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"The distribution and morphology of dermal and dental denticles in different stages of ontogeny in *Chiloscyllium cf. punctatum*, *Scyliorhinus canicula* and *Scyliorhinus retifer*"

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Abstract

This study documents the overall distribution and morphology of dermal and dental denticles in different stages of ontogeny in *Chiloscyllium cf. punctatum*, *Scyliorhinus canicula* and *Scyliorhinus retifer*. The distribution, morphology, and development of dermal and dental denticles in *Scyliorhinus* and *Chiloscyllium* have never been described in detail before but are important for conclusive classification and for understanding behavioural attributes. In addition, the association of dermal denticles with pit organs and ampullae of Lorenzini is documented. Chromatophores are shown to be located in the pulp cavity of a tooth.

The morphology of dermal denticles in *Chiloscyllium cf. punctatum*, *Scyliorhinus canicula* and *Scyliorhinus retifer* are consistent with Oeffner and Lauder (2012), who state that dermal denticles are constructed in ways to reduce frictional drag and mechanical abrasion. As claimed by Motta et al. (2012), placoid scales were found to have different morphologies across the body. In all specimens ovate, leaf-shaped dermal denticles could only be detected along the ventral side, in the ventral snout area, and along the edges of all fins. Pointy, monocuspid or tricuspid denticles developing later in ontogeny cover the dorsal and lateral sides of the shark species as well as both sides of the fins. The eruption of teeth out of dentigerous cysts could be observed in all three species, as could the eruption of placoid scales from papillae. Moreover, all three species develop Palaeozoic denticles, while large dorsal denticles (LDD) develop only in *Scyliorhinus*.

In *Chiloscyllium cf. punctatum* (TL= 138 mm; TL=223 mm) the number of dental series in the oral cavity increases with maturity, whereas the number of dental rows decreases. In *Scyliorhinus retifer,* the youngest specimen (TL=80 mm) has 13 rows of teeth in one to two series, whereas the eldest specimen (TL=155 mm) has 18-19 rows of teeth in two to three series. Thus, *Scyliorhinus retifer* show an increase in rows and series with maturity. A few younger specimens of *Scyliorhinus retifer* display a higher number of rows (25 rows in TL=141 mm) and series (up to five series at some rows in TL=142 mm) than the eldest specimen. Moreover, specimens of *Scyliorhinus retifer* illustrated that teeth transform from tricuspid to pentacuspid during ontogeny, which is consistent with the development of teeth studied in Rasch et al. (2016). The two largest specimens in *Scyliorhinus canicula* reveal an increase in dental rows with development. *Scyliorhinus canicula* with a TL=103 mm has 16 rows of teeth in one to two series, whereas *Scyliorhinus canicula* with a TL=105 mm displays 21 rows of teeth. Not all pit organs as described in Peach and Marshall (2000) could be located, but an association of dermal denticles with pit organs is noticeable. In addition, ampullae of Lorenzini are either surrounded or covered by dermal denticles. Chromatophores could be detected in many parts of a tooth, in all three species.

Zusammenfassung

Diese Studie befasst sich mit der Verteilung und Morphologie von Zahnstrukturen in der Haut und im Mund, in verschiedenen Phasen der Ontogenie, von *Chiloscyllium cf. punctatum*, *Scyliorhinus canicula* und *Scyliorhinus retifer*. Die Verteilung, Morphologie und Entwicklung von Zahnschuppen und Zähnen von *Chiloscyllium* und *Scyliorhinus* wurde noch nie im Detail beschrieben, obwohl diese Strukturen zusätzliche Eigenschaften für eine zuverlässige Identifikation darstellen und ebenso viel über Verhaltensweisen aufdecken. Zusätzlich wird die Assoziation von Zahnschuppen mit Grubenorganen (pit organs) und Lorenzinischen Ampullen dokumentiert. Chromatophoren sind vermutlich in der Pulpahöhle der Zahnschuppen zu finden.

Die Morphologie der Zahnschuppen von *Chiloscyllium cf. punctatum*, *Scyliorhinus canic*ula und *Scyliorhinus retifer* stimmt mit Oeffner and Lauder (2012) Hypothese überein, dass Zahnstrukturen der Haut spezifisch geformt sind, um die Reibung und den Widerstand im Wasser zu reduzieren. Zusätzlich behauptet Motta et al. (2012), dass Zahnschuppen unterschiedlich geformt sind entlang des Körpers. Alle Individuen zeigten eiförmig-lanzettartig, blattgeformte Zahnschuppen auf der ventralen Seite, an der Schnauze und entlang der Ränder der Finnen. Zugespitzte, einhöckerige oder dreihöckerige Zahnschuppen, welche erst später in der Ontogenie gebildet werden, bedecken die dorsalen und lateralen Regionen, aber auch beide Seiten der Finnen.

In allen Arten gehen die Zähne im Mund aus dentogenen Zysten hervor, wobei Zahnschuppen sich aus Papillen der Haut erheben. Alle Arten entwickeln sogenannte paläozoische Zahnschuppen entlang des posterioren Endes der Schwanzflosse, wohingegen große dorsale Zahnschuppen (LDD) nur bei *Scyliorhinus* ersichtlich sind.

Die Anzahl an Zahnserien in *Chiloscyllium cf. punctatum* (TL=138 mm; TL=223 mm) erhöht sich, wobei die Anzahl an Zahnreihen mit zunehmenden Alter abnimmt. Das jüngste Individuum von *Scyliorhinus retifer* (TL=80 mm) hat 13 Zahnreihen in ein bis zwei Zahnserien und das älteste Individuum (TL= 155 mm) weist 18-19 Zahnreihen in zwei bis drei Zahnserien auf. *Scyliorhinus retifer* zeigt somit eine Erhöhung der Zahnreihen und Zahnserien mit zunehmenden Alter. Jedoch haben jüngere Individuen als TL=155 eine höhere Anzahl an Zahnreihen (25 Reihen in TL=141 mm) und Zahnserien (fünf Zahnserien in TL=142 mm). Die Entwicklung der Zähne in *Scyliorhinus retifer* zeigte eine Transformation von einhöckerigen zu dreihöckerigen Zähnen im Laufe der Ontogenie, was mit der Untersuchung von Rasch et al. (2016) übereinstimmt.

Die ältesten Individuen von *Scyliorhinus canicula* zeigen eine Erhöhung der Zahnreihen mit fortschreitender Ontogenie. *Scyliorhinus canicula* mit TL=103 mm hat 16 Zahnreihen in ein bis zwei Zahnserien und *Scyliorhinus canicula* mit TL= 105 mm hat 21 Zahnreihen.

Nicht alle Grubenorgane konnten lokalisiert werden, wie in Peach and Marshall (2000) dokumentiert, jedoch konnte eine Assoziation von Zahnschuppen mit Grubenorganen festgestellt werden. Zudem zeigten die Lorenzinischen Ampullen auch eine Assoziation mit den umliegenden Zahnschuppen. Chromatophoren wurden in der Pulpahöhle einer Art entdeckt, sowie in anderen Teilen der Zahnschuppe.

Keywords: placoid scales, teeth, pit organs, ampullae of Lorenzini, *Scyliorhinus retifer*, *Scyliorhinus canicula*, *Chiloscyllium punctatum*, ontogeny, distribution of denticles, arrangement of teeth, development of denticles, Palaeozoic denticles, chromatophores

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Introduction

In this study, the overall distribution and morphology of dermal and dental denticles in different stages of ontogeny in *Chiloscyllium cf. punctatum* (J. P. Müller & Henle, 1838), *Scyliorhinus canicula* (Linnaeus, 1758) and *Scyliorhinus retifer* (Garman, 1881) was addressed. The aim of this study was to understand the development of chondrichthyan dermal and dental denticles across their body from young embryonic specimens without denticles to specimens with erupted and fully exposed denticles. The distribution, morphology, and development of dermal and dental denticles in *Scyliorhinus* and *Chiloscyllium* have never been described in detail before, but are significant for conclusive classification and for understanding behavioural attributes. In addition, the association of dermal denticles with pit organs and ampullae of Lorenzini was documented. Chromatophores are reported to be within the pulp cavity of a tooth, which was also investigated.

Detailed knowledge of the morphology of dermal and dental denticles in *Chiloscyllium* and *Scyliorhinus* would be beneficial to classifying specimens of these genera without relying only on anatomical and morphological characteristics. Valenzuela et al. (2008) proposed that if external morphology is not conclusive for classification then dermal denticles and teeth could be used instead as reliable characters. Moreover, dermal and dental denticles which accumulate in the oceanic bed could be classified with increased efforts of descriptive dental science in elasmobranchs. The classification of denticles could help to understand which elasmobranch species encountered specific marine habitats long after they have disappeared (Gravendeel et al., 2002).

The morphology of denticles is also of great interest to aerodynamics as the skin texture of elasmobranchs is applied to the outer surfaces of aircrafts in order to reduce drag during flight (Ball, 1999).

Additionally, the type of oral dentition reveals important information about the type of prey elasmobranchs consume, namely whether they prey on soft or hard bodied tissue. Each type of elasmobranch has a specific type of dentition for a specific type of prey (Shimada, 2002)

Scyliorhinus canicula is a very good model organism for this specific study as its size and accessibility make imaging and analysis easier. *Scyliorhinus canicula* is harvested along most coastal areas in Europe and is the only known species within the elasmobranchs from which individuals at any stage of development can be obtained in abundance at any time of the year. Even though fertilisation is internal, eggs are laid at an early stage of development and are able to develop normally in oxygenated water in laboratories (Ballard et al., 1993). In contrast, *Chiloscyllium punctatum* has only recently been introduced as a model organism for jaw and tooth development. *Chiloscyllium punctatum* also occurs in coastal areas of the Indo-West Pacific from Japan to northern Australia, and also breeds in captivity and lays eggs (Fritz et al., 2015).

Chondrichthyan fishes are equipped with teeth and dermal denticles (Figure 1), also known as placoid scales, which cover the skin and are very similar to teeth in all vertebrates (Parker, 1999). Teeth contain a core of dentin covered by an enamelled layer formed by odontoblasts and ameloblasts (Pole, 1976, as cited by Whitenack et al., 2011). Some elasmobranchs have a pulp cavity (orthodont), whereas others feature a root extending to the centre of the crown (osteodont) (Cappetta, 1987 and Compagno, 1988, as cited by Whitenack et al., 2011). Enamel contains 96% inorganic material and dentin 70%. The inorganic material contains hexagonal hydroxyapatite and small quantities of sodium and magnesium. While dermal denticles are odontodes located outside the buccal cavity, dental denticles are odontodes located inside the buccal cavity (Gutiérrez-salazar and Reyes-gasga, 2003).

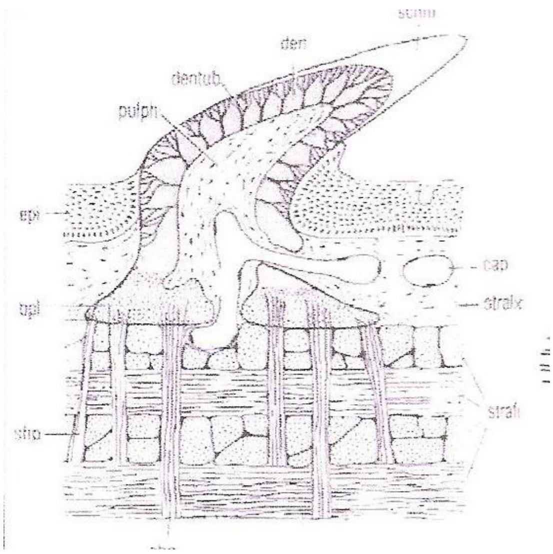

Figure 1. Placoid scale: contains basal plate, pulp cavity, dentine and enamel; embedded into the stratum laxum by Sharpey’s fibres (Mickoleit, 2004).

Elasmobranch skin is divided into a thin layered epidermis and a thick layered dermis consisting of the top layer, stratum laxum and the bottom layer, stratum compactum with collagen I fibres (Figure 2). Placoid scales feature a crown, neck, and base and the enameloid layer covers the dentin from the tip of the crown down to the neck. The bony base is embedded within the stratum laxum by attachment fibres called Sharpey’s fibres. Additionally, a pulp cavity is located within the denticle (Kimura et al., 1981; Lingham-Soliar, 2005a, b; Hwang et al., 2007; Meyer and Seegers, 2012; as cited by Motta et al., 2012).

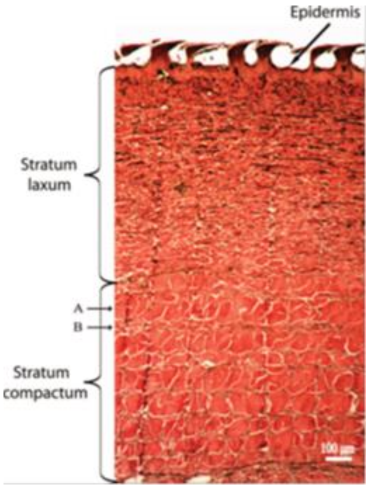

Figure 2. Sagittal section through the skin of *Isurus oxyrinchus* (shortfin mako): collagen fibres are stained red (Motta et al., 2012).

The colouration of the integument depends on pigment cells referred to as chromatophores, which occur either in stratum laxum, subcutis, or in both layers of the integument (Figure 3). Chromatophores might have been located within the pulp cavity of dermal denticles. All poikilothermic fish have chromatophores including various types such as melanophores (black and brown pigments), xanthophores (yellow and orange pigments), erythrophores (red, orange, yellow pigments) and cyanophores (blue pigments) (Farrell, 2011).

Melanophores produce melanin, which yields a darkish colouration, but in albinism, a lack of melanophores produces light pigmentation as has been described in *Chiloscyllium plagiosum*. Other pigment cells such as xanthophores are not reduced in the retinal pigment epithelium and therefore albinos display red eye colouration (Clark, 2002).

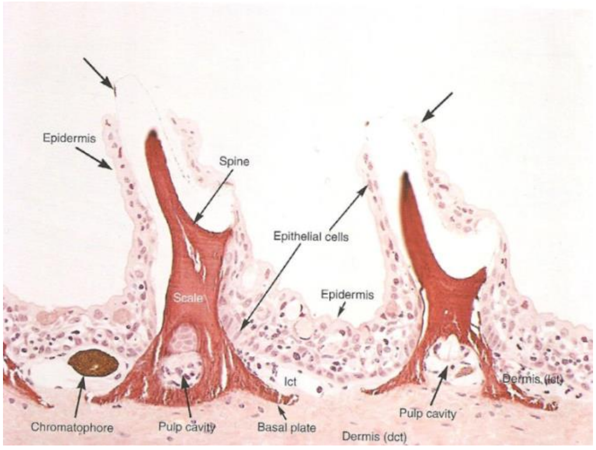

Figure 3. Histological section of fin tissue from a chondrichthyan containing chromatophores within the dermal skin layer (Farrell, 2011).

Additional morphological structures imbedded within the epidermis and closely related to dermal denticles are pit organs. Pit organs are sensory organs and they are scattered all over the body, but are mainly located dorsally. The pit organs are classified as part of the lateral line system, which is responsible for water motion detection. The lateral lines are arrangements of several linked neuromasts, which are mechanoreceptors, found either in the skin surface or enclosed in subsurface canals. Only in elasmobranchs, free neuromasts are referred to as pit organs. Neuromasts consist of one kinocilium and several stereocilia anchored within a hair cell body and covered by a gelatinous structure called a cupula (Peach and Rouse, 2000).

According to (Peach and Marshall, 2000) grey spotted catshark *(Asymbolus analis)* should have spiracular pit organs (spo), mandibular pit organs (mpo), dorsal pit organs (dpo), umbilical pit organs (upo), and supratemporal pit organs (stpo). Spiracular pit organs are located behind the spiracles. Mandibular pit organs are located laterally in front of the gills and ventrally below the jaw in shape of a slope. Dorsal pit organs occur along the dorsal side right towards the end of the caudal fin. Umbilical pit organs are located ventrally across the pectoral fin and supratemporal pit organs behind the orbital bone (Figure 17). In contrast, *Chiloscyllium punctatum* does not possess any supratemporal pit organs (Figure 4a).

In 1978, Mathewson (1978) depicted the lateral line canals and ampullae of Lorenzini of *Scyliorhinus canicula* in *Sensory Biology of Sharks, Skates and Rays* (Figure 4b). Ampullae of Lorenzini, bottle-shaped pits filled with jelly, are scattered all over the shark's head. These ampullae are capable of detecting a change in voltage and thereby sensing electrical pulses emitted by another animal's muscle activity (Parker, 1999).

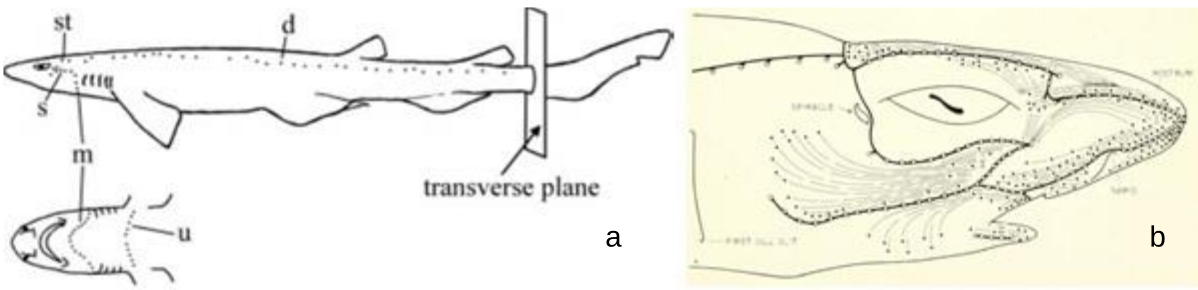

Figure 4a. Distribution of pit organs in *Asymbolus analis* (Peach and Marshall, 2000); 4b. Lateral line and ampullae of Lorenzini in *Scyliorhinus canicula* (Mathewson, 1978).

Pit organs are often associated with dermal denticles (Figure 5): pit organs are found posterior to a denticle or in between denticles as in *Heterodontus*. In carcharinid sharks, denticles are arranged anterior-posteriorly in the anterior-ventral region of the gills.

Sometimes pit organs are not associated with denticles, as in *Pastina*. *Rhinobatos* has two to seven denticles arranged around the pit organ whereas *Trygonorrhina* is surrounded by tightly packed denticles (Peach and Marshall, 2009)

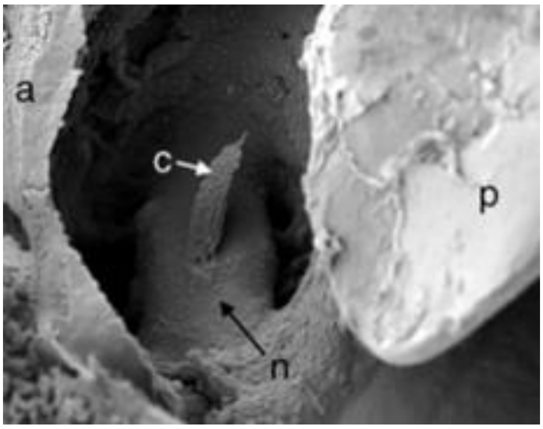

Figure 5. Morphology of pit organs: cupula (c), neuromast (n), anterior (a) and posterior (p) denticle (Peach and Marshall, 2009).

Placoid scales are very different in morphology across the body. The snout features dermal denticles that are roundish with nerve connections, the front edges of the fins are diamond-shaped to cut through the water, the ventral side has shield-shaped denticles to prevent getting hooked on the substrate, and the flanks feature keeled denticles (Parker, 1999).

More specifically, *Isurus oxyrinchus* (shortfin mako shark) features smooth denticles on the leading edge of the pectoral and caudal fin, whereas long narrow scales are seen at the trailing base of the pectoral fin and ventral side (Figure 6 and 7). Scales with a triangular shaped base are visible on both lateral sides (Motta et al., 2012).

In contrast, all scales in *Carcharinus limbatus* (blacktip reef shark) have a rhomboid shaped base except for the scales on the leading edge of pectoral and pelvic fin, which feature a smooth surface. The trailing edge scales of the pectoral fin possess riblets (Motta et al., 2012). Contributing to drag reduction, diverging riblets across the scale surface increase flow velocity as fluid medium flows towards the centre (Koeltzsch, 2002).

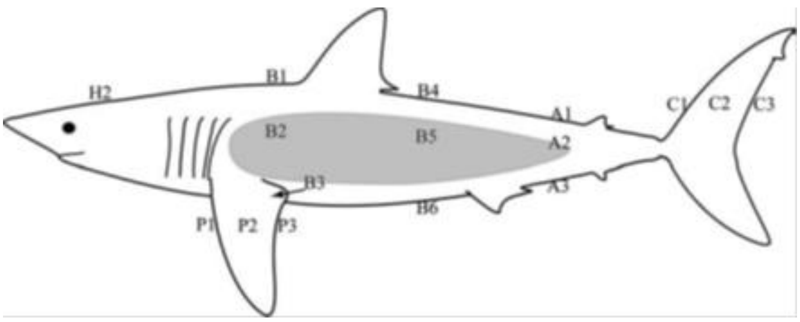

Figure 6. Lateral view of shortfin mako (*Isurus oxyrhinchus*): showing the regions of dermal denticles sampled (Motta et al., 2012).

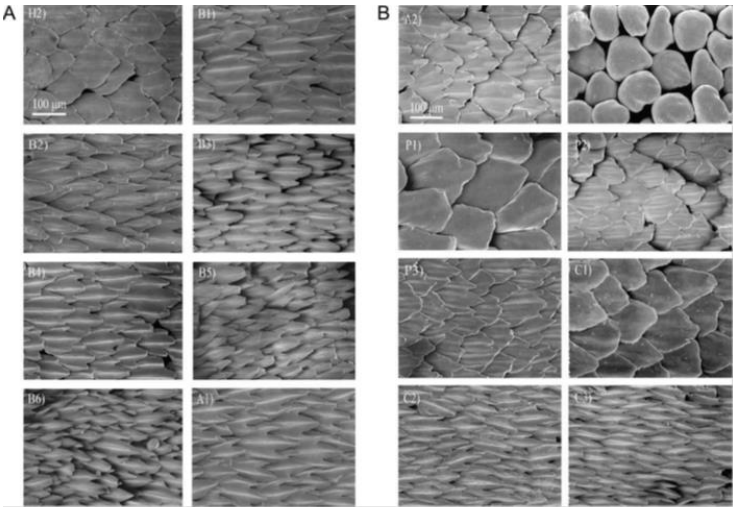

Figure 7. Dorsal view of placoid scales from a 158 cm TL male shortfin mako (*Isurus oxyrinchus*) obtained from sixteen regions outlined in Figure 6 above (Motta et al., 2012).

Additionally, the angle of dermal denticles determines the swimming speed. Thus *Isurus oxyrhincus* have highly erectable scales on their flanks (greatest flexibility of scale at 50°), which allow rapid burst swimming speed (Bechert et al., 1985; Lang et al., 2011, as cited by Motta et al., 2012).

Shark skin is constructed in ways that reduce frictional drag and mechanical abrasion and protects against ectoparasites and predators (Parker, 1999). Dermal denticles reduce drag in so far as they establish a suction force which enhances thrust and reduces drag while swimming (Oeffner and Lauder, 2012).

Southall and Sims (2003) discovered in *Scyliorhinus canicula* that shark skin also had a function in food processing. *Scyliorhinus canicula* feature posterior pointing and hook-like dermal denticles on the caudal peduncle. Thus, by grabbing the prey item with its mouth and getting it hooked in the denticles simultaneously, a rapid return flick would result in a tiny piece of prey item being bitten off. This should help embryos to process food items too big to handle.

The tooth germ develops from ectodermal cells of the first pharyngeal arch and from ectomesenchymal cells from the neural crest. The tooth germ consists of three parts: the enamel organ, the dental papilla as well as the dental sac. The dental papilla contains cells that develop into odontoblasts. Mesenchymal cells in the dental papilla form the tooth pulp (Thesleff et al., 1995). The dental sac contains osteoblasts and cementoblasts which develop the alveolar bone and the cementum of the tooth (Ross and Wojciech, 2010).

Tooth development is divided into the initiation stage, bud stage, cap stage, bell stage and the final maturation (Figure 8a). The first evidence of tooth development is the dental lamina, a band of epithelial tissue. The dental lamina develops when the oral ectoderm proliferates more than the adjacent cells. The development of the dental lamina is characterised as the initiation stage. When the epithelial cells proliferate and invaginate into the ectomesenchyme of the jaw the bud stage is introduced establishing a tooth bud. Once the ectomesenchymal cells stop producing extracellular substances they are referred to as the dental papilla and initiate the cap stage. Subsequently, the tooth bud grows around these ectomesenchymal cells establishing a cap which becomes the enamel organ. The enamel organ will produce enamel and the dental papilla will produce dentin and pulp. During the bell stage the enamel organ becomes bell-shaped and histodifferentiation and morphodifferentiation take place. The next stage, the maturation stage, produces the hard tissue such as dentin and enamel (Nanci, 2013). Tucker and Sharpe (2004) also outlined the stages of tooth development (Figure 8b).

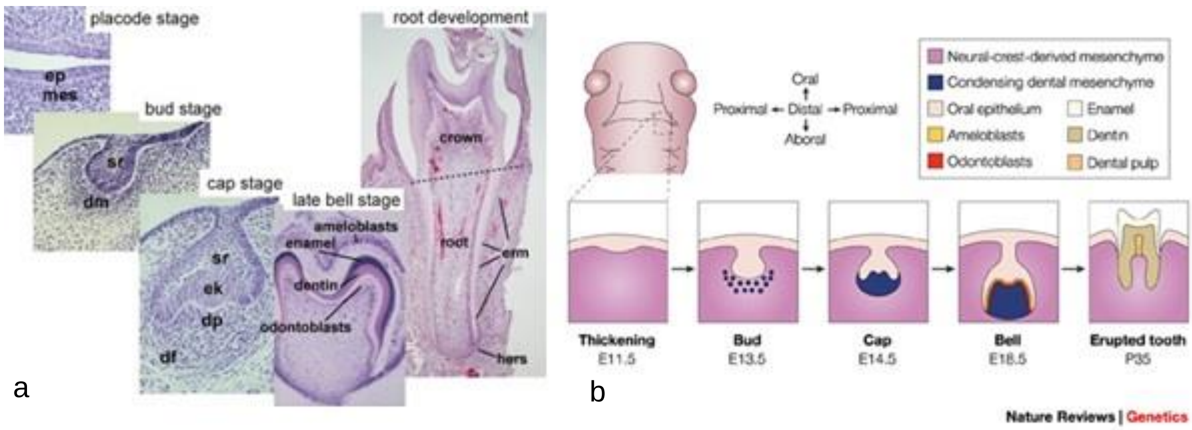

Figure 8a. Stages of tooth development (Thesleff et al., 1995). Figure 8b. Stages of tooth development (Tucker and Sharpe, 2004)

Polyphyodont dentition is a plesiomorphic characteristic within the chondrichthyans and describes that sharks and rays display a “many for one system” in which teeth are constantly replaced with multiple denticles (Tucker and Sharpe, 2004). An increase of proliferation of the oral epithelium establishes the termed dental lamina from which a tooth emerges (Buchtová et al., 2012) Originally, dental lamina is not essential for odontogenesis, yet for tooth regeneration it indeed is crucial (Rasch et al., 2016).

The growth and morphogenesis of cusps is regulated by the enamel knot, which represents the organising centre and expresses sonic hedgehog, bone morphogenetic proteins, and fibroblast growth factors for regulation of tooth development (Vaahtokari et al., 1996). In terms of deficiency the bone morphogenetic protein ectodin is responsible for highly enlarged enamel knots, highly altered cusp patterns, and extra teeth in mice (Yoshiaki et al., 2005).

The skin of chondrichthyans is constantly replaced with new dermal denticles. Accordingly, dermal denticles are discarded and are immediately exchanged by new dermal denticles

(Ebert et al., 2006). As a result, not only dental denticles but also dermal denticles are replaced, although not steadily.

The development of dermal and dental denticles have been studied in detail in embryonic specimens of *Scyliorhinus canicula* (Reif, 1980). The denticles were characterised in 45 stages, from the stage where the epidermis started to thicken to the stage where the specimens hatched.

Recently, Rasch et al. (2016) used *Scyliorhinus spp*. as a model organism in order to look at the expression of genes homologous to those expressing tooth initiation, morphogenesis and regeneration in bony vertebrates. These authors state that the first generation of teeth functions as a place maker for forthcoming generations of teeth, and are incomplete in their morphology; only teeth arriving later are complete and regularly shaped. The first teeth emerge tricuspid on either side of the symphysis (Figure 9) and then continue to develop to pentacuspid in juveniles and multicuspid in adult specimens (Rasch et al., 2016).

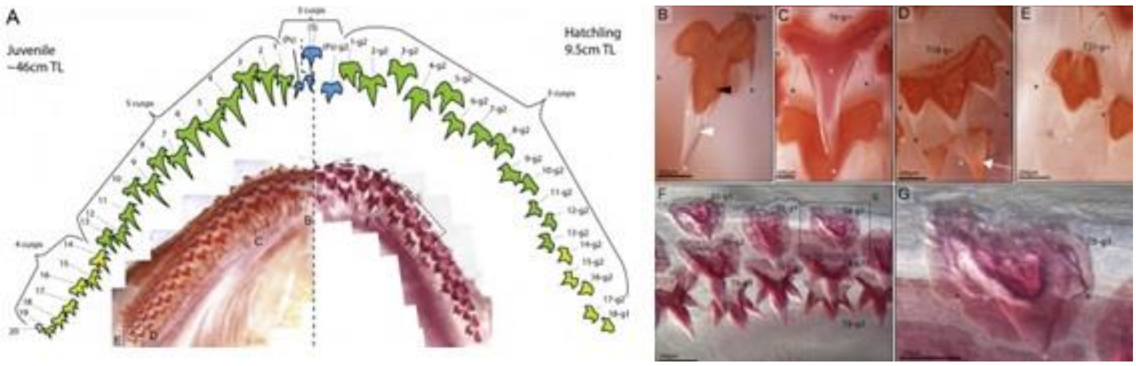

Figure 9. Dental formula based on body length in *Scyliorhinus spp*. (Rasch et al., 2016)

Additionally, Shimada (2002) described the dental denticles in embryonic and adult lamniformes including *Carcharias taurus* (sand tiger shark), *Pseudocarcharias kamoharai* (crocodile shark), *Alopias pelagicus* (pelagic thresher), *Alopias superciliosus* (bigeye thresher), *Alopias vulpinus* (common thresher), *Isurus oxyrinchus* (short fin mako shark), and *Lamna nasus* (porbeagle). Valenzuela et al. (2008) characterised the dermal and dental denticles in *Schroederichthys chilensis* (redspotted catshark), *Halaelurus canescens* (dusky catshark) and three Squaliforms, *Centroscyllium granulatum* (granular dogfish), *Deania calcea* (birdbeak dogfish) and *Squalus acanthias* (spiny dogfish).

Chiloscyllium plagiosum has even developed a strategy to prey on soft and hard bodied organism termed duophagy. This species has flattened cusps for consuming hard prey and spike-like cusps for grabbing soft tissue. The rotation and depression of denticles in the oral cavity demands tooth attachment to be flexible within the gum tissue, and thus allows dental movement in the sagittal and frontal planes. While processing harder prey, the spike-like cusps, which are functional for soft bodied prey, are passively depressed (Figure 10). After the cusp has rotated inwards towards the oral cavity with its broader labial face parallel to the surface of the jaw, it forms a crushing surface in order to consume hard prey. This strategy allows cusps to stay unharmed (Ramsay and Wilga, 2007).

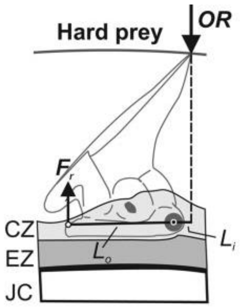

Figure 10. *Chiloscyllium plagiosum*: Force of hard prey on cusp apex pushing denticle into a flat position (Ramsay and Wilga, 2007).

Johanson et al. (2008) located irregular shaped dermal denticles at the posterior end of the caudal fin in specimens of *Scyliorhinus canicula*. These dermal denticles are referred to as Palaeozoic denticles in reference to shark specimens from Ordovician to Silurian, which featured these denticles permanently. Specimens of *Scyliorhinus canicula* briefly develop these dermal denticles early in ontogeny before they disappear again. Thus Palaeozoic denticles in *Scyliorhinus* may be considered vestigial dermal denticles at the posterior end of the caudal fin. Palaeozoic denticles occur in two rows on each side along the posterior end of the notochord. Usually the upper dorsal row develops one additional irregular shaped dermal denticle compared to the lower ventral row (Figure 11a). The crown of each Palaeozoic denticle overlaps more ventrally than dorsally emphasising their characteristic irregular shape (Figure 11b).

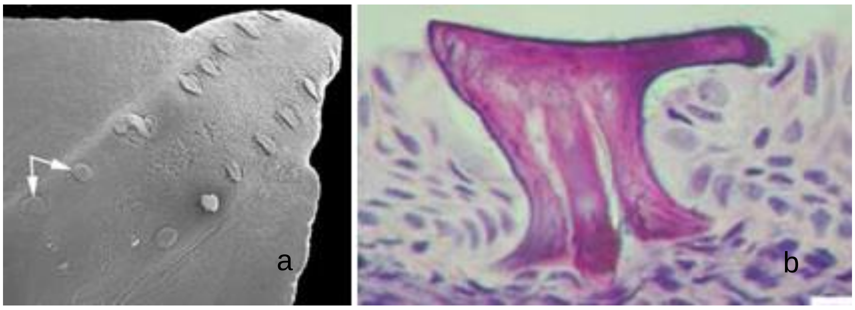

Figure 11a. Palaeozoic denticles in *Scyliorhinus canicula:* Dorsal and ventral row along posterior end of notochord. 11b. Crown of each Palaeozoic denticle overlaps more ventrally (Johanson et al., 2008).

Materials and Methods

To analyse the distribution, development, and morphology of dermal and dental denticles in catsharks and bamboo sharks, seven specimens of *Scyliorhinus canicula*, eight specimens of *Scyliorhinus retifer*, and five specimens of *Chiloscyllium cf. punctatum* were closely studied. The specimens of *Chiloscyllium cf. punctatum* were brought from the Department of Palaeontology, University of Vienna which were initially bred in the “House of the Sea,” a Viennese aquarium. The species of *Chiloscyllium* could not be determined with certainty but was identified as brown banded bamboo shark (*Chiloscyllium punctatum*) based on morphological characteristics. *Scyliorhinus canicula* and *Scyliorhinus retifer* belong to the embryonic fish collection in the Theoretical Biology Department. Some specimens of *Scyliorhinus* were previously stained with iodine in water or 95% ethanol. All specimens were transferred to 70% ethanol in order to make Alizarin red S staining consistent. The *Chiloscyllium cf. punctatum* remained in an aqueous solution and reliably stained with Alizarin red S.

Ethanol-Alizarin red S stains bony structures such as dermal and dental denticles, as it dyes the calcium-containing tissues red (Figure 12 a). Thus, all specimens were put into a basin filled with 70% ethanol and enough ethanol-Alizarin red S to give the solution a urine-yellow colour (Springer and Johnson, 2000). This was intended to make all denticles better visible for inspection under the binocular microscope. The documentary evidential images were taken with the ProgRes C5 camera attached to a binocular microscope.

The total length of each embryonic specimen in *Chiloscyllium* and *Scyliorhinus* was measured in order to determine the stage of development, as body length in animals is a reliable indicator for maturity. Reif (1980) staged each embryonic specimen according to its total body length in the interest of documenting the development of denticles in *Scyliorhinus canicula*. The ages of some specimens of *Scyliorhinus retifer* were originally labelled in months. The total length of specimens in *Scyliorhinus canicula* reached from TL=46 mm to TL=105 mm, in *Scyliorhinus retifer* from TL=80 mm to TL=155 mm and *Chiloscyllium cf. punctatum* from TL=33 mm to TL=223 mm.

Every specimen was subjected to an inspection of its oral denticles in which the type of morphology and dental formula was noted. Thus, the number of dental rows and dental series in upper and lower jaw were documented (Figure 12 b). Dental rows are defined as the teeth along the line of the jaw and dental series as the teeth counted from the front of the jaw inwards (Heemstra and Heemstra, 2004). According to Smith et al. (2013) dental rows are referred to as vertical rows or tooth families and dental series as horizontal rows (Figure

12c). In young and embryonic specimens this required either high-resolution microCT (Zeiss Xradia MicroXCT) scans or lower resolution scans with the Skyscan 1174 (Bruker).

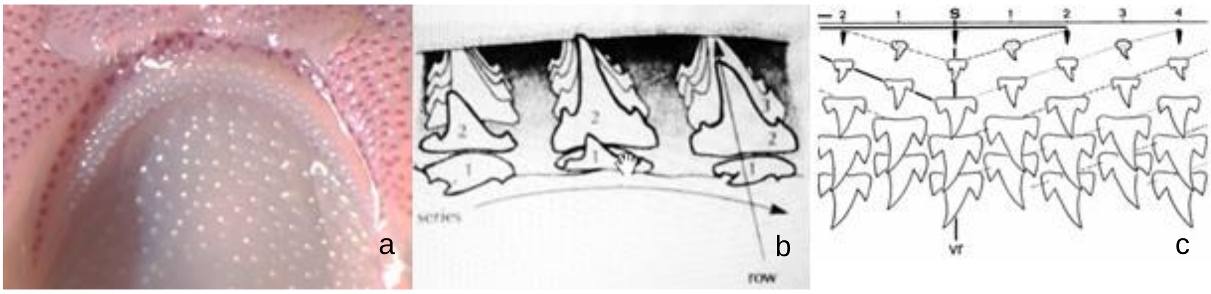

Figure 12a. Alizarin red S staining in *Scyliorhinus canicula* TL=105 mm (stereomicroscope). 12b. Dental rows and dental series in cartilaginous fish (Heemstra and Heemstra, 2004). 12c. Dental arrangement: dental rows are shifted in the oral cavity (Smith et al., 2013).

For microCT scans, specimens were stained in PTA (phosphotungstic acid) solution and imbedded in agarose to immobilise them (Metscher, 2009 and Schulz-Mirbach et al., 2013). Larger specimens were studied with a binocular microscope and the mouth was spread open with a self-made mouth spread, namely a tweezers or piece of sponge. Additionally, scans without lower jaw dissection enabled a closer look at the occlusion of embryonic specimens in catsharks and bamboo sharks.

MicroCT scans were reconstructed in XMReconstructor and viewed in XM3DViewer in order to take snapshots of the morphology of dermal and dental denticles.

Dermal denticles were inspected at eight different locations across every specimen's body including snout, dorsal fin, pectoral fin, pelvic fin, anal fin and caudal fin (Figure 13). Moreover, the development of distinctive dermal denticles such as Palaeozoic denticles and large dorsal denticles (LDD) along the dorsal side of the back, resembling two ridges in *Scyliorhinus,* were documented. In the process, skin tissue was dissected and scanned with microCT, in case Alizarin red S staining was not consistent. Otherwise a binocular microscope was sufficient to inspect denticles.

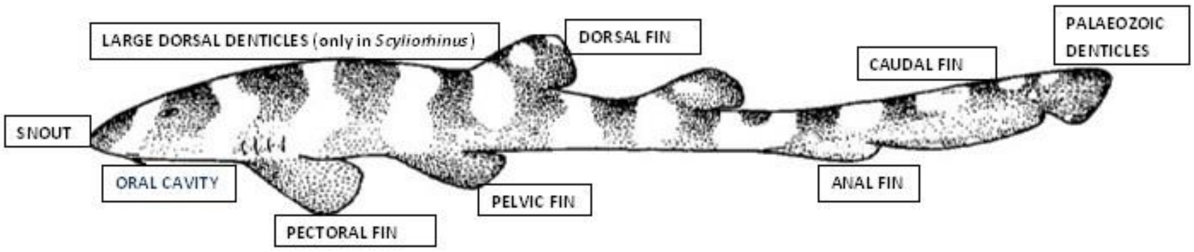

Figure 13. Sample sites of denticles in *Chiloscyllium cf. punctatum*: snout, dorsal fin, pectoral fin, pelvic fin, anal fin and caudal fin (Compagno, 1984)

Likewise, the association of pit organs or ampullae of Lorenzini with dermal denticles was inspected with a binocular microscope and microCT. The chromatophores were studied with a compound microscope (Zeiss Axio Imager). Tissue samples for detecting chromatophores in dermal denticles were dissected dorsally behind the head in one specimen of each species.

Results

Additional pictures and notes are available on the University of Vienna Phaidra resource at http://phaidra.univie.ac.at/o:454143

Dermal and dental denticles in *Chiloscyllium cf. punctatum*

The development of dermal and dental denticles in *Chiloscyllium cf. punctatum* was inspected on five specimens (Figure 14).

The youngest specimen with a TL= 33 mm features a completely smooth-surfaced skin. Dermal and dental denticles have not developed yet. Palaeozoic denticles can be detected underneath the skin. In *Chiloscyllium cf. punctatum* with a TL= 60 mm, Palaeozoic denticles which are pear-shaped at this stage, are fully exposed (Figure 15a). Dermal and dental denticles have still not emerged and the skin remains smooth.

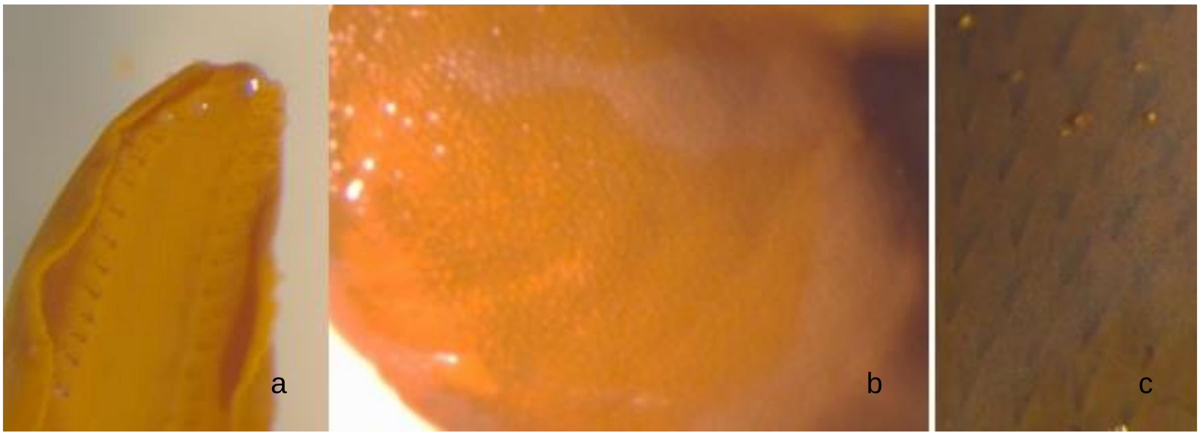

Figure 15a. Palaeozoic denticles in *Chiloscyllium cf. punctatum* with a TL=60 mm; 15b. Papillae in *Chiloscyllium cf. puncatum* (TL=94 mm); 15c. Cusps lying flat underneath the skin on the dorsal side in *Chiloscyllium cf. punctatum* with a TL=94 mm (stereomicroscope).

In *Chiloscyllium cf. punctatum* with a TL= 94 mm the skin is characterised by a goose pimple-like structure, referred to as papillae (Figure 15b) from which dermal denticles will erupt. All fins are covered with papillae and the dorsal side shows cusps lying flat underneath the skin (Figure 15c). The oral cavity contains several dentigerous cysts and taste buds, but no teeth. The Palaeozoic denticles have developed additional lateral cusps and feature a tricuspid morphology. The dorsal row has 20 Palaeozoic denticles imbedded whereas the ventral row reveals 14.

Chiloscyllium cf. punctatum with a TL=138 mm is covered with dermal denticles. The snout area is covered with ovate to roundish leaf-shaped dermal denticles in which the denticles located dorsally are highly erected, and the denticles ventrally are lying flat. At the tip of the snout the denticles radiate from the centre into all directions and with increasing distance from the centre they turn to face the posterior end. The dental arrangement in one quadrant of the lower jaw displays 13 rows in four series and the upper jaw 15 rows in two series (Figure 16). The first dental row starts at the dental midline. All teeth in the oral cavity are tricuspid and feature one longish central cusp and one short lateral cusp on each side. Skyscans of the dental occlusion discovered that the basal plates of teeth in the upper jaw touch those of the lower jaw. Thus, the cusps of all oral denticles point inwards.

Moreover, the dental rows are shifted as described in Smith et al. (2013). The dental row at the symphysis is shifted from the second neighbouring dental row. However, the third dental row is in alignment with the first row. This pattern does not necessarily continue to the posterior denticles. Sometimes two or more neighbouring rows are in alignment. Likewise, *Scyliorhinus* display an offset of dental rows in the oral cavity.

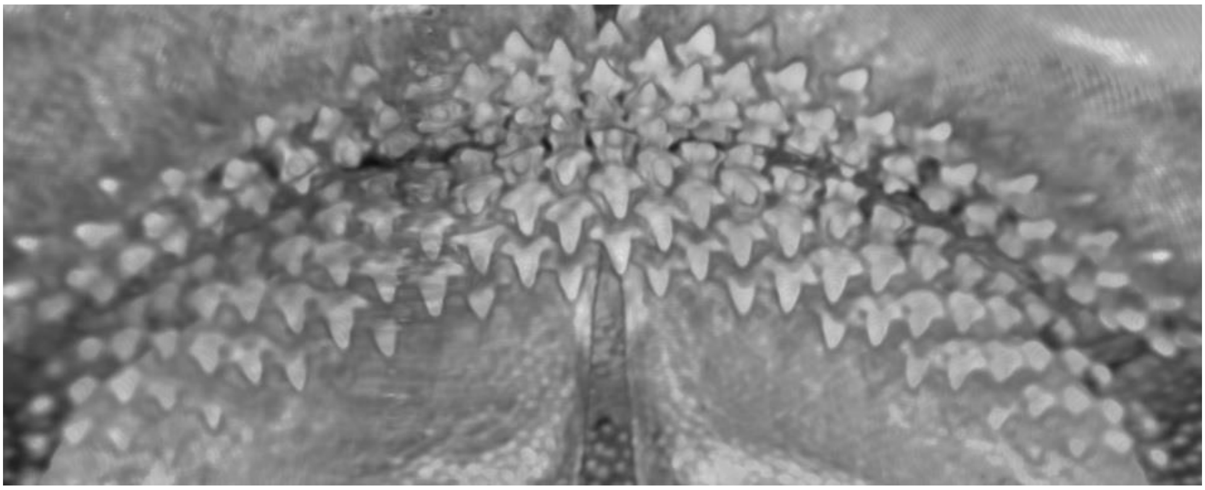

Figure 16. Dental arrangement of upper and lower jaw in *Chiloscyllium cf. punctatum* with a TL=138mm (microCT volume rendering)

All fins including dorsal fin, pectoral fin, pelvic fin, anal fin and caudal fin are covered with two types of ovate, leaf-shaped dermal denticles with a central ridge. The lateral sides of the fins are covered with very pointy leaf-shaped dermal denticles, whereas the edges are covered with more roundish denticles. Furthermore, the denticles on the edge overlap each other. On each side of the posterior end of the caudal fin 20 Palaeozoic denticles are displayed dorsally and 15 denticles ventrally.

Chiloscyllium cf. punctatum with a TL=223 mm indicate no Palaeozoic denticles anymore. All fins are still covered with the same type of dermal denticles as in *Chiloscyllium cf. punctatum* with TL=138 mm. The upper jaw features 14 rows in three series while the lower jaw features 14 rows in three to four series (Figure 17).

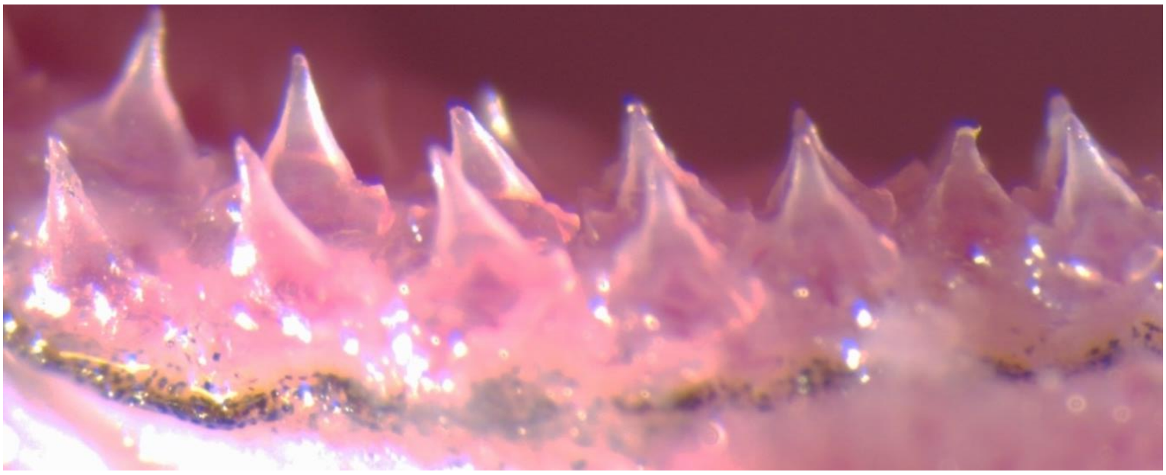

Figure 17. Oral denticles in *Chiloscyllium cf. punctatum* with a TL=223 mm (stereomicroscope)

In *Chiloscyllium cf. punctatum* no large dorsal denticles (LDD), as displayed in *Scyliorhinus sp.*, can be detected.

Observations on the morphology, distribution, and development of dermal and dental denticles in *Chiloscyllium cf. punctatum* are displayed in the Appendix, Table 1.

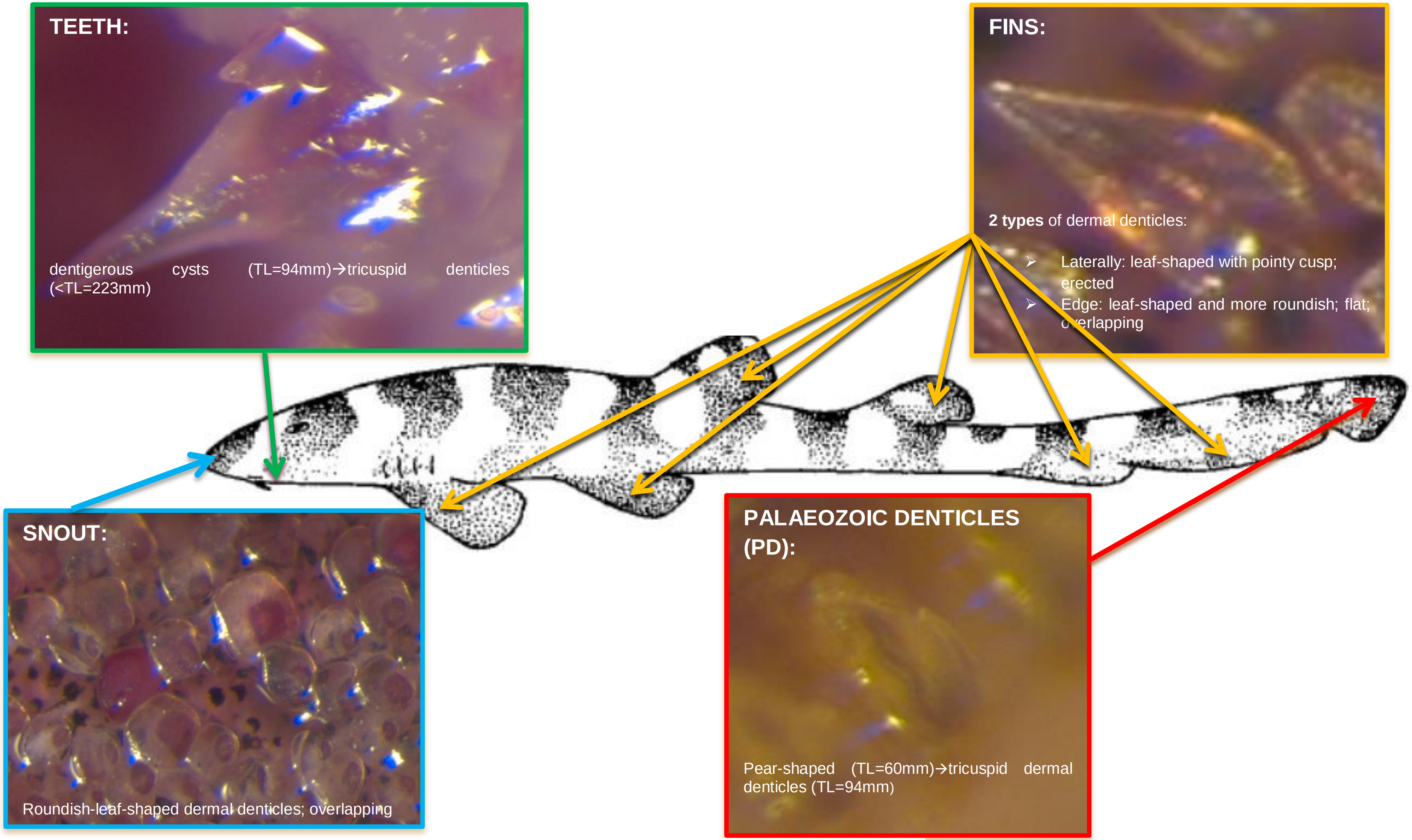

Figure 14. Dermal and dental denticles (stereomicroscope) in *Chiloscyllium cf. punctatum* (Compagno, 1984)

Association of pit organs and ampullae of Lorenzini with dermal denticles in *Chiloscyllium cf. punctatum*

In *Chiloscyllium cf. punctatum* with a TL= 138 mm, three main locations of pit organs are detectable such as spiracular pit organs, mandibular pit organs and dorsal pit organs. Every pit organ was covered by two dermal denticles in which the cusp would bend over the pit and occasionally would break in response.

In *Chiloscyllium cf. puncatum* with a TL= 223 mm spiracular, mandibular and dorsal pit organs could be spotted, but not as characterised in Peach and Marshall (2000) (Figure 18). Spiracular pit organs were clearly visible whereas only a few mandibular and dorsal pit organs could be localised. In this specimen a few dermal denticles surround each pit organ but do not cover or bend over as in younger *Chiloscyllium* specimens.

Instead, in *Chiloscyllium cf. punctatum* with a TL= 138 mm, three dermal denticles would cover the ampullae of Lorenzini dorsally and ventrally. Again in *Chiloscyllium cf. punctatum* (TL=223 mm) several dermal denticles are positioned next to the ampullae. Ampullae of Lorenzini, located on the ventral side of the head, are surrounded by leaf-shaped dermal denticles and cover each ampullae entirely. Dorsal dermal denticles only cover each ampullae with the tip of their cusp.

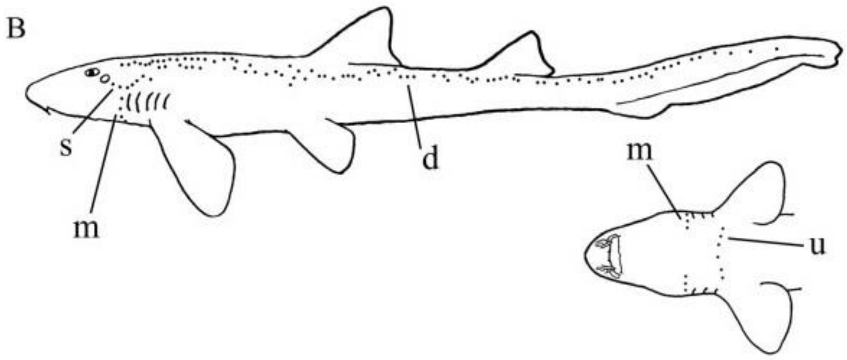

Figure 18. Distributions of pit organs in *Chiloscyllium cf. punctatum* (Peach and Marshall, 2000)

Chromatophores within the dermal denticles of *Chiloscyllium cf. punctatum*

The skin of *Chiloscyllium cf. punctatum* (TL=138 mm) contains chromatophores. The skin sample was dissected from the dorsal region behind the head. Additionally, dermal denticles contain chromatophores within the neck region along the edges and within the cusp of the denticle (Figure 19).

Figure 19. Compound microscope: Chromatophores are located within the skin (left), neck region (centre) and cusp (right) of dermal denticles (chromatophores are indicated with a black arrow).

Dermal and dental denticles in *Scyliorhinus canicula*

The development of dermal and dental denticles in *Scyliorhinus canicula* was inspected on seven specimens (Figure 20).

In *Scyliorhinus canicula* with a TL=46mm no dentigerous cysts, taste buds or teeth could be discovered within the oral cavity. Besides, the skin surface was completely smooth without any dermal denticles covering the body. Additionally, this stage does not feature any large dorsal denticles along the back. The only visible dermal denticles in this specimen are the Palaeozoic denticles. The dorsal row represents six Palaeozoic denticles and the ventral row five. As a result, Palaeozoic denticles develop before the large dermal denticles (LDD) along the dorsal side.

In *Scyliorhinus canicula* with a TL=59 mm the skin surface features papillae, which indicates that dermal denticles will erupt soon. This specimen has no oral denticles developed, but two rows of dentigerous cysts. The dorsal region does not display any large dorsal denticles either. Likewise, the only dermal denticles visible in this specimen are the Palaeozoic denticles. Nine Palaeozoic denticles represent the dorsal row and four the ventral row. All fins in *Scyliorhinus canicula* with TL=76 mm are covered with two types of dermal denticles. Elongated and monocuspid dermal denticles cover both sides of the fins, whereas the edges are covered with ovate, leaf-shaped dermal denticles with a single central ridge.

The posterior ends of all fins are not covered with any dermal denticles. The density of dermal denticles decreases from the anterior to the posterior region in all fins. The oral cavity does not contain any teeth but features dentigerous cysts and taste buds. Therefore, dermal denticles evolve earlier and faster than teeth throughout ontogeny. The snout is covered with ovate, leaf-shaped dermal denticles with a single central ridge. The arrangement of the snout denticles is equal to the snout denticles in *Chiloscyllium cf. punctatum* in which the denticles located dorsally are highly erected and the denticles ventrally are lying flat.

At the tip of the snout, denticles radiate from the centre into all directions and step by step turn to face the posterior end. This specimen has eleven Palaeozoic denticles representing the dorsal row and eight the ventral row.

At this point, the large dorsal denticles (LDD) are visible for the first time and are larger than the other dorsal denticles distributed along the dorsal side. Moreover, the monocuspid LDD have a slim neck part and feature a roundish instead of a pointy tip (Figure 22). They occur in two rows along the dorsal side which extend from the pectoral fins to the first dorsal fin. The left row features 30 LDD and the right 29 LDD. Placing the shark on its ventral side with the head facing frontwards determines the left and right row.

Scyliorhinus canicula with a TL= 85 mm has not developed any oral teeth but only dentigerous cysts, which indicates that dental eruption will take place soon. The morphology of dermal denticles remains the same in fins and the snout area. The number of Palaeozoic denticles is decreasing from this point on. The dorsal row features nine Palaeozoic denticles and the ventral row eight. The left LDD row features 27 and the right row 28.

The eruption of teeth is conspicuous in *Scyliorhinus canicula* with a TL= 99 mm. The dental denticles have a tricuspid morphology. The upper jaw depicts a dental arrangement in each quadrant containing 15 rows of denticles in two to four series. The number of series shows a tendency of reduction in posterior denticles. The number of series is higher in the anterior region of the oral cavity and lower in the posterior region. The dermal denticles covering the edges of all fins have three ridges instead of a single ridge now. The first row of dental denticles starts exactly at the dental midline. This specimen features less LDD in which 23 large denticles are located on both sides. Furthermore, a reduction of Palaeozoic denticles is noticeable. The dorsal row contains four Palaeozoic denticles and the ventral row only two.

Each quadrant of the upper jaw in *Scyliorhinus canicula* with TL=103 mm has 16 rows in one to two series whereas *Scyliorhinus canicula* with TL=105 mm reveals 21 rows. In both specimens dentigerous cysts are present within the oral cavity. The morphology of dermal denticles in fins and the snout coincides with *Scyliorhinus canicula* (TL=76 mm). The dorsal row of Palaeozoic denticles in *Scyliorhinus canicula* (TL=103 mm) has nine denticles imbedded and the ventral row seven, whereas *Scyliorhinus canicula* (TL=105 mm) has seven dorsally and five Palaeozoic denticles ventrally (Figure 21).

Scyliorhinus canicula (TL=103 mm) shows 17 large dorsal denticles on its left and 26 on its right, less than *Scyliorhinus canicula* (TL=105 mm) with 29 denticles on each side (Figure 22). In course of ontogeny, LDD blend in with other dorsal denticles and become less conspicuous as the gaps between the LDD along the dorsal side fill up with more dorsal denticles.

Figure 21. Ventral row of Palaeozoic denticles in *Scyliorhinus canicula* with a TL=103 mm (microCT volume rendering)

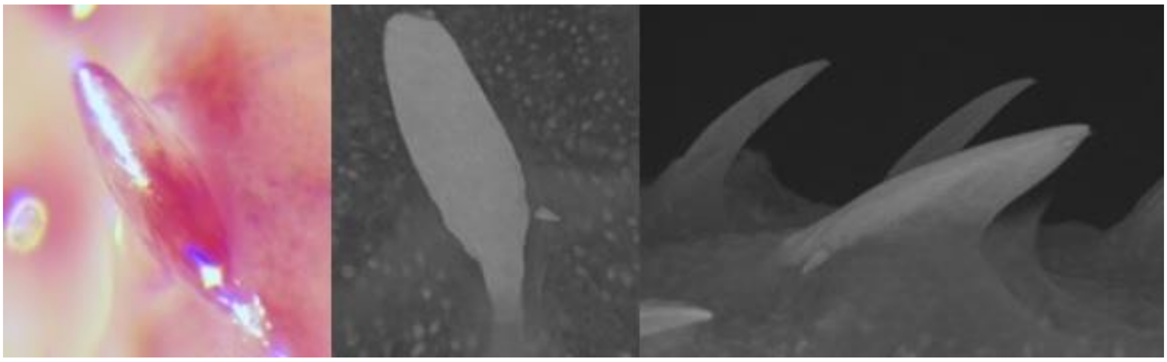

Figure 22. Large dorsal denticles in *Scyliorhinus canicula* with a TL=103 mm from different angles (with stereomicroscope and microCT volume rendering)

According to the results obtained, the number of Palaeozoic denticles first increases and then decreases throughout ontogeny until the denticles are fully discarded. However, the number of LDD remains consistent after its development before becoming inconspicuous.

Observations on the morphology, distribution, and development of dermal and dental denticles in *Scyliorhinus canicula* are displayed in the Appendix, Table 2.

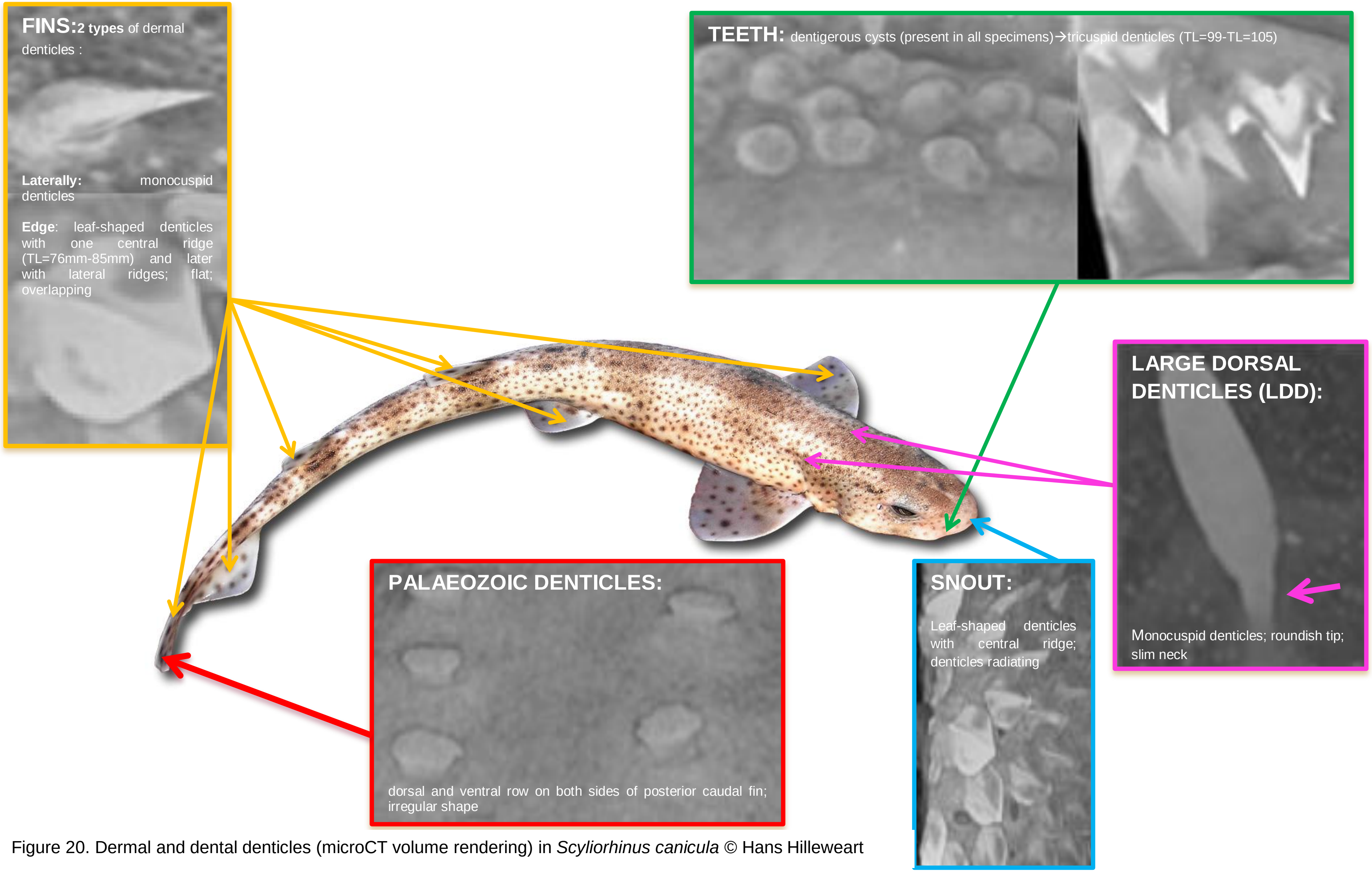

Figure 20. Dermal and dental denticles (microCT volume rendering) in *Scyliorhinus canicula* © Hans Hilleweart

Association of pit organs and ampullae of Lorenzini with dermal denticles in *Scyliorhinus canicula*

In *Scyliorhinus canicula* with a TL= 99 mm, three types of pit organs could be detected including spiracular, supratemporal and dorsal pit organs. Pit organs are surrounded by dermal denticles, but the denticles do not cover the pit organs. Only one dorsal pit organ could be detected along the dorsal side to the caudal peduncle which does not agree with the description of dorsal pit organs in Peach and Marshall (2000). Umbilical pit organs and mandibular pit organs are not visible in this specimen either.

In *Scyliorhinus canicula* with a TL= 103 mm, dorsal pit organs are detectable as described in Peach and Marshall (2000). Along the dorsal side the large dorsal denticles (LDD) are associated with the dorsal pit organs in which the denticles occur around the pit (Figure 23a). Moreover, spiracular and supratemporal pit organs are visible in which dermal denticles surround the pit organ and possibly have their cusp leaning over. In this specimen, umbilical and ventral mandibular pit organs are not visible. A single mandibular pit organ could be located laterally.

Instead, in *Scyliorhinus canicula* with TL= 105 mm spiracular, mandibular and dorsal pit organs are detectable. All pit organs are surrounded by several dermal denticles. Mandibular pit organs could only be located on one side of the mandible and not the other. Supratemporal pit organs and umbilical pit organs are not conspicuous in this specimen.

The ampullae of Lorenzini imbedded in the ventral snout are surrounded by ovate, leaf-shaped dermal denticles and at times cover the ampullae with the tip of their cusp (Figure 23b). The denticles located on the dorsal side of the snout are erected and do not cover the ampullae with their presence.

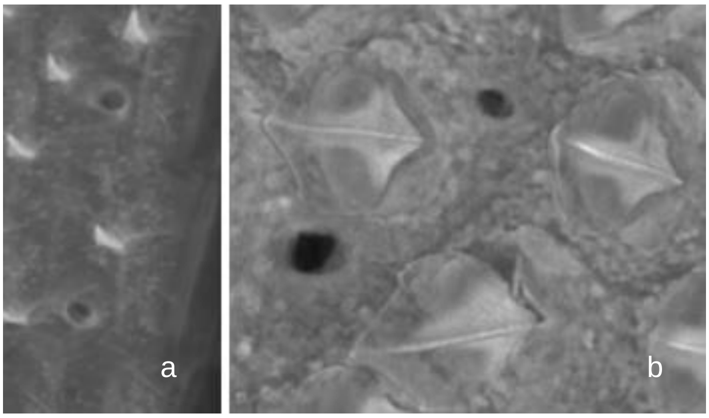

Figure 23a. Association of LDD with dorsal pit organs. 23b. Ampullae of Lorenzini (imbedded in the ventral snout) surrounded by dermal denticles (microCT volume rendering).

Chromatophores within the dermal denticles of *Scyliorhinus canicula*

The skin of *Scyliorhinus canicula* (TL=103 mm) contains chromatophores. Additionally, dermal denticles contain chromatophores along the edges of the cusps (Figure 24).

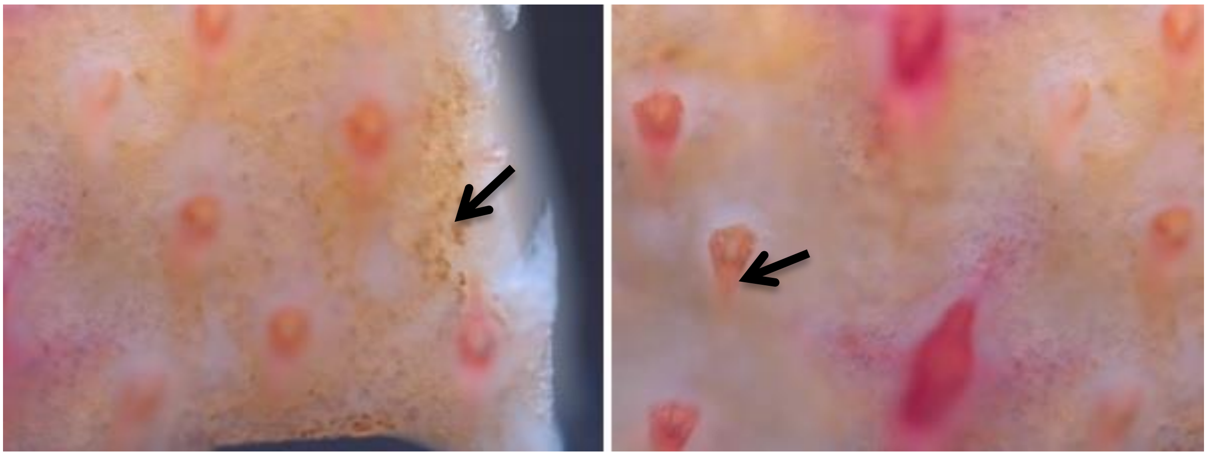

Figure 24. Compound microscope: Chromatophores imbedded within the skin (left) and in the cusps (right) in *Scyliorhinus canicula* with a TL=103 mm; the black arrows indicate the location of chromatophores;

Dermal and dental denticles in *Scyliorhinus retifer*

The development of dermal and dental denticles in *Scyliorhinus retifer* was inspected on eight specimens including an additional sample of ½ head (Figure 25).

The head sample of *Scyliorhinus retifer* with a TL= 78 mm displays tricuspid denticles in the oral cavity and leaf-shaped dermal denticles with a central ridge across the snout in which each denticle keeps a distance to its neighbouring denticles.

A similar sized specimen, *Scyliorhinus retifer* with a TL= 80 mm, reveals 13 rows in one to two series of tricuspid denticles in each quadrant of the upper jaw and roundish leaf-shaped denticles with a central ridge in the snout area. The oral cavity also contains dentigerous cysts. The teeth start at the dental midline. The dorsal and pectoral fins are covered with two types of dermal denticles. Both sides of the fins are coated with monocuspid denticles and the edges feature ovate, leaf-shaped denticles with a central ridge. The pelvic fins, anal fin and caudal fin did not display any ovate, leaf-shaped dermal denticles along the edges, but monocuspid denticles on both sides. The posterior end of all fins is not covered with dermal denticles. The dorsal side shows two rows of LDD in which the right row consists of 31 and the left row of 33 large denticles (Figure 26). The posterior end of the caudal fin displays eight Palaeozoic denticles establishing the dorsal row and four the ventral row.

Figure 26. Large dorsal denticles in *Scyliorhinus retifer* with a TL=80 mm (microCT volume rendering).

In *Scyliorhinus retifer* with a TL= 89 mm labelled as three months, features a similar dermal and dental denticle coverage as in the previous specimen. The oral cavity contains 18 rows in one to three series of tricuspid denticles (Figure 27). Additionally, the pelvic fins, anal fin and caudal fin possess leaf-shaped dermal denticles along the edges. At this point in ontogeny, Palaeozoic denticles are not visible anymore. However, two rows of LDD are conspicuous with 33 large denticles on both sides.

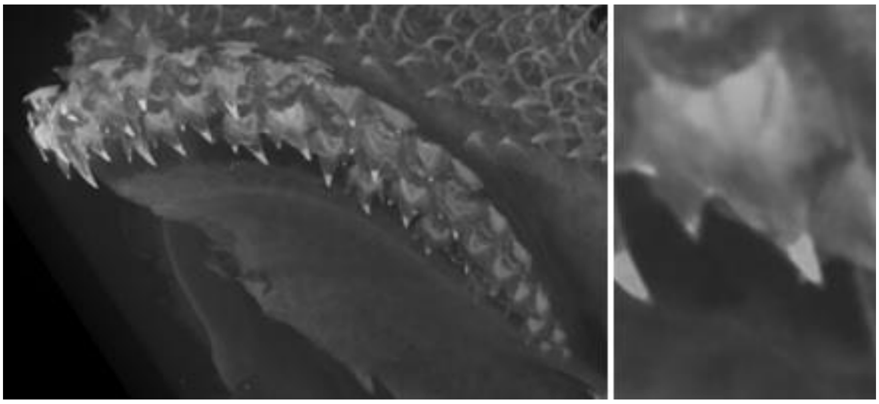

Figure 27. Arrangement (left) of tricuspid (right) dental denticles in *Scyliorhinus retifer* with a TL=89 mm (microCT volume rendering).

The oral cavity in *Scyliorhinus retifer* with a TL= 88 mm labelled as five months, shows 15 dental rows in two to four series in the lower jaw and 16 rows in the upper jaw (Figure 28). The dorsal side has 29 LDD imbedded on the left hand side and 30 on the right hand side.

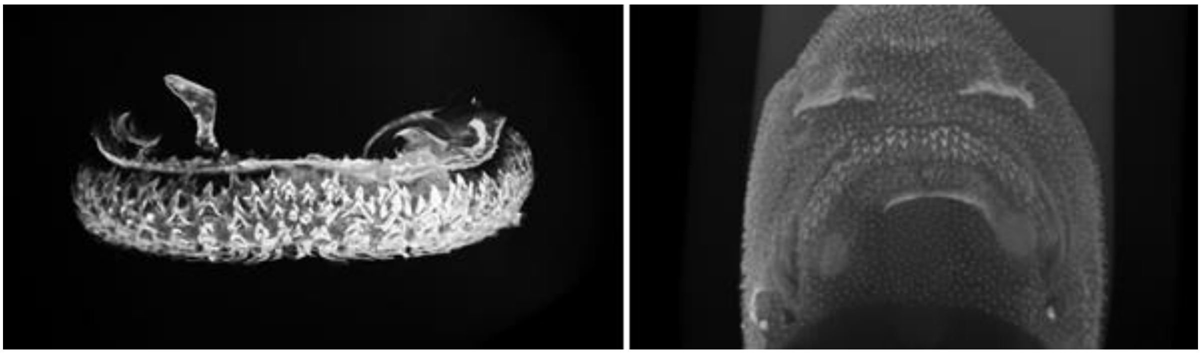

Figure 28. Arrangement of dental denticles in the lower jaw (left) and upper jaw (right) of *Scyliorhinus retifer* with a TL=88 mm (microCT volume rendering)

The morphology of dermal denticles of all fins takes a turn in *Scyliorhinus retifer* with a TL= 132 mm labelled as seven months, in which the previous monocuspid dermal denticles turn into tricuspid denticles. All fins are covered by two types of dermal denticles. The sides of all fins are covered by erected tricuspid denticles with a long central cusp and one central ridge. The edges are covered by more roundish tricuspid denticles with a shorter central cusp and one central ridge. The denticles along the edges are flattened and overlap each other. The posterior ends of all fins still have no dermal denticles developed. The oral cavity displays a dental arrangement of 21 dental rows in two to three series in the upper jaw and 17 in the lower jaw (Figure 29). At the midline in the lower jaw the dental denticles are arranged V-like. The left row of LDD is accompanied by an additional denticle compared to *Scyliorhinus retifer* with TL=88 mm.

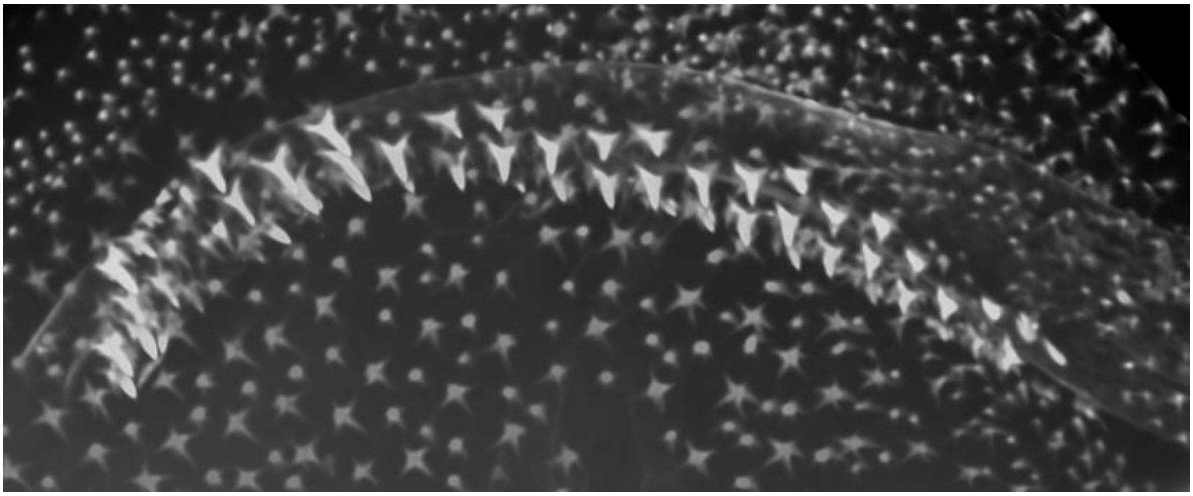

Figure 29. Arrangement of dental denticles in the upper jaw of *Scyliorhinus retifer* with a TL=132 mm (microCT volume rendering)

Additionally, *Scyliorhinus retifer* with a TL= 138 mm has developed tricuspid dermal denticles with three ridges, instead of only one ridge alongside the denticle described in younger specimens. This morphology of dermal denticles remains consistent until *Scyliorhinus retifer* (TL= 155 mm). The dental denticles in the oral cavity remain tricuspid and the dental arrangement in the upper jaw shows 23 rows in three to four series whereas the lower jaw contains 18 rows in two to three series (Figure 30). This specimen features a large number of LDD with 32 on its right and 34 on its left dorsal side.

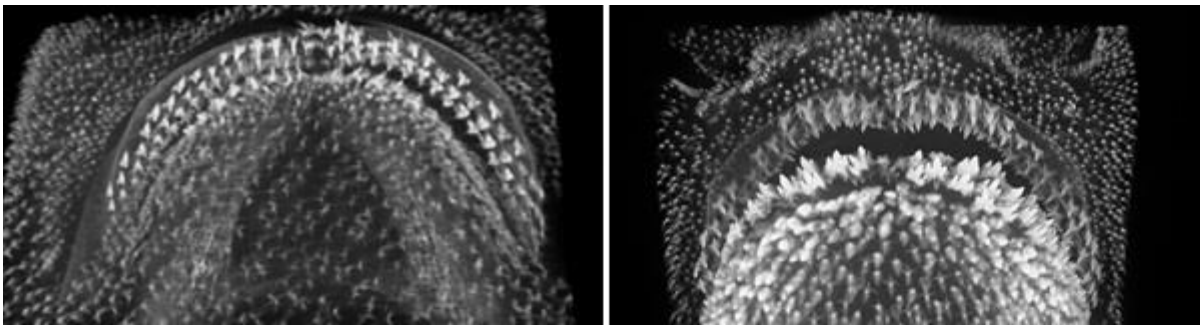

Figure 30. MicroCT volume rendering: Arrangement of dental denticles in *Scyliorhinus retifer* with a TL=138 mm (left) and with a TL=141 mm (right).

The following specimen, *Scyliorhinus retifer* TL=141 mm features pentacuspid dental denticles with seven ridges within the oral cavity. Thus, in *Scyliorhinus retifer* dental denticles develop additional cusps between TL=138 mm and TL=141 mm becoming pentacuspid. The lower jaw contains 22-23 rows of teeth in three to four series while the upper jaw has 24-25 rows in one to three series (Figure 30). This specimen has 29 LDD on its right and 31 LDD on its left. However, small teeth within the oral cavity still remain tricuspid.

Likewise, *Scyliorhinus retifer* with TL=142 mm features pentacuspid teeth with seven ridges. The lower jaw displays twelve rows in four to five series and the upper jaw twelve rows in three to four series (Figure 31). The LDD are the same as in *Scyliorhinus retifer* (TL=138 mm).

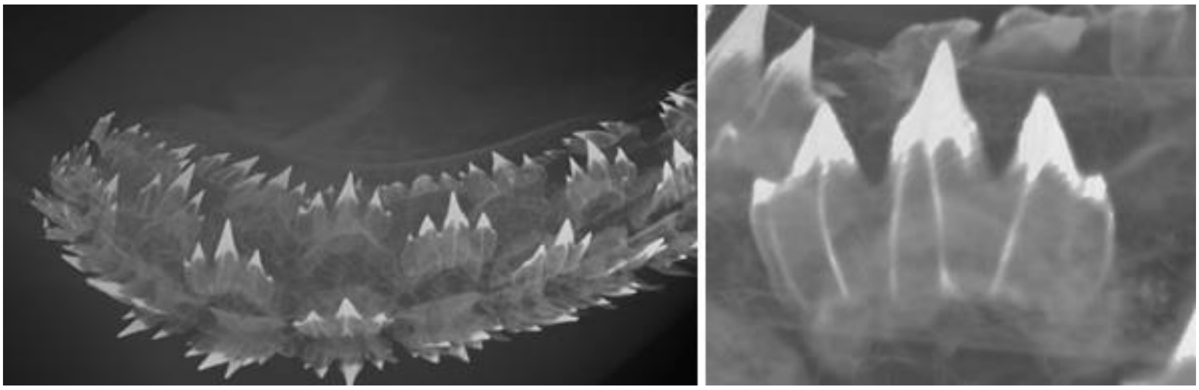

Figure 31. MicroCT volume rendering: Arrangement of dental denticles in *Scyliorhinus retifer* with a TL=142 mm (left) and pentacuspid tooth with seven ridges (right).

The most mature specimen of *Scyliorhinus retifer* in this study (TL=155 mm) has no conspicuous LDD anymore. The upper jaw features 19 dental rows in two to three series in each quadrant. Instead, the lower jaw has 18 rows of teeth. The teeth are pentacuspid (Figure 32).

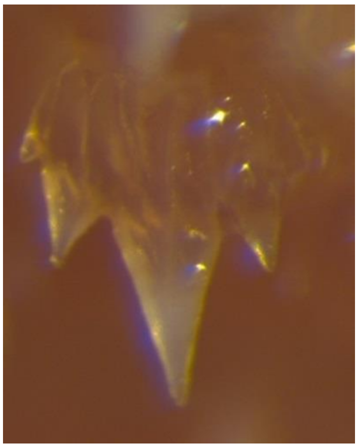

Figure 32. The teeth in *Scyliorhinus retifer* with a TL=155 mm are pentacuspid (stereomicroscope)

Observations on the morphology, distribution, and development of dermal and dental denticles in *Scyliorhinus retifer* are displayed in the Appendix, Table 3.

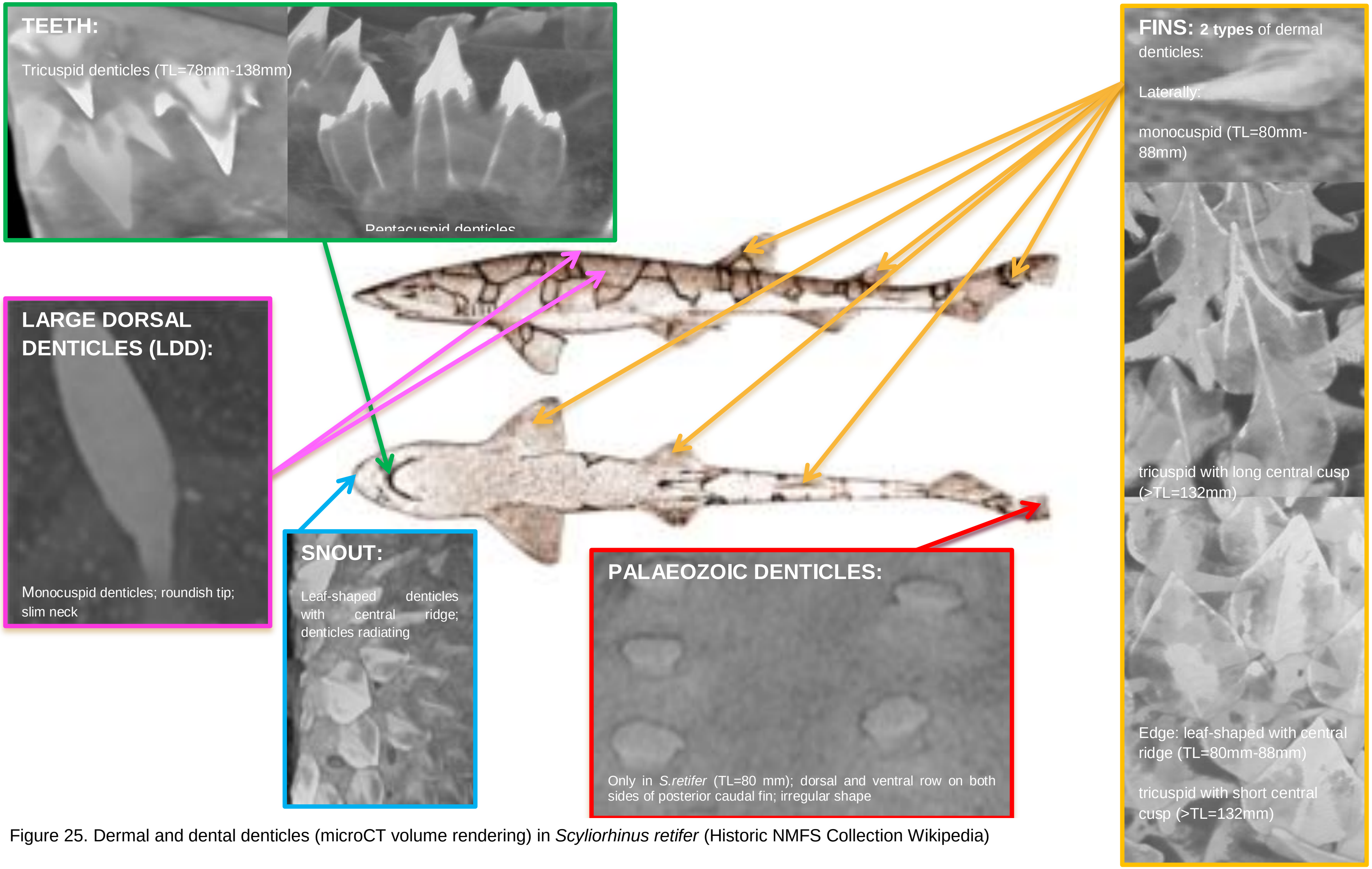

Figure 25. Dermal and dental denticles (microCT volume rendering) in *Scyliorhinus retifer* (Historic NMFS Collection Wikipedia)

Association of pit organs and ampullae of Lorenzini with dermal denticles in *Scyliorhinus retifer*

In *Scyliorhinus retifer* with TL= 138 mm mandibular and supratemporal pit organs are visible, whereas dorsal pit organs and spiracular pit organs are not conspicuous. Pit organs are surrounded by dermal denticles.

Nevertheless, spiracular pit organs are detectable in *Scyliorhinus retifer* (TL=141 mm) in which dermal denticles cover the pit with their cusp tip. Supratemporal pit organs are also covered by the cusp tip of dermal denticles. Mandibular, dorsal and umbilical pit organs could not be located in this specimen.

In *Scyliorhinus retifer* (TL=155 mm) only supratemporal pit organs could be detected in which dermal denticles gather around the pit and occasionally cover the pit with their cusp. The supratemporal pit organs are clustered at the temples. The other pit organs such as dorsal, mandibular, umbilical and spiracular pit organs could not be localised, but according to Peach and Marshall (2000) should be present.

The ampullae of Lorenzini are surrounded by dermal denticles. In *Scyliorhinus retifer* (TL=138 mm; TL=155 mm) dorsal dermal denticles cover the ampullae with their cusps only, whereas ventral dermal denticles cover the ampullae slightly more. The ventral dermal denticles are roundish leaf-shaped and the dorsal denticles pointy and monocuspid. In *Scyliorhinus retifer* with a TL=141 mm dorsal denticles are located around the ampullae, but do not cover the pit organ.

Chromatophores within the dermal denticles of *Scyliorhinus retifer*

Dermal denticles are imbedded in the skin which contain chromatophores. Moreover, the basal plates, pulp cavities and the cusps of dermal denticles in *Scyliorhinus retifer* (TL=141 mm) contain chromatophores. The accumulation of dark spots in the centre of the cusp (Figure 33) resembles chromatophores within the pulp cavity. The dark spots in the image are located between the cusp and basal plate and therefore can only be chromatophores located within the pulp cavity.

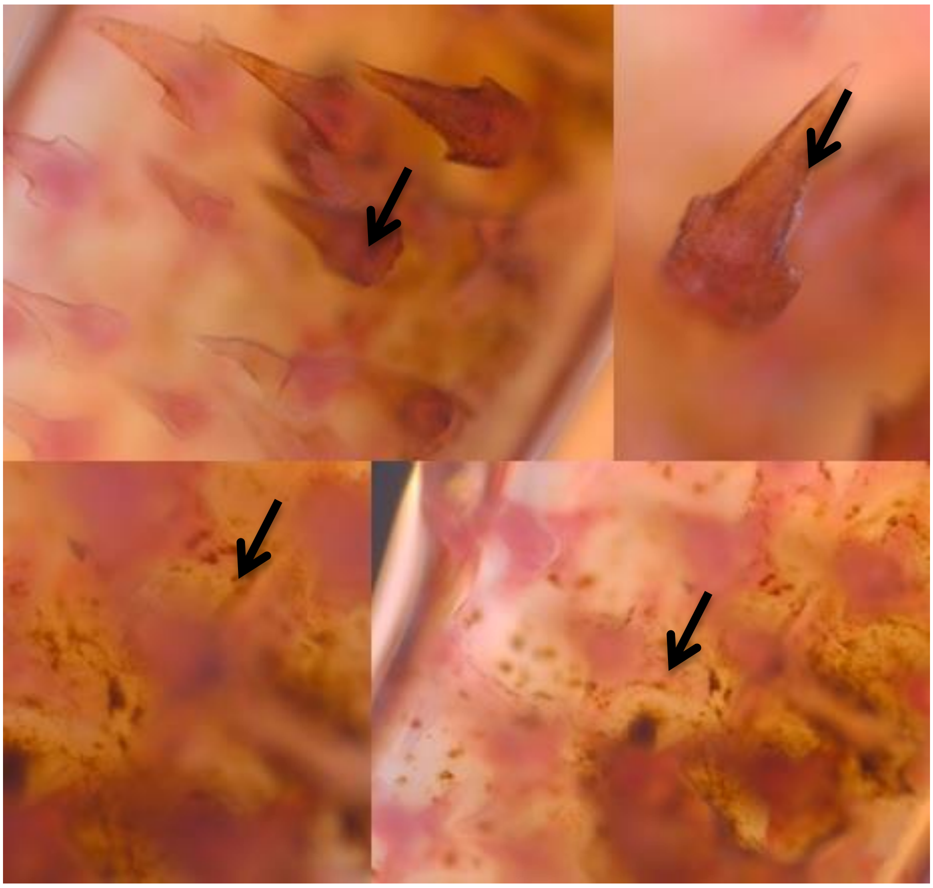

Figure 33. Compound microscope: Chromatophores detected in skin (bottom left), basal plate (bottom right), pulp cavity (top left) and cusp (top right); the black arrows indicate the location of chromatophores;

Discussion

The morphology of dermal denticles in *Chiloscyllium cf. punctatum*, *Scyliorhinus canicula* and *Scyliorhinus retifer* are in accordance with Oeffner and Lauder (2012), who state that dermal denticles are constructed in ways to reduce frictional drag and mechanical abrasion. Motta et al. (2012) stated that placoid scales have a different morphology across the body. In all specimens ovate to roundish leaf-shaped dermal denticles could only be detected along the ventral side, the ventral snout area, and along the edges of all fins. Pointy, monocuspid or tricuspid denticles cover the dorsal and lateral sides of the shark species as well as both sides of the fins. Leaf-shaped denticles covering the ventral side make it impossible for species to become entangled in sedimentary structures while feeding or resting on the bottom of the seafloor (Parker, 1999). All three species are bottom dwelling sharks and occur in coastal areas amongst coral reefs or tidal pools. Besides, leaf-shaped dermal denticles densely overlap each other ventrally and along edges producing a cover for ambient water to stream across the fins and body. The flanks of all three species are covered with highly erected, cusped dermal denticles to attain a rapid increase in swimming speed as mentioned by Motta et al. (2012) (Bechert et al., 1985; Lang et al., 2011, as cited by Motta et al., 2012).

At the tip of the snout, denticles radiate from the centre into all directions and with increasing distance from the centre turn to face the posterior end. In the process, dermal denticles change in morphology with increasing distance from the centre, being cusped on the dorsal side and remaining leaf-shaped on the ventral side, which is consistent with the snout tip. With regard to drag reduction, even dermal denticles around the snout establish coverage for the ambient water to stream across.

The development, distribution and morphology of dermal and dental denticles in *Chiloscyllium cf. punctatum*, *Scyliorhinus canicula* and *Scyliorhinus retifer* are documented by looking at all denticles in each embryonic specimen of different developmental age (see Appendix). The development of dentigerous cysts to teeth in the oral cavity could be observed in all three species as well as the development of papillae to placoid scales. Moreover, all species develop Palaeozoic denticles while LDD only develop in *Scyliorhinus*. Differential filiation or secondary loss of large dorsal denticles in bamboo sharks could explain why *Chiloscyllium cf. punctatum* do not possess large dorsal denticles. The morphology of Palaeozoic denticles in *Chiloscyllium cf. punctatum* differs from *Scyliorhinus*. *Scyliorhinus* has Palaeozoic denticles which are simultaneously roundish and squared, whereas *Chiloscyllium* first develops pear-shaped and later tricuspid Palaeozoic denticles.

The appearance and disappearance of Palaeozoic denticles is based upon gene expression. The sequence of gene expression most probably remained continuously in the caudal fin from the Palaeozoic Era until today. Johanson et al. (2008) suggests that the caudal fin is an independent developmental unit excluded from the rest of the body and that chondrichthyan scales on different regions of the body can be considered as independent developmental modules.

Accordingly, Palaeozoic denticles in *Chiloscyllium cf. punctatum* erupt anytime from a TL=33 mm to 60 mm. These denticles remain at the posterior end of the caudal fin at least until a TL=138 mm is reached. Afterwards, Palaeozoic denticles are either resorbed or discarded as they are not present in the largest specimen (*Chiloscyllium cf. punctatum* with a TL=223 mm). *Chiloscyllium punctatum* hatch at a TL=130 mm (Compagno, 1984) which shows that Palaeozoic denticles are only lost after the egg case stage which indicates that denticles would not accumulate within the egg case in terms of denticle discard. According to Moya Smith (personal communication) Palaeozoic denticles are most likely not resorbed as chondrichthyans are not capable of resorption.

The development of teeth in *Chiloscyllium cf. punctatum* (TL=138 mm; TL=223 mm) displayed that the number of series increase with maturity, however the number of rows decrease. *Chiloscyllium* feature a slit-like mouth in which an increase in number of series rather than number of rows is the only anatomical way possible and more beneficial for seizing prey. Interestingly, the number of series differs in upper and lower jaw, thus the lower jaw features more series compared to the upper jaw. The number of rows in each quadrant is equal in *Chiloscyllium*, but in *Scyliorhinus* this is not always the case. The morphology of teeth in *Chiloscyllium cf. punctatum* is equivalent to the morphology of teeth displayed in Ramsay and Wilga (2007), of which the teeth are tricuspid.

In *Chiloscyllium cf. punctatum* only three main locations of pit organs in association with dermal denticles could be analysed whereas according to Peach and Marshall (2000), umbilical pit organs should also be present from a TL=205 mm. However, this is not the case in the largest specimen of *Chiloscyllium cf. punctatum* (TL=223 mm). In this specimen no umbilical pit organs and only a few mandibular and dorsal pit organs are noticeable. This could result from dermal denticles covering the pit organs which makes them inconspicuous and difficult to localise. On the other hand, pit organs might have not developed in *Chiloscyllium* (TL=138mm and TL=223 mm) yet. In consequence, any association of pit organs with dermal denticles remains undetectable. Ampullae of Lorenzini are easier to detect which makes the association with dermal denticles simpler to analyse. The increase of dermal denticles around the pit organs and ampullae of Lorenzini during maturation could be based upon the concurrent development of synapses in terms of water displacement reception and electroreception in *Chiloscyllium*. In general, a higher coverage of pit organs and ampullae with dermal denticles could make shark specimens less susceptible to electro and water displacement reception. Dermal denticles surrounding or covering pit organs and ampullae with their cusp tip only could be a preliminary stage before dermal denticles cover everything to induce sensory regulation.

In *Scyliorhinus canicula* the number of dental rows increases with total length. *Scyliorhinus canicula* with a TL= 99 mm has 15 rows of teeth in two to four series in the upper jaw. The lower jaw features a V-shaped dental arrangement at the dental midline (symphysis). Instead, *Scyliorhinus canicula* with a TL= 103 mm contains 16 rows and TL= 105 mm 21 rows. Perhaps a larger mouth in *Scyliorhinus* allows teeth to extend horizontally and therefore the number of rows increase with age. The morphology of teeth in Scyliorhinus corresponds to the teeth depicted in Rasch et al. (2016). Early in ontogeny, teeth are tricuspid in shape, whereas later the number of cusps increase, and the teeth become pentacuspid. Anterior dental denticles feature more cusps, being closer to the symphysis than the posterior dental denticles. Possibly, teeth are expressed and produced at the symphysis. The number of dental rows and dental series increases in response to denticles being conveyed out of the symphysis. Therefore, anterior denticles show a higher cusp number than the posterior denticles as they have just recently been produced. Rasch et al. (2016) stated that a medio-lateral initiation of teeth take place in *Scyliorhinus* meaning that denticles develop at the symphysis and subsequently migrate laterally. On the contrary, studying the location of dentigerous cysts distributed across the gum tissue in younger specimens of *Scyliorhinus* reveals that the primary teeth have their genetic make-up in the gum tissue. Subsequently, the number of teeth in the oral cavity increases in response to secondary denticles migrating from the symphysis and increasing the number of dental rows and dental series.

In *Scyliorhinus canicula* Palaeozoic denticles are already present at a TL= 46 mm. In *Scyliorhinus retifer* only specimens from a TL=80 mm were available. Taking *Chiloscyllium cf. punctatum* into account, Palaeozoic denticles erupt sometime between a TL=33 mm and a TL=46 mm. The disappearance of Palaeozoic denticles varies in all three species. *Chiloscyllium cf. punctatum* with a TL=223 mm does not possess these denticles anymore. Instead, *Scyliorhinus retifer* features no Palaeozoic denticles from a TL=89 mm and *Scyliorhinus canicula* still possess them up to a TL=105 mm.

In *Scyliorhinus canicula* large dorsal denticles erupt at a TL= 76 mm and in *Scyliorhinus retifer* at a TL=80 mm. In *Scyliorhinus canicula* (TL=59 mm) LDD are not exposed yet concluding that LDD develop sometime between a TL= 59 mm and TL=80 mm in *Scyliorhinus*. According to *Scyliorhinus retifer*, large dorsal denticles become inconspicuous sometime in between TL=142 mm and TL=155 mm. In this study specimens of *Scyliorhinus* *canicula* were only available up to a TL= 105 mm in which evidential support of LDD becoming inconspicuous in this species could not be further analysed.

In Peach and Marshall (2000), the pit organs of catsharks were studied in catshark species *Asymbolus analis* using specimens five times larger than *Scyliorhinus* specimens available to this study. In specimens of *Scyliorhinus canicula*, no significant numbers of spiracular, supratemporal and dorsal pit organs could be detected. However, *Scyliorhinus canicula* with a TL=103 mm shows that even large dorsal denticles are associated with dorsal pit organs. In *Scyliorhinus retifer* mandibular, supratemporal and spiracular pit organs are associated with dermal denticles, but not all specimens depict all detected pit organs.

Scyliorhinus specimens of this study are much younger than the catsharks of Peach and Marshall (2000), and the distribution of pit organs could in fact differ in different species within a family. Besides, supratemporal pit organs are very similar to temporally located ampullae of Lorenzini therefore a possible confusion of both should be taken into account. Nevertheless, in both species of *Scyliorhinus*, ampullae of Lorenzini are associated with dermal denticles.

In *Scyliorhinus retifer* dermal denticles across the body first develop as elongated and pointy monocuspid denticles. Later these monocuspid denticles become tricuspid during development. Each denticle develops secondary cusps next to its central cusp. In this study specimens of *Scyliorhinus canicula* only reach a TL=105 mm, but based upon its close phylogenetic relationship towards *Scyliorhinus retifer*, conclusions about a similar development of dermal denticles can be expected. In addition, not only the number of cusps increases during ontogeny, but also the number of ridges alongside the denticle. At the beginning denticles feature a central ridge along the central cusp whereby later the neighbouring secondary cusps develop ridges as well.

In the course of development, dermal denticles are replaced by new dermal denticles as described in Ebert et al. (2006). Possibly dermal denticles do not develop lateral cusps but instead change their dental morphology during ontogeny by discarding old denticles and replacing them with new denticles. Certainly this would show a mixture of monocuspid and tricuspid dermal denticles across the body in specimens, which could not be detected, as each specimen is covered in the same type of dermal denticle whether its monocuspid or tricuspid. Moreover, discarded dermal denticles would accumulate within the egg case, which is unlikely as a pile of dermal denticles have never been detected within an egg case. In contrary dermal denticles could be resorbed by the skin in order to make place for new dermal denticles, but this alternative is associated with high costs. Therefore specimens would be without denticles or feature large gaps between denticles which are not noticeable amongst specimens of this study. However dermal denticles are covered by an epidermis as depicted in Figure 3 (Farrell, 2011) therefore the underlying ectomesenchyme could be responsible for producing lateral cusps the same way it produces primary teeth.

In *Scyliorhinus retifer* with a TL=80 mm only one type of dermal denticle instead of two cover the pelvic, anal and caudal fin. Even though this specimen is the youngest of *Scyliorhinus retifer* it is unusual that the edges of these fins are not covered in roundish, leaf-shaped denticles, but in monocuspid denticles. Possibly a deficiency in gene expression could have induced monocuspid denticles to be expressed instead of leaf-shaped denticles.

The number of rows and series steadily increases in *Scyliorhinus retifer* during ontogeny. The youngest specimen, *Scyliorhinus retifer* (TL=80 mm) features 13 rows in one to two series of dental denticles whereas the eldest specimen (TL=155 mm) contains 18-19 rows in two to three series. Some specimens of *Scyliorhinus retifer* display a larger amount of rows (25 rows in TL=141 mm) and series (up to five series at some rows in TL=142 mm) than the eldest specimen. The number of series listed in this study is not consistent throughout all rows. Only some rows within the oral cavity display the listed amount of series. Mostly anterior teeth have more series than posterior teeth. Reasons for outliers in the number of dental rows and series in *Scyliorhinus retifer* could be human error, as at times, counting the number of denticles within the oral cavity was challenging. Taking this into account would have caused miscalculations of one to two denticles which would not have caused this noticeable bias in number of rows and series listed in the results. Besides, shark specimens could develop denticles in their own time and not based upon a specific pattern. In this particular case no significant increase of rows and series could be expected. Furthermore, the interpretation of microCT images was difficult at times, as dermal and dental denticles were small and close together.

Conclusions

Further research in describing the morphology of dermal and dental denticles, not only in specimens of catsharks and bamboo sharks but also in all other shark species, could assist in identifying shark species more reliably. The morphology of dermal and dental denticles is a distinct trait in each shark species and should be added on to already listed characteristics of species in identification books. Besides studying the development of dermal and dental denticles in shark species would increase our knowledge about sharks in general. Knowing the exact morphology of denticles could give us information on nutrition and habitat requirements without having to observe species in the field. Understanding the transformation of monocuspid denticles into multiple cuspid denticles, whether dermal denticles are discarded or resorbed or whether secondary cusps develop from the ectomesenchyme, should be further analysed. Moreover, reason for Palaeozoic denticles occurring and disappearing in the caudal fin region compared to large dorsal denticles remaining along the dorsal side throughout ontogeny should be clarified. Further research on teeth in *Scyliorhinus* should explain why the number of dental rows and dental series do not significantly increase during ontogeny and whether this could be in terms of catshark specimens having unique mode of tooth development.

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Appendix: Development of dermal and dental denticles in *Chiloscyllium cf. punctatum* (Table 1)

Specimens	Oral cavity	Snout	Dorsal fin	Large DD	Pectoral fin	Pelvic fin	Anal fin	Caudal fin	Palaeozoic D
C.punctatum_33 Smooth STAGE	No teeth	No dermal denticles have developed yet	No dermal denticles have developed yet		No dermal denticles have developed yet	No dermal denticles have developed yet	No dermal denticles have developed yet	No dermal denticles have developed yet	Maybe underneath skin
C.punctatum_60 Start of PD STAGE	No teeth; no taste buds; no dentigerous cysts	No dermal denticles have developed yet	No dermal denticles have developed yet		No dermal denticles have developed yet	No dermal denticles have developed yet	No dermal denticles have developed yet	No dermal denticles have developed yet	**Palaeozoic denticles VISIBLE:** 14 PD dorsally and 13 PD ventrally; **Pear-shaped**
C.punctatum_94 Papillae STAGE	No teeth; dentigerous cysts and taste buds visible;	Papillae visible	Papillae visible on fin; Instead dorsal side has triangular denticles showing, which are still underneath the skin;		Papillae visible	Papillae visible	Papillae visible	Papillae visible; NO dermal denticles visible besides Palaeozoic denticles covering posterior end of caudal fin	Dorsal row: 20 PD Ventral row: 14 PD **Tricuspid**
C.punctatum_138	Tricuspid teeth Dental arrangement: **Lower jaw:** 4 series; 13 rows; 13th row only 3 series; 1-12th row with 4 series and shifted **Upper jaw:** 2 series of denticles; 15 rows; 9,11,13,14,15 single denticle	Leaf-shaped dermal denticles Dorsal snout: 90° erected; tip of snout: denticles radiating; ventral snout: lying flat	**2 types** of leaf-shaped dermal denticles: Laterally: leaf-shaped with pointy cusp and central ridge Edge: leaf-shaped and roundish Leaf-shaped without any lateral cusps		**2 types** of leaf-shaped dermal denticles: Laterally: leaf-shaped with pointy cusp and central ridge Edge: leaf-shaped and roundish Leaf-shaped without any lateral cusps	**2 types** of leaf-shaped dermal denticles: Laterally: leaf-shaped with pointy cusp and central ridge Edge: leaf-shaped and roundish Leaf-shaped without any lateral cusps	**2 types** of leaf-shaped dermal denticles: Laterally: leaf-shaped with pointy cusp and central ridge Edge: leaf-shaped and roundish Leaf-shaped without any lateral cusps	**2 types** of leaf-shaped dermal denticles: Laterally: leaf-shaped with pointy cusp and central ridge Edge: leaf-shaped and roundish Leaf-shaped without any lateral cusps	Dorsal row: 20 PD Ventral row: 15 PD Pit organ: 2 leaf-shaped dermal denticles covering pit Ampullae of Lorenzini: 3 leaf-shaped dermal denticles covering ampullae

***C.punctatum*_223** No PD STAGE	**Tricuspid denticles**; Upper jaw: 3 series and 14 rows of denticles Lower jaw: 3-4 series and 14 rows of denticles Dental arrangement **upper jaw:** at midline 3 series set in; row 2-10 3 rows set out; row 11-14 3 series set in; **lower jaw:** at midline 4 series set in; row 2-5 4 series set out; row 6-9 3 series set in; 10th row with 4 series set in; row 11-14 3 series set in;	**Leaf-shaped** dermal denticles Dermal denticles overlapping each other Dermal denticles with central ridge	**2 types** of leaf-shaped dermal denticles: Laterally: leaf-shaped with pointy cusp and central ridge Edge: leaf-shaped and roundish Leaf-shaped without any lateral cusps		**2 types** of leaf-shaped dermal denticles: Laterally: leaf-shaped with pointy cusp and central ridge Edge: leaf-shaped and roundish Leaf-shaped without any lateral cusps	**2 types** of leaf-shaped dermal denticles: Laterally: leaf-shaped with pointy cusp and central ridge Edge: leaf-shaped and roundish Leaf-shaped without any lateral cusps	**2 types** of leaf-shaped dermal denticles: Laterally: leaf-shaped with pointy cusp and central ridge Edge: leaf-shaped and roundish Leaf-shaped without any lateral cusps	**2 types** of leaf-shaped dermal denticles: Laterally: leaf-shaped with pointy cusp and central ridge Edge: leaf-shaped and roundish Leaf-shaped without any lateral cusps	No Palaeozoic denticles anymore

*NO large dorsal denticles (LDD) in *Chiloscyllium punctatum*, but do OCCUR in *Scyliorhinus canicula* and *Scyliorhinus retifer*

*Palaeozoic denticles (PD) OCCUR in *Chiloscyllium punctatum*, *Scyliorhinus canicula* and *Scyliorhinus retifer*

Appendix: Development of dermal and dental denticles in *Scyliorhinus canicula* (Table 2)

Specimens	Oral cavity	Snout	Dorsal fin	Large DD	Pectoral fin	Pelvic fin	Anal fin	Caudal fin	Palaeozoic D
***S.canicula*_46mm**	NO dentigerous cysts and taste buds visible; **NO oral denticles**	NO dermal denticles	NO dermal denticles	HAVE **NO LARGE DORSAL DENTICLE**S DEVELOPED YET	NO dermal denticles	NO dermal denticles	NO dermal denticles	NO dermal denticles	**Irregular in shape: roundish but squared** **Palaeozoic denticles** PRESENT; Dorsal row: 6 PD Ventral row: 5 PD
***S.canicula*_59mm**	Dentigerous cysts (2 series of dentigerous cysts) Taste buds **NO oral denticles**	Papillae	No papillae on fin **Only dorsal side** has papillae	HAVE **NO LARGE DORSAL DENTICLES** DEVELOPED YET	Papillae	Papillae	Papillae	Papillae	**Irregular in shape: roundish but squared** **Palaeozoic denticles** PRESENT; Dorsal row: 9 PD Ventral row: 4 PD
***S.canicula*_76mm**	Highly dentigerous cysts→almost ready to erupt PLUS Normal dentigerous cysts Taste buds	Leaf-shaped denticles with central ridge	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	**monocuspid** denticle with roundish tip **2 rows:** Left: 30 Right: 29	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	**Irregular in shape: roundish but squared** **Palaeozoic denticles** PRESENT; Dorsal row: 11 PD Ventral row: 8 PD

***S.canicula*_85mm**	Highly dentigerous cysts→almost ready to erupt Taste buds	Leaf-shaped denticles with central ridge	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	**monocuspid** denticle with roundish tip **2 rows:** Left: 27 Right: 28	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	**Irregular in shape: roundish but squared** **Palaeozoic denticles** PRESENT; Dorsal row: 9 PD Ventral row: 8 PD
***S.canicula*_99mm**	**Tricuspid** dental denticles 2-4 dental series Upper jaw: Parallel midline; 15 rows in one quadrant from midline distally Lower jaw: V-midline; Dentigerous cysts	Leaf-shaped denticles with central ridge	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with 3 dental ridges Posterior end is not covered by any dermal denticles	**monocuspid** denticle with roundish tip **2 rows:** Left: 23 Right: 23	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with 3 dental ridges Posterior end is not covered by any dermal denticles	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with 3 dental ridges Posterior end is not covered by any dermal denticles	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with 3 dental ridges Posterior end is not covered by any dermal denticles	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with 3 dental ridges Posterior end is not covered by any dermal denticles	**Irregular in shape: roundish but squared** **Palaeozoic denticles** PRESENT; Dorsal row: 4 PD Ventral row: 2 PD
***S.canicula*_103mm**	**Tricuspid** dental denticles 1-2 dental series 2:1:2 pattern 16 rows in upper right quadrant from midline distally Dentigerous cysts	Leaf-shaped denticles with central ridge Denticles radiating from tip and later facing posterior end	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with 3 dental ridges Posterior end is not covered by any dermal denticles	**monocuspid** denticle with roundish tip **2 rows:** Left: 17 Right: 26	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with 3 dental ridges Posterior end is not covered by any dermal denticles	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with 3 dental ridges Posterior end is not covered by any dermal denticles	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with 3 dental ridges Posterior end is not covered by any dermal denticles	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with 3 dental ridges Posterior end is not covered by any dermal denticles	**Irregular in shape: roundish but squared** **Palaeozoic denticles** PRESENT; Dorsal row: 9 PD Ventral row: 7 PD

***S.canicula*_105mm**	**Tricuspid** dental denticles 1-2 dental series 2:1:2 pattern 21 rows in one quadrant from midline distally Dentigerous cysts	Leaf-shaped denticles with central ridge	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with <u>3 dental ridges</u> Posterior end is not covered by any dermal denticles	**monocuspid** denticle with roundish tip **2 rows:** Left: 29 Right: 29	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with <u>3 dental ridges</u> Posterior end is not covered by any dermal denticles	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with <u>3 dental ridges</u> Posterior end is not covered by any dermal denticles	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with <u>3 dental ridges</u> Posterior end is not covered by any dermal denticles	**2 types** of dermal denticles: **Laterally:** elongated and monocuspid denticles **Edge:** leaf-shaped denticles with <u>3 dental ridges</u> Posterior end is not covered by any dermal denticles	**Irregular in shape: roundish but squared** **Palaeozoic denticles** PRESENT; Dorsal row: 7 PD Ventral row: 5 PD

Appendix: Development of dermal and dental denticles in *Scyliorhinus retifer* (Table 3)

Specimens	Oral cavity	Snout	Dorsal fin	Large DD	Pectoral fin	Pelvic fin	Anal fin	Caudal fin	Palaeozoic D
***S. retifer*_78mm (only ½ head sample)** **TRICUSPID STAGE**	Tricuspid denticles	leaf-shaped denticles with central ridge Huge gap between denticles							
***S. retifer*_80mm**	Tricuspid denticles 1-2 series of denticles (shifted) 13 rows PLUS Dentigerous cysts	Leaf-shaped denticles with central ridge Some exposed and others still imbedded	**2 types** of denticles: Laterally: **monocuspid** denticles Edge: leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	Large DD present **2 rows of LDD:** Right row: 31 LDD Left row: 33 LDD Roundish tip and monocuspid denticles; larger than dorsal denticles	**2 types** of denticles: Laterally: **monocuspid** denticles Edge: leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	**1 type** of denticle: Monocuspid and elongated dermal denticles covering edge and lateral sides No leaf-shaped denticles on edge Posterior end is not covered by any dermal denticles	**1 type** of denticle: Monocuspid and elongated dermal denticles covering edge and lateral sides No leaf-shaped denticles on edge Posterior end is not covered by any dermal denticles	**1 type** of denticle: Monocuspid and elongated dermal denticles covering edge and lateral sides No leaf-shaped denticles on edge Posterior end is not covered by any dermal denticles	Palaeozoic denticles present: 2 rows of PD; roundish but edged (irregular in shape) Dorsal row: 8 denticles Ventral row: 4 denticles
***S. retifer*_89mm (3 months)**	Tricuspid denticles 1-3 series 18 rows	Leaf-shaped denticles with central ridge	**2 types** of denticles: Laterally: **monocuspid** denticles Edge: leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	Large DD present **2 rows of LDD:** Right row: 33 LDD Left row: 33 LDD	**2 types** of denticles: Laterally: **monocuspid** denticles Edge: leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **monocuspid** denticles Edge: leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **monocuspid** denticles Edge: leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **monocuspid** denticles Edge: leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	NO Palaeozoic denticles visible
***S. retifer*_88mm (5 months)**	Tricuspid denticles 2-4 series of denticles (shifted) **Upper jaw:** 16 rows **Lower Jaw:** 15 rows Midline V-shaped in lower jaw	Leaf-shaped denticles with central ridge	**2 types** of denticles: Laterally: **monocuspid** denticles Edge: leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	Large DD present **2 rows of LDD:** Right row: 29 LDD Left row: 30 LDD	**2 types** of denticles: Laterally: **monocuspid** denticles Edge: leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **monocuspid** denticles Edge: leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **monocuspid** denticles Edge: leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **monocuspid** denticles Edge: leaf-shaped denticles with central ridge Posterior end is not covered by any dermal denticles	NO Palaeozoic denticles visible

***S. retifer*_132mm (7 months)**	Tricuspid denticles 2-3 series of denticles (shifted) **Upper jaw:** 21 rows **Lower jaw:** 17 rows	Leaf-shaped denticles with central ridge	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat Posterior end is not covered by any dermal denticles	Large DD present **2 rows of LDD:** Right row: 29 LDD Left row: 31 LDD	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat Posterior end is not covered by any dermal denticles	NO Palaeozoic denticles visible
***S. retifer*_138mm (9 months)**	Tricuspid denticles **Upper jaw:** 3-4 series; >17 rows left; 23 rows right; posteriorly 2 series; last row 1 denticle **Lower jaw:** 2-3 series; anteriorly 3 series; posteriorly 2 series;18 rows in each quadrant	Leaf-shaped denticles with central ridge	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat; 3 ridges Posterior end is not covered by any dermal denticles	Large DD present **2 rows of LDD:** Right row: 32 LDD Left row: 34 LDD	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat; 3 ridges Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat; 3 ridges Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat; 3 ridges Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat; 3 ridges Posterior end is not covered by any dermal denticles	No Palaeozoic denticles visible
***S. retifer*_141mm (11 months)** **PENTACUSPID STAGE**	**Pentacuspid denticles** **Lower jaw:** Teeth start at midline 22 rows left quadrant; 23 rows right quadrant; 2-4 series; small teeth tricuspid; large teeth; pentacuspid **Upper jaw:** Teeth start at dental midline; 24 rows left quadrant**;** 25 rows right**;** 1-3 series**;** small teeth tricuspid**; l**arge teeth; pentacuspid	,	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat; 3 ridges Posterior end is not covered by any dermal denticles	Large DD present **2 rows of LDD:** Right row: 29 LDD Left row: 31 LDD	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat; 3 ridges Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat; 3 ridges Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat; 3 ridges Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat; 3 ridges Posterior end is not covered by any dermal denticles	No Palaeozoic denticles visible

***S. retifer*_142mm (13 months)**	**Pentacuspid denticles** Pentacuspid denticles with 7 ridges; **Lower jaw:** 4 series of denticles and 5 series exactly along midline; 12 rows from midline **Upper jaw:** 3-4 series; 12 rows from midline	Leaf-shaped denticles with central ridge (tip and ventral side) Dorsal side has tricuspid denticles	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat; 3 ridges Posterior end is not covered by any dermal denticles	Large DD present **2 rows of LDD:** Right row: 32 LDD Left row: 34 LDD	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat; 3 ridges Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat; 3 ridges Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat; 3 ridges Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat; 3 ridges Posterior end is not covered by any dermal denticles	No Palaeozoic denticles visible
***S. retifer*_155mm (15 months)** NO LARGE DD	Pentacuspid **Upper jaw:** 19 rows left; 19 rows right; 2-3 series **Lower jaw:** 18 rows left; 18 rows right; 2-3 series	Leaf-shaped denticles with central ridge	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat; 3 ridges Posterior end is not covered by any dermal denticles	**NO Large DD present**	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat; 3 ridges Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat; 3 ridges Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat; 3 ridges Posterior end is not covered by any dermal denticles	**2 types** of denticles: Laterally: **tricuspid** denticles with long central cusp Edge: **tricuspid** denticles with short central cusp; denticles overlapping and lying flat; 3 ridges Posterior end is not covered by any dermal denticles	No Palaeozoic denticles visible

Additional pictures and notes are available on the University of Vienna Phaidra resource at http://phaidra.univie.ac.at/o:454143