depth of 30 m in the artisanal fishery using a bottom longline with 3600 m of the mainline, 1200 snoods with circle hooks baited with Atlantic thread herring (*Opisthonema oglinum*). According to Castro (2011), the identification was based

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Table 1. Reported cases of bicephalia in sharks and rays. ND: no determinate.

Species	Sex	Locality	Reference
Squalus acanthias	Male	São Paulo, Brazil	Queiroz-Lopes et al. (2020)
Squalus blainvillei	ND	Palma de Mallorca, Spain	Lozano-Cabo (1945)
Squalus blainvillei	Female	Eastern Mediterranean Sea, Syria	Capapé & Ali (2017)
Galeus atlanticus	Early embryo	Alboran Sea, Western Mediterranean Sea	Sans-Coma et al. (2016)
Galeorhinus galeus	Male	Mar del Plata, Argentina	Delpiani et al. (2011)
Mustelus higmani	Male	Nueva Esparta, Venezuela	Ehemann et al. (2016)
Carcharhinus leucas	Female	Key West, Florida, USA	Wagner et al. (2013)
Carcharhinus limbatus	Male	Southern Gulf of Mexico	This study
Prionace glauca	Female	Punta Arenas, Baja California Sur, Mexico	Galván-Magaña et al. (2011)
Prionace glauca	2 Females; 1 Male	Baja California Sur, Mexico	Bejarano-Álvarez et al. (2011)
Prionace glauca	Female	Nueva Esparta, Venezuela	Ehemann et al. (2016)
Prionace glauca	2 Females	Canary Island, Spain	Ramírez-Amaro et al. (2019)
Rhizoprionodon acutus	Female	Arabian Sea	Gopalan (1971)
Rhizoprionodon lalandii	Female	Parana State, Brazil	Prado et al. (2020)
Rhizoprionodon porosus	Female	Bahia State, Brazil	Hirata-Dos Santos & Fazzano-Gadig (2014)
Pseudobatos percellens	Female	Southern coast of Brazil	Bornatowski & Abilhoa (2009)
Trygonorrhina dumerilii	Female	Swan Bay, Victoria, Australia	Guida et al. (2014)
Rhinoptera steindachneri	Male	Puerto Adolfo López Mateos, Baja California Sur, Mexico	Castro-Aguirre & Torres-Villegas (1979)

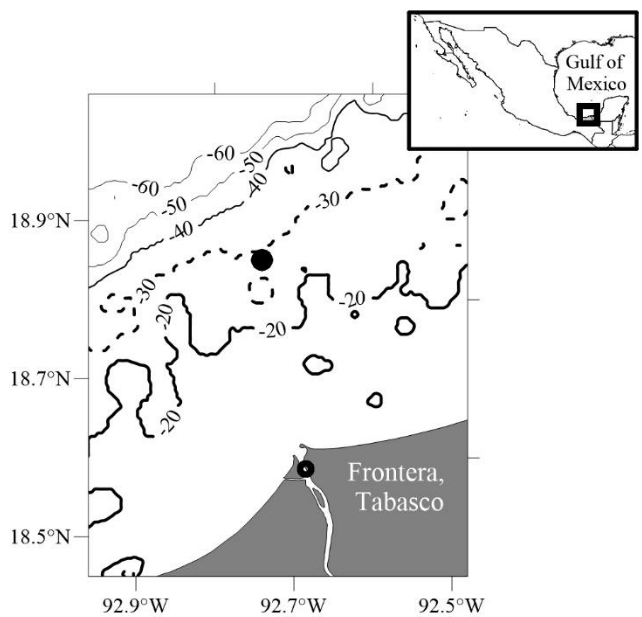

Figure 1. Sampling area to catch the bicephalic male embryo of *Carcharhinus limbatus* in the southeastern Gulf of Mexico, Tabasco, Mexico. Open dot: fishery camp, dark dot: caught point.

on having a nose as long as the width of the mouth, the origin of the first dorsal fin near the middle of the inner margin of the pectoral fin. Tips of the dorsal and pectoral fins and the lower lobe of the caudal fin are black. Upper teeth are narrow, triangular, erect, and slightly oblique with finely serrated edges; lower teeth are erect and narrower than upper teeth with finely serrated edges.

The fishermen donated the abnormal embryo, but unfortunately, due to the quick processing of meat to obtain fillets, they did not record the measurements of the pregnant shark, no photographs or other important data. The embryo was fixed in formaldehyde (10%), preserved in ethyl alcohol (70%), and deposited in the Ichthyological Collection of the Facultad de Estudios Superiores Iztacala (CIFI, by its Spanish acronym),

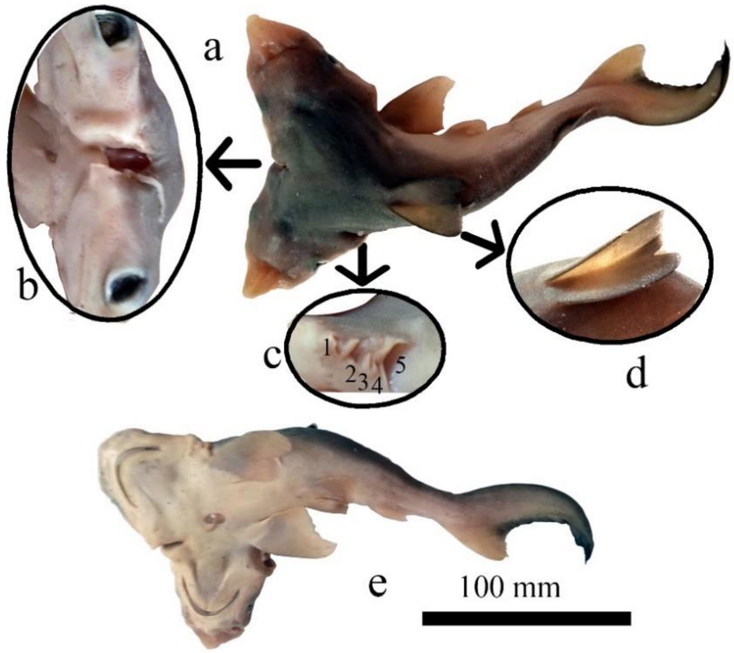

Figure 2. External anatomy of the bicephalic male embryo of *Carcharhinus limbatus* collected in the southeastern Gulf of Mexico. a) View of the dorsal side, b) frontal approach to the gill opening at the junction of the heads, c) approach to the gill openings on the free side of the left head, d) approach to the first dorsal fins, e) view of the ventral side. Photographs by A.T. Wakida-Kusunoki.

Universidad Nacional Autónoma de México (UNAM) under catalog number CIFI-1964. The morphometric measures were taken according to Compagno (2001) using a 30 cm ichthyometer (±0.1 cm). The evaluation for determinate bicephaly (two well-defined skulls in a single body) or diprospia (duplication of craniofacial structures in a single skull) and some anomalies in the spine and internal organs (Spenser 2000) has been based on X-radiogram analysis.

RESULTS

The bicephalic embryo was a male with a TL of 14.7 cm measured from the right head. The embryo had two well-developed heads of similar size and shape (Table 2, Figs. 2a,e). The specimen has a single gill opening at the junction of the two heads (Fig. 2b), while there are five gill openings on the sides (Fig. 2c). On the dorsal side, there are two first dorsal fins and a second dorsal fin, all well-developed (Figs. 2a,d). In contrast, on the ventral side, the pectoral, pelvic, and anal fins are normal both in shape and number (Fig. 1e). On X-radiogram (Fig. 3) observed two heads (*dicephalous*) with well-developed two vertebral columns (*dipus*) conjoined in pelvic region (*parapage*), two esophagi empty in a single stomach (*dibrachius*); according with Spencer (2000) classification these anomalies are typical of conjoined twins with ventro-lateral attach of *parapage dicephalous dibrachius dipus* twins.

DISCUSSION

Bicephaly has been recorded in 14 species of elasmobranchs, although the phenomenon is rare (Table 1). The presence of this anomaly has been attributed to various causes, such as an incomplete division of the embryonic disc or secondary fusion of adjacent embryos (Ehemann et al. 2016), the number of embryos that exceed the uterine capacity (Galván-Magaña et al. 2011, Rodríguez-Romero et al. 2019), abiotic factors such as poor nutrition, contamination, or diseases (Escobar-Sánchez et al. 2014, Rodríguez-Romero et al. 2019), and overfishing of elasmobranch, which significantly reduces the density of reproductive stock, decreasing genetic diversity (Pereyra et al. 2010).

The elasmobranch species reported with bicephalia are of commercial importance in the areas where these abnormalities have been reported (Chiaramonte 1998, Melendez & Macias 2007, Bizarro et al. 2009, Bornatowski et al. 2009, Arocha et al. 2016, Marquez

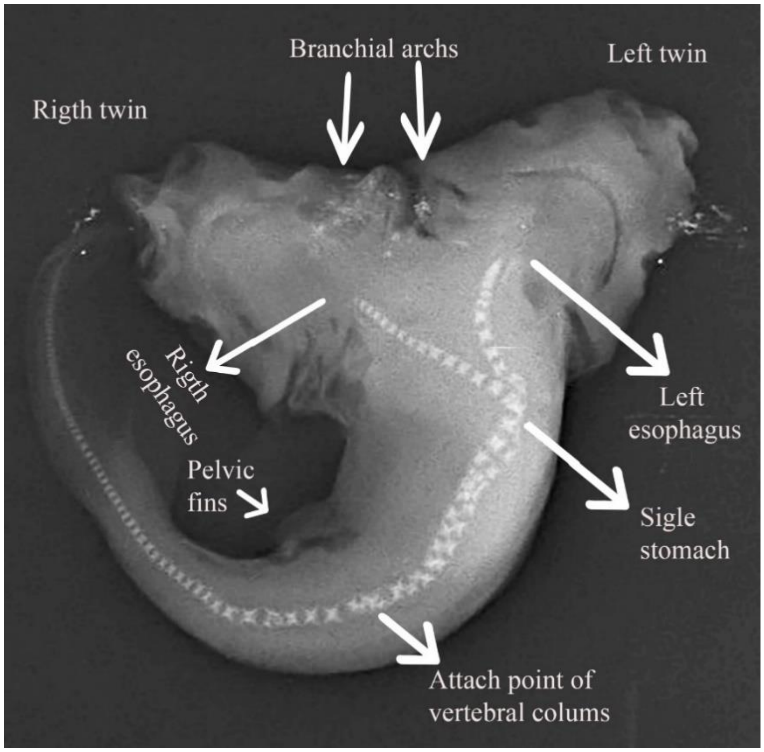

Figure 3. X-radiogram of the ventral side of the bicephalic male embryo of *Carcharhinus limbatus* collected in the southeastern Gulf of Mexico. X-Radiogram by E. Cadena.

Table 2. Measurements and meristics of the bicephalic male embryo of *Carcharhinus limbatus* collected in coastal waters of Tabasco, Mexico.

Measurements and meristics	cm	Measurements and meristics	cm
Total length measured from the right head	14.7	Pre-dorsal length of the right head	5.1
Left head length	3.4	Base of the left dorsal fin	1.0
Right head length	4.0	Base of the right dorsal fin	1.0
Left pre-dorsal length	6.9	Height of the left dorsal	1.4
Right pre-dorsal length	7.1	Height of the right dorsal	1.2
Eye diameter of the left head	0.4	Anterior margin of the left dorsal fin	2.0
Eye diameter of the right head	0.4	Anterior margin of the right dorsal fin	2.0
Pre-mouth length of the left head	2.7	Length of the anterior point of the dorsal fins to the upper lobule of caudal fin	9.6
Pre-mouth length of the right head	2.6	Length of the anterior point of the dorsal fins to the fork	6.8
Width of mouth of the left head	1.8	Length of the anterior point of the dorsal fins to the caudal pit	5.7
Width of mouth of the right head	1.7	Inter-dorsal length	3.1
Inter-narinal space of the left head	1.1	Number of open gills on the free side of the left head	5.0
Inter-narinal space of the right head	1.0	Number of open gills on the free side of the right head	5.0
Pre-dorsal length of the left head	5.1	Number of open gills on the joint side	1.0

et al. 2019). However, at the time of these records, only three species with bicephalia cases are subject to overfishing (Tavares 2005, Fordhand et al. 2016, Pollom et al. 2020), which could rule out overfishing as a possible cause of this abnormality.

The species with the highest number of bicephaly reports is *Prionace glauca*, with 33% of total cases. This oceanic species is the most abundant and has the widest distribution and catch volumes of commercial shark species. However, its global status of "near threatened" implies that it is not currently overfished (Camhi et al. 2009, Carvalho & Winker 2015, Rigby et al. 2019). This shark has the highest fecundity of the elasmobranchs (Cortés 2000), which may support the hypothesis that limited uterine carrying capacity could be the most feasible explanation for this phenomenon. On *C. limbatus* bicephalous embryo, this proceeds the biggest reported litter size (10 embryos) for this species (6 to 8 embryos, Capapé et al. 2004). The lack of environmental data prevents the possible correlation of this phenomenon to other causes, i.e. global climatic change, high or low temperatures, water acidifications, and anoxic conditions observed in reptiles (Cooper 2009, de Carvalho et al. 2017).

In bony fishes, the twin's anomalies were reported in the larval of poecilids, and the cause is the chloramphenicol treatment (Petrescu-Mag et al. 2011). Still, these anomalies are not scientifically reported in wildlife bony fishes, which may be because the larvae or juveniles are rarely exploited commercially, reducing or impossibility their records.

Of all records of two-headed elasmobranchs, 28.6% (6 of 21) are in Mexico. Five are located in the fishing area with the greatest effort (CONAPESCA 2021); four are cases in *P. glauca*. Of the above, the cause holding greater explanatory weight is intrauterine competence derived from high atypical fertility. Other abnormalities have been recorded in the blacktip shark. In the Gulf of Mexico, an embryo presented eyes in a forward position and a vertebral column with scoliotic and lordosis (Driggers III et al. 2012).

The origin of bicephaly in *C. limbatus* is unknown. It may be due to multifactorial reasons, such as malnutrition, parasitism, genetic anomalies (Delpiani et al. 2011, Driggers III et al. 2012), high embryo production, and environmental degradation (Mancini et al. 2006). Additional sampling and long-term monitoring are needed to determine these abnormalities possible causes and ecological impacts.

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