Rostral tooth microwear and ultrastructure of the barbels and rostrum tip in the Common Sawshark *Pristiophorus cirratus* and their proposed behavioural implications

Pristiophorus cirratus (© Ryan Nevatte)

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Summary

Although sawsharks are frequent by-catch in commercial deep-water fisheries, our understanding of their behaviour is limited; in particular with regards to how they use their barbels and rostrum. This project employed a variety of techniques to infer how sawsharks use their barbels and rostrum. Evidence from histology and preliminary fluorescent microscopy work suggests that the barbels serve a tactile function, since they lack sensory structures such as taste buds, free neuromasts and ampullae, and contain neurons in the epidermis that may be connected to tactile receptors or free nerve endings. Regression analysis also provided evidence to suggest that sawsharks may have a preference as to whether they use the left or right barbel to sense the environment (behavioural lateralisation). Microwear (i.e. scratches) on the rostral teeth of sawsharks and sawfishes was found to be similar, suggesting that both elasmobranchs use their rostrum for a similar purpose. Analysis of stomach contents from recently caught sawsharks showed that diet consisted of small fish and prawns; however, whole prey items did not show signs of impalement by rostral teeth. Histological investigation of the unusual structure on the tip of the rostrum (named the "fingerprint") regarding a possible sensory purpose was inconclusive. These results provide important first steps in understanding the biology and behaviour of sawsharks and may prove useful for the management of threatened sawshark species, as well as deep-water fisheries in general.

Declaration

This thesis is written in the form of a journal article for the *Journal of Fish Biology*, and presented for the degree of Master of Research at Macquarie University in 2014.

I hereby declare that the material presented in this thesis is my own original work, unless otherwise stated. It has not been submitted in any form to another university or institution for any other higher degree.

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Chapter 1: General Introduction

1.1 Rationale of Study:

The ability for an organism to sense and perceive its environment is critical for its survival. Sensory systems and their specific adaptations to a species' lifestyle and ecology enable organisms to carry out essential tasks such as locate food, avoid predators, navigate, identify conspecifics, prey or predators, and to exploit new ecological niches. The type and complexity of sensory systems have been shaped by natural selection to enable an individual to effectively inhabit and reproduce within its environment (Stevens, 2013). Therefore, understanding the capabilities of a sensory system that an organism possesses can elucidate how and why an organism behaves in a particular manner and lends insight into why it may be successful in some ecosystems rather than others. In teleosts, for example, the overall brain size, as well as the size of specific regions of the brain involved in the processing of different sensory stimuli and the fishes' reliance on particular senses over others, are related to three key factors: water depth, habitat structure and lifestyle (benthic or pelagic) (Kotrschal *et al.*, 1998).

Information regarding the sensory capabilities of elasmobranchs is important in understanding their behaviour due to their wide dispersal, both in distance and depth, within oceans (Collin, 2012). Moreover, it is difficult to make behavioural observations for many species of elasmobranchs in their natural environment or to maintain them in captivity for controlled experiments because of their predatory nature, often with large foraging ranges and large body sizes (Gruber and Myrberg, 1977; Nelson, 1977). Often, this results in aspects of their behaviour being assumed based on their external morphology (Gruber and Myrberg, 1977) or the behaviour of morphologically similar or closely related organisms. For example, the manner by which the Frilled Shark *Chlamydoselachus anguineus* (Garman 1884) capture their prey is currently unknown; however it has long been believed that the serpentine body allows it to strike at prey like a snake (Garman, 1884; Ebert, 2013). Similarly, the mating behaviour of the Whale Shark *Rhincodon typus* (Smith 1828) has yet to be observed, and so inferences as to how courtship occurs are derived from the behaviour of closely related species (Martin, 2007).

While drawing behavioural conclusions from such methods may be the only option available for some species, it is important to recognise that these conclusions may not always be correct. For example, due to the rake-like appearance of the rostrum, it was long assumed that

sawfishes use the rostrum to search for and uncover prey hidden in the sediment (e.g. Nichols, 1929; Schaeffer, 1963; Compagno, 1990). However, a recent anatomical study on the rostrum of sawfishes demonstrated that it has a high density of electroreceptors (Wueringer *et al.*, 2011a), which could play a role in prey detection. Behavioural investigation of this hypothesis found that sawfishes use the electroreceptive rostrum to actively hunt for prey in the water column (Wueringer *et al.*, 2012); with Wueringer *et al.* (2012) suggesting that sediment raking by sawfishes may not occur. Similarly, the Freshwater Whipray *Himantura dalyensis* (Last and Manjaji-Matsumoto 2008) was once thought to only inhabit environments of low salinity; however, the structure of *H. dalyensis*' ampullary system has been shown to be similar to marine whiprays, suggesting that it is capable of living in environments with a range of salinities (Marzullo *et al.*, 2011). Thus, studying the sensory systems of elasmobranchs through alternative means such as anatomical examinations may lead to more informed hypotheses regarding selected behaviours.

One elasmobranch family in particular that would benefit from an investigation of their sensory systems is the Pristiophoridae (sawsharks). Sawsharks inhabit deeper waters of the continental shelf and slope and are characterised by elongated barbels on the ventral side of a long, toothed rostrum (Figure 1.1) (Compagno, 1984; Last and Stevens, 2009; Castro, 2011). Although these sharks frequently comprise a substantial amount of by-catch for a variety of commercial deep water fisheries, including trawling, gill netting and long lining (Walker *et al.*, 2005; Last and Stevens, 2009; Braccini *et al.*, 2012), knowledge of their biology is limited. Behavioural observations of these sharks are scarce due to their benthic-associated deep-water habitat and the difficulty in maintaining them in captivity (T. Guttridge, pers. comm.). Consequently, many aspects of their behaviour are currently inferred from observations of other organisms with similar structures and/or habits, especially those possessing barbels and elongated rostra (Schaeffer, 1963; Compagno, 1990; Ebert, 2013).

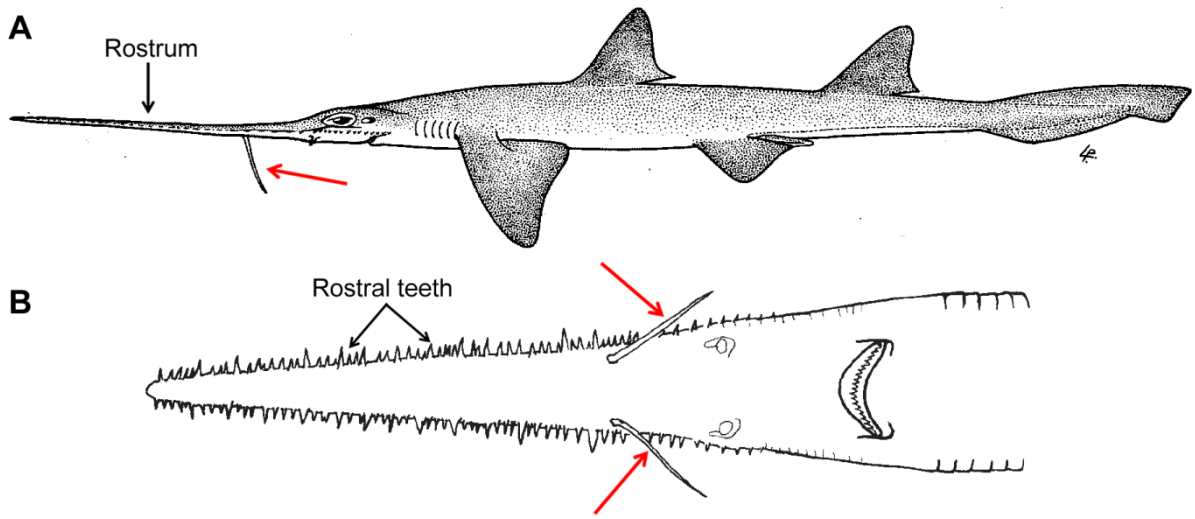

Figure 1.1: Lateral view (A) and ventral view of the head region (B) of the Sixgill Sawshark *Pliotrema warreni* (Regan 1906). Red arrows indicate the elongated barbels. Diagrams of *P. warreni* sourced from Compagno (1984), Page 132.

The purpose of the barbels in sawsharks is not known. They are thought to enable the detection of prey buried in the sediment (Last and Stevens, 2009; Castro, 2011) through chemo- (Compagno, 1984) or mechanoreception (Hoffmann, 1912; Sato, 1937; Goto *et al.*, 1994). Moreover, the toothed rostrum on which the barbels sit is thought to be used to uncover buried prey and then impale or stun prey with a slashing action (Springer, 1961; Schaeffer, 1963; Compagno, 1990). However, none of these assumptions have been validated. Determining the manner in which sawsharks utilise their rostrum and its associated structures such as the barbels and rostral teeth would therefore provide necessary information for understanding the biology of this group of sharks. Furthermore, as the majority of sawshark species are either listed as Data Deficient or Near Threatened (IUCN, 2013), such information could prove vital to their conservation. For example, understanding how sawsharks detect and capture prey would assist with the management of captive sawsharks as it is currently difficult to get them to feed (T. Guttridge, pers. comm.). Development of appropriate feeding protocols may then allow for captive breeding to be conducted should it be required. Finally, such information regarding sawshark behaviour may also help with the development of by-catch mitigation strategies.

The concept of using sensory biology data to facilitate management in fisheries is a relatively new one. A recent review, however, highlighted the importance of linking sensory biology to by-catch reduction, not only for conservation of elasmobranchs but also to ensure that fisheries are able to harvest sustainably (Jordan *et al.*, 2013). In the Australian Southern and Eastern Scalefish and Shark Fishery, Common *Pristiophorus cirratus* (Latham 1794) and Southern *Pristiophorus nudipinnis* (Günther 1870) Sawsharks caught as by-catch are rarely

discarded (Braccini *et al.*, 2012). Furthermore, the meat is easily marketable (Stead, 1963), thus indicating that these species provide an additional source of revenue for deep water fisheries. Understanding the biology, sensory capabilities and behaviour of sawsharks, therefore, is of importance to both 'pure' and 'applied' shark research, and to both conservationists and managers.

1.2 Biology of Sawsharks:

There are currently eight recognised species of sawshark in two genera (*Pliotrema* and *Pristiophorus*) globally (Ebert and Wilms, 2013). Of these eight, three are endemic to Australia (Last and Stevens, 2009) and are currently listed by IUCN (2013), but in the category of Least Concern. The remaining five species are listed as either Data Deficient or Near Threatened, or have yet to be formally assessed (IUCN, 2013). Despite their frequent capture through commercial fishing operations (Walker *et al.*, 2005; Braccini *et al.*, 2012), very little is known about the biology of sawsharks, and most of the pertinent literature is either relatively old (e.g. Hoffmann, 1912; von Bonde, 1933; Slaughter and Springer, 1968), not focused directly on sawsharks (e.g. Coleman and Mobley, 1984; Goto *et al.*, 1994), or related to the discovery of fossil rostral and oral teeth (e.g. Gottfried and Rabarison, 1997; Underwood and Schlögl, 2013).

Sawsharks of the genus *Pristiophorus* have existed since the Late Cretaceous (approximately 100 – 66 Ma) (Gottfried and Rabarison, 1997; Maisey, 2012) and rostral tooth morphology suggests that they have remained relatively unchanged throughout time (Gottfried and Rabarison, 1997). Members of the *Pliotrema* genus have existed since the Palaeocene (approximately 66 – 56 Ma) (Maisey, 2012), whilst a third extinct genus, *Ikamauius*, occurred during the Cenozoic Era (approximately 56 – 2.6 Ma) in New Zealand (Keyes, 1979; Maisey, 2012).

Sawsharks can grow to a maximum size of approximately 1.5 metres, are ovoviviparous and, depending on the species, can have between five to twenty-two pups in a litter (Last and Stevens, 2009). Developing pups have their rostral teeth folded down against the rostrum to prevent harming the mother during birth (Whitley, 1940; Gudger, 1951) and are born biennially after a species-dependent gestation period of between 12 to 15 months (Last and Stevens, 2009). Life span is considered between nine to fifteen years (Last and Stevens, 2009) and age at which maturity is reached is believed to be five years. However, accurate ageing of sawsharks is difficult with current ageing methods. Diet consists of bentho-pelagic fish,

crustaceans and cephalopods (Coleman and Mobley, 1984; Last and Stevens, 2009; Ebert, 2013).

The morphologies of the dermal denticles or placoid scales (tooth-like structures that cover the surface of elasmobranchs (Meyer and Seegers, 2012)) can differ between species of sawsharks. For example, the denticles of the African Dwarf Sawshark *Pristiophorus nancyae* (Ebert and Cailliet 2011) and the Bahamas Sawshark *Pristiophorus schroederi* (Springer and Bullis 1960) are morphologically similar but completely different to those of the two Australian species *P. cirratus* and *P. nudipinnis* (Weigmann *et al.*, 2014). The rostral teeth, which are modified dermal denticles, are replaced regularly throughout life (Slaughter and Springer, 1968). They are found on both the lateral edges and ventral surface of the rostrum (Ebert, 2013) and most species possess a single ventral rostral tooth before the nostrils (Springer and Bullis, 1960; Ebert and Cailliet, 2011; Ebert and Wilms, 2013).

The skull and cranial nerves of sawsharks are highly modified to accommodate the elongated rostrum (von Bonde, 1933). The foramen magnum (the large opening in the occipital bone in the cranium) is unusual in that it is surrounded by crescent-shaped occipital condyles (von Bonde, 1933), and the cranium is reinforced by modifications of the first five vertebrae (Springer and Bullis, 1960) to allow for controlled sideways movement of the head and rostrum. Electroreception and mechanoreception appear to be of great importance to sawsharks since the medulla, the region of the brain that receives sensory information from the ampullae of Lorenzini and lateral lines, is greatly enlarged when compared with other benthic sharks (Kajiura *et al.*, 2010).

1.3 Function of Barbels in Teleosts and Elasmobranchs:

Barbels are sensory structures containing innervations of the trigeminal (V) and/or facial (VII) cranial nerves that protrude from an animal's body (Fox, 1999). These skin covered structures are found in many species of fishes, amphibians and reptiles and can vary in size, number and location on the body of the animal. They most commonly occur, however, in the cephalic region (Fox, 1999) and, in teleosts, are capable of regenerating after having been lost (Fox, 1999; LeClair and Topczewski, 2010). Due to the widespread occurrence of barbels in teleosts (Fox, 1999), much of the research has been focused on this group and inferences on other species have been made from this research.

By far the most commonly cited function of barbels in teleosts is chemoreception since histological and morphological studies have shown that barbels in many species are replete

with taste buds (Ovalle and Shinn, 1977; Fox, 1999; Kiyohara *et al.*, 2002; Saadatfar and Shahsavani, 2007). The concept of barbels playing a role in prey detection has been supported by a number of eclectic studies. For example, behavioural experiments on Whitesaddle Goatfish *Parupeneus porphyreus* (Jenkins 1903) that had their barbels removed and were then presented with chemical cues from prey demonstrated that the barbels were the main organ used to detect and orient to food. *Parupeneus porphyreus* without barbels had slower arousal times than those with barbels, and failed to initiate food searching behaviour in the presence of the cues (Holland, 1978). Anatomical work on other goatfish species suggests that goatfishes are also able to discern the depth at which a prey item is hidden through chemoreception (Kiyohara *et al.*, 2002). The nocturnal Southern Bastard Cod *Pseudophycis barbata* (Günther 1863) uses the chemoreceptors distributed over its chin and pectoral barbels to locate prey that do not produce hydrodynamic signals (Bassett and Montgomery, 2011), thus providing support for the notion that barbels are important sensory structures for species inhabiting turbid or low light environments. Recently it was shown that Japanese Sea Catfish *Plotosus japonicus* (Yoshino and Kishimoto 2008) use their barbels to detect the changes in the pH of the seawater surrounding respiring polychaete prey (Caprio *et al.*, 2014), whilst the barbels of the Hardhead Catfish *Ariopsis felis* (L. 1766) are sensitive to the ink excretions of the California Sea Hare *Aplysia californica* (Cooper 1863) and serve as a means of detecting and avoiding unpalatable prey (Nusnbaum *et al.*, 2012).

Chemical detection by barbels can serve purposes other than prey detection. For example, there is the suggestion that males of some fish species utilise their chemoreceptive barbels to determine the receptivity status of potential mates (Zanger and Greven, 2013).

Teleost barbels can also play a mechanoreceptive (tactile) role, and many have specialised structures such as Merkel cells and free nerve endings for this purpose (Lane and Whitear, 1977; Kasumyan, 2011). Mechanoreception is often utilised in tandem with chemoreception to enable fishes to track and capture prey efficiently. For example, the chin barbel of the Abyssal Grenadier *Coryphaenoides armatus* (Hector 1875) contains taste buds and is innervated by the trigeminal nerve (V), enabling *C. armatus* to detect nearby prey via chemoreception (taste buds) and mechanoreception (trigeminal nerve) when the chin barbel contacts the seafloor (Bailey *et al.*, 2007). Other species of teleosts, however, lack taste buds in their barbels. Such examples include the Striped Dwarf Catfish *Mystus vittatus* (Bloch 1794) (Agarwal and Rajbanshi, 1965) and Artedidraconidae plunderfishes (Eastman and Lannoo, 2003; Eakin *et al.*, 2006). In the case of the plunderfish *Dolloidraco longedorsalis* (Roule 1913), it is proposed that the tactile information received by the barbel on the lower

jaw, which is held at 45°, supplements that received by the lateral line whilst ambush-hunting polychaetes (Eastman and Lannoo, 2003). Currently, it is unclear as to whether mechanoreception via free neuromasts is possible for teleost barbels. In the last review of barbels and their functions, Fox (1999) stated that these were not present. To the best of the author's knowledge, free neuromasts on the barbels of teleosts have not been reported in the intervening years.

It has also been proposed that barbels facilitate other senses and functions. Bailey *et al.* (2007) suggests that the chin barbel of *C. armatus* could carry nocisensory information in addition to chemo- and mechanosensory information. The ability to perceive pain in the barbels may enable early detection of potentially harmful prey and allow the fish to escape. Experiencing pain and possibly losing the barbel would be a small cost to pay when compared to death, especially in light of the regenerative ability of many barbels (Fox, 1999; LeClair and Topczewski, 2010). The barbels of goatfishes are used to probe crevices in which prey are hiding to encourage them to flee (Strübin *et al.*, 2011) and are also capable of proprioception (Ono, 1979). Barbels of deep-sea fishes, such as the Broomfin Dragonfish *Thysanactis dentex* (Regan and Trewavas 1930) and female *Linophryne* anglerfish, have bioluminescent properties that are used to lure prey (Hansen and Herring, 1977; Jørgensen and Munk, 1979).

Barbels are found in a number of shark species in addition to sawsharks. They commonly occur in benthic shark species (Timm and Fish, 2012), especially those belonging to the orders Orectolobiformes and Squatiniformes (Fox, 1999; Last and Stevens, 2009). Barbels are also present in a few members of the orders Squaliformes (Garrick and Paul, 1971; White *et al.*, 2007) and Carcharhiniformes (Fox, 1999). However, information regarding how sharks possessing barbels utilise them is limited.

The nasal barbels of sharks have been attributed to several functions. For example, the Epaulette Shark *Hemiscyllium ocellatum* (Bonnaterre 1788) possesses a high concentration of electroreceptive pores on its nasal barbels (Winther-Janson *et al.*, 2012), suggesting that they are used to detect the electric fields of prey in the benthos. In contrast, the nasal barbels of wobbegongs lack electroreceptive pores (Theiss *et al.*, 2011) and may function in some other capacity. Nurse Sharks *Ginglymostoma cirratum* (Bonnaterre 1788), after detecting the odour of prey, will contact the substrate with their nasal barbels when searching for food (Demski, 1977; Compagno, 1984), possibly indicating that they serve a tactile function. Recent computed tomography on the nasal cavities of sharks living in different positions of the water

column suggests that, in addition to a sensory function, nasal barbels can aid in directing water flow into the nasal cavity (Timm and Fish, 2012).

Another type of barbel present in sharks is the throat barbel. Histological work on the throat barbels of the Saddle Carpetshark *Cirrhoscyllium japonicum* (Kamohara 1943) has shown that these are likely to be used for mechanoreception as they lack taste buds or other sensory cell types (Goto *et al.*, 1994). The specific mechanoreceptive function of the throat barbels is unknown but may relate to the detection of prey, objects on the substrate, changes in current and/or maintenance of balance (Goto *et al.*, 1994). Thus it is clear that the precise function of barbels in sharks is species-specific and may heavily depend on the morphology and life history characteristics (e.g. benthic versus pelagic, type of prey consumed) of the species.

The specific function of the rostral barbels in sawsharks is currently unclear. Shark identification guides such as Last and Stevens (2009) and Castro (2011) only state that sawsharks use their barbels to locate prey in the sediment and do not disclose the sensory modality. Compagno (1984) proposes that sawsharks use their barbels for chemoreception. Histological studies on the rostral barbels of *Pristiophorus nudipinnis* and the Japanese Sawshark *Pristiophorus japonicus* (Günther 1870), however, suggest that they lack taste buds and thus function in a mechanosensory (tactile) capacity only (Hoffmann, 1912; Sato, 1937; Goto *et al.*, 1994). The issue is complicated further through a possible mistranslation by Schaeffer (1963) of Hoffmann (1912), taking the German word "Tastsinne" to mean taste and stating that the barbels function as chemosensory organs when the correct translation is "sense of touch". The hypertrophied medulla of sawsharks (Kajiura *et al.*, 2010) could also indicate that the barbels function in an electroreceptive capacity, although Hoffmann (1912) was unable to locate any electroreceptive ampullae in the barbels. Thus, there is a clear need to resolve the debate over the sensory capabilities of barbels in sawsharks.

1.4 Function of Elongated Rostra in Teleosts and Elasmobranchs:

An elongated rostrum, defined as a lengthening of the skull's rostral process (Schultze, 1993), occurs in a number of fish species; especially those belonging to the families Xiphiidae and Istiophoridae (billfishes). The function of this structure has been heavily debated, and there is a wide range of hypotheses as to its purpose. Many attribute the role of the rostrum to the capture of prey (Schneider and Fierstine, 2004; Shimose *et al.*, 2007; Domenici *et al.*, 2014). Support for this concept comes from examination of the stomach contents of Atlantic Blue Marlin *Makaira nigricans* (Lacépède 1802) where whole prey items occurred, still bearing wounds supposedly inflicted by the rostrum (Shimose *et al.*, 2007). Moreover, holes in the

vertebrae of fossil tuna have been matched in size to the rostra of extant billfishes (Schneider and Fierstine, 2004). Recent behavioural studies on the Atlantic Sailfish *Istiophorus albicans* (Latreille 1804) show that they are able to capture prey with their bill via two methods, lateral swipes on a school of fish and short taps on a single targeted fish the latter of which was more successful (Domenici *et al.*, 2014).

A variety of additional functions of the rostrum in billfishes have also been proposed. Shortfin Mako *Isurus oxyrinchus* (Rafinesque 1810) and *M. nigricans* have been found with the rostrums of billfishes buried in their bodies, suggesting that billfish are able to use their rostrum for self-defence (Fierstine, 1997; Fierstine *et al.*, 1997). Sea turtles have also been found with rostrums in their bodies; however, it is thought that the billfishes were targeting the surrounding fish and impaled them by mistake (Frazier *et al.*, 1994). The rostrum of billfishes has also been hypothesised to enable fast swimming speeds through a reduction in drag (Wisner, 1958; Videler, 1993), although Sagong *et al.* (2013) suggest otherwise.

Two other groups of fishes that possess elongated rostra include the sturgeon fishes and paddlefishes. Both groups use the electroreceptive capabilities of their rostrum to find prey in turbid water (Miller, 2005). Sturgeon fishes swim with their barbels and rostrum close to the substrate to locate buried prey using a combination of electroreception (rostrum) and chemoreception (barbels) (Miller, 2005). Similarly, paddlefishes identify the electric signals and associated stochastic resonance of planktonic prey with their rostrum (Russell *et al.*, 1999; Freund *et al.*, 2002) and are also able to detect metallic objects in their environment and avoid them (Wilkens *et al.*, 2002). There is also evidence that the rostrum of paddlefishes provide a hydrodynamic function during filter feeding by offsetting the drag encountered when water is swallowed, thus preventing paddlefishes from nose diving (Allen and Riveros, 2013).

Several other elasmobranchs in addition to sawsharks possess elongated rostra. As with the paddlefishes, members of the extinct genus *Bandringa* and the extant Goblin Shark *Mitsukurina owstoni* (Jordan 1898) are thought to locate prey via rostrum electroreception (Duffy, 1997; Parsons *et al.*, 2002; Sallan and Coates, 2014). The extension of the electroreceptive rostrum away from the body allows for a wider area of the environment to be sensed, as was recently demonstrated in sawfishes (Wueringer *et al.*, 2012). In addition to sensing prey, Wueringer *et al.* (2012) showed that sawfishes could use their toothed rostrum to both stun and impale prey in the water column and then manipulate it once on the substrate (e.g. turning fish for head-first ingestion or pinning it to the substrate). This study also

proposes that it is unlikely that sawfishes use their rostrum to remove sediment from buried prey, and thus challenges this long-held notion of its function (e.g. Nichols, 1929; Schaeffer, 1963; Compagno, 1990).

The rostrum of sawfishes is also thought to serve a self-defence purpose. Indian fisherman have reported that sawfishes have been responsible for the deaths of Dugongs *Dugong dugon* (Müller 1776) that have foraged too close to them (Lal Mohan, 1986), and Bull Sharks *Carcharhinus leucas* (Müller and Henle 1839) in Central America have been found bearing the marks of rostral teeth on their bodies (Tuma, 1976). There is also anecdotal evidence of sawfishes injuring or killing human swimmers and fisherman (McCormick *et al.*, 1963; White, 1975; Lal Mohan, 1986). A claimed third function of the rostrum is for combat between large males during the breeding season (Stead, 1963; Grant, 1993).

As was once thought for sawfishes, the function of the toothed rostrum of sawsharks is generally assumed to enable the shark to clear sediment away from prey in the benthos and then stun or kill the prey with lateral swipes of the rostral teeth (Springer, 1961; Schaeffer, 1963; Compagno, 1990). Although it is now thought that the toothed rostrums of sawsharks and sawfishes evolved independently (Wueringer *et al.*, 2009), it is plausible that sawsharks utilise their rostrums in a manner similar to sawfishes; for predation, or self-defence or both. Moreover, perhaps the rostra of sawsharks are used in a sensory capacity such as mechanoreception (Kasumyan, 2011).

1.5 Thesis Objectives and Chapter Outline:

The current knowledge regarding the behaviour of sawsharks is poor. Due to their deep water habitat and the difficulty in maintaining them in captivity, the behaviour of sawsharks needs to be inferred through an examination of their anatomy. This thesis employs a variety of methods to conduct an examination of two anatomical structures, the barbels and elongated rostrum, in order to develop better informed hypotheses regarding their functions as they may be involved in a variety of behaviours and could also bear sensory structures. Such information will contribute greatly to understanding the general biology of sawsharks and assist with the conservation of threatened species of sawsharks. Also, by-catch mitigation strategies could potentially be developed from these findings to aid in the management of deep-water fisheries.

Chapter 2 presents a histological investigation of the sensory structures contained within the barbels of sawsharks for specimens preserved as soon after capture as possible. Also

presented are the preliminary results of fluorescent Nissl staining to detect the presence of neurons in the barbels of sawsharks, and the findings from regression analyses on morphometric data. The results indicated that the barbels of sawsharks do not contain taste buds, free neuromasts or ampullary receptors and are thus hypothesised to function in a tactile capacity, although other senses such as thermoception and nociception are also possible. Nissl staining may have proved successful in highlighting neurons in the epidermis which may be connected to tactile receptors or free nerve endings. However, it was unclear whether the stained cells were neurons or cells of developing dermal denticles. Regression analyses suggested that sawsharks may have a preference for using either the right or left barbel when sensing the environment (i.e. behavioural lateralisation).

Chapter 3 examines the function of the elongated rostra in sawsharks through a variety of methods, including a comparison of rostral tooth microwear between sawsharks and sawfishes with micro computed tomography (micro-CT) and scanning electron microscopy (SEM), a comparison of rostral tooth composition with Raman spectroscopy, and an examination of the stomach contents of recently captured sawsharks. Also presented are the preliminary findings of a histological study on a recently discovered structure on the tip of the rostrum, named the "fingerprint", which may be connected to the canals of the lateral line. Microwear analysis suggested that the rostra of sawsharks and sawfishes are used for a similar purpose, whilst Raman spectroscopy indicated that the rostral teeth were composed of hydroxyapatite but differed in their collagen content. Histological investigation of the "fingerprint" structure for a possible sensory function was inconclusive; however it is proposed that this structure is a dense collection of epidermal cells. Based on the results of this study, it is proposed that sawsharks use their rostrum for predation, both to sense and capture prey, and possibly for self-defence.

Chapter 4 presents a general discussion of the findings of this thesis in the context of conservation and fisheries management, and highlights areas of future research.

Chapter 2: Ultrastructure of *Pristiophorus cirratus* barbels

2.1 Abstract:

The sensory structures present within the barbels of the Common Sawshark *Pristiophorus cirratus* were histologically evaluated with both light and fluorescent microscopy. The barbels were found not to contain chemosensory structures such as taste buds or structures for other senses such as ampullary receptors (electroreception) and free neuromasts (lateral line mechanoreception), and are thus proposed to function in a tactile capacity. Additionally, the barbels may convey nocisensory and thermosensory information. Fluorescent staining of the barbels for the presence of neurons may have been successful; however, it is unclear whether the observed staining pattern was the result of the Nissl stain highlighting neurons or cells of developing dermal denticles. Regression analyses on *P. cirratus* morphometric data showed that the width of the rostrum at the barbels and at the tip was not significantly correlated with total length, which may be due to the tapering form of the rostrum. The regression analyses also suggested that the barbels of *P. cirratus* may be lateralised.

2.2 Introduction:

Understanding the types of sensory systems an organism possesses and how they are utilised is important for inferring their behaviour and ecology (Weissburg and Browman, 2005; Stevens, 2013). This is because the sensory systems and the associated sensory structures of an organism have been moulded by natural selection to enable successful reproduction and habituation within its environment (Stevens, 2013). For species which are difficult to observe in their natural habitat or to maintain in captivity, examination of their sensory structures can therefore serve as means of developing more informed hypotheses regarding their behaviour.

One sensory structure that has been heavily researched, especially in teleosts, is the barbel. Barbels are skin covered protrusions from an animal's body that contain innervations of the cranial nerves and can vary in size, number and location on the body (Fox, 1999). Most commonly situated on the head of fishes, barbels have been shown to confer two main senses – chemoreception and/or mechanoreception (Fox, 1999; Kasumyan, 2011). The primary role of these two senses in teleost barbels is attributed to the detection of prey (e.g. Holland, 1978; Eastman and Lannoo, 2003; Bailey *et al.*, 2007; Caprio *et al.*, 2014). However, there is also evidence that barbels may serve additional chemoreceptive roles such as the detection and subsequent avoidance of noxious substances (Nusnbaum *et al.*, 2012), or checking the receptivity status of potential mates (Zanger and Greven, 2013). Other sensory functions such

as proprioception (Ono, 1979) and nociception (Bailey *et al.*, 2007) are also proposed, whilst barbels bearing photophores, as seen in deep sea fishes, function as lures for potential prey (Hansen and Herring, 1977; Jørgensen and Munk, 1979).

In contrast to teleosts, the purpose of barbels in elasmobranchs is less understood. Although common in species of the orders Orectolobiformes, Squatiniformes and Pristiophoriformes (Fox, 1999; Last and Stevens, 2009), and being present in a few species of the orders Squaliformes (Garrick and Paul, 1971; White *et al.*, 2007) and Carcharhiniformes (Fox, 1999), there is a paucity of research on the function of barbels in sharks. From the available evidence, suggested sensory functions include electroreception (e.g. Winther-Janson *et al.*, 2012) and mechanoreception (e.g. Goto *et al.*, 1994). It is also thought that in addition to a sensory function, barbels may aid in directing water flow into the nasal cavity of some species (Timm and Fish, 2012). The exact function of the barbels in sharks appears to be species-specific and heavily dependent on the species' morphology and life history characteristics.

Sawsharks are benthic, deep-water elasmobranchs that possess prominent, elongated barbels on the ventral side of their toothed rostrum (Compagno, 1984; Last and Stevens, 2009; Castro, 2011). Despite forming a substantial amount of the by-catch in commercial deep-water fisheries (Walker *et al.*, 2005; Last and Stevens, 2009; Braccini *et al.*, 2012), understanding of the behaviour of sawsharks is limited because of the difficulties in studying them *in situ* and their low survivability in captivity (T. Guttridge, pers. comm.). Due to this, many aspects of the behaviour of sawsharks, such as the function of the barbels, can therefore only be assumed, based on the results of studies on other species.

There are currently two hypotheses regarding the sensory capabilities of barbels in sawsharks: one chemosensory and the other mechanosensory. The chemosensory hypothesis, as proposed by Compagno (1984), suggests that the barbels contain chemoreceptors and are utilised in the same manner as those of catfishes and sturgeon fishes. The mechanosensory hypothesis suggests that the barbels lack taste buds and serve a tactile function only and is supported by a few histological studies on the barbels of two sawshark species, the Southern *Pristiophorus nudipinnis* (Günther 1870) (Hoffmann, 1912) and Japanese Sawshark *Pristiophorus japonicus* (Günther 1870) (Sato, 1937; Goto *et al.*, 1994). This hypothesis is supported further by anatomical studies which indicate that the barbels are innervated by branches of the trigeminal (V) (von Bonde, 1933) and/or facial (VII) cranial nerves (Hoffmann, 1912; Goto *et al.*, 1994). However, confusion as to the sensory capabilities of barbels in sawsharks persists, as Ebert (2013) writes that the barbels may contain receptors for taste, touch or other senses.

Furthermore, the conclusions drawn from current histological studies are problematic given the methodologies employed and the material used (e.g. unclear fixation methods (e.g. Sato, 1937) and low numbers of replicates (e.g. Goto *et al.*, 1994)).

This study presents a histological evaluation of the sensory structures present within the barbels of the Common Sawshark *Pristiophorus cirratus* (Latham 1794) with both light and fluorescent microscopy. In addition, due to the lack of morphometric data available for sawsharks in south-east Australia, especially in relation to a targeted fishery, various morphometrics of *P. cirratus* are presented and correlations between them examined. Also, in light of evidence for animals with prominent sensory structures such as whiskers (Aggestam and Cahusac, 2007) or elongated ventral fins (Bisazza *et al.*, 2001) to exhibit a preference as to which side of the body is used to sense the environment (i.e. behavioural lateralisation), this study investigates the possibility of lateralisation in the barbels of sawsharks. It is hypothesised that the barbels of *P. cirratus* contain both chemo- and mechanoreceptors to detect prey in the substrate, and are not lateralised.

2.3 Methods:

Morphometrics

Fresh but dead specimens of *Pristiophorus cirratus* were collected on the 29th April and 2nd June from a trawl fishery operator in Wollongong, Australia, immediately placed on ice, and transported to laboratories at Macquarie University for processing. Specimens were kept at 4°C and were processed on arrival. The following information was recorded for each specimen: total length (L_T), pre-caudal length (L_{PC}), rostrum length (L_R; defined as the distance from the tip of the rostrum to the nostrils), rostrum width at three locations (at the nostrils (W_{RN}), at the barbels (W_{RB}) and at the first pair of enlarged rostral teeth from the rostrum tip (W_{RT})), left and right barbel length (L_{BL} and L_{BR} respectively), mouth width (W_M; measured from one corner of the mouth to the other), and mouth length (L_M; mouth was opened as much as possible and the distance from the top jaw to the bottom jaw was measured). Photographs of each *P. cirratus* with a scale bar present were taken and information regarding sex and sexual maturity of the sawsharks recorded. Sexual maturity for males was assessed by checking the firmness (calcification) of the claspers (Clark and von Schmidt, 1965), whilst maturity for females was assessed by the presence or absence of mature follicles/ova during dissections (Holden and Raitt, 1974).

Regression analyses on the morphometric data were performed using the Excel analysis tool pack (Microsoft Excel 2010). The variable L_T was compared in separate analyses with all variables listed above (i.e. L_T vs L_{PC}; L_T vs L_R; etc.). A regression analysis was also performed on the L_{BL} and L_{BR} data to test for possible lateralisation in the barbels of *P. cirratus*. Differences between left and right barbel length were tested using the Mann-Whitney U test in Minitab (Version 16) (Minitab Inc., www.minitab.com), as it was not possible to transform these data to meet the assumptions of normality.

Magnetic Resonance Imaging (MRI) of sawshark heads

To visualise the neural pathways connecting the barbels to the brain in sawsharks, heads of *Pristiophorus cirratus* embryos that had been obtained during dissections were scanned with the Siemens MAGNETOM Verio 3T MRI machine at Macquarie Medical Imaging. The heads of the embryos were chosen for scanning over those of adult sharks as their translucent tissue may have allowed for improved visualisation of the nerves. Four heads were preserved in 70 % ethanol whilst another three were preserved in 10 % formaldehyde solution for one month. The two different preservatives were used to asses which method maintained the nerves best. Prior to scanning the heads were washed with water (3x 20 minutes) and placed into zip-locked bags. Scans took approximately 30 minutes to complete.

Histology of sawshark barbels

Histological samples, consisting of whole barbels, excised from each sawshark and preserved in 10 % formaldehyde solution, were taken within hours of collection. Samples were refrigerated at 4°C for two weeks. The barbels were then decalcified in Gooding and Stewart's Fluid (Culling, 1963) for two weeks. During these two weeks, decalcification progress was checked by adding 1 ml of 5 % ammonium oxalate to 5 ml of the decalcification fluid that had contained the tissue samples. When decalcification was complete, each sample was washed in distilled water (3x 20 minutes) and dehydrated in an ascending ethanol series (70 %, 80 %, 90 %, 100 %, 100 % and 100 %) for one hour each. The samples were then cleared with HistoChoice Clearing Agent (AMRESCO, Solon, Ohio, USA) and embedded in paraffin wax at 60°C in a Thermoline VORS vacuum oven. Transverse sections of a single barbel from 10 individuals were cut using a Spencer "820" rotary microtome set to a thickness of 4 µm. Sections were then stained with haematoxylin and eosin, and mounted onto glass slides with HistoChoice Mounting Media (AMRESCO, Solon, Ohio, USA). These were then viewed with an Olympus BX53 compound microscope and photographed with the attached Olympus DP26 digital camera in the accompanying cellSens imaging software.

Images from three sections of the barbel – the tip, middle and base (i.e. the thickest part of the barbel) – were used to assess the diameter of the cartilage core, and the number and diameter of the nerve fibre bundles on the left and right sides of the cartilage core, throughout the length of the barbel. Measurements of core and nerve bundle diameter were made with the imaging software ImageJ (Version 1.49g) (Abràmoff *et al.*, 2004). Due to the sectioning causing circular structures, such as the core and nerve bundles, to become elliptical in shape, diameter was defined as the average of the long and short axis of the ellipse. Hence the long axis and short axis was measured for each cartilage core and nerve bundle.

A total of approximately 30 sections (10 sections each from the tip, middle and base of the barbel) were also inspected for the presence of nerves and sensory structures. The number of sections was dependent on the ease of sectioning. These sections were initially examined at 10x magnification and any structures that could not be identified immediately were investigated further at 20x and 40x magnification.

The change in the diameter of the cartilage core throughout the length of the barbel was assessed using a one-way analysis of variance (ANOVA) on $\log_{10}$ transformed data. One-way ANOVAs were also performed on the data pertaining to the number of nerve bundles and their diameters. Since the diameter of a nerve bundle would be dependent on those nerve bundles surrounding it, and the number of nerve bundles left and right of the cartilage core was often uneven, the average diameter of the nerve bundles at each section of the barbel was analysed to avoid biasing the results. For some analyses with these data, it was necessary to perform a $\log_{10}$ transformation for the assumptions of normality to be met. Results of all ANOVAs were considered significant at $\alpha = 0.05$, and those displaying significance were tested further with a Tukey HSD post hoc test. All analyses were conducted in Minitab.

Fluorescent microscopy

Several histological sections were used to test the effectiveness of fluorescent Nissl staining on the barbels of sawsharks. Sections were deparaffinised with HistoChoice Clearing Agent, rehydrated in a descending ethanol series (2x 100 % for 5 minutes; 2x 85 % for 3 minutes; and 1x 70 % for 3 minutes) and rinsed with tap water. They were then washed with 1x phosphate buffered saline (PBS) for three minutes, PBS with 0.5 % Triton X-100 (PSBT) for 10 minutes, and PBS for a further 10 minutes before being stained with NeuroTrace® 530/615 red fluorescent Nissl stain (Invitrogen, catalogue number N21482). Three dilutions (1:20, 1:140 and 1:300) were tested at two staining times (20 minutes and one hour). After the staining time elapsed, the sections were rinsed with PBST for 10 minutes and a series of PBS

(2x five minutes followed by 2x 20 minutes) before being counterstained with 4`,6-diamidino-2-phenylindole 1:1000 dilution (DAPI; Life Technologies, catalogue number D3571) for five minutes. The tissue sections were then washed with PBS (3x five minutes) and mounted onto glass slides. Slides were viewed using an Olympus Fluoview FV1000 IX81 inverted confocal microscope and images taken with the accompanying FLUOVIEW Viewer software (Version 3.1a). Sections that were not stained with NeuroTrace® or DAPI acted as controls. Figures of the stained sections were created with the free software ScientiFig (Aigouy and Mirouse, 2013).

2.4 Results:

Morphometrics

A total of 41 *Pristiophorus cirratus* with an average total length of 91.44 cm (± 5.47 S.D.) were obtained from the Wollongong trawl fishery (39 males and two females). Clasper firmness indicated that all males were sexually mature. Dissections on the two females showed that one was either maturing or mature, whilst the other was sexually mature due to the presence of eight developing embryos (Figure 2.1) in the oviduct.

Figure 2.1: *Pristiophorus cirratus* embryos recovered from a pregnant female, with a 30 cm ruler below. Average total length of embryos = 15.65 cm (± 1.03 S.D.).

Total length (L_T) of *P. cirratus* was significantly correlated with most of the variables tested, except for rostrum width at the barbels (W_{RB}) and rostrum width at the tip (W_{RT}) (Table I). Of those significant correlations, the three strongest positive correlations based on the coefficient of determination (r^2) were pre-caudal length (L_{PC}), rostrum length (L_R) and mouth width (W_M) whilst the weakest correlation was rostrum width at the nostrils (W_{RN}). There was no significant difference between the median left barbel length (L_{BL}) and the median right barbel length (L_{BR}) (W = 1566.5, p = 0.609). When L_{BL} and L_{BR} for each individual *P. cirratus* were

plotted against each other, a significant correlation was found ($F_{1,38}$ = 36.389, p = < 0.001, r^2 = 0.489), however this was not a 1:1 relationship (Figure 2.2). The 99% confidence interval for the slope of the regression line was less than one (lower limit = 0.302; upper limit = 0.797), suggesting that the barbels of *P. cirratus* may be lateralised. Furthermore, the number of *P. cirratus* that had barbels of even lengths (n = 11) was less than the number that had barbels of uneven lengths (n = 29). Of the 29 individuals with one barbel longer than the other, the right barbel was longer in 14 individuals while the left barbel was longer in 15 individuals, indicating that two types of laterality may exist in *P. cirratus* – left side lateralised individuals and right side lateralised individuals.

Table I: Regression analyses comparing total length (L_T) of *Pristiophorus cirratus* with other measurements. Correlation coefficient (r) is presented with the 95% confidence interval (CI). L_{PC} = pre-caudal length; L_R = rostrum length; W_{RN} = rostrum width at nostrils; W_{RB} = rostrum width at barbels; W_{RT} = rostrum width at tip; L_{BL} = left barbel length; L_{BR} = right barbel length; W_M = mouth width; L_M = mouth length.

Comparison	F statistic	d.f.	p value	r (95% CI)	r^2
L_T vs L_{PC}	1536.082	1,39	< 0.001	0.988 (0.977, 0.993)	0.975
L_T vs L_R	113.261	1,39	< 0.001	0.862 (0.756, 0.924)	0.744
L_T vs W_{RN}	6.353	1,39	0.016	0.374 (0.076, 0.611)	0.140
L_T vs W_{RB}	0.882	1,39	0.353	0.149 (-0.436, 0.166)	0.022
L_T vs W_{RT}	3.983	1,39	0.053	0.304 (-0.003, 0.559)	0.093
L_T vs L_{BL}	8.578	1,39	0.006	0.425 (0.135, 0.647)	0.180
L_T vs L_{BR}	18.925	1,38	< 0.001	0.577 (0.324, 0.752)	0.332
L_T vs W_M	65.482	1,39	< 0.001	0.792 (0.640, 0.884)	0.627
L_T vs L_M	10.690	1,39	0.002	0.464 (0.183, 0.675)	0.215

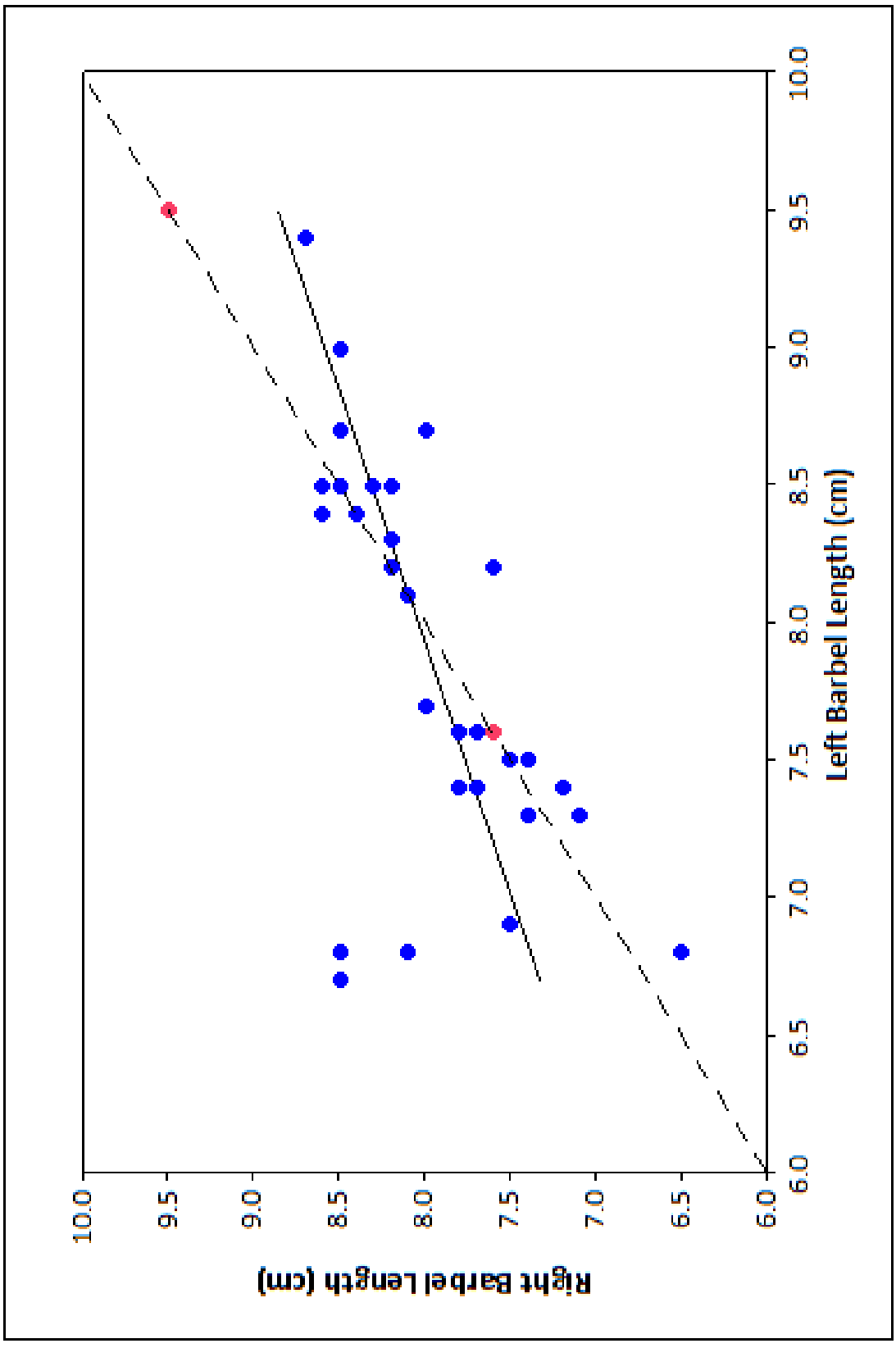

Figure 2.2: Relationship between left barbel length and right barbel length in 40 adult *Pristiophorus cirratus* (38 males (blue circles) and two females (pink circles)). The black dotted line represents a 1:1 relationship between the lengths of the left and right barbels (i.e. no lateralisation).

Magnetic Resonance Imaging (MRI) of sawshark heads

Despite multiple attempts, it was not possible to visualise the neural pathway connecting the barbels to the brain in the *P. cirratus* embryos using the available MRI equipment. The small size and low water content of the samples prevented the resolution required to visualise such structures from being achieved. For samples of this size, it is recommended that they are scanned in an ultra-high field MRI to achieve the best results.

Histology of sawshark barbels

The external surface of the barbel was covered in broad, leaf-shaped dermal denticles that occurred along its entire length (Figure 2.3). When the internal structure of the barbel was examined, it was found that the base of each denticle was anchored in the dermis and extended outwards to cover the epidermis (Figure 2.4). Bundles of nerve fibres were situated on the left and right of an elastic cartilage core surrounded by a thick perichondrium; with a blood vessel positioned in between the bundles of nerve fibres left of the core, and a second blood vessel located anterior to the bundles of nerve fibres right of the core.

When barbel sections were examined for any sensory structures, such as taste buds, free neuromasts or ampullary receptors none could be found in either the epidermis or dermis. However, structures positioned between the epidermis and dermis, and between dermal denticles, (Figures 2.5 and 2.6) were investigated further to determine whether these could be taste buds. After inspection at 20x and 40x magnification, and comparison with published literature (e.g. Kemp, 1999; Miyake *et al.*, 1999; Debiais-Thibaud *et al.*, 2011), it was concluded that these structures were developing dermal denticles. These arrowhead-shaped structures consisted of a central mass of odontoblasts (dentin secreting cells) separated by a layer of enamel from the enamel secreting ameloblasts at the dorsal surface.

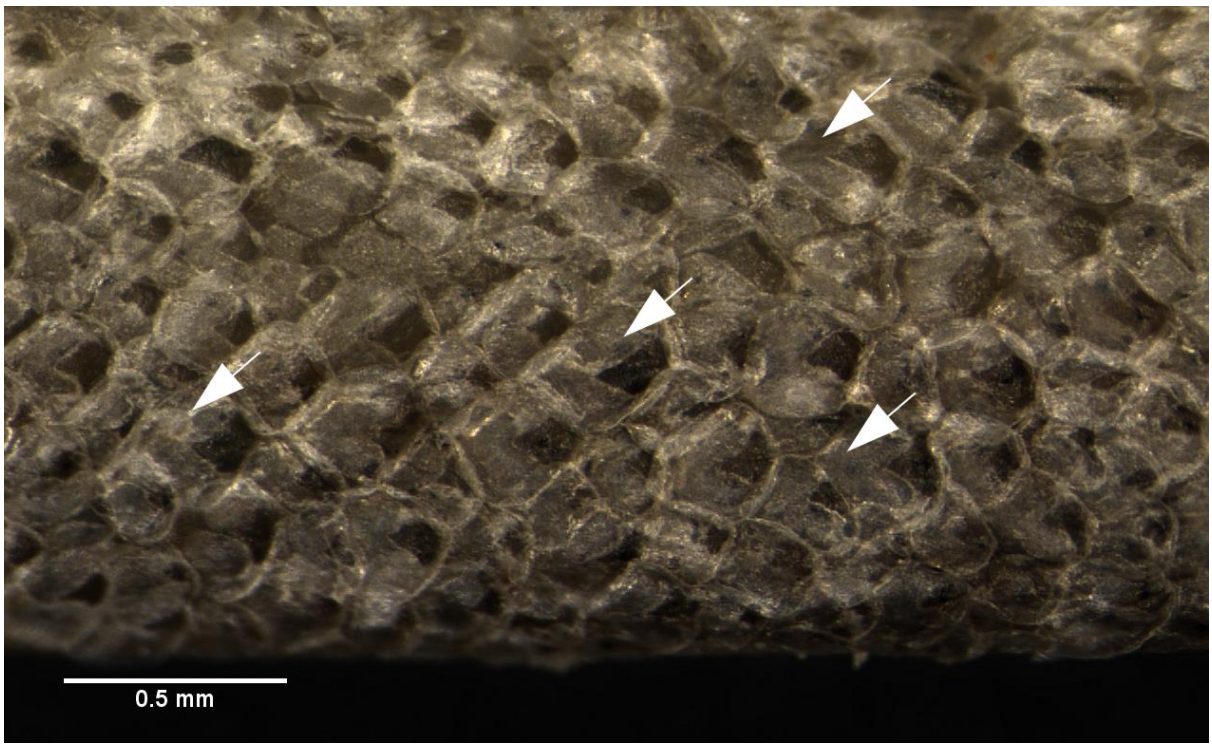

Figure 2.3: Dermal denticles covering the external surface of a *Pristiophorus cirratus* barbel. Arrows indicate individual denticles.

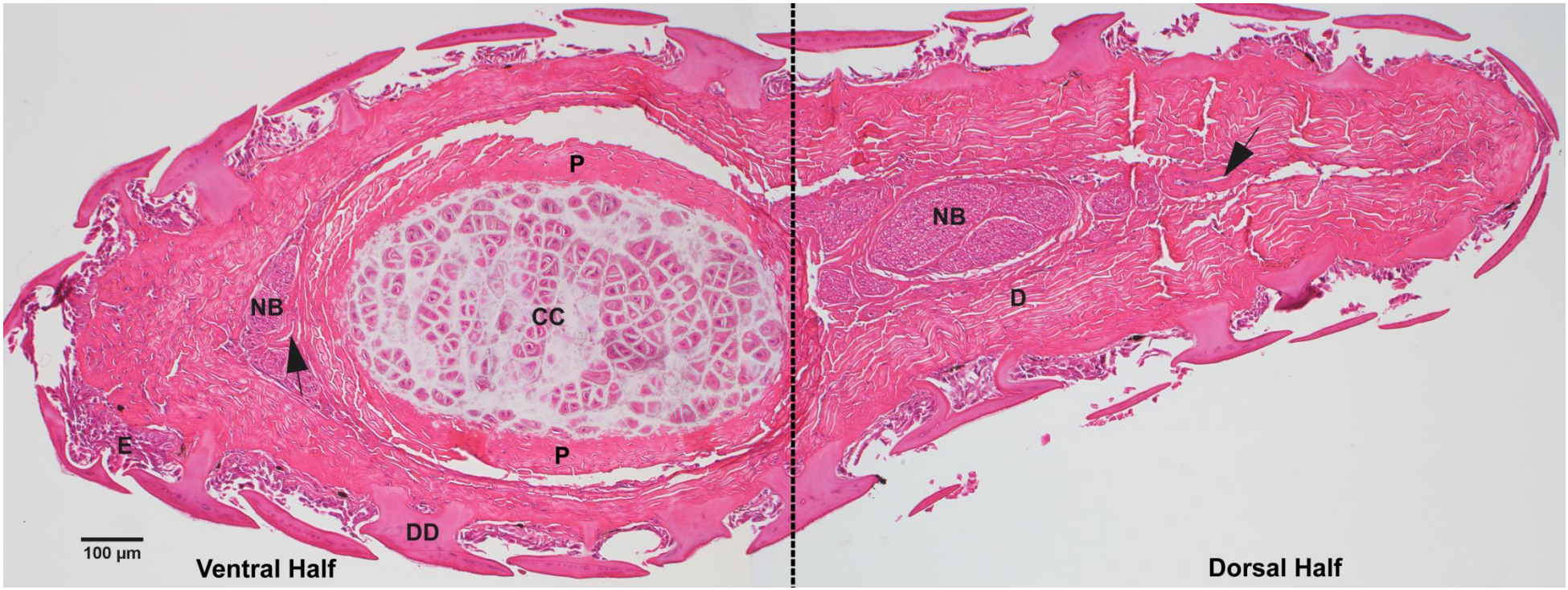

Figure 2.4: Transverse section (4 µm thick) of a *Pristiophorus cirratus* barbel stained with haematoxylin and eosin. Black arrows = blood vessels; CC = cartilaginous core; D = dermis; DD = dermal denticle; E = epidermis; NB = bundles of nerve fibres; P = perichondrium. Dashed line indicates the division used when quantifying the number of nerve bundles on the left and right of the cartilaginous core.

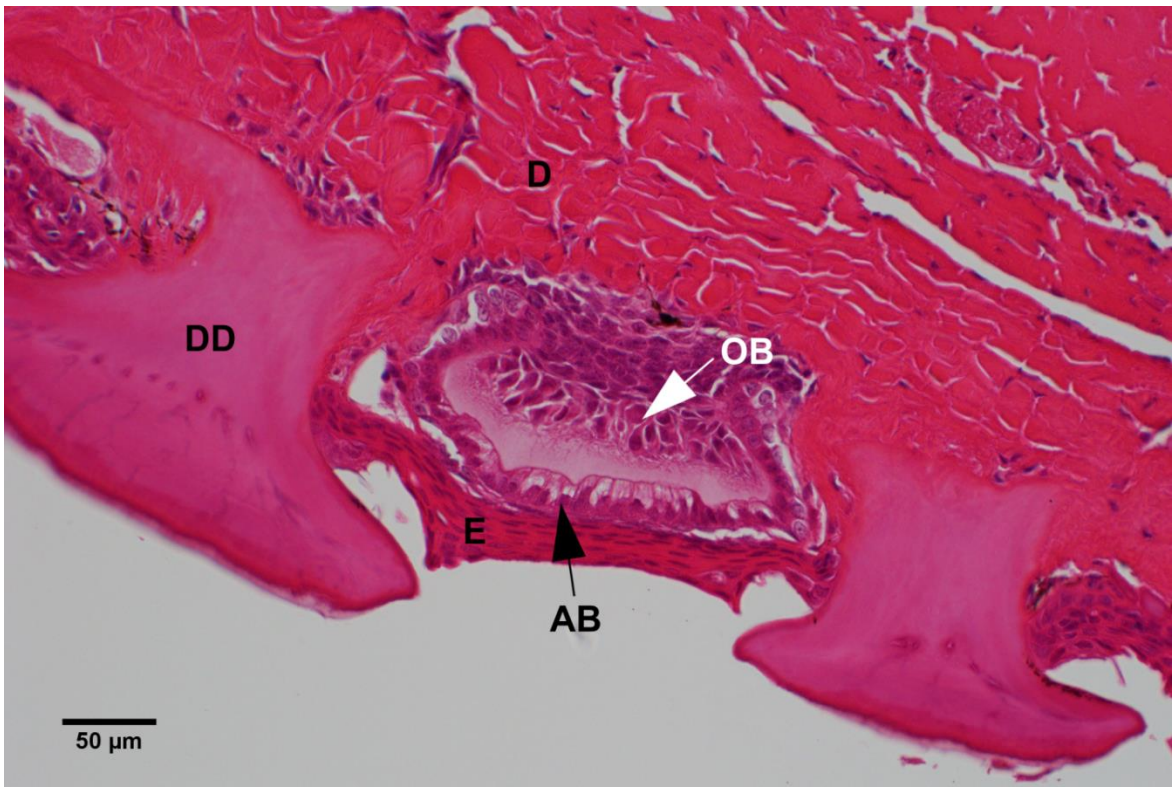

Figure 2.5: Developing dermal denticle situated between two erupted dermal denticles in the barbel of *Pristiophorus cirratus*. The boundary between the enamel and the dentin layer is not yet visible. Section thickness = 4 µm. AB = ameloblasts; D = dermis; DD = dermal denticle; E = epidermis; OB = odontoblasts.

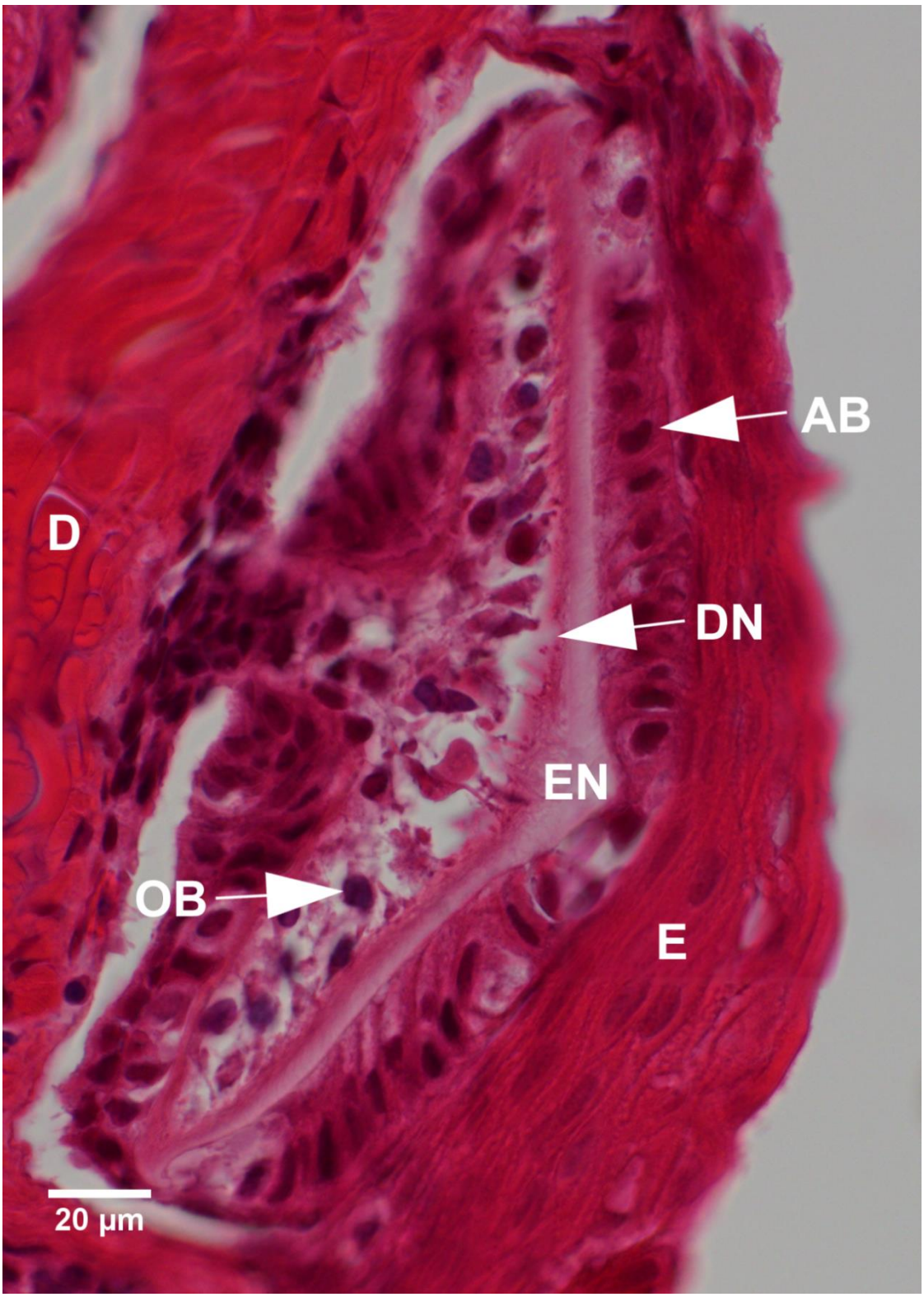

Figure 2.6: Developing dermal denticle in the barbel of *Pristiophorus cirratus*, showing the boundary between the enamel and the dentin layer. Section thickness = 4 µm. AB = ameloblasts; D = dermis; DN = dentin; E = epidermis; EN = enamel; OB = odontoblasts.

The diameter of the cartilage core changed significantly throughout the length of the barbel ($F_{2,26}$ = 62.78, p = < 0.001). The Tukey HSD post hoc test showed that there were significant differences in the diameter of the cartilage core at each section of the barbel. On average, the diameter of the core was found to be smallest at the tip and over three times larger at the base (Figure 2.7).

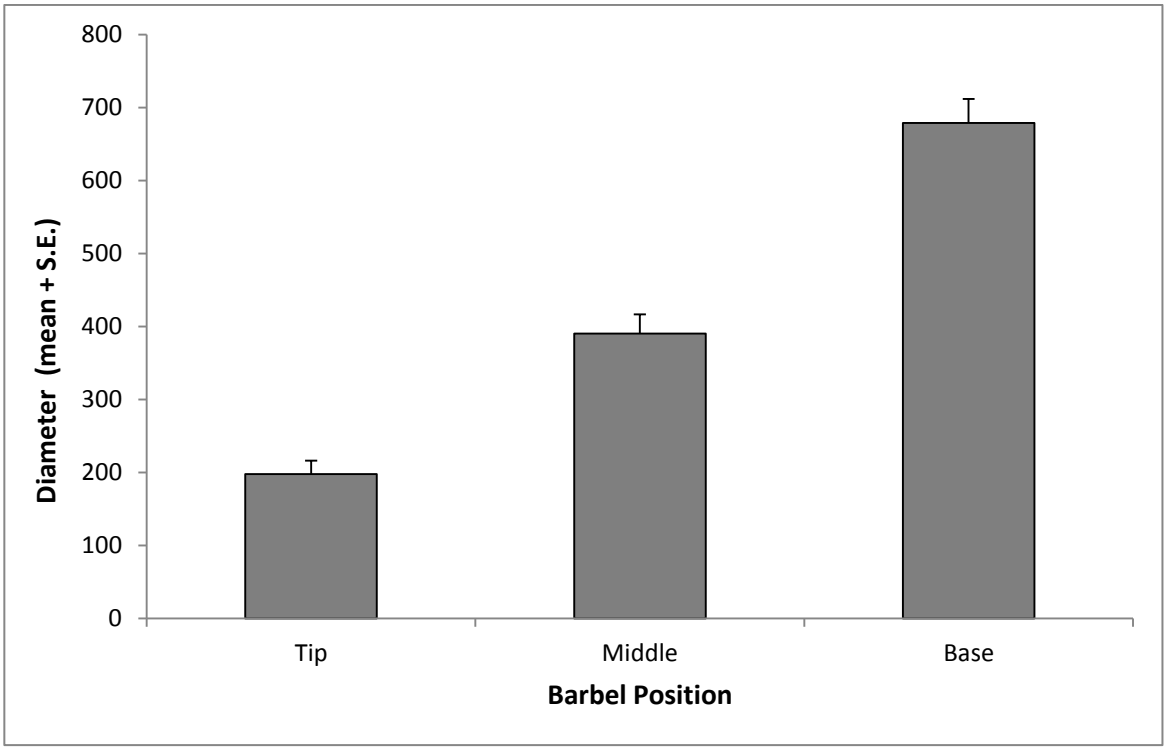

Figure 2.7: Bar chart showing the mean (+ S.E.) diameter (in µm) of the cartilage core at three sections of *Pristiophorus cirratus* barbels.

The number of nerve bundles in each half of the barbel was compared throughout the length of the barbel. The dorsal half of the barbel was defined as the half where the blood vessel was located anterior to the bundles of nerve fibres, whilst the ventral half was defined as the half where the blood vessel was in between the bundles of nerve fibres (Figure 2.4). There was no significant change in the number of nerve bundles in the dorsal half of the barbel throughout its length ($F_{2,24}$ = 1.24, p = 0.308), however a significant change was found for the ventral half ($F_{2,24}$ = 14.08, p = < 0.001). In this half, the average number of nerve bundles decreased significantly from the tip of the barbel towards the base (Figure 2.8). However, the average number of nerve bundles did not differ significantly between the tip and middle of the barbel. The number of nerve bundles present in the dorsal and ventral halves was also compared at the three sections of the barbel (i.e. tip, middle and base). Significant differences were found between the two halves at the tip ($F_{1,14}$ = 8.03, p = 0.013) and base ($F_{1,16}$ = 9.96, p = 0.006), but not in the middle of the barbel ($F_{1,18}$ = 2.62, p = 0.123) (Figure 2.8).

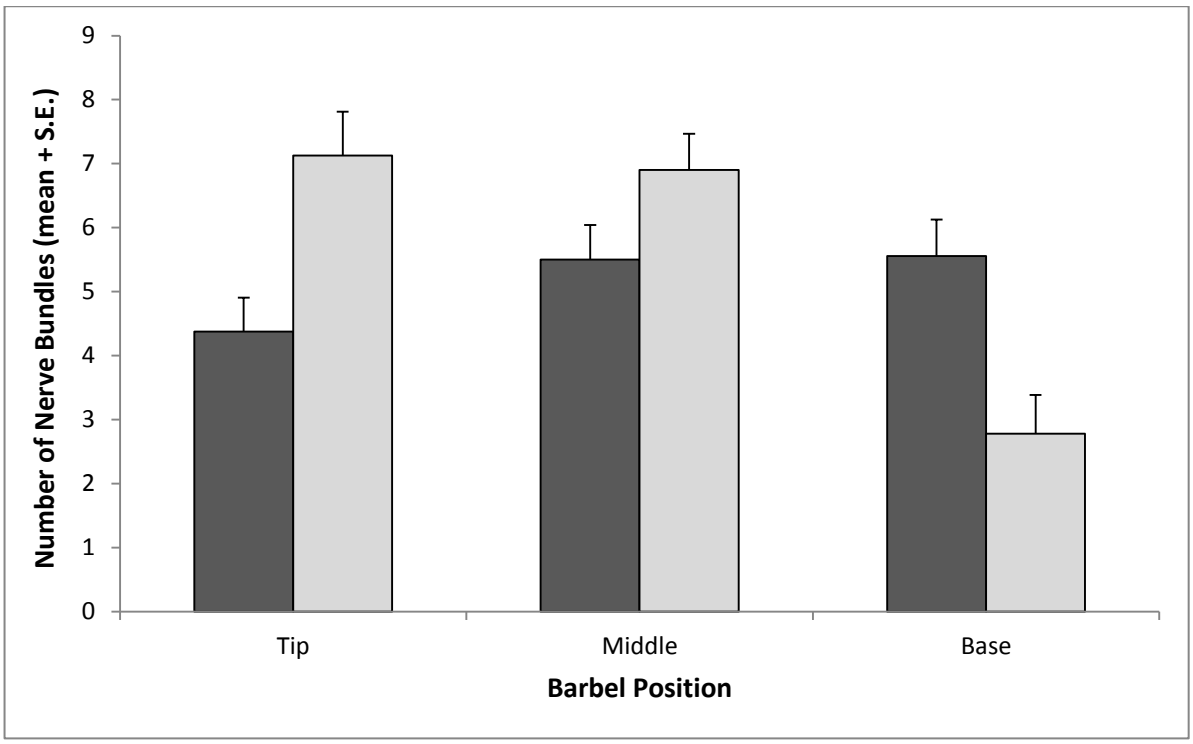

Figure 2.8: Bar chart showing the mean (+ S.E.) number of nerve bundles in each half of the barbel of *Pristiophorus cirratus* at three sections. Dark grey bars represent the dorsal half of the barbel; Light grey bars represent the ventral half of the barbel.

Throughout the length of the barbel, the diameter of the nerve bundles in the dorsal half was found to change significantly ($F_{2,24}$ = 33.53, p = < 0.001). A Tukey HSD post hoc test revealed that the diameters of the nerve bundles measured at the tip, middle and base of the barbel were all significantly different in the dorsal and ventral halves, with the diameter at the tip being the smallest and the diameter at the base the largest (Figure 2.9). A significant difference was also found for the nerve bundles in the ventral half ($F_{2,24}$ = 15.55, p = < 0.001), however this difference only applied to the nerve bundles located at the base of the barbel (Figure 2.9). At each section of the barbel, a significant difference in nerve bundle diameter between the two halves was shown for the middle ($F_{1,18}$ = 38.02, p = < 0.001) and base ($F_{1,16}$ = 41.74, p = < 0.001), but not at the tip ($F_{1,14}$ = 0.23, p = 0.640) (Figure 2.9).

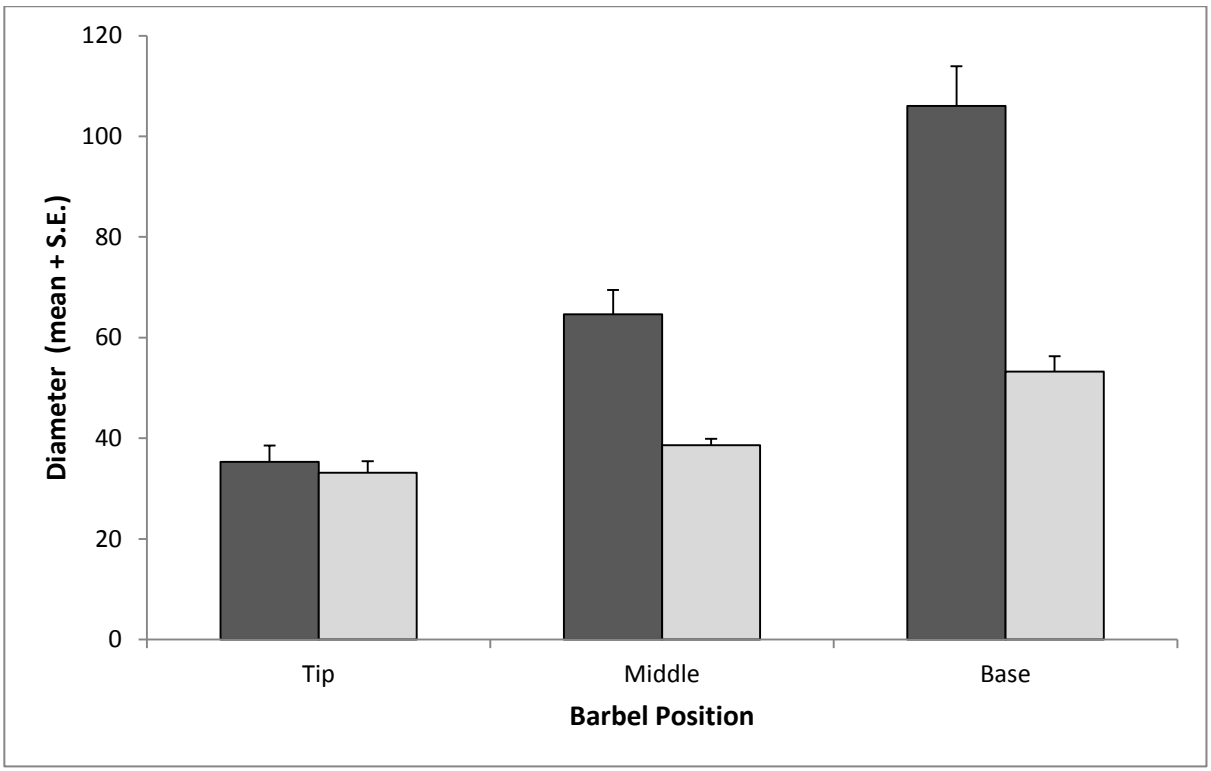

Figure 2.9: Bar chart showing the mean (+ SE) diameter (in µm) of the nerve bundles on each side of the cartilage core at three sections of the *Pristiophorus cirratus* barbel. Dark grey bars represent the dorsal half of the barbel; Light grey bars represent the ventral half of the barbel.

Fluorescent microscopy

Barbel sections of *Pristiophorus cirratus* that were stained with NeuroTrace® in phosphate buffered saline (PBS) at a dilution of 1:20, 1:140 and 1:300 for an hour each did not show fluorescence, nor did sections stained at dilutions of 1:140 and 1:300 for 20 minutes. Fluorescence was observed; however, for sections stained at a dilution of 1:20 for 20 minutes. NeuroTrace® staining in these sections occurred in the dermis and epidermis of the barbel, thus suggesting the presence of neurons. The intensity of the fluorescence was strongest in the cells of the epidermis (Figure 2.10), in particular those epidermal cells near or in between dermal denticles (Figure 2.11).

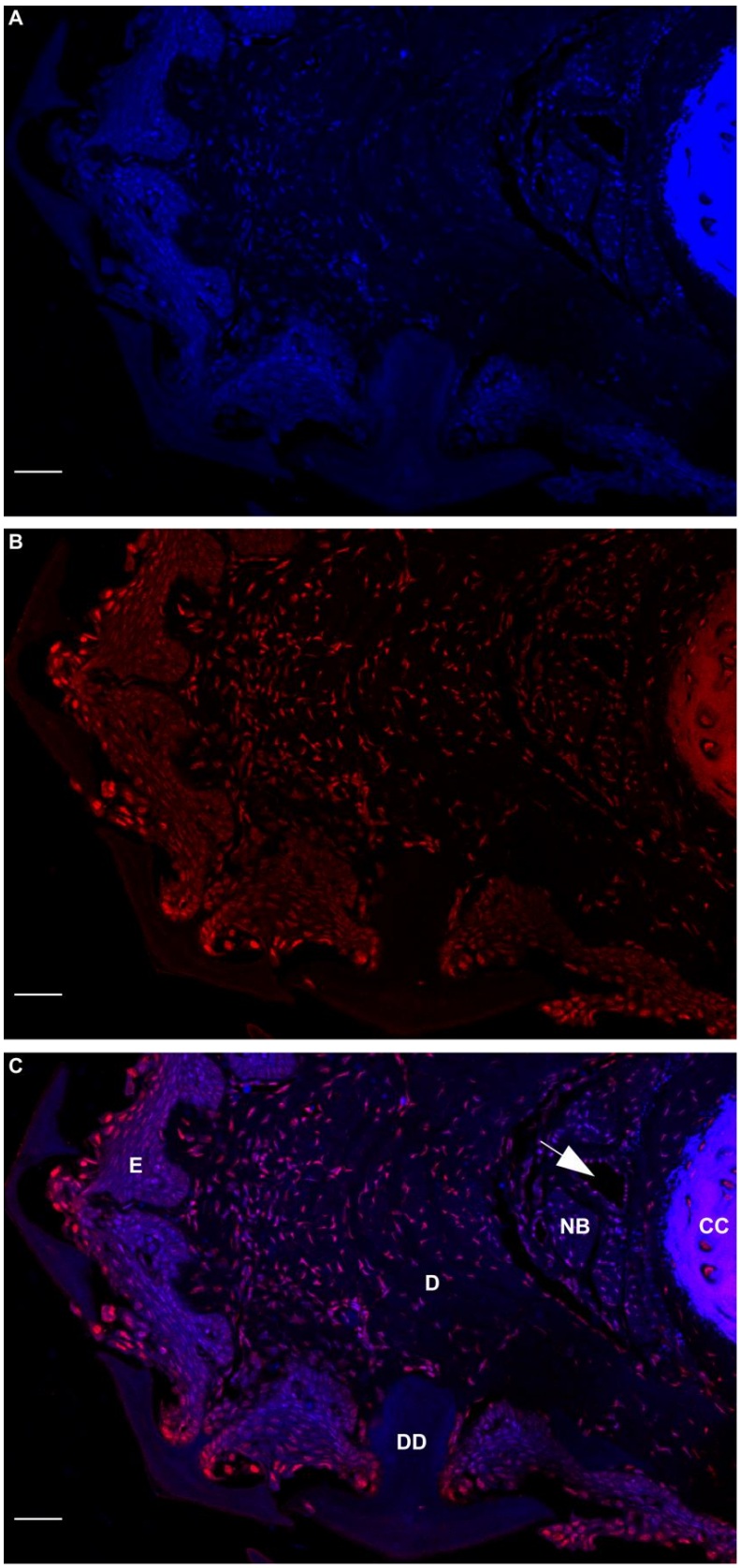

Figure 2.10: Transverse section (4 µm thick) of a *Pristiophorus cirratus* barbel stained with fluorescent dyes to detect neurons in the barbels. A) DAPI (counter stain); B) NeuroTrace®; C) DAPI and NeuroTrace® stains combined. CC = cartilaginous core; D = dermis; DD = dermal denticle; E = epidermis; NB = bundles of nerve fibres; White arrow = blood vessel. Scale bars = 40 µm.

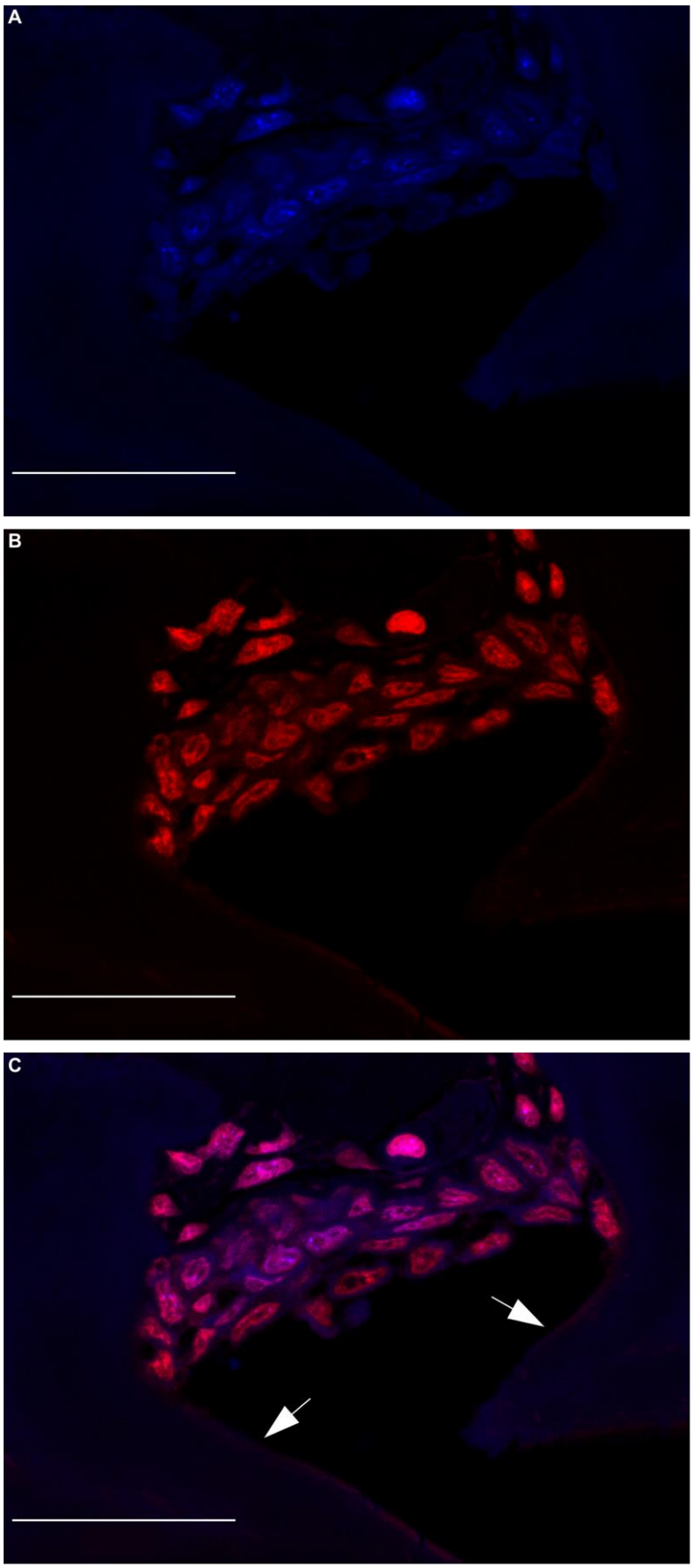

Figure 2.11: Epidermal cells between two dermal denticles stained with fluorescent dyes to detect neurons in *Pristiophorus cirratus* barbels sectioned at 4 µm. A) DAPI (counter stain); B) NeuroTrace®; C) DAPI and NeuroTrace® stains combined. White arrows = dermal denticles. Scale bars = 40 µm.

2.5 Discussion:

The current hypotheses regarding the sensory capabilities of the barbels of sawsharks include chemoreception (Compagno, 1984) and mechanoreception (Hoffmann, 1912; Sato, 1937; Goto *et al.*, 1994). Although the latter hypothesis is supported by histological studies, confusion as to the sensory capabilities of barbels in sawsharks has persisted (e.g. Ebert, 2013). The results of this study provide further evidence in support of the mechanoreception hypothesis, since chemoreceptive structures such as taste buds were not found in the histological sections of *Pristiophorus cirratus* barbels. Other receptors such as ampullae and free neuromasts could also not be located. This study also presents evidence to suggest that the barbels of sawsharks may be lateralised and contain neurons in the epidermis that may be connected to tactile receptors or free nerve endings.

The internal features of the barbel identified in this study were mostly consistent with previous histological investigations (Table II). The one discrepancy between this and previous studies was regarding the composition of the barbel's core. Goto *et al.* (1994) stated that the core is composed of a non-cartilaginous substance, whilst other studies have found that it is composed of cartilage (Hoffmann, 1912; Sato 1937; this study). The reason for this discrepancy is unclear and it is not possible to compare the findings of the present study with those of Goto *et al.* (1994) as no images of their barbel sections are presented. However, the identification of the core as cartilaginous in this study is consistent with elastic cartilage since the cells had irregular shapes and a nucleus in the centre (Sato, 1977) and the core was surrounded by a thick perichondrium (Benjamin, 1990).

Study	Species	Structures within the Barbel		Cranial Nerve Innervation	No. of Replicates	Section Thickness
Hoffmann (1912)	*Pristiophorus nudipinnis*	Cartilaginous core	(+)	Ramus buccalis (VII)	Not stated*	Not stated*
		Blood vessels	(+)			
		Nerve fibres	(+)			
		Taste buds	(-)			
		Ampullae of Lorenzini	(-)			
		Tactile sensory cells on the epidermis	(+)			
Sato (1937)	*Pristiophorus japonicus*	Cartilaginous core	(+)	N/A	Not stated	10 µm
		Blood vessels	(+)			
		Nerve fibres	(+)			
		Taste buds	(-)			
		Mucous cells	(-)			
Goto *et al.* (1994)	*Pristiophorus japonicus*	Cartilaginous core†	(-)	Ramus buccalis (VII)	1	10 µm
		Muscles	(-)			
		Taste buds	(-)			
This study	*Pristiophorus cirratus*	Cartilaginous core	(+)	N/A	10	4 µm
		Blood vessels	(+)			
		Nerve fibres	(+)			
		Taste buds	(-)			
		Ampullae of Lorenzini	(-)			
		Free neuromasts	(-)			
		Tactile sensory cells on the epidermis	(?)			

Table II: Summary of the findings from previous histological investigations of the barbels of three species of sawsharks, including the internal structures, cranial nerve innervation, number of replicates (i.e. individual sharks) for each study and the section thickness utilised. The listed internal structures are based on what was reported in each study. The symbol (+) denotes that the structure was found; (-) denotes that the structure was not found; and (?) denotes that the structure may have been found. N/A indicates that the study did not examine the innervation of the cranial nerves. (Note (*): such information could not be found in the small section of this paper that has been translated from German to English. Note (†): Goto *et al.* (1994) stated that the core is composed of a non-cartilaginous substance.)

The section thickness utilised in this study (4 µm) was thinner than had been previously attempted (e.g. Sato, 1937; Goto *et al.*, 1994). However, sensory structures such as taste buds, free neuromasts or ampullae could still not be located. When the arrowhead-shaped structures found within the barbel (Figures 2.5 and 2.6) were compared with the work of Miyake *et al.* (1999) and Debiais-Thibaud *et al.* (2011), they more closely resembled developing dermal denticles rather than taste buds or other sensory cell types. The structures were not the characteristic bulbous shape of vertebrate taste buds (Takashima and Hibiya, 1995) and lay between the epidermis and dermis, rather than being embedded in the epidermis. Thus, these structures were concluded to be dermal denticles. Given the absence of sensory structures in the histological sections, and that previous anatomical studies have indicated that the barbels are innervated by branches of the trigeminal (V) (von Bonde, 1933) and/or facial (VII) cranial nerves (Hoffmann, 1912; Goto *et al.*, 1994), it is hypothesised that the barbels of sawsharks serve a tactile function. It is also likely that these barbels convey thermo- and nocisensory information due to the innervation of the trigeminal nerve (V). In order to exclude the possibility of the barbels being involved in chemoreception, it is recommended that further histological work in the form of transmission electron microscopy (TEM) be conducted to determine if solitary chemosensory cells (Whitear, 1965; Kotrschal, 1991) are present. This would also allow for the presence of receptors of other senses to be investigated simultaneously.

A predominantly tactile role for the barbels is unusual amongst fishes. Thus far only a small number of species have been found to lack taste buds in their barbels, including the Striped Dwarf Catfish *Mystus vittatus* (Bloch 1794) (Agarwal and Rajbanshi, 1965) and members of the plunderfish family Artedidraconidae (Eastman and Lannoo, 2003; Eakin *et al.*, 2006). The manner in which these fishes utilise the tactile sense of their barbels has yet to be determined; however, in the case of the plunderfish *Dolloidraco longedorsalis* (Roule 1913), it is hypothesised that the barbel on the lower jaw supplements the information received by the lateral line when hunting polychaetes (Eastman and Lannoo, 2003). In fishes whose barbels are capable of both chemo- and mechanoreception, the tactile sense appears to play a key role in determining the precise location of prey. For example, Stone Loaches *Barbatula barbatula* (L. 1758) with their barbels removed were less successful at retrieving food pellets than those which retained their barbels (Kasumyan *et al.*, 2010). Thus it appears that one of the main roles of the tactile sense in barbels relates to the detection and/or location of prey.

Tactile detection of prey is hypothesised to be the main function of the barbels in sawsharks. The observed change in the diameter of the cartilage core throughout the length of the barbel

suggests that the barbel is conical in shape, which has recently been shown to be the ideal shape for tactile reception in mouse whiskers (Hires *et al.*, 2013). The diet of *P. cirratus* consists of demersal teleosts and crustaceans (Last and Stevens, 2009), including members of the Cynoglossidae and Platychephalidae families (Chapter Three) which partially bury themselves in the sediment. Given that the barbels lack muscles (Goto *et al.*, 1994), it is unlikely that sawsharks probe the sediment for prey like goatfishes (Gosline, 1984; Kim *et al.*, 2001). Rather it is hypothesised that the barbels contact the sediment as the shark swims and any prey-related changes in the surface texture of the benthos are detected.

A further function of the barbels may be to facilitate positive rheotaxis. Sawsharks are often photographed in a head-up orientation with the rostrum held at a 45° angle above the substrate (personal observation). This raising of the rostrum and the attached rostral barbels into the water column may allow changes in current direction to be detected so that the shark can position itself against the direction of flow, and thus ensure water passes over its gills when resting on the substrate (Hodgson and Mathewson, 1971). Whether these are indeed the purposes of the barbels in sawsharks requires behavioural experiments to be conducted once adequate husbandry protocols have been developed.

The preliminary fluorescent microscopy work performed in this study may have revealed the method by which the barbels of sawsharks receive mechanosensory information from the environment. Unlike terrestrial vertebrates, the epidermis of most fishes and sharks is composed of living cells since it is protected from the external environment by scales or dermal denticles (Kemp, 1999). Given that the entire external surface of the barbel is covered by dermal denticles, it is possible that the presence of neurons in the epidermis, in particular in the spaces between dermal denticles, enables tactile stimuli to be received. These neurons could be the epidermal tactile cells mentioned by Hoffmann (1912) when examining the barbels of *Pristiophorus nudipinnis*. Alternatively, the neurons could be associated with the aforementioned epidermal tactile cells or are connected to free nerve endings. It is recommended that these results be treated with caution since the fluorescent Nissl staining was only conducted once on a small number of sections; it was not possible to determine as to what sensory receptors or free nerve endings the neurons were attached; and it is unclear whether the cells stained are indeed neurons. The staining pattern observed with NeuroTrace® could be due to the high protein synthesis activity of elasmobranch epidermal cells (Meyer and Seegers, 2012); a feature shared by neurons (Kourtis and Tavernarakis, 2009). Furthermore, a study on embryo Small-Spotted Catsharks *Scyliorhinus canicula* (L. 1758) utilised the cresyl violet (Nissl) stain to visualise the development of dermal denticles

(Debiais-Thibaud *et al.*, 2011). Therefore, the cells stained by the NeuroTrace® may also be part of developing dermal denticles. It has been suggested that the teeth of Great White Sharks *Carcharodon carcharias* (L. 1758) possess a pore at their base to allow nerves to pass into the root of the tooth and thus enable mechanoreception (Hammerschlag *et al.*, 2012). Whether this occurs with dermal denticles as well is unknown, and so further research is required to determine if the cells stained in this study are both structural and sensory.

Examination of the morphometrics of the *P. cirratus* caught as a result of a targeted trawl fishery has provided some interesting results. Firstly, two of the collected measurements (rostrum width at the barbels and rostrum width at the tip) were not significantly correlated with the total length of the sawsharks. It is possible that these non-significant correlations are due to the tapering form of the rostrum. Secondly, there appears to be a bias in the gender of the sawsharks that were captured in the trawls. The majority of the *P. cirratus* examined in this study were mature males which could indicate that segregation by maturity and sex occurs in this species. It has been noted that adults and juveniles of the Sixgill Sawshark *Pliotrema warreni* (Regan 1906) are partially segregated by depth, with juveniles inhabiting shallower water (Bass *et al.*, 1975). Further research is needed to determine whether this is also the case for *Pristiophorus cirratus*. The observed male bias may be related to the seasons, as the breeding season for *P. cirratus* has been reported to be during winter (Whitley, 1940) and the sharks in this study were collected in late autumn and early winter. Thus it is possible that the large number of mature males caught in the trawl reflects the breeding behaviour of this species, as at this time males may be more abundant and active in order to locate mates.

Finally, the comparison of the lengths of the left and right barbels at the individual level provided evidence to suggest that the lateralisation may be present in the barbels of sawsharks. To the best of the author's knowledge, this is the first time that lateralisation has been reported in barbels; although lateralisation has been reported in similar tactile sensing structures. For example, rats preferentially use the whiskers on either the left or right side of the head when assessing the roughness of a surface and are unable to effectively assess surface roughness when the whiskers ipsilateral to their preference are trimmed (Aggestam and Cahusac, 2007). Similarly, Three Spotted Gourami *Trichogaster trichopterus* (Pallas 1770), which possess elongated ventral fins, prefer to use their left ventral fin to investigate inanimate objects (Bisazza *et al.*, 2001). In this study, 15 *Pristiophorus cirratus* had a longer left barbel while 14 had a longer right barbel, suggesting that two types of laterality (left side lateralised individuals and right side lateralised individuals) may be present in this species and that the preference for which barbel is used to sense the environment is dependent on the

individual. To test this hypothesis of lateralised barbels in sawsharks, behavioural studies and/or comparisons of receptor density in left and right barbels are required. It is possible that lateralised barbels exist in other fishes and may have thus far been overlooked.

In conclusion, this study has provided histological evidence in support of the mechanosensory hypothesis for the barbels of sawsharks. With the inclusion of this study, three species of sawsharks have now had the sensory structures within their barbels histologically evaluated, namely *Pristiophorus nudipinnis*, *P. japonicus* and *P. cirratus* (Table II). Thus it is likely that the barbels of all sawsharks serve a tactile function. It is also possible that the barbels could convey thermo- and nocisensory information. Additionally, this study offers evidence to suggest that the barbels of sawsharks may be lateralised. In order to test these hypotheses further, behavioural studies are required.

Chapter 3: Comparison of rostral tooth microwear to assess rostrum function in sawsharks

3.1 Abstract:

The potential roles of the rostrum of sawsharks - including predation, self-defence, or both - were assessed through a variety of inferential methods. Comparison of microwear on the surface of the rostral teeth of sawsharks and sawfishes showed that microwear patterns were alike, and suggested that the elongated rostra in the two elasmobranchs were used for a similar purpose (predation). Raman spectroscopy revealed that the rostral teeth of sawsharks and sawfishes were composed of hydroxyapatite, but differed in their collagen content. Sawfishes were found to possess collagen throughout their rostral teeth whereas collagen was present only in the middle of the rostral teeth of sawsharks, which may relate to differences in ecology. Analysis of the stomach contents of the Common Sawshark *Pristiophorus cirratus* indicated that the most important component of the diet was pandalid shrimp. Comparison of proportional rostrum length between *P. cirratus* and two sawfishes showed that this was significantly different for the two elasmobranchs. Histological analysis of the unusual structure on the tip of the *P. cirratus* rostrum, named the "fingerprint", for a sensory role was inconclusive; however it is proposed that this structure is a dense collection of epidermal cells. In light of all the results, it is hypothesised that sawsharks use their rostrum for predation (sensing and capturing prey) and possibly for self-defence.

3.2 Introduction:

Investigation of the different forms of elongated rostra suggests that a single form may serve multiple purposes. Defined as a lengthening of the skull's rostral process (Schultze, 1993), the forms of elongated rostra include long and slender (e.g. billfishes), broad and flat (e.g. paddlefishes), or tooth bearing (e.g. sawfishes and sawsharks; Figure 3.1). In billfishes, the role of the long, pointed rostrum has been attributed to the wounding and capture of prey (Schneider and Fierstine, 2004; Shimose *et al.*, 2007; Domenici *et al.*, 2014); however there is also evidence of the rostrum being used for self-defence (Fierstine, 1997; Fierstine *et al.*, 1997), and/or for enhancing swimming speed by reducing drag (Wisner, 1958; Videler, 1993; but see Sagong *et al.*, 2013). Similarly, the broad, flat rostrum of paddlefishes has been shown to facilitate the detection of planktonic prey via electroreception (Russell *et al.*, 1999; Freund *et al.*, 2002), but may also be employed in navigation (Wilkens *et al.*, 1997) and assist in stabilising paddlefishes whilst filter feeding (Allen and Riveros, 2013).

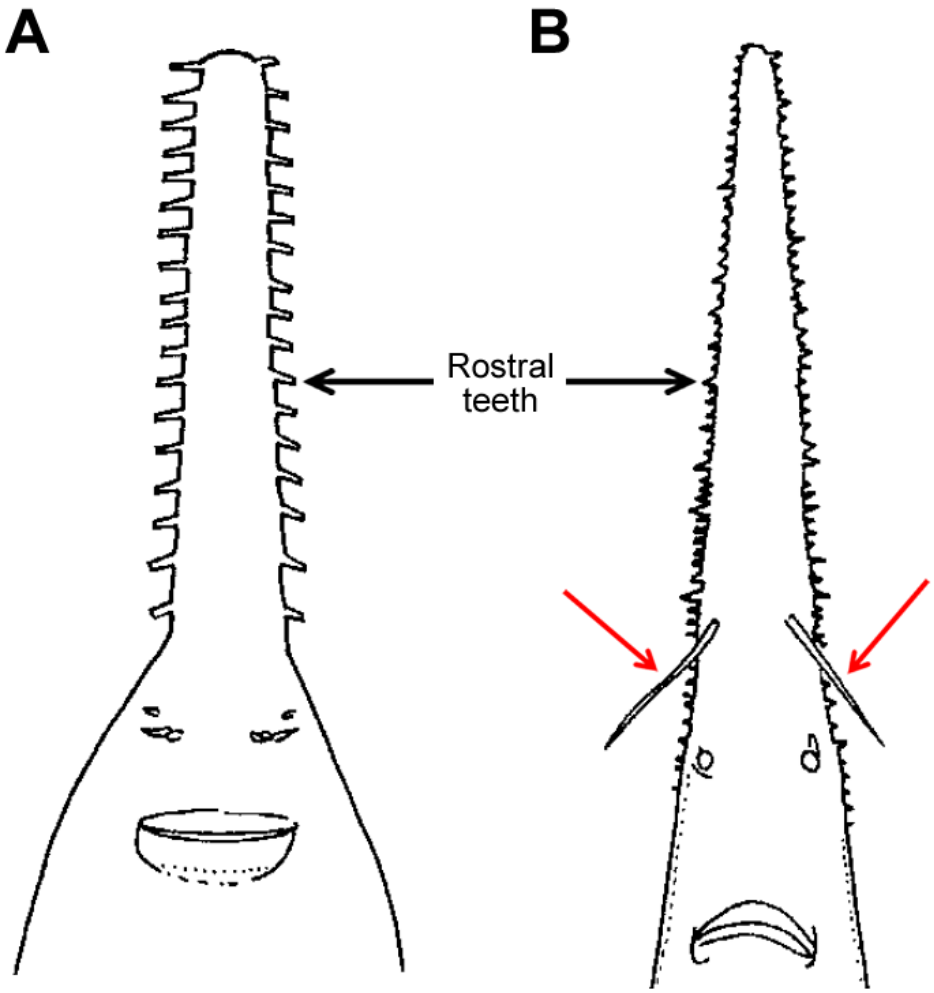

Figure 3.1: Ventral view of the tooth bearing rostrum form as seen in A) sawfishes and B) sawsharks. Note the differences in the size of the teeth and shape of the rostrum. Red arrows indicate the barbels. Diagrams sourced from Compagno (1990), Figure 19, Page 54.

The purpose of the tooth bearing rostrum form has long been the subject of debate. Possessed by sawfishes and sawsharks, the toothed rostrum was once believed to be used for uncovering prey buried in the sediment and then impaling the prey on the rostral teeth with a slashing motion (Nichols, 1929; Springer, 1961; Schaeffer, 1963; Compagno, 1990). Recent work on sawfishes, however, has shown that the rostrum contains a high density of electroreceptors (Wueringer *et al.*, 2011a) and allows for the sensing and hunting of prey in the water column (Wueringer *et al.*, 2012). Additionally, this rostrum can be used for manipulating prey once it has reached the substrate (Wueringer *et al.*, 2012). Self-defence is another role proposed for the rostra of sawfishes since it is claimed that they have been responsible for the killing of Dugongs *Dugong dugon* (Müller 1776) and wounding of predators, such as Bull Sharks *Carcharhinus leucas* (Müller and Henle 1839) (Tuma, 1976; Lal Mohan, 1986). Combat between male sawfishes during the breeding season has also been proposed as a function of the toothed rostrum (Stead, 1963; Grant, 1993).

While the function of the toothed rostrum in sawfishes is becoming clearer, the function of the toothed rostrum in sawsharks is still assumed to be used for stirring up sediment and impaling prey (Springer, 1961; Schaeffer, 1963; Compagno, 1990). This is because the feeding behaviour of sawsharks has yet to be documented due to the difficulties of observing them in their deep-water marine habitat and with maintaining them in captivity (T. Guttridge, pers. comm.). Although it is now thought that the toothed rostrums of sawsharks and sawfishes evolved independently (Wueringer *et al.*, 2009), it is possible that sawsharks use their rostrum in a similar manner to sawfishes; for predation, or self-defence or both. It is also possible that the rostrum has a sensory role, such as mechanoreception (Kasumyan, 2011), since it is innervated by branches of the trigeminal (V) and facial (VII) cranial nerves (von Bonde, 1933). Previous research suggests that the barbels of sawsharks, which are located on the ventral side of the rostrum, serve a tactile purpose (Chapter 2) and together with the rostrum may play a key role in sensing and capturing prey. Therefore, information elucidating the true role of the rostrum in sawsharks will enable better inferences of their feeding behaviour, and thus a clearer understanding of appropriate management techniques.

This study investigates the potential roles of the rostrum in sawsharks. As it is not currently possible to conduct behavioural experiments on sawsharks, this study employs a range of alternative methods such as examination and comparison of microwear on the rostral teeth of sawsharks and sawfishes, and assessment of the stomach contents of recently caught sawsharks for prey type and whether any prey bear rostral tooth wounds. The amount of tiny abrasions on the teeth, known as microwear, as a reflection of potential rostrum function is also assessed. If microwear is found to be similar for the rostral teeth of sawsharks and sawfishes, then one can expect similar usages of the rostrum. In addition, the composition of the rostral teeth of sawsharks and sawfishes is assessed through Raman spectroscopy to determine if the rostral teeth are composed of different substances. Furthermore, a recently discovered structure located on the tip of the rostrum of sawsharks, which has been named the "fingerprint" because of its superficial resemblance to the pattern of a human fingerprint (sensu Wueringer, unpublished), is histologically evaluated and described to determine whether any sensory structures are present as an indication of sensory function. Although the term fingerprint implies a unique feature that identifies an individual, it is unknown whether the pattern of this structure is unique to individual sawsharks. It was beyond the scope of the current study to assess this aspect. It is hypothesised that the rostrum of sawsharks is used for both predation and self-defence; thus the amount of microwear on the rostral teeth of sawsharks and sawfishes is expected to be similar. It is also hypothesised that the rostral teeth

of both groups of elasmobranchs will be composed of the same material. The "fingerprint" is hypothesised to serve a mechanosensory function as it may be connected to the canals of the lateral line (Wueringer, pers. comm.).

3.3 Methods:

Rostral teeth samples

Rostral tooth samples were collected from the rostra of *Pristiophorus cirratus* transported to Macquarie University for histological investigation (Chapter Two). Tooth samples of *Pristiophorus nudipinnis*, common sawfish *Pristis prisitis* (L. 1758) and knifetooth sawfish *Anoxyprisitis cuspidata* (Latham 1794) still embedded in the rostrum were obtained from B.E. Wueringer (BEW). The *P. pristis* and *A. cuspidata* samples comprised both adults and juveniles (i.e. within the first year of life) and were collected under permit by commercial fishery observers in various locations in Queensland for a previous study on the sensory morphology of sawfishes (see Wueringer *et al.* (2011b) for permit numbers). They had been stored in phosphate buffer with less than 3 % neutrally buffered formalin for several years. One tooth of an adult *P. pristis* that had never been placed in formaldehyde or phosphate buffer was donated to BEW for this study from a private collection.

Raman spectroscopy

To identify the composition of the rostral teeth of sawsharks and sawfishes, a single tooth sample from the rostra of two sawsharks (*Pristiophorus cirratus* and *P. nudipinnis*) and two sawfishes (*Anoxypristis cuspidata* and *Pristis pristis*) was placed into a HORIBA LabRAM HR Evolution Raman spectrometer. Spectra were obtained with the accompanying LabSpec 6 software and the resulting output background corrected (interpolated) in Origin (OriginLab, Northampton, MA).

Micro computed tomography (micro-CT) of rostral teeth

All samples were air dried prior to being scanned in an Xradia MicroXCT-400 X-ray micro-tomography system at the University of Sydney. Rostrum sections with embedded teeth and a single *P. cirratus* rostral tooth were scanned at a source energy of 100 ke V with a flux of 5 µA. As the size of the rostrum differed between samples, the positions of the source and detector away from the sample were selected based on rostrum size to maximise the field of view (see Table III for details). Scans were taken with the cooled CCD camera set to the binning 2 mode, an exposure time of 1.0 s and a total of 451 projection images. The

projection images were taken at 16 bit grey-scale depth. Each scan took approximately an hour and 45 minutes to complete. Images were reconstructed using the built-in filtered back projection algorithm in the scanner's hardware and, if necessary, corrections were made for images that presented issues such as beam hardening, ring artefacts and rotational misalignments (i.e. centre shifts). Reconstructions took approximately 10 minutes to complete. The images were then visualised using Avizo Fire (www.vsg3d.com/avizo/fire). Upon completion of the scans, rostrums were placed back into phosphate buffer.

Table III: Distances between the examined tooth/rostrum samples and the source and detector, with the resulting pixel resolution. R = rostrum; T = tooth.

Species	Sample	Source to Sample Distance	Detector to Sample Distance	Pixel Resolution
Pristiophorus cirratus	Adult T	65	200	13.719
Pristiophorus nudipinnis	Adult R	65	200	13.719
Anoxypristis cuspidata	Juvenile R	65	67	27.931
Anoxypristis cuspidata	Adult R	300	100	42.715
Pristis pristis	Juvenile R	NOT SCANNED		
Pristis pristis	Adult R	NOT SCANNED		

Scanning electron microscopy (SEM) of rostral teeth

A single rostral tooth of *Pristis pristis*, *Anoxypristis cuspidata* and *Pristiophorus nudipinnis* was extracted from the samples provided by BEW for inspection with SEM. A rostral tooth of *Pristiophorus cirratus* was extracted from one of the specimens used for histological investigation in Chapter Two. All samples were washed in 100 % ethanol, air dried and mounted onto aluminium stubs (12.6 mm and 25 mm diameter stubs for small and large teeth respectively) with adhesive carbon tabs (small teeth) or carbon/graphite paint (large teeth). Mounted samples were gold coated for two minutes in an Emitech K550 gold sputter coater and imaged with a JEOL JSM-6480 LA scanning electron microscope at an accelerating voltage of 3 kV. Due to the range of tooth sizes, the magnification at which each tooth sample was imaged was standardised based on similarities in size: large teeth of adult sawfishes at 22x, teeth of sawsharks and the juvenile *Anoxypristis cuspidata* at 85x, and the juvenile *Pristis pristis* tooth at 500x.

Images of the rostral teeth were examined and measured with the free software ImageJ (Version 14.9g) (Abràmoff *et al.*, 2004). The length of each tooth was measured and used to

divide to the tooth into three equal sections – the tip, middle and base – and the number of scratches in each section was counted. A scratch was defined as a narrow line with a minimum length of 34 µm etched into the surface of the tooth. If a scratch was found to cross into two sections, then it was assigned to the section where the majority of scratch's length occurred and was not counted in the other section. Chi-square tests conducted in Minitab (Version 16) (Minitab Inc., www.minitab.com) were used to assess differences in the total number of scratches over the entire tooth between sawsharks and sawfishes, and adults and juveniles. Uniformity in the number of scratches in the three sections of the rostral tooth was also assessed in this manner. Chi-square tests were used for analysis since the scratch data were counts from a single tooth sample.

Analysis of stomach contents

Specimens of *Pristiophorus cirratus* collected for histological investigation (Chapter Two) were stored at 4°C for the later removal of stomach contents. Whole stomachs were removed from each shark by cutting the upper end of the oesophagus and lower end of the intestines whilst pinching the oesophagus and intestinal sphincter to prevent any contents from spilling out. Once removed, a small incision was made in the stomach to allow for eversion and all contents were stored in 70 % ethanol and at -20°C until examination. Stomachs were classified on a stomach fullness scale, where 1 represented an empty stomach and 5 represented a full stomach containing undigested prey. Contents were examined with a Leica S6 D Greenough stereo microscope and each prey item was identified to the lowest possible taxonomic level. The number of prey items in each stomach was recorded and wet weight of each prey item was measured with an electronic balance. Whole/intact prey items had their total length recorded and were inspected for evidence of rostral tooth marks.

The lowest possible taxonomic level to which the majority of prey items could be identified to was family. Hence this level was used in all analyses of stomach contents. The number (*N*), frequency of occurrence (*F*) and wet weight (*W*) of each prey item and their percentages (*%N*, *%F* and *%W* respectively) were calculated to assess the diet of *P. cirratus* with the Index of Relative Importance (IRI) (Pinkas *et al.*, 1971). In this study, the volumetric percentage of the IRI equation was replaced with *%W*, thus IRI = (*%N* + *%W*) × *%F*.

Histological analysis of the "fingerprint" structure

The first centimetre of the rostrum tip from each *P. cirratus* was processed and photographed using the methods outlined in the barbel histology section of Chapter Two.

Comparison of proportional rostrum length

To assess for similarities in rostrum use, the proportion of the total length (L_T) comprised by the length of the rostrum (L_R) in sawsharks and sawfishes was compared using the *Pristiophorus cirratus* morphometric data from Chapter Two and morphometric data for two sawfishes (*Pristis pristis* and *Anoxypristis cuspidata*) supplied by BEW. These data were collected from samples donated for research to BEW under permit (see Wueringer *et al.* (2011b) for permit numbers). Rostrum length to total length was expressed as a ratio (L_R/L_T), logit transformed and analysed using the non-parametric Kruskal-Wallis test and Dunn's multiple comparison test in Minitab with the KruskMC.mac macro (available from the Minitab website).

3.4 Results:

Raman spectroscopy

Raman spectroscopy was used to assess whether there were differences in the composition of the rostral teeth of sawsharks and sawfishes. The spectra produced for the rostral teeth of sawfishes (*Anoxypristis cuspidata* and *Pristis pristis*) and sawsharks (*Pristiophorus cirratus* and *P. nudipinnis*) were consistent with a form of hydroxyapatite (the main mineral component of dentin and enamel in vertebrate teeth and bone), as these showed peaks between the 430 to 450 cm^{-1} wavelengths, the 587 to 604 cm^{-1} wavelengths and the 960 to 961 cm^{-1} wavelengths (Nishigori *et al.*, 1986; Tsuda *et al.*, 1996). When examined separately, the spectra produced for the rostral teeth of the two species of sawfish were almost identical to each other (Figure 3.2), with the peak in the spectra situated between the 2750 and 3000 cm^{-1} wavelengths indicating the presence of collagen (Penel *et al.*, 2005). In contrast, the Raman spectra of the teeth of the sawsharks indicated that collagen was present in the centre of the tooth but not along its edges (Figure 3.3). These differences in tooth composition were reflected by the colouration on the surface of the sawsharks' rostral teeth (Figure 3.4).

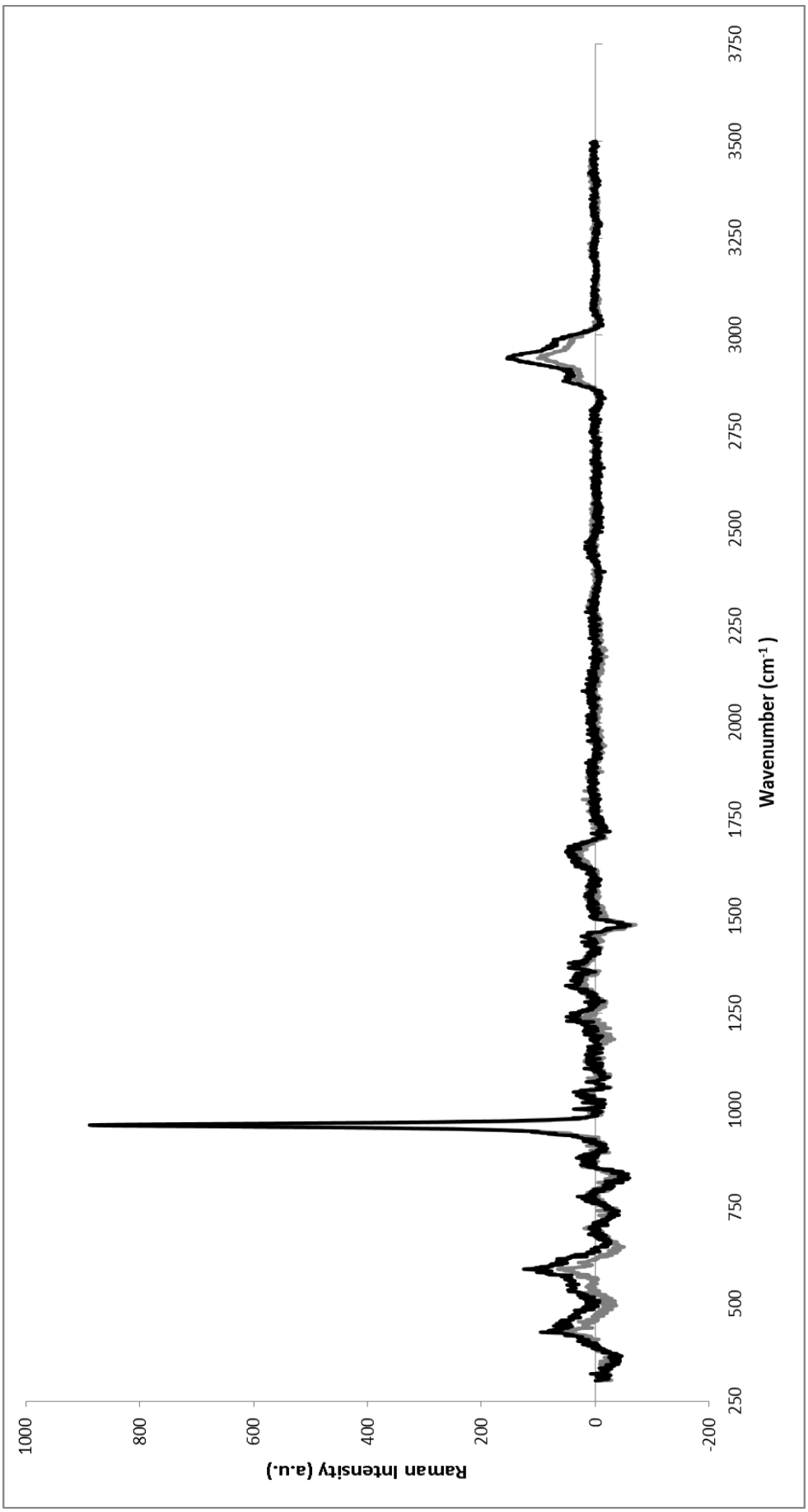

Figure 3.2: Raman spectra of *Anoxypristis cuspidata* (black line) and *Pristis pristis* (grey line) rostral teeth.

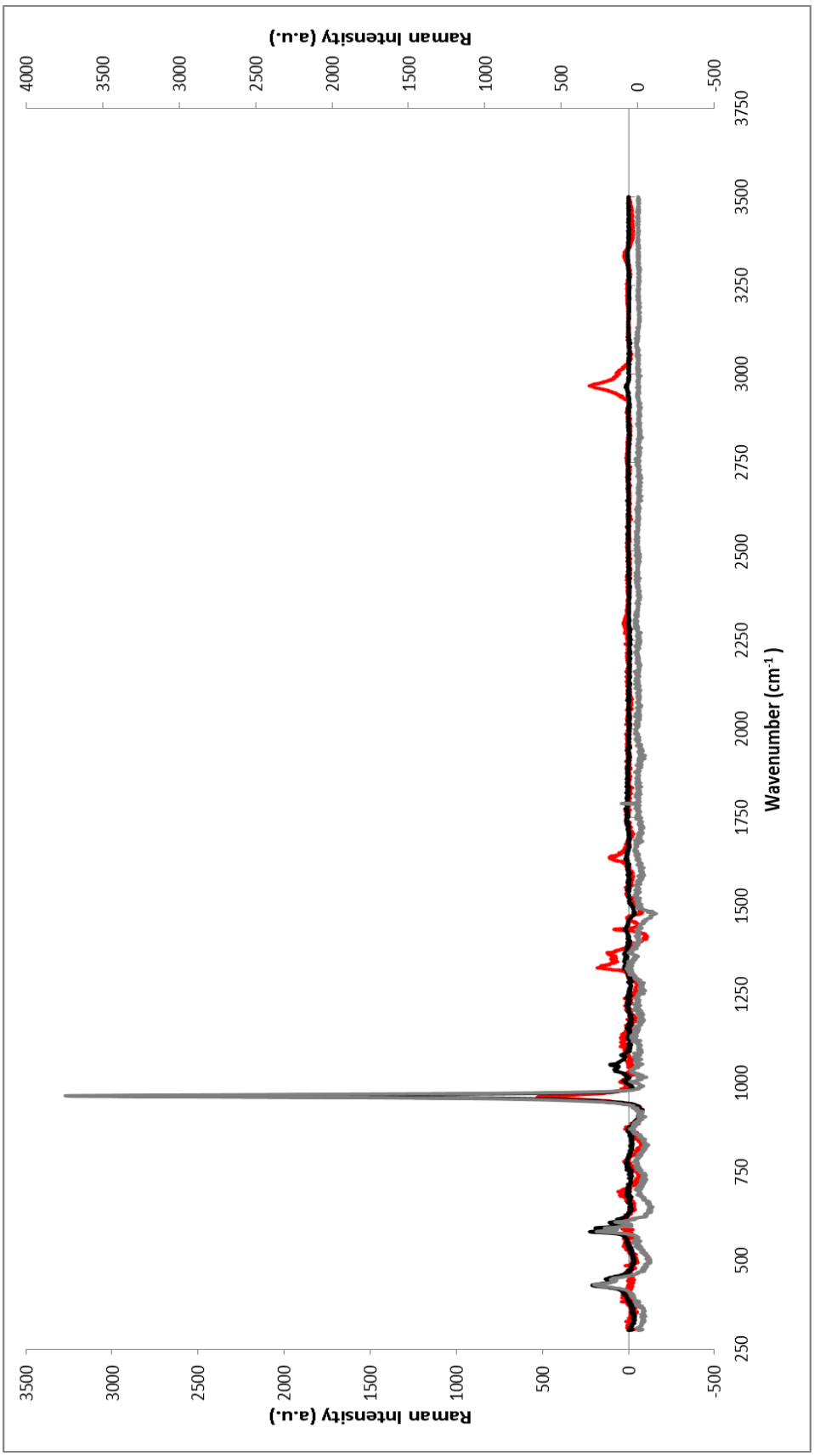

Figure 3.3: Raman spectra of *Pristiophorus nudipinnis* (primary axis) and *P. cirratus* (secondary axis) rostral teeth. Black line = edge of *P. nudipinnis* tooth; Grey line = edge of *P. cirratus* tooth; Red line = centre of *P. nudipinnis* tooth.

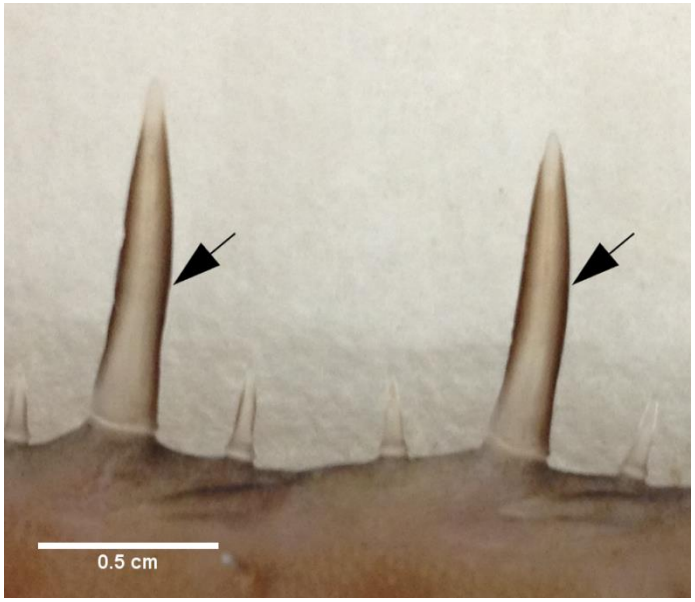

Figure 3.4: Rostral teeth of *Pristiophorus cirratus*. Black arrows highlight the black edges of the tooth where Raman spectroscopy indicated the absence of collagen. The centre of the tooth (white) contains collagen.

Micro-computed Tomography (micro-CT) and Scanning Electron Microscopy (SEM) of rostral teeth

Microwear patterns on the surface of the rostral teeth of sawsharks (*Pristiophorus cirratus* and *P. nudipinnis*) were compared with those of sawfishes (*Anoxypristis cuspidata* and *Pristis pristis*) to assess whether the toothed rostrum was used for the same purpose in these two types of elasmobranchs. In addition to the rostral teeth of adult sawfishes, those of juvenile sawfishes were also included in this study so that the microwear patterns from the teeth of an animal similar in size to an adult sawshark could be compared. To visualise microwear, two different methods were trialled. Micro-CT was initially chosen as this method could produce three-dimensional images of the teeth without the samples needing to be processed (e.g. coated with gold). However, microwear on the surface of the rostral teeth of sawsharks and sawfishes was not visible in the reconstructed images produced by this method; but was visible in images produced through SEM (Figure 3.5).

When the total number of scratch marks present on the rostral teeth of all sawfishes were combined and compared with the combined total for all sawsharks, it was found that the rostral teeth of sawfishes possessed significantly more scratch marks than those of sawsharks (χ^2 = 130.720, d.f. = 1, p = < 0.001, n = 6). The same held true when the total number of scratch marks counted on the teeth of only adult sawfishes was compared with sawsharks (χ^2 = 32.766, d.f. = 1, p = < 0.001, n = 4). There was, however, no significant difference in the

total number of scratch marks present on the rostral teeth between juvenile sawfishes and sawsharks (χ^2 = 3.042, d.f. = 1, p = 0.081, n = 4). Also, adult sawfishes were found to possess significantly more scratch marks on their rostral teeth than juveniles (χ^2 = 54.911, d.f. = 1, p = < 0.001, n = 4). For most specimens, the number of scratch marks in each section of the tooth was not uniform (Table IV). In both sawsharks and sawfishes, the average number of scratches was greatest in the middle of the tooth (Table V).

Scratch marks on the examined rostral teeth of sawfishes and sawsharks were generally orientated diagonally across the surface of the tooth (Figures 3.6 – 3.8). For most teeth, the majority of these scratches were angled towards the base of the tooth. However, several of the longer scratches on the tooth of the juvenile *Pristis pristis* were angled towards the tip.

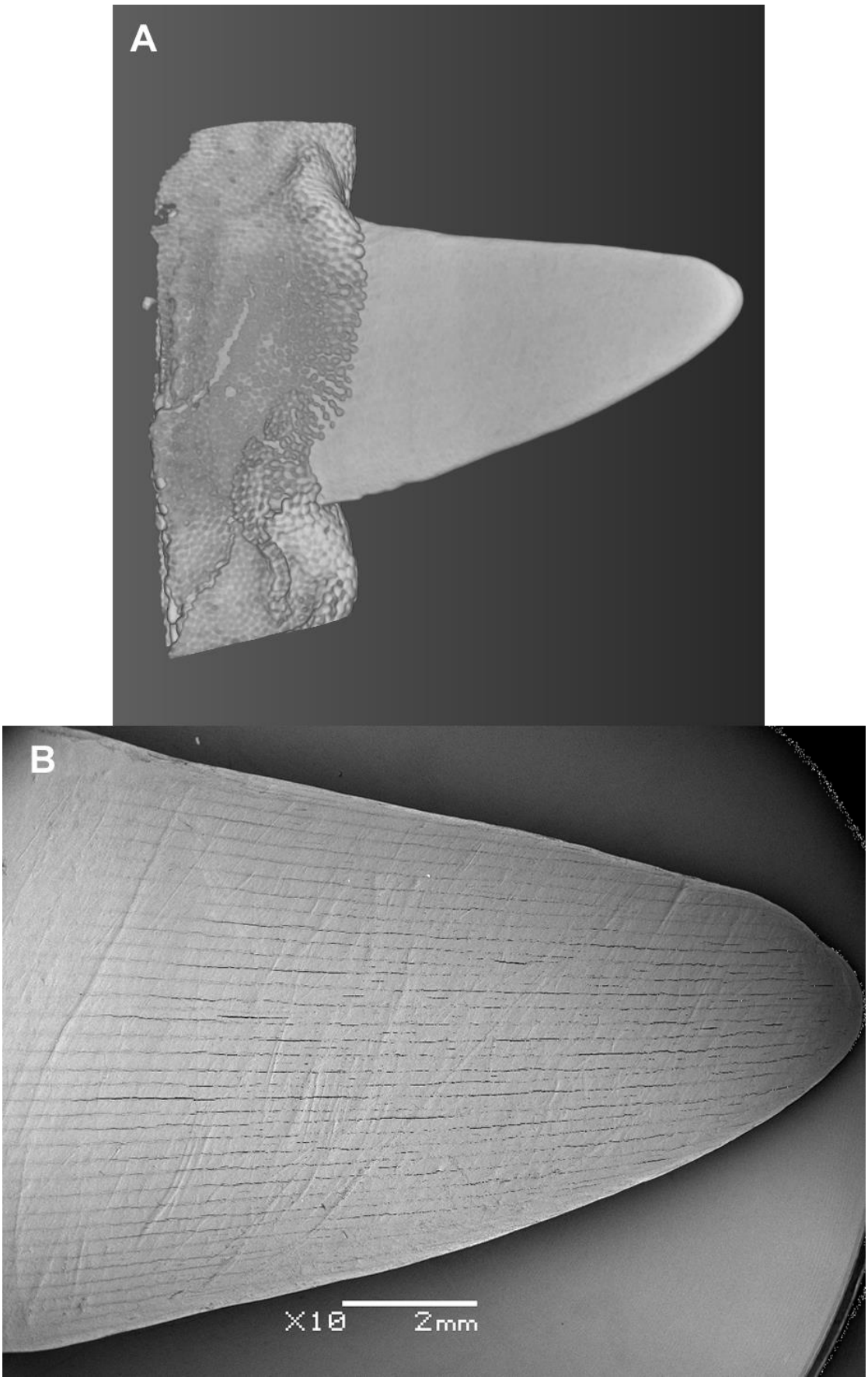

Figure 3.5: Comparison of visible microwear on the rostral tooth of *Anoxypristis cuspidata* when scanned with A) micro computed tomography and B) scanning electron microscopy.

Table IV: Summary of the Chi-square tests conducted on each rostral tooth to assess uniformity in microwear across the tooth's surface. The Chi-square (χ^2) value column refers to the test statistic for the test comparing microwear in all three sections of the tooth. The last three columns refer to the *p* value obtained when the Chi-square value for each section of the tooth is compared against the Chi-square distribution with two degrees of freedom. A = adult; J = juvenile.

Species	Maturity	Chi-square (χ^2) value	*p* value	*p* value (Tip)	*p* value (Middle)	*p* value (Base)
Pristis pristis	A	8.167	0.017	1	0.130	0.130
Pristis pristis	J	80	< 0.001	< 0.001	0.001	0.001
Anoxypristis cuspidata	A	18.380	< 0.001	0.007	0.015	0.974
Anoxypristis cuspidata	J	0.737	0.692	0.809	0.859	0.995
Pristiophorus cirratus	A	36.737	< 0.001	0.277	0.005	< 0.001
Pristiophorus nudipinnis	A	35.216	< 0.001	0.683	0.001	< 0.001

Table V: Mean (± S.D.) number of scratches on each section of the rostral tooth for sawfishes and sawsharks.

Group	Mean number of scratches (Tip)	Mean number of scratches (Middle)	Mean number of scratches (Base)
Sawfishes	40.5 (± 23.685)	66 (± 58.129)	51.333 (± 49.803)
Sawsharks	42 (± 18.385)	54.5 (± 16.263)	7 (± 7.071)

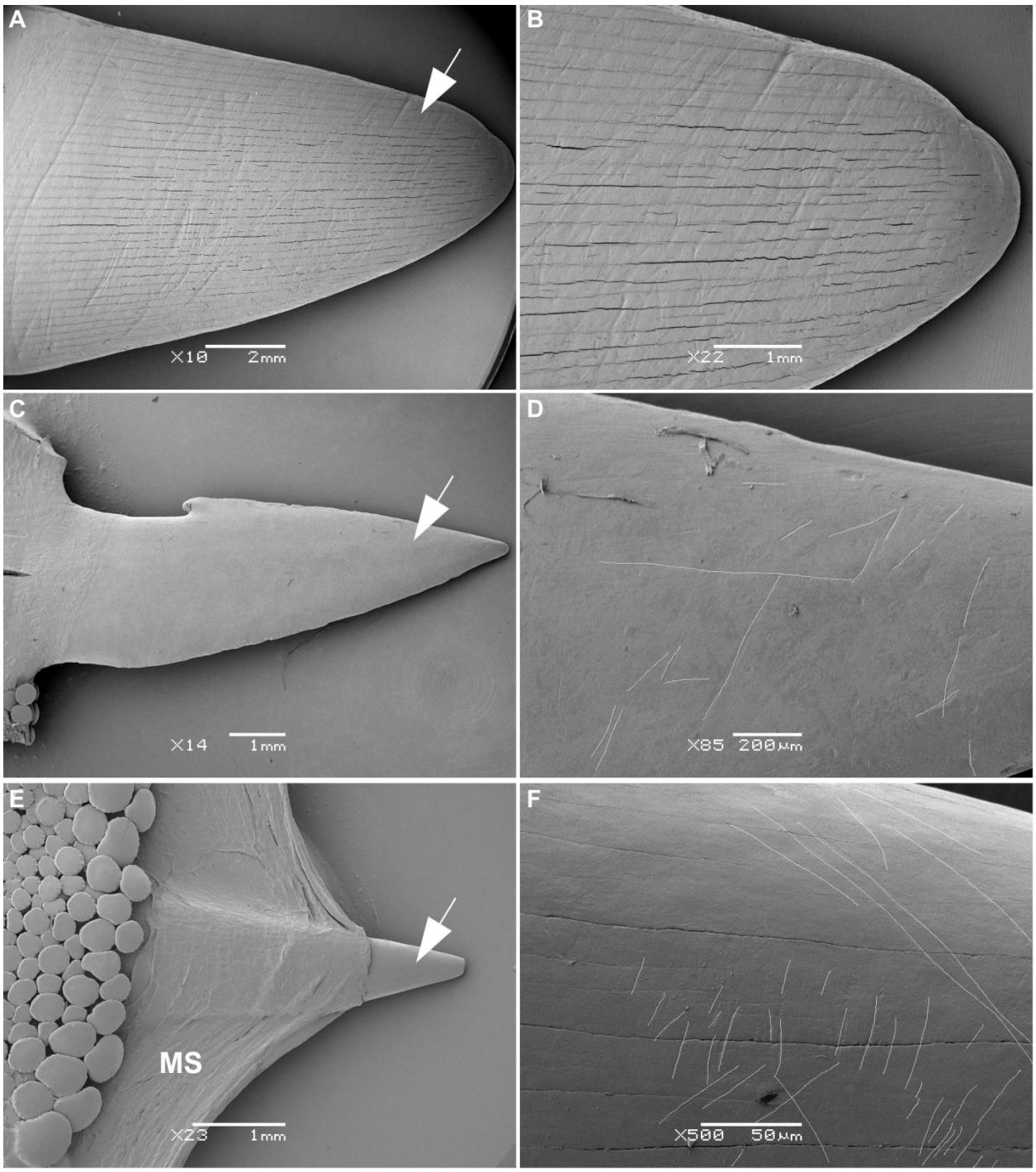

Figure 3.6: Rostral teeth of sawfishes scanned with scanning electron microscopy. A) Whole rostral tooth of an adult *Anoxypristis cuspidata*; B) Close-up of the area denoted by the arrow in A); C) Whole rostral tooth of a juvenile *Anoxypristis cuspidata*; D) Close-up of the area denoted by the arrow in C); E) Whole rostral tooth of a juvenile *Pristis pristis*, with a membrane sheath (MS) covering the majority of the tooth; F) Close-up of the area denoted by the arrow in E). Scratches in D) and F) have been highlighted for clarity. Magnification indicated in the close-up images was that used to count the number of scratches for that tooth. Figure created with ScientiFig (Aigouy and Mirouse, 2013).

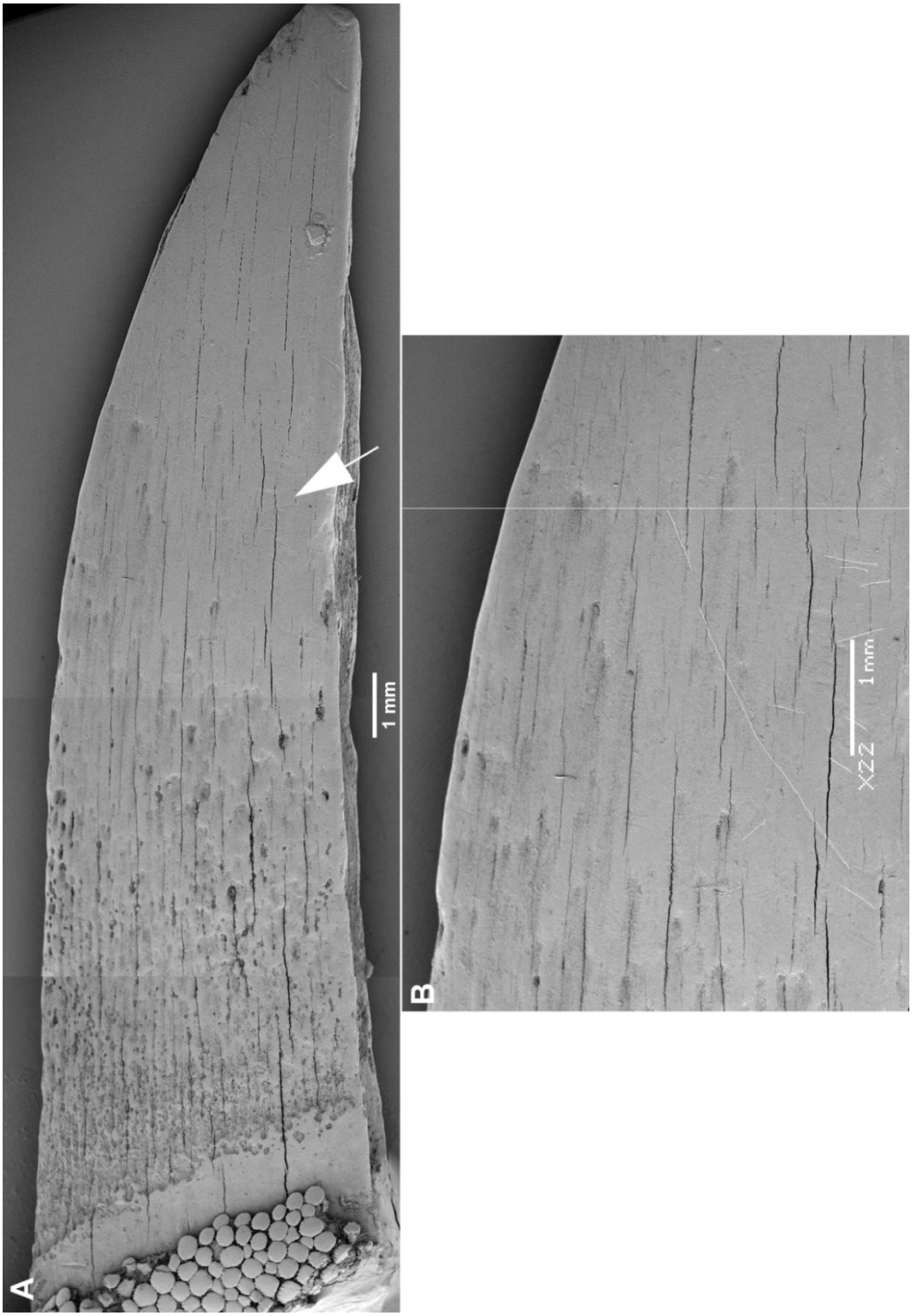

Figure 3.7: Rostral tooth of an adult *Pristis pristis* sawfish scanned with scanning electron microscopy. A) Whole rostral tooth; B) Close-up of the section denoted by the white arrow in A). Scratch marks in B) have been highlighted for clarity. Magnification indicated in B) was that used to count the number of scratches for that tooth. Figure created with ScientiFig (Aigouy and Mirouse, 2013).

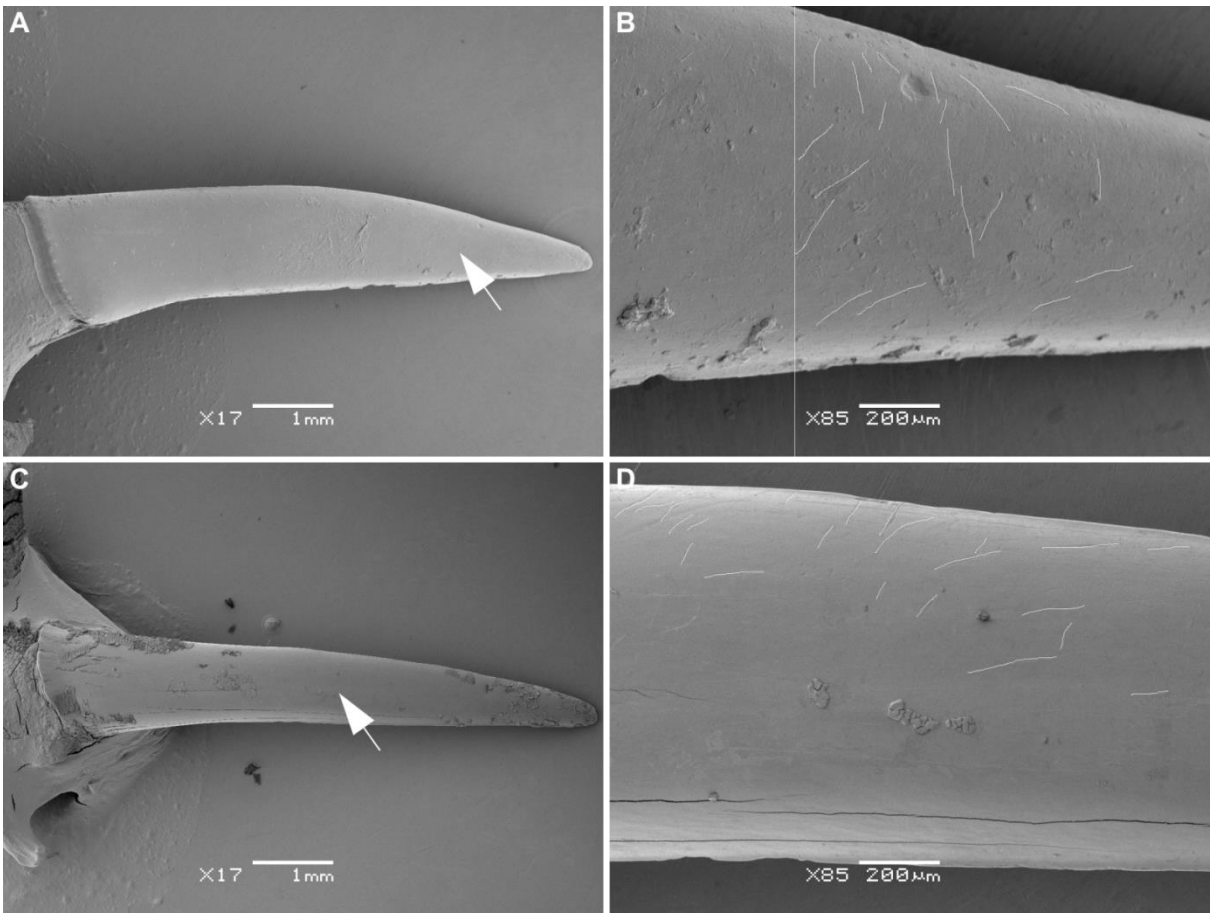

Figure 3.8: Rostral teeth of sawsharks scanned with scanning electron microscopy. A) Whole rostral tooth of *Pristiophorus cirratus*; B) Close-up of the area denoted by the arrow in A); C) Whole rostral tooth of *Pristiophorus nudipinnis*; D) Close-up of the area denoted by the arrow in C). Scratch marks in B) and D) have been highlighted for clarity. Magnification indicated in the close-up images was that used to count the number of scratches for that tooth. Figure created with ScientiFig (Aigouy and Mirouse, 2013).

Analysis of stomach contents

A total of 41 stomachs from *Pristiophorus cirratus* were examined for the number of prey items consumed. Nine (21.95%) of the stomachs were empty. Of the remaining 32 stomachs that contained prey, 43.75% contained a single prey item, 37.50% contained two prey items, 12.50% contained three, and 6.25% contained four or five prey items. No stomach was found to contain more than five prey items.

Prey items that were not too heavily digested could be identified to the level of family and comprised three families of crustaceans and five families of teleosts (Table VI). These included: pandalid shrimp, penaeid shrimp, sicyonid shrimp, garden eels (fam. Congridae), toungefishes (fam. Cynoglossidae), lanternfishes (fam. Myctophidae), flatheads (fam. Platycephalidae) and lizardfishes (fam. Synodontidae). Two prey items that could be identified to species level were the crustaceans *Sicyonia australiensis* (Hanamura and Wadley 1998) and *Heterocarpus sibogae* (de Man 1917). Pandalid shrimp were the most numerous (35.59%) and frequently occurring (40.63%) prey item and also comprised the majority (33.19%) of the total wet weight for all prey items. The percentage Index of Relative Importance (%IRI) for this prey family indicated that it is an important component of the diet of *P. cirratus*. The next two important prey items based on %IRI were penaeid shrimp (3.22%) and lizardfishes (1.42%) respectively. No prey items showed evidence of impalement by rostral teeth.

Table VI: Stomach contents of 32 *Pristiophorus cirratus* caught by a Wollongong trawl fishery. *N* = number of prey belonging to each family; *F* = frequency of occurrence for each prey family; *W* = wet weight (in grams) of each prey family; IRI = Index of Relative Importance of each prey family.

Taxa	Family	*N*	*%N*	*F*	*%F*	*W*	*%W*	IRI	%IRI
Crustaceans	Pandalidae	21	35.59	13	40.63	83.71	33.19	2794.44	48.01
	Penaeidae	4	6.78	3	9.38	33.36	13.23	187.56	3.22
	Sicyoniidae	1	1.69	1	3.13	1.25	0.49	6.84	0.12
	Unidentified	9	15.25	8	25	20.93	8.30	588.87	10.12
Teleosts	Congridae	3	5.08	2	6.25	9.51	3.77	55.35	0.95
	Cynoglossidae	3	5.08	1	3.13	14.71	5.83	34.12	0.59
	Myctophidae	1	1.69	1	3.13	0.86	0.34	6.36	0.11
	Platycephalidae	1	1.69	1	3.13	4.48	1.78	10.85	0.19
	Synodontidae	2	3.39	2	6.25	24.88	9.87	82.84	1.42
	Unidentified	14	23.73	14	43.75	58.50	23.20	2053.1	35.27
Total		**59**	**100**	**46**	**143.75**	**252.18**	**100**	**5820.34**	**100**

Histological analysis of the "fingerprint" structure

The "fingerprint" is a structure located on both the dorsal and ventral surface at the tip of the rostrum in sawsharks (Figure 3.9). This unusual structure comprises darkly pigmented ridges in epidermal tissue that covers approximately the first 3 mm of the rostrum tip. Histological investigation of this structure showed that at the distal end, lamellae in the pattern of the "fingerprint" were interspersed amongst the epidermis (Figure 3.10). Cutting towards the medial part of the "fingerprint", these lamellae became wider and exposed areas of the dermis (Figure 3.11). From there, the dermis widened further as sectioning continued towards the caudal end of the "fingerprint" and revealed what are believed to be the rostral canals described by von Bonde (1933) that contain innervations of the trigeminal (V) and facial (VII) cranial nerves, and the distal portion of the elongated rostral process (Figure 3.12). The number of lamellae also increased. The "rostral canals" contained a collection of cells which are proposed to be the neuromasts of the lateral line (Figure 3.13). It was not possible from the obtained images to determine whether the "fingerprint" has a sensory purpose; however, it appears that the "fingerprint" is a dense collection of epidermal cells around the margins of the dermis (Figure 3.14).

Comparison of proportional rostrum length

The proportion of the animal's total length (L_T) that was comprised by the length of the rostrum (L_R), that is L_R/L_T, was significantly different between *Pristiophorus cirratus* and the sawfishes (H = 17.53, d.f. = 2, p = < 0.001). The Dunn's multiple comparison test indicated a significant difference between *P. cirratus* and *Anoxypristis cuspidata* (Z = 3.970, p = < 0.001) and *P. cirratus* and *Pristis pristis* (Z = 1.99, p = 0.046). There was no significant difference in proportional rostrum length between the two sawfishes. The average L_R/L_T for *Pristiophorus cirratus* was 0.246 (± 0.008 S.D., n = 41), 0.286 (± 0.032 S.D., n = 9) for *Anoxypristis cuspidata* and 0.254 (± 0.010 S.D., n = 8) for *Pristis pristis*. The non-significant result observed when the proportional rostrum length was compared for the two sawfishes could be due to the small sample sizes.

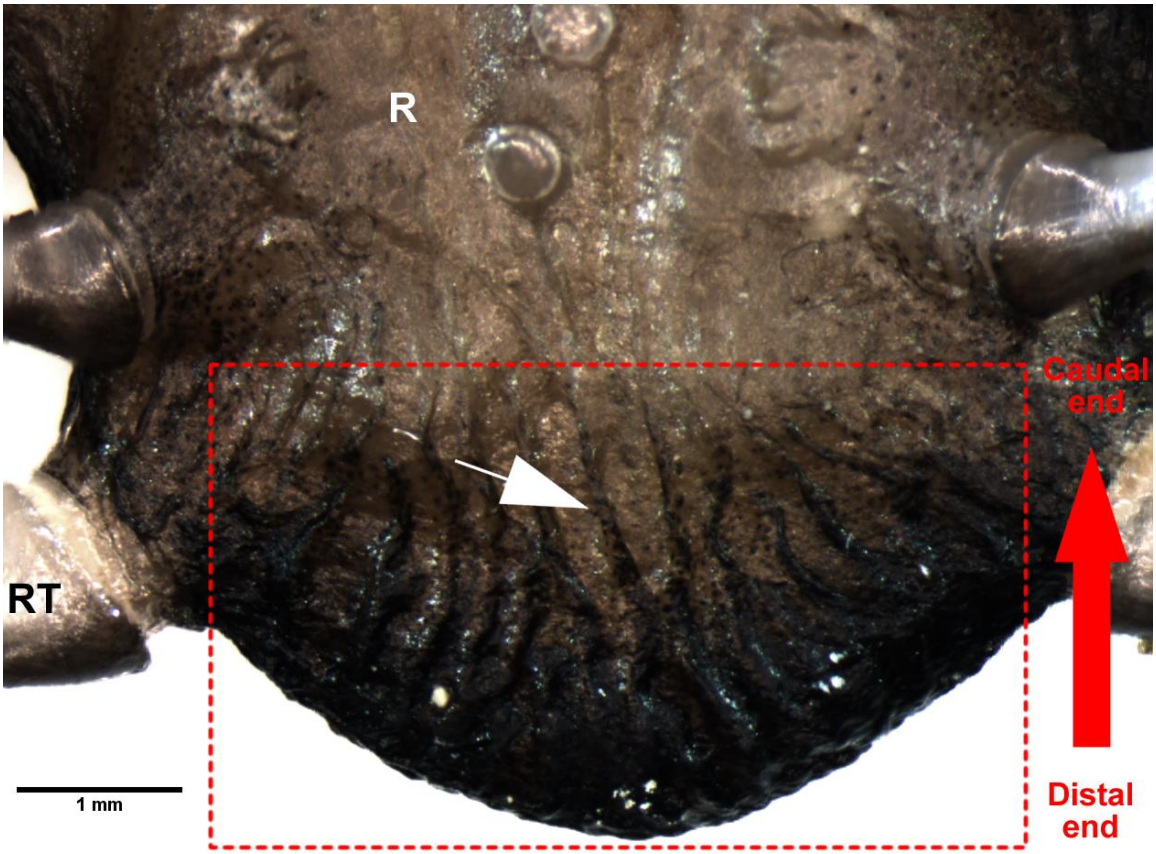

Figure 3.9: Tip of the rostrum of *Pristiophorus cirratus*, with the "fingerprint" indicated by the white arrow. The red dashed box indicates the approximate area where sectioning occurred and the red arrow shows the direction of sectioning. R = rostrum; RT = rostral tooth.

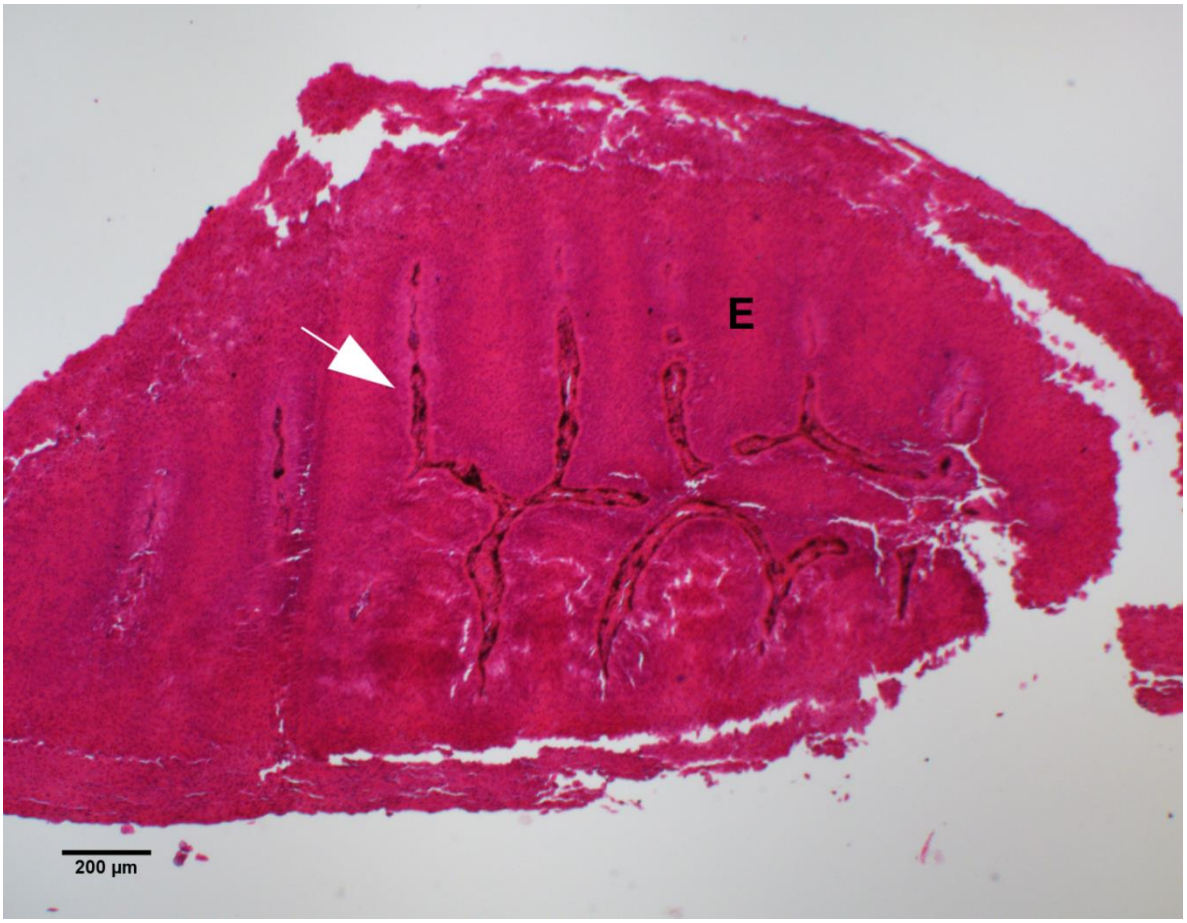

Figure 3.10: Transverse section (4 µm thick) of the rostrum of *Pristiophorus cirratus* at the distal end showing lamellae interspersed amongst the epidermis. E = epidermis; White arrow = lamellae in the pattern of the "fingerprint".

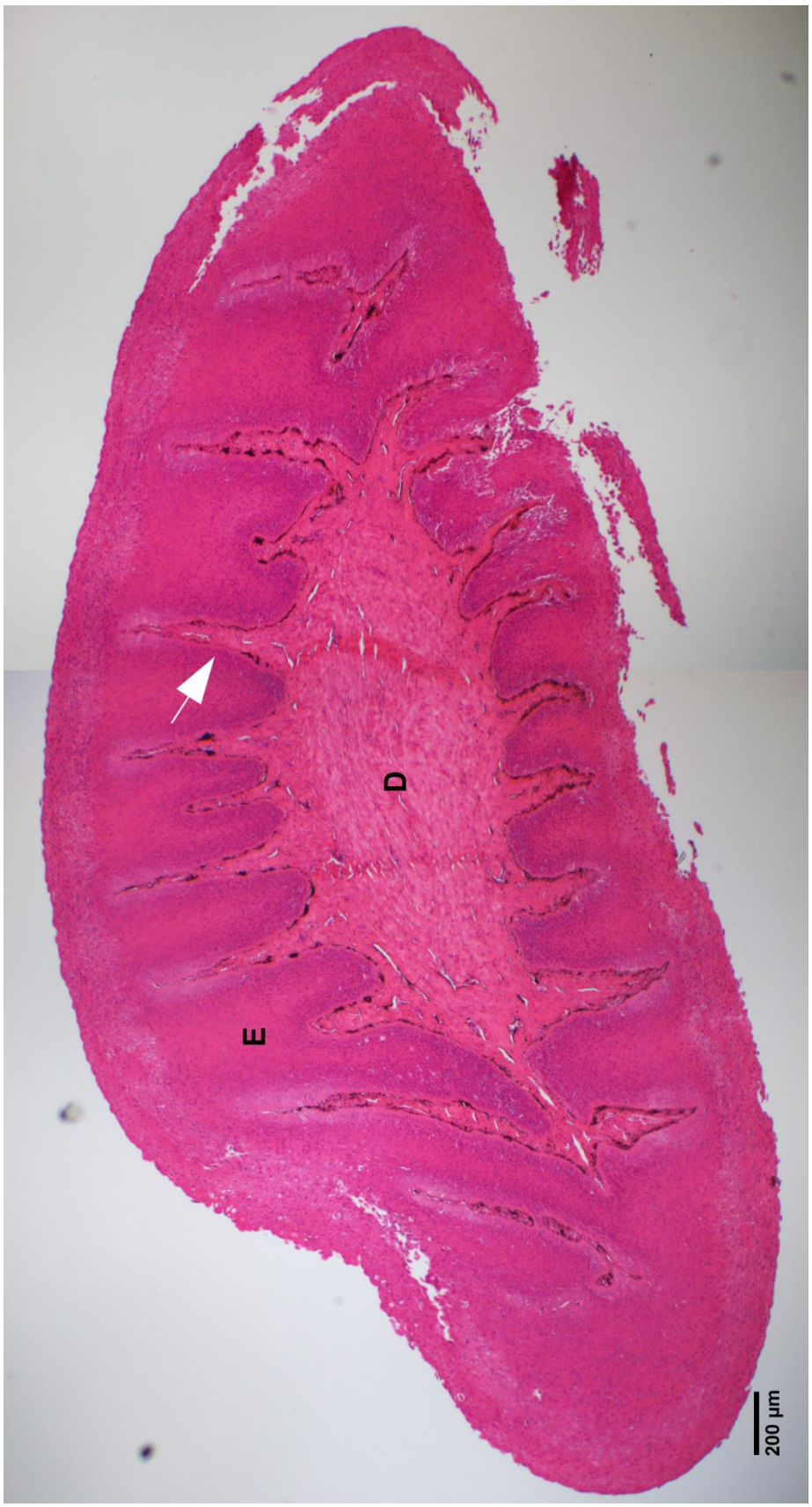

Figure 3.11: Transverse section (4 µm thick) of the rostrum of *Pristiophorus cirratus* in the medial part of the "fingerprint", showing widening of the lamellae. D = dermis; E = epidermis; White arrow = lamellae in the pattern of the "fingerprint".

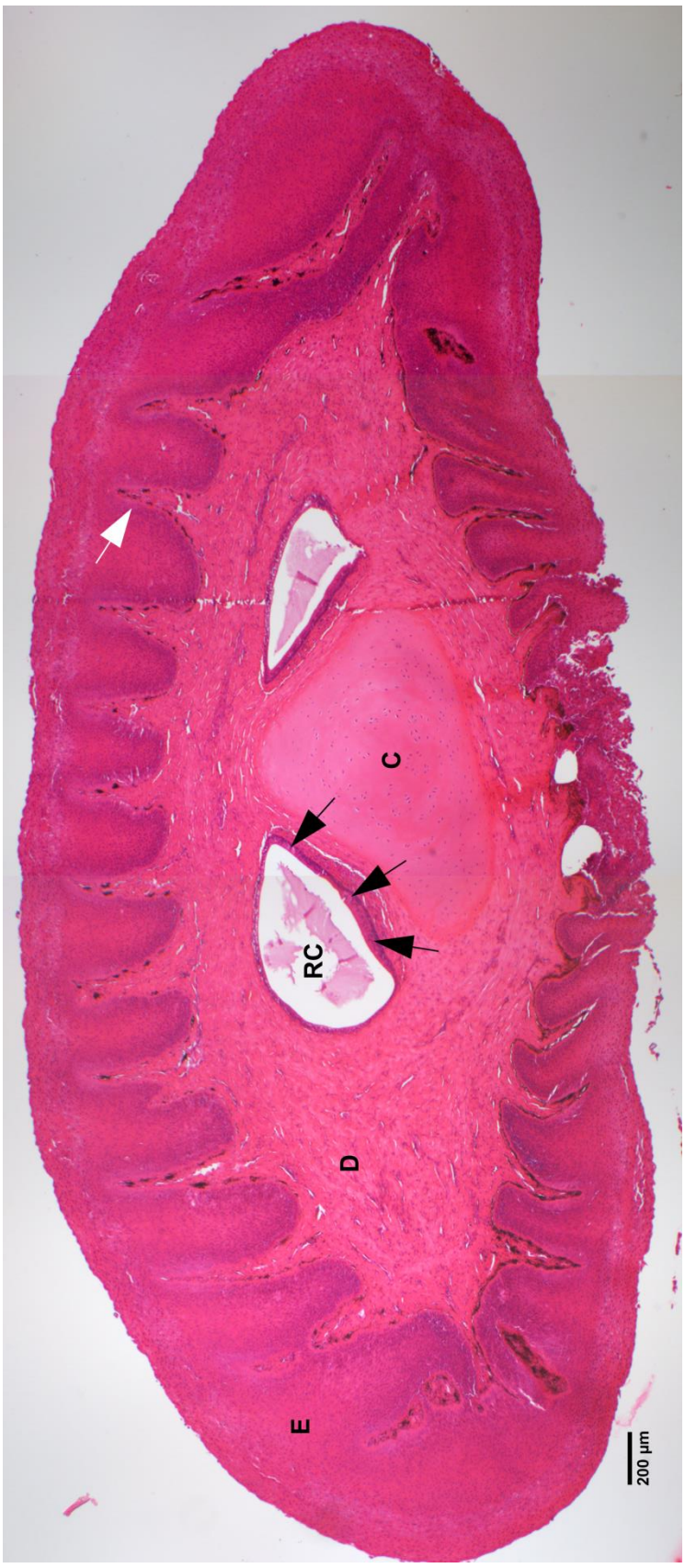

Figure 3.12: Transverse section (4 µm thick) of the rostrum of *Pristiophorus cirratus* at the caudal end of the "fingerprint". C = cartilage of the elongated rostral process; D = dermis; E = epidermis; RC = rostral canal; Black arrows = hair cells of neuromasts; White arrow = lamellae in the pattern of the "fingerprint".

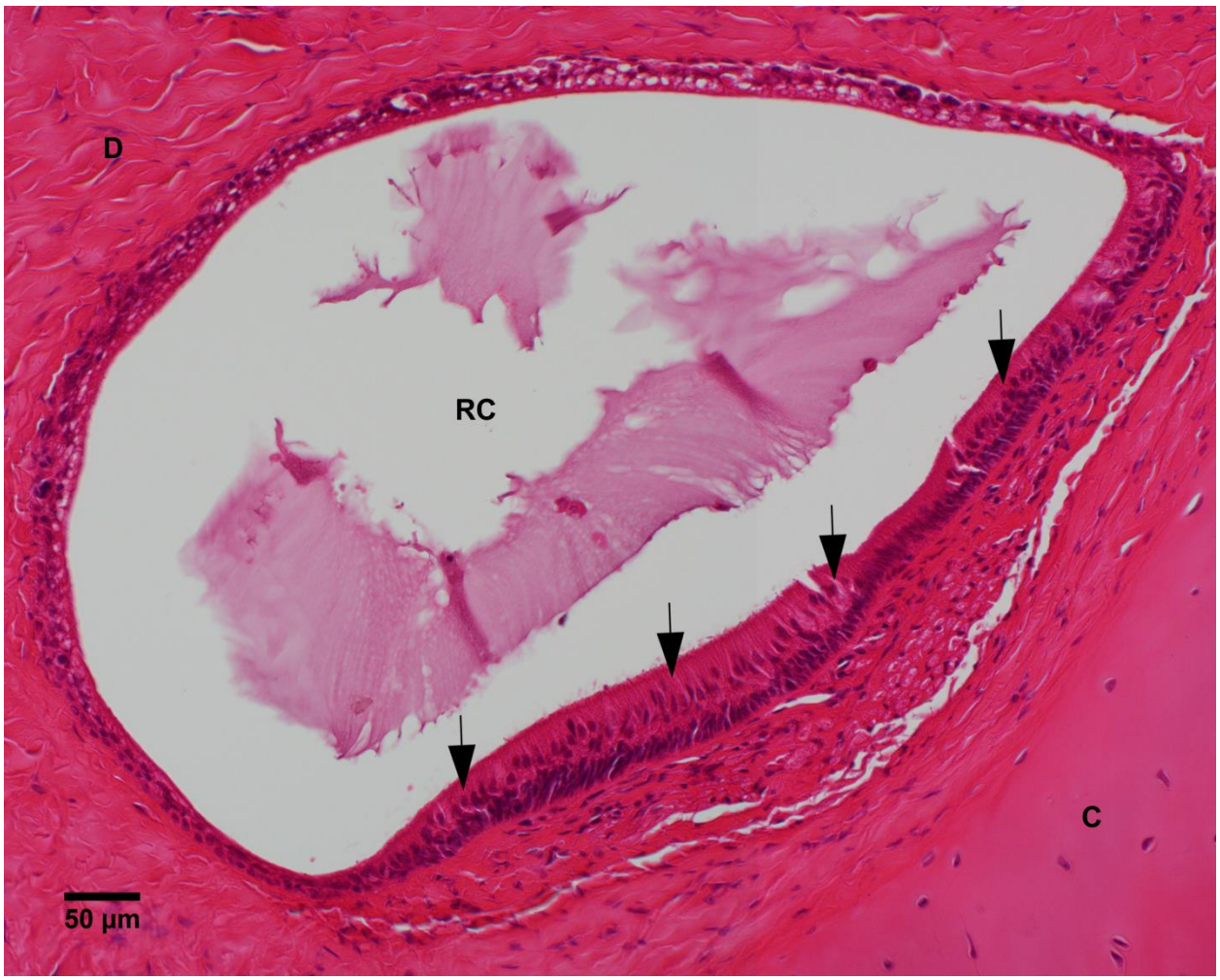

Figure 3.13: Close up of a rostral canal in the rostrum of *Pristiophorus cirratus*. Hair cells of the neuromasts located on the bottom of the canal are indicated with black arrows. Section thickness = 4 µm. C = cartilage of the elongated rostral process; D = dermis; RC = rostral canal.

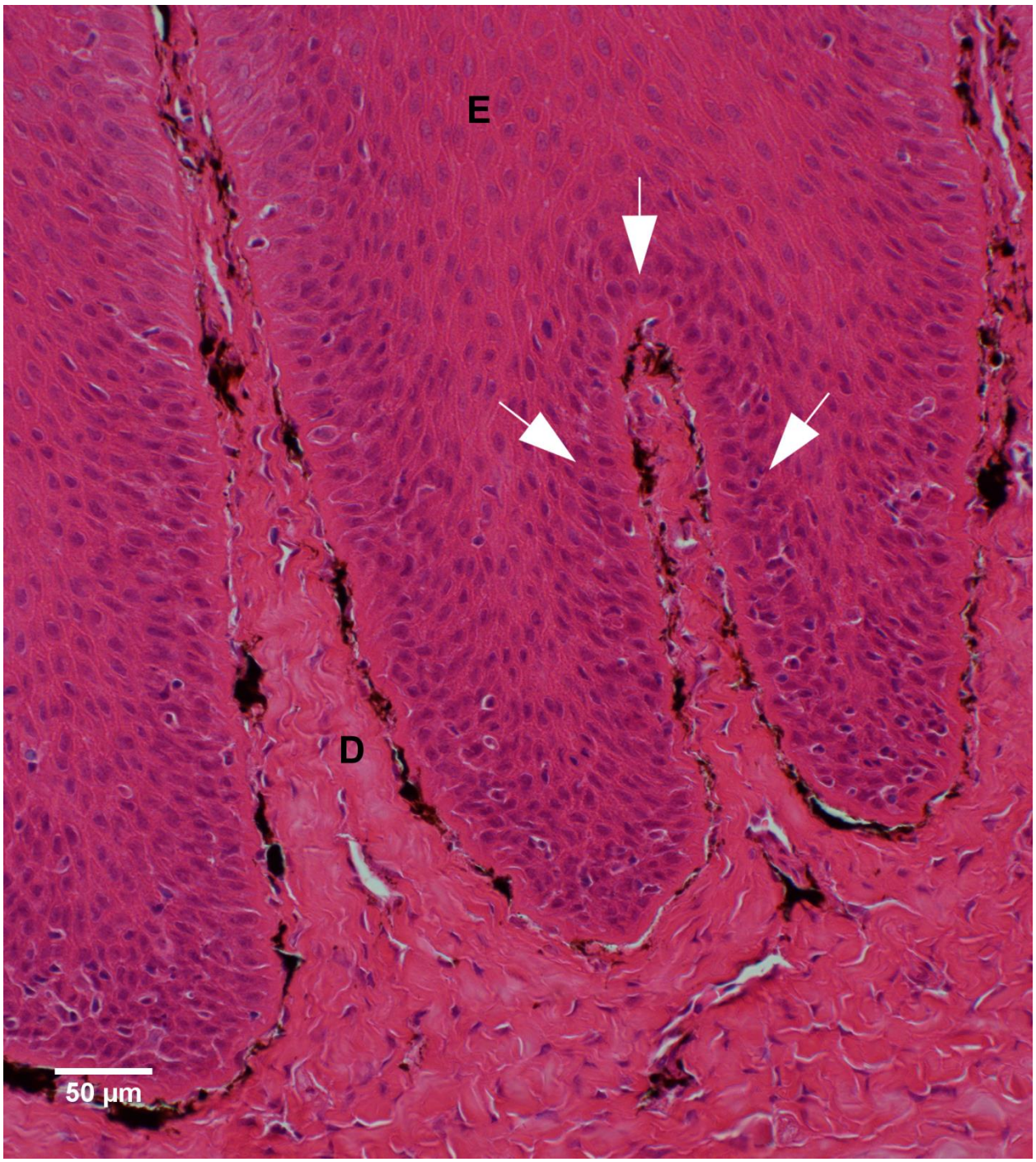

Figure 3.14: Large collection of epidermal cells (white arrows) surrounding the margins of the dermis (D) in the rostrum of *Pristiophorus cirratus*. Section thickness = 4 µm. E = epidermis.

3.5 Discussion:

The function of the toothed rostrum in sawsharks has long been held to be for the uncovering of prey buried in the sediment and then impaling it on the rostral teeth (Springer, 1961; Schaeffer, 1963; Compagno, 1990). In light of recent work on sawfishes (e.g. Wueringer *et al.*, 2009; Wueringer *et al.*, 2012), this study employed a variety of alternative methods to test hypotheses regarding the likely roles of the toothed rostrum in sawsharks; including predation, self-defence or both. Microwear patterns on the surface of the rostral teeth of sawsharks and sawfishes were found to be alike, thus suggesting that sawsharks utilise their rostrum in a manner similar to sawfishes. This study has also shown that there is a difference in the composition of the rostral teeth and the proportional rostrum length between sawsharks and sawfishes.

Based on the results of this study, it is concluded that one likely function of the toothed rostrum in sawsharks is predation. In both sawsharks and sawfishes, the greatest number of scratch marks occurred in the middle of the tooth and generally had a diagonal orientation. This wear pattern may be indicative of impaling prey on rostral teeth given that this behaviour has been observed in sawfishes (Wueringer *et al.*, 2012). The non-significant difference in the total number of scratch marks between juvenile sawfishes and sawsharks supports this hypothesis further, since the body length of juvenile sawfishes is similar to adult sawsharks (Compagno and Last, 1999; McEachran and de Cavarlho, 2002; Last and Stevens, 2009) and would thus feed on prey of comparable size. Furthermore, the diets of sawsharks and sawfishes both consist of demersal crustaceans and teleosts (Last and Stevens, 2009; Peverell, 2010; this study), therefore suggesting that the microwear observed is also related to similarities in diet. As the total number of scratch marks in sawsharks was significantly less than sawfishes, and it is unlikely that sawfishes exhibit sediment raking behaviour (Wueringer *et al.*, 2012), it is hypothesised that sawsharks do not search for buried prey by raking the sediment with their rostrum. In order to test this hypothesis, it is suggested that the normal microwear on rostral teeth is compared with microwear created by deliberately raking rostral teeth through sediment.

Other findings from the analysis of microwear on the rostral teeth were as to be expected. The significant difference observed in the total number of scratches on the entire rostral tooth between adult sawfishes and sawsharks, and adult sawfishes and juvenile sawfishes, is because sawfishes are unable to replace their rostral teeth once lost (Slaughter and Springer, 1968). Adults would therefore be in possession of the teeth for a longer period of time allowing for more scratches to accumulate. The contradictory results obtained when

examining the uniformity of the scratches along the tooth of the adult *Pristis pristis* (Table IV), where the overall test statistic indicates a significant difference while the test statistics for each section of the tooth indicate a non-significant difference, was due to an unusually low number of scratches being found. Similarly, the significant difference in uniformity reported for the juvenile *P. pristis* was because of the membrane sheath covering the majority of the tooth (Figure 3.6E). Hence only the tip of the tooth was exposed and could accumulate scratches.

In addition to capturing prey, it is likely that the rostrum of sawsharks enables the sensing of prey. The broad, flat rostra of paddlefishes and sawfishes have been shown to contain sensory receptors such as neuromasts or ampullae that enable the detection of prey (Wilkens *et al.*, 2002; Wueringer *et al.*, 2011a; Wueringer *et al.*, 2011b). Although the rostra of sawsharks are tapering, an anatomical investigation of the rostrum has indicated the presence of canals that are innervated by branches of the trigeminal (V) and facial (VII) cranial nerves (von Bonde, 1933); nerves which are involved in carrying tactile (Kasumyan, 2011) and lateral line (i.e. mechanosensory and electrosensory (Boord and Campbell, 1977)) information respectively. The head-up orientation often seen in photographs of sawsharks (personal observation), with the rostrum held at a 45° angle above the substrate, may allow them to sense prey in the water column via electro- or mechanoreception (Kasumyan, 2011) and then ambush it with lateral swipes of the rostrum. A similar dual sensing and capturing role for the toothed rostrum was recently shown in sawfishes (Wueringer *et al.*, 2012). The difference in the proportional rostrum length between the two sawfishes (*Anoxypristis cuspidata* and *Pristis pristis*) and *Pristiophorus cirratus* observed in this study (which should be treated with caution as the sample sizes for both sawfishes were small) may indicate that the sensory capabilities of the rostra are different for the two elasmobranchs. However, an examination of the abundance and distribution of sensory receptors along the rostra of sawsharks and a comparison with previously published work on sawfishes (e.g. Wueringer *et al.*, 2011a; Wueringer *et al.*, 2011b) needs to be performed to assess this.

Another possible function of the rostrum in sawsharks may be self-defence. It has been noted that some sawsharks brought to the surface bear scars or puncture marks on their bodies, which are thought to be have been sustained from the rostral and oral teeth of other sawsharks (Ebert and Cailliet, 2011; Ebert, 2013). Specimens of *Pristiophorus cirratus* examined in this study also displayed such marks; however whether these were the result of deliberate or accidental strikes by surrounding sawsharks in schools or during feeding aggregations is unclear. Defence from predators, in particular large sharks as has been suggested for

sawfishes (Tuma, 1976; Wueringer *et al.*, 2009), is another possibility since a Sixgill Sawshark *Pliotrema warreni* (Regan 1906) has been recovered from the stomach of a Tiger Shark *Galeocerdo cuvier* (Péron & Lesueur 1822) (Ebert, 2013). However, as the encounter rate between a sawshark and a large predatory shark is likely to be lower than that of a sawfish and a predatory shark, such as in Central America (Tuma, 1976), it is proposed that sawsharks could use the rostrum for self-defence when necessary.

The differences in the composition of the rostral teeth in sawsharks and sawfishes could reflect differences in their ecology. The finding that the rostral teeth of both elasmobranchs were composed of a form of hydroxyapatite (the main mineral component of dentin and enamel in vertebrate teeth and bone) is consistent with previous studies (Slaughter and Springer, 1968; Miller, 1974). However, Raman spectroscopy also indicated that the rostral teeth of sawfishes contained collagen throughout, whereas in sawsharks this was restricted to the middle of the tooth. The presence of collagen would make the tooth less mineralised and thus softer (D. Jacob, pers. comm.), which may have important ecological implications. Sawfishes have been observed sharpening their continuously growing rostral teeth on the bottom of aquaria (Wueringer *et al.*, 2012), and may therefore require the malleability of the collagen to ensure that the tooth is sufficiently sharp for impaling the hard flesh of shallow-water fishes (Slaughter and Springer, 1968). In contrast, sawsharks are able to replace their rostral teeth when lost (Slaughter and Springer, 1968); thus the difference in collagen may indicate that maintenance of their rostral teeth is unnecessary. It is recommended that hardness tests are conducted on the rostral teeth to determine if hardness is related to the observed differences in composition.

Stomach contents analysis of the collected *P. cirratus* showed that the diet comprised of demersal crustaceans and teleosts (Stevens and Wiley, 1986; Hanamura and Wadley, 1998; Simpfendorfer, 1998), with pandalid shrimp being the most important component based on the Index of Relative Importance (Pinkas *et al.*, 1971). For the nine *P. cirratus* found with empty stomachs, possible explanations include: they had not eaten recently, digestion and excretion may be rapid in sawsharks, or contents were regurgitated following capture. Although no whole prey item was found to bear rostral tooth marks, it does not preclude that the rostrum is employed in predation as these marks may have been too small to see or were obscured by digestive processes. The large number of intact prey items does suggest however that minimal processing occurs prior to ingestion and that sawsharks may use suction feeding (Ebert, 2013), as do sawfishes (personal observation).

The histology on the "fingerprint" structure was inconclusive in determining whether it could serve a sensory function. Sections of 4 µm were too thick to indicate if it was connected to the rostral canals (believed to contain neuromasts of the lateral line) or reveal the relationship between the lamellae and the epidermis or dermis. It was not possible to produce sections thinner than 4 µm because this was the smallest section size that could be consistently achieved with the available embedding medium (paraffin wax) and the material may not have been decalcified sufficiently (rostrum samples with the "fingerprint" were decalcified for two weeks). Also, interpretation of the internal features of the rostrum at the location of the "fingerprint" proved difficult as the obtained images did not closely resemble previously published diagrams of the internal features of the rostra of sawsharks in cross-section (e.g. Hoffmann, 1912; Kirkland and Aguillón-Martínez, 2002; Wueringer *et al.*, 2009). Based on the available evidence, it is hypothesised that the "fingerprint" is a dense collection of epidermal cells around the margins of the dermis, perhaps to protect the rostrum tip. Alternatively, the lamellae could be the location of cartilage secretion. It is recommended that future histological investigations of the "fingerprint" use thinner sections (e.g. 1 µm thickness), a different embedding medium and decalcify the structure for longer than two weeks.

In conclusion, this study has presented evidence to suggest that the rostra of sawsharks are used in a manner similar to sawfishes, based on an analysis of microwear and stomach contents. The analysis of tooth microwear is a technique that has been largely restricted to mammals, but has recently been used to examine the feeding ecology of extinct and extant Three-Spined Stickleback *Gasterosteus aculeatus* (L. 1758) (Purnell *et al.*, 2006; Purnell *et al.*, 2007; Baines *et al.*, 2014). Thus, this study has also shown another group of fishes in which microwear analysis is suitable for investigating feeding ecology. However, the results presented here are preliminary as only a limited number of tooth samples were available. It is recommended that a larger sample size is utilised to allow for a more accurate assessment of rostra function and that microwear resulting from raking rostral teeth through sediment is compared with normal microwear to test the notion of sediment raking behaviour in sawsharks. Based on the results of the current study, it is proposed that sawsharks utilise their rostrum for predation, both in the form of capturing and sensing of prey, and possibly for self-defence.

Chapter 4: General Discussion

The results presented in this thesis provide information that lends important insights into the biology and behaviour of sawsharks. Firstly, the histological evaluation of the sensory structures in the barbels of the Common Sawshark *Pristiophorus cirratus* (Latham 1794) demonstrated that these lack structures such as taste buds (chemoreception), free neuromasts (lateral line mechanoreception) or ampullae of Lorenzini (electroreception) and provides further evidence in support of a tactile function for the barbels of sawsharks, which is hypothesised to be involved in the detection of prey on the substrate. Secondly, the regression analyses on the *P. cirratus* morphometric data suggest that the barbels could be lateralised and thus sawsharks may exhibit a preference for which barbel is utilised to sense the environment. Thirdly, microwear patterns on the rostral teeth of sawsharks and sawfishes were found to be alike, suggesting that the rostrum in both elasmobranchs serve a similar purpose. It is proposed that sawsharks use their rostrum for predation, both to sense and capture prey in the water column, and possibly for self-defence.

This knowledge regarding the behaviour of sawsharks could help to explain why they are frequent by-catch for commercial fishing practices such as trawling, long lining and gill netting (Walker *et al.*, 2005; Last and Stevens, 2009; Braccini *et al.*, 2012). The purpose of trawls is to exploit the anti-predator behaviour of the target organisms, which is often avoiding the predator by fleeing (Newland and Chapman, 1989; Ryer, 2008). However the speeds at which the trawl nets travel are often too fast for the target organisms to escape and they are thus captured regardless (e.g. Newland and Chapman, 1989). In the case of sawsharks, it is likely that they too attempt to flee from approaching trawl nets but once overtaken, may utilise the proposed self-defence function of the rostrum as a last resort to escape. This however would cause them to become entangled in the fishing gear; a problem also encountered by sawfishes (Peverell, 2005; Wueringer *et al.*, 2009). Similarly, the proposed mechanosensory capabilities of the barbels and rostrum could allow the detection of struggling prey either hooked on demersal long lines or ensnared in gill nets, and thus attract sawsharks to the fishing gear.

These behavioural inferences could prove vital for the conservation of sawsharks and the management of deep-water fisheries. Previous behavioural research has shown that elasmobranchs only respond to trawl gear once in contact with it (Queirolo *et al.*, 2012), thus for by-catch mitigation to be successful, the range at which elasmobranchs can detect the gear needs to be extended. Since the tactile sense of the barbels and rostrum is likely to play a key

role in the feeding behaviour of sawsharks, by-catch reduction strategies utilising this sensory modality may be the most effective. Although research in this area is still in its infancy, one proposed solution is to place water jets on fishing gear (Jordan *et al.*, 2013). However, the effectiveness of such a technique, in terms of by-catch reduction without significantly affecting catch of target species, requires testing.

Knowledge of the current population size is also key to successful conservation and management. Of the eight currently recognised species of sawsharks, five are listed as either Data Deficient or Near Threatened, or have yet to be formally assessed (IUCN, 2013). Harvesting of these species without adequate knowledge of stock size or at an unsustainable rate could lead to their extinction and alterations in the structure of deep marine communities. In turn, this could lead to the decline of the fishery (Norse *et al.*, 2012). Thus, in addition to the application of by-catch reduction strategies, it is recommended that population surveys and catch rate assessments be conducted for these potentially threatened species of sawsharks.

Although the findings presented in this thesis are important steps in understanding the behaviour of sawsharks, much of their biology is still unknown. For example, the majority of the *Pristiophorus cirratus* collected from the Wollongong trawl fishery were male, suggesting that segregation by sex could occur in this species. However, whether this is related to the season or depth at which trawling takes place is unclear. Other areas of future research include assessing the sensory capabilities of the rostrum, collection of population and catch rate data for potentially threatened species and developing adequate husbandry protocols for sawsharks in captivity. Continued research into the biology of sawsharks will ensure the sustainability of both deep marine communities and their associated fisheries.

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