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RESEARCH PAPER

Radiographic and tomographic description of marlin sucker *Remora osteochir*, Pisces: Echeneidae—preliminary data of one specimen

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Abstract

Remoras are commensal fish of various marine species, such as sharks, swordfishes, turtles, dolphins, manta rays and whales. One specimen of marlin sucker *Remora osteochir* was evaluated using computed tomography for skeletal digital reconstruction, digital radiology for general evaluation and bone counting, and the double contrast technique to distinguish coelomic organs. In radiographic images, it was possible to observe otoliths in the center of the neurocranium, to count 27 vertebrae, nine pairs of ribs, and to detect the presence of the hipural and epural bones near of the caudal fin. In double contrast images, it was possible to visualize the swim bladder, gills, heart, liver, stomach and intestines. From the tomographic images and reconstructions, it was possible to identify the intercalar bones of the cephalic disc; the spine with vertebral bodies composed of neural arches and ventral ribs; and the pectoral girdle formed by posttemporal, cleithrum and scapulocoracoid bones. It was concluded that digital radiology and computed tomography were able to describe anatomical structures of marlin sucker *R. osteochir* without the need for dissection.

Keywords Teleost fish · Double contrast · Computed tomography · Digital radiology · Skeleton

Introduction

The marlin sucker *Remora osteochir* is a cosmopolitan fish found in all tropical and temperate oceans, with some reports in less common places, such as Turkey (Tuncer et al. 2012) and South Korea (Myoung et al. 2015). *R. osteochir* is commensal comprising almost exclusively of marlins, sailfish and swordfish (Castro Pampillón 1996; Morota and Fujita 1995). Fixed on their hosts, marlin suckers feed on fish remains the marlin ectoparasite *Nesipus* sp. and zooplankton (Vaske Junior 1995). Both females and males begin their reproductive cycle when they reach 13–14 cm of furcal length (Morota and Fujita 1995), and can reach up to 40 cm in total length (Figueiredo and Menezes 1985).

One of the most striking features of the remoras is the first modified dorsal fin into a cephalic disc in a craniodorsal position, extending from the nostrils to the skull (Britz and Johnson 2012). The cephalic disc has several distinct characteristics to achieve the reversible bond: an external fleshy lip, an array of lamellar compartments, and spines that protrude from the pectinated lamellae (Beckert et al. 2015). The attachment of the remoras to the surfaces possibly occurs as follows: when the cephalic disc is applied to the surface of

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the host, the pectinated lamellae are redirected increasing the space between them, leading to decompression inside the organ. The spinules of the lamellae upper face rub against the surface of the host skin, and the friction holds the remora in place (Beckert et al. 2015; Grassé 1967).

Historically, the first study using radiographs in fish was conducted by Guyénot and Plattner (1939) for the evaluation of the swim bladder. Later, Gosline (1948) reported his experience with the use of X-rays in studies of fish anatomy, reflecting that the ease and low cost of X-ray images would save time with dissection. Recently, the use of Computed Tomography scan (CT) is a revolution in fish anatomy research by providing 3D imaging and skeletal reconstruction (Lauridsen et al. 2011). With CT, it was possible to study the complete anatomy of the coelacanth (*Latimeria chalumnae*) (Schultze and Cloutier 1991); pacu (*Colossoma macroponum*) (Carr et al. 2014) and meagre (*Argyrosomus regius*) (Gisbert et al. 2012). In addition, this technique assisted the description of anatomical characteristics in specimens of zoological collections resulting in the classification of new species of fish, such as *Iracema caiana* (Carvalho and Albert 2011) and *Gymnotus inaequilabiatus* (Maxime and Albert 2014). Moreover, X-rays provide us with only 2D images of the skeleton, but organs can be enhanced with using positive and negative contrast media. The advantage of using CT is to acquire 3D images of organs or regions, and even the entire specimen. Using those imaging techniques, a multiprofessional group composed of biologists, veterinarians and zoologists may use those techniques to evaluate the anatomy of animals at risk of extinction, and avoid damages to rare specimens deposited in centennial zoological collections (Chanet and Guintard 2012). Thus, this work contributes to the research and anatomical description of marlin sucker *R. osteochir* using radiologic and tomographic techniques.

Results

The neurocranium is a compact structure formed by small bones, which hampers a reliable image. The overlapping of the structures near the neurocranium in the right lateral recumbency, such as the pectoral fins and the intercalar bones of the cephalic disc, hinders the identification of the bones of those structures. However, in the center of the skull, the otoliths were observed in the right lateral recumbency (Fig. 1a).

Radiographic images allowed the counting of 27 vertebrae (13 pre-caudal and 14 caudal) and nine pairs of ribs (Fig. 1a). In the cranial region, the eight pairs of intercalar bones of the cephalic disc were distinguished, and the dorsal and ventral ribs were seen distally (Fig. 1b).

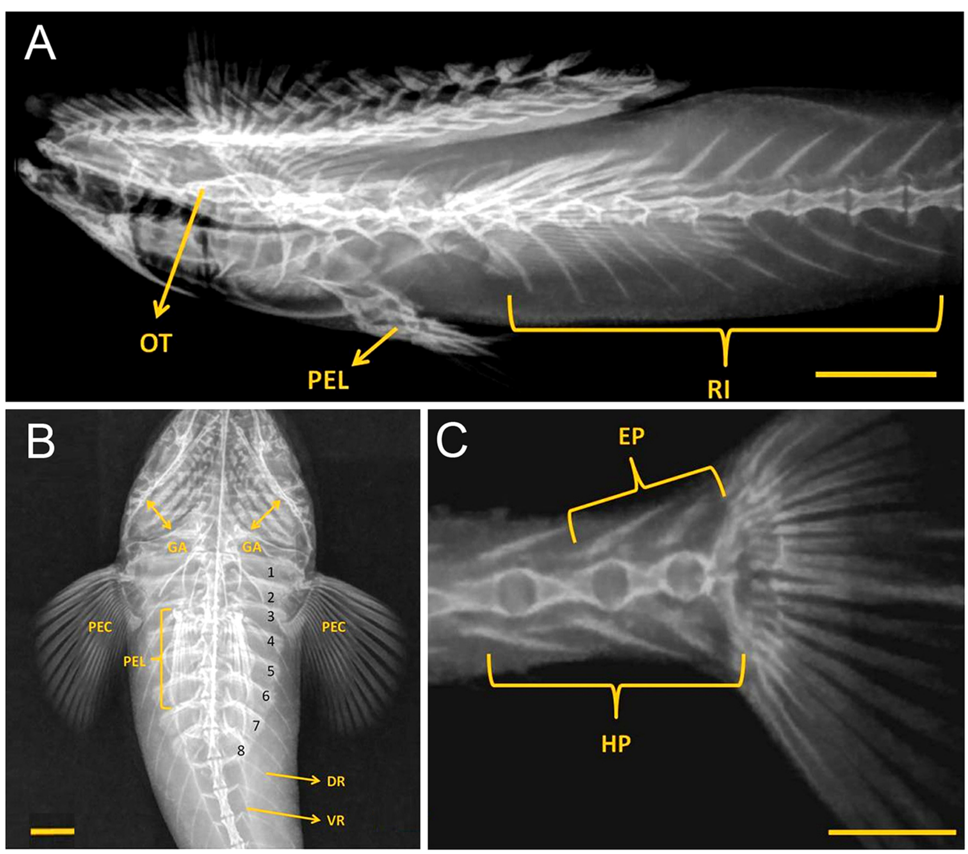

Fig. 1 **a** Right lateral recumbency radiograph of a marlin sucker *R. osteochir*. The otoliths (OT), the pelvic fin (PEL) and the nine pairs of ribs (RI) are observed in the center of the neurocranium. **b** Ventrodorsal projection of marlin sucker *R. osteochir*. Protected by opercular bones, it is possible to distinguish the two sets of gill arches (GA) with four gills in each. Superimposed on the intercalar bones (1 to 8) are the pelvic fins (PEL) and, laterally, the rays that make up the pectoral fins (PEC) are visible. Distal to intercalar bones, the dorsal (DR) and ventral (VR) ribs are visualized. **c** End of the caudal skeleton of marlin sucker *R. osteochir*, where it is possible to observe the caudal vertebrae and the epural (EP) and hipural (HP) bones. Radiographic technique for figure **a**: 55 kVp and 10 mAs. Radiographic technique for figures **b** and **c**: 60 kVp and 6 mAs. Scale bar: 1 cm

At the caudal skeleton, bones of the spine were observed composed of two epural and three hipural bones (Fig. 1c).

In double-contrast radiographs, it was possible to observe pericardial and abdominal coelomic cavities. In the cranial region, gill arches and the pericardial cavity were highlighted, where the heart ventriculum (Fig. 2a) was laid between the transverse septum and the axial ventral musculature. The abdominal coelomic cavity (Fig. 2b) has an inverse scalene triangle format, with the basis between the cranioventral region of the swim bladder to the cloaca (6.38 cm length), from the cloaca until pelvic fins insertion (6.31 cm length) and from pelvic fins to ventral region of the swim bladder (1.83 cm). Swim bladder measurements were 5 cm length and 3.3 cm height. Liver, stomach and intestines were detected by the double contrast technique. In the coelomic cranial region, the liver was detected by iohexol circumvention as well as the stomach and intestines filled by air.

From the tomograms, we counted nine pairs of radiodense pectinated lamellae (Fig. 3a). Using reconstructions in a range of different views, it was possible to identify the bones composing the cephalic disk region. At the caudal end of the neurocranium, parietal bone and eight pairs of intercalar bones were observed (Fig. 3b, c). In the same region, the pectoral girdle was constituted by the cleithrum, scapulocoracoid and posttemporal bones (Fig. 3c, d).

Ventral to the disc, vertebral bodies with neural (dorsal) spines and ventral ribs (hemal arches) were seen. These superimposed structures have generated an image with the “less than” sign shape. Due to the curvature of the remora body after its fixation, it was not possible to determine the total number of vertebral bodies and ribs using the tomograms.

Discussion

The biometric data of the remora used in this study are within the standards recorded by Tuncer et al. (2012), such as the number of rays in the dorsal fins (20–26) and in the anal fins (20–25). Using regression formulae for weight and length published for *R. osteochir* of the Mediterranean Sea, it was estimated the weight of the specimen between 42.75 g (Garibaldi and Orsi Relini 2003 as reported by Tuncer et al. 2012) and 66.23 g (Battaglia et al. 2016).

According to Britz and Johnson (2012), the number of pairs of pectinated lamellae and intercalar bones is similar during the growth of different species of remora. In this work, the similarity was also maintained, with the counting of nine pairs of pectinated lamellae and eight pairs of intercalar bones. Indeed the number of pairs of intercalar bones and pectinated lamellae were similar both in the external anatomical evaluation and in radiographic and tomographic examinations (Britz and Johnson 2012). However, it is possible that some cartilaginous structures were not detected either reflecting the size of the specimen or by the characteristics inherent to X-rays. The outcome could lead to a false result of anomalous development in marlin suckers *R. osteochir* when compared to other remora species. By the standard (total) length of the specimen, it was regarded as still in the growth phase, and cartilage calcification was not finished yet at the time of collection.

Conversely, taking into account the relationship between the total length and age of marlin suckers *R. osteochir* (Battaglia et al. 2016), the specimen evaluated in this research should be aged around 3–4 years when collected. Estimating the age could be achieved through dissection and ex-situ otolith ring analysis (Campana 2001). Fish otoliths are located in the inner ear at the base of each neurocranium and are organized in three pairs: *sagitta*, *lapillus* and *asteriscus*. In most species, the *sagittae* are the pair with the larger size,

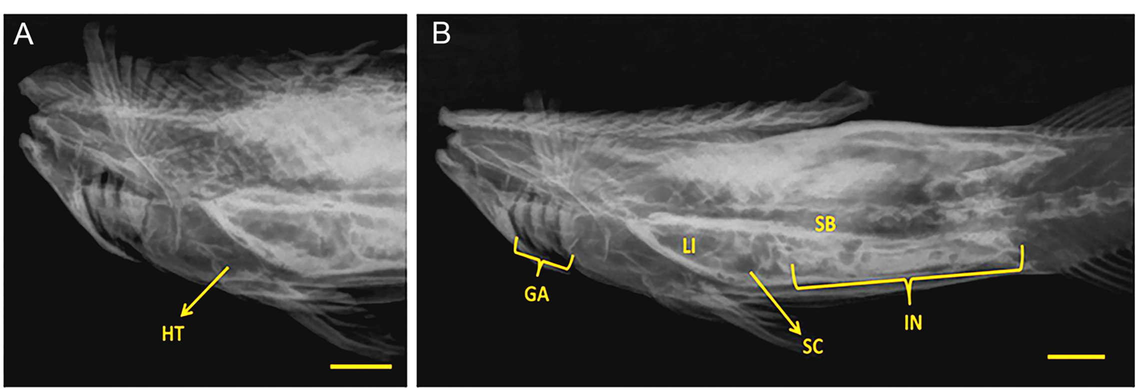

Fig. 2 Double contrast radiographs. **a** the heart ventriculum (HT) was seen between the axial ventral musculature and the transverse septum. **b** iohexol and air injections highlighted the gill arches (GA), liver (LI), stomach (SC), swim bladder (SB) and the intestines (IN). Scale bar: 1 cm

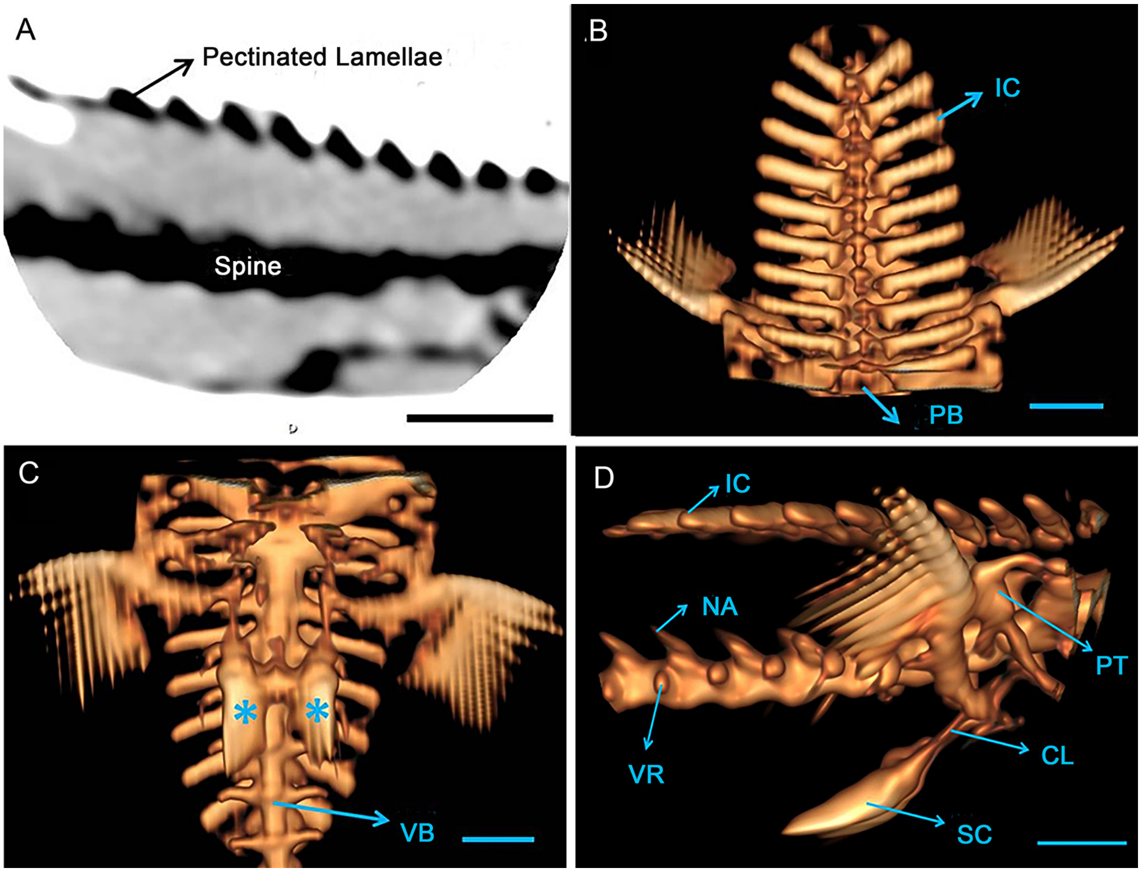

Fig. 3 **a** An inverted color tomogram of the cephalic disc region of marlin sucker *R. osteochir* facilitates the distinction between the pectinated lamellae and the spine. **b, c** and **d** Tomographic reconstructions of the cephalic disc. **b** In the dorsal view of the cephalic disc where it is possible to observe the parietal bone (PB) of the neurocranium and the intercalar bones (IC) of the cephalic disk. **c** Ventral view showing the vertebral bodies (VB) and the overlapping of the cleithrum and posttemporal bones (asterisks). **d** In the lateral view, the intercalar bones (IC) of the base of the cephalic disk are observed. In vertebral bodies, there are projections of the neural arches (NA) and the ventral ribs (VR). In the cranial region, pectoral girdle compounds, such as posttemporal (PT), cleithrum (CL) and scapulocoracoid (SC) bones, may be discerned. Scale bar: 1 cm

allowing taxonomic identification of fish, and age estimation (Morales-Nin 2000; Popper and Lu 2000).

On the total number of vertebrae and costal arches, Myoung et al. (2015) reported that *R. osteochir* and *R. brachyptera* present 27 vertebrae, which is the same for *R. remora* (Ford 1937). Such similarity may be characteristics inherited from the common ancestor †*Opisthomyzon* (Friedman et al. 2014). In the last vertebra, there is the insertion of the caudal fin, which is dorsally supported by the slope of the vertebral column and by epural bones. Ventrally, the caudal fin is supported by hemal spines, known as hipural bones (Romer and Parsons 1985). According to Ford (1937) and O'Toole (2002), fish belonging to the genus *Remora* have two epural and three hipural bones holding the truncated caudal fin.

The peculiar feature of bonefish is the presence of two pairs of ribs (dorsal and ventral ribs) in each side where connective tissue septa (myocommata) are anchored, and suffer most of the muscular strength (Romer and Parsons 1985). The ribs, together with the bones of the intermuscular system, delimit the shape of the myomers of the axial musculature. Due to the fact that the dorsal ribs were thinner than the ventral ribs, the overlapping of the compact clusters led only the identification of the ventral ribs in the right lateral recumbency radiograph. In this way, ribs are discriminated from intermuscular bones mainly because of their thickness and the emergence of the ribs from vertebral bodies in sagittal axis tomograms, and their number in ventrodorsal radiographs (Patterson and Johnson 1995).

In relation to the pectoral girdle, O'Toole (2002) described the phylogeny of fish bones of the Echenoidea superfamily (families Echeneidae, Rachycentridae and Coryphaenidae), characterizing the bones that make up the pectoral fins of *R. osteochir* by the absence of postcleithra bones; the supracleithrum is in a reduced size and forming a side plate between the posttemporal and the cleithrum;

fused articulation between the radial and scapula bones and cleithrum in contact with the coracoid bone throughout its length. Both the radiographs and the CT scan were not sufficient for a complete evaluation.

We recommend the double contrast technique in cases of long-time specimen storage in alcohol and posterior anatomical radiographic and/or tomographic description. Positive contrast medium is valuable to distinguish coelomic organs of fish, as used in companion animals and humans for diagnostic imaging. Indeed, using magnetic resonance images of a *R. remora* available at the Digital Fish Library (www.digitalfishlibrary.org) (Berquist et al. 2012), it was possible to check the topography of the remora *R. osteochir* organs and to compare them in double contrast radiographies. Finally, sex determination was not possible in double-contrast radiographic images. Radiographic, tomographic and magnetic resonance imaging procedures of rare fish from zoological collections are recommended to enlighten and solve doubts in the systematics of those species.

Materials and methods

Acquisition of *Remora osteochir*

The specimen was found attached to a blue marlin *Makaira nigricans* caught by the commercial longline fishing fleet of Itajaí, Brazil, in the South Atlantic Ocean at 35º58′S and 33º59′W isobaths, in April 2015. The remora was euthanized by the excess of benzocaine (1 g/L) (Ribas et al. 2017), total and furcal length measurements were taken using a tape, before fixation in 10% formalin solution and storage in 70% alcohol until anatomical evaluation.

Taxonomic confirmation of the specimen as *R. osteochir* occurred after counting of 20 rays in the dorsal and anal fins, in addition to 17 pairs of lamellae in the cephalic disc. The marlin sucker presented 19 cm furcal length and 22 cm total length, weighing around 54 g and of undetermined sex.

Radiographic and tomographic evaluation

The acquisition of the radiographic images occurred in October 2018 at the Diagnostic Imaging Service of the Veterinary Hospital of the School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil. The AGFA DX-D 400 digital radiography (AGFA Healthcare, AGFA-Gevaert, Belgium) was used with right lateral—55 kVp and 10 mAs and at ventrodorsal recumbencies—60 kVp and 6 mAs.

Visualization of the organs was performed using a double contrast technique. A 4Fr veterinary urethral catheter was inserted by the cloaca, and the air was injected to play as a negative contrast agent. Four ml of positive contrast agent iohexol was injected into the abdominal coelomic cavity at a concentration of 300 mg iodine/ml (Omnipaque, GE Healthcare, China). Finally, ~ 1 ml of iohexol was dropped out by the opercula to highlight the gills. Right lateral recumbency radiographs were acquired using 50 kVp and 5 mAs technique.

The tomographic evaluation of the marlin sucker was carried out in a private diagnostic clinic in the municipality of São Sebastião, Brazil in July 2017. For the tomographic evaluation, a HiSpeed CT/e Dual helical tomograph (GE Medical Systems, GE Healthcare Life Sciences, USA) was utilized, which operated at a power level of 120 kVp and intensity of 80 mA, with a voxel resolution of 1 mm, integration of 1000 ms.

The radiographs and tomograms were evaluated in dicom format in PACS Aurora (Pixeon, Brazil) and MicroDicom Viewer (Micro DICOM, Bulgaria) software, respectively. RadiAnt DICOM Viewer (Medixant, Poland) was used for tomographic reconstructions.

Anatomical description

For the identification of bone components, the descriptions of bone fish of Romer and Parsons (1985), Patterson and Johnson (1995), O'Toole (2002) and Britz and Johnson (2012) were taken into account. For the topographic description, double-contrast radiographs were compared to magnetic resonance images of a *R. remora* available at the Digital Fish Library (www.digitalfishlibrary.org) (Berquist et al. 2012).

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Author contributions André Luiz Veiga Conrado, Renata Stecca Iunes and Aline Tiemi Shiraishi Rocha evaluated and described the marlin sucker anatomy using radiographies and tomographic images, and wrote the manuscript. Carlos Eduardo Malavasi Bruno collected the marlin sucker and identified the specimen as *Remora osteochir*. José Roberto Machado Cunha da Silva advised all the manuscript confection. The final manuscript was approved by all the authors.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Animal and human rights statement This work was approved by the Ethics Commission of the School of Veterinary Medicine and Animal Science of the University of São Paulo, Brazil # 9396271113, and the collection was authorized by the Brazilian Institute of Environment and Renewable Natural Resources # 47691-1.

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