

Comparative morphology, histology, and cytology of odontocete cetaceans prostates

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ABSTRACT

The prostate is the only male accessory gland in cetaceans. However, little is known about this organ in these species. Anatomical and histological characteristics of the prostate have been described in only a few cetacean species, further, one study reported a high incidence of prostatic pathologies in cetaceans that may impair reproduction. The objective of this work was to describe and compare the morphological, histological, and cytological characteristics of the prostate in different odontocete cetaceans. To this end, the prostate glands of 47 animals from nine different species of cetaceans were macroscopically and microscopically studied. Members of the families Delphinidae, Ziphiidae, and Physeteridae were included. In general, the prostate appeared as a musculo-glandular organ with two distinct parts—the *Corpus prostaticae* and the *Pars disseminata prostaticae*. In the pygmy sperm whale (*Kogia breviceps*) and the Cuvier's beaked whale (*Ziphius cavirostris*), the prostate was a discrete gland with a small *Corpus prostaticae*. Microscopically, the prostates of different delphinids species shared similarities; however, the prostate of the pygmy sperm whale revealed significant histological differences compared to those of the delphinids. Immunohistochemical analysis was performed using low- and high-molecular-weight cytokeratin, vimentin, and prostatic specific antigen commercial antibodies. Electron microscopy analysis was performed on the prostate of a bottlenose dolphin and the cytomorphological differences among the major epithelial components of the prostatic epithelium were described. *Anat Rec*, 303:2036–2053, 2020. © 2019 American Association for Anatomy

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The reproductive system of cetaceans exhibits a number of unique morphological and physiological adaptations to aquatic life. These include intra-abdominal testes, a completely concealed retractile fibroelastic penis for maintaining hydrodynamics, and an arterio-venous counter-current heat exchanger that reduces the temperature of the gonads (Reynolds and Rommel, 1999). The prostate, being the only male accessory gland in cetaceans, has undergone modifications in these animals. However, little is known about the morpho-physiological characteristics of this organ in cetaceans. In delphinids, the prostate is located between the two vestigial pelvic bones, close to the root of the penis, and it is supported by skeletal muscles (Cozzi et al., 2017). This organ comprises a diffuse part and a lobular part and is externally surrounded by the *muscle cromptressor prostatae* (Matthews, 1950).

Anatomical and histological properties of the prostate in dolphins and beluga whales (*Delphinapterus leucas*) have been described; however, no information on the prostates of other families of cetaceans is available (Matthews, 1950; Harrison et al., 1972; Yablokov et al., 1974; De Guise et al., 1994; Cozzi et al., 2017). Furthermore, most studies mentioned above focused on a single species. In addition, there are very few studies on the comparative aspects of prostatic anatomy in different cetaceans.

The prostate plays a major role in animal reproduction systems as it contributes to the production of the seminal fluid essential for sperm viability and motility (Clapp and Croker, 2012). For instance, alterations in the prostatic gland may lead to infertility in animals (Foster, 2017). Recently, it has been reported that lesions in the prostate gland may impair reproduction in cetaceans (Suarez-Santana et al., 2018). However, detailed knowledge of the topography, morphology, and histology of this organ is essential to better understand its role in the reproduction of such species and to characterize possible prostatic lesions. The objective of this work was to detail the gross morphology, histology, and cytology of the prostate in different odontocete cetaceans.

MATERIAL AND METHODS

Animals

For this study, prostates were dissected from carcasses of odontocete cetaceans. The examined animals (Supplemental table 1) included males of free-ranging cetaceans stranded in the Canary Islands ($n = 44$) and Mallorca ($n = 1$), as well as captive cetaceans ($n = 2$). A total of 47 animals from nine different species of cetaceans were included: striped dolphin (*Stenella coeruleoalba*, $n = 16$), Atlantic spotted dolphin (*Stenella frontalis*, $n = 12$), pygmy sperm whale (*Kogia breviceps*, $n = 5$), common dolphin (*Delphinus delphis*, $n = 4$), bottlenose dolphin (*Tursiops truncatus*, $n = 4$), short-finned pilot whale (*Globicephala macrorhynchus*, $n = 2$), Cuvier's beaked whale (*Ziphius cavirostris*, $n = 2$), rough-toothed dolphin (*Steno bredanensis*, $n = 1$), and sperm whale (*Physeter macrocephalus*, $n = 1$). In each case, a complete standardized necropsy procedure was performed. Life history data (age

class, body condition, decomposition code, morphometry, and stranding condition) of the examined animals were systematically recorded. The age class was determined using the total body length, physiomorphological parameters, and gonadal development, and it was categorized into four groups: neonate, calf, juvenile/subadult, and adult (Kuiken and García-Hartmann, 1993; IJsseldijk and Brownlow, 2018). The decomposition code of the examined animals was classified as very fresh, fresh, moderate autolysis, advanced autolysis, or very advanced autolysis (Kuiken and García-Hartmann, 1993; IJsseldijk and Brownlow, 2018).

Morphological Study

The standardized protocol published by Suarez-Santana et al. (2018) was followed for the dissection and sampling of the prostate. First, the peritoneal and pleural cavities were opened. Then the thoracic viscera, spleen, liver, adrenal glands, and gastrointestinal system were extracted. Subsequently, the genitourinary system was entirely removed, including kidneys, ureters, urinary bladder, testicles, the penis root with pelvic bones and penis. Following inspection and sampling of the upper urinary system (kidneys, ureters, and bladder), the pelvic bones were detached, and a transversal cut was made sectioning the prostatic gland, prostatic urethra, *crura* of the penis and nearby musculature. Routine histological samples of the prostate were obtained. These protocols were modified to obtain a more complete morphological description, including sagittal, coronal, and oblique sections of the prostate.

In one animal (Case 3, striped dolphin), a vascular repletion was performed by injecting red and blue latex through a canalized artery and vein, respectively, filling the vascular system (Rivero et al., 2009). The carcass of this animal was frozen posteriorly and serial transverse sections were obtained from this case for topographic studies.

Histology, Histochemistry, and Immunohistochemistry

The prostatic samples were fixed in 10% buffered formalin, embedded in paraffin wax, sectioned at 5 μm and routinely stained with hematoxylin and eosin (Leia Autostainer XL 2V3 RevC, Leica Biosystems Nussloch GmbH, Nussloch, Germany). In addition, periodic acid-Schiff (PAS), PAS-diacetate, Masson's trichrome, Gram, and Alcian Blue (pH 2.5) stains were performed in all cases.

Fresh prostates from different species were selected for immunohistochemical analysis (Cases 8, 11, 17, 20, and 31); sections at 4 μm were obtained, immunolabeled with cytokeratin (CK) CK5 + 8, CK8 + 18 (Euro-Diagnostica, Arnhem, The Netherlands), CK7, CK20, anti-prostate-specific antigen (anti-PSA), and vimentin (Dako, Glostrup, Denmark) primary antibodies. These sections were visualized using the Dako EnVisionTM system (Dako, Denmark). We used previously published methodologies for immunolabeling the sections with CK 5 + 8, 8 + 18, 7, 20, and vimentin (Suárez-Santana et al., 2016). For

anti-PSA immunohistochemistry, antigenic retrieval was performed by immersing the slides in a Tris-EDTA buffer and then heating them in the microwave (800 W) for 10 min, in two cycles of 5 min each. Positive controls included canine mammary glands for CK5 + 8, CK8 + 18, and CK7, and lung from a short-finned pilot whale for CK20, respectively. For anti-PSA, positive control was a chimpanzee prostate (Karr et al., 1995). For vimentin, there was an internal control (vessel walls). For the negative control, the same methodology was followed; however, the addition of the primary antibody was omitted.

Ultrastructural Analysis

The prostatic samples from a bottlenose dolphin (Case 31) were fixed in 2% buffered glutaraldehyde (0.1 M, pH 7.4) for 24 h at 4°C. Osmium tetroxide (1%) was used as the second fixative for 4 h before dehydration in graded ethanol series and they were embedded in oxide-Ebbed812 resin. After the embedding, the samples were ultrasectioned at 80 nm using an Ultracut S Microtome (Leica, Vienna, Austria) and stained with uranyl acetate in 1% methanol and lead citrate solution. A JEOL JEM-1400 electron microscope (JEOL Ltd., Tokyo, Japan) was used for observing the ultrastructural morphology of the prostate and for taking microphotographs.

RESULTS

Table 1 summarizes the comparative aspects of the prostates of different species of odontocetes.

Morphology

Relationship with other structures. The prostate was closely related to the different structures of the penis root (Figure 1A–C). The penis root is the anatomical region that provides a firm anchorage for the penis and provides its blood supply and innervation. The penis root is always located in the caudal abdomen, ventral to the rectum, caudal to the urinary bladder, and cranial to the anus. In most odontocetes, the penis root had an osseous component (the pelvic bones), a muscular component (*ischiocavernosus* and *bulbospongiosus* muscles), and a fibrovascular component (*crura* of the penis). However, in some species (i.e., pygmy sperm whales and Cuvier's beaked whales) the pelvic bones were absent.

The *crura* of the penis were composed of a prominent vascular component (*corpus cavernosum*) and a thick fibroelastic tissue (*tunica albuginea*) that medially fused each. In most odontocetes, the *crura* of the penis were attached strongly to the pelvic bones. However, in the absence of pelvic bones, the *crura* of the penis by itself acted as the rigid support of the penis root. The *m. ischiocavernosus* were bilateral and well developed in all species. They originated in the rudimentary pelvic bones and inserted into the *crura penis*. When pelvic bones were absent (i.e., in the pygmy sperm whale and Cuvier's beaked whale), the *ischiocavernosus* muscle originated and inserted into the *Tunica albuginea corporum cavernosorum*. The *bulbospongiosus* muscle was an unpaired muscle that ran ventromedially to the *ischiocavernosus* muscle and the *crura* of the penis, covering the bulb of the penis and the penis retractor muscle.

The rectum was dorsal and caudal to the prostate, separated by the *excavatio rectogenitalis*, which corresponds to the fascia of the muscle *crompressor prostatae*, peritoneum

and a variable amount of fat. The prostate was ventrally supported by the *crura of the penis* and laterally enveloped by the muscle *crompressor prostatae*. The prostate was intimately related to the preprostatic urethra cranially, which was found to be very short in delphinids, but it was longer in the other odontocetes (i.e., pygmy sperm whale, Cuvier's beaked whale, and sperm whale). In delphinids, fibers of smooth muscles from the bladder neck (external urethral sphincter) ran through a larger cranial portion of the prostate and gradually disappeared in the caudal direction.

Anatomy of the prostate of odontocete cetaceans. The prostate was the only accessory gland identified in the genital tract of all 47 males. In all cases, it was musculo-glandular with an elliptical geometry that was closely related to the urethra.

The urethra of cetaceans twisted by 180° after the bladder changed from a caudal (preprostatic urethra) to a cranial course in the penis urethra. This curve was situated in the part of the urethra that was enveloped by the prostate (prostatic urethra). The interior of the prostatic urethra contained the *Colliculus seminalis*, a prominence that emerged from the urethral crest and supported the outlet of the deferent ducts into the urethra. In the caudal wall of the prostatic urethra and immediately ventral to the *Colliculus seminalis* was a double row of tiny orifices, corresponding to the exit of the main prostatic ducts (*Ductuli prostatici*) to the urethra.

The glandular parenchyma of the prostate had a white-pink color and firm consistency in fresh animals, whereas the gland became softer and more reddish with autolysis. Two parts of the glandular component of the prostate were noted in all species of cetaceans: the *Pars disseminata prostatae* and the *Corpus prostatae*. The *Pars disseminata prostatae* encompassed most of the prostatic urethra, whereas the *Corpus prostatae* contained the majority of the prostatic parenchyma. The *Corpus prostatae* began approximately at the level of the *Colliculus seminalis* and expanded caudally to the prostatic urethra. In the *Corpus prostatae*, bands of fibrous tissue, corresponding to the prostatic trabeculae, that projected radially to the urethra could be distinguished. The prostatic ducts, which drained the prostatic fluid from the acini to the prostatic urethra, converged near the urethra in the *ductuli prostatici*, and this was macroscopically visible when a transverse cut was made close to the prostatic urethra in the most cranial part of the *Corpus prostatae*.

Differences by species. In the analyzed odontocetes, the delphinids had the most developed prostate of the analyzed odontocetes, characterized by a notorious *Corpus prostatae* and a more discrete *Pars disseminate prostatae* (Figure 2). In the delphinids, the prostate was completely enveloped by a well-developed *muscle crompressor prostatae*, which was thicker at the proximity of the bladder and became progressively thinner in the caudal direction. Among the examined delphinids, the Atlantic spotted dolphin appeared to have the larger prostate in relation to the total body length, whereas that of the striped dolphin was considerably smaller in relation to the total body length.

The prostates of the pygmy sperm whale and Cuvier's beaked whale were less developed than those in delphinids. The organ was a small and poorly delineated musculo-glandular structure that enveloped the urethra. Furthermore, a small *Corpus prostatae* was observed. In the pygmy sperm whale, a majority of the prostatic

TABLE 1. Comparative morphologic and histologic aspects of the prostate of odontocete cetaceans

	Morphology			Histology and histochemistry			
	Supportive structures	Glandular parenchyma	<i>Muscle compressor prostatae</i>	Immature prostate	Secretory epithelium	Ductal epithelium	Immature prostate
Delphinids	Between pelvic bones Dorsal to prominent <i>crura</i> of the penis	Well developed	Well developed	Scarcer glandular parenchyma and more discrete <i>muscle compressor prostatae</i>	Low-to-high cuboidal epithelium frequently vacuolated Intracytoplasmic granules: -PAS (+) -PAS-diastase (+) -Alcian Blue (+)	Low cuboidal epithelium: -PAS (-) -PAS-diastase (-) -Alcian Blue (-)	Smaller prostatic lobules Greater number of basal cells In neonates: -Immature prostatic ducts -Clusters of transitional epithelia within the acini -Prostatic urethral epithelium with PAS (+), PAS-diastase (-) inclusions Smaller prostatic lobules and relatively thicker prostatic trabeculae Immature ducts
Kogia breviceps	No pelvic bones Prominent <i>crura</i> of the penis Prominent penis root muscles	Discrete	Discrete	Small gland with scant glandular parenchyma	Low cuboidal to columnar epithelium Intracytoplasmic granules: -PAS (+) -PAS-diastase (+) -Alcian Blue (+)	Columnar epithelium: -PAS (+) -PAS-diastase (+) -Alcian blue (+)	No data
Physeter macrocephalus	Between pelvic bones Dorsal to prominent <i>crura</i> of the penis	Well developed	Discrete	No data	Lobular architecture with thick trabeculae	No data	No data
Ziphius cavirostris	No pelvic bones Dorsal to less-developed <i>crura</i> of the penis Prominent penis root muscles	Discrete	Discrete	No data	Lobular architecture with thick trabeculae	No data	No data

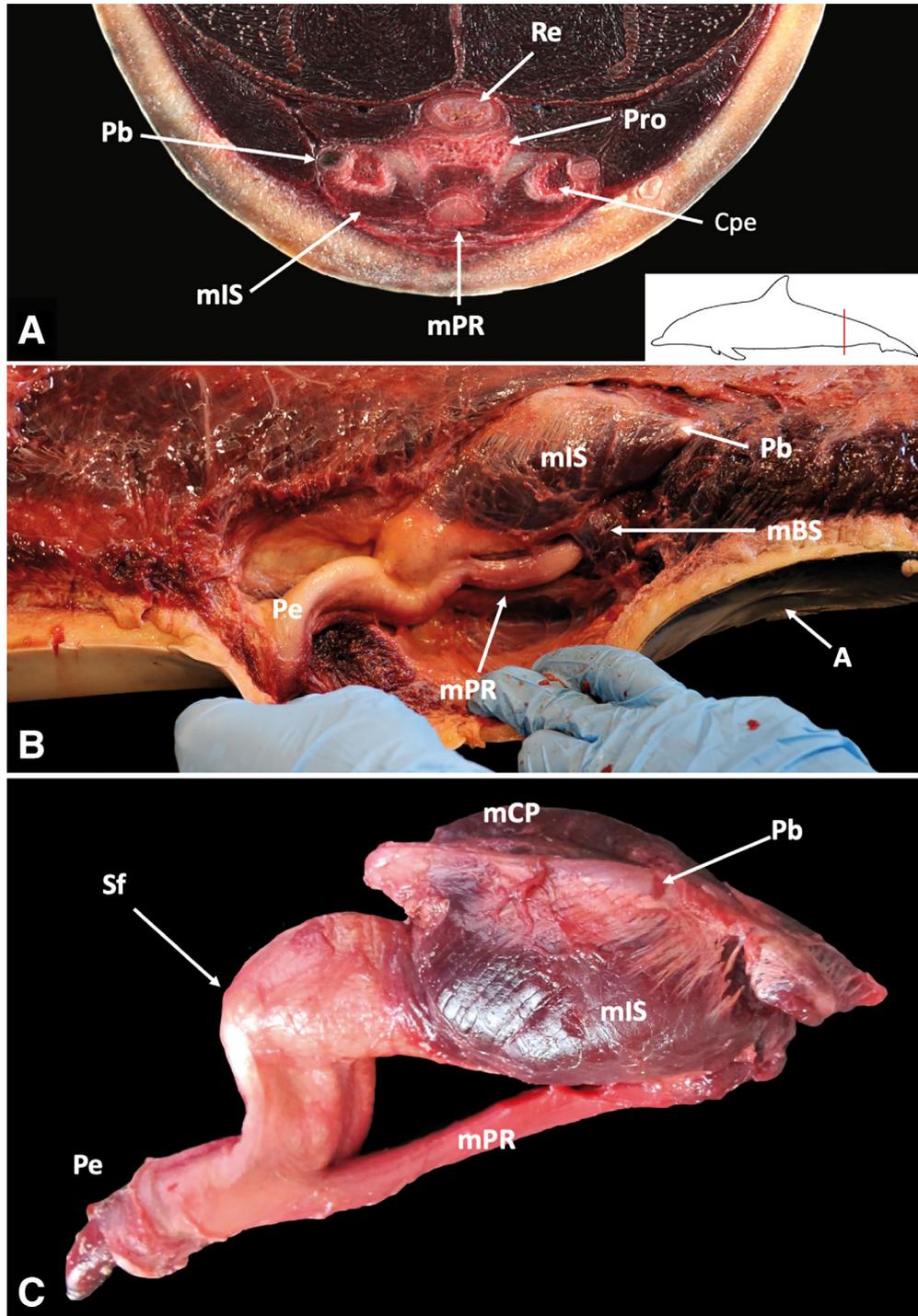


Fig. 1. Topography of the delphinid prostate. (A) Striped dolphin (Case 3). Transverse cut of the prostate *in situ*. Inset: Approximate level of the cut. (B) Striped dolphin (Case 2). Aspect of the penis root *in situ*. (C) Striped dolphin (Case 32). Dissection of the penis root and penis. A, anus; Cpe, Crura of the penis, Pb, pelvic bones; mCP, muscle compressor prostatae; mBS, m. *bulbospongiosus*; mIS, m. *ischiocavernosus*; Pe, penis; mPR, m. penis retractor; Pro, prostate; Re, rectum; Sf, sigmoid flexure

parenchyma was disposed caudal to the urethra and the prostate was closely attached to the surrounding muscles and connective tissue of the penis root (Figure 3). The *muscle compressor prostatae* was present in the pygmy sperm whale, but it was made up of a few, poorly defined

muscle fibers surrounding the gland. The muscles related to the penis root (i.e., m. *ischiocavernosus* and the m. *bulbospongiosus*) were well developed in this species (Figure 3, A), accounting for a robust anchorage for a prominent penis.

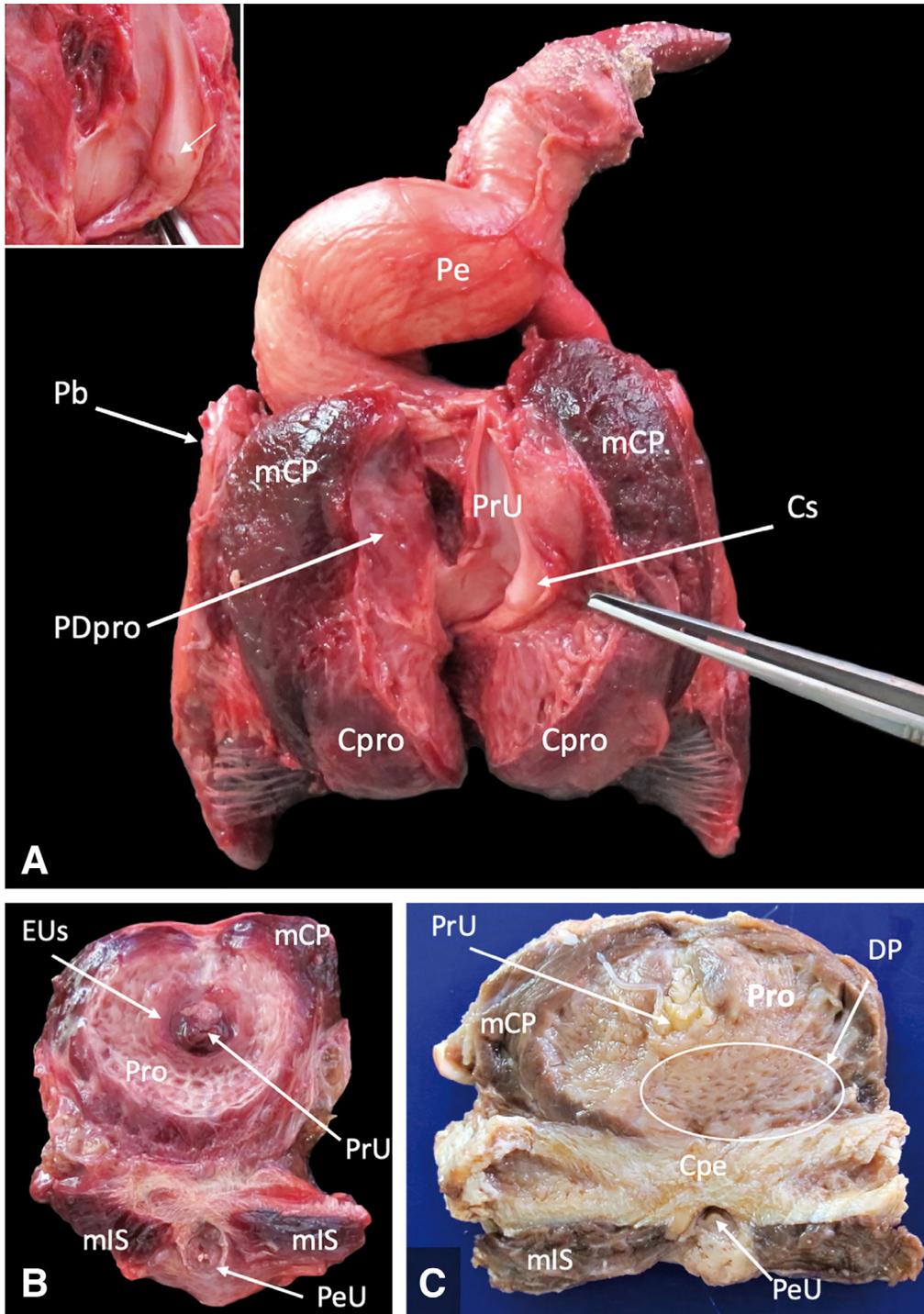


Fig. 2. Morphology of the delphinid prostate. (A) Striped dolphin (Case 32). Dorsal view of a sagittal cut of the prostate, showing the *Corpus prostaticae* and the *Pars disseminata prostaticae*. Inset: Detail of the *Colliculus seminalis* (arrow). (B) Atlantic spotted dolphin (Case 34). Proximal transverse cut of the *Pars disseminata prostaticae*. (C) Bottlenose dolphin (Case 29). Proximal cut of the *Corpus prostaticae* and *crura* of the penis. Cpe, *Crura* of the penis; Cpro, lobular part of the prostate; Cs, *Colliculus seminalis*; DP, *Ductuli prostatici*; EUs, external urethral sphincter; Pb, pelvic bones; mCP, muscle compressor prostaticae; mIS, m. *ischiocavernosus*; PDpro, *Pars disseminata prostaticae*; Pe, penis; PeU, penile urethra; Pro, prostate; PrU, prostatic urethra.

Only the prostate of a juvenile/subadult individual of the sperm whale was analyzed (Figure 4). In this animal, the prostate was a well-developed organ, with a conspicuous

Corpus prostaticae and a *Pars disseminata prostaticae* as seen in delphinids. Dorsally the prostate was surrounded by a thick layer of connective tissue corresponding to the caudal

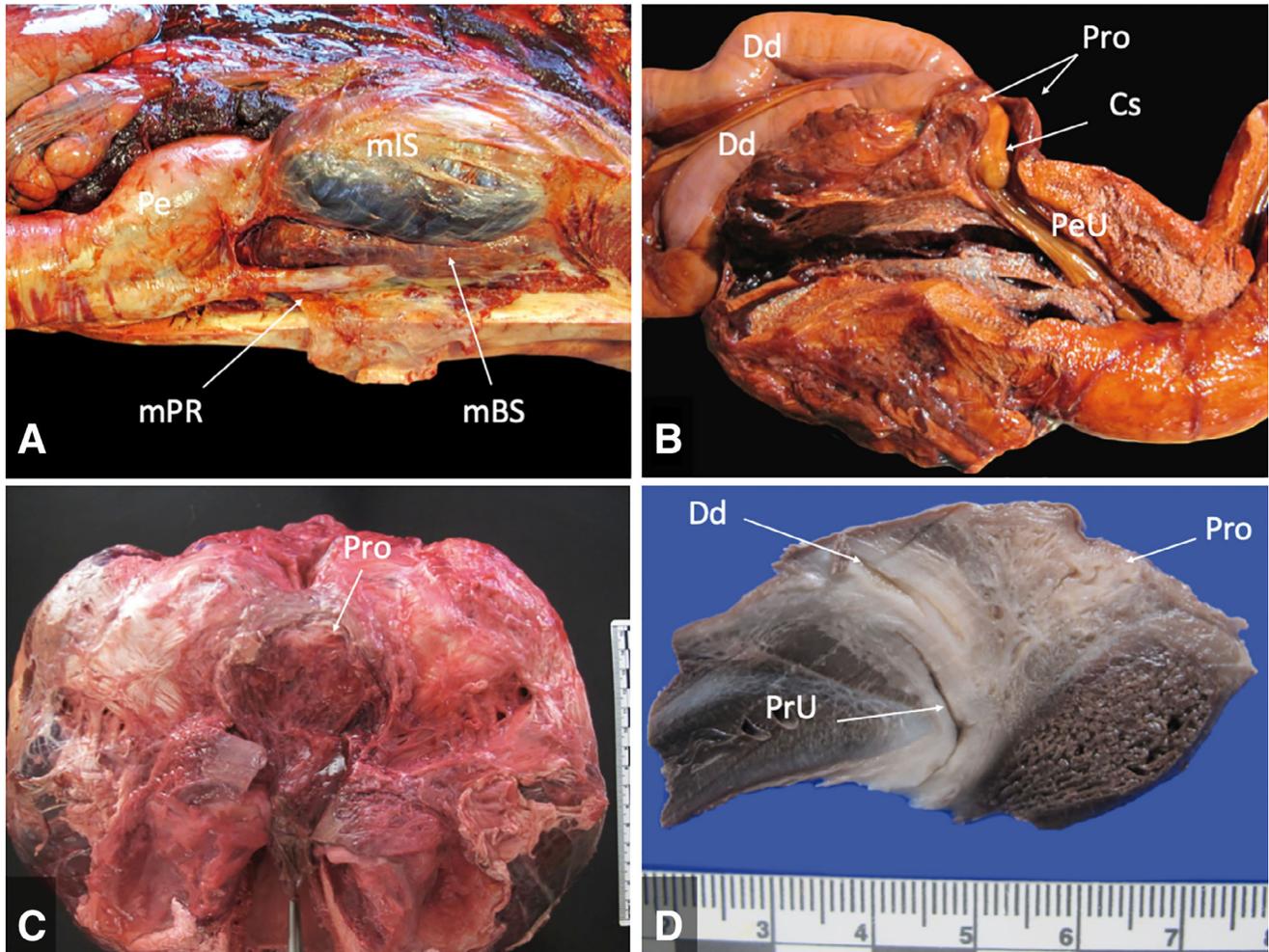


Fig. 3. The prostate of the pygmy sperm whale. (A) The penis root *in situ*. The muscles of the penis root are prominently developed; note the absence of pelvic bones (Case 11). (B) The prostate of the pygmy sperm whale is a small gland located around the Colliculus seminalis (Case 11). (C) Dorsal view of the prostate of an adult individual (Case 44). (D) Sagittal cut of the prostate of a calf (Case 47, formalin-fixed specimen). Cs, colliculus seminalis; Dd, deferent ducts; mBS, m. bulbospongiosus; mIS, m. ischiocavernosus; Pe, penis; PeU, penile urethra; PRm, penis retractor muscle; Pro, prostate; PRu, prostatic urethra.

peritoneum (*excavatio rectogenitalis*). The transversal sections of the gland showed a discrete *muscle compressor prostatae* with a laterodorsal disposition.

Prostatic development with age. Among the 47 individuals included in this study, two were categorized as neonates, 11 as calves, 11 as juvenile/subadult, and 21 individuals were adult animals (Table 1).

In the neonates and calves, the glandular parenchyma of the prostate was scanty but uniform. Furthermore, the radial distribution of the prostatic trabeculae at the *Corpus prostatae* was not observed. The gland was pinker in color and its consistency was firmer than that in adults. In the juvenile/subadults, the trabecular aspect of the *Corpus prostatae* was more evident. In addition, the glandular parenchyma was developed and whiter than in neonates. With sexual maturity, the trabeculae became more pronounced, the glandular parenchyma, the *muscle compressor prostatae*, and the penis root became fully developed, increased

in size and became further differentiated; the gland acquired a white appearance and a firm consistency.

Histology

General histology of the odontocete prostate. The prostatic glandular parenchyma was composed of seromucous acini, which were supported by a thin stroma of connective tissue and capillaries (Figure 5A). The acini were made up of two cell types: acinar secretory epithelium (ASE) and basal cells. The ASE was low to high simple cuboidal epithelium, with round, basal, euchromatic nuclei. The cytoplasm was moderate in amount and frequently vacuolated in different areas of the gland. A majority of the ASE contained numerous basophilic intracytoplasmic granules, which were more evident in the apical portion of the cytoplasm. However, a few acinar cells, often distributed at the periphery of the gland, did not contain these granules. Histochemical analysis showed that these granules were intensely PAS+ (magenta), PAS-diastase+, and Alcian blue-positive

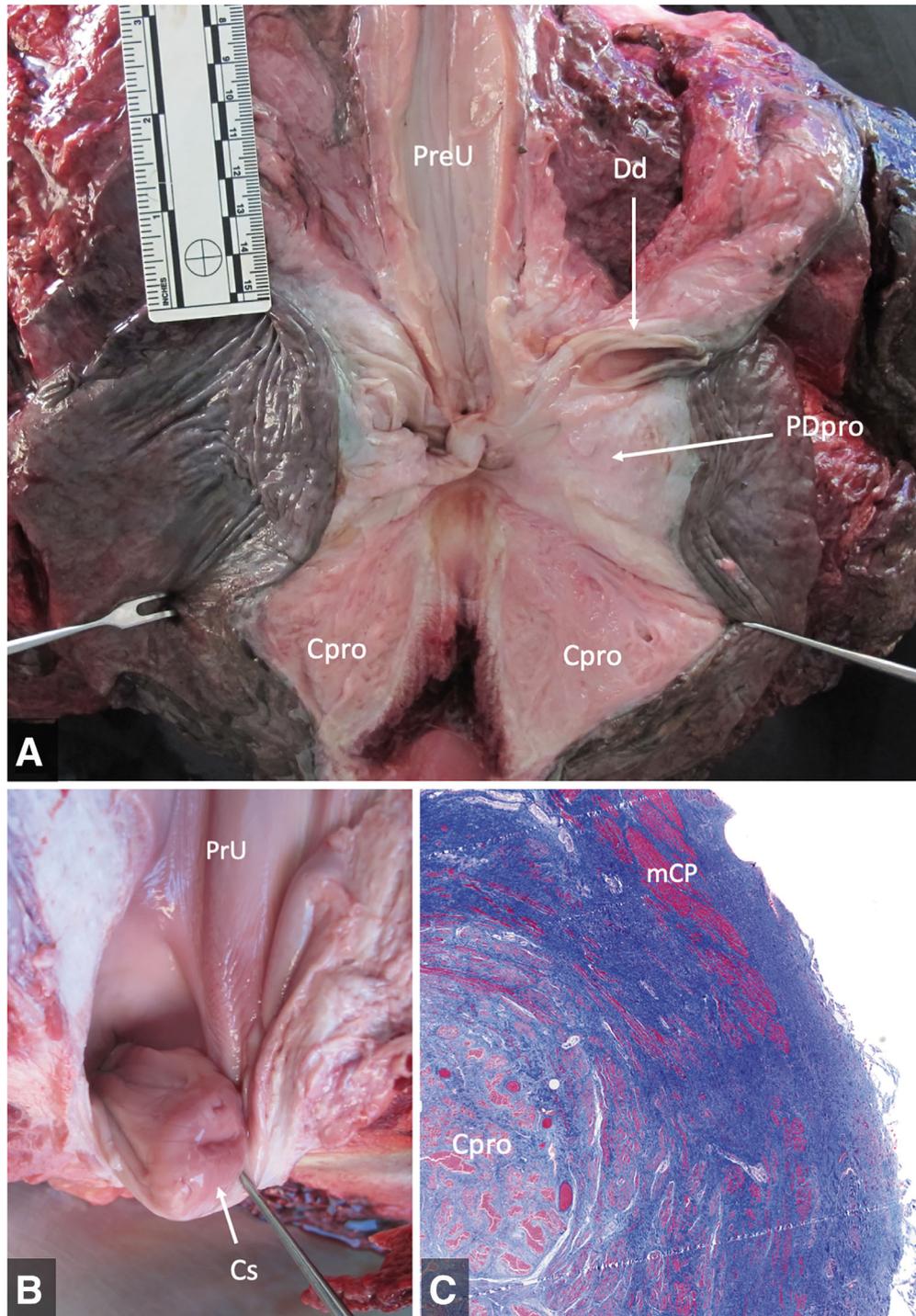


Fig. 4. The prostate of the sperm whale (Case 46). (A) The prostate is a well-developed gland with a *Corpus prostatica* and a *Pars disseminata prostatica*. (B) Detail of the Colliculus seminalis and the prostatic urethra. (C) The prostate is surrounded by a thick capsule and various layers of fibres of the *muscle compressor prostatica*. Masson's trichrome. Cpro, *Corpus prostatica*; Cs, Colliculus seminalis; Dd, deferent ducts; mCP, *muscle compressor prostatica*; PDpro, *Pars disseminata prostatica*; PreU, preprostatic urethra; PrU, prostatic urethra.

(blue) (Figure 5B–D). The basal cells, which were usually located close to the basement membrane of the acini, were characterized by oval, hyperchromatic nuclei, and scant cytoplasm. Their number varied between individuals.

Acini directly drained into the prostatic ducts (Figure 5B), that is, collector structures that drain the secretion produced by the ASE into the *ductuli prostatici* and finally into the prostatic urethra. These prostatic

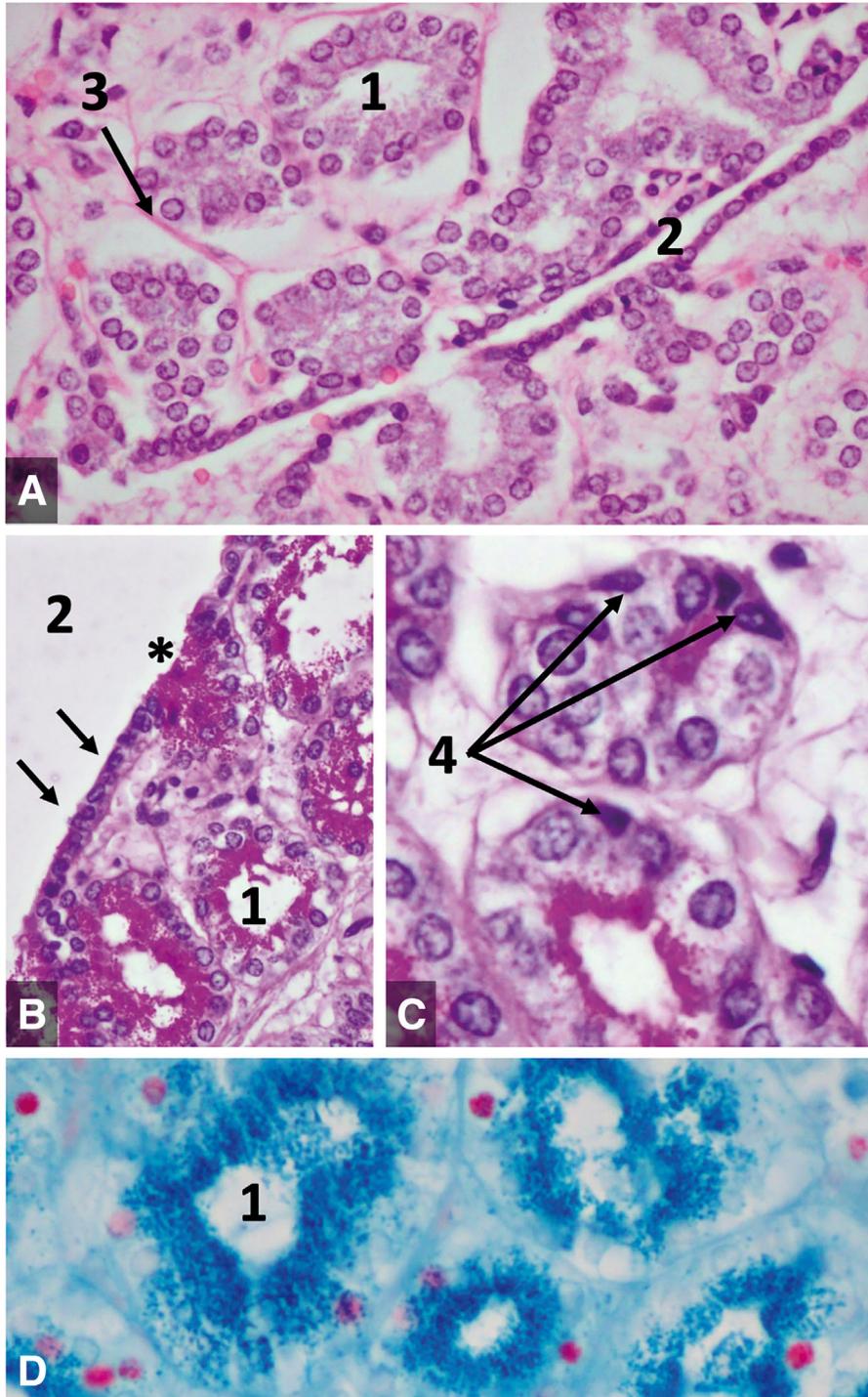


Fig. 5. Prostatic glandular parenchyma of the delphinid prostate. Bottlenose dolphin (Case 31). (A) Histological aspect of the prostatic gland; note the cytological differences between the epithelium of the acini and the prostatic ducts. H&E, 40 \times . (B) The acinar secretory epithelium (ASE) has abundant intracytoplasmic, PAS-positive granules (asterisk), which are absent in the epithelium of the prostatic ducts (arrows). PAS, 40 \times . (C) Detail of the basal cells. PAS, 60 \times . (D) The intracytoplasmic granules of the ASE positively stain with the Alcian Blue technique. Alcian Blue, 60 \times . 1 acinar secretory epithelium; 2 prostatic duct; 3 stroma; 4 basal cells

ducts were lined with simple low cuboidal epithelium cells, which had oval basal hyperchromatic nuclei and scant cytoplasm without intracytoplasmic granules. However, since the acini and prostatic ducts formed an

indivisible functional unit, the acinar epithelium was frequently observed to be intermixed with the ductal epithelium. When the prostatic ducts approached the prostatic urethra, numerous ducts converged into the *ductuli*

prostatici, and they were distinguished by transitional epithelium. The transitional epithelium of the *ductuli prostatici*, in their most proximal areas to the urethra, had an outer layer of cylindrical cells, with vacuolated cytoplasm, resembling caliciform cells.

Prostatic secretions inside the acini and prostatic ducts were frequently observed. These secretions were characterized by amorphous PAS+ material, which appeared acidophilic in the H&E staining. A few desquamated epithelial cells and detritus were often intermixed with this secretion.

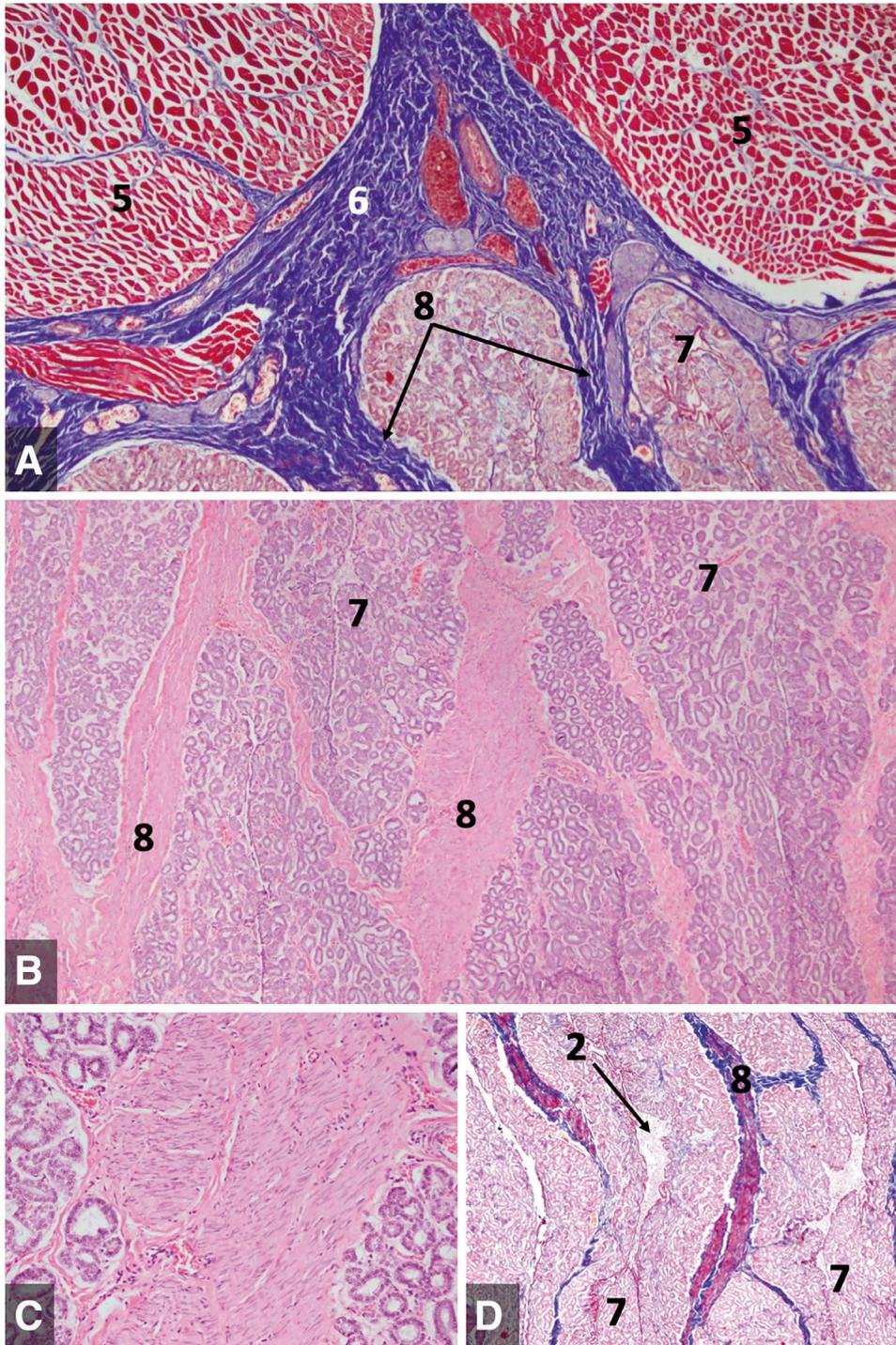


Fig. 6. Non-glandular parenchyma of the delphinid prostate. Bottlenose dolphin (Case 31). (A) Histology of the prostatic capsule. Masson's trichrome, 4x. (B) Trabeculae and prostatic lobules. H&E, 4x. (C) Detail of the smooth muscle of the trabecula. H&E, 10x. (D) With the Masson's trichrome technique, the muscle of the prostatic trabecula is stained in red. Masson's trichrome, 4x. 2 prostatic duct; 5 muscle compressor *prostatæ*; 6 prostatic capsule; 7 prostatic lobule; 8 prostatic trabecula

The trabeculae were formed by bundles of connective tissue, intermixed with smooth muscle cells that separated the acini in prostatic lobules (Figure 6A–D). They followed a radial course that originated from the connective tissue supporting the prostatic urethra and *ductuli prostatici* and ended in the prostatic capsule and frequently joined one another to form a network. This organization is possibly instrumental at the moment of ejaculation, as it causes glandular extrusion. Vessels and nerves of medium caliber were occasionally observed within the trabeculae.

The prostate was covered externally by the prostatic capsule and the *muscle compressor prostatae*. The prostatic capsule (Figure 6A) was a connective tissue envelope that completely surrounded the prostatic glandular parenchyma. Externally, the prostatic capsule joined the perimysium that separated the different fascicles of the *muscle compressor prostatae*, whereas internally it was continuous with the prostatic trabeculae. Occasionally, vessels and nerves of medium caliber were observed within the capsule. The *muscle compressor prostatae* (Figure 6A) was formed by skeletal muscle fibers, with variable orientation. This muscle totally enveloped the prostate in most cetacean species and was intimately related with the prostatic capsule.

The *ductuli prostatici* and prostatic urethra were supported by abundant interstitium, made up of dense connective tissue intersected with numerous capillaries, medium-caliber vessels, and nerves (Figure 7A).

In the *Pars disseminata prostatae* (Figure 7B) the trabeculae were scarcer than in the *Corpus prostatae*. These were composed of thin bundles of connective tissue and smooth muscle fibers, aligned longitudinally to the prostatic urethra. Thus, the organization of the lobules was not so well delineated, and acini were disposed more homogeneously.

Histology of the immature prostate. In the sexually immature examined animals, the prostate had smaller prostatic lobules with fewer glandular acini, compared with those of the adults (Figure 8A,B). With age, the acini multiplied, and the prostate became enlarged.

The neonatal prostate had some distinctive cytological characteristics. Often, the glandular acini presented with two or more layers of epithelial cells containing a large number of basal cells. The ASE had round basal euchromatic nuclei with occasional mitosis. The cytoplasm was moderate and had few intracytoplasmic granules in the H&E staining (Figure 8C). However, histochemical analysis (PAS, PAS-diastase, and Alcian blue) revealed more granules in the apical part of the acinar and ductal cells. Big vacuoles occupying the majority of the cytoplasm were often visualized in the ASE of the neonate prostate. The calves had proportionally more intracytoplasmic granules in the ASE than the neonates.

The prostatic ducts were not totally developed in the neonates. At birth, they appeared as dilations in the prostatic lobules lined with cells showing the same characteristics as acinar cells (Figure 8D). In contrast, the prostatic ducts were totally mature in calves, resembling the same cytomorphological and histochemical features as in adults. Furthermore, the *ductuli prostatici* appeared to be completely mature in both neonates and calves.

The transitional epithelium of the prostatic urethra of the neonates showed numerous intracytoplasmic PAS+ inclusions. These inclusions were round to oval in shape, coalescent, had a diameter of 1–3 μm and stained negative



Fig. 7. (A) Histological aspect of a *Ductuli prostatici*. Bottlenose dolphin (Case 31). The *Ductuli prostatici* are lined by a transitional epithelium and sustained by dense connective tissue. PAS, 4x. (B) Striped dolphin (Case 32). *Pars disseminata prostatae*. Note that acini are not organized into lobules and that prostatic trabeculae are thin and longitudinally oriented. H&E, 4x. 6 prostatic capsule; 8 prostatic trabecula; 9 *Ductuli prostatici*; 10 periurethral interstitium

for PAS-diastase and Alcian blue, suggesting glycogen accumulation. These structures were not observed in the *ductuli prostatici*. In the calves, these inclusions were absent. In addition, clusters of transitional epithelia intercalated with the normal glandular epithelium of acini and prostatic ducts were frequently observed in the prostate of neonates.

Even in the newborn animals, the prostate gland seemed productive; secretion was frequently observed in the lumen of the acini, immature ducts, and the *ductuli prostatici*, as a moderate amount of amorphous material, which was slightly eosinophilic in the H&E and positive in the PAS stain, and scattered with few cellular debris.

In general, both the prostatic capsule and the *muscle compressor prostatae* were less developed in the young animals than in the adults. With sexual maturity, the fibers of the *muscle compressor prostatae* became enlarged resulting in a substantial thickness of this muscle. The capsule and the trabeculae tended to increase in thickness and longitude.

Histology of the prostate of the pygmy sperm whale and sperm whale. The prostates of the five pygmy sperm whales were histologically evaluated and

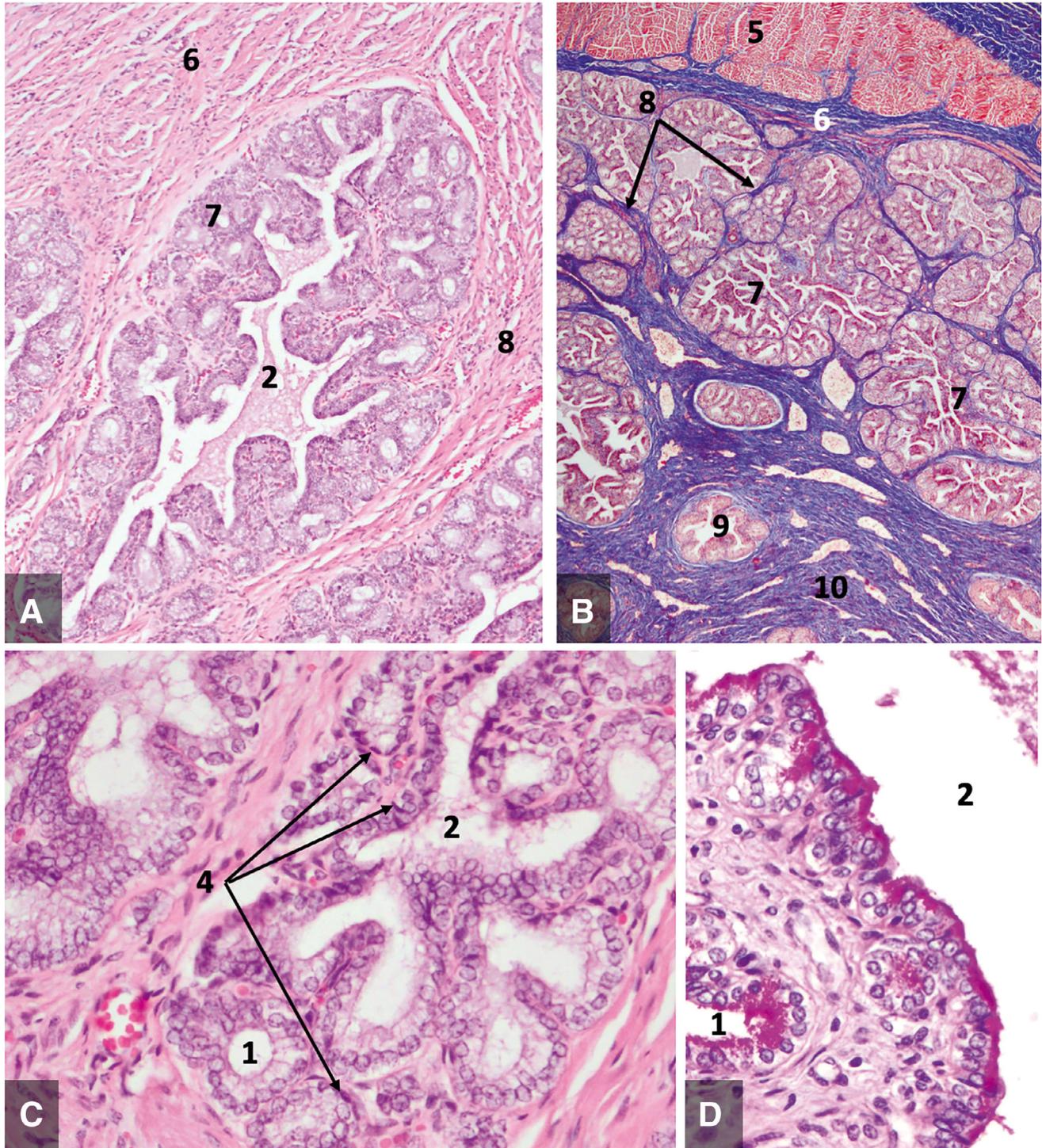


Fig. 8. Histology of the immature prostate of delphinids. Bottlenose dolphin (Case 20). (A) Prostatic lobule composed of few acini and an immature prostatic duct. (B) Architecture of the lobular part of the prostate. Masson's trichrome, 4x. (C) Detail of the acini. H&E, 40x. (D) Immature prostatic duct. Note the PAS+ reaction in the ductal epithelium. 1 acinus; 2 prostatic duct; 5 *muscle compressor prostatae*; 6 prostatic capsule; 7 prostatic lobule; 8 prostatic trabeculae; 9 *Ductuli prostatici*; 10 periurethral interstitium

they showed a number of differences compared to the prostates of the delphinids. The glandular part of the prostate was represented by seromucous acini, sustained by a moderate stroma that was slightly thicker than that in the

delphinid prostate. The secretory epithelium of acini varied morphologically, ranging from low cuboidal to columnar epithelium (Figure 9A,B). The more cylindrical acinar cells frequently displayed a vacuolated cytoplasm. Despite these

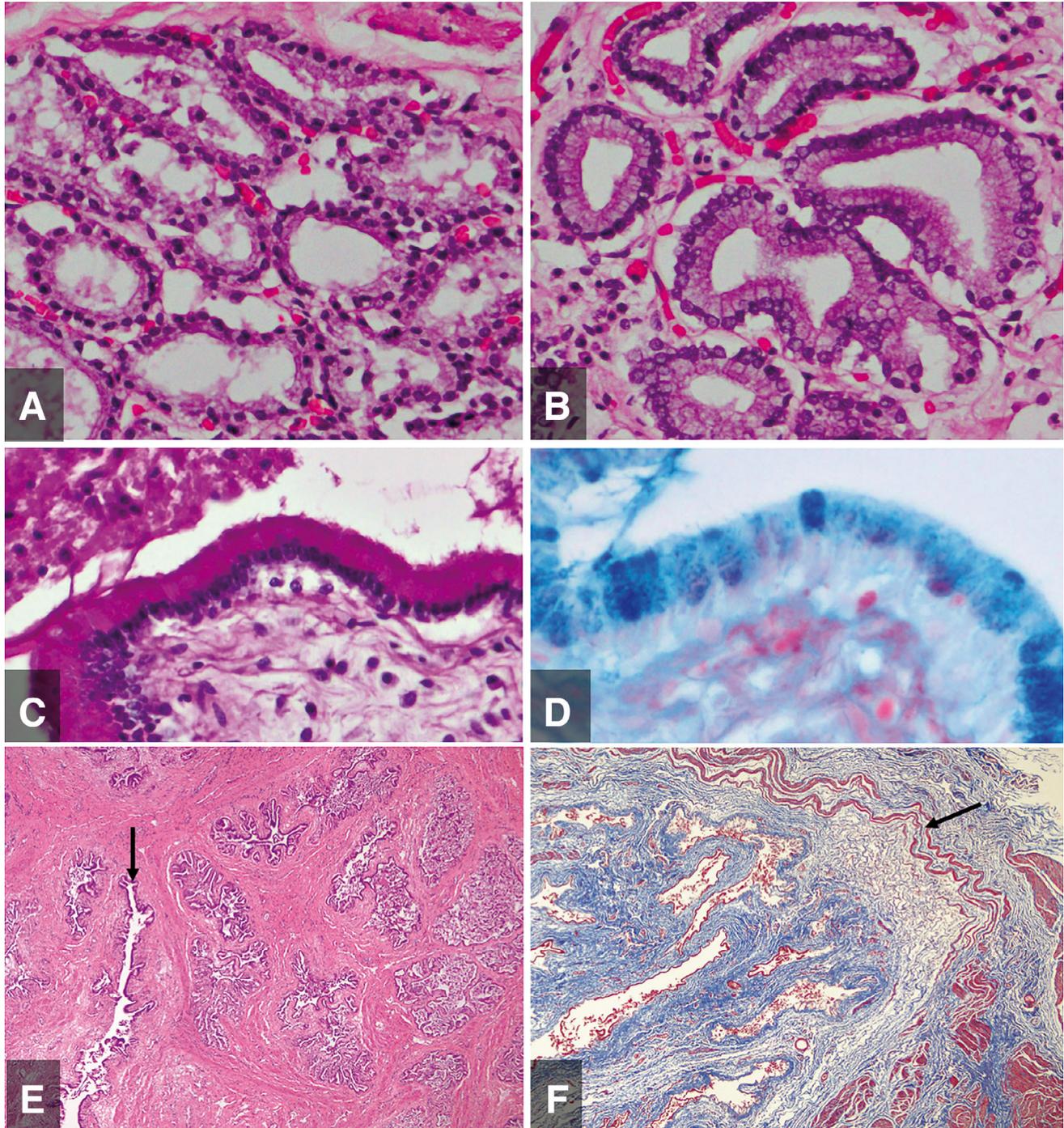


Fig. 9. Histology of the prostate of the pygmy sperm whale. (A–B) Heterogeneity of the prostatic epithelium (Case 11). PAS, 40 \times . (C) Prostatic duct with a PAS+ cylindrical epithelium (Case 11). PAS, 40 \times . (D) Same prostatic duct as in (C). Intracytoplasmic vacuolations of the ductal epithelium stain positive with Alcian blue. Alcian Blue, 60 \times . (E) *Corpus prostaticae* of a calf of pygmy sperm whale (Case 47) revealing small prostatic lobules and thick prostatic trabeculae. The arrow points to a deferent duct. H&E, 4 \times . (F) Sagittal section of the prostate of a calf (Case 47) revealing the *muscle compressor prostaticae* (arrow). Masson's trichrome, 4 \times .

differences, the cytological and histochemical characteristics of the ASE resembled those observed in the delphinids. The morphology and dimensions of the acini varied in the different regions of the gland, ranging from small circular/oval to larger and irregular acini. The prostatic ducts were

lined by a PAS+ and Alcian blue-positive columnar epithelium, differing from the delphinid prostatic ductal epithelium (Figure 9C–F). The prostatic capsule was thicker than in other species, whereas the *muscle compressor prostaticae* was represented by fewer fascicles of skeletal muscle

TABLE 2. Immunohistochemistry results for major epithelial components of the prostate of selected odontocete cetaceans

Primary antibodies	<i>Tursiops truncatus</i> and <i>Stenella coeruleoalba</i>			<i>Tursiops truncatus</i> (neonate)			<i>Kogia breviceps</i>		
	ASE	PDE	Uro	ASE	PDE	Uro	ASE	PDE	Uro
CK5+8	+++	+++	+++	++	+++	+++	-	-	NT
CK7	-	++	+++	-	-	-	-	-	NT
CK8+18	-	-	-	-	-	-	-	-	NT
CK20	-	-	-	-	-	-	+	+	NT
Vimentin	-	-	-	-	-	-	-	-	NT
PSA	-	-	-	-	-	-	-	-	NT

ASE, acinar secretory epithelium; CK, cytokeratin; NT, not tested; PDE, prostatic duct epithelium; PSA, prostatic specific antigen; Uro, urothelium; +, weak positiveness; ++, moderate positiveness; +++, strong positiveness; -, negative.

intermixed in between the collagen bundles of the prostatic capsule. The prostatic trabeculae had a variable orientation and thickness, which resulted in multiple dimensions and arrangements of the prostatic lobules. Small lobules were frequently observed next to larger ones. The smooth muscle of the trabeculae was less developed in this species, which, together with the less developed *muscle compressor*

prostaticae, could suggest that the extrusion of the gland in the pygmy sperm whale may have less importance compared to delphinids. In the immature prostate, the prostatic lobules were smaller, with fewer acini, and they resulted in relatively thicker prostatic trabeculae (Figure 9E,F).

The prostate of the analyzed individual of the sperm whale was moderately autolytic, and details of the

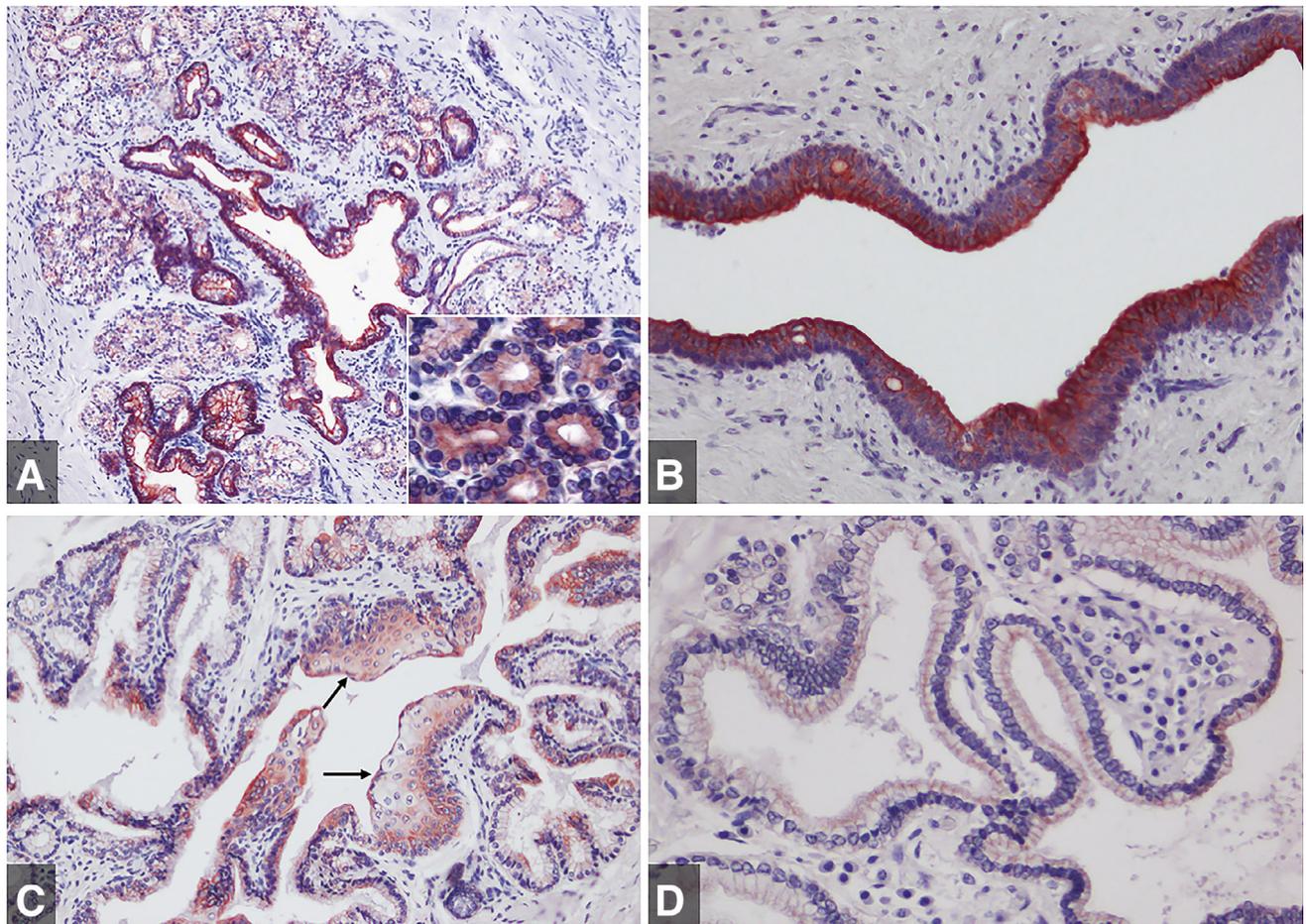


Fig. 10. Cytokeratin (CK) immunostaining of the prostate. (A) Striped dolphin, (Case 8). Strong intracytoplasmic and membranous immunostaining for CK5. IHC CK5 + 8, 4x. Inset: 60x. (B) Striped dolphin (Case 8). The transitional epithelium displays strong intracytoplasmic immunostaining for CK7. IHC CK7, 40x. (C) Bottlenose dolphin, (Case 20). Inclusions of transitional epithelium in the immature prostate of a neonate (arrows). IHC CK5 + 8, 10x. (D) Pygmy sperm whale (Case 11). The prostatic acinar epithelium shows weak membranous immunostaining for CK7. IHC CK7, 40x.

prostatic epithelium were lacking. However, similarities with the prostate of delphinids were observed, such as a lobular architecture as confirmed by the presence of thick prostatic trabeculae. Externally, the gland was surrounded by fascicles of the *muscle cromptressor prostatae* that, similar to that in the pygmy sperm whale, was interspersed with capsular collagen bundles.

Immunohistochemistry and ultrastructure

For the immunohistochemical analysis, various sections of the prostate from very fresh individuals were tested: an adult and a juvenile/subadult of striped dolphin (Case 8 and Case 17, respectively), a neonate and an adult of bottlenose dolphin (Case 20 and Case 31, respectively), and an adult of pygmy sperm whale (Case 11). The results of this immunohistochemical analysis are summarized in Table 2. In all tested delphinids, strong membranous and cytoplasmic immunolabeling for CK5 + 8 was observed for the ASE, the epithelium of the prostatic ducts, the urothelium of the *ductuli prostatici*, and the transitional epithelium of the prostatic urethra, in all delphinids tested. In the neonate animals, the ASE showed a more moderate labeling for CK5 + 8 than in the adult animals (Figure 10A–C). In contrast, each of the epithelial components of the prostate of the pygmy sperm whale was labeled

with this antibody. For CK7, immunolabeling was detected in the urothelium of the prostatic urethra and *ductuli prostatici* of the juvenile/subadult and adult delphinids, but not in the delphinid neonate or the pygmy sperm whale. Labeling for CK8 + 18 and CK20 was consistently negative in all cases, except in the pygmy sperm whale prostate, in which a weak membranous positive reaction for CK20 was observed in the ASE and prostatic duct epithelium (Figure 10D). Using anti-vimentin primary antibody, we detected strong cytoplasmic labeling (positive internal control) in the vascular endothelium, the tunica media of vessels, and the connective tissue in all cases; however, any prostatic epithelium displayed positivity to vimentin in all cases. The prostate of all tested individuals exhibited a negative immunostaining for the anti-PSA antibody.

The ultrastructural study was performed on the prostate of an adult bottlenose dolphin (Case 31). Electron microscopy analysis revealed numerous electrodense and circular structures (0.5–1.5 μm of diameter) in the cytoplasm of the ASE, which consisted of secretory granules (Figure 11A). These epithelial cells contained few mitochondria that were more concentrated at the basal parts. The nucleus of the secretory cells displayed dispersed heterochromatin that was frequently confined to the lateral border and the nucleoli were occasionally evident. The lateral borders of the cellular membrane of the ASE were interdigitated and folded with the adjacent cells. Basal cells closely related to the basement membrane were observed; they had scant cytoplasm with no secretory granules and the nucleus contained more concentrated chromatin than that in the ASE (Figure 11A and inset of B). The ductal epithelium was characterized by cells with moderate cytoplasm, no secretory granules, an oval to polygonal nucleus, and a few organelles (Figure 11B).

DISCUSSION

In this work, we have analyzed the morphology and histology of the prostate in nine different species of odontocete cetaceans, including members of the families Delphinidae, Physteridae, and Ziphiidae. In addition, immunohistochemical and ultrastructural analyses of the prostate of selected animals were performed to better characterize the cytological aspects of this organ.

Literature data on the prostate of cetaceans are limited to a series of studies published in the second half of the last century (Matthews, 1950; Harrison et al., 1972; Yablokov et al., 1974; De Guise et al., 1994). In these studies, the topography of the gland was defined. In addition, the presence of the *muscle cromptressor prostatae* as well as the division of the organ into a lobular part and a diffuse part were described. Our results are in accordance with these observations. Based on the Nomina Anatomica Veterinaria, we have used the terms *Corpus prostatae* and *Pars disseminata prostatae* to refer to the lobular and diffuse parts, respectively. Matthews, in 1950, described the prostate of the Atlantic spotted dolphin (*Stenella frontalis*), and Harrison et al. (1972) later published various observations about the prostate of the bottlenose dolphin. On the other hand, De Guise et al. (1994) observed only a *Pars disseminata prostatae* in beluga whales. The anatomies of the prostate of the pygmy sperm whale, Cuvier's beaked whale and the sperm whale have not been previously reported in the literature. The prostate

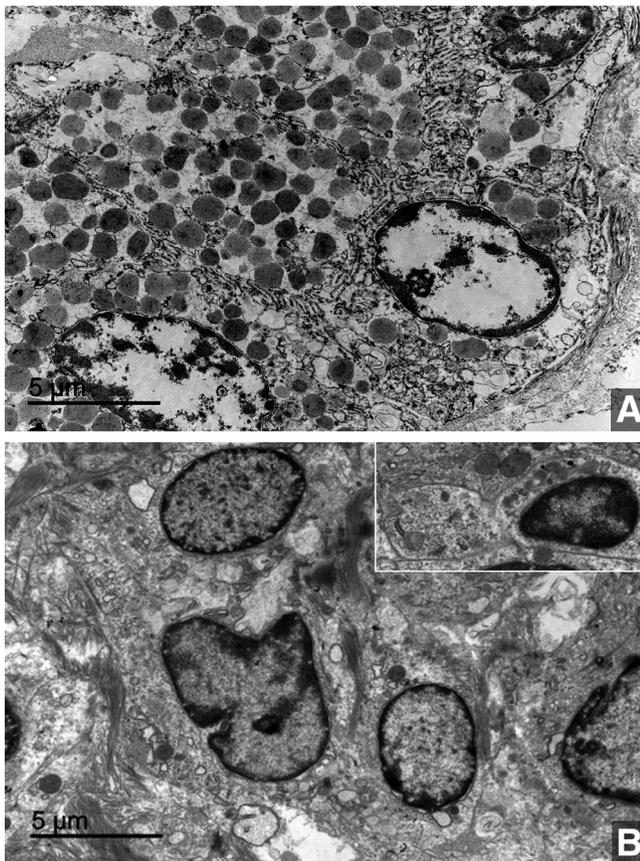


Fig. 11. Ultrastructure of the prostate of a *bottlenose dolphin* (Case 31). (A) Acinar secretory epithelium with numerous secretory granules. On top of the image, there is a basal cell. (B) The prostatic duct epithelium lacks secretory granules. Inset: Detail of a basal cell.

anatomy of the delphinids has been briefly compiled by Cozzi et al. (2017) in their book “*Anatomy of Dolphins*.”

One of the singularities of the odontocete prostate is that, unlike in other mammals (Nickel et al., 1979), it is not divided into a left and a right lobe, but instead is a single gland with a round to oval shape. Carnivores only have a *Corpus prostatae* but no *Pars disseminata*, whereas ruminants, which are phylogenetically closer to cetaceans (Spaulding et al., 2009), only have a *Pars disseminata prostatae*, which surrounds the urethra, before the prominent bulbourethralis glands (Nickel et al., 1979). Both portions, the *Pars disseminata prostatae* and the *Corpus prostatae*, were observed in all cetacean species studied. Another marked adaptation of the cetacean prostate was the development of the *muscle cromptressor prostatae*, especially in dolphins, whereas it was much less developed in Ziphiids and Kogiids. Cozzi et al. (2017) referred to the *muscle cromptressor prostatae* as *m. bulbocavernosus*. In other species, it has been described as *substantia muscularis* since it consists of scattered fibers of smooth muscle around the gland (Nickel et al., 1979; Bacha and Bacha, 2001). However, in cetaceans, this muscle is composed of skeletal fibers, and due to this difference, together with the grade of development, we have opted to maintain the term *muscle cromptressor prostatae*, which was used for the first time by Matthews (1950). The supposed function of this muscle is to compress the prostate during the ejaculation (Matthews, 1950; Harrison et al., 1972; Yablokov et al., 1974); however, given the fact that this muscle is better developed in the proximal part of the gland, near the bladder's neck, it probably also has a function in avoiding the retrograde flush of the semen to the bladder. The *muscle cromptressor prostatae* is a unique adaptation of the cetacean prostate that has not been reported in any other marine mammals such as pinnipeds (Colegrove et al., 2009).

Comparatively, we found that the prostate of the Atlantic spotted dolphins was well developed, while those of other delphinids, such as the bottlenose dolphin and the striped dolphin, were relatively small. These data agree with the observations of De Smet (1977) and Matthews (1950), who described the prostate of the spotted dolphin as large and well developed, and that of the bottlenose dolphin as a small organ. The prostates of the pygmy sperm whale and Cuvier's beaked whale were smaller than the delphinid prostate. These differences in the development of the prostate could be related to different ancestors of the families Kogiidae and Ziphiidae with respect to Delphinidae (Spaulding et al., 2009; Berta et al., 2015), or to different reproductive strategies. For example, Kogiids tend to live solitary or in pairs, although small groups of 6–10 individuals have occasionally been observed, whereas delphinids usually live in large groups of even hundreds of individuals (Yamada, 1954; Cadwell and Cadwell, 1989). The males of pygmy sperm whales are characterized by a well-developed reproductive system, including testicles, epididymis, deferent ducts, and a penis of large dimensions (Yamada, 1954). However, their prostates were relatively small in relation to the other reproductive structures. Additionally, we observed that the deferent ducts in the adults of this species are histologically lined by a secretory epithelium (unpublished data). We hypothesize that, in this species, other tissues, such as the deferent ducts, may contribute to seminal fluid production to a larger extent than the prostatic gland. The sperm whale together with the kogiids (i.e., the pygmy sperm whale) are members of the family

Physeteridae (Cadwell and Cadwell, 1989). However, in the sperm whale, the prostate is a well-developed gland with a conspicuous *Corpus prostatae*, that is, it is more similar to that observed in delphinids than to that seen in their closest relatives from the genus Kogiidae.

We additionally described the changes in the organ occurring with age and showed how the prostate became larger and more productive with the manifestation of sexual maturity. Bryden (1988) confirmed that this organ was small in immature cetaceans but large and active in the adults, whereas Harrison et al. (1972) reported that the delphinid prostate was completely developed by the age of 18–24 months. Our observations were in accordance with these findings.

Histologically, the prostates of the different species of odontocetes were highly similar, although that of the pygmy sperm whales showed architectural and cytological differences. Matthews (1950), who were the first to describe the histology of the prostate in dolphins, reported that the glandular acini of this organ were lined by polygonal epithelium, whereas other authors defined this epithelium as cylindrical. According to our observations, the ASE in delphinids ranged from high cuboidal to cylindrical. However, the morphology of the ASE may depend on seasonal variation and reproductive status as also is the case with other animal species (Abou-Elhamd et al., 2013; Chaves et al., 2015). Domestic animals exhibit a large variation in the cytomorphology of the prostatic acinar epithelial cells (Bacha and Bacha, 2001). In most animal species, the prostate is a seromucous gland; however, in dogs, this gland is only serous. In boars, calciform cells are frequently found in the prostate, and chromophobic cells (which are not stained by usual histochemical staining) are occasionally observed within the acini (Nicaise et al., 1991). In ruminants and boars, the ASE displayed a positive reaction to PAS, as we also observed in odontocetes. In many species, including humans and dogs, the epithelium of the prostatic ducts shares the same cytological characteristics with the ASE, both of which are referred to as luminal epithelium (Bacha and Bacha, 2001; Clapp and Croker, 2012). The prostatic luminal epithelium of humans comprises cylindrical cells with a large vacuolated and clear cytoplasm (Clapp and Croker, 2012), whereas in the dog this epithelium has a large acidophilic cytoplasm. In contrast to what we reported in cetaceans, no intracytoplasmic granules are histologically observed in human or canine prostatic luminal cells. In our study, we also observed marked cytological differences between acinar epithelium and ductal epithelium in the odontocete prostate; therefore, we opted to differentiate between them as ASE and ductal epithelium. The human prostate is divided into different parts: a peripheral zone, a transitional zone, and a central zone, with minimal histological differences (Clapp and Croker, 2012). In this study, we clearly observed two different parts in the prostate of delphinids, that is, a *Pars disseminata* and a *Corpus prostatae*. In addition, the *Corpus prostatae* of delphinids contained an area in which the *ductuli prostatici* adopted a horizontal disposition, with a caudocranial orientation. This area was particularly visible in a transversal cut of the proximal region of the *Corpus prostatae*. We suggest naming this particular region of the delphinid's gland the ventral *ductuli prostatici* zone (the encircled zone of Figure 2D).

Immunohistochemical analysis of the prostate has been extensively studied in humans, dogs, and rats. Some differences in cytokeratin expression have been reported

among these species (Sherwood et al., 1991; Hayward et al., 1996; Grieco et al., 2003). In men, dogs, and rats, luminal cells express CK8 and CK18. In humans and rats, basal cells express CK14 and CK5, whereas in dogs, only CK5 expression has been observed. Vimentin expression has been observed in both luminal and basal cells in the human prostate but not in canine prostate (Sherwood et al., 1991). In our study, we demonstrated CK5 expression in the ASE, ductal epithelium, and transitional epithelium of urethra and *ductuli prostatici* of the prostate of bottlenose dolphin and striped dolphin, as well as CK7 expression in the urothelium of the prostatic urethra and the *ductuli prostatici* of these species. Basal cells did not display CK5, CK7, CK8, CK18, or CK20. However, differences were observed in the pygmy sperm whale, in which only the expression of CK20 was detected in both the ASE and ductal epithelium. Vimentin expression was consistently negative in all epithelial components of the prostate of all tested individuals. In the rat, CK expression changes with the development of the prostate, and in neonates, some CK are absent, although they are normally expressed in adult rats (Hayward et al., 1996). This evolution also appears to be present in our study because the neonate bottlenose dolphin, unlike the adult animals, did not express CK7 in its prostatic urothelium.

The ultrastructure of the prostate of the bottlenose dolphin displayed a number of differences compared with that of other mammals. In humans and other primates, the luminal cells of the prostate showed several electron-lucent secretory vesicles (El-Alfy et al., 2000), whereas in dogs, as in bottlenose dolphins, the vesicles are electron-dense. These changes in the ultrastructural component of the gland may reflect changes in the secretion of the gland in the different species.

In humans, the prostate accounts for about 20%–30% of the total ejaculate volume and the prostatic fluid is composed of spermine, citric acid, cholesterol, phospholipids, fibrinolysin, fibrinogenase, zinc acid, phosphatase, and prostate-specific protein (PSA) (Lawrentschuk and Perera, 2016). In other species, the prostatic contribution to the seminal fluids is variable and prostatic fluid composition is yet to be completely determined (Gobello et al., 2002). PSA has a major role in the physiology of human reproduction in that it produces the liquefaction of the semen and as such promotes seminal fluidity (Clapp and Croker, 2012). In dogs, rodents, and ungulates, the prostatic epithelium does not produce PSA; however, there are other closely related prostatic specific kallikreins whose functions seem to be similar to that of PSA (Gobello et al., 2002; Olsson et al., 2004; Oliveira et al., 2016). Despite structural differences, prostatic kallikreins in dogs (canine arginine esterase) have been shown to display cross-reactions with anti-PSA antibodies in normal and cancerous prostatic tissue due to antigenic similarities (Lai et al., 2008). In the present work, PSA was not labeled in the prostatic tissue of odontocete cetaceans. However, the possible presence of prostatic kallikrein in cetaceans needs to be further investigated. The histochemical characteristics of the ASE in the odontocete prostate point to a rich mucin production of the gland, which in turn may favor fertilization by producing the attachment of the seminal fluid near the cervix during copulation, or by plugging the entrance to the cervix and thus preventing ulterior insemination by another male. Further investigations into the composition of the prostatic fluid and seminal physiology in cetaceans are needed to

better understand the reproduction of this globally endangered animals.

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