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Development of the Goose Tongue Filiform Papillae: Could it be Tooth-Like Sense Organs?

Desarrollo de las Papilas Filiformes de la Lengua de Ganso: ¿Podrían ser Órganos Sensoriales Parecidos a los Dientes?

Stéphane Louryan[1]; Myriam Choa-Duterre[1]; Gabriel Destoop[1]; Marie Lejong[1,2]; Caroline Matte-Allain[3] & Nathalie Vanmuylder[1]

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SUMMARY: The geese's tongue filiform papillae are particularly long, and exhibit the same morphology of a tooth, evoking the lingual teeth of several fishes. In adult animals, they contain numerous mechanical Herbst's corpuscles but no taste buds. In the embryo, they appear since stage 38 and acquire their definitive shape between stages 38 and 42. They express several proteins associated with mammalian tooth development (BMP4, β-catenin, SHH, PITX2, PAX9), also known to be linked to parrot's pseudoteeth and goose's denticulations development. Neurofilaments are early present in the papillae primordia, and appear particularly numerous in adult papillae. Our results suggest that these papillae constitute a mechanical organ with a « tooth shape » derived from ancestral odontodes, whose development is controlled by numerous genes involved in classical odontogenesis.

KEY WORDS: Tongue papillae; Goose; Development; Genes; Embryo.

INTRODUCTION

Bird tongue papillae are classified as filiform and do not contain taste buds. They can be found in some species in the posterior, pharyngeal part of the tongue, without any papilla associated (Lindemaier & Kase, 1959; Bradley, 1971).

Geese papillae appear to be particularly large and long; they are involved in some functions during food intake and are called « mechanical papillae » (Skieresz-Szewczyk *et al*., 2012; Skieresz-Szewczyk & Jackowiak, 2017).

Some of these papillae have a « tooth appearance ». However, geese exhibit numerous denticulations on their beak, suggesting the possibility of common functions. Furthermore, several fishes, as the salmon trout, possess tongue teeth (Berkovitz, 1978; Berkovitz & Shellis, 2017). It could thus be interesting to correlate the anatomy and development of the goose filiform papillae to those of bird denticulations and pseudoteeth, which exhibit during their development some gene expressions associated with odontogenesis (Louryan *et al*., 2021, 2023). The morphological features of papillae development in geese (Skieresz-Szewczyk *et al*., 2012; Skieresz-Szewczyk & Jackowiak, 2017) and in duck (Skieretz-Szeczyk *et al*., 2014) were described using histology and scanning electron microscopy.

The aim of this study is to re-explore the morphological development of this papilla using traditional histologic examination, to explore protein expression associated with some developmental genes involved in mammalian tooth induction, and to analyze the histologic structure of adult papillae, with special reference to the presence of mechanical corpuscles.

[1] Laboratoire d'Anatomie, Biomécanique et Organogenèse (Dir: Prof. S. Louryan), Faculté de Médecine, Université Libre de Bruxelles, 808, route de Lennik (C.P. 619), B1070 Bruxelles, Belgique.
[2] UpVet Universita Popolare, Roma, Italia.
[3] Hospital Clinico Universidad de Chile, Independencia, Chile.

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MATERIAL AND METHOD

Eggs purchased from stockbreeders were incubated at 38.5°C. Four goose embryos (stages 35, 39, 40 and 42) were removed, drawn, and photographed. Goose embryo developmental stages were determined as described by Li *et al*. (2019).

The embryos were fixed in Serra's fixative medium (Ethanol 95 %: 6 parts, formalin 40 %: 3 parts; acetic acid 10 %: 1 part), dehydrated in a graded alcohol series, and embedded in paraffin. Coronal 5-µm sections were fixed onto slides for staining and immunohistochemistry. The slides were rehydrated and stained with Masson's trichrome or Haematoxylin-Eosin. For antigen retrieval, the slides were placed in citrate buffer and microwaved for 20 min. Sections were washed in distilled water for 5 min, then in 0.01 M phosphate-buffered saline (PBS) at pH 6 containing 0.1 % Triton for 5 min to permeabilize the cytoplasmic membrane, and then washed three times for 5 min in PBS. Endogenous peroxidase activity was blocked with 0.3 % H_2O_2 in methanol before the sections were washed three times for 5 min in PBS and incubated for 1 h in an immunohistochemistry blocking reagent (product number 20773, Sigma-Aldrich, Darmstadt, Germany). Slides were incubated overnight in a humidified chamber with one of the following antibodies: rabbit anti-PITX2 (1/200 in PBS; Biorbyt Ltd., Cambridge, UK), rabbit anti-SHH (1/500; Abcam, Cambridge, UK), goat anti-b-catenin (75/1000; Bio-Techne Ltd., Abingdon, UK), , rabbit anti-BMP4 (1/50; MyBioSource, San Diego, CA, USA), or rabbit anti-PAX9 (1/250; LSBio, Seattle, WA, USA). The tissue sections were washed and incubated with biotinylated goat anti-rabbit IgG (product 21537, Sigma-Aldrich, Darmstadt, Germany) or bovine anti-goat IgG (Jackson Immunoresearch, Cambridge, UK) for 10 min. After washing three times for 5 min in PBS, the sections were incubated for 10 min with streptavidin (product 20774, Millipore, Darmstadt, Germany). Diaminobenzidine staining was performed using the Highdef DAB kit (Enzo Life Sciences, Farmingdale, NY, USA). For neurofilaments staining, sections were incubated with anti-PGP9.5 (1/200; GeneTex-Bio-connect, TE Huissen, Nl) or rabbit polyclonal antiserum to neurofilament M (150 kDa; primate) (1/1000, Biomol, Hamburg, Germany, counterstained by Haematoxylin-Eosin) and anti-and biotinylated goat anti-rabbit IgG (product 21537, Sigma-Aldrich, Darmstadt, Germany). The procedure was the same as that used for previously described immunostaining procedures. Negative control experiments were performed by omitting the primary antibodies and suppress and provided in the supplementary material section. In the positive control experiments, primary antibodies were incubated with tissues known to express the corresponding proteins as described previously by Louryan *et al*. (2021).

According to European and Belgian law, the sampling of "*non-free living larval forms*", which include pre-viable embryos, does not require ethical committee advice and thus, no permission was required for this study.

Adult geese (*Anser anser*) were obtained from a slaughterhouse. The tongues were divided into 4 different areas (Fig. 1) following the shape and the locations of the papillae. The specimens were fixed in 4 % formalin buffered with PBS for 24 to 48 h, depending on their size.

The tongue specimens were sectioned in coronal and sagittal planes and then stained with classical histological technique (Masson's trichrome).

RESULTS

Morphological features. In the adult tongue, giant filiform papillae are present on the lateral margin of the tongue. Numerous similar keratinized papillae surround the laryngeal vestibule in the pharyngeal part of the tongue. We divided these locations into several areas (Fig. 1).

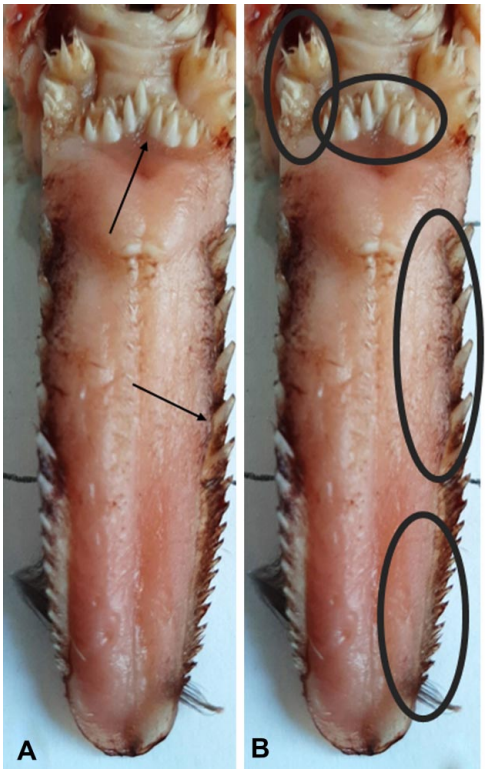

Fig. 1. Upper view of a adult goose's tongue. The long filiform papilla are shown (arrows) along the lateral aspect of the tongue, and around the laryngeal aperture (A). In B, the different locations of papillae are defined by circles.

These papillae are composed of an epithelial layer, divided into basal, intermediate, and apical sub-layers, that contains keratin and surrounds a dermal layer (Fig. 2). The epidermal layer is filled with numerous sensory tubules, containing essentially Herbst's corpuscles and less frequent Grandry's corpuscles (Fig. 3). These tubules are more numerous in the posterior papillae and were intensively stained by anti-neurofilaments antibodies (Fig. 4).

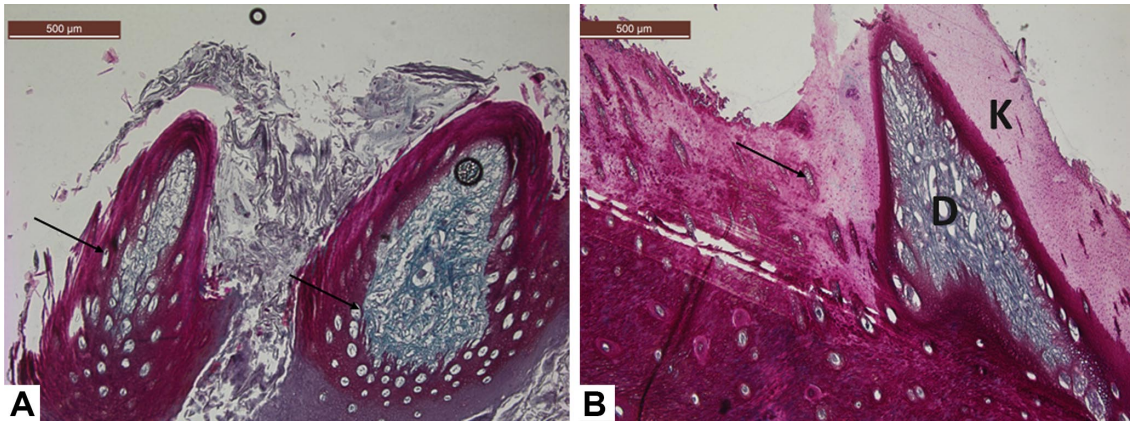

Fig. 2. Adult histologic sections of papillae (Masson's trichrome). Arrows indicate sensory tubule present in the peripheral part of the papillae. D : dermal content of papilla, K : keratinized part.

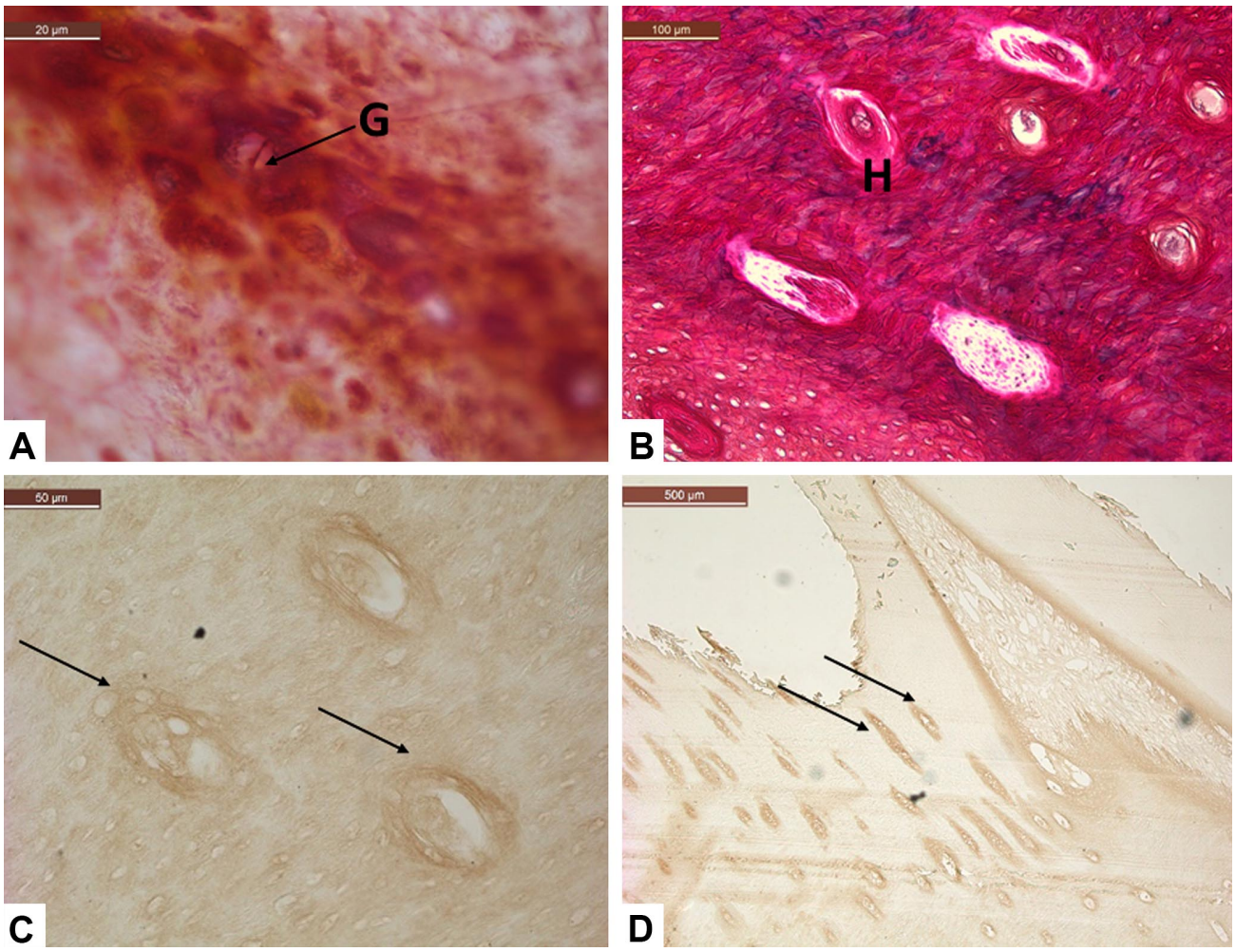

Fig. 3. Adult histologic sections of papillae. A and B : Masson's trichrome. C and D : anti-neurofilament staining. The numerous Herbst's corpuscles (H) (arrows on C) and the sensory tubules (arrows on D) are intensively stained. Rare Grandry's corpuscles (G) are present.

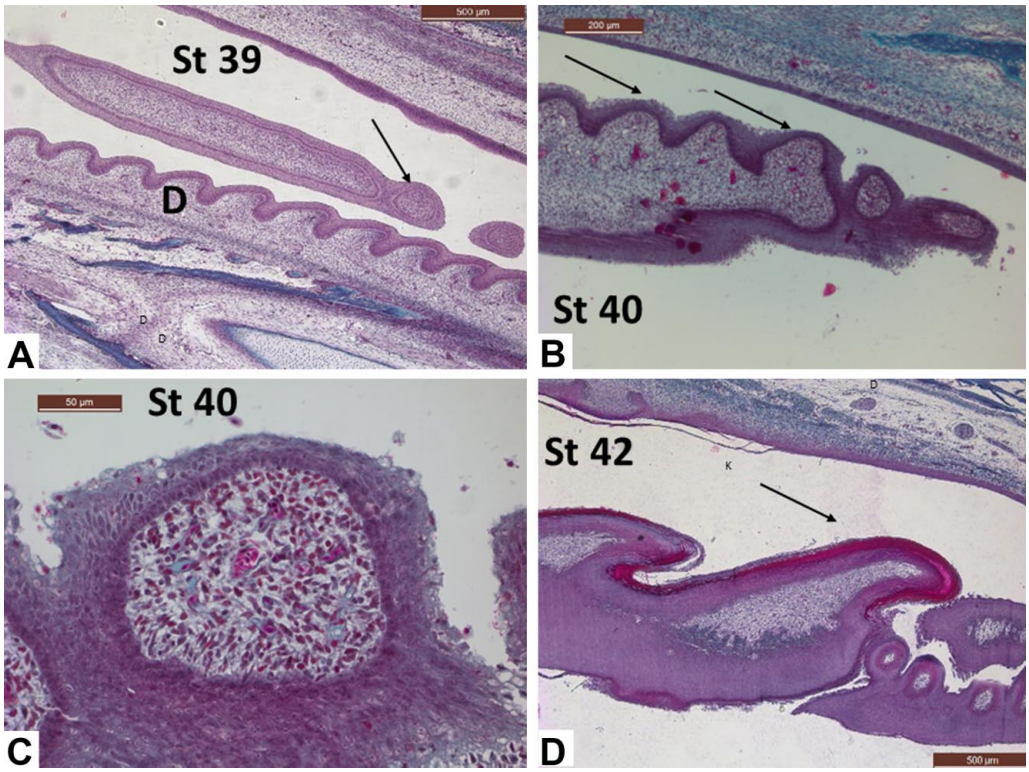

Fig. 4. successive stages of papillae development on embryonic histologic sections (Masson's trichrome). The arrows indicate the papillae on low-magnification sections. At stage 40, the basal and middle layers of the epithelial component differentiate, at stage 42, the keratinized apical part is well visible.

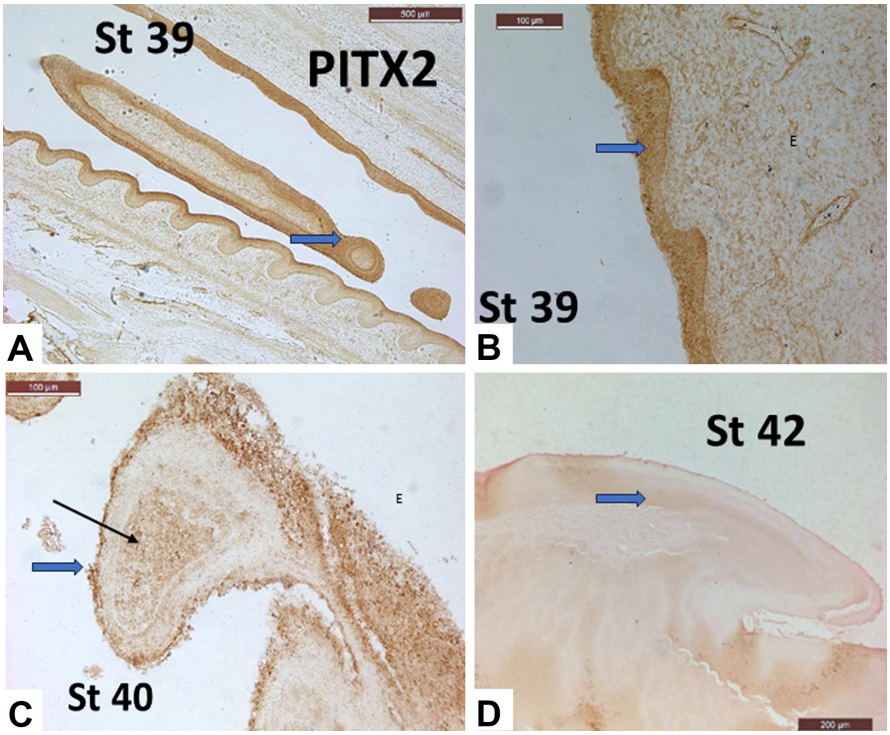

Fig. 5. Expression of PITX2 at each developmental stage. Large arrows indicate epithelial staining, and thin arrows the mesenchymal staining. As for SHH, the protein is only present in the mesenchyme at stage 40.

During the development (Fig. 3), the posterior papillae appeared at stage 39; followed by the more rostral ones at stage 40. The epithelium of these papillae divides during this stage into different layers. Between stages 40 and 42, the papillae elongate progressively and acquire their horny covering. Despite their different locations, all papillae exhibited the same development features.

Immunohistochemistry. The labelings are summarized in Table I and appear in Figures 5 to 10.

We observed that every protein was present both in epithelium and mesenchyme from a time-specific manner, except for β-catenin, whose expression was limited to the epithelium at every stage (Fig. 8). Mesenchymal expression of SHH is transient, limited to stage 40, in which the papilla was growing to acquire their definitive shape at stage 42.

Table I. Summary of labeling patters during papillae development.

	Stage 39	Stage 40	Stage 42
PIXT2			
epithelium	+	+	-
mesenchyme	-	+	-
PAX9			
epithelium	-	-	+
mesenchyme	-	+	-
BMP4			
epithelium	+	+	+
mesenchyme	-	+	+
β-catenin			
epithelium	+	+	+
mesenchyme	-	-	-
SHH			
epithelium	+	+	+
mesenchyme	-	+	-
PGP and NFM			
epithelium	+	+	+
mesenchyme	+	+	-

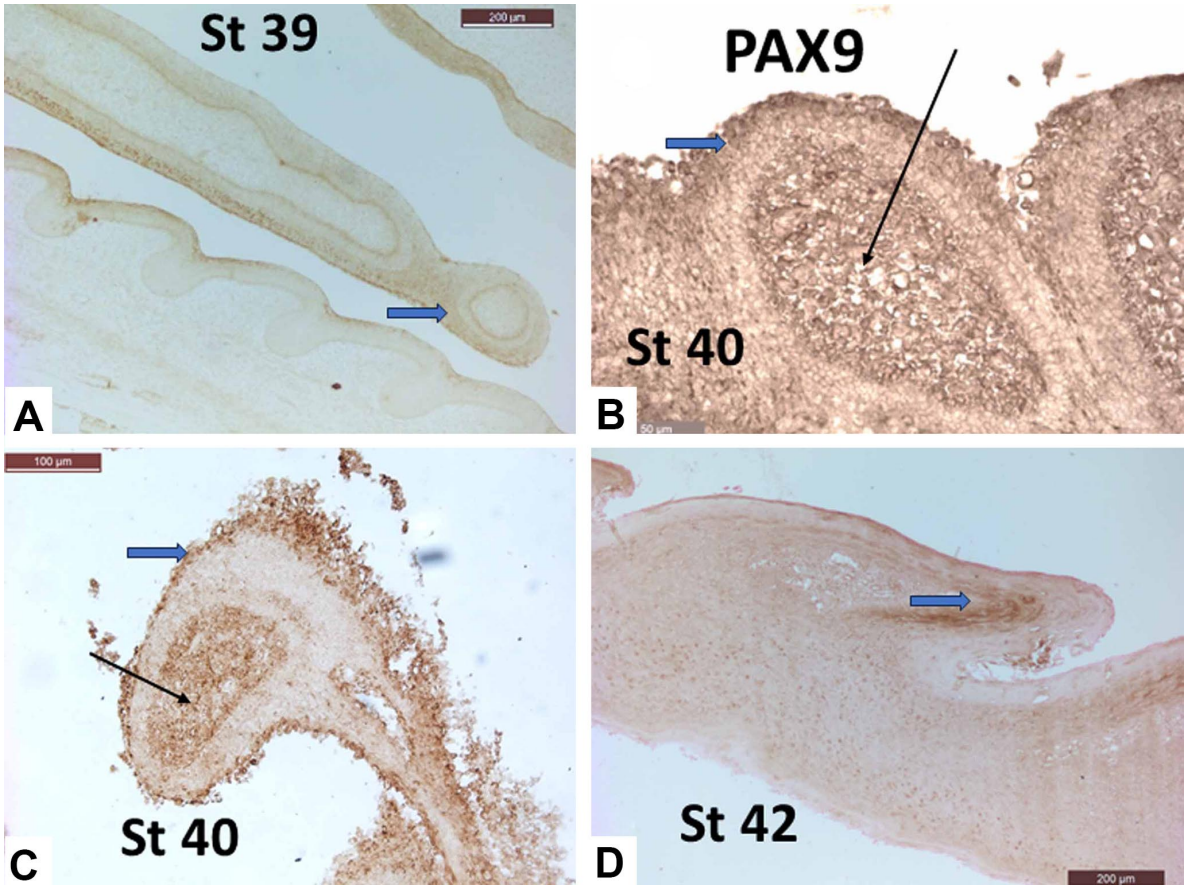

Fig. 6. Expression of PAX9. Its expression is initially restricted to the epithelial layer (A, large arrow), becomes ubiquitary at stage 40 (B), and is later restricted to the basal layer of the epithelium (C). Large arrows indicate epithelial staining and thin arrows the mesenchymal staining.

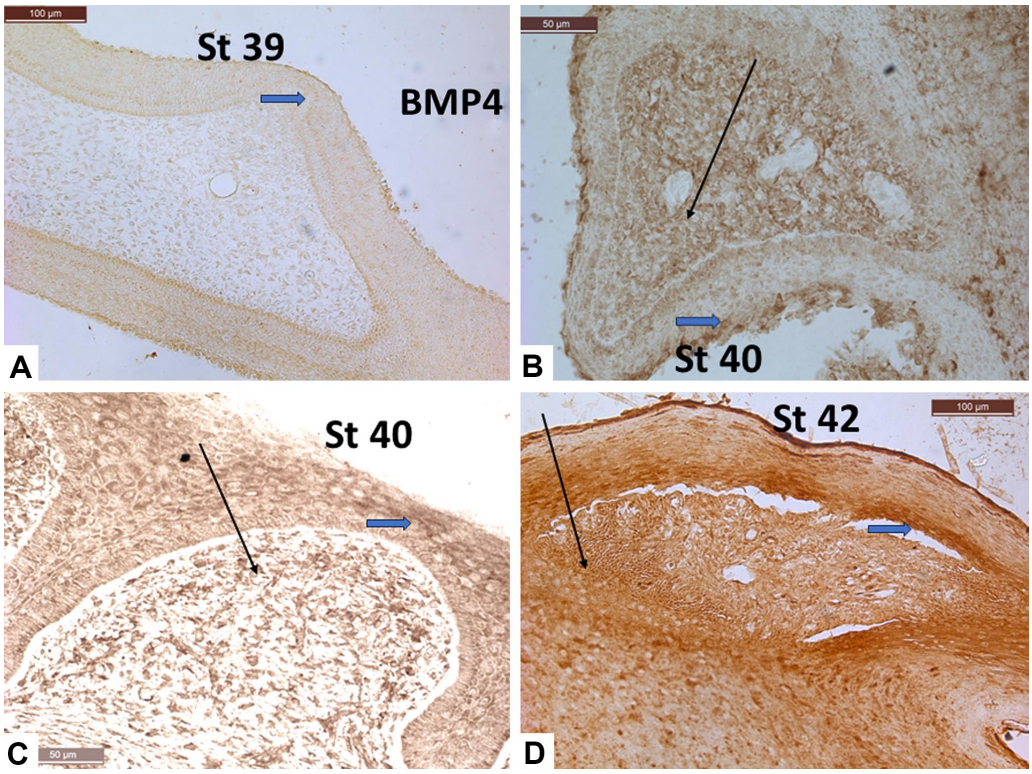

Fig. 7. Expression of BMP4. Initially epithelial (A), the labeling extends later to the mesenchyme (B and C), and keeps present in the basal layer of the epithelium (C). Large arrows indicate epithelial staining and thin arrows the mesenchymal staining.

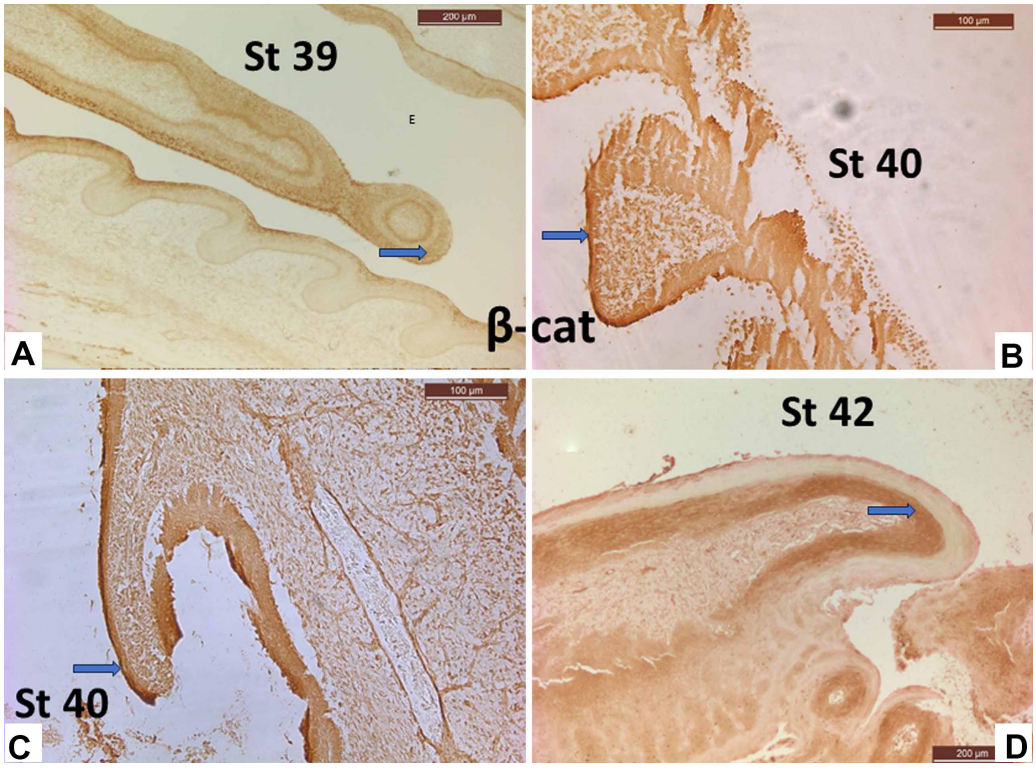

Fig. 8. Expression of b-catenin during papillae formation. The labeling concerns only the epithelial layer (large arrows).

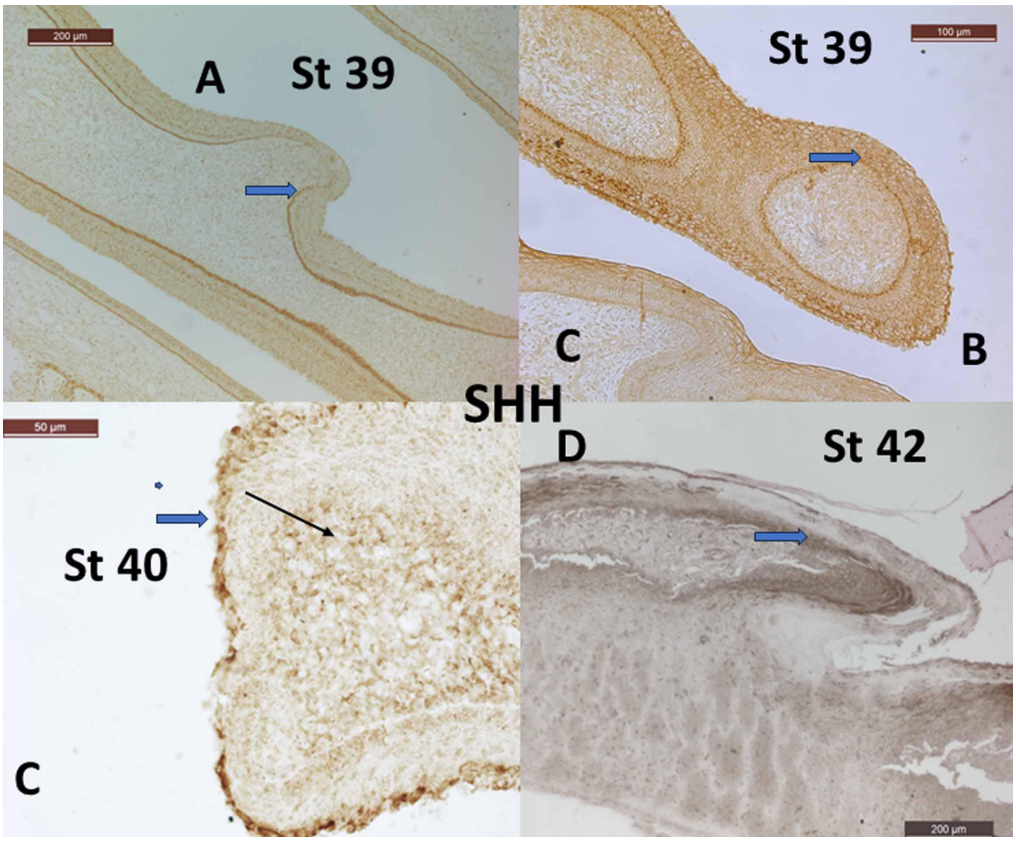

Fig. 9. Expression of SHH at each stage. Large arrows indicate epithelial staining and thin arrows the mesenchymal staining, limited to stage 40.

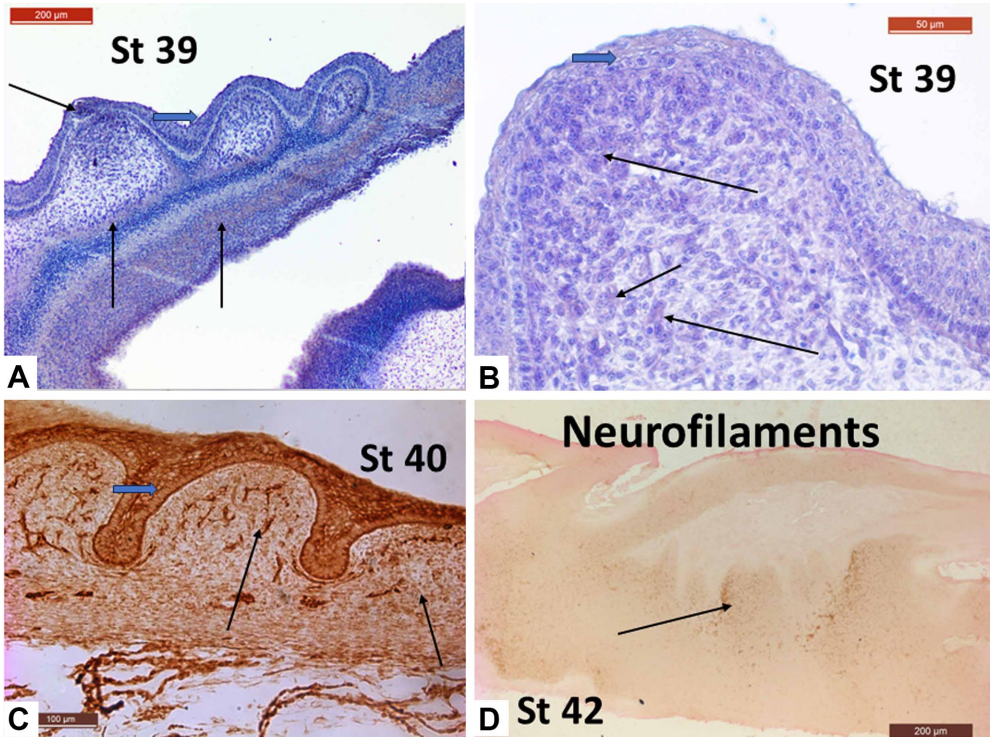

Fig. 10. Presence of neurofilament proteins during papillae development. Sections A and B were counterstained by H.E. At the initial stages, primordia of fibers appear in the mesenchymal layer (arrows), and some spots are visible in the epithelium at the apex of the papillae. (large arrows). A large staining is detectable at the basal floor of the tongue (inferior arrow). At stage 40, the epithelium is strongly positive (C, large arrow) and fibers are clearly visible in the mesenchyme (arrows) and keep only visible at stage 42.

DISCUSSION

The morphologic development features are similar to those described by Skieresz-Szewczyk *et al*. (2012) and Skieresz-Szewczyk & Jackowiak (2017). Despite several differences, gene expressions are very similar to our previous observations in geese denticulations and bill tip organ, with a more intense expression of some markers such as BMP4, PITX2, PAX9, and SHH in the mesenchyme (Louryan *et al*., 2023). However, no specific protein expression was observed prior to the first apparition of papillae. These results could also be compared to similar data obtained during the parrot's pseudoteeth development (Louryan *et al*., 2021). These genes are known to be highly involved during mammalian tooth development (Peters & Balling, 1999). In mammals, BMPs, Shh, and Wnt (closely associated with β-catenin) express also during taste papilla development (Iwatsuki *et al*., 2007; Kawasaki *et al*., 2012).

Morphology and immunohistochemistry suggest, both together, a deep homology between keratinized tongue's papillae and birds' pseudoteeth and denticulations, reinforced by several similarities between papillae development and fish tongue teeth ontogeny (Berkowitz, 1978; Meunier *et al*., 2013).

The general morphology of tongue papillae, pseudoteeth and denticulations evokes odontodes, the ancestral unique rudiment of teeth, scales and feathers. Parrot's pseudoteeth, scales and feathers express some common genes involved in epithelial-mesenchymal interactions (Louryan *et al*., 2021), and tongue papillae are classically considered as odontode derivatives (Dhouailly, 2009). Furthermore, the histological morphology of the embryonic papilla is very similar to the fetal keratinized teeth of cyclostomes (Weichert, 1970).

We have also previously shown that bird bill tip organ, pseudoteeth, and denticulations contained a lot of mechanical tubules, associated with Herbst and Grandry corpuscules, suggesting a sensory role (Louryan *et al*., 2023). The Herbst corpuscles correspond to the mammalian Pacini corpuscle, while Herbst is the equivalent of the Meissner one, with similar functions. The present results, including the early presence of neurofilaments in the papillae anlagen, support the hypothesis of a common touch sensory function of the geese's beak and tongue appendages already called mechanical papilla (Skieresz-Szewczyk & Jackowiak, 2017). The presence of neurofilaments in the terminal axons of corpuscles is consistent with the observations of Watanabe *et al.* (1985), in the duck's palate beek. The numerous tubules present in the papilla as retrait well in denticulations and parrot pseudoteeth, filled by numerous Herbst's corpuscles, are similar to those observed in the duck bill tip organ (Avilova, *et al*., 2018) and clearly refer to a tactile function (Ziolkowski *et al*., 2022). However, we observed relative lack of Grandry's corpuscles, which are classically associated with velocity and low-frequency vibration perception (Gottschaldt & Lausmann, 1974; Berkhoudt, 1980; Ziolkowski *et al*., 2022). This difference can support the hypothesis that the geese tongue could be less involved in the analysis of velocity and vibrations than the beak.

These results highly suggest complementary studies about bird beak and tongue appendages development.

LOURYAN, S.; CHOA-DUTERRE, M.; DESTOOP, G.; LEJONG, M.; MATTE-ALLAIN, C. & VANMUYLDER, N. Desarrollo de las papilas filiformes de la lengua de ganso: ¿Podrían ser órganos sensoriales parecidos a los dientes? *Int. J. Morphol., 41(6)*:1631-1639, 2023.

RESUMEN: Las papilas filiformes de la lengua de los gansos son particularmente largas y exhiben la morfología de un diente, evocando los dientes linguales presentes en varios peces. En los animales adultos, contienen numerosos corpúsculos de Herbst mecánicos, aunque una ausencia de papilas gustativas. En el embrión, aparecen a partir del estadio 38 y adquieren su forma definitiva entre los estadios 38 y 42. Expresan varias proteínas asociadas al desarrollo dentario de los mamíferos (BMP4, β-catenina, SHH, PITX2, PAX9), también conocidas por estar asociadas al desarrollo de pseudodientes en el loro y denticulaciones en el ganso. Los neurofilamentos están presentes tempranamente en los primordios de las papilas y aparecen particularmente numerosos en las papilas adultas. Nuestros resultados sugieren que estas papilas constituyen un órgano mecánico con «forma de diente» derivado de odontoides ancestrales, cuyo desarrollo está controlado por numerosos genes implicados en la odontogénesis clásica.

PALABRAS CLAVE: Papilas de la lengua; Ganso; Desarrollo; Genes; Embrión.

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Corresponding author:
Stéphane Louryan
Laboratoire d'Anatomie
Biomécanique et Organogenèse
Faculté de Médecine
Université Libre de Bruxelles, 808
route de Lennik (C.P. 619)
B1070 Bruxelles
BELGIQUE

E-mail: Stephane.louryan@ulb.be