Morphology of the Lung of the Red-eared Terrapin *(Trachemys scripta elegans)*

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Respiration

- The respiratory system consists of the organs that effect the exchange of CO_2 and O_2 between the blood of the organism and the external environment[1]

- The respiratory organs are divided into two portions[2]:
  - Conducting portion
  - Respiratory portion

- Respiration is divided into 2 phases[2]:
  - External respiration
  - Internal respiration

- **Blood-gas barrier** separates the external from the internal environment[3]

Evolution

- Comprehensive changing of traits[3]:
  - Structural
  - Physiological and
  - Behavioural
- Intimate relationship between the animal and its environment[4,5]
- Extreme variation[1]

The Reptilian lung

- Locomotion and breathing constraints[6]
- Change in the lung structure lead → pulmonary vein and artery their branches[7]
- Terminal gas exchange units are directly supplied with air, via a large central lumen[8]
- Large central lumen held open via smooth muscle network[7]
- The two cranial thirds: gaseous exchange tissue[9]
- Caudal third: hypothesised to store air[10]

The reptilian lung

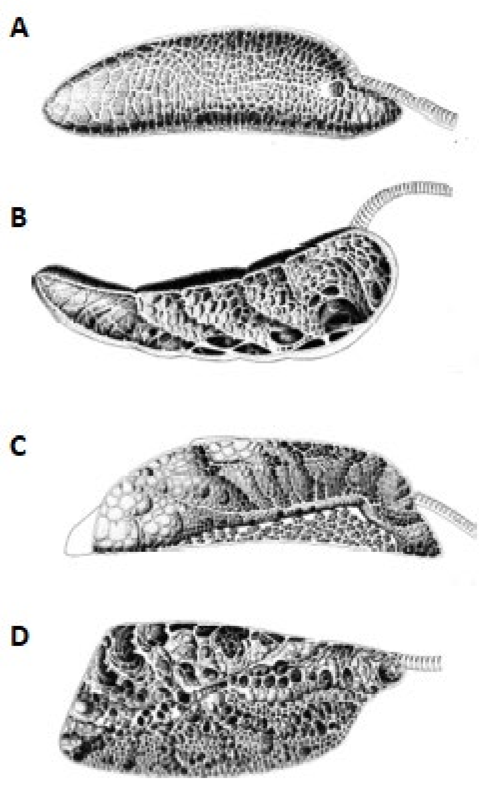
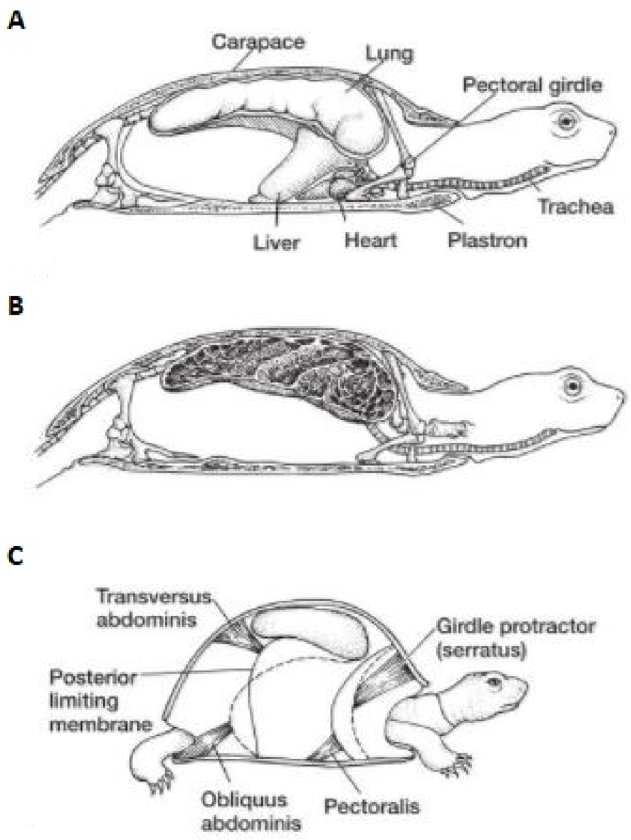


Figure 1: The 3 different classes of reptilian lungs A unicameral; B and C paucicameral and D multicameral[7]

- Numerous degrees of internal subdivision[7]
- Multicameral lung[7]:
    - Extremely heterogenous
    - Internal primary pulmonary bronchus
    - IPPB connecting chambers to EPPB
    - Cartilaginous IPPB and EPPB

Testudines lung



- Incorporation of the ribs into the shell negates the movement of the costal muscles[11]

Figure 2: **A** The position of lungs inside the turtle shell **B** A longitudinal section through the lung of the turtle showing the internal structure of the lung [7]. **C** The muscles that will aid in respiration in the tortoise[12]

Red-eared terrapin

- Indigenous to North America[13]
- Semi aquatic reptiles[13]
- Antipredator behaviour: flight into water[14]
- Multicameral lung[7]
- Periods of respiratory activity $<$ apnoea periods[15]
- Diving and buoyancy

Materials and Methods

Ethical Clearance number: 2018/03/13A

DEA permit Number: 5069218120609 1228

1. Collection of specimens
2. Euthanasia and intratracheal instillation
3. Gross anatomical photography
4. Sampling of tissue
5. Processing
6. Microscopy

Figure 3: An overview of the methods

Materials and Methods

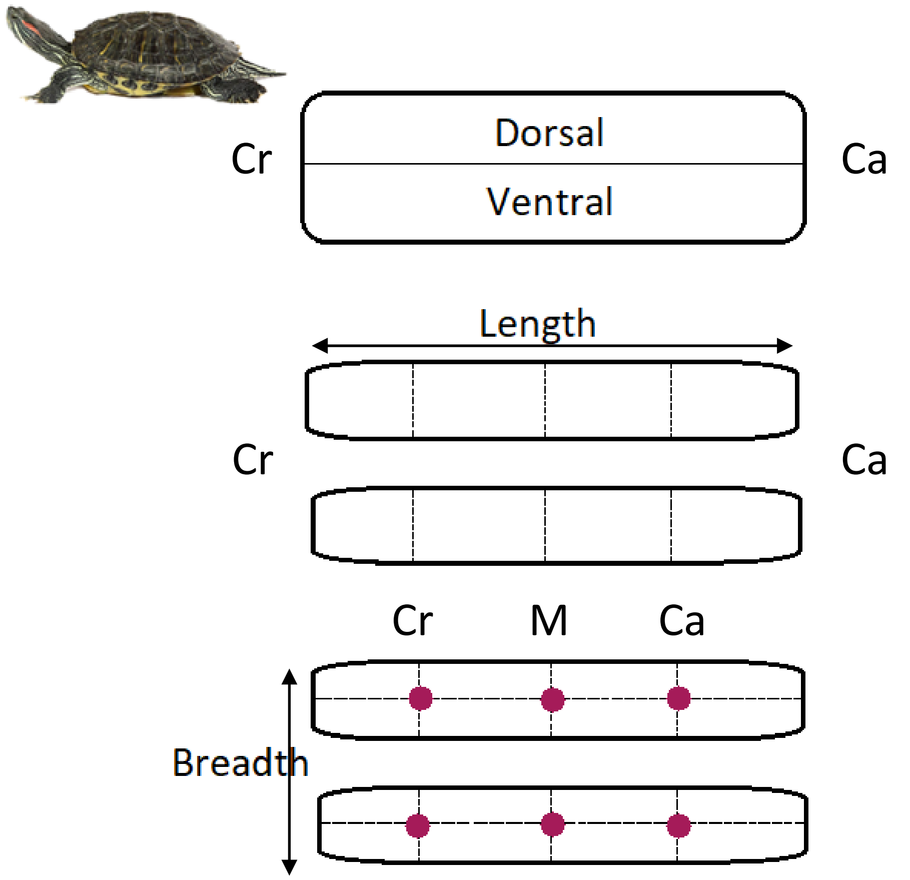


Figure 4: A flow diagram illustrating the systematic sampling procedure[16] (Cr- cranial, M-middle, Ca-caudal)

Materials and Methods

Histology

a. Histological processing using standard techniques[17]
   - Tissue processing
   - Wax embedding
   - Cutting → 5μm

b. Staining
   - New Pentachrome[18]

c. Lundvall Technique[19]
   - The Lundvall technique stains cartilage while clearing all surrounding tissue

Microscopy:

- Brightfield
- Stereo fluorescence

Materials and Methods

SEM Processing:

1. OTO-method[20]
2. Dehydrated in graded alcohol
3. 100% hexamethyldisilazane
4. Desiccator with silica gel
5. Mounted on aluminium stub
6. Sputter coated with gold-palladium

Figure 5: An overview of the SEM processing methods

Microscopy:

- 30Kv with SE

Results and Discussion:

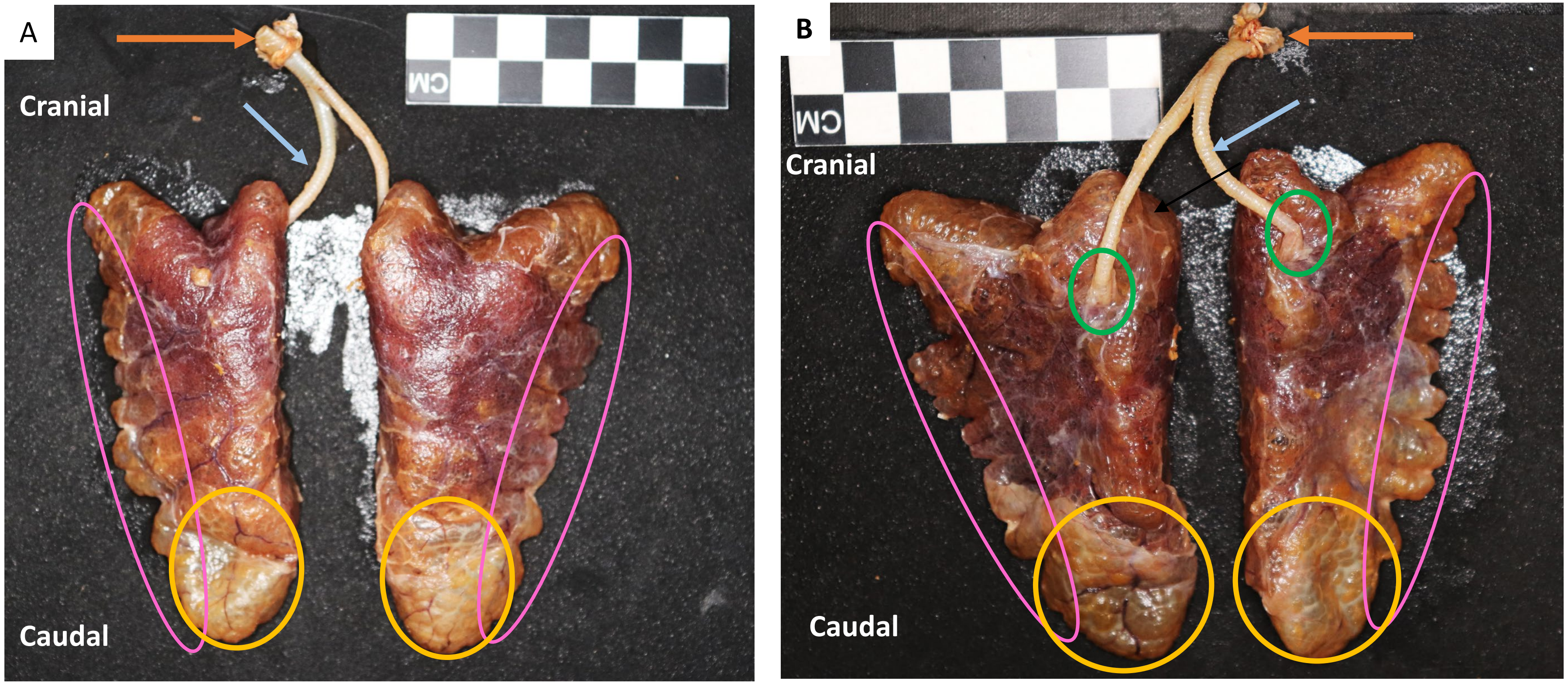


Figure 6: The gross anatomy of the lung of the red eared slider. A- Dorsal view; B- Ventral view.

Key: intercostal sulci; bronchus; large air chamber; trachea; entry of bronchi

Results and Discussion:

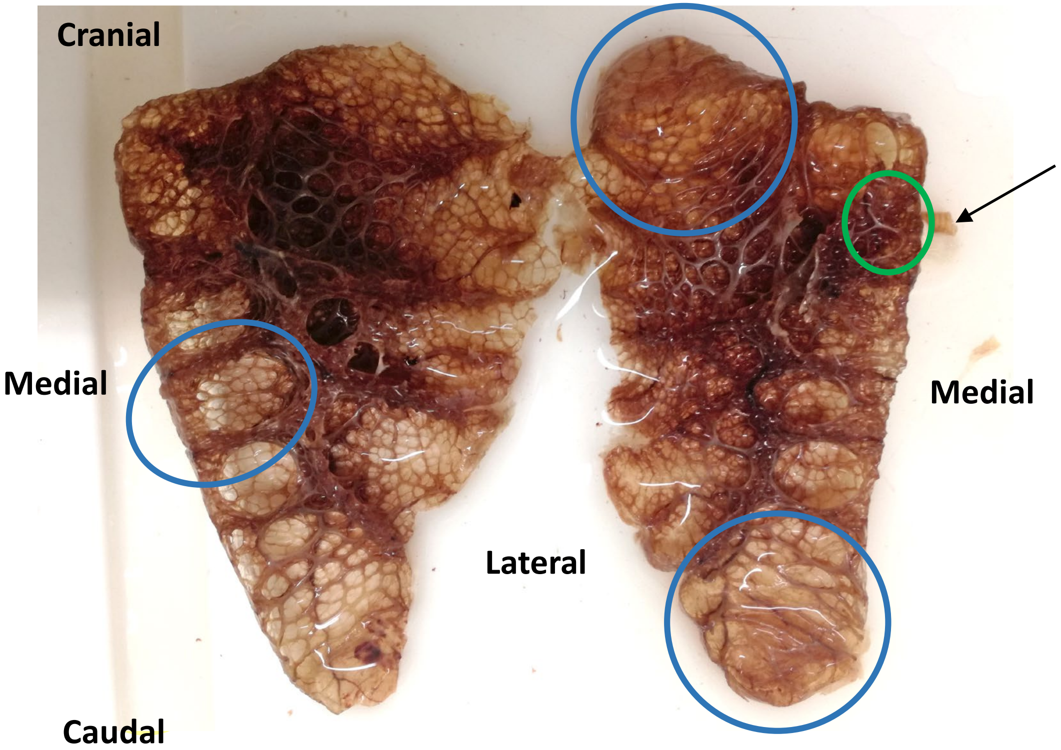


Figure 7: The internal gross anatomy of the left lung of the red eared slider

Key: large air chamber; entry of bronchi

Results and Discussion:

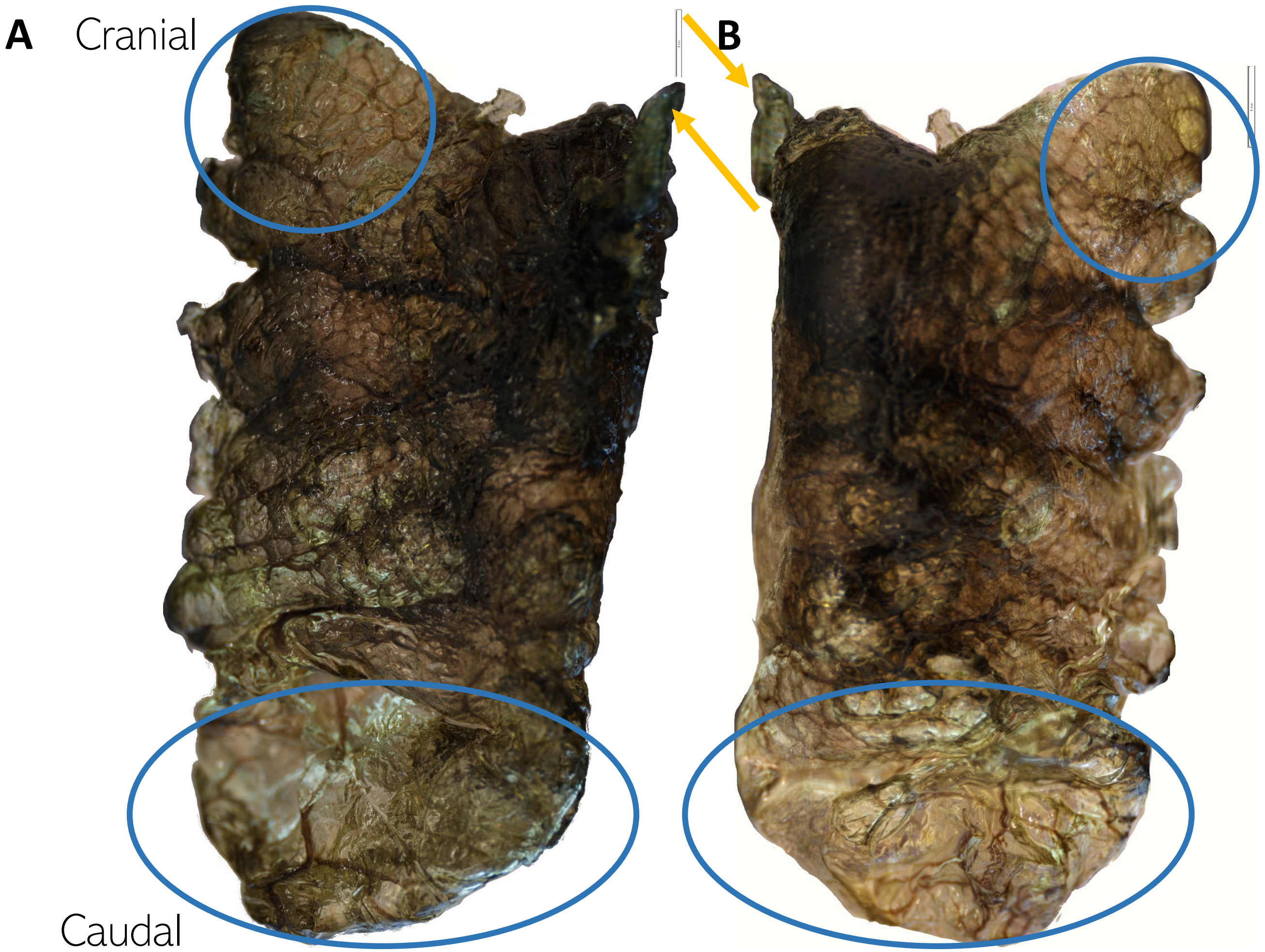


Figure 8: The dorsal view of the right lung using the Lundvall technique A: Ventral; B: Dorsal

Key: bronchus; large air chamber

Results and Discussion:

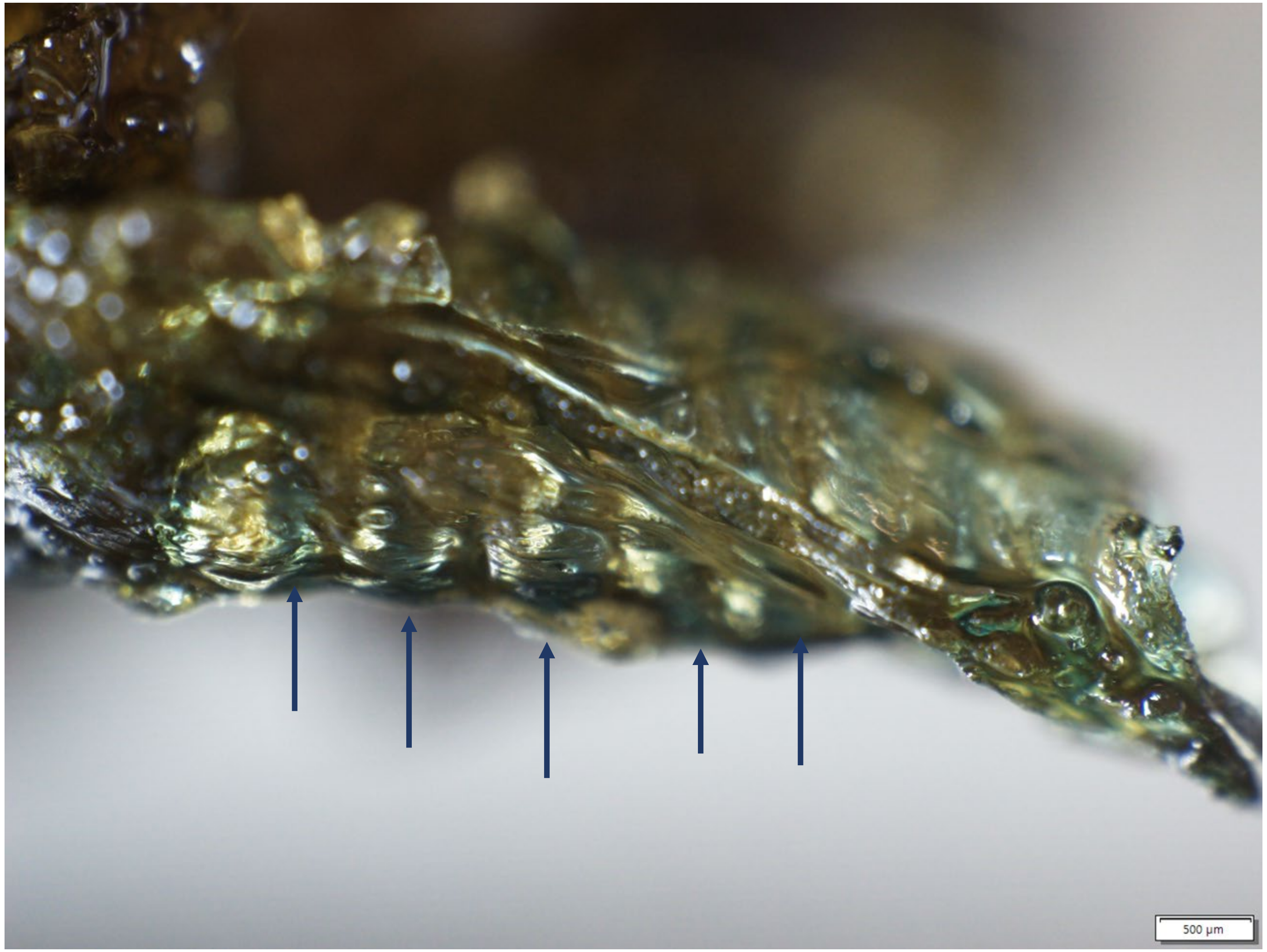


Figure 9: Micrograph of the bronchus of the Lundvall lung. The blue arrows pointing to the cartilaginous rings found in both the trachea and bronchi **Key:** cartilage

Results and Discussion:

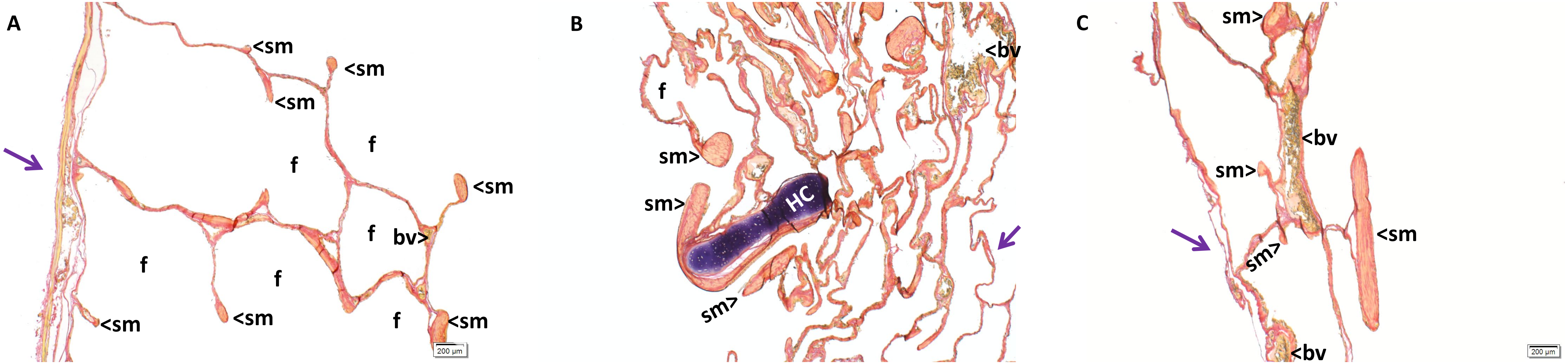


Figure 10: Overview of the tissue found in the various regions of the lung
A - cranial; B - middle; C - caudal.
key: f: faveolus; sm: smooth muscle; bv: blood vessel; HC: Hyaline Cartilage; pleura

Results and Discussion:

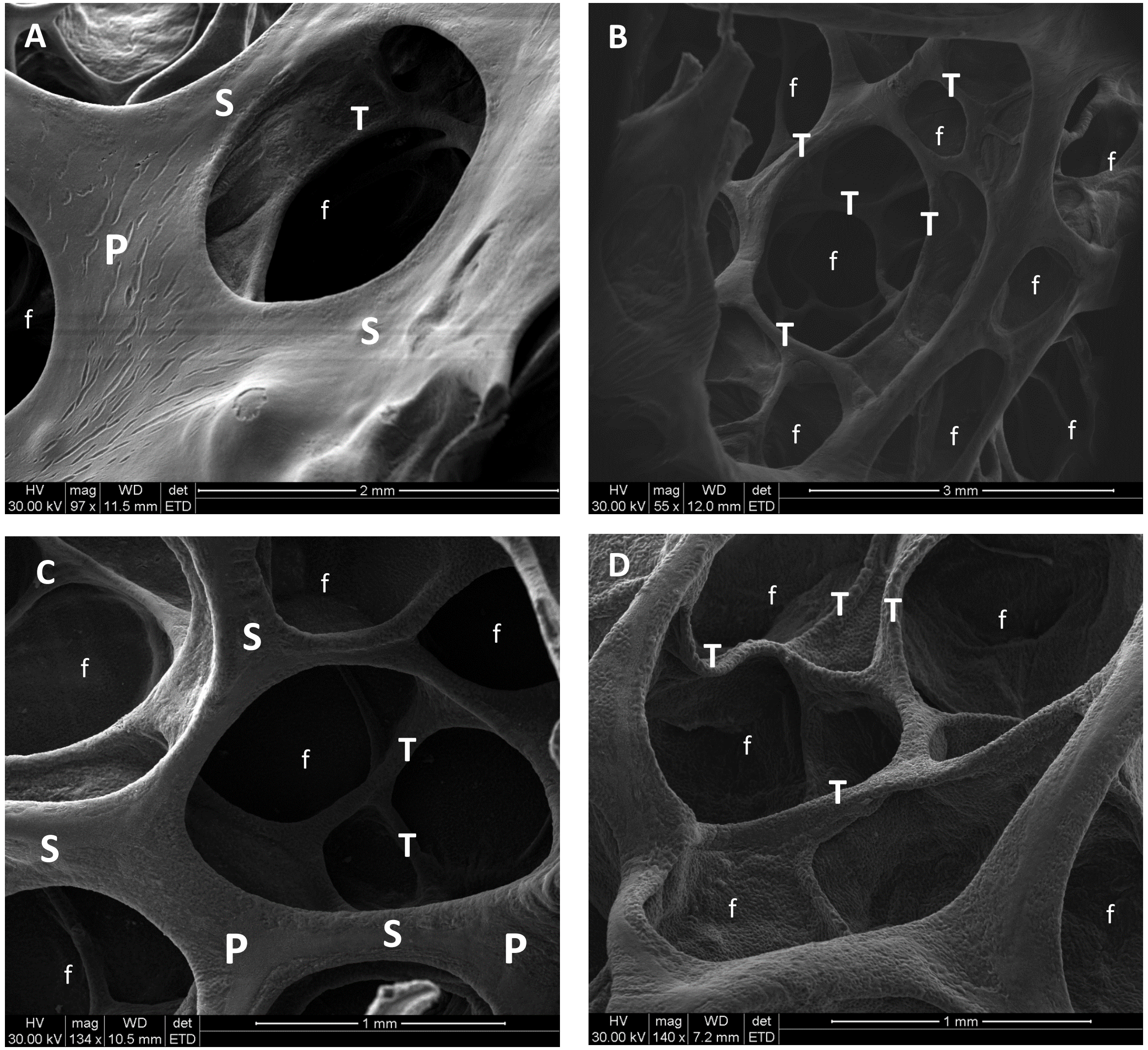


Figure 11: Septa found in the various regions of the lung

Key: f: faveoli, P: primary septa; S: secondary septa; T: tertiary septa

Results and Discussion:

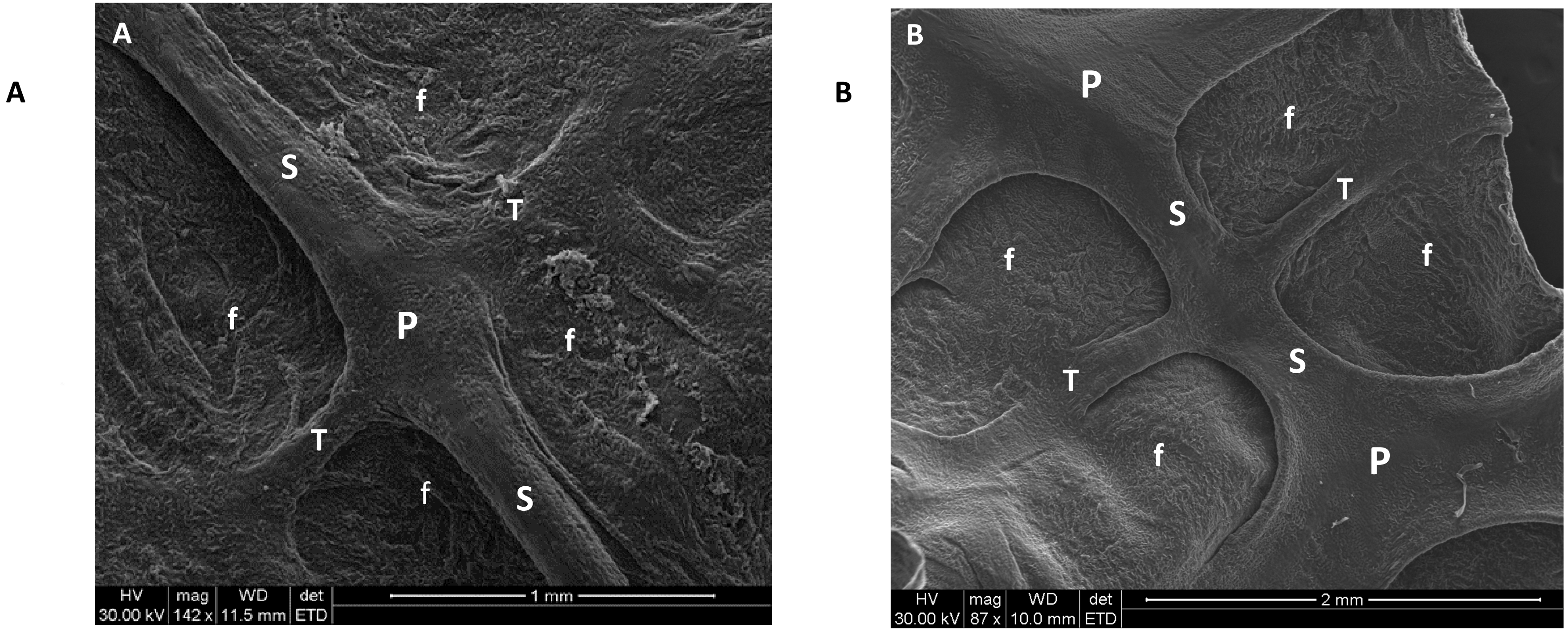
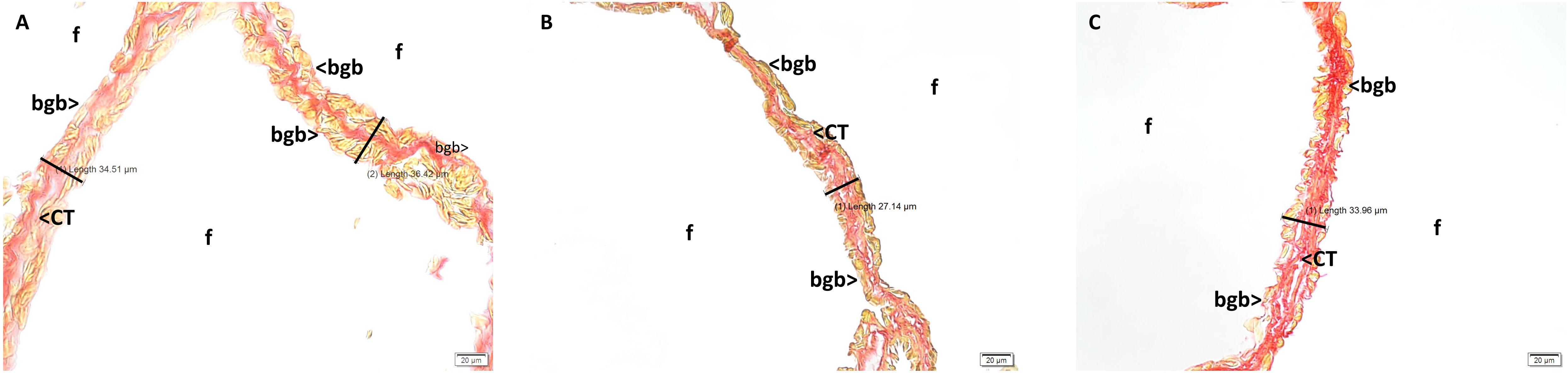


Figure 12: Septa and faveoli found in the caudal region
Key: f: faveoli, P: primary septa; S: secondary septa; T: tertiary septa; sm: smooth muscle; pleura

Results and Discussion:



34.51µm 36.42µm 27.14µm 33.96µm

Figure 13: Septa found in the various regions of the lung

A - cranial; B - middle; C - caudal.

Key: f: faveoli; sm: smooth muscle; CT: connective tissue; bgb: blood-gas barrier

Results and Discussion:

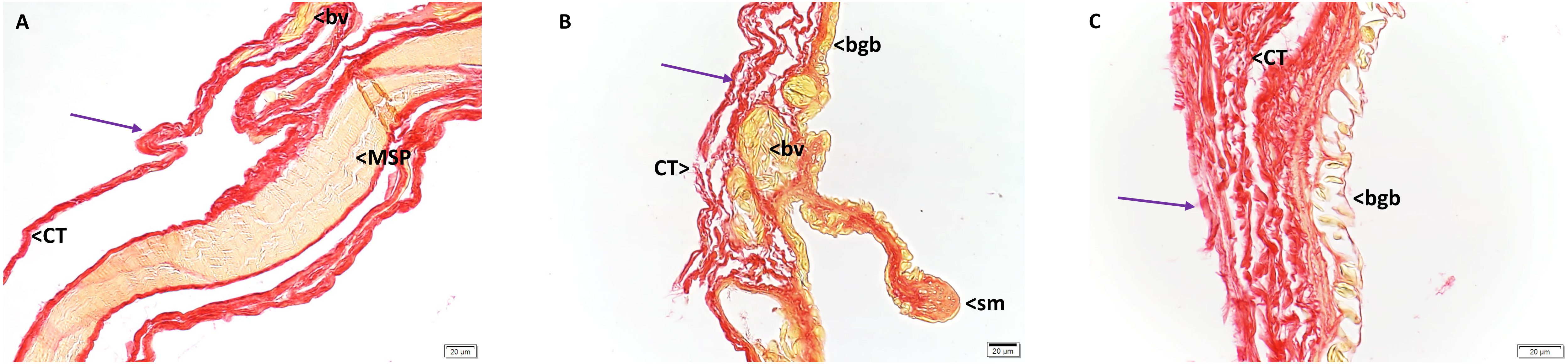


Figure 14: Micrographs of the pleura found in the various regions of the lung.
A: cranial; B: middle; C: caudal.
Key: pleura; bv: blood vessel; sm: smooth muscle; bgb: blood-gas barrier; MSP: *Muscularis Striatum Pulmonale*

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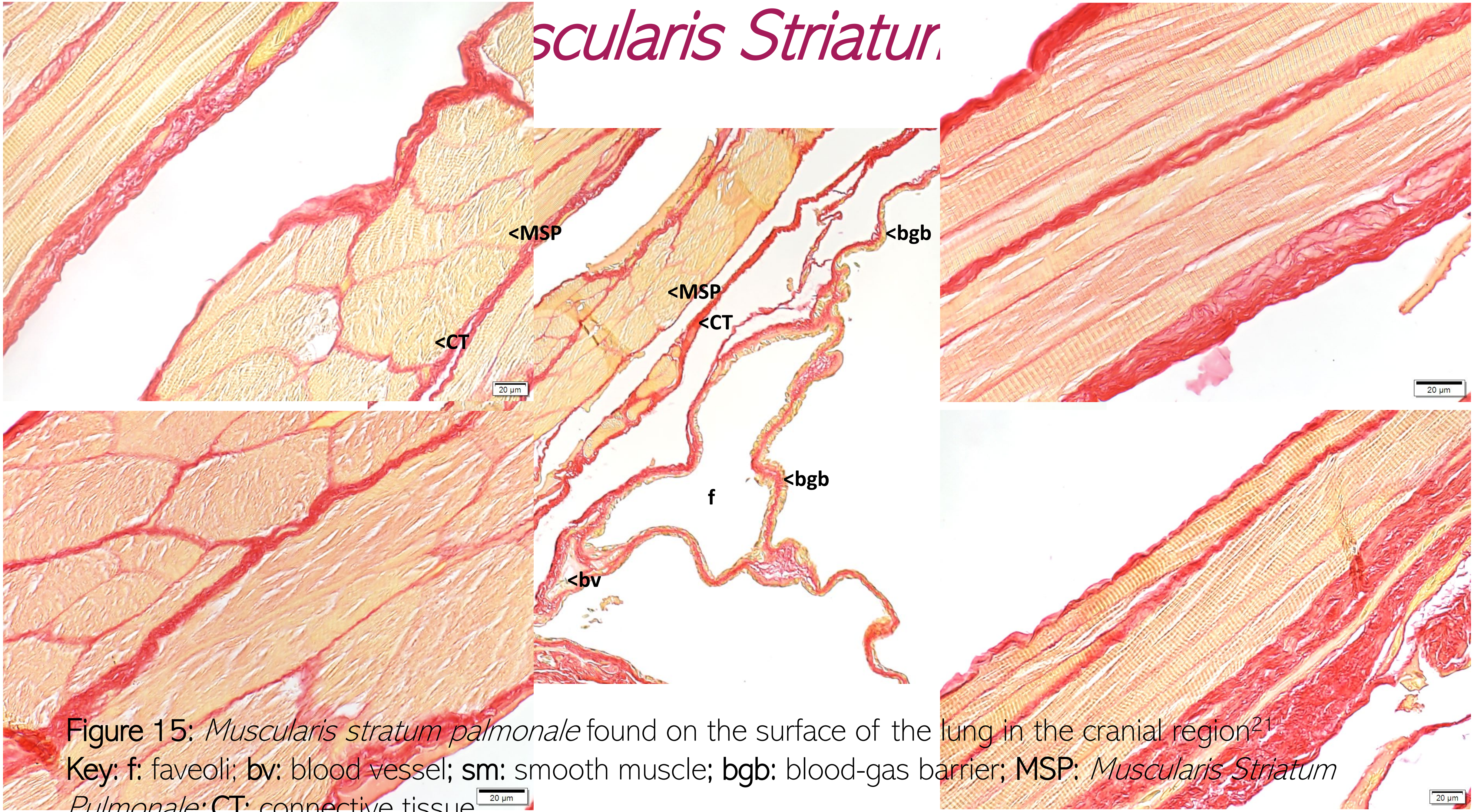

Figure 15: *Muscularis stratum palmonale* found on the surface of the lung in the cranial region[21]
Key: f: faveoli; bv: blood vessel; sm: smooth muscle; bgb: blood-gas barrier; MSP: *Muscularis Striatum Pulmonale;* CT: connective tissue

Results and Discussion

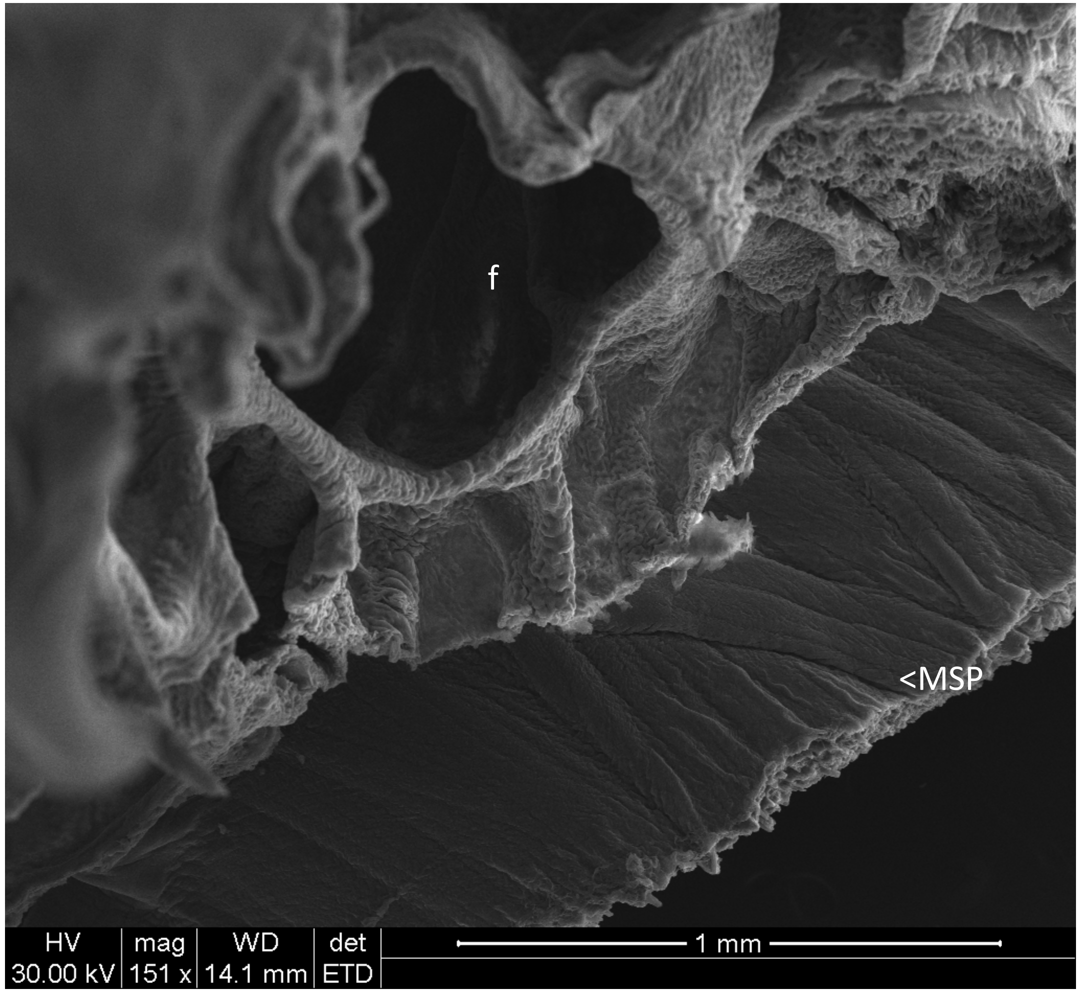

Figure 16 : SEM micrograph of the MSP
Key: f: faveolus; MSP: *Muscularis Striatum Pulmonale*

Results and Discussion:

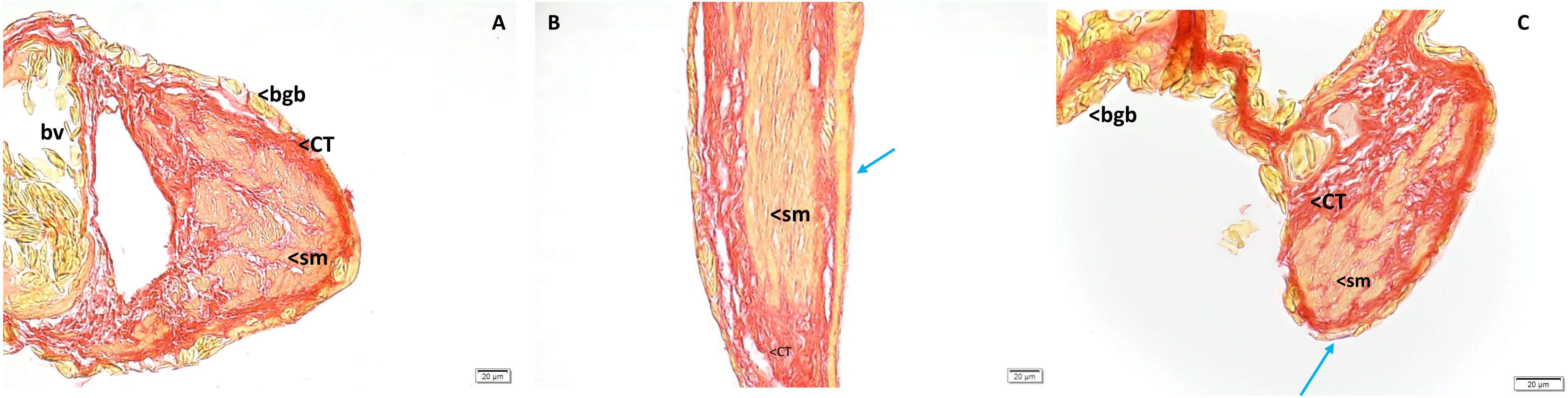

Figure 17: Smooth muscle knobs and plates found all of the regions of the lung. Connective tissue layers were found around and between muscle bundles.

Key: Cilia; sm: smooth muscle; CT: connective tissue; bgb: blood-gas barrier; bv: blood vessel

Results and Discussion:

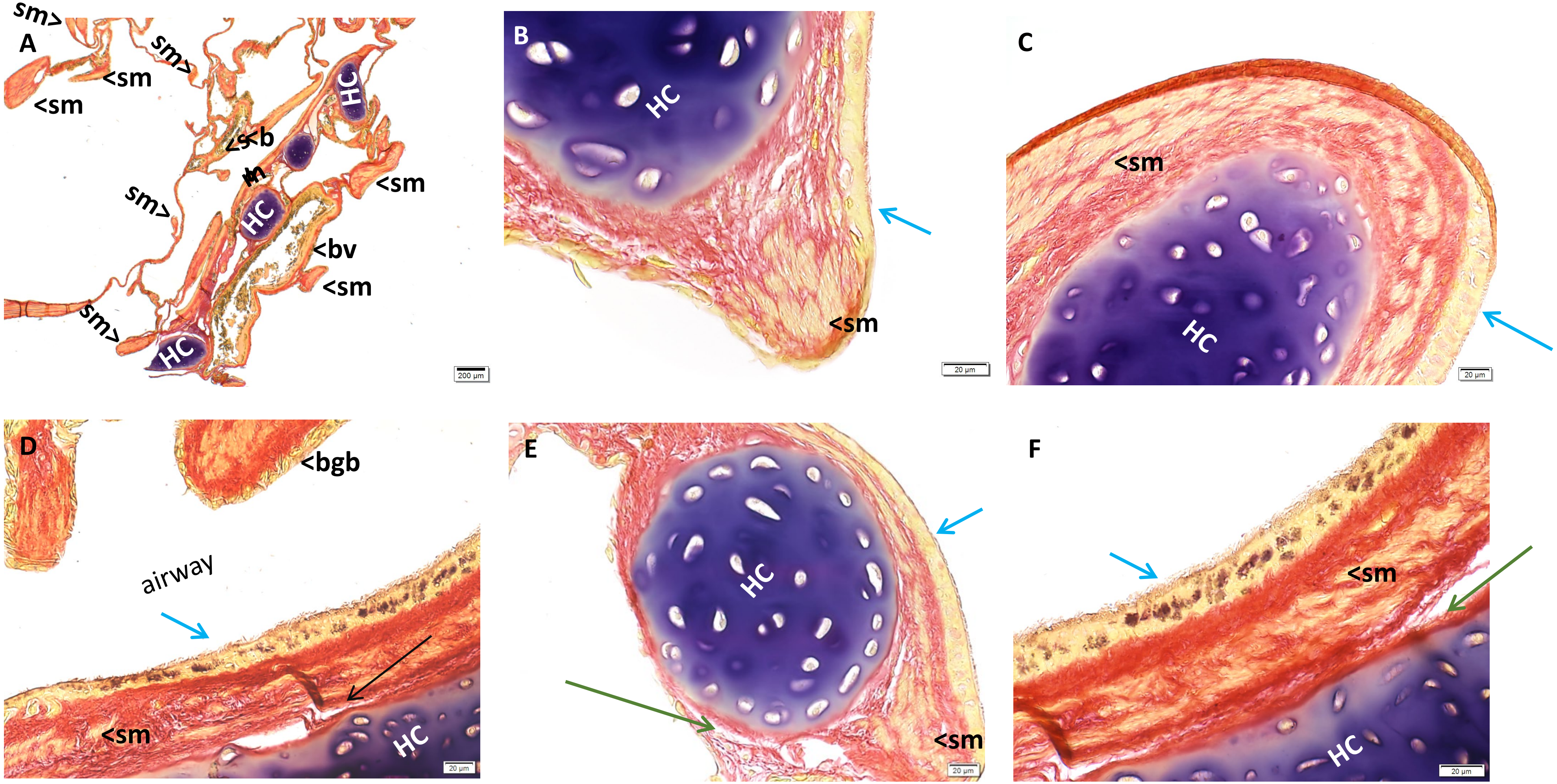

Figure 18: Hyaline cartilage found in the middle and caudal regions, found with a fibrous perichondrium (black arrow), in close relation to smooth muscle

Key: Cilia; sm: smooth muscle; HC: Hyaline cartilage; bv: Blood vessel; **perichondrium.**

Results and Discussion:

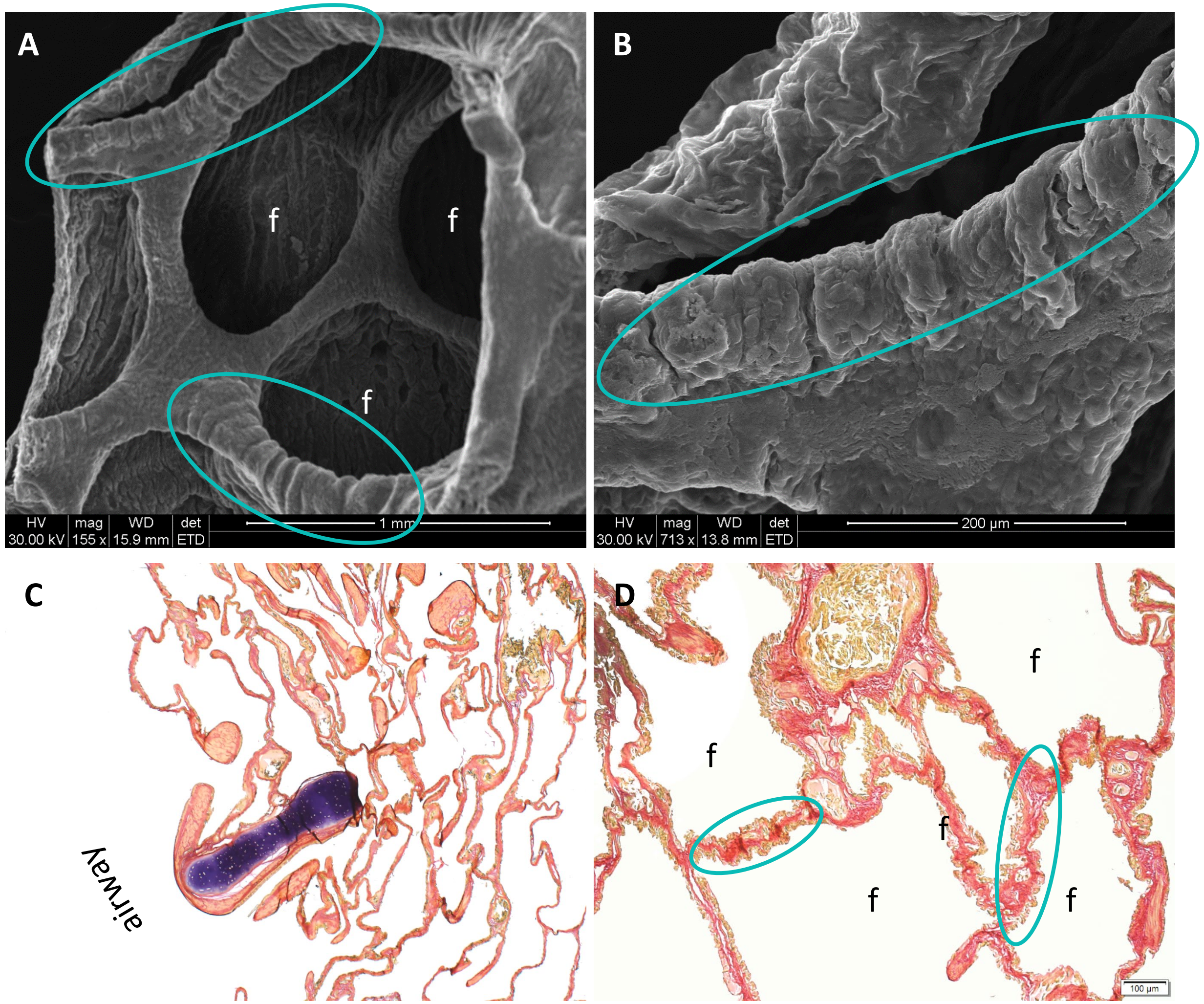

Figure 19: Septal foldings found in the cranial, middle and caudal regions of the lung in order to increase the surface area. Key: f: faveoli; foldings

Results and Discussion

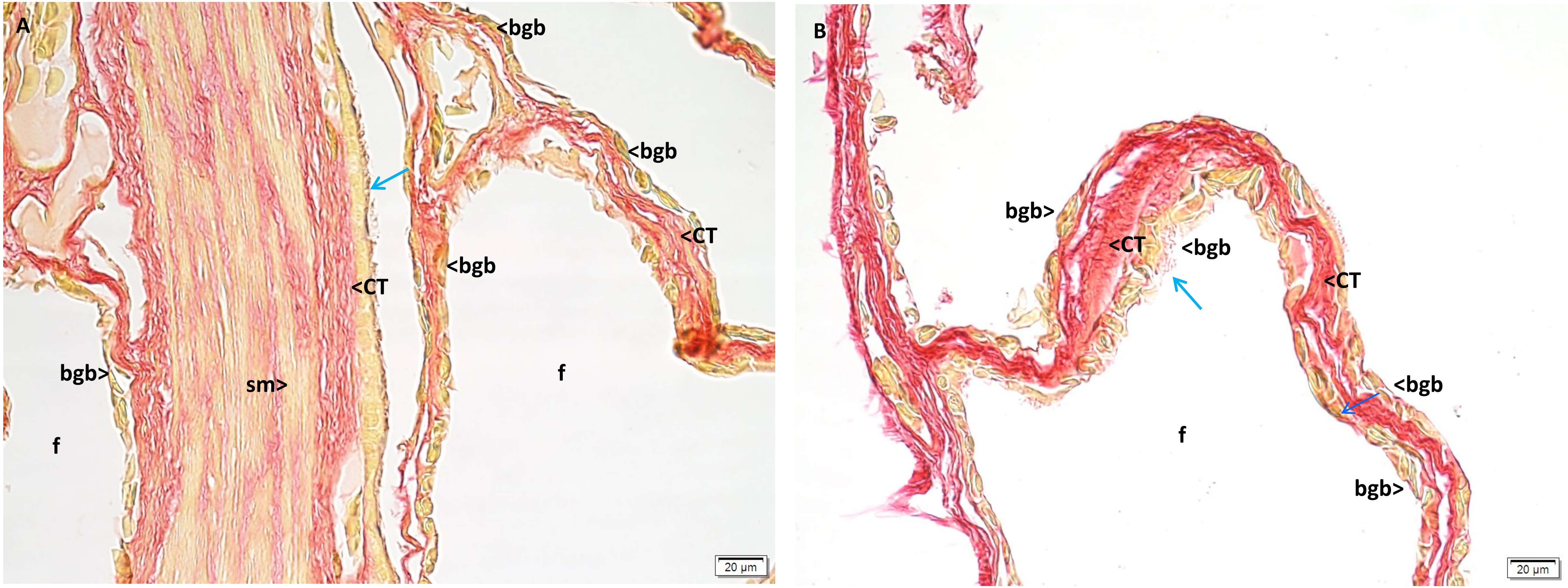

Figure 20: Epithelium found in the lung.

Key: bgb: blood-gas barrier; sm: smooth muscle; CT: connective tissue; cilia

Results and Discussion:

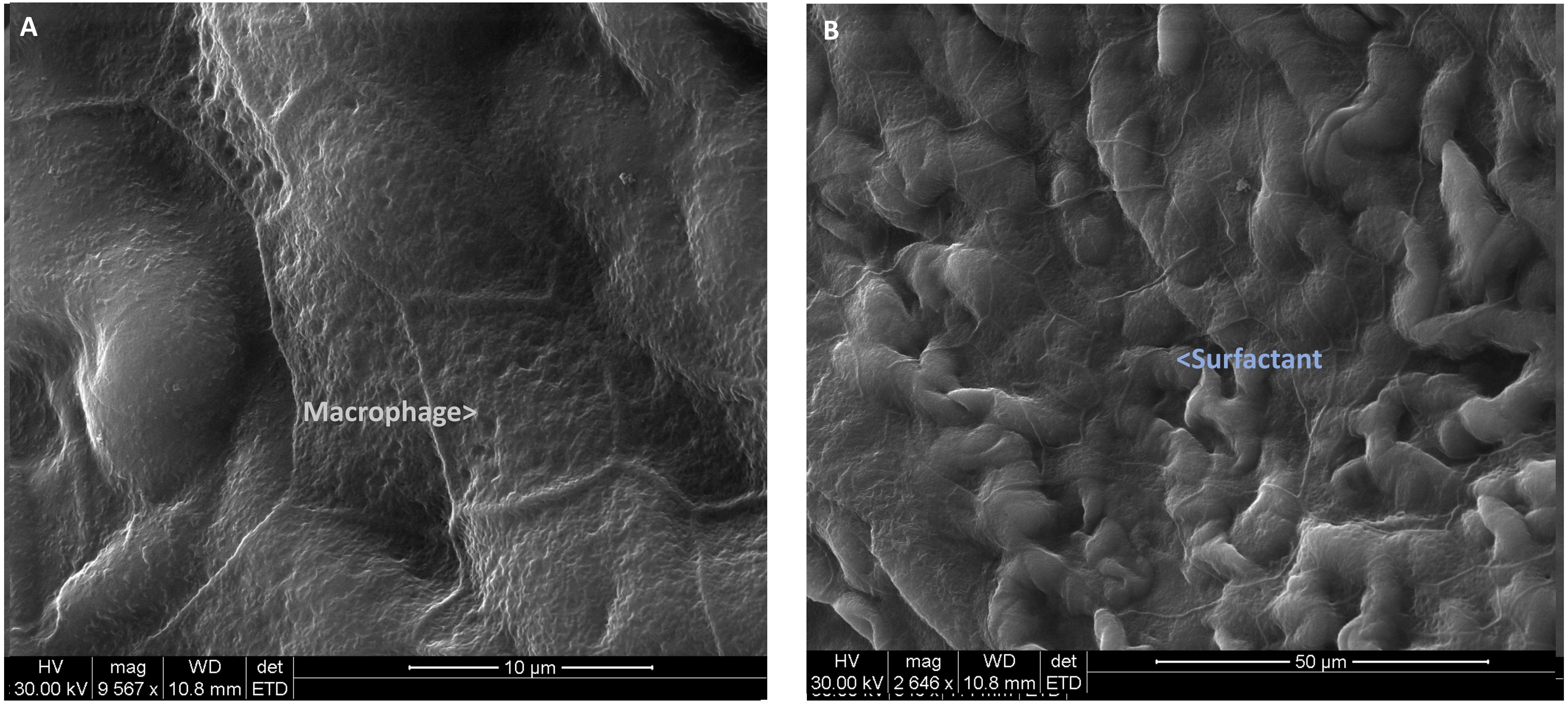

Figure 21: A surfactant and a type II pneumocyte. B surfactant found on a slanted surface.

Figure 22: Type I pneumocyte junctions found on the capillary surface.

Results and Discussion:

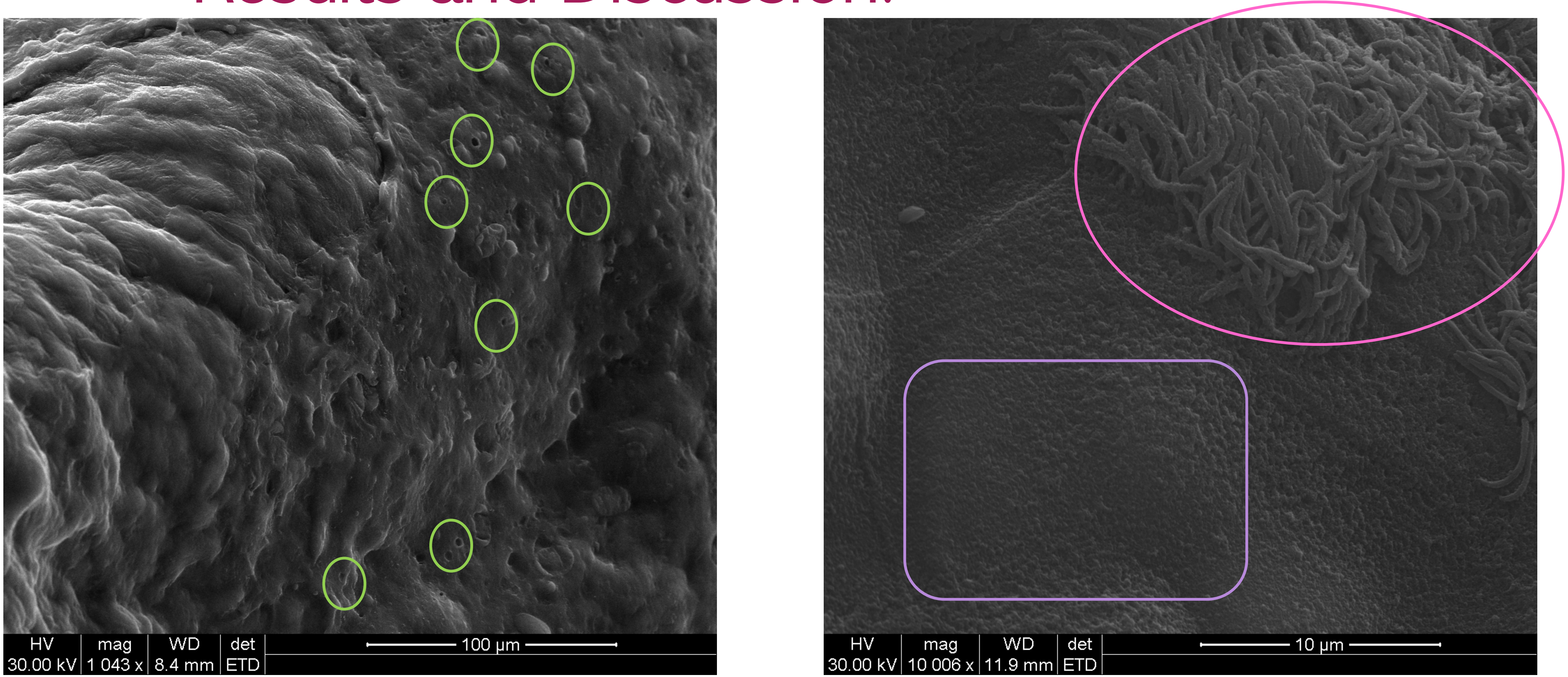

Figure 23: A Pores and B microvilli and stereocilia found in various regions of the lung

Key: pores; stereocilia; microvilli

LUNG
STORY
SHORT

Conclusion

- Extreme heterogeneity throughout the lung
  - Cranial → Caudally
  - Dorsal → Ventrally

- Structure and size of the cranial region → Gas exchange

- Path of the bronchi
- Muscle in middle region

Conduit

- Little tissue in caudal region and air chambers → Air storage

Conclusion

- ↑ Surface Area: volume → ↑ Gas exchange capacity

- Movement of air for buoyancy
- Control of lung volume for buoyancy

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