

X-Ray Radiological Study for Skeleton of Rounded Fantail Stingray *Taeniura grabata* from Susah, Libya

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دراسة صورة أشعة لهيكل غضروفي لسمكة بقرة مدورة *Taeniura grabata* من ساحل سوسة، ليبيا

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Abstract

A sample of Round Fantail Stingray *Taeniura grabata* was brought, from Susah harbor in east Libya, to establish radiographically, it was situated Dorsal-ventrally, to diagnose skeleton and tooth plate, using Siemens X-ray System (Multix Fusion). In the Multi-graded radiograph, the specimen skeleton was so pale white in most of the axial skeleton and parts of the cranium, and poorly calcified. 88 pectoral radials: 41 propterygials, 15 mesopterygial, and 32 metapterygial radials, with 22 pelvic radials counted. Fin radials were attached to the scapulocoracoid by three enlarged basal radials. The superficial muscles were darker in coloration. About 80 Pre-sting vertebrae, 50 Post-sting with 62 pre-caudal vertebrae, were collected into 192 vertebrae in the tail. There were 32 upper teeth rows, and 36 lower teeth rows, they were small, blunt, and arranged into flattened surfaces. The neurocranium is slightly elongated, longer than 1.5 times in width. Nasal capsules process about 30% of neurocranial desk length. Meckel's cartilages were broadly triangular. The bronchial skeleton comprises five arches. Also, a single small bridge project ventrally from the medial plate. Jaws are very robust and small.

Keywords: *Taeniura grabata*, Radiograph, Skeleton, Susah coast, Libya.

الملخص

تم تثبيت عينة من سمكة البقرة المدورة *Taeniura grabata* من ميناء سوسة ليبيا، في وضع ظهري-بطني، بُغية تشخيص الهيكل العظمي وصفائح الأسنان، باستخدام منظومة سمينز للأشعة السينية (Multix Fusion). وبدراسة التدرج اللوني للصورة، كان الهيكل العظمي أبيضاً باهتاً للغاية ناحية الهيكل العظمي المحوري وأجزاء من الجمجمة، مشيراً إلى تكلس ضعيف، بينما كانت الألوان الداكنة من العضلات السطحية، كما حصرت 88 شعاعياً صدرياً: 41 شعاعياً شعاعياً و15 متوسطاً و32 شعاعياً متحولاً مع 22 شعاعاً حوضياً، كما لوحظ ربط شعاعي الزعانف بالكتف بواسطة ثلاثة إشعاعات قاعدية مكبرة، تم حصر 80 فقرة قبل الابرة و50 بعدها مع 62 فقرة قبل الذيلية، في 192 فقرة بالذيل. كان هناك 32 صفّاً علوياً و36 صفّاً سفلياً من الأسنان الصغيرة والغير حادة، والمرتبة في أسطح مفلطحة، بينما كان القحف العصبي مستطيل قليلاً، أطول من عرضه بمرة ونصف، كانت الجيوب الخيشومية حوالي 30% من القحف العصبي طويلاً، كما كانت غضاريف ميكيل عريضة وواسعة ومثلثة، وقد تألف الهيكل الخيشومي من خمسة أقواس، مع بروز جسر صغير واحد بطني من اللوحة الوسطى، الفكوك قوية جداً وصغيرة وينطبق عليها شكل كسارة اللوز، مثل هذا العمل قد يفيد في مزيد من الدراسات الفسيولوجية لأسمك الساحل الليبي في المستقبل.

الكلمات الدالة: البقرة المدورة، *Taeniura grabata*، صورة اشعاعية، ساحل سوسة، ليبيا.

1. Introduction

Batoids are shark relatives with flattened bodies dorso-ventrally and expanded and attached pectoral fins to their heads and trunks, in a disk-shaped body (Compagno, 1999; Aschliman, 2011 and Franklin *et al.*, 2014). In the Mediterranean, there are 36 species approximately (Bradai, *et al.*, 2012; Buzaid & El-Mor, 2015; Buzaid, 2019; and Buzaid *et al.*, 2020). The Round Fantail Stingray *Taeniura grabata* colonizes the southern Mediterranean Sea (Serena 2005). Including the Libyan coast (Ben-Abdalla *et al.*, 2012). This species was recorded in the Turkish Seas (Başusta *et al.*, 1998), and the Syrian coast (Ali *et al.*, 2013) in the Levant Basin according to Golani *et al.*, (2006). It can grow to be 1 m across and 1.5 m long (Bauchot, 1987; Serena 2005; Golani *et al.*, 2006; and Ben-Abdalla *et al.*, 2012), and weigh up to 150 kg (Francis, 1968; Capapé & Desoutter, 1990). A predator of bottom-dwelling benthic fishes, crustaceans and mollusks (Dulvy & Reynolds, 1997; Jensen *et al.*, 2000; Golani *et al.*, 2006; and Ben-Abdalla *et al.*, 2012). Spines grow in front of the primary spine. A sharp serrated spine on its tail can cause painful wounds (Golani *et al.*, 2006). Schwartz (2005) mentioned that there is a deep fin fold running beneath the tail from the level of the spine, almost to the tip. Recently, it is described as a vulnerable species by Serena *et al.* (2009), due to exposure from over-fishing for many industries (Serena, 2005; and Bradai *et al.*, 2012).

Anatomical studies of these species are essential to appreciating their biology, Enault *et al.* (2016) indicated that the skeleton of cartilaginous fishes is notoriously difficult to study, and the literature caping this subject is very rare compared to other vertebrates. The importance of skeletal specimens in research collections, using a few ways to study, which including radiographs. Especially the skeletal system and vertebral column; that plays an important mechanical role in movement of these fishes (Learn, 1976; Lindsey, 1978; and Weihs, 1989). Nowroozi and Brainerd, (2012) indicated that the vertebral column plays a dichotomous role during locomotion across vertebrate taxa, providing both the stiffness and flexibility required for locomotion (Symmons, 1979; Smeathers, 1981; Hurov, 1987; Gal, 1993; Schmitz, 1995; Long *et al.*, 2011; Porter *et al.*, 2009). To some extent, different mechanical properties in different regions of the vertebral column can meet the conflicting demands of stiffness and flexibility (White & Panjabi, 1978; and Panjabi *et al.*, 2001). Ultimately, the structure of the individual vertebra and its interaction with the adjacent vertebra via the inter-vertebral joint (IVJ) determine the mechanics of not only individual segments but also across entire regions, and along the full length of the column as well (Smeathers, 1981; Hurov, 1987; Gal, 1993; Bond, 1996; Ward & Brainerd, 2007; Porter *et al.*, 2009). The Vertebral column varies in regionalization degrees across vertebrates (Qasim, 1995; and Al-Shubka, 2009). These degrees can be revealed by biometrical studies (Kubo & Asano, 1987; 1990; Desse *et al.*, 1989). In batoid fishes, wing vessels are located between the ceratotrichia, which are cartilaginous ray-like projections that make up the wings (Culpepper and Myniczenko, 2017). Stepanek and Kriwet (2012) mentioned that the shape of the neuro-cranium (the skull) is related to functional aspects of the jaws and locomotion. It encloses the brain and the olfactory, auditory, and visual organs. The tesserae in the skull are functionally important in stiffening the parts of the cranium;

such as jaws as adaptation to durophagy, when arranged in several layers; because of the high level of kineticism in fish skulls, and more than 20 movable skeletal elements in the pharyngeal apparatus in these skulls (Dean and Summers, 2006), the primary cranial skeletal elements couplings involved in feeding behavior are presented here. Westneat (2006) mentioned that batoids are characterized by a spectacular diversity of skull form and feeding mechanisms, from sit and wait predators that use high suction forces to engulf their prey species, even fishes; that get remove pieces of them to feed in a biting strategy. This pattern is the widespread use of suction during prey capture as a strategy to transport food into the mouth. Suction feeding is the most used mode to prey capture of bony fishes (Liem, 1980; Muller & Osse, 1984; Lauder, 1985; Alfaro *et al.*, 2001; Ferry-Graham *et al.*, 2003 and Westneat, 2006). Most batoids employ jaw protrusion and suction feeding; thus a current area of active research is focused on the mechanics and evolution of these behaviors, and how the suction profiles compare to those of other fishes.

This study aims to use X-ray graphs for the vertebral column, jaws, and teeth of the round fantail stingray; because there is no such information available in the literature for this species on the Libyan eastern coast, as well as, this study could be as a prelude to future anatomical studies of elasmobranches.

2. Materials and Methods

2.1. Study Area [Susah Harbor (21°58' 00"E, 31°54' 18"N)]

Offshore harbor as shore base and permanent fish-landing site; with old jetty as a barrier, and moorage for small artisanal fishing units (Reynolds *et al.*, 1995; MBRC, 2005; Abu-Grarah, 2008; Abu-Madinah, 2008) (Figure 1.A).

2.2. Identification of the Species

In the Marine Sciences lab, Omar Al-Mukhtar University, Albayda. A specimen of *Taeniura grabata* (E. Geoffroy Saint-Hilaire, 1817) (Figure 1.B) had been identified according to (Serena, 2005; Golani *et al.*, 2006; Iglésias, 2006; Abdallah, 2007; and Ben-Abdalla *et al.*, 2012).

2.3. Skeletal radiology

2.3.1. Radiograph Machine: A radiograph of frozen specimen was made on Siemens X-ray System (Multix Fusion) (Figure 1.C).

2.3.2. Positioning: It was in horizontal beam (Ventre-dorsal view), with sagittal section of whole (Summers, 2000; and Alhamroni, 2018 - *personal meeting*).

2.3.3. Radiographic density: of the studied specimen in the radiograph-desk had an image, with different densities (black, dark grey, light grey, white, and so on) in the scale of contrast (Thrall *et al.*, 2013).

2.3.4. Vertebrae counting: Vertebrae and fin rays were counted according to De Carvalho & Ragno (2011), even tooth rows on preserved specimens and the exposed on the radiograph; following Stehmann *et al.* (1978).

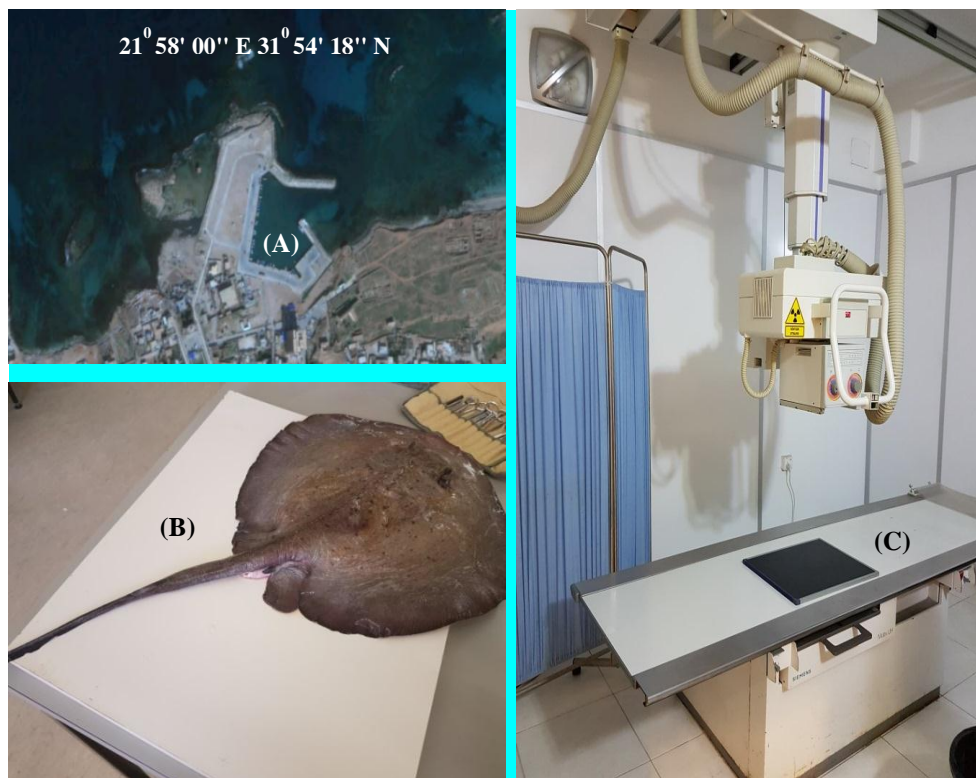


Figure 1. [A] Susah Harbor; [B] A specimen of *Taeniura grabata* (E. Geoffroy Saint-Hilaire, 1817); and [C] Siemens X-ray System (Multix Fusion) in Tyba center for medical imaging, Albayda, Libya.

3. Results and Discussion

3.1. Biometrically

A male Rounded stingray *Taeniura grabata* from Susah harbor, Libya, it was measured the total length, disk length, width and depth and Tail length as well as 770, 370, 40, 38, and 400 mm, respectively.

3.2. Radiographically

In Figure (2), talking about the calcified cartilages in the skeleton of study specimen was so pale white in most the axial skeleton and parts of the cranium, and poorly calcified, obscured in radiographs. According to denticles on overlying integument, there is multiplication in the mineralized tessellate layers in the jaws of this batiod, and it was analogous to cortical thickening (Summers, 2000). These tesserae are perichondrial in origin. In general, there is mineralized and un-mineralized tissue, and the response of the element to load is determined by both materials (Wroe *et al.*, 2008; and Liu *et al.*, 2010, 2014). These higher degrees of

stiffness and differing Poisson's ratios were seen even though uniform cubes of bone were used for compression testing. The difference between axes of compression is due to the sub-structural properties of the bone, such as the orientation of the mineralized collagen fibril bundles (Weiner & Wagner, 1998; and Shahar *et al.*, 2007). However, this issue has effect to investigate the elemental composition of vertebral bands, and by association, the factors that may influence their deposition, elemental distribution within vertebrae was assessed in five diverse species of sharks using Scanning X-ray Fluorescence (Raoult *et al.*, 2016).

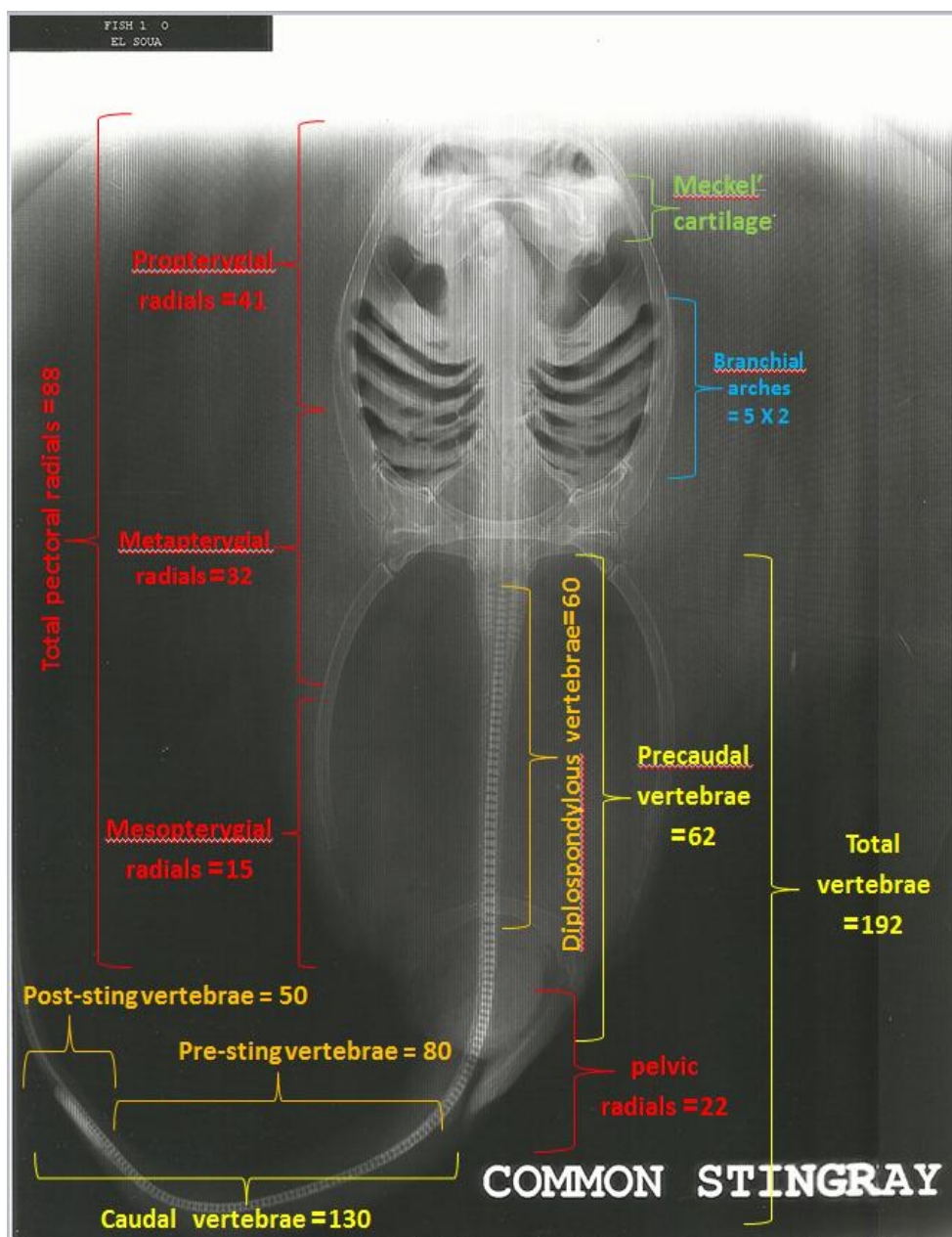


Figure 2. Radiograph (Dorsal-ventral position) of male Rounded Stingray *Taeniura grabata*, from Susah Harbour, Libya.

3.3. Pectoral and caudal radials count

In this work: Pectoral radials (88 in total): Propterygial radials, Mesopterygial radials and Metapterygial radials in count to 41, 15, and 32 units, respectively. As well as Pelvic radials (22) were counted in *Taeniura grabata* in this work, in general cartilaginous radials highly branched distally and extend to margins of pectoral fin (displacing ceratotrichia) (Compagno, 1999). As shown in Table (1), Pectoral-fin radials were counted from the radiograph of *T. grabata*, with number of, with a count of 88 pectoral radials, this is much fewer than what De Carvalho and Ragno (2011) recorded (78) in freshwater species. Pelvic radials were 22 in *T. grabata* in this work. This result is less significantly than was counted (108-113) by Cowley and Compagno (1993) in *Dasyatis chrysonota*. The pelvic radials in male are more than in females. The pectoral girdle is unique in batoids, with the scapulacoracoid either fusing together dorsally by means of the suprascapula or articulating directly to the synarcual (fused vertebra) by means of a ball-and-socket joint (Compagno, 1999). In *T. grabata*, the fin radials are attached to the scapulacoracoid via three enlarged basal radials (Figure 2), the same subject has been found in *Taeniura lymma* (Rosenberger and Westneat, 1999).

Table 1. Radiographic characters in skeleton of male Rounded Stingray *Taeniura grabata*, from Susah Harbor.

Character	Count	Character	Count	Character	Count
Precaudal vertebrae	62	Diplospondylous vertebrae	60	Mesopterygial radials	15
Caudal vertebrae	130	Upper tooth rows	32	Metapterygial radials	32
Total vertebrae	192	Lower tooth rows	36	Total pectoral radials	88
Pre-sting vertebrae	80	Propterygial radials	41	Pelvic radials	22
Post-sting vertebrae	50				

The muscles of *T. grabata* are darker in coloration to approximately half-way to the fin margin, where the superficial muscles end (Figure 2). This coloration has not been documented in any other stingray species (Rosenberger and Westneat, 1999). Lying proximally on top of the dorsal superficial muscle are thick bands of tendons that run along the anteroposterior axis of the pectoral fin. The mesopterygium, (Mesopterygial radials) in most stingrays is a single element that articulates medially with the scapulacoracoid and laterally with pectoral-fin radials. The mesopterygium, in *Gymnura* and *Myliobatis*; consists of several distinct components that all articulate with the scapulacoracoid (Nishida, 1990). In this work it was counted to 15 in *T. grabata* (Table 1). A hypothesis for this character is based on the progressive fragmentation and appearance of the mesopterygium. In this work, caudal fins are absent in this species, matching for Heemstra & Smith (1980). The absence of cartilaginous fin-fold radials in some other stingrays is considered the derived state, out-group analysis of caudal radials reveal their presence or absence a plesiomorphic condition and thus be of little significance in defining groups (Lovejoy, 1996).

3.4. The vertebral count

In *T. grabata*; compare to De Carvalho and Ragno (2011). It was relatively high caudal vertebrae count, with a modal count of 130; In *Dasyatis chrysonota*, it was stated similar values, according to (Cowley and Compagno, 1993). As more details, about 80 Pre-sting vertebrae and 50 Post-sting vertebrae, with 62 for the pre-caudal vertebrae, they were counted in *T. grabata* of this study (Table 1 & Figure 2). Le Port *et al.* (2013) recorded higher insignificantly number (71 - 88) Pre-sting diplospondylous vertebrae and less than 50 Post-sting vertebrae in *Dasyatis brevicaudata*. This to prove the significant higher variation in Dasyatids.

In the study specimen, there was 60 diplospondylous vertebrae (in front or behind the pelvic girdle), they were less than De Carvalho and Ragno (2011) recorded in the species of freshwater stingray. This greater extension does not have direct influence on the number of total vertebrae (Tables 2), but is associated to the presence of a longer cartilaginous rod which supports the distal part of tail posterior to vertebral centra (individual centra do not occur posterior to caudal stings). Generally, Officer *et al.* (1996) stated that location in the vertebral column in species can have a statistically significant effect on increment counts.

3.5. Teeth rows count

In the specimen of *T. grabata* by presenting the following characters (Table 2): adult specimen with numerous tooth rows, ranging from about 32 upper tooth rows and 36 lower tooth rows (Figure 2). These teeth were small, blunt, and arranged into flattened surfaces, not rounded or oval in section as Underwood *et al.* (2016) described the typical shape of teeth for the non-squaliformes. Round ribbontail ray *Taeniura meyeni* has much fewer of tooth rows till 46 in the upper jaw and to 45 in the lower jaw. De Carvalho and Ragno (2011) indicated to higher counts (40 – 64) in freshwater species.

3.6. Neurocranium

Which locates dorsally in most stingrays, is rectangular broadening anteriorly because of the large nasal capsules in *T. grabata* as a box-like lateral view, with horizontal base (Figure 2). Stepanek and Kriwet (2012) mentioned that fresh-water stingrays lack a rostral cartilage. In our round fantail stingray, a dorsal-ventrally position, neurocranium is slightly elongate, longer than 1.5 times of width, it is wider at postorbital processes and nasal capsules (Figure 2). In *Dasyatis violacea*, as pelagic stingray, nasal capsules and rest of the neurocranium were formed an angle, whereas the non-pelagic stingrays had the nasal capsules and the rest of the neurocranium are in the same plane. Nasal capsules are relatively large, oval, broadly rounded slightly toward midline; its length posterior to postorbital processes about 30% of neurocranial desk length (Figure 2). Meckel's cartilage in this specimen is stout, dorsally projecting lateral process low and broadly triangular, not slender and elongate. Angular cartilage is less straight, slightly thicker closer to Meckel' cartilage (De Carvalho and Ragno, 2011). The examined specimen has the same character; other studies showed nasal capsules ventro-laterally, that shape is difficult to identify (Miyake, 1988; and Lovejoy, 1996).

Looking to the mandibular arch and hyomandibulae; the wing-shaped mandibular process in the examined specimen, which is close to be attached between mandibular arch and angular overlap both jaws, which are also overlapped well in *Dasyatis* Spp. and *Himantura* Spp. (Lovejoy, 1996). Also, jaws are very robust and smaller than those of other stingrays (Figure 2). The mandibular symphysis and the hyomandibular symphysis are entirely fused (Summers, 2000). The wing-shaped gill rays were 10 at the epibranchials.

3.7. The bronchial skeleton

Which is part of the viscerocranium, comprises five arches in *T. grabata* (Figure 2). The ventral bronchial skeleton consists of an enlarged central medial plate, which resulted from the fusion of the basibronchial copula and the basibronchial components (De Carvalho *et al.*, 2004), a short and transversely directed basihyal, a pair of short and anteriorly directed hypobronchials, and five pairs of ceratobronchials. Duncan *et al.* (2015) indicated to the gills are a multifunctional organ involved in gas exchange, acid-base, and ion regulation; where the structure and dimensions of gills of the potamotrygonid are important to assess their function. Gill measurements include gill filament's length and abundance, number of respiratory lamellae on the filaments, surface area of lamellar bilateral, total gill surface area, mass-specific gill area, and the water-blood diffusion barrier, are species-specific as reported from Hughes *et al.* (1986). In addition, some respiratory factors; such as the anatomical diffusion and diffusing coefficient, are obtained from these measurements, and may reflect the gills' performance under specific environmental conditions (Perry, 1990).

In the examined *T. grabata*; a single small bridge projects ventrally from the medial plate (Figure 2). According to Lovejoy (1996), this bridge forms a shelter for the aorta and afferent bronchial vessels. Such projections also are present in *Plesiobatis*, *Hexatrygon*, *Urobatis*, *Urotrygon*, *Urolophus*, and *Gymnura*, but are absent in some potamotrygonids and some other stingrays; such as *Dasyatis* spp. and pelagic *Myliobatis* spp.

3.8. The tessellated cartilage (Jaws and Teeth)

To describe it in a typical batoid jaw, as exemplified by a rounded fantail stingray: Two thick, parallel-fibered ligaments limit the relative mobility of the upper and lower jaws to just a little portion of freedom; as a block of that size would not fit between the jaws for an open mouth radiograph. The upper and lower jaws' left and right sides are not firmly connected, meanwhile teeth are tiny and sharply pointed.

A radiograph of a stingray *Dasyatis sabina* elaborated an independent motion in sides of the jaws during prey processing (Summers, 2000). However, it is not suited to exerting the large forces needed for crushing hard prey. Nishida (1990) mentioned Myliobatid stingrays are particularly interesting clade of pelagic stingrays; to examine the evolution of morphological novelties related with eating hard prey.

Talking about crushing prey; in batoids (propterygia) the skeletal element associated with the pelvic girdle, has tessellated cartilage, and is used to punt or to push off of the sea floor with the appendages (Dean & Summers, 2006, and Macesic & Summers, 2012); with more

stiffness with higher mineralization levels, and greater stiffness in the propterygia cartilages of benthic species that punt. This hyper-mineralization strategy is reminiscent of the thick outer layers, and even trabeculation of the jaw cartilages in hard prey crushing elasmobranches (Summers, 2000; and Summers *et al.*, 2004).

The dentition is set in an elastic ligament that may absorb energy as the tooth plates, relatively to one another during crushing. To amplify the force of the jaw adductors; the fused mandibular and palatiquadrates symphyses, and the reduced mobility of the upper and lower jaw relative to each other, are combined.

Summers (2000) suggested the “nutcracker” to clarify morphology and function of the jaw of stingrays, with muscle acting at a large force advantage. In a lever system; force advantage occurs when the input lever arm is longer than the output lever arm (Withers, 1992). The nutcracker model could be tested directly, by measuring force production in live animals, with simultaneous confirmation that the jaw adductors are firing asynchronously. Its difficulties are associated with eliciting a natural behavior like feeding under experimental conditions as stated by (Liem, 1976; and Motta *et al.*, 1991). There are dietary records on the hard prey specialists, including an eagle ray crushed a clam, that weighed about 1360 g according to Coles (1910) and Summers (2000). This shows the complex architecture of the adductors, including at least six separate slips of muscle, would make the computation of effective cross-sectional area difficult.

4. Conclusion

This study is considered a first step to elucidate the skeleton anatomy of stingrays in general and to assemble reliable anatomical characters for inferring relationships and evolutionary aspects of this highly interesting group, motivating for more chondrichthyan skeletal specimens, especially the rare species, in more extensive biological studies of these species and other cartilaginous fishes in the Libyan coast.

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