


# Labial and buccal minor salivary glands of the dog – location, three-dimensional arrangement and histology

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## Abstract

**Objective:** Transplantation of minor salivary glands (MSGs) to the conjunctiva is a treatment option for patients suffering from dry eye disease. As there is not enough information about labial and buccal MSGs in dogs, the aim of this study was to provide evidence of the presence of these glands and to investigate their spatial arrangement and excretory ducts.

**Methods:** The oral mucosa of the lower lip of 4 dogs and the whole lower jaw of 1 dog were used for histological and microCT analysis. Presence, number, volumes and the tissue depth of MSGs were assessed.

**Results:** Histological analysis showed that compact tubulo-acinar glands were located in the submucosal connective tissue. MicroCT images revealed that 9 to 21 MSGs were arranged in a single row at the level of the dental alveolae. The volume of the MSGs increased from rostral to caudal and the total volume of glandular tissue per animal ranged from 35.01 mm<sup>3</sup> to 549.43 mm<sup>3</sup>. The mean tissue depth of MSGs ranged from 0.57 mm to 1.37 mm (upper surface of glands) and between 1.43 mm and 3.09 mm (lower surface of the glands). Excretory ducts left the dorsal part of the glands and ran in dorso-rostral direction.

**Conclusions:** The location, number and volume of the labial and buccal MSGs in the dog could be detected and described using microCT scans and histology. The present results can provide valuable information for future transplantation of labial MSGs as therapeutic measure against keratoconjunctivitis sicca.

## KEYWORDS

3D reconstruction, autotransplantation, canine, dry eye disease, keratoconjunctivitis sicca, microCT

## 1 | INTRODUCTION

Keratoconjunctivitis sicca (KCS) in dogs has been thoroughly studied and described elsewhere.<sup>1–11</sup> Many causes have been established and they may be grouped into two major

categories, primary KCS, which is thought to be a complex immune mediated disease and secondary KCS, which may be due to numerous causes.<sup>12,13</sup> Independent of the etiology, KCS can lead to keratitis and ulceration and may result in the loss of the eye and vision in severe cases.<sup>5,14</sup>

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Different treatment modalities are available including tear stimulants (ie, ciclosporin, tacrolimus, pimecrolimus) and tear supplements (ie, topical humectants, mucinomimetics and/or lipid containing substances used to support the ocular surface).<sup>12</sup> Surgical therapy has been developed with parotid duct transposition leading to mitigation of the symptoms.<sup>15,16</sup> Recently, the transplantation of oral mucosa containing minor salivary glands (MSGs) was described with favorable results in humans<sup>17–19</sup> and in one study in dogs.<sup>20</sup>

However, the presence or absence of MSGs in labial and buccal mucosa is described controversially in recent literature. Cherry et al. investigated the oral mucosa in dogs and could not detect MSGs or other secreting cells in the examined locations.<sup>21</sup> Hence, the authors claimed that MSGs could not be associated with alleviation of canine KCS symptoms following labial mucosa transplantation.<sup>21</sup> In contrast to these findings, some general anatomy and histology textbooks describe the presence of MSGs in different species including dogs in a variety of locations in the submucosa of the oral cavity.<sup>22,23</sup>

The aim of this study was to present further detailed evidence of the presence, localization, arrangement and histology of labial and buccal MSGs and their excretory ducts in the dog, with the goal to help inform MSG transplantation surgery in patients with KCS resistant to other therapeutic interventions.

## 2 | MATERIAL & METHODS

### 2.1 | Tissue samples

The oral mucosa of the lower lip of four dogs (dogs 1–4) and the whole lower jaw including the mandible and full thickness lip of one dog (dog 5) were used for histological and microscopic x-ray computed tomography (microCT) analysis (Table 1). The dogs were euthanized for reasons unrelated to this study and showed no obvious, grossly visible lesions in the oral cavity. Samples from dog 1 were taken immediately after euthanasia. The entire body of dogs 2–4 were kept frozen at  $-20^{\circ}\text{C}$  and dog 5 was kept formalin-fixed between euthanasia and sample collection. Paraffin wax blocks containing oral mucosal tissue of the lower lip spanning from the canine to the third molar tooth were prepared by carefully removing the skin and separating

the oral mucosa at the mucogingival border. Afterward, the tissue was cut in 3–5 pieces (depending on the size of the dog) to fit the size of the embedding cassette (Figure 1A,B). Samples were fixed in 4% buffered formalin for 48 h and embedded in paraffin.

### 2.2 | Histology

Paraffin wax blocks of the right lip were used for histological evaluation. Serial sections of 4  $\mu\text{m}$  in thickness were stained with hematoxylin and eosin (H&E) and analyzed for the histological appearance of the glandular tissue as well as the location of MSGs in relation to the rest of the lip. Special attention was paid to the presence and the course of the excretory ducts. In addition, periodic acid-Schiff staining (PAS) and Alcian blue staining (pH 2.5) were performed to evaluate the composition of glandular secretion, as previously described by Romeis.<sup>24</sup>

### 2.3 | MicroCT imaging of formalin-fixed and paraffin-embedded (FFPE) tissue samples

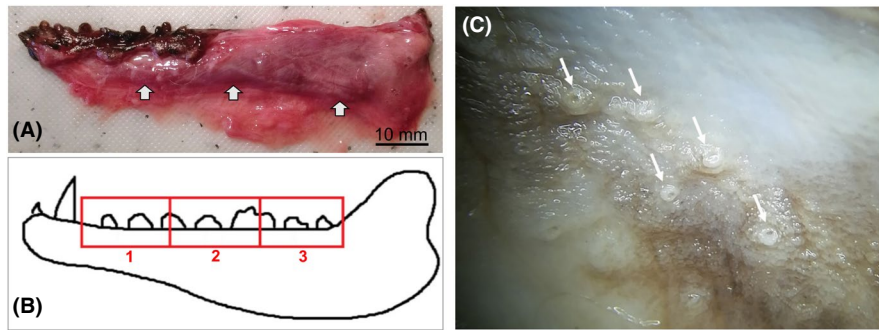
Paraffin wax blocks containing tissue samples from the left lip were mounted for microCT scanning by gluing the block carrier to the sample stage adapter using adhesive tape. MicroCT scans were acquired with an XRadia MicroXCT-400 (Carl Zeiss X-Ray Microscopy) using the 0.4X detector assembly. Projection images were recorded at 40 kVp voltage and 200  $\mu\text{A}$  current with 13 s exposure time per projection and an angular increment of  $0.225^{\circ}$  between projections over a  $360^{\circ}$  sample rotation. Tomographic slices were reconstructed with the XMReconstructor software supplied with the scanner. Isotropic voxel size in the reconstructed volumes was 17.51  $\mu\text{m}$ . Reconstructed volumes were exported in \*.TXM format.

### 2.4 | MicroCT imaging of a contrast-enhanced lower jaw

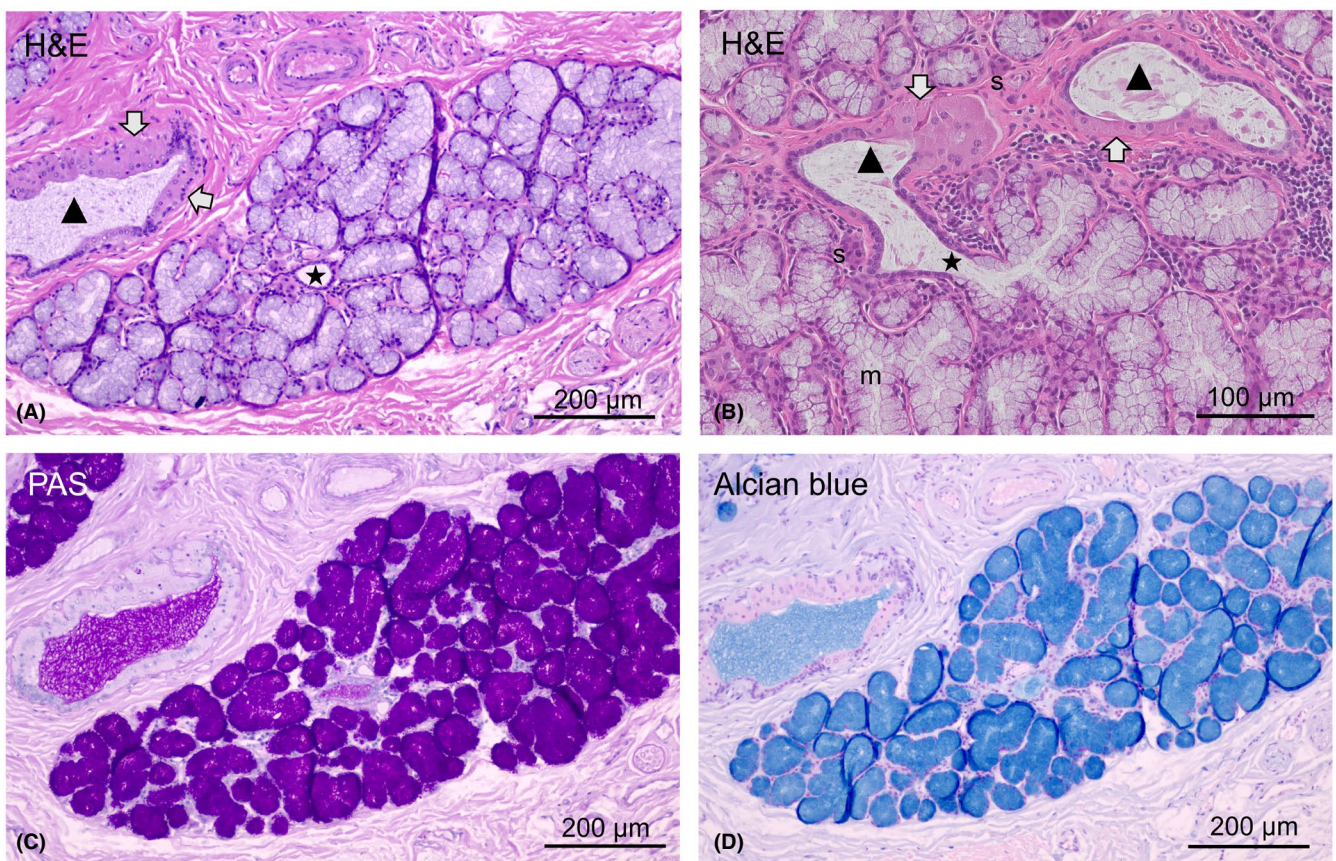
In order to image MSGs in their in situ tissue context next to the mandible, the complete lower jaw of dog 5 was fixed

TABLE 1 Dogs used in this study

Dog ID	Breed	Age (years)	Sex
Dog 1	Maltese	11	Female spayed
Dog 2	American Staffordshire Terrier	14	Male
Dog 3	Poodle	Unknown	Female
Dog 4	Miniature Pinscher	14	Female spayed
Dog 5	Braque mixed breed	6	Male



**FIGURE 1** Oral mucosal tissue of the left lower lip from the canine to the third molar tooth with the skin carefully removed. Larger glands were already visible macroscopically (arrows) (A). Cutting scheme: to fit the embedding cassette tissue was cut in three pieces, resulting in three paraffin wax blocks (B). On the mucosal surface, duct openings with small papillae (arrows) were detected during sample preparation (C)



**FIGURE 2** In labial minor salivary glands of the dog, mucous (m) tubules dominated over serous (s) acini or demilunes (A, B). The excretory intralobular ducts (asterisk) were lined with cuboidal epithelium. Groups or patches of simple columnar epithelial cell with basal striation (arrows) were frequently present within small interlobular secretory ducts (triangles). The secretion is composed of PAS positive neutral and Alcian blue positive acid mucopolysaccharides (C, D)

in 4% buffered formalin for 7 days. Afterward, the two jaw halves were separated in the *symphysis mandibulae*, and both *rami mandibulae* and the skin were removed. The right lower jaw was then washed in distilled water, dehydrated to absolute ethanol, and stained for 10 days in 1% (w/v) elemental iodine in absolute ethanol.<sup>25</sup> Subsequently, the specimen

was washed in absolute ethanol, wrapped in tissue paper and mounted in a plastic container containing absolute ethanol. The contrast-enhanced lower jaw specimen was scanned again with an XRadia MicroXCT-400 using the 0.4X detector assembly. Projection images were recorded at 140 kVp voltage and 56 µA current with 4 s exposure time per

projection and an angular increment of  $0.225^\circ$  between projections over a  $360^\circ$  sample rotation. Isotropic voxel size in the reconstructed volumes was  $27.44\ \mu\text{m}$ . Since the length of the specimen exceeded the size of the detector assembly, two scans were acquired with a vertical detector offset, and later merged using the `Plugin_Stitch.dll` of the `XMController` software. The stitched volume was exported in `*.TXM` format.

## 2.5 | Image processing, image analysis and visualization

TXM files were imported into the 3D software package Amira 2019.1 (FEI SAS, Mérignac, France, part of Thermo Fisher Scientific™). Image volumes were filtered using a 3D bilateral filter followed by a 3D Gaussian filter for noise reduction. MSGs and ducts were manually segmented using the Amira segmentation editor, and visualized using combined volume and surface renderings. Number of MSGs were counted manually based on the segmented microCT datasets. Gland volumes were directly extracted from the segmentation masks using the *MaterialStatistics* tool. Distances from the mucosal surface to the upper and lower surface of glands were measured manually at five different positions per block to assess the tissue depth of MSGs.

## 3 | RESULTS

Minor salivary glands were found in varying numbers and sizes in the lower lip in all dogs investigated. Small papillae could be seen on the mucosal surface at the opening of excretory ducts during sample preparation, mounding into the oral vestibule (Figure 1C).

### 3.1 | Histology

Compact tubulo-acinar glands with lobular composition were located in the submucosal connective tissue, surrounded solely by a very delicate layer of connective tissue that separated the single glands (Figure 2A). Mucous tubules were predominant, whereas serous cells formed either demilunes at the end of tubules or rarely serous acini (Figure 2B). The composition of the secretion was dominated by PAS positive neutral and Alcian blue positive acid mucopolysaccharides (Figure 2C,D).

The excretory intralobular ducts were lined with a cuboidal epithelium resembling short intercalated ducts. Actual striated ducts were rare (Figure 2A,B). However, groups or patches of simple columnar epithelial cell with basal striation were frequently present within small interlobular secretory ducts (Figure 2A,B). Larger interlobular ducts left each gland

and opened independently on the mucosal surface. They were lined by a two-layered epithelium still containing patches of striated cells (Figure 2A) that changed to a stratified squamous epithelium shortly before their orifice at the inner surface of the lip.

MicroCT scans of the FFPE tissue blocks from the left lower lip were used for analysis because it would have been highly impractical to assess the total number of MSGs, as well as their volume, through the analysis of serial histological sections (Figure 3A).

### 3.2 | MicroCT analysis

MicroCT images of the tissue revealed that MSGs were irregularly shaped and flattened in a mesio-lateral direction. The MSGs were arranged in a single row with occasionally overlapping glands on the level of the dental alveolae of the mandible (Figure 3B,C; Figure 4). In the rostral part, the MSGs lay in the submucosal connective tissue medial to the muscular tissue, whereas they were embedded in the muscular tissue in the caudal part of the lip and the cheek (Figure 3A). The buccal MSGs took a medio-dorsal course following the local anatomical structures (Figure 4).

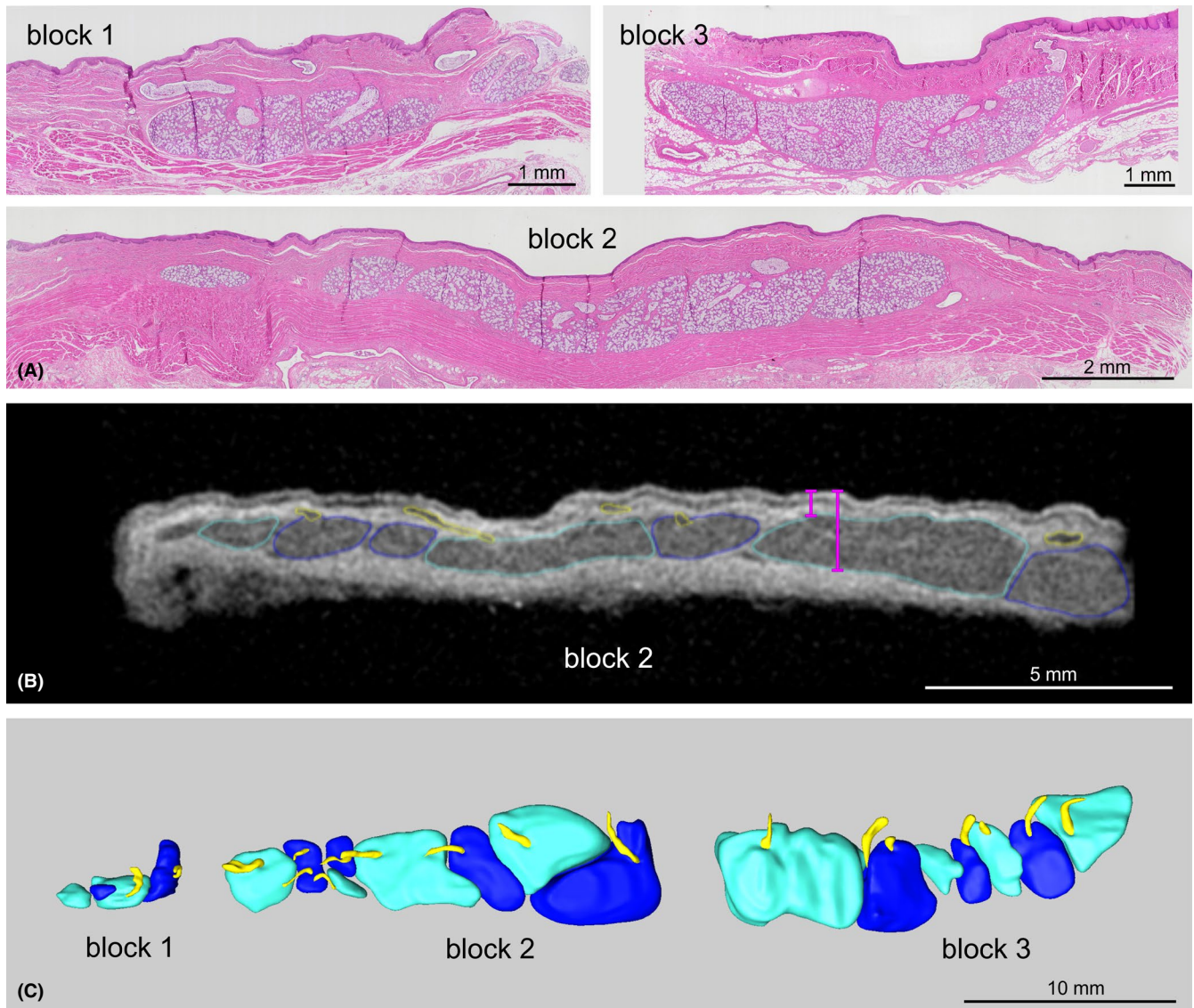
The number of labial and buccal MSGs in the left lower jaw ranged from 9 to 21 (Table 2). Minor salivary glands were either absent or only present in the caudal part of the most rostral block of every animal. Minor salivary glands started at the level the third premolar tooth in the whole jaw scan of dog 5 (Figure 4).

The volume of the MSGs increased from rostral to caudal with a mean volume per gland between  $3.89$  and  $26.40\ \text{mm}^3$  (Table 2). The total volume of glandular tissue of one side per animal ranged from  $35.01\ \text{mm}^3$  to  $549.43\ \text{mm}^3$  (Table 2). The mean distance from the mucosal surface to the upper surface of the glands (Figure 3B) was between  $0.57\ \text{mm}$  and  $1.37\ \text{mm}$  and ranged from  $1.43\ \text{mm}$  to  $3.09\ \text{mm}$  to the lower surface of the glands in FFPE specimens (Table 2).

Excretory ducts could be depicted in microCT images of the paraffin wax blocks (Figure 3B). However, it was not always possible to trace the ducts to their openings at the mucosal surface. One excretory duct could be assigned to each gland in most cases (Figure 3C). Rarely, glands with no or two excretory ducts were observed (Figure 3C). The ducts left the glandular tissue in the dorsal part and ran in dorsal or dorso-rostral direction to enter the mucosal surface sometimes at the level of the rostral neighboring gland (Figure 3A-C).

## 4 | DISCUSSION

Minor salivary glands were found in the tissue samples of the lower lip of all dogs investigated. These observations were in



**FIGURE 3** Visualization of minor salivary glands in dog 3. H&E stained histological sections of all three paraffin wax blocks (A). Note that not all glands can be seen on the respective section (eg, glands in the rostral and caudal part of block 2). Virtual section of the microCT scan of paraffin wax block 2 with manually segmented glands (turquoise and blue) and excretory ducts (yellow, B). Measurements of distances from the mucosal surface to the upper and lower surface of glands are depicted in magenta. MicroCT based 3D surface renderings of glands (turquoise and blue) and excretory ducts (yellow). For better visualization, a view directly facing on the oral mucosa surface angle was chosen (C)

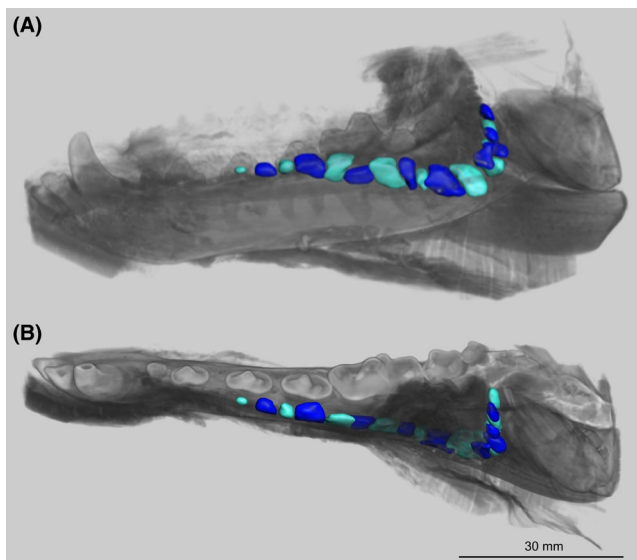
line with early anatomic findings of Ellenberger and Baum and Hartig, who described the labial and buccal salivary glands of various species including dogs in detail.<sup>22,26</sup> No glandular tissue is present in the upper lip and dorsal buccal glands are unified to form the zygomatic gland in carnivores.<sup>22,23,26</sup> Castanho et al.<sup>20</sup> transplanted labial glands into the fornices of the upper eye lid in 17 dogs with dry eye.<sup>20</sup> These authors vaguely described the anatomical site from where the graft was taken as tissue from the lower or upper lip in the vicinity of the labial commissure and dissected to the depth of the muscle layer.<sup>20</sup> Irritation with iodine was used for visualization of what Castanho et al. referred to as “labial glands”.<sup>20</sup> Cherry et al.<sup>21</sup> were not able to find glandular tissue in punch

biopsies from 5 locations of the upper and lower lip and the cheek (upper rostral labial mucosa at midline, lower rostral labial mucosa at midline, upper labial mucosa near the labial commissure, lower labial mucosa near the labial commissure, buccal mucosa approximately 1cm caudal to the commissure).<sup>21</sup> This discrepancy is most probably due to the rather superficial and random sampling technique.

The histological features of the canine MSGs resemble the description of human and rat MSGs showing prominent mucous and less serous glandular endpieces.<sup>27,28</sup> In contrast, Hartig described the MSGs in the dog as exclusively mucous glands.<sup>26</sup> Concordant with the present results, the ductal system was found to be less developed than that of

**TABLE 2** Number, volume and tissue depth of MSGs of one side of the lower jaw

	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5
Number of glands	9	21	19	13	19
Mean volume per gland (mm <sup>3</sup> ) [Min/Max]	3.89 [1.01/9.98]	18.97 [0.26/91.68]	6.74 [0.25/32.23]	7.53 [1.94/17.83]	26.40 [2.18/74.78]
Total volume of glandular tissue (mm <sup>3</sup> )	35.01	549.43	128.07	97.85	501.54
Mean distance from mucosal surface to upper surface of glands (mm) [Min/Max]	1.05 [0.87/1.29]	1.37 [0.69/2.66]	0.57 [0.25/1.03]	0.69 [0.23/1.16]	Not assessed
Mean distance from mucosal surface to lower surface of glands (mm) [Min/Max]	1.69 [1.33/2.33]	3.09 [1.87/5.07]	1.43 [0.91/2.27]	1.65 [0.93/2.20]	Not assessed



**FIGURE 4** Combined volume (complete microCT scan) and surface (segmented MSGs) renderings of the whole lower jaw and lip of dog 5 in lateral (A) and dorsal (B) view. Labial and buccal minor salivary glands (turquoise and blue) are located on the level of the dental alveolae starting at the level of the third premolar tooth

major salivary glands. Actual striated ducts were missing in human MSGs although single or groups of striated columnar cells were observed in larger intralobular ducts.<sup>27</sup> This was similar to the observations made in the present study.

The glands found in the present study were arranged in a row forming a ribbon-like string of glands parallel to the labial margin, which was consistent with previous findings.<sup>26</sup> Glandular tissue could not be detected at the level of the canine tooth in any of the dogs investigated, but it was found more caudally. These findings were in

concordance with the literature, where labial glands were described near the labial commissure.<sup>22,26</sup> The present results showed that the labial MSGs merged with the buccal MSGs without clear demarcation. Anatomical nomenclature identifies glands rostral to the labial commissure as *glandulae labiales*, those between the labial commissure and the masseter muscle positioned glands as *glandulae buccales ventrales*.<sup>13,17</sup>

The present study showed that the number of glands per dog correlated with the size of the dog, with larger dog breeds having a higher number of glands. As no brachycephalic breeds were examined, no statement concerning the impact of skull conformation on the anatomical location of MSGs can be made. Further studies on potential differences in MSGs between brachycephalic, mesocephalic, and dolichocephalic dog breeds are needed. Hartig pointed out that the number of glands varied between dog breeds, but described only 6–8 labial glands and one large ventral buccal gland with multiple ducts.<sup>26</sup> A potential reason for this discrepancy might be how authors differentiate between glands and glandular lobules. In theory, each gland should possess one excretory duct.<sup>23,27</sup> In the present study, the majority of glands showed one duct. All single glands with two excretory ducts were most likely two neighboring glands that could not be separated. Rarely, a duct could not be detected, which might have been an error due to technical reasons, as the identification of the lumen of the duct was limited by the scanning resolution that was used to identify the glandular tissue.

The volume of a single gland and the total glandular volume also appeared to be related to the body size of the dog in the present study. However, the actual glandular volume measured must be interpreted with caution as formalin-fixed tissue samples were used, which were subsequently either paraffin-embedded or contrast-enhanced. Fixation and paraffin embedding was found to result in tissue shrinkage up to 33%.<sup>29</sup> Therefore, surgeons considering

gland transplantation would need to take this possible size difference into account when planning a surgical approach. The innervation of MSGs was not subject of the present study. However, Geerling et al<sup>17</sup> transplanted MSGs in 17 human patients and found that over 90% of the transplanted grafts remained viable.<sup>17</sup> The authors also reported that although the grafts probably lost their ability to react to stimuli, basal secretion remained for up to 36 months.<sup>17</sup> A similar study in dogs is warranted.

The present study detected and described the position, number and volume of the labial and buccal MSGs using microCT scans and histology in a selected number of dogs. While surgeons must bear in mind the procedural artefacts possibly affecting gland volume, the results presented here may inform future efforts to transplant labial MSGs to the conjunctiva of dogs with an indication for this procedure. The histological analysis and the 3D reconstruction of the topography of labial and buccal MSGs demonstrated that the glands were caudo-ventral from the ductal mound, that no glands were present rostrally in the lip and that glands of relevant size are located in the vicinity of the labial commissure.

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## CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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