


Lacunae rostralis: A new structure on the rostrum of sailfish *Istiophorus platypterus*

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Abstract

Recent comparative studies of billfishes (Istiophoridae and Xiphiidae) have provided evidence of differences in the form and function of the rostra (bill) among species. Here, we report the discovery of a new structure, lacuna rostralis, on the rostra of sailfish *Istiophorus platypterus*, which is absent on the rostra of swordfish *Xiphias gladius*, striped marlin *Kajikia audax* and blue marlin *Makaira nigricans*. The lacunae rostralis are small cavities that contain teeth. They were found on the ventral rostrum surface of all *I. platypterus* specimens examined and dorsally in half of them. Ventrally, the lacunae rostralis were most prominent in the mid-section of the rostrum. Dorsally, they occurred closer to the tip. The density of lacunae rostralis increased towards the rostrum tip but, because they are smaller in size, the percentage of rostrum coverage decreased. The teeth located within the lacunae rostralis were found to be different in size, location and orientation from the previously identified micro-teeth of billfish. We propose two potential functions of the lacunae rostralis that both relate to the use of the bill in feeding: mechanoreception of prey before tapping it with the bill and more efficient prey handling via the creation of suction, or physical grip.

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KEYWORDS

billfishes, lacunae rostralis, prey-handling, rostrum, sailfish

1 | INTRODUCTION

Billfishes are pelagic apex predators that are characterized by the name-giving elongated upper rostrum (Nakamura, 1985). Within the billfishes, pronounced differences in rostrum morphology exist between the two extant families: Istiophoridae and Xiphiidae (Fierstine & Voigt, 1996; Nakamura, 1985). The istiophorid rostrum has a round cross-section, spear-like shape, is covered in villiform micro-teeth and measures up to 30% of body length (SL) (Habegger et al., 2020; Nakamura, 1985; Ovchinnikov, 1971). The rostrum of the swordfish *Xiphias gladius* L. 1758, the only extant species of the Xiphiidae, has a lenticular section, sword-like shape, bears no micro-teeth and can measure up to 44% of body length (SL) (Habegger et al., 2020; Nakamura, 1985; Ovchinnikov, 1971).

Different functions for the rostrum have been proposed, including prey capture and hydrodynamic benefits (Domenici et al., 2014; Videler et al., 2016). It has been suggested that flatter rostra are more suitable for horizontal slashing at prey, whereas rounder rostra might also be used for vertical motion (Habegger et al., 2015). Recent studies on morphological microstructures of rostra, that is, micro-teeth (Hansen et al., 2020) and oil pores (Dhellemmes et al., 2020), have increased our knowledge of the variation in rostrum morphology within the billfishes and linked this to functional aspects. For example, sailfish *Istiophorus platypterus* (Shaw 1792) showed a higher density of micro-teeth on the dorsal and ventral sides of their rostra, and a higher amount of micro-teeth regrowth compared to striped marlin *Kajikia audax* (Philippi 1887), which is consistent with the greater amount of rostrum use in *I. platypterus* during prey capture (Hansen et al., 2020). The situation is more complex regarding the presence of oil pores, which release fatty acids on the forehead and dorsal part of the rostrum in the four billfish species (*X. gladius*, *K. audax*, blue marlin – *Makaira nigricans* Lacépède 1802, *I. platypterus*) that have been studied so far (Dhellemmes et al., 2020). The oily substance itself was found to be of similar consistency in all species, but the region of release extended much further towards the rostrum tip in *I. platypterus* and *K. audax* compared to *M. nigricans* and *X. gladius*. The release of the oil on the forehead (i.e., the area that experiences the greatest drag) in all four species is consistent with the hydrodynamic benefits of the oil (Dhellemmes et al., 2020; Videler et al., 2016). A functional explanation of the extension of the oil pores further forward towards the tip remains elusive, although it might be linked to the antibacterial and anti-inflammatory properties of the oil or to lowering drag on the slashing rostrum (Dhellemmes et al., 2020).

Here we report the discovery of a new rostral structure, the ‘lacuna rostralis’ (plural: lacunae rostralis). Lacunae rostralis are cavities in the rostrum's surface characterized by the presence of tooth-like protrusions within the cavity itself (Figure 1) (the protrusions were identified as teeth and are henceforth called lacunal teeth, see Section 3). The aim

of this study was to examine four different billfish species for the presence or absence of lacunae rostralis, to characterize their distribution and general morphology, and to discuss potential functions.

2 | MATERIALS AND METHODS

2.1 | Presence or absence of lacunae rostralis

The rostra of four billfish species (*X. gladius*, *K. audax*, *M. nigricans* and *I. platypterus*) were examined for the presence of lacunae rostralis using a stereomicroscope (Wild MZ8, Leica, Wetzlar, Germany). The specimens examined were collected by commercial fishermen in Sardinia, Italy (*X. gladius*, $N = 4$), Cancun, Mexico (*I. platypterus*, $N = 3$) and Reunion, France (*K. audax*, $N = 4$ /*M. nigricans*, $N = 4$ /*I. platypterus*, $N = 10$) (see Supporting Information Table S2 for body length range estimates). One single *I. platypterus* head of unknown geographic origin was also included. Lacunae rostralis were exclusively found in *I. platypterus* and were present in every *I. platypterus*

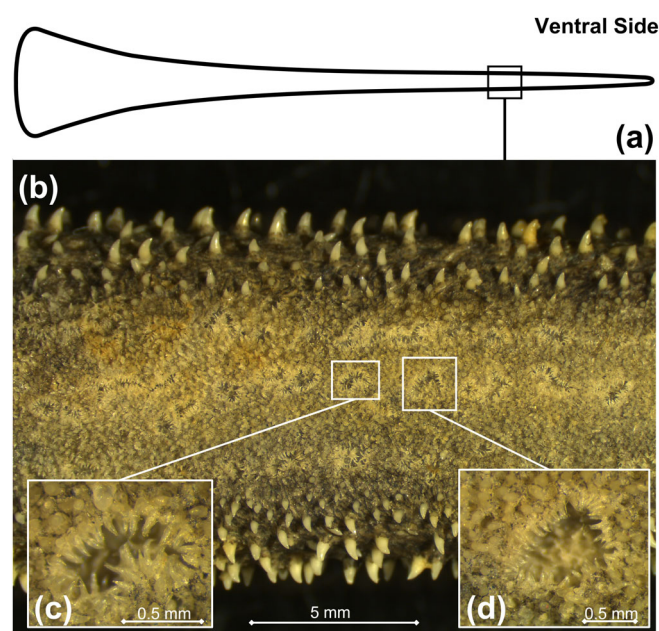


FIGURE 1 Lacunae rostralis on the ventral surface of an *Istiophorus platypterus* rostrum. The location of the photographed area is indicated by a schematic drawing (a). Multiple lacunae rostralis with characteristic lacunal teeth, which are much smaller than the lateral micro-teeth (b). Images of higher magnification (c, d) highlight their shape and orientation. Lacunal teeth were found to be of smaller size, a more slender shape and more translucent than micro-teeth and were tilted towards the cavity centre, while micro-teeth are oriented distally. Lacunal teeth were present throughout the lacunae rostralis, including the bottom of these cavities

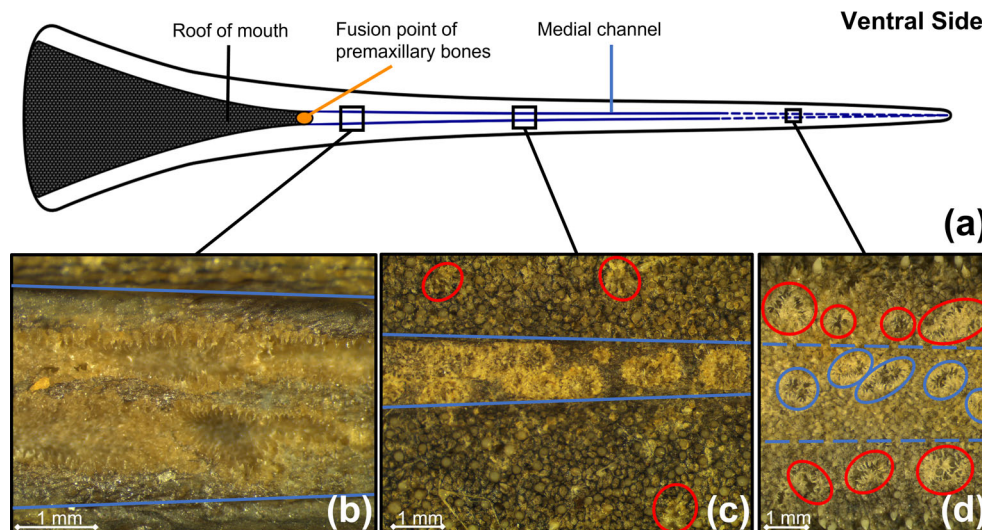


FIGURE 2 Schematic drawing of *Istiophorus platypterus* rostrum highlighting landmarks and variation in lacunae rostralis morphology. Schematic drawing indicating the position of landmarks on the ventral rostrum surface of *I. platypterus* (a). The roof of the mouth ends at an elevated area associated with the fusion point of the premaxillary bones (FPPM). Anterior a channel-like structure termed the medial channel, which is characterized by an absence of micro-teeth and the presence of lacunae rostralis, can be found and is highlighted by blue lines. Pictures of the ventral surface at different positions along the anterior–posterior axis (b–d) show large groove-like lacunae rostralis within the medial channel close to the FPPM (b), strings of lacunae rostralis (some potentially fused) within the medial channel located midway (c) and the absence of a visible medial channel close to the rostrum tip (d). Lateral lacunae rostralis are highlighted using red ellipses (c, d). In segments without a visible medial channel, a blue dotted line marks the innermost third of the rostrum (a, d) and medial lacunae rostralis are highlighted by blue ellipses (d). Lateral lacunae rostralis were generally round, small and unfused along the whole rostrum while the morphology of medial lacunae rostralis on the ventral side of the rostrum was found to depend on the location along the anterior–posterior axis (also see Supporting Information Morphology of the lacunae rostralis)

specimen. The remaining Section 2 concerns the analysis of *I. platypterus* specimens only.

2.2 | Medial and lateral lacunae

Lacunae rostralis were found to differ in their morphology along the medial–lateral axis of the rostrum (Figure 2 and Supporting Information Figure S1). For that reason, we discriminated between medial and lateral lacunae rostralis, and used rostral landmarks to define these two types of lacunae rostralis (the differences and definitions are described in the Supporting Information Definition of medial and lateral lacunae rostralis as well as Figure 2 and Supporting Information Figure S1).

2.3 | Lacunae rostralis: Extension

The maximum extent of lacunae rostralis along the anterior–posterior axis was examined in each specimen by measuring the distance between the rostrum tip and the most anterior and most posterior lacuna rostralis. This was done for both medial and lateral lacunae rostralis, on both the ventral and dorsal side of the rostra. To standardize between individuals, we divided the measurements of the maximum extension by the rostrum length (the length of the rostrum was measured as the distance from the anterior eye edge to the rostrum tip following Fierstine and Voigt 1996; Ovchinnikov 1971). For

four specimens with incomplete heads, the rostrum length was inferred based on linear regression using the distance between the rostrum tip and the fusion point of the premaxillary bones (FPPM) (Supporting Information Figure S2).

2.4 | Lacunae rostralis: Density, coverage and lacunal tooth length

For a subset of six *I. platypterus* specimens (four from Reunion Island and two from Mexico), we took morphological measurements at six equidistant medial positions (labelled 1 to 6 from posterior to anterior) between the tip of the rostrum and the FPPM (on both the ventral and dorsal side). A similar framework had previously been employed by Dhellemmes *et al.* (2020) to investigate oil pores. At each of the six positions, we counted the number of individual lacuna rostralis present within a 1 cm² square to calculate the lacunal density (lacunae rostralis cm^{−2}). We also calculated the percentage of the 1 cm² surface area covered by lacunae rostralis (lacunal coverage, %). Lacunal coverage and density were extrapolated if less than 1 cm² of area was available for measurement, which occurred at anterior positions in a few specimens. At each position, we calculated the average lacunal tooth length from 16 to 25 individually measured lacunal teeth (average lacunal tooth length, mm). The length of an individual lacunal tooth was measured as a straight line from the centre of the base to the tip. For this, lacuna rostralis were horizontally aligned with the

image plane of the microscope. Length measurements were exclusively done on lacunal teeth for which the base and tip were in focus and unobscured. Measurements were done using Image J (Freeware, Version 1.53 h) and Fiji (Freeware).

Data analysis was performed in R (v 4.0.5, <https://cran.r-project.org/>). For the three investigated variables (lacunal density, lacunal coverage, average lacunal tooth length) we tested whether the variables varied along the length of the bill and if this variability was different between the dorsal and ventral side of the rostrum. We used linear mixed models with the 'lme4' package (Bates *et al.*, 2015), with the maximum likelihood estimation method, for the variable average lacunal tooth length. For lacunal density, a zero-inflated generalized linear mixed model was used in the 'glmmTMB' package (Brooks *et al.*, 2017), with the maximum likelihood estimation method and with the probability distribution set to Poisson. Finally, for coverage, a beta zero-inflated generalized linear model was fitted using Bayesian inference in the package 'brms' (Bürkner, 2017, 2018). The model was set with a thinning interval of 10, a burn-in of 2000 and an iteration number of 12,000, resulting in posterior Monte Carlo Markov chains of length 1000. In each model we included a sampling point, as a fixed effect, in an interaction with side (ventral or dorsal). To account for repeated measurements within samples, we always included 'sample' as a random effect. For models that use the frequentist approach, the fixed effects were backwards simplified using chi-squared tests until the most parsimonious model was found. For the Bayesian models, we achieved model simplification using leave-one-out cross-validation in the 'loo' package (Vehtari *et al.*, 2017). The leave-one-out information criterion (LOOIC) was calculated for model selection. Similarly,

to the Akaike information criterion (AIC), the LOOIC estimates the expected log predictive density for a new dataset but is more suitable for Bayesian analyses as it does not make distributional assumptions. When the response was not transformed (*i.e.*, average lacunal tooth length), effects were considered significant if their 95% confidence intervals (hereafter 95% CIs) did not overlap zero. If a log transformation was performed (density) or the model produced a log-odds output (coverage), effects were considered significant if the 95% CI did not overlap one. Statistical assumptions were checked by plotting residuals, Q-Q plots and with the library DHARMA (<https://cran.r-project.org/web/packages/DHARMA/>). For the Bayesian model, we visually inspected the posterior chains.

2.5 | Morphological observations

The anatomy of the lacunae rostralis was investigated using a range of observation methods and imaging techniques [microscopy, histology, scanning electron microscopy and phase-contrast enhanced micro-computed tomography]. Pictures of the unaltered rostrum surface were taken using a microscope camera (MC190, Leica, Wetzlar, Germany) attached to a stereomicroscope (Wild MZ8, Leica) and processed using the software LAS X (Leica, version 3.7.2). For histological observations, two segments were cut from the rostrum of a single individual and decalcified in an aqueous EDTA solution (25%, pH 7.4). Subsequently, the segments were dehydrated using a two-step ethanol procedure (50% and 70%). Paraffin infiltration was done

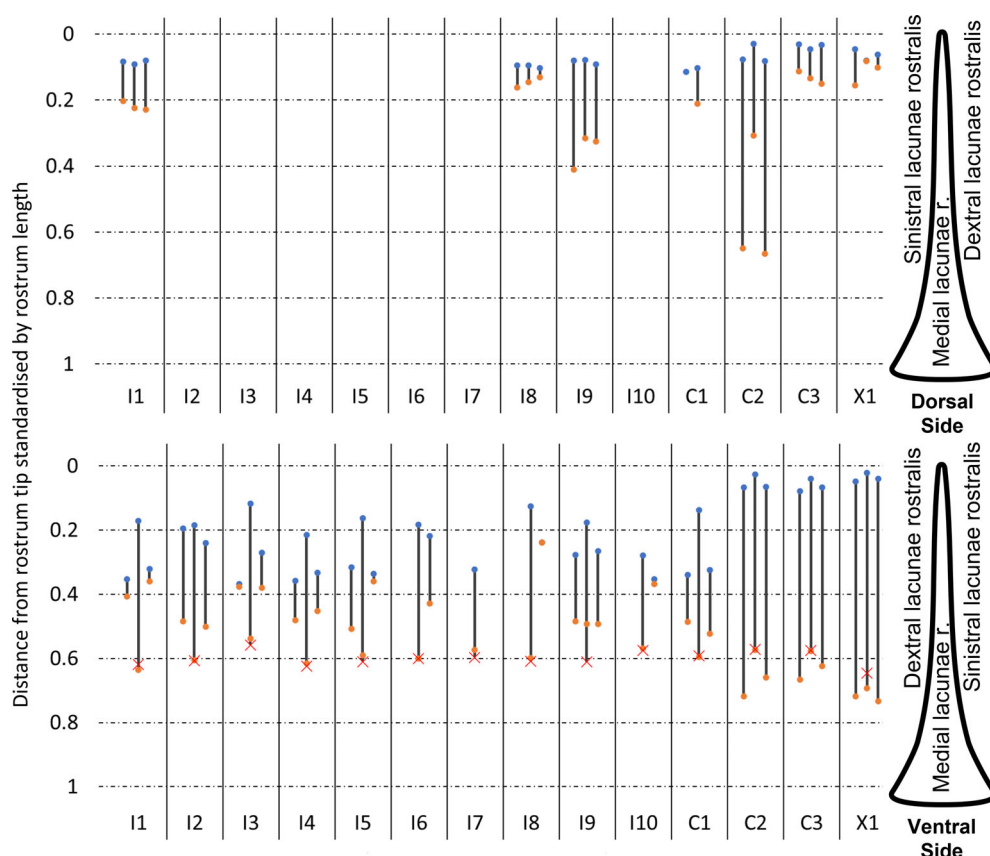
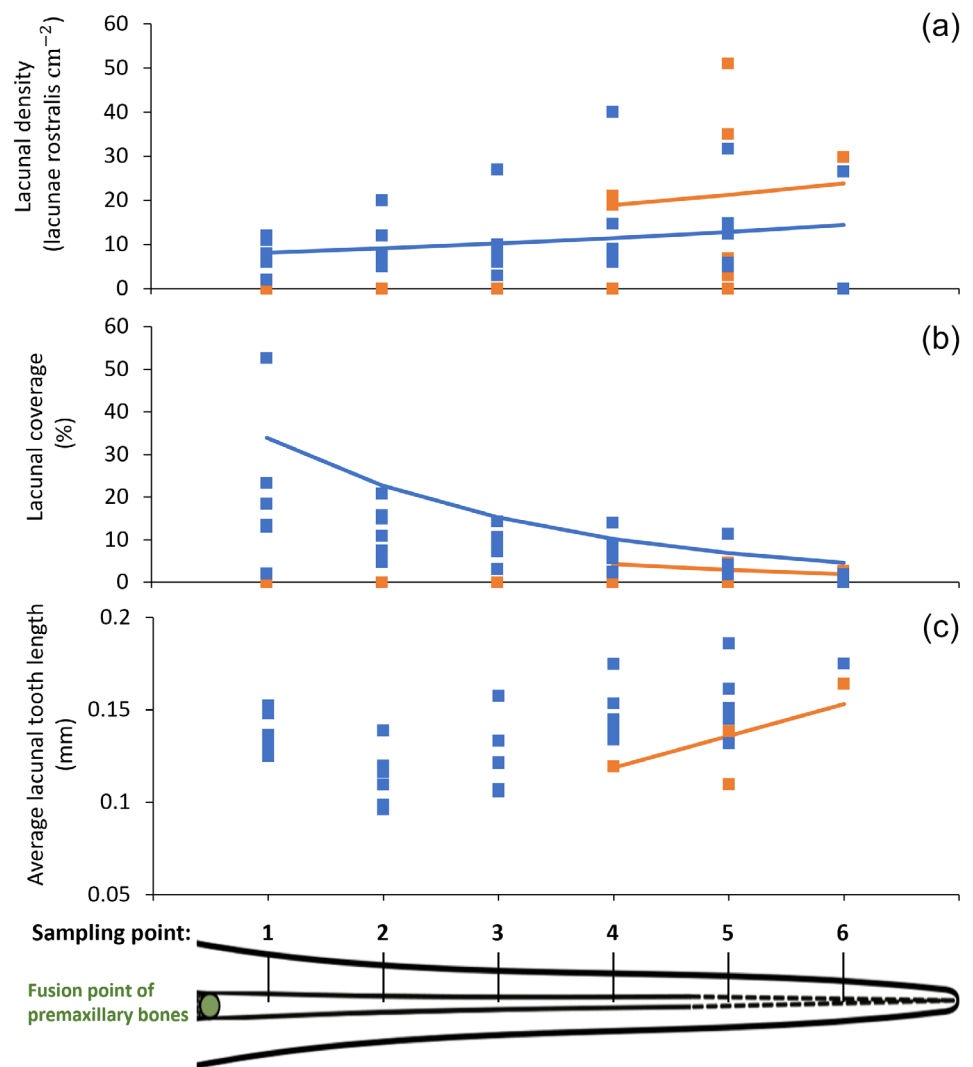


FIGURE 3 Individual variation in *Istiophorus platypterus* lacunae rostralis extension. Location of measured lacunae rostralis indicated by schematic drawings. Differences in maximal anterior-posterior position of lacunae rostralis between individuals were identified. I1-10 originated from Reunion Island, C1-3 originated from Cancun and X1 was of unknown origin. Some individuals had no lateral lacunae rostralis (I7) and others only had lateral lacunae rostralis on one side (I6, I8, I10). The fusion point of premaxillary bones was close to the most posterior medial ventral lacuna rostralis in most individuals. Dorsal lacunae rostralis were found in seven specimens and were generally located anteriorly. ● Most anterior lacunae rostralis; ● Most posterior lacunae rostralis; × Fusion point of premaxillary bones

FIGURE 4 Change of lacunae rostralis variables along the *Istiophorus platypterus* rostrum. Lacunae rostralis were measured at six equidistant medial positions between the fusion point of premaxillary bones and rostrum tip, as indicated by the schematic drawing. Along the whole bill, towards the tip, these main trends were identified as significant by statistical analysis (significant model estimates plotted as solid lines, for details see Section 2): lacunal density was found to increase on both sides (dorsal and ventral) (a), lacunal coverage decreased on both sides (dorsal and ventral) (b) and average lacunal tooth length increased on the dorsal side (c).
 ■ Dorsal; ■ Ventral



using an embedding machine (TP 1020, Leica). Thereafter the segments were cut into 6 μm sections using a sliding microtome (SM 2010R, Leica) and stained with hematoxylin and eosin. The sections were then mounted and photographed using an AxioCam MRc5 camera (Zeiss, Oberkochen, Germany) attached to an AxioScope.A1 microscope (Zeiss) and the imaging software Axio Vision (Zeiss, version V4.8.2). Scanning electron microscope images were taken using a scanning electron microscope (Phenom XL G2, Thermo Fisher Scientific, Waltham, MA, USA) at 490 \times magnification (chamber pressure = 60 Pa, accelerating voltage = 10 kV). For the micro-computed tomography imaging small segments of the rostrum were cut using a water-cooled diamond saw (EXAKT, Otto Herrmann, Nordstedt, Germany) and mounted for phase-contrast enhanced micro-computed tomography (PCE-CT) on the BAMline imaging beamline of BESSY-II of the synchrotron storage ring at the Helmholtz-Zentrum Berlin (HZB, Berlin, Germany). A total of 1440 projections were collected for several bone segments, rotated 360° around the sample axis, each exposed for 1 s. An energy of 24.5 kV was used, with an effective pixel resolution of 2.2 μm , and propagation-based phase-contrast enhancement was induced using a sample-detector distance of 50 mm. The projection radiographs were normalized by empty beam (flat field) images obtained before and after each scan, and

normalized datasets were reconstructed using Octopus V8.7 (XRE, Ghent, Belgium) employing moderate ring artefact filtering. The data were visualized using Fiji/ImageJ and CTvox (Bruker, Kontich, Belgium).

3 | RESULTS

Lacunae rostralis were found in *I. platypterus* but were absent in *K. audax*, *M. nigricans* and *X. gladius*. They were present on the ventral surface of the rostrum in all *I. platypterus* specimens, and dorsally in seven out of 14 individuals. Ventrally, the lacunae rostralis were most prominent in the mid-section of the rostrum (at c. 0.2–0.6 relative rostrum length, measured from the rostrum tip; Figure 3) whereas dorsally – when present – they occurred close to the tip (Figure 3). On the ventral side, lacunae rostralis were present both medially (all specimens) and laterally (in all but one specimen) (Figure 3). The most posterior ventral medial lacunae rostralis were near the FPPM in most of the rostra. Large groove-shaped lacunae rostralis were found ventrally within the posterior medial channel (Figure 2b and Supporting Information Figure S3). In general, the morphology of medial lacunae rostralis on the ventral side of the rostrum seemed to depend on the location along the anterior–posterior axis, an observation that does not apply to lateral

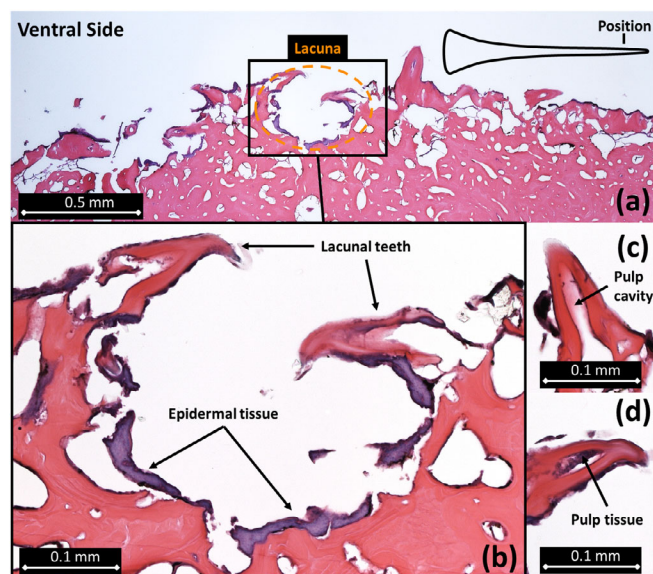


FIGURE 5 Transverse histological sections of an *Istiophorus platypterus* rostrum stained with hematoxylin and eosin. Photographs (a) and (b) are from the same section but of different magnification, whereas the photographs (c) and (d) are from different sections and show details of lacunal tooth anatomy. The location of the photographed area is indicated by a schematic drawing. The internal structure of the ventral rostrum is visible (a) and a single lacuna rostralis can be observed in detail (b). Epidermal tissue on top of the rostral surface, and within the lacuna, can be identified by its blue colouration due to the staining of epidermal cell nuclei by haematoxylin. The extracellular matrix of the rostral bone tissue, and the lacunal teeth, is stained red. The lacuna rostralis clearly constitutes a cavity in the rostral bone (a, b). Two lacunal teeth are visible on the rim of the lacuna rostralis and are stained red like the bone surrounding them (a, b). Lacunal teeth have pulp cavities (a–d) and pulp tissue was found in some of them (d). They thus are anatomically similar to micro-teeth. The pulp cavity and tissue of a micro-tooth can be observed in (a), located directly right to the highlighted lacuna rostralis. The rostral bone appears to be filled with internal cavities, which seem to be especially large at more distal positions

lacunae rostralis on the ventral side or lacunae rostralis on the dorsal side (see Supporting Information Morphology of the lacunae rostralis and Figure 2). Differences existed in the extension of lacunae rostralis between *I. platypterus* sourced from Reunion Island and Mexico (Supporting Information Figure S4). For instance, dorsal lacunae rostralis were present in all specimens from Mexico but only in three specimens from Reunion Island (Figure 3). However, the small number of rostra from Mexico prevented statistical analyses by location.

3.1 | Lacunae rostralis: Density, coverage and lacunal tooth length

Lacunal density increased on both sides of the rostrum towards the tip by an estimated 12% per unit of the independent variable sampling point (95% CI 1.0485, 1.1972) (Figure 4a). Lacunal coverage decreased by 33% per unit change along the bill with no effect of side (95% CI 0.59, 0.77) (Figure 4b). Thus, lacunae rostralis closer towards

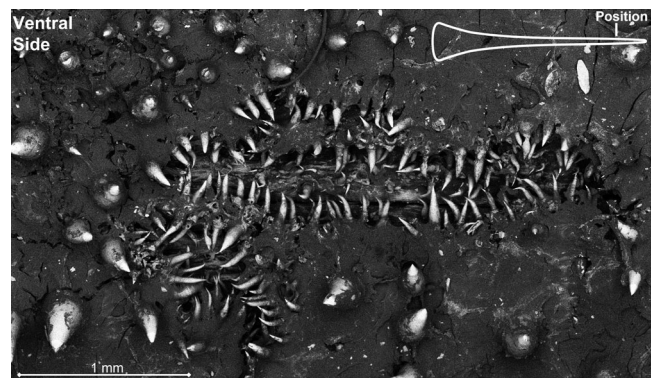


FIGURE 6 Lacunae rostralis on an *Istiophorus platypterus* rostrum visualized by scanning electron microscopy. Numerous lacunal teeth are visible in detail and are present throughout the lacunae rostralis, seemingly ‘filling’ the cavities. Epidermal tissue covering the lacunal teeth appears dark and seems to be partially eroded and cracked, likely a result of transport and storage. On the bottom of the largest lacuna rostralis lightly coloured bone tissue and multiple lacunal teeth can be observed. The tissue between neighbouring lacuna rostralis seems to be especially eroded and allows the observation of spongy bone and the attachment of the lacunal tooth to the bone. The position is indicated by a schematic drawing

the rostrum tip were more numerous and smaller than those closer to the mouth. Side influenced the change of the average lacunal tooth length along the rostrum, which increased towards the tip on the dorsal side (95% CI 0.0097, 0.025, estimate 0.017), but not on the ventral side (95% CI –0.007, 0.02, estimate 0.007) (Figure 4c, see Supporting Information for details on model selection).

3.2 | Morphological observations

Lacunae rostralis were found to be structures of the rostral bones in both histological sections as well as scanning electron microscope and micro-computed tomography images (Figures 5–7) and were covered in epidermal tissue (Figures 6 and 7). The protrusions found in lacunae rostralis were identified as teeth in micro-computed tomography images and histological sections since pulp cavities and pulp tissue were identified within them (Figures 5 and 7). The colouration of lacunae rostralis, and their teeth, in hematoxylin and eosin stained histological sections and scanning electron microscope images is consistent with that of bones and teeth (Figures 5 and 6). The bone surrounding the lacunae rostralis appeared to be spongy and filled with cavities in both histological sections and micro-computed tomography images (Figures 5 and 7). In the micro-computed tomography images, a clear difference in porosity between the bone directly surrounding the lacuna rostralis and that below them is apparent (Figure 7a,c).

4 | DISCUSSION

Our comparative investigation of four species of billfish showed that only *I. platypterus* exhibited lacunae rostralis on their rostra, whereas none were found in *X. gladius*, *K. audax* or *M. nigricans*. Crucially, not

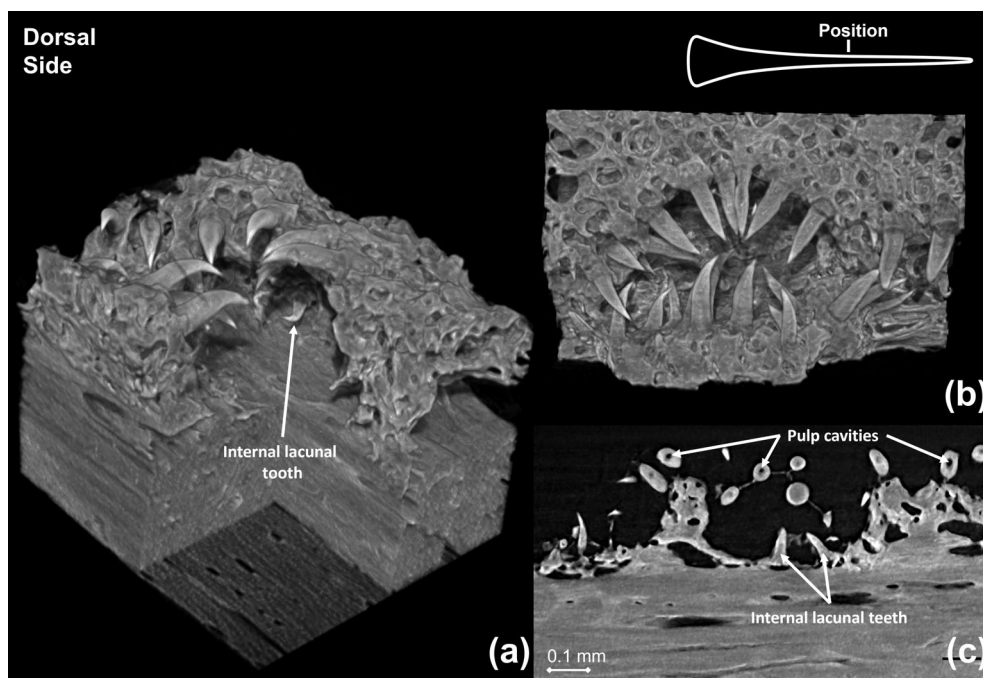


FIGURE 7 Three-dimensional reconstructions and a two-dimensional slice of phase-contrast enhanced micro-computed tomography scans of a typical lacuna rostralis on an *Istiophorus platypterus* rostrum. The scanned region containing the lacuna rostralis is indicated by the schematic drawing. (a) Three-dimensional rendering of the reconstructed lacuna rostralis with a corner cut-out reveals teeth attached directly to the underlying porous bone at the rostrum surface. (b) Three-dimensional outer view rendering of the reconstructed lacuna rostralis with inward-facing lacunal teeth. (c) A virtual longitudinal slice depicts the attachment site of lacunal teeth relative to the surrounding bone tissue. Internal lacunal teeth are observed to be less numerous and smaller than those at the surface (a, c). The outer region of the rostral bone appears to be porous, comprising an elaborate network of connected internal cavities. The inner bone has a more regular, layered appearance with sporadic, elongated pores, presumably blood vessels. The transition between these two types of bone tissue, the outer more spongy bone and the inner more dense bone, coincides with the maximal depth of the lacunae rostralis (a, c). Similar to observations by histology, the lacunal teeth contain pulp cavities (c). Additional details on the bone structure and lacunal morphology are observable in a three-dimensional video showing the same lacuna rostralis as above (Supporting Information Video S2)

only was *I. platypterus* the only species to have lacunae rostralis, but lacunae rostralis were present in every specimen examined, suggesting they may have a functional significance particular to this species. The general function of the billfish rostrum is under continued discussion and has been proposed to relate to defence (Fierstine, 1997), locomotion (Kozlov, 1973) and feeding (Shimose et al., 2007). While the billfish rostrum may have multiple functions, similar to the beak of birds (Friedman et al., 2019) or the saw of the sawfish (Wueringer et al., 2012), in *I. platypterus* and *K. audax* (Domenici et al., 2014; Hansen et al., 2020) the evidence for rostrum use in prey capture is unequivocal.

I. platypterus are unique among the billfishes regarding their rostral morphology and their feeding behaviour. *I. platypterus* have the ability to insert their rostrum into a prey school without dispersing the fish (Domenici et al., 2014), which enables them to perform directed short-range strikes at individual prey fish (tapping behaviour). This kind of rostrum insertion has not been observed in other billfish species. *I. platypterus* attack behaviour has been linked to the morphology of two rostral structures common in the billfishes: the micro-tooth (Hansen et al., 2020; Kurvers et al., 2017) and the oil pore (Dhellemmes et al., 2020). Oil pores are thought to function in drag

reduction during swimming (Dhellemmes et al., 2020; Videler et al., 2016) and might also reduce drag on the slashing rostrum during hunting (Dhellemmes et al., 2020). Micro-teeth are thought to function by increasing the damage to the prey's body surface during contact and potentially, along with oil pores, may reduce drag on the slashing billfish rostrum (Hansen et al., 2020; Krause et al., 2017).

Lacunae rostralis and their lacunal teeth are similar in structure to oil pores and micro-teeth, as they represent different types of cavities (lacunae rostralis and oil pores) and teeth (lacunal teeth and micro-teeth) on the *I. platypterus* rostrum. How lacunae rostralis and their lacunal teeth differ in size and distribution from their structural counterparts may relate to their function. Regarding their position, we found some overlap between oil pores and lacunae rostralis. The maximum extension of oil pores in *I. platypterus* (0.16–1, anterior to posterior) found by Dhellemmes et al. 2020 overlaps that of lacunae rostralis (0.03–0.67, anterior to posterior; Figure 3) found in this study. An important difference between the two is that oil pores are exclusively found dorsally whereas lacunae rostralis were mostly found on the ventral surface (Figure 3). Lacunae rostralis on the dorsal side were found only near the tip and only in half of all specimens examined (Figure 3). They were specifically not found on the

forehead, an area that experiences great drag during swimming and has a high density of oil pores in several billfish species, including *I. platypterus* (Dhellemmes *et al.*, 2020). Therefore, it is unlikely that the function of lacunae rostralis is to reduce drag via oil retention. Lacunal teeth are a newly described type of teeth, and like lacunae rostralis have only been found on the rostra of *I. platypterus*. They are similar to micro-teeth in their conical shape and anatomy. Both contain pulp cavities and pulp tissue. Pulp cavities were identified by both histology (Figure 5) and micro-computed tomography (Figure 7), while pulp tissue was exclusively identified by histology (Figure 5). However, micro-teeth are distinguishable from lacunal teeth regarding their length, position and orientation. The average length of micro-teeth (0.27 mm; Hansen *et al.*, 2020) is about two-fold that of lacunal teeth (Supporting Information Table S1). Micro-teeth are furthermore distributed along most of the rostrum, while lacunal teeth have a more restricted extension. Most importantly lacunal teeth point towards the cavity centre of lacunae rostralis while micro-teeth are oriented outward. Based on these findings it is unlikely that lacunal teeth play a role in damaging prey.

Changes in micro-teeth and oil pore morphology and distribution along the *I. platypterus* rostrum have been identified and been used to elucidate the functional significance of these structures (Dhellemmes *et al.*, 2020; Hansen *et al.*, 2020). In a similar manner, we were able to find changes in the morphology and distribution of lacunae rostralis and used them to develop some initial hypotheses on lacunae rostralis function. Specifically, we found that the density of lacunae rostralis increased towards the rostrum tip, whereas the percentage of rostrum surface area covered by lacunae rostralis decreased in the same direction.

One hypothesis we propose regarding the function of lacunae rostralis is that they are accessory structures of a mechanoreceptive sensory organ, enabling *I. platypterus* to sense where their prey is relative to the rostrum, thus enhancing the precision of movement and the manipulation of prey. In that context, lacunae rostralis might be crucial to the unique ability of *I. platypterus* to insert their rostrum into a prey school without dispersing the fish (Domenici *et al.*, 2014). The existence of rostral cavities with a sensory function is known for the southern sawshark *Pristiophorus nudipinnis*. This species has pores on its rostrum – surrounded by rings of dermal denticles – which are accessory structures to the ampullae of Lorenzini (Wueringer *et al.*, 2021) and thus may support the sensing of prey. The morphology of the anterior lacunae rostralis in *I. platypterus*, which have been found to be more numerous and smaller than posterior ones (Figures 2 and 4a,b), could be suitable for shielding sensory organs within the cavities in a similar way. Whether lacunae rostralis house mechanoreceptive neuromast cells, similar to the trunk canals on lateral line scales (Wada *et al.*, 2014), is a key future research question. A range of methods, for example, histological methods (Wonsettlar & Webb, 1997) or fluorescent dye labelling (Brown *et al.*, 2013), could potentially be used to investigate this hypothesis but were beyond the scope of this work due to the limited availability of fresh *I. platypterus* rostra.

Another potential function of lacunae rostralis relates to prey handling. Such a function has been proposed for a rostral structure, that is, the mandibular canal, in the saw fishes Pristidae (Wueringer *et al.*, 2012, 2021). In the Pristidae, prey handling with the mandibular canal is assumed to aid in swallowing prey headfirst and might thereby prevent injuries from the pointed and venomous spines of some prey species. A notable finding of our study is that the most posterior lacunae rostralis of *I. platypterus*, on the ventral side closer to the mouth, cover a greater surface area compared to those located towards the tip of the rostrum or on the dorsal side (Figure 4b and Supporting Information Figure S3). Based on their location, these lacunae rostralis likely contact prey during prey handling leading up to ingestion (see Supporting Information Video S1). *I. platypterus* hunt in groups and prey is usually not consumed on immediate contact, but instead incapacitated with slashes and taps of the rostrum and then handled and ingested (Domenici *et al.*, 2014; Hansen *et al.*, 2020; Herbert-Read *et al.*, 2016). Quick and efficient prey handling may therefore be important to reduce the risk of kleptoparasitism. Lacunae rostralis may help manipulate prey orientation by creating suction or physical gripping onto prey tissue. To test this hypothesis future morphological work should be complemented with examinations of flow visualization around the bill, alongside the analysis of *I. platypterus* rostral contacts with prey using high-resolution video.

In conclusion, lacunae rostralis were found on every *I. platypterus* specimen examined, but not in the three other billfish species examined (*K. audax*, *M. nigricans* and *X. gladius*). Pronounced interindividual variation in lacunae rostralis morphology and distribution was found and was related to the location along the rostrum. Based on location, and circumstantial evidence, lacunae rostralis may have a function in prey handling or have a mechanoreceptive function. Unquestionably, more research is required to shed light on the functional significance of lacunae rostralis in *I. platypterus*.

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Conceptualization: J.H., M.J.H., K.P., F.D., P.D., J.F.S. and J.K. Methodology: J.H., M.J.H., F.D., M.B., S.K., P.Z., J.M., K.M., M.S., G.F., T.B.H. and J.K. Formal analysis: J.H., K.P., F.D. and M.J.H. Resources: P.S.S., P.B., P.D., J.F.S., M.J.H. and J.K. Data

curation: J.H. and K.P. Writing – original draft: J.H., M.J.H. and J.K. with contributions from all authors.

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