

Article

The Cranial and Locomotory Musculature and Associated Skeletal System of the Epaulette Shark *Hemiscyllium ocellatum* (Bonnaterre, 1788): A Morphological Framework for Understanding Fin-Based Substrate Locomotion

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Abstract

Orectolobiform sharks are predominantly benthic, and members of the genus *Hemiscyllium* are further distinguished by a unique walking locomotion driven by coordinated movements of the paired pectoral and pelvic fins. Although this locomotion has been described repeatedly in the epaulette shark, *Hemiscyllium ocellatum* (Bonnaterre, 1788), a morphological framework linking musculoskeletal structures to these movement patterns is still lacking. Here, we combine manual dissection with diffusible iodine-based contrast-enhanced computed tomography (diceCT) to describe the cranial and locomotory musculature and the associated skeletal system of *H. ocellatum*, with particular emphasis on the pectoral, pelvic and dorsal fin musculature and adjacent axial structures. Both the pectoral and pelvic fins possess a well-developed musculature with robust skeletal support, consistent with a weight-bearing role during substrate-based locomotion. Although not in contact with the substrate, the dorsal fins possess a likewise well-developed and clearly differentiated musculature, the *musculi inclinatores dorsales*, suggesting a functional specialisation beyond passive stabilisation. These findings expand current knowledge of locomotor adaptations in orectolobiform sharks and establish a morphological basis for future comparative and functional studies on fin-assisted locomotion in neoselachians. They may further provide insight into morphological adaptations analogous to the fin-to-limb transition in early tetrapods.



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Keywords: Chondrichthyes; diceCT; dissection; Galeomorphii; Hemiscylliidae; micro-CT; Orectolobiformes

Key Contribution: This study provides the first comprehensive description of the cranial and locomotory musculature, as well as the associated skeletal system, of the epaulette shark, *Hemiscyllium ocellatum*, based on manual dissection and diffusible iodine-based contrast-enhanced microcomputed tomography. We found well-differentiated pectoral and pelvic fin musculature, as well as a robust skeletal system that together support the typical substrate-bound walking locomotion. We further identified a two-layered composition of the *musculi inclinatores dorsales*, which suggests a potential functional specialisation beyond mere passive stabilisation. Using two morphological investigation methods in an integrative approach is instrumental in providing a holistic picture of

neoselachian morphology, as combining the respective strengths and weaknesses of the two methods compensates for the limitations of each method alone. Our study establishes a morphological foundation for future functional and comparative research on neoselachian locomotion modes and enhances our understanding of the impact of substrate-based locomotion on appendicular morphology.

1. Introduction

Carpet sharks (order Orectolobiformes) represent a relatively small but ecologically diverse order comprising 45 extant species arranged into 13 genera in seven families [1,2]. Most are benthic, inhabiting shallow tropical and warm-temperate waters, with some exceptions like the pelagic whale shark, *Rhincodon typus* Smith, 1828 [3–6]. Their benthic lifestyle is reflected in specialised feeding strategies, primarily suction feeding [7–9]. Several orectolobiform species show distinct adaptations to a benthic lifestyle, with the epaulette shark studied herein being the most prominent [10,11].

The members of one genus, *Hemiscyllium* Müller and Henle, 1838 [12], are known for not only swimming continuously as all other orectolobiforms but also for their specific substrate-based walking locomotion. Within this genus, nine extant species of comparatively small, benthic sharks are currently recognised [1,13]. These species are distributed across the Indo-Australian Archipelago and northern Australia, including New Guinea and several satellite islands [13,14].

All known species are characterised by their very distinctive substrate-based locomotion, in which coordinated movements of the paired fins are used to propel the body across the substrate, a locomotor behaviour commonly referred to as “walking” [4,13,14]. Phylogeographic analyses indicate a recent radiation during the Mio-Pliocene, mediated by tectonic changes and sea level fluctuations across the Indo-Australian region [14].

The epaulette shark, *Hemiscyllium ocellatum* (Bonnaterre, 1788) [15], is small, reaching up to 107 cm in total length, and native to shallow coastal reefs, lagoons, and tidal pools of the tropical Western Pacific [4,6,13,16]. It is characterised by an elongated, flexible body, large muscular paired fins, and a subterminal mouth with barbels [11,13]. This species has attracted repeated scientific attention due to its unique walking locomotion using coordinated pectoral and pelvic fin movements [10,17], and its remarkable hypoxia tolerance linked to activity on exposed reef flats during low tides [18–20]. Kinematic analyses showed that during submerged walking, *H. ocellatum* alternates its pectoral and pelvic fins in a diagonal-couplets lateral sequence pattern that shares fundamental similarities with the locomotor patterns observed in salamanders and other basal tetrapods [10,11]. This functional convergence positions *Hemiscyllium* as a valuable extant model for investigating the morphological prerequisites of fin-based substrate locomotion.

The musculoskeletal morphology of walking sharks is of broader evolutionary significance because it provides insight into the functional demands placed on appendicular structures during weight-bearing, substrate-based locomotion in aquatic vertebrates and also in early finned tetrapods. The fin-to-limb transition, a key event in vertebrate evolution, required substantial remodelling of the musculoskeletal system to support body weight, generate propulsive thrust against a solid substrate, and coordinate multi-joint movements [21–25]. While sharks and tetrapods are phylogenetically distant, the convergent evolution of substrate-based locomotion in hemiscylliid sharks offers a natural experiment in which analogous functional demands have shaped the appendicular morphology independently [10,11]. Detailed morphological descriptions of the musculoskeletal system of walking sharks therefore can serve as a comparative framework for understanding how fin

morphology may be functionally adapted to substrate-based locomotion, complementing palaeontological and developmental data on the fin-to-limb transition.

Despite the recurring scientific interest in the peculiar walking locomotion of *H. ocellatum*, the documentation of the musculoskeletal morphology remains incomplete. Previous studies have focused on isolated morphological aspects such as paired fin kinematics [10,11], electrosensory morphology [26,27], and dorsal fin musculature in related hemiscylliids [28], but a comprehensive, morphological description of the cranial, axial, and appendicular musculoskeletal system is missing. Diffusible iodine-based contrast-enhanced computed tomography (diceCT) has emerged as a powerful tool for non-destructive, high-resolution three-dimensional imaging of soft tissues in preserved vertebrate specimens [29]. By staining specimens with iodine-based solutions prior to micro-CT scanning, diceCT enables the simultaneous visualisation of muscles, connective tissues, and skeletal elements in their natural spatial relationships, without the destruction associated with traditional dissection [29,30]. Conversely, manual dissection provides direct access to superficial structures and allows stepwise documentation of muscle layering but inevitably disrupts three-dimensional spatial relationships and is limited in resolving deeply situated structures such as the layered dorsal fin musculature. The combination of both methods thus provides a complementary, integrative approach that capitalises on the respective strengths of each technique and compensates for their individual limitations [29,30].

The present study aims to provide a comprehensive morphological description of the musculoskeletal system of *H. ocellatum*, encompassing the cranial, axial, and appendicular skeleton and associated musculature. Using an integrative approach that combines manual dissection with diceCT imaging, we document the locomotory and cranial musculature and the associated skeletal system, with particular emphasis on the pectoral, pelvic, and dorsal fin regions. In addition to the paired fins, which drive walking, we examined the dorsal fins. Although they do not contact the substrate, the dorsal fins of bamboo sharks are more than passive stabilisers [28,31]. This study importantly establishes a detailed morphological framework that can serve as a basis for future functional, comparative, and evolutionary investigations.

2. Materials and Methods

2.1. Materials

Two specimens of *Hemiscyllium ocellatum* were used for the morphological investigation: a subadult female (EMRG-Chond-H-2; total length 57.55 cm, Evolutionary Morphology Research Group, University of Vienna, Austria) and a subadult male (EMRG-Chond-A-5; total length 66.78 cm). Both specimens were captive-bred individuals obtained from De Jong Marinelife (Spijk, The Netherlands) and collected after natural death on 10 March 2023. They were stored frozen at -20°C at the Department of Palaeontology, University of Vienna.

Additional comparative material includes another two subadult males (ERB 1190, total length 70.6 cm; ERB 1191, total length 54.0 cm, ERB = Elasmobranch Research Belgium, Bonheiden, Belgium), obtained from the same source and in analogue conditions, collected on 30 October 2017 and handled following the protocol by [32]. A tissue sample of the first specimen (ERB 1190) was sent to Gavin Naylor's Lab (Gainesville, FL, USA) and registered under sample number GN 19559.

2.2. Methods

2.2.1. Dissection

The female specimen was used for manual dissection following the protocol of De Iuliis [33]. The specimen was thawed prior to each examination step and refrozen after-

wards. The dissection was always performed on the right side first, so that the other side could serve as a reference. In the next step, the left side was dissected to provide a clearer view of any damaged or overlooked structures, or variations. The procedure started with the removal of the skin, followed by the stepwise removal of connective tissue, individual muscle groups (progressing from head to fins to torso), and organs, until the cartilaginous skeleton was fully exposed. Progress was documented photographically at each stage.

2.2.2. Micro-CT Scanning and Staining

The male specimen was used for micro-CT examination. The scanning was applied specifically to the dorsal fin regions, as the multilayered musculature in this area was difficult to resolve through manual dissection alone. Two transverse body sections at the level of the first and second dorsal fins were extracted and prepared for diceCT scanning [29]. The sections were immersed in a 50 g/L iodine–ethanol (70%) solution for 22 days, following the protocol established by Staggl et al. [30]. Samples were placed on a laboratory shaker (40 rpm) and shielded from light during staining to avoid iodine degradation. Scanning was performed using a SkyScan 1173 High Energy micro-CT (Bruker microCT Systems, Kontich, Belgium) at the Department of Palaeontology of the University of Vienna (Austria) with identical scanning parameters applied for both dorsal fin sections (Table 1).

Table 1. Scanning parameters used for the micro-CT scans of the first and second dorsal fin of male specimen (EMRG-Chond-A-5).

kV	mA	Exposure [ms]	Voxel Size [μm]	Filter	Scanning Time [min]	Number of Images
130	61	1180	35.763	0.25 mm brass	1:59:32	1200

2.2.3. Image Processing

Dissection photographs during each preparatory step were processed and arranged as composite figures using Adobe Photoshop CS6 (Adobe Inc., San Jose, CA, USA). Scale bars were calibrated against the original specimen scale and applied to each panel. Morphological structures were labelled with abbreviations and indicated by arrows. Micro-CT images were selected to complement and verify the dissection results, particularly for structures that were difficult to expose during dissection (e.g., the layered dorsal fin musculature).

2.2.4. Morphological Terminology

Morphological terminology for the cranial and axial musculature follows Goto [34], with minor modifications where necessary. The term *m. radialis* (*m.* = *musculus*) was used following Flammang [35], as it is the commonly used terminology for this muscle. Muscle nomenclature for the pectoral and pelvic fin musculature follows Goto et al. [11]. Dorsal fin muscle terminology follows Medeiros et al. [28], who described the *mm. inclinadores dorsales* (*mm.* = *musculi*) as comprising a *profundus* and a *superficialis* component in galeomorph sharks. Skeletal terminology for the paired fins again follows Goto et al. [11], and for the cranial and axial skeleton, Goto is followed once again [34]. The skeleton of the dorsal fins follows Medeiros et al. [28].

3. Results

The two subadult specimens of *H. ocellatum* exhibit an elongated, cylindrical body with a proportionally long, robust tail and the characteristic large, white-edged dark ocellus (epaulette spot) just behind the pectoral fins (Figure 1A,C). No external sexual dimorphism is evident apart from the presence of claspers in the male specimen.

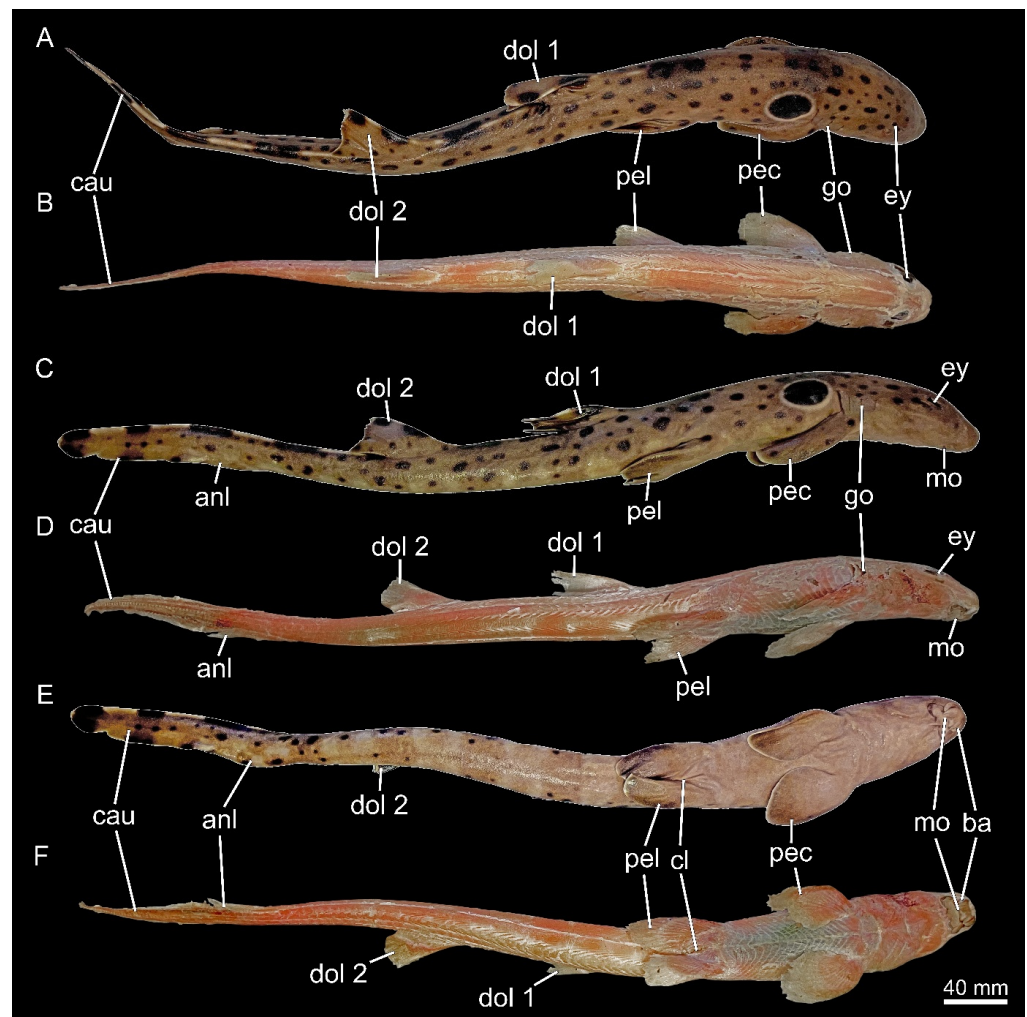


Figure 1. *Hemiscyllium ocellatum*, subadult female (EMRG-Chond-H-2): Different perspectives of the whole body with and without skin. (A) dorsal view showing the epaulette spot, (B) dorsal view without skin, (C) lateral view, (D) ventral view without skin, (E) ventral view, and (F) ventral view without skin. Abbreviations: anl, anal fin; ba, barbell; cau, caudal fin; cl, cloaca; dol 1, first dorsal fin; dol 2, second dorsal fin; ey, eye; go, gill opening; mo, mouth opening; pec, pectoral fin; pel, pelvic fin.

Compared to the closely related species *Chiloscyllium punctatum* Müller and Henle, 1838 [12], the head appears narrower and rounder, though still dorso-ventrally flattened with a ventrally pointing snout [30]. The eyes are positioned more dorsally compared to pelagic sharks (Figure 1). The caudal fin is strongly heterocercal with a distinct dorsal lobe exhibiting a subterminal notch and a notch separating the caudal from the anal fin (Figures 1C–F and 2A,B). Both dorsal fins are positioned posteriorly, with the first dorsal fin located posterior to the pelvic fins (Figure 1C,D). Both pectoral and pelvic fins are relatively large and muscular (Figure 1E,F).

3.1. Locomotory Muscles

The *m. epaxialis* originates at the chondrocranium posterior to the eyes and extends dorsal to the horizontal septum and dorsolateral to the vertebral column into the caudal fin (Figures 1B,D and 3A). The left and right portions of the *m. epaxialis* appear as a single massive bundle except in the precaudal region, where they are separated by the neural arches, becoming progressively thinner posteriorly (Figures 1B,D, 2B and 3), which is consistent with the description provided by Goto [34]. In our specimen, the *m. hypaxialis* extends below the horizontal septum, forming a thin layer surrounding the abdominal

cavity ventrally (Figures 1F and 3B,C), with its posterior portion converging into a single longitudinal strand similar to the *m. epaxialis*, which also matches the specifications of Goto [34]. The *m. radialis* originates at the notch separating the anal from the caudal fin and extends to the end of the vertebral column (Figure 2B), which is consistent with descriptions for different shark species [35].

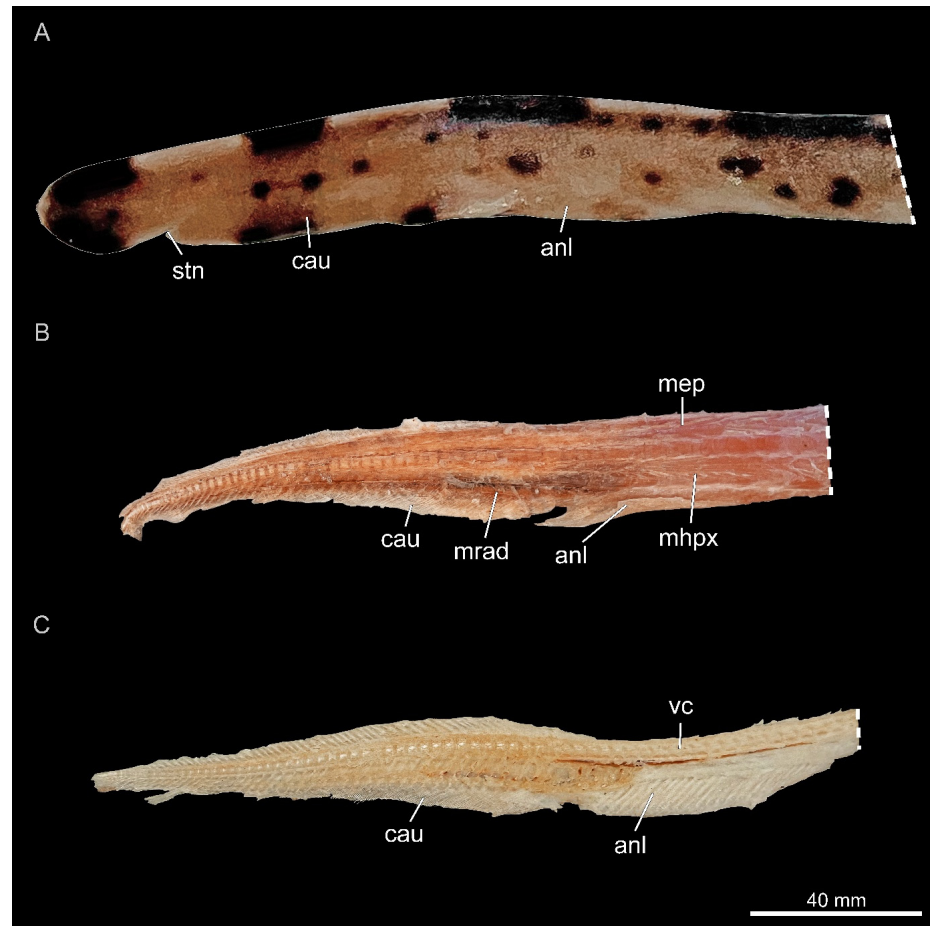


Figure 2. *Hemiscyllium ocellatum*, subadult female (EMRG-Chond-H-2): Lateral views of caudal fin throughout the dissection process. (A) with skin, (B) muscular system, and (C) cartilaginous skeleton. Abbreviations: anl, anal fin; cau, caudal fin; mep, m. epaxialis; mhp, m. hypaxialis; mrad, m. radialis; stn, subterminal notch; vc, vertebral column.

3.2. Cranial Musculature

The cranial musculature, which is associated with jaw and eye movements as well as gill ventilation, is distributed across seven visceral arches: the mandibular, hyoid and five branchial arches [34]. Although not directly involved in locomotion, documenting the cranial muscles establishes a comprehensive anatomical framework for *H. ocellatum* and offers insight into the species' specialised suction feeding mechanism, a defining functional adaptation closely tied to its benthic lifestyle.

3.2.1. Mandibular Arch Musculature

The *m. adductor mandibulae* represents the most prominent muscle of the jaw apparatus, bridging the posterior portions of Meckel's cartilage and the palatoquadrate (Figures 3B,C and 4A). Four distinct subdivisions (parts I–IV) are identified in the here investigated specimen. Part I originates at the anteroventral margin of the complex, starting at the Meckel's cartilage and attaching to the connective tissue associated with the

m. suborbitalis (Figure 4B,C). Part II is situated in the anterior region of the complex. Part III was the most voluminous subdivision and continued directly from the posterior region of part II, running obliquely between the posteroventral margin of Meckel's cartilage and the dorsal palatoquadrate. Part IV is separated from part III, covering the quadratomandibular joint. This four-part arrangement corresponds to the condition reported for orectolobiform sharks by Goto [34].

The *m. suborbitalis* is strongly attached dorsally to the chondrocranium. It originates in the ethmoid region, protrudes over the cranium in contact with the *m. epaxialis* (separated by connective tissue; Figure 3A), surrounds the anterior orbita and overlaps the palatoquadrate (Figures 3C and 4A–C).

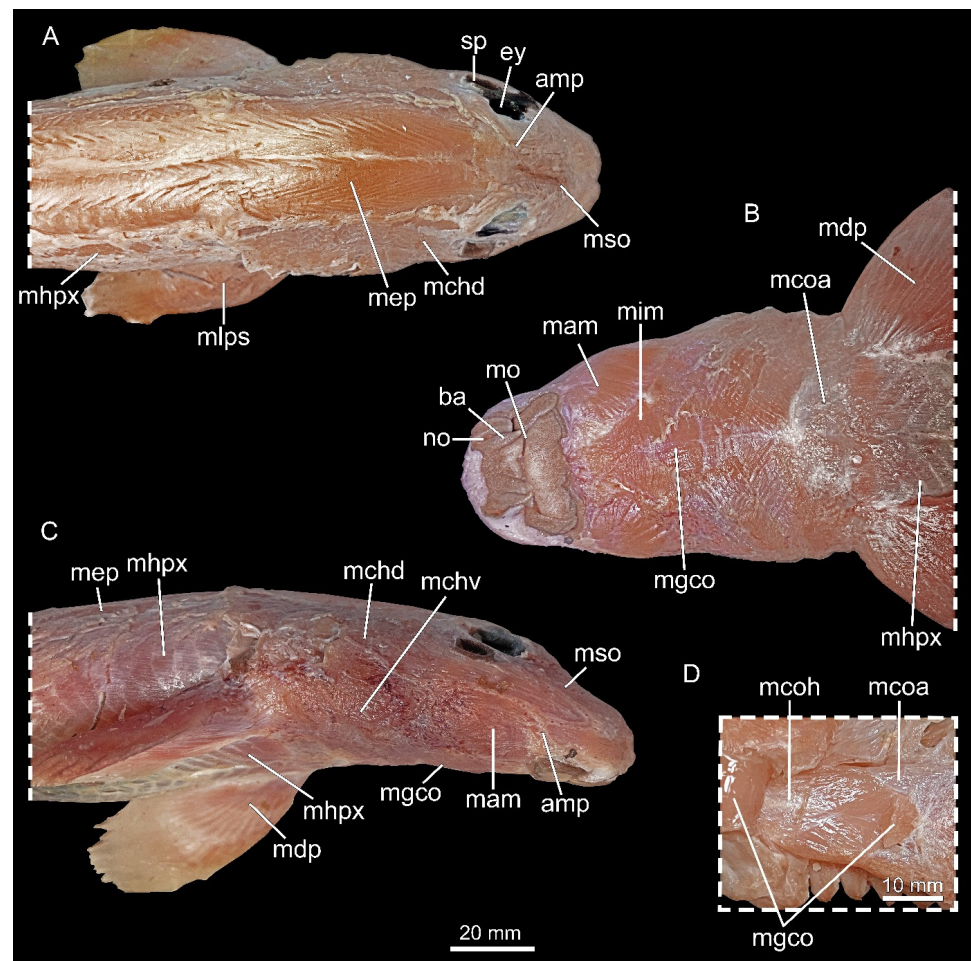


Figure 3. *Hemiscyllium ocellatum*, subadult female (EMRG-Chond-H-2): Different perspectives of the head region anterior to the pelvic girdle. (A) dorsal, (B) ventral, (C) dorsolateral, and (D) ventral detailed views. Abbreviations: amp, ampullae of Lorenzini; ba, barbell; ey, eye; mam, *m. adductor mandibulae*; mchd, *m. constrictor hyoideus dorsalis*; mchv, *m. constrictor hyoideus ventralis*; mcoa, *m. coraco arcualis*; mcoh, *m. coraco-hyoideus*; mdp, *m. depressor pectoralis*; mep, *m. epaxialis*; mgco, *m. genio coracoideus*; mhp, *m. hypaxialis*; mim, *m. intermandibularis*; mlps, *m. levator pectoralis superficialis*; mo mouth opening; mso, *m. suborbitalis*; no, nostril; sp, spiracle.

The *m. constrictor dorsalis* could not be fully exposed without significant damage. In *C. punctatum* and *H. ocellatum*, it consists of the *m. levator palatoquadrati* and the *m. spiracularis* [34,36]. In the closely related *C. punctatum*, the *m. levator palatoquadrati* runs on the inside and the *m. spiracularis* on the outside [30]. The paired *m. intermandibularis* originates at subcutaneous tissue of the *m. genio-coracoideus*, fuses anteriorly, and inserts along the

ventral surface of the Meckel's cartilage with a tight connection to the *m. constrictor hyoideus ventralis* (Figure 3B) [34,36].

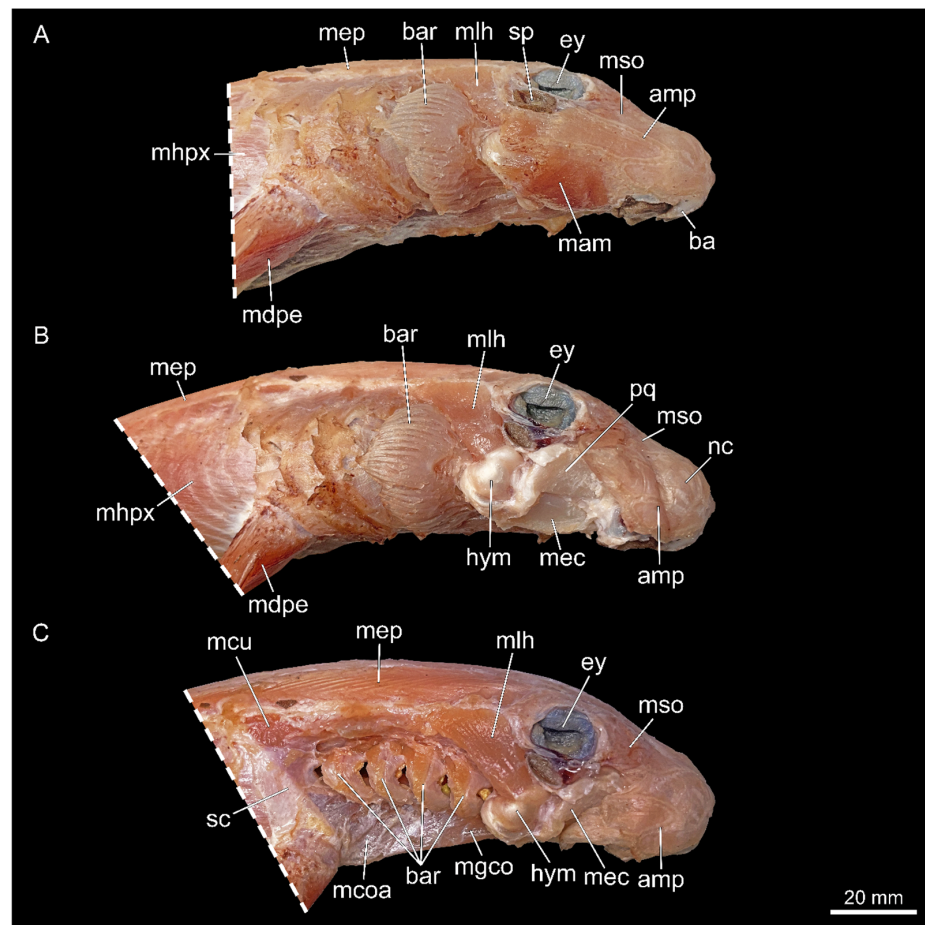


Figure 4. *Hemiscyllium ocellatum*, subadult female (EMRG-Chond-H-2): Different layers of the head region anterior of the pectoral girdle in lateral view. (A) removed *m. constrictor hyoideus dorsalis*, (B) removed *m. adductor mandibulae* and (C) exposed visceral arches. Abbreviations: amp, ampullae of Lorenzini; ba, barbell; bar, branchial arches; ey, eye; hym, hyomandibula; mam, *m. adductor mandibulae*; mgco, *m. genio coracoideus*; mcoa, *m. coraco arcualis*; mcu, *m. cucullaris*; mdpe, *m. depressor pectoralis*; mec, Meckel's cartilage; mep, *m. epaxialis*; mhp, *m. hypaxialis*; mlh, *m. levator hyomandibulae*; mso, *m. suborbitalis*; nc, nasal capsule; pq, palatoquadratum; sc, scapula; sp, spiracle.

3.2.2. Hyoid Arch Musculature

The *m. constrictor hyoideus* comprised a dorsal and ventral part. The dorsal part was subdivided into the *m. constrictor hyoideus dorsalis* (originating from the otic capsule and *m. epaxialis*, inserting at the hyomandibula) and the *m. levator hyomandibulae* (originating from the otic capsule, inserting at the lateroventral hyomandibula), which is in line with the structure of other galeomorph sharks [34,36]. The dorsal part superficially covered parts of the first branchial arch (Figures 3C and 4A). The *m. constrictor hyoideus ventralis* originated from subcutaneous tissue at the lateral edge of Meckel's cartilage [36] and inserted on the ceratohyal [34]. In the branchial region, the dorsal and ventral parts were fused (Figure 3C).

3.3. Hypobranchial Muscles

In the examined specimens, the *m. genio-coracoideus* originate from the fascia of the *m. coraco-hyoideus* and the aponeurotic septum, inserting on the ventral side of the Meckel's cartilage near the symphysis (Figures 3B–D and 4C). The *m. rectus cervicis* consists of the paired *m. coraco-arcualis* (originating from the coracoid; Figures 3B,D and 4C) and the

m. coraco-hyoideus (originating from the anterior m. coraco-arcualis and the pericardial membrane; Figure 3D), fusing medially. The five pairs of mm. coraco-branchiales originate from the coracoid and pericardial membrane, inserting into the ventromedial side of the branchial arches. The overall structure aligns with the descriptions of Goto [34] and Shirai [37] in general.

3.4. Pectoral Fin Musculature

The pectoral fin musculature of *H. ocellatum* comprises four distinct muscles that can be functionally divided into the depressor (one muscle) and the levators (three muscles) (Figure 5A–D). As observed in the examined specimen, pectoral muscles are supported by the pectoral girdle and two basal cartilages (metapterygium and a propterygium fused with the mesopterygium), with the pectoral girdle capable of greater rotation than in other shark species [10]. The three layers of levator muscles consist of the (1) fan-shaped *m. levator pectoralis superficialis* (outermost, originating from the scapula, inserting on radial cartilages), (2) *m. levator pectoralis inferior* (originating from the coracoid, inserting on the posterior mesopterygium), and (3) thin *m. levator pectoralis distalis* (originating from distal basal cartilages, inserting on radial cartilages) (Figure 5B). The single ventral *m. depressor pectoralis* is fan-shaped, thicker than its dorsal counterpart, and becomes increasingly massive posteriorly. It originates from the coracoid and inserts onto the mesopterygium and radial cartilages (Figure 5C,D). The results confirm the observations made by Goto et al. [11].

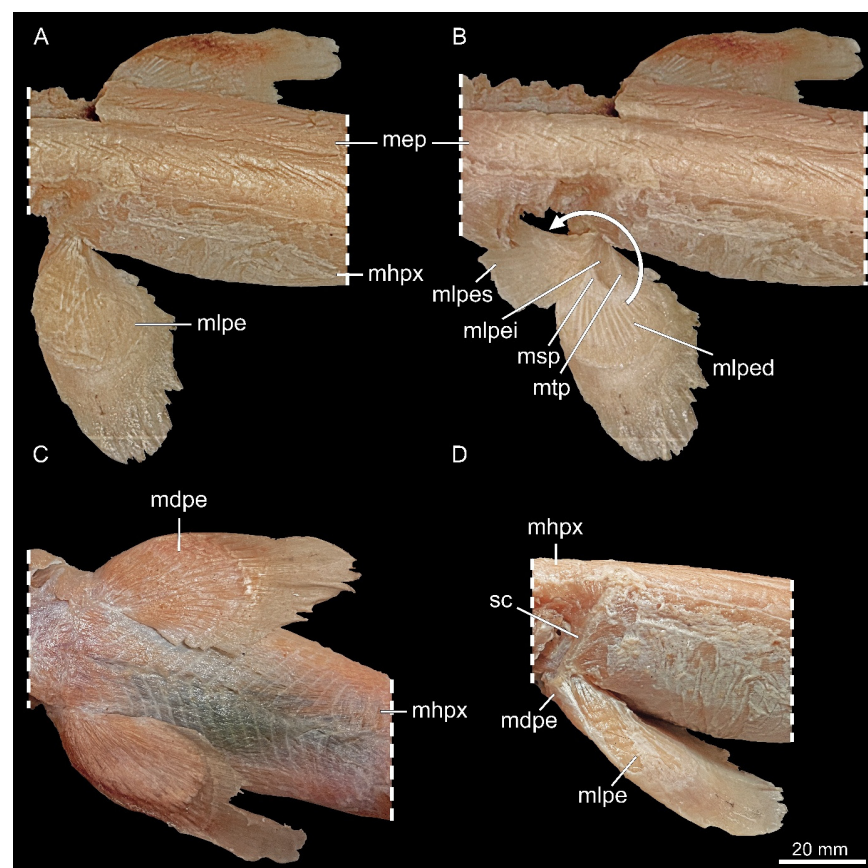


Figure 5. *Hemiscyllium ocellatum*, subadult female (EMRG-Chond-H-2): Different views on the pectoral girdle region. The arrow demonstrates the opening of the superficial muscle layer. (A) dorsolateral, (B) dorsolateral with folded-out *m. levator pectoralis*, (C) ventral and (D) lateral view. Abbreviations: mdpe, *m. depressor pectoralis*; mep, *m. epaxialis*; mhp, *m. hypaxialis*; mlpe, *m. levator pectoralis*; mlpd, *m. levator pectoralis distalis*; mlpei, *m. levator pectoralis inferior*; mlpes, *m. levator pectoralis superficialis*; msp, *mesopterygium*; mtp, *metapterygium*; sc, *scapula*.

3.5. Pelvic Fin Musculature

We observed that the pelvic muscles include a dorsal levator, a lateral depressor, and a ventral adductor muscle (Figure 6A–C), which is consistent with the findings by Goto et al. [11]. The sheet-like *m. levator pelvici* originates from the hypaxial body muscle fascia and inserts on the radial cartilages and pelvic basiptyrgium (Figure 6A,B). The *m. depressor pelvici* comprises anterolateral and posterolateral portions, originating from the prepelvic process and basiptyrgium respectively, inserting on basal cartilages and ventral radials (Figure 6B,C) also described by Goto et al. [11]. The *m. adductor pelvici* originates from the puboischiadic bar and inserts onto the ventral basiptyrgium.



Figure 6. *Hemiscyllium ocellatum*, subadult female (EMRG-Chond-H-2): different views of the pelvic region. (A) lateral, (B) lateral with removed *m. levator pelvici* superficialis, and (C) ventral. Abbreviations: cl, cloaca; map, *m. adductor pelvici*; mdp, *m. depressor pelvici*; mep, *m. epaxialis*; mhp, *m. hypaxialis*; mids, *m. inclinator dorsalis superficialis*; mlp, *m. levator pelvici*.

3.6. Dorsal Fin Musculature and Skeleton

We observed that in *H. ocellatum* the dorsal fins contain only the *mm. inclinatores dorsales*, divided into an outer (*m. inclinator dorsalis superficialis*) and an inner layer (*m. inclinator dorsalis profundus*) (Figures 7–9), which is in line with observations in other galeomorph sharks [28]. Unlike the condition reported for some species (e.g., *Rhincodon typus*) by these authors, where the *profundus* extends between the epaxial muscles, in *H. ocellatum* both layers had the same length and overlapped completely (Figure 9B,C). The *m. inclinator dorsalis*

superficialis generally originates from an aponeurosis on the *m. epaxialis* and inserts onto the fascia of the *profundus*, while the *profundus* originates from the fin basals and inserted on distal radials, which is also consistent with other galeomorph sharks [28]. The two muscle layers showed a clearly segmented organisation, consisting of distinct individual muscle bundles that are separated from one another by epimysial connective tissue (Figure 7B,C). Furthermore, both *superficialis* and *profundus* layers extend beyond the main body of the fin and continue into the free posterior tip of the dorsal fins, indicating that their influence is not restricted to the proximal regions, but also contributes to the structure and potentially the movement of the distal fin portions (Figures 7A,B and 8A–C). This was also observed in other orectolobiform sharks by Medeiros et al. [28].

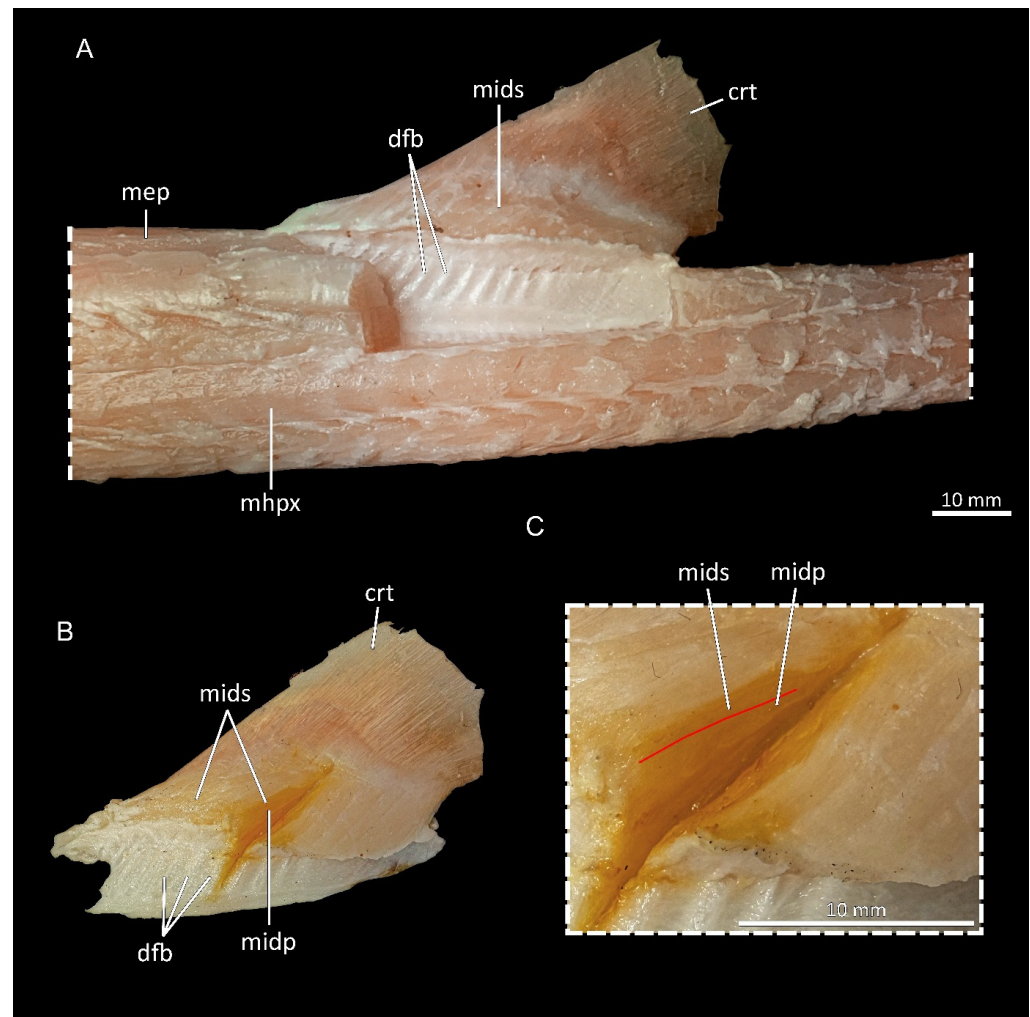


Figure 7. *Hemiscyllium ocellatum*, subadult female (EMRG-Chond-H-2): In situ, ex situ and detailed views of the second dorsal fin. The red line indicates the boundary between the superficial and the profund layer of the *mm. inclinatores dorsales*. (A) lateral in situ, (B) lateral ex situ and (C) detailed view. Abbreviations: crt, ceratotrichia; dfb, dorsal fin basals; mep, *m. epaxialis*; mhp, *m. hypaxialis*; midp, *m. inclinator dorsalis profundus*; mids, *m. inclinator dorsalis superficialis*.

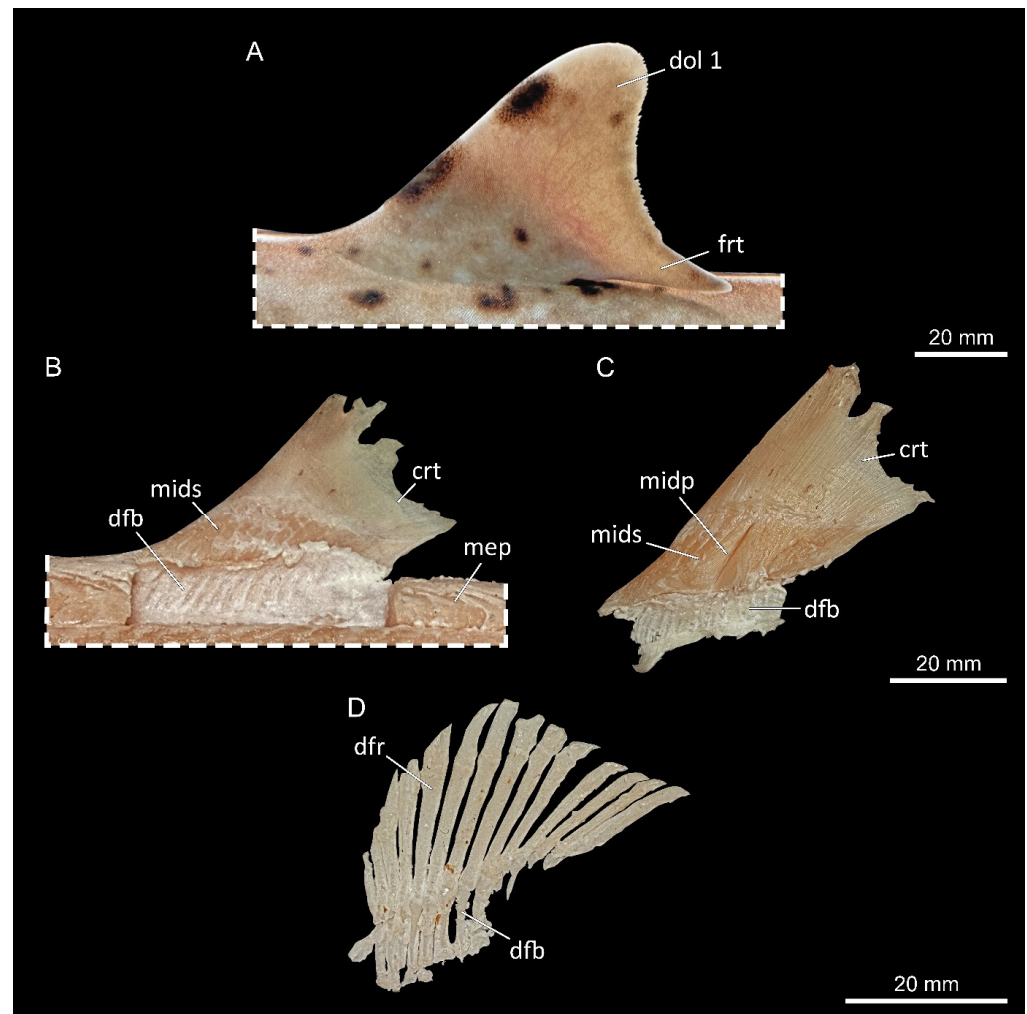


Figure 8. *Hemiscyllium ocellatum*, subadult female (EMRG-Chond-H-2): Different stages of the dissection of the first dorsal fin. (A,B) lateral in situ, (C) lateral ex situ and (D) lateral cartilaginous skeleton. Abbreviations: crt, ceratotrichia; dfb, dorsal fin basals; dfr, dorsal fin radials; dol 1, first dorsal fin; frt, free rear tip; mep, m. epaxialis; midp, m. inclinator dorsalis profundus; mids, m. inclinator dorsalis superficialis.

The dorsal fin skeleton consists of basals and radials, with posterior basals and radials being fused in *H. ocellatum*. The basals are shorter than the radials and penetrate the *m. epaxialis* (Figures 7A,C and 8B–D). The radials consist of a long, slender proximal element and a shorter, thicker distal element (Figure 8D).

3.7. Micro-CT Cross Sections of the Dorsal Fin Region

Micro-CT imaging of the male specimen (EMRG-Chond-A-5) revealed the detailed arrangement of muscular and skeletal structures in the dorsal fin region (Figure 9A–C). The boundary between the *m. inclinator superficialis* and *profundus* layers accordingly is extremely thin and therefore difficult to distinguish during manual dissection, as it is separated only by a delicate layer of connective tissue. In contrast, this subtle interface is clearly resolved in the diceCT cross-sections, where the two layers can be distinctly differentiated (Figure 9B,C).

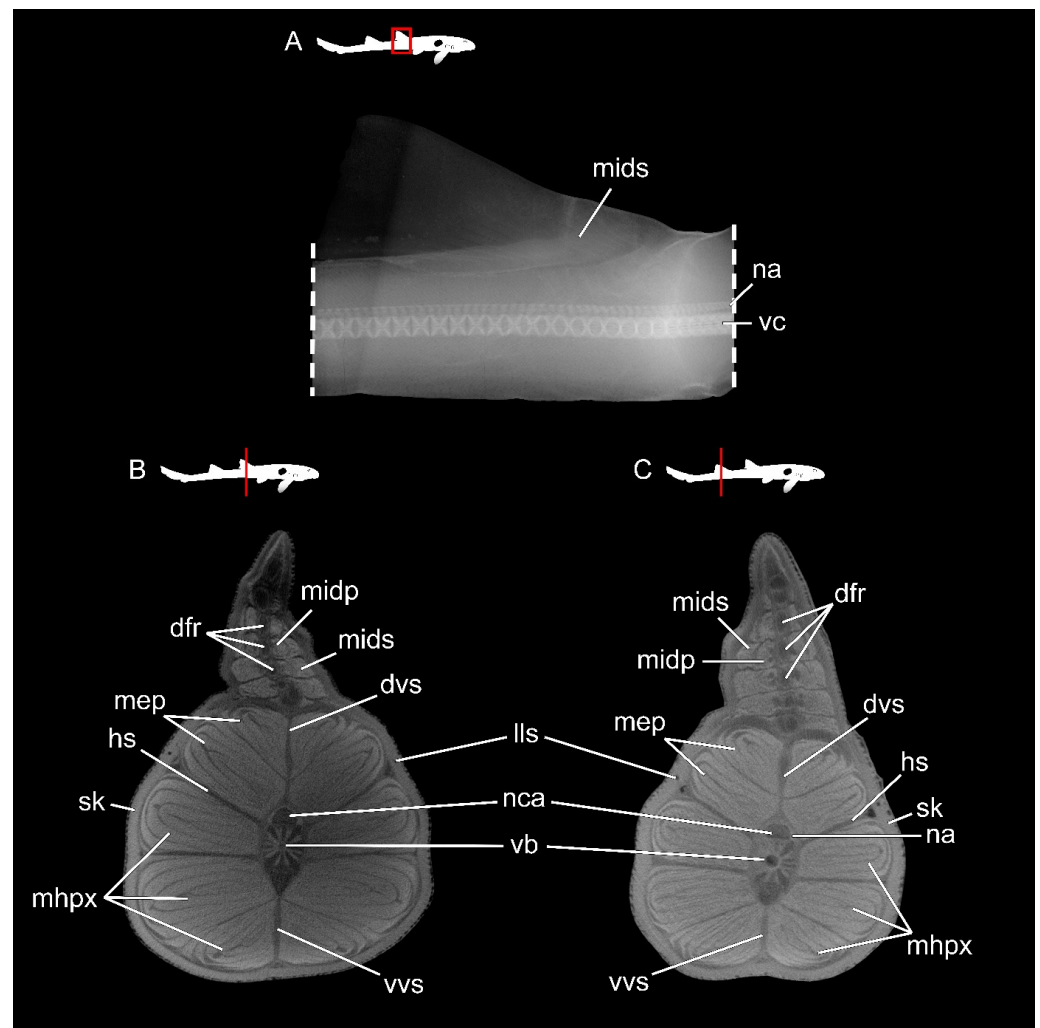


Figure 9. *Hemiscyllium ocellatum*, subadult male (EMRG-Chond-A-5): Micro-CT scans of the first and second dorsal fin regions. (A) Radio image of first dorsal fin (lateral view), (B) first dorsal fin, transversal cross-section and (C) second dorsal fin, transversal cross-section. Abbreviations: dfr, dorsal fin radials; dvs, dorsoventral septum; hs, horizontal septum; lls, lateral line system; mep, m. epaxialis; mhp, m. hypaxialis; midp, m. inclinator dorsalis profundus; mids, m. inclinator dorsalis superficialis; na, neural arch; nca, neural canal; sk, skin; vb, vertebral body; vc, vertebral column; vvs, ventral vertical septum.

Furthermore, the cross sections show that the dorsal fin radials are surrounded by the *mm. inclinatores dorsales*. A distinct aponeurosis separates the *mm. inclinatores dorsales* from the *m. epaxialis*. The *m. epaxialis* and *m. hypaxialis* surround the vertebral column and are separated by the horizontal septum. The *epaxialis* is further divided by the dorsoventral septum and the *hypaxialis* by the ventral vertical septum (Figure 9B,C).

3.8. Cranial Skeleton

3.8.1. Chondrocranium

The chondrocranium is a single, moderately calcified cartilaginous capsule, curved in lateral view with an elongated anterior part comprising the rostral process and paired nasal capsules (Figures 10 and 11). A prefrontal fontanelle is clearly visible dorso-medially (Figure 11A,C). The orbital region contains two large, dorsally positioned eye sockets with a supraorbital crest (Figure 11A,E). Multiple foramina are identified for cranial nerves II, V, VII, IX, and X, the pituitary vein, and the orbital and internal carotid arteries (Figure 11B–E). The otic and occipital regions are outlined dorsally by the sphenopterotic ridge, with a

large foramen magnum (Figure 11D). Overall, our observations of the chondrocranium are comparable to those in previous studies [34,38,39].

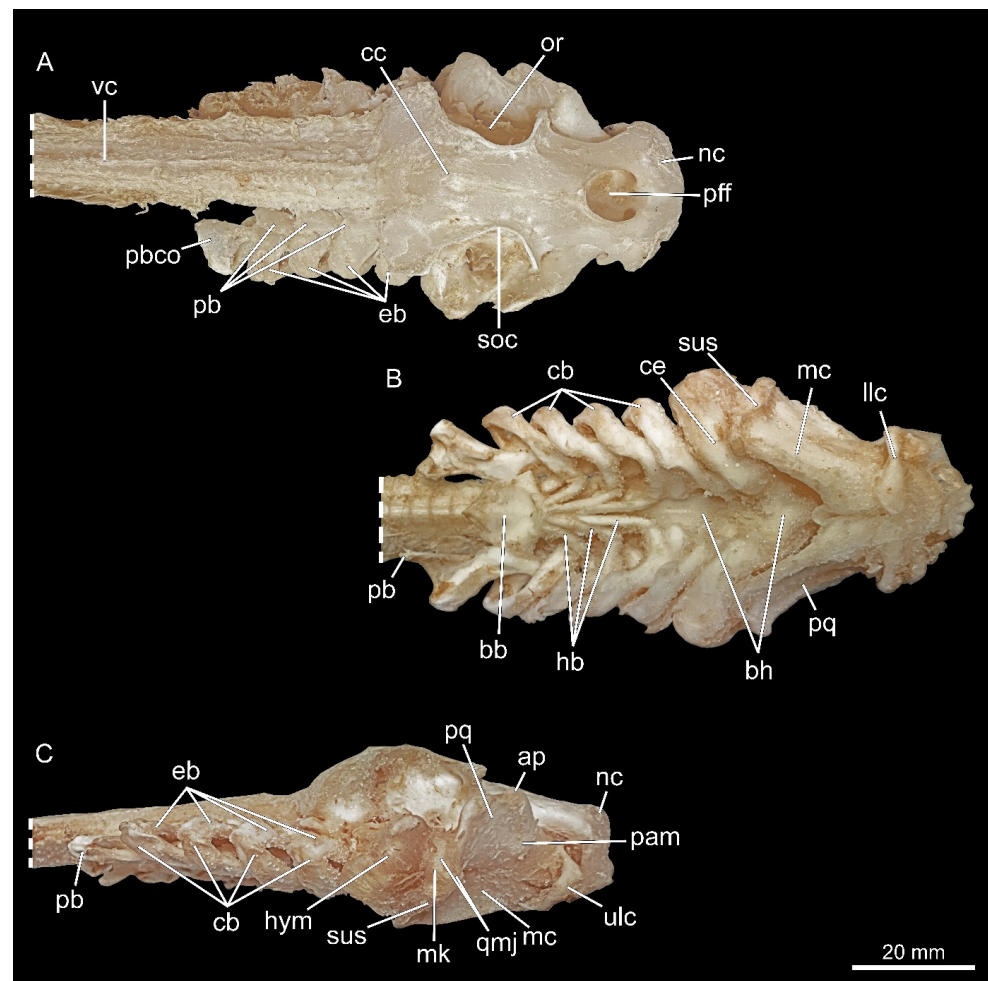


Figure 10. *Hemiscyllium ocellatum*, subadult female (EMRG-Chond-H-2): Different views of the chondrocranium and the visceral arches. (A) dorsal, (B) ventral and (C) lateral view. Abbreviations: ap, ascending process; bb, basibranchiale; bh, basihyal; cb, ceratobranchiale; cc, chondrocranium; ce, ceratohyal; eb, epibranchial; hb, hypobranchial; hym, hyomandibula; llc, lower labial cartilage; mc, Meckel's cartilage; mk, mandibular knob; nc, nasal capsule; or, orbita; pam, processus for m. adductor mandibulae; pb, pharyngobranchial; pbco, pharyngobranchial complex; pff, praefrontal fontanelle; pq, palatoquadrate; qmj, quadratomandibular joint; soc, supraorbital crest; sus, sustentaculum; ulc, upper labial cartilage; vc, vertebral column.

3.8.2. Splanchnocranium

The splanchnocranium consists of superficially calcified cartilaginous arches representing the only segmented part of the skull [6,40,41]. In the here dissected individual, the mandibular arch comprises the paired palatoquadrates (upper jaw) and paired Meckel's cartilages (lower jaw), plus four pairs of labial cartilages (Figure 10A–C). The palatoquadrates meet anteriorly along a vertical symphysis, with an adductor mandibular process and an ascending process connecting them to the chondrocranium via the ethmopalatine ligament; see also Goto [34] and Wilga [42]. The Meckel's cartilage is triangular in lateral view with a sustentaculum connecting it to the palatoquadrate via the quadratomandibular joint, and a mandibular knob connecting it to the hyomandibula. Two pairs of labial cartilages on the upper and lower jaws, respectively, are present (Figure 10B), which verifies observations made by Wu [7] and Klimpfinger and Kriwet [43].

The hyoid arch consisted of an unpaired basihyal, paired ceratohyals, and paired hyomandibulas (Figure 10B,C) [44]. The hyomandibula suspends the jaw from the chondrocranium, articulating with a knob-like structure on the Meckel's cartilage and the hyomandibular fossa on the chondrocranium, respectively. The five branchial arches are each composed of paired pharyngobranchials, epibranchials, ceratobranchials, hypobranchials, and an unpaired basibranchial, confirming the results of Goto [34]. The pharyngobranchials of the last two arches are fused with the epibranchial of the last arch to form the so-called gill pickaxe [34,45]. Orectolobiforms possess four or more branchial rays in the first four arches, but fewer in the fifth [34].

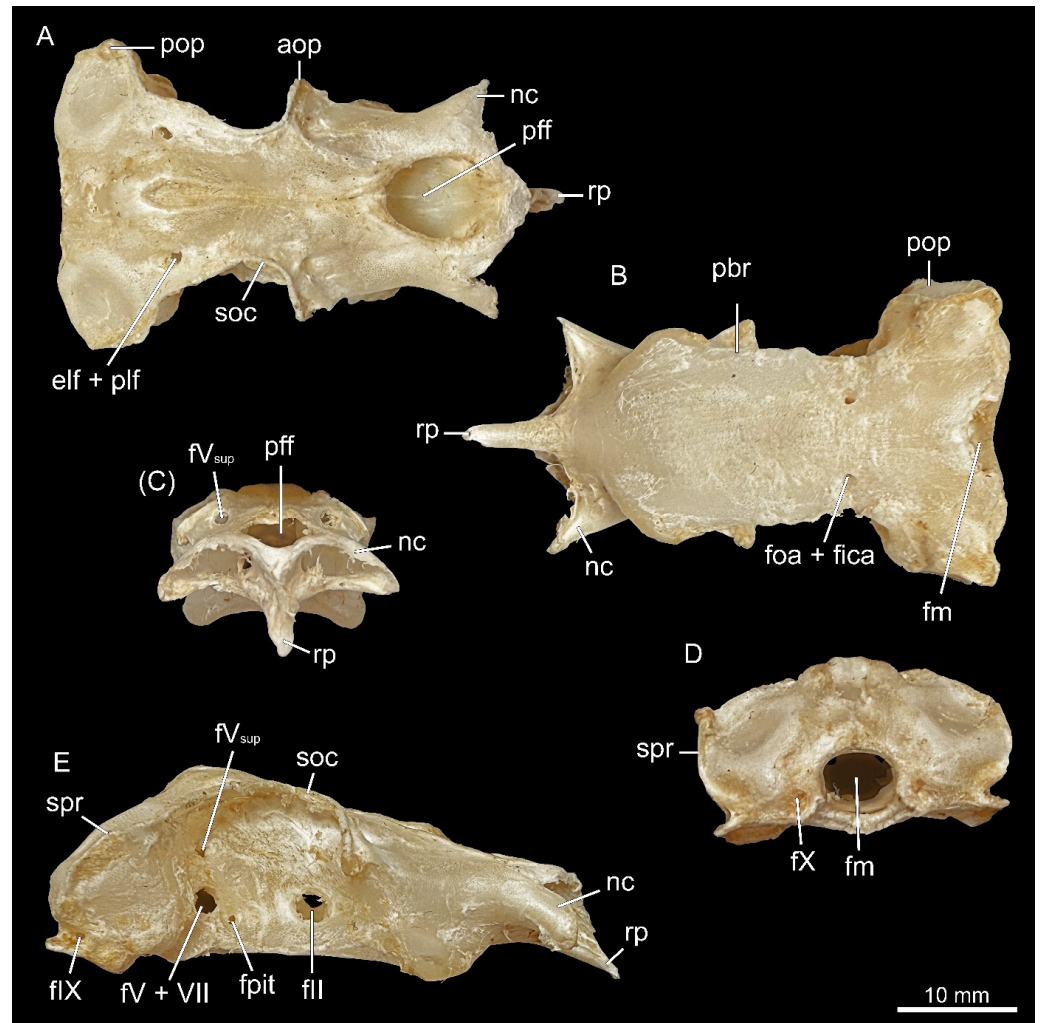


Figure 11. *Hemiscyllium ocellatum*, subadult female (EMRG-Chond-H-2): Different views of the chondrocranium. (A) dorsal, (B) ventral, (C) anterior, (D) posterior and (E) lateral view. Abbreviations: aop, antorbital process; elf, endolymphatic foramen; fica, foramen of internal carotid artery; flI, foramen of cranial nerve II; fv(sup), foramen of (superficial branch of) cranial nerve V; fvII, foramen of cranial nerve VII; flX, foramen of cranial nerve IX; fx, foramen of cranial nerve X; fm, foramen magnum; foa, foramen of orbital artery; fpit, foramen of pituitary vein; nc, nasal capsule; pbr, palatobasal ridge; pff, praefrontal fontanelle; plf, perilymphatic foramen; pop, postorbital process; rp, rostral process; soc, supraorbital crest; spr, sphenopterotic ridge.

3.9. Pectoral Girdle

The pectoral girdle consists of fused scapulocoracoids forming a U-shaped trough in anterior view with a dorsal scapular process and horizontal coracoid bar and an apron supporting the pericardial cavity (Figure 12A,B). In *H. ocellatum*, the propterygium is fused with the mesopterygium, resulting in only two distinct, paddle-like basal cartilages (Figure 12A,B). The pectoral fin radials consist of proximal, medial, and distal elements. The anterior series are shorter, and some posterior radials lack distal or medial elements. Our description of the pectoral girdle matches the one from Goto et al. [11].

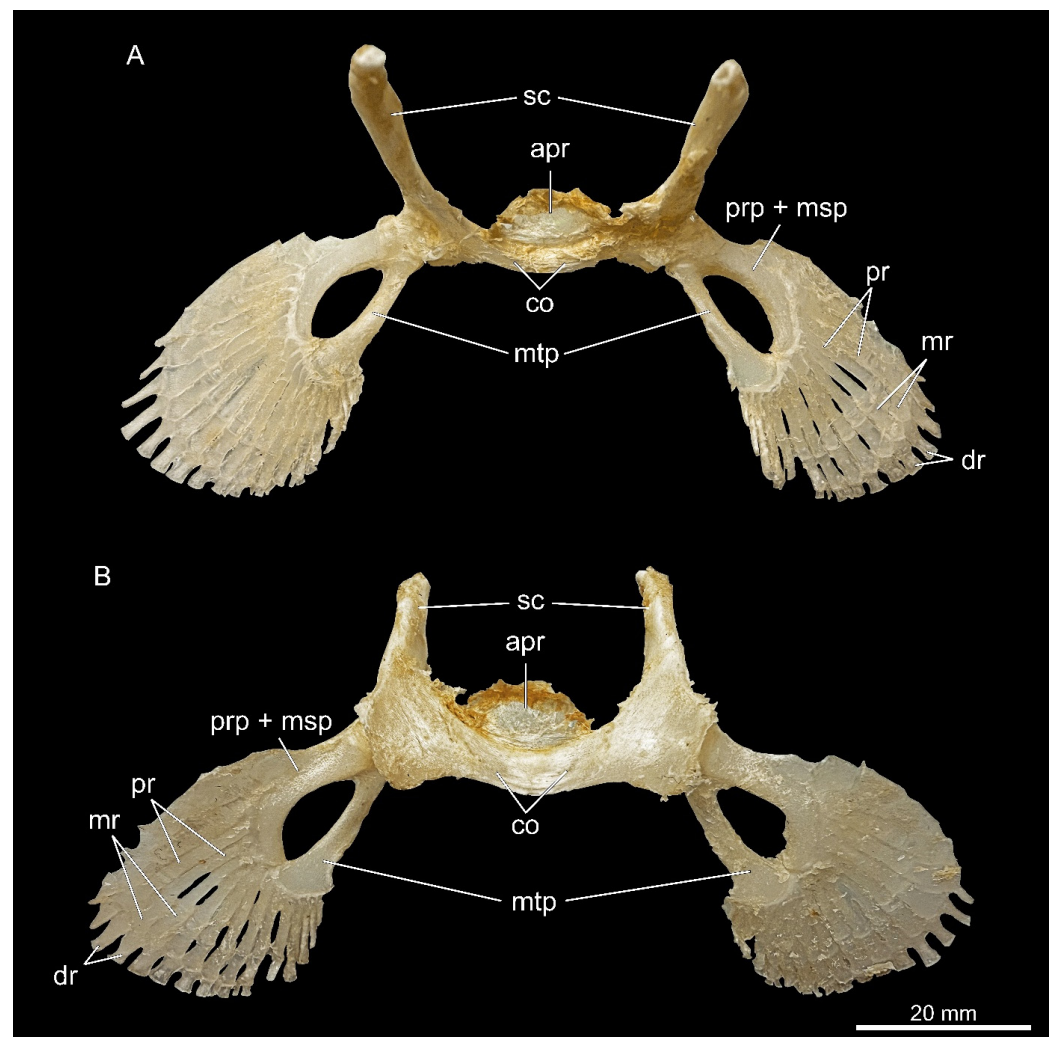


Figure 12. *Hemiscyllium ocellatum*, subadult female (EMRG-Chond-H-2): Different views of pectoral girdle. (A) dorsal and (B) ventral view. Abbreviations: apr, apron; co, coracoid; dr, distal radials; mr, medial radial; msp, mesopterygium; mtp, metapterygium; pr, proximal radials; prp, propterygium; sc, scapula.

3.10. Pelvic Girdle and Fins

The pelvic girdle consists of a slightly arched, fused puboischiadic bar with prepelvic processes and a pelvic basipterygium at the base of each fin (Figure 13A,B). The anterior pelvic basal cartilage shows a slight bifurcation as noted by Goto et al. [11]. Radials consist of long proximal and short, pointed distal elements (Figure 13A,B) [46].

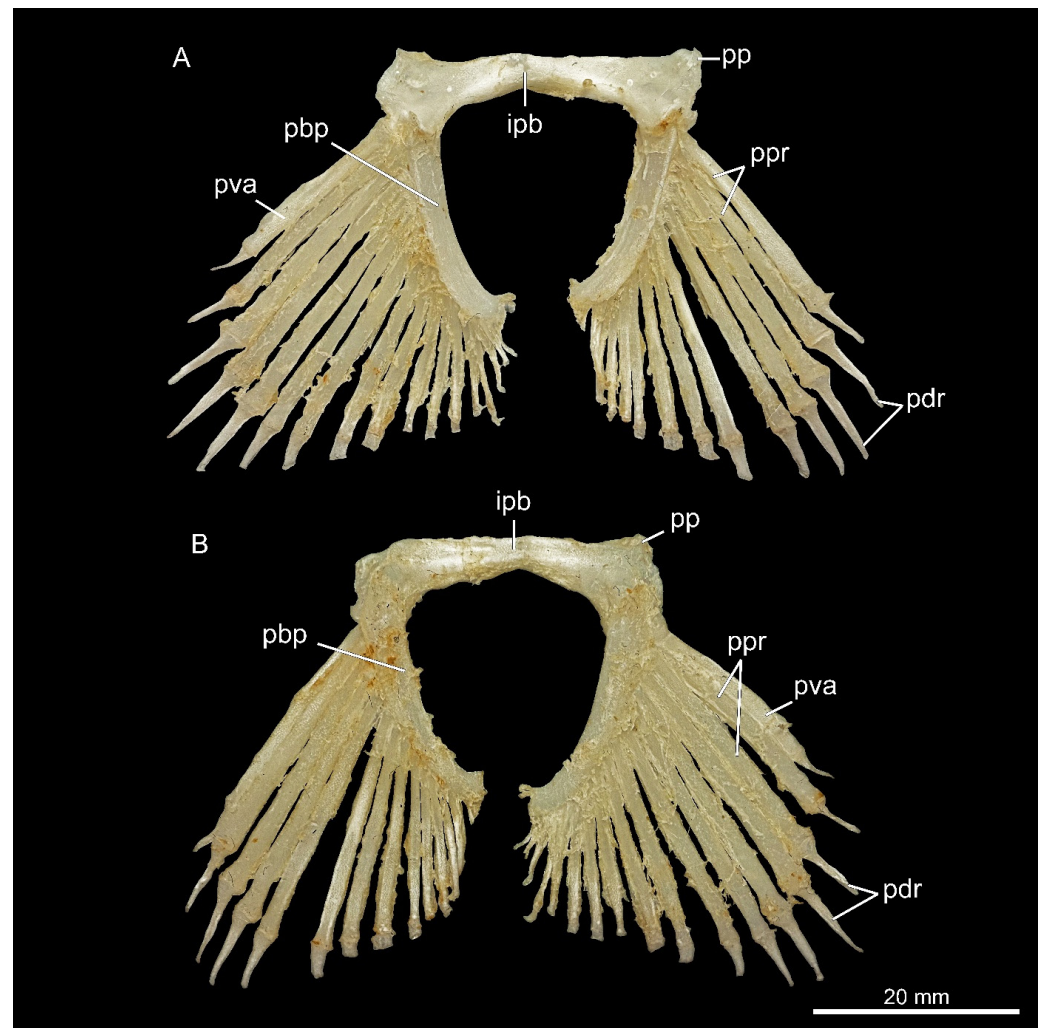


Figure 13. *Hemiscyllium ocellatum*, subadult female (EMRG-Chond-H-2): Different views of pelvic girdle. (A) dorsal and (B) ventral view. Abbreviations: ipb, puboischiadic bar; pbp, pelvic basiptyrgium; pdr, pelvic distal radials; pp, prepelvic process; ppr, pelvic proximal radials; pva, anterior pelvic basal cartilage.

3.11. Vertebrae

Species of *Hemiscyllium* typically possess 180–195 vertebrae [6], with *H. ocellatum* having 183–197 vertebral elements [13,44,47]. The amphicoelous vertebral centra exhibit the typical asterospondyl calcification pattern of orectolobiforms (compare also Goto [34]) (Figure 14C). The vertebral column is separated into abdominal (vertebrae without haemal arch), precaudal (vertebrae with incomplete haemal arch), and caudal (vertebrae with complete haemal arch) portions (Figure 14A–C). Monospondylous vertebrae occur in the abdominal and precaudal regions, while diplospondylous vertebrae are present posteriorly. The neural arch consists of a basidorsal process, dorsal intercalary plate, and supraneural, forming the neural canal (Figure 14A–C). Again, our observations align with those of Goto [34].

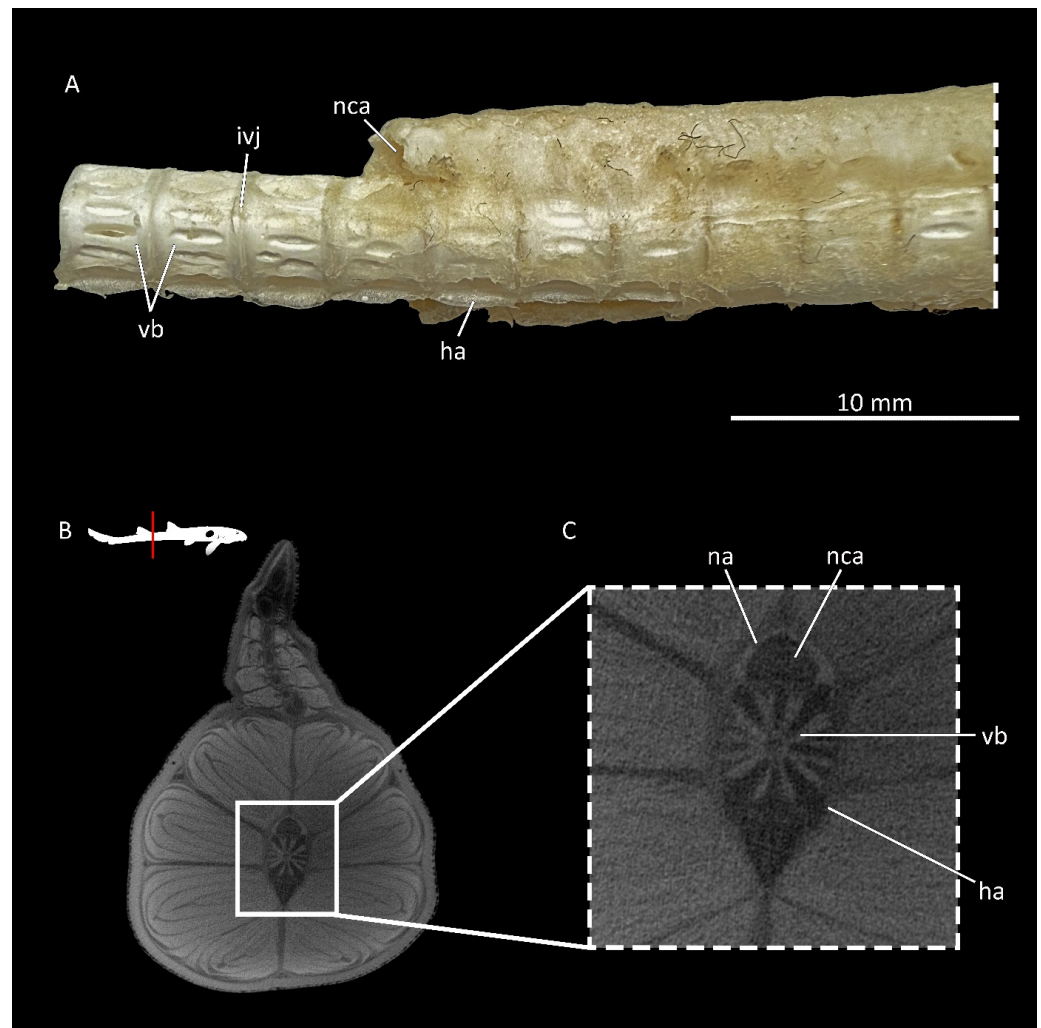


Figure 14. *Hemiscyllium ocellatum*, (A) subadult female (EMRG-Chond-H-2), B-C male (EMRG-Chond-A-5): Dissected vertebral column and CT scan of the first dorsal fin region. (A) lateral view, (B) CT scan overview and (C) detail of vertebral column in CT scan. Abbreviations: nca, neural canal; ivj, intervertebral joint; vb, vertebral body; ha, haemal arch; na, neural arch.

4. Discussion

4.1. Methodological Discussion

The present study combines traditional dissection with diffusible iodine-based contrast-enhanced micro-CT (diceCT) to investigate the musculoskeletal system of *H. ocellatum*. Each method carries distinct strengths and limitations, and their combination proved essential for a comprehensive morphological description.

The successful staining of the dorsal fin sections of a male specimen with iodine, following the protocol of Stagg et al. [30], enabled non-destructive, high-resolution micro-CT imaging that differentiated between various soft and hard tissue types. Heavily calcified skeletal elements, such as the vertebral centra and neural arches, appeared as bright structures in the scans, while muscles showed intermediate grey values due to iodine accumulation, and connective tissue, as well as uncalcified cartilage (e.g., dorsal fin radials), appeared in darker tones. These results are consistent with previous applications of the iodine-enhanced micro-CT approach in neoselachian studies [30,48] and confirm its advantages over non-stained micro-CT imaging. This finding is relevant for future studies, as it indicates that the staining duration and concentration may need to be adapted depending on tissue thickness and preservation conditions. Callahan et al. [49] demonstrated that

optimal staining time for diceCT generally increases linearly with specimen size, which aligns with the prolonged staining period required here for the relatively large dorsal fin cross-sections in the present study. Furthermore, Gignac et al. [29] noted that the iodine penetration rate varies substantially depending on tissue density and composition, which may explain the initially poor contrast observed after the first staining period.

The dissection of the female specimen allowed direct tactile examination of muscle texture, fibre orientation, and articular properties that are not accessible through CT imaging alone. The direct manipulation of joints during dissection provided insights into the range of motion at the pectoral and pelvic girdles that purely digital methods cannot replicate. However, the destructive nature of dissection imposes an inherent limitation, as damaged structures cannot be revisited. Particularly in the fin regions, the strong adhesion of skin and connective tissue to the underlying musculature made it challenging to expose certain structures without causing damage. To mitigate this, at first only one side of the specimen was dissected, preserving the contralateral side as a reference, a strategy that has proven useful in comparable studies [30,50].

Micro-CT imaging, in contrast, enables repeated, non-destructive scanning and thus preserves the specimen for future analyses and independent verification. The cross-sectional scans of both dorsal fin regions clearly revealed the boundary between the *m. inclinator dorsalis superficialis* and *profundus*, a distinction that was barely visible during dissection due to the tight adhesion between these muscle layers. This demonstrates the complementary nature of both approaches: dissection provides unmatched insight into surface-level morphology, tissue properties, and articulation mechanics, while diceCT excels at revealing internal spatial relationships in situ. The combination of both methods thus minimises the potential loss of essential morphological information and enables a more complete and reproducible description of the functional morphology.

4.2. Morphological Discussion

4.2.1. General Body Plan and Ecological Context

Epauvette sharks are commonly encountered in shallow waters, lagoons, and tidal pools of tropical coral reefs [4,6]. Coral reefs constitute complex, three-dimensional habitats characterised by overhangs, tunnels, and crevices [51], which support not only high species richness [52,53] but also a high diversity of locomotor strategies [54,55]. The body plan of *H. ocellatum* reflects a suite of adaptations to this structurally complex environment. The slender, elongated body and the proportionally large, muscular paired fins allow efficient manoeuvring through narrow reef spaces, while the posteriorly positioned dorsal fins and the strongly heterocercal caudal fin are characteristic of benthic orectolobiforms [6,34,56]. Compared to pelagic sharks, the more dorsally positioned eyes and the dorso-ventrally flattened head are consistent with a benthic lifestyle where visual surveillance of the environment above the animal is advantageous. The absence of observable sexual dimorphism beyond the male claspers is consistent with descriptions for other hemiscylliids [13,34].

4.2.2. Cranial Morphology and Adaptations for Suction Feeding

The cranial musculature described here broadly conforms to the general orectolobiform pattern established by Goto [34] and among others refined by Soares and de Carvalho [36] and Stagg et al. [30]. The *m. adductor mandibulae* complex, consisting of four distinct portions with varying fibre orientations, is massive and firmly connects the Meckel's cartilage with the palatoquadrate, reflecting the need for a powerful jaw during suction feeding [7,34,36].

Of particular functional relevance are the hypobranchial muscles, especially the *m. coraco-branchialis* and the *m. rectus cervicis* (comprising the *m. coraco-arcualis* and the

m. coraco-hyoideus). These muscles are primarily responsible for the rapid expansion of the pharyngeal cavity during suction feeding [57], as demonstrated in the nurse shark *Ginglymostoma cirratum* (Bonnaterre, 1788) [15] by Motta et al. [8] and in the white-spotted bamboo shark *Chiloscyllium plagiosum* (Bennett, 1830) [58] by Wilga and Sanford [9]. In *H. ocellatum*, the paired *m. coraco-arcualis* originates from the anteroventral surface of the coracoid, and the *m. coraco-hyoideus* is closely associated with the pericardial membrane, suggesting a tight mechanical coupling between the pectoral girdle and the hypobranchial feeding apparatus. This linkage could have implications for the simultaneous use of paired fins during substrate-based prey capture, as the pectoral girdle must remain stable while the hypobranchial muscles generate the powerful suction forces needed to draw in prey.

The chondrocranium of *H. ocellatum* shows the typical orectolobiform features, including the prominent prefrontal fontanelle, the dorsally positioned orbits, and the paired nasal capsules connected by a thin internasal septum [34,38,39]. Several cranial nerve foramina were identified, including those for the nervus opticus (II), nervus trigeminus (V), nervus facialis (VII), nervus glossopharyngeus (IX), and nervus vagus (X). The arrangement of these foramina is consistent with descriptions in *C. plagiosum* [34] and *C. punctatum* [30], indicating a conserved bauplan across hemiscylliids. The jaw suspension in *H. ocellatum*, mediated by the hyomandibula connecting the mandibular arch to the otic capsule region, corresponds to the hyostylic type typical of orectolobiforms [34,42]. This suspension type allows some degree of jaw protrusion, which, together with the labial cartilages that funnel water flow during mouth opening, likely enhances suction performance.

4.2.3. Musculi Inclinatores Dorsales

One of the most significant findings of this study is the first detailed description of the *mm. inclinatores dorsales* in *H. ocellatum*. This muscle complex, consisting of a superficial (*m. inclinador dorsalis superficialis*) and a deep layer (*m. inclinador dorsalis profundus*), was recently redescribed by Medeiros et al. [28] for several galeomorph taxa, but their study did not include *H. ocellatum*. The present results confirm the general two-layered organisation that was reported by Medeiros et al. [28] for other galeomorphs and extend their comparative framework to Hemiscylliidae.

In the epaulette shark, both muscle layers overlap completely and are of similar length, a condition that differs from some other galeomorph taxa where the deeper layer extends in between the epaxial muscles [28]. The tight adhesion between the two layers, separated only by a thin layer of connective tissue, made their distinction during dissection particularly challenging. Here, the micro-CT scans proved indispensable, as the boundary between the two layers was clearly visible in cross-sectional images, a finding that underscores the complementary value of diceCT in myological studies of neoselachians.

The functional significance of these muscles likely lies in their capacity for fine motor control of the dorsal fins. With the superficial layer connecting the epaxial aponeurosis to the deep layer, and the deep layer in turn attaching to the fin radials, the *mm. inclinatores dorsales* form a system capable of precisely modulating dorsal fin position. This is consistent with findings from Maia and Wilga [31], who demonstrated, through electromyographic recordings, that the dorsal fins of *C. plagiosum* are actively controlled during turning manoeuvres. While the external morphology and arrangement of these muscles are broadly similar in *H. ocellatum* and *C. plagiosum*, the unique locomotor repertoire of the epaulette shark, including its pronounced walking gait, may place different functional demands on these muscles. The presence of the *mm. inclinatores dorsales* extending into the free rear tip of the dorsal fins was noted by Medeiros et al. [28] for galeomorphs in general. Our observations confirm this also for *H. ocellatum*, which may suggest that even the distal portions of the fin might be under active muscular control.

4.2.4. Pectoral Girdle and Fin Morphology

The pectoral girdle of *H. ocellatum* consists of fused scapulocoracoids with dorsally directed scapular processes and horizontal coracoid bars representing the general orectolobiform pattern [11,34]. The U-shaped structure with its central apron supports the pericardial cavity and is firmly embedded within the body musculature by connective tissue. Our findings confirm the observations of Goto et al. [11] in that the propterygium and mesopterygium are fused in *H. ocellatum*, resulting in only two distinct basal cartilages that are notably elongated and paddle-like [11,44]. This fusion, which is not observed in all orectolobiform genera [59], creates a broader articular surface that may increase the effective range of motion of the fin base—a feature potentially important for the large-amplitude fin excursions required during walking.

The pectoral fin musculature is well-developed, consistent with the flexible, mobile fin architecture that is characteristic for benthic sharks [11]. This contrasts with the stiffer, less mobile pectoral fins in fast-swimming pelagic species [60]. The morphology found in *H. ocellatum* thus aligns with its functional demands of substrate-based locomotion. The three-layered levator musculature (*m. levator pectoralis superficialis*, *inferior*, and *distalis*) is a distinctive feature compared to many other sharks, where only two layers are typically described (compare [11]). This additional subdivision provides increased degrees of freedom for fin elevation and rotation, enabling the graded postural adjustments necessary during a walking gait. The outermost *m. levator pectoralis superficialis*, originating from the scapula and inserting on the radial cartilages, acts as the primary dorsal elevator, while the underlying *m. levator pectoralis inferior* connects the coracoid to the mesopterygium, adding a medial pulling component. The innermost *m. levator pectoralis distalis*, though very thin, merges with the ventral part of the superficial layer and likely fine-tunes the position of the distal fin tip.

The strongly developed *m. depressor pectoralis* is particularly noteworthy. This fan-shaped muscle is thicker than its dorsal counterpart and becomes increasingly massive posteriorly [11]. In the context of walking locomotion, the depressor plays a critical role: it presses the pectoral fin against the substrate, generating the reaction force needed to pull the body forward. In comparison, the pectoral fins of more pelagic bamboo sharks, i.e., *C. plagiosum*, are also relatively flexible and serve for benthic station-holding, but their depressor musculature is less developed [61]. Hoffmann et al. [62] recently demonstrated that despite limited differences in external pectoral fin morphology, internal skeletal support differs considerably between benthic and pelagic sharks, with benthic species showing shorter and more rounded fins. In *H. ocellatum*, the elongated basal cartilages and multiple radial series provide a skeletal framework that enables the paddle-like fin to function as a weight-bearing propulsive organ during benthic locomotion, a functional role that is fundamentally different from the lift-generating function of pectoral fins in pelagic species [44,63,64].

4.2.5. Pelvic Girdle and Fin Morphology

The pelvic girdle of *H. ocellatum* follows the basic neoselachian bauplan, with a fused puboischiadic bar positioned ventrally and anterior to the cloaca [11,46]. The pelvic basiptyrgium is elongated and slightly curved, providing a broad base for the attachment of multiple radials and the anterior pelvic basal cartilage [44]. Unlike the pectoral fins, which possess a three-layered levator system, the pelvic fins exhibit single-layered levator and depressor muscles, supplemented by a distinct *m. adductor pelvicius*. This organisational difference between pectoral and pelvic fin musculature suggests that the two fin pairs play distinct but complementary roles during locomotion.

The *m. levator pelvicius* is notable for its connection to the fascia of the hypaxial body musculature, forming a direct mechanical link between the lateral body wall and the dorsal surface of the fin [46]. This arrangement likely enables a powerful pulling force that, during locomotion, helps to retract the fin and transfer propulsive force from the body wall to the substrate. The *m. depressor pelvicius*, divided into anterolateral and posterolateral portions, provides both robust anterior support and finer distal control. The anterolateral portion originates from the prepelvic process and extends over the anterior edge of the fin as a strong bundle, while the posterolateral portion is thinner and originates from the basipterygium. This dual architecture may allow the pelvic fins to produce both the forceful substrate contact needed for propulsion and the subtle rotational adjustments required for steering.

The *m. adductor pelvicius*, which is absent in the pectoral fin, covers the medial ventral side of the pelvic fin. Its presence adds a component of medial adduction that, together with the depressor, enables a more complex range of fin movements. In the context of walking, this additional muscle may help control the degree of lateral splay during the stance phase and facilitate the precise fin placement required for effective substrate-based locomotion.

The elongated basal cartilages, robust articulations, and highly developed musculature in both paired fin systems are consistent with the greater rotational potential of the pectoral and pelvic girdles described by [10]. Collectively, these features support the interpretation that the paired fin morphology of *H. ocellatum* reflects specific functional specialisations for a terrestrial-like mode of walking.

4.2.6. Vertebral Column

The vertebral column of *H. ocellatum* exhibits the typical asterospondyl calcification pattern of Orectolobiformes, giving the centra a star-like appearance in cross-section [34]. The division into monospondylous abdominal (associated with ribs), diplospondylous precaudal vertebrae, and caudal vertebrae with complete haemal arches follows the general shark pattern [34]. With 183–197 centra [13,44,47], *H. ocellatum* possesses a relatively high vertebral count compared to its body length, which contributes to the pronounced axial flexibility required for anguilliform undulation during swimming and the sinusoidal body bending observed during walking.

4.2.7. Evolutionary and Comparative Implications

The morphological features described in this study place *H. ocellatum* within a broader evolutionary context of locomotor adaptation in sharks. The majority of extant selachians are benthic [65], and the evolution of pectoral fin morphology was shown to be closely linked to habitat use, with benthic species possessing shorter, more rounded fins optimised for manoeuvring and station-holding, and pelagic species evolving longer, narrower fins for efficient lift generation [61–64]. Within Hemiscylliidae, *H. ocellatum* appears to represent an extreme along this benthic continuum, with morphological modifications in the paired fins, dorsal fin musculature, and cranial structures that go beyond what is typically observed in closely related taxa such as *C. punctatum* [30,34].

The substrate-based locomotion of *H. ocellatum* should be interpreted as a functional analogy rather than a homologous condition in comparison to early tetrapod locomotion [10]. Although the underlying musculoskeletal systems differ fundamentally, the locomotor strategy observed in *Hemiscyllium* represents a case of convergent evolution. As such, it provides a valuable extant model for exploring the biomechanical requirements of weight-bearing, substrate-associated locomotion. This convergence highlights how similar functional constraints can lead to the development of similar solutions in phylogenetically distant taxa [10,23,66,67].

However, it should be noted that the study is based on a limited number of specimens, which restricts the ability to assess intraspecific variation. Furthermore, the subadult developmental stage of the specimens may not fully represent the adult morphological condition. Future studies should include adult specimens and, ideally, quantitative morphometric analyses to determine the extent to which ontogenetic changes influence the structures described here.

5. Conclusions

By combining manual dissection with diceCT imaging, this study provides the most comprehensive musculoskeletal description of *H. ocellatum* to date, fulfilling the aim of establishing a detailed morphological framework for functional analyses. The pectoral and pelvic fin musculature is robust and well-differentiated, with skeletal support structures consistent with the functional demands of substrate-based, weight-bearing locomotion. The cranial musculature conforms to the general orectolobiform bauplan while exhibiting features associated with suction feeding, a key trait of the species' benthic lifestyle. In the dorsal fins, the *mm. inclinatores dorsales* are organised into two clearly distinct layers, a *superficialis* and a *profundus*, indicating a degree of functional specialisation that extends beyond passive stabilisation.

The integrative methodological approach proved essential for resolving structures that neither dissection nor micro-CT could adequately capture alone. In particular, the boundary between the *superficialis* and *profundus* layers of the *mm. inclinatores dorsales*, which was difficult to resolve during dissection, was clearly delineated in the diceCT cross-sections. This demonstrates the complementary value of combining dissection and contrast-enhanced micro-CT for anatomical investigations of chondrichthyans.

The detailed anatomical framework presented herein provides a foundation for future quantitative and functional investigations. Moreover, the data enable comparative work across Oreotolobiformes and, more broadly, contribute to understanding how appendicular morphology is shaped by the functional demands of substrate-based locomotion in aquatic vertebrates.

Author Contributions: R.-C.P., M.A.S. and J.K. conceived the study. F.H.M. provided the specimen. M.A.S. performed the specimen pre-treatment (iodine staining). R.-C.P. performed the dissection, processed the scan data and produced the figures with advice from M.A.S. R.-C.P. performed the morphological investigation and wrote the manuscript. M.A.S. advised throughout. M.A.S., J.K. and F.H.M. edited and added to the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Ethical review and approval were waived for this study because no live animals were used. All specimens were captive-bred individuals obtained from De Jong Marinelife (Spijk, The Netherlands) after natural death, and all analyses were conducted on frozen post-mortem material.

Data Availability Statement: The original contributions presented in this study are included in the article. The raw data supporting the conclusions of this article will be made available by the authors on request.

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