

# SCANNING ELECTRON MICROSCOPY OF THE BASILAR PAPILLA OF THE LIZARD (*ANOLIS CAROLINENSIS*)

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## Résumé

Le lézard est un modèle utile pour l'étude de la biophysique de la fonction auditive périphérique, notamment parce qu'il possède un organe auditif relativement simple par rapport à la cochlée mammalienne. Pour bien comprendre les mécanismes de l'oreille interne, une description précise et détaillée de l'anatomie est nécessaire. À cette fin, nous décrivons la morphologie et la disposition des cellules ciliées le long de la papille basilaire de l'espèce de lézard *Anolis carolinensis*, telle que révélée en utilisant la microscopie électronique à balayage. Nous fournissons également des détails sur les méthodes d'obtention et de préparation d'échantillons pour le microscope électronique.

**Mots clefs :** labyrinthe des reptiles, papille basilaire, anolis.

## Abstract

The lizard is a useful model for study of the biophysics of peripheral auditory function, not least because it has a hearing organ that is relatively simple compared to the mammalian cochlea. To fully understand inner ear mechanisms, an accurate and detailed description of anatomy is required. To that end we describe morphology and arrangement of haircells along the basilar papilla of the lizard species *Anolis carolinensis*, as revealed using scanning electron microscopy. We also provide details of the methods for obtaining and preparing specimens for the electron microscope.

**Keywords:** reptile inner ear, auditory papilla, anoles.

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## 1 Introduction

In biological studies of hearing mechanisms, a wide range of animal models has been used. For studies relating the human condition, the mammalian cochlea has been widely studied, however many non-mammalian vertebrates have an ear structure that appears to be more simple and thus suitable for understanding some basic principles of auditory signal detection [5, 8]. Reptiles in particular have highly evolved inner ear structures and excellent hearing, and within this group the lizards (Lacertilia) have been used in a number of studies of auditory function. In many respects the lizard is an excellent animal model for elucidating biophysical principles of peripheral auditory function. The inner ear is easily accessible and the cochlea equivalent, the basilar papilla, has a less complex structure compared to the mammalian organ of Corti. There have been studies of the variations in hearing sensitivity and frequency range between different lizard types and this animal has been used to study middle and inner ear mechanisms [e.g. 2, 3, 7, 11]. In the present study, we describe some anatomical features of the lizard *Anolis carolinensis* (fig 1). This species is useful as a model in

many scientific studies due to their low cost in breeding and also because the entire genome has been sequenced [1]. The *Anolis* is particularly useful because it exhibits robust otoacoustic emissions [2, 3, 4].



**Figure 1:** The anolis lizard.

In order to fully understand inner ear function, detailed study of the anatomy and morphology of the sensory epithelium is required. There have been useful descriptions of the anatomy of the basilar papilla using light microscopy, not least the comprehensive studies by Wever [11]. However to obtain an accurate analysis of stereocilia bundle

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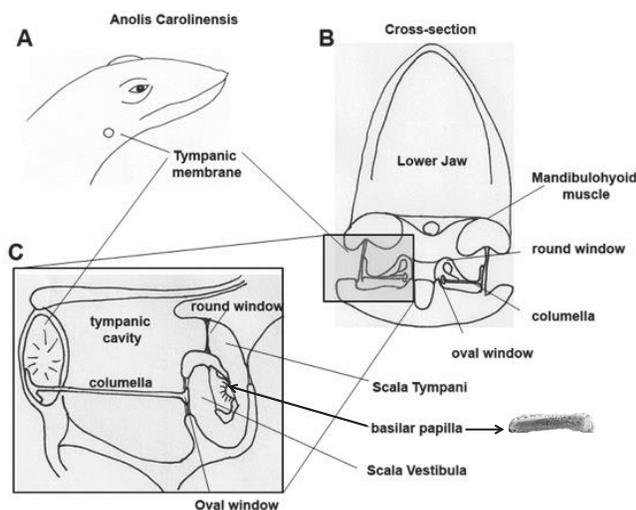
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structure and the geometric arrangement of haircells, scanning electron microscopy (SEM) is perhaps the best technique available. There have been SEM studies of the inner ear in various lizard species [e.g. 7, 10] but no clear description of the basilar papilla of *Anolis carolinensis*. Furthermore there has been no detailed description of the methodology for obtaining and preparing specimen for SEM study. We provide these methods here, and discuss some aspects of basilar papilla haircell arrangements as revealed using scanning microscopy.

## 2 Methods

### 2.1 Inner ear specimen preparation

To prepare the *Anolis carolinensis* lizard for electron microscopy the subject is deeply anesthetized. A 1-2 ml solution of 2.5% glutaraldehyde in sodium cacodylate buffer is injected through the tympanic membrane into the middle ear. Immediately after, the head of the lizard is removed and placed in a solution of the same fixative at 4°C overnight. Once fixed, the head is washed in phosphate buffered saline (PBS) and then dissected under a microscope to remove the inner ear. This is achieved by surgical removal of the lower mandible to expose the mandibulo-hyoid muscle. This muscle is cut away to expose the bony structure housing the inner ear. The view of structures is schematically represented in figure 2B.



**Figure 2:** Anatomical landmarks to locate the inner ear of the lizard, and the position of the basilar papilla. Adapted from Wever [11].

From this position, the middle ear columella bone (the equivalent of the mammalian middle ear ossicles) can be seen connecting the tympanic membrane to the oval window (Fig 2B, C). With this exposure the inner ear round window can also be located. At this time, all surrounding tissue is removed, before gently pulling away the columella to expose the oval window of the inner ear. Cacodylate buffer

is then flushed through the round window using a 30G needle. The sample is immersed in sodium cacodylate buffer at 4° C overnight.

The following day, the sample is washed in fresh sodium cacodylate for 15 mins. The inner ear is flushed via the round window with 2% buffered osmium tetroxide, 2-3 times using a 30G needle, and then immersed in a solution of 2% buffered osmium tetroxide for 1.5 hours. The specimen is then briefly washed in cold PBS and is ready for a gradual dehydration process.

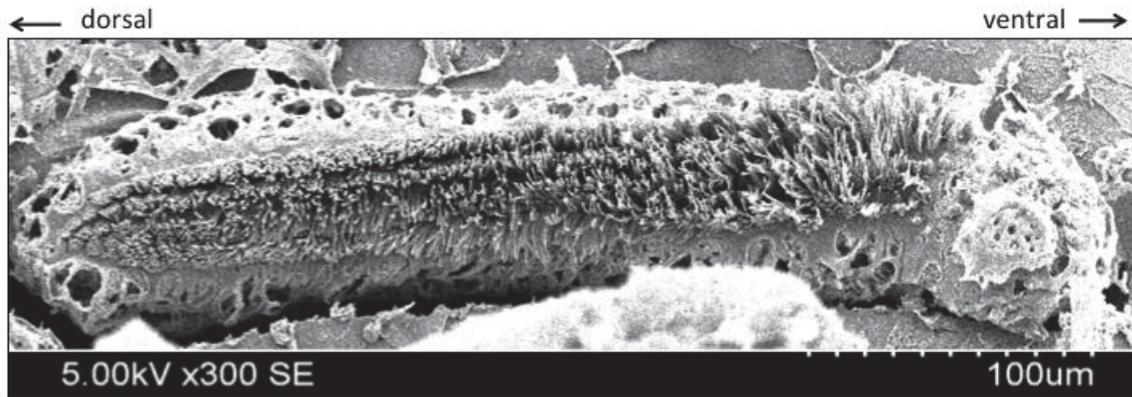
The sample is submerged and gently shaken for 15 mins in, sequentially, 35%, 50% and 70% solutions of ethanol. Once the dehydration process begins, the specimen should not be exposed to air. Removing most of the fluid from the glass vial, but leaving some to cover the sample can achieve this. The vial is then filled with the next ethanol strength solution. Once the sample is submerged in 70% ethanol, further dissection can be made to open the inner ear and expose the basilar papilla. With very fine forceps, the bone surrounding the round window is gently chipped away in the direction of the oval window, thereby opening up the bony structure of the inner ear. This will expose the tissue that forms a basilar membrane-like structure to which the basilar papilla is attached (fig. 2C). Very gently, this small (and fragile) organ can be removed and transferred to a vial of 90% alcohol on a shaker. The specimen is then further dehydrated in 90% and 95% ethanol for 15 mins each and then washed in 100% ethanol three times for 15 mins. Rinses are done in the same manner as before without exposing the sample to air.

### 2.2 Scanning electron microscopy

For electron microscopy the basilar papilla specimen undergoes critical point drying, whereby all moisture is removed from the sample by replacing water with liquid carbon dioxide at very high pressure and temperature. The specimen is then mounted on a stub, and gold sputter-coated. This creates a conductive layer of metal on the specimen that reduces thermal damage and improves the electron signal in the microscope. The basilar papilla is imaged at high resolution (5 kV accelerating voltage; magnification 200X) using the Hitachi 3400 microscope (Hitachi, Ltd., Chiyoda-ku, Tokyo, Japan).

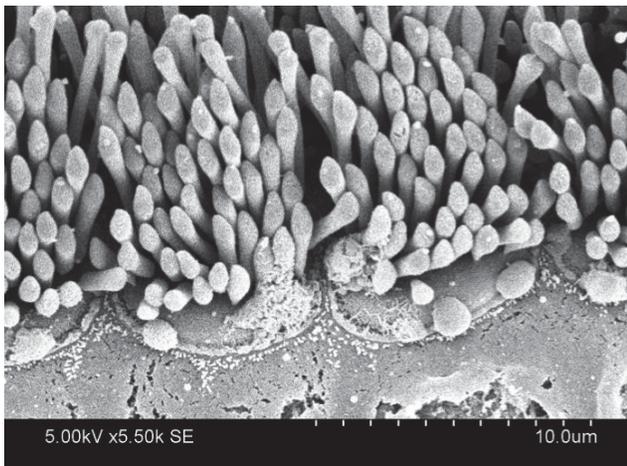
## 3 Results and discussion

An image of the whole basilar papilla is shown in figure 3. Such whole specimens are often obtained, but on occasion there can be breakage. Because the sensory epithelium is not a coiled cochlear structure, apex to base descriptions are not useful. Here we use dorsal-ventral co-ordinates as adopted by Wever [11] and others. In figure 3, dorsal is left, and ventral is right. The length of the organ is 3.5 mm and the width of the haircell-bearing region is about 400 µm.



**Figure 3:** Scanning electron image of the whole basilar papilla of the lizard (*Anolis carolinensis*). In this image, the dorsal region is to the left, ventral to the right.

The organ has two distinct regions, with a long tapering dorsal section having four rows of haircells, and a ventral section showing evidence of a tectorial membrane structure (see discussion below). The dorsal region, making up 85% of the papilla has, almost uniformly, four rows of haircells. Figure 4 shows the close packing of stereocilia in individual haircells in the dorsal region of the papilla. Each haircell has a bundle of about 50 stereocilia with length gradation, the longest being towards the midline of the papilla.



**Figure 4:** Stereociliar bundles of individual haircells in the dorsal section of the basilar papilla.

Along most of the length of the papilla two rows of haircells have bundle orientation opposite from the other two rows as illustrated in figure 5. From the SEM images there appears to be no tectorial membrane or other overlying structure in this region. This is consistent with other descriptions of lizard ears [11]. However we should note that preparation artifacts (especially dehydration) can shrink or distort delicate tectorial tissue.

Along the dorsal segment of the organ, the length of the longest stereocilia changes significantly as shown in figure 6. At the extreme dorsal tip of the tapering papilla, the (largest) stereocilia are less than 5 µm. It is not clear whether the very small haircell bundles at the extreme tip of the papilla are mature haircells or new cells being generated. It is possible that this is an area of regeneration of the sensory epithelium. In the ventral direction stereocilia length progressively increases, and we note (fig 6) long 20-30 µm stereocilia at the end of this region. The progression in stereocilia length along the papilla is not strictly linear; there appear to be sectional changes. For the haircells with long stereocilia we can often see a longer thinner kinocilium. At the ventral boundary edge of this region of long stereocilia we see smaller haircells; as with the dorsal tip of the papilla these might be newly generated haircells.

At the ventral end of the basilar papilla is an almost separate sensory epithelium in which the haircells are not in four orderly rows, and have relatively short stereocilia (5-10 µm). As shown on figs. 3 and 7 we note a tectorial plate structure that overlies some of the haircells. We suggest that this whole area is normally covered with a tectorial membrane but only this plate or sallet remains in this SEM specimen.

In figure 8 we illustrate this dorsal region in another specimen where the tectorial plate or sallet has been removed, showing the (somewhat disrupted) haircell bundles beneath. In this image we can note that some of the haircells have lost some of all of their stereocilia. This results from the close attachment of the hair bundle to the tectorial plate that, when removed, takes with it some of the stereocilia. Wever [11] has used a lizard family classification system based on the tectorial membrane characteristics of the basilar papilla, and has defined the Lacertidae as having a “combined tectorial and sallet system of ciliary restraint”. This is consistent with our observations here in *Anolis carolinensis*.

stereocilia bundle orientation

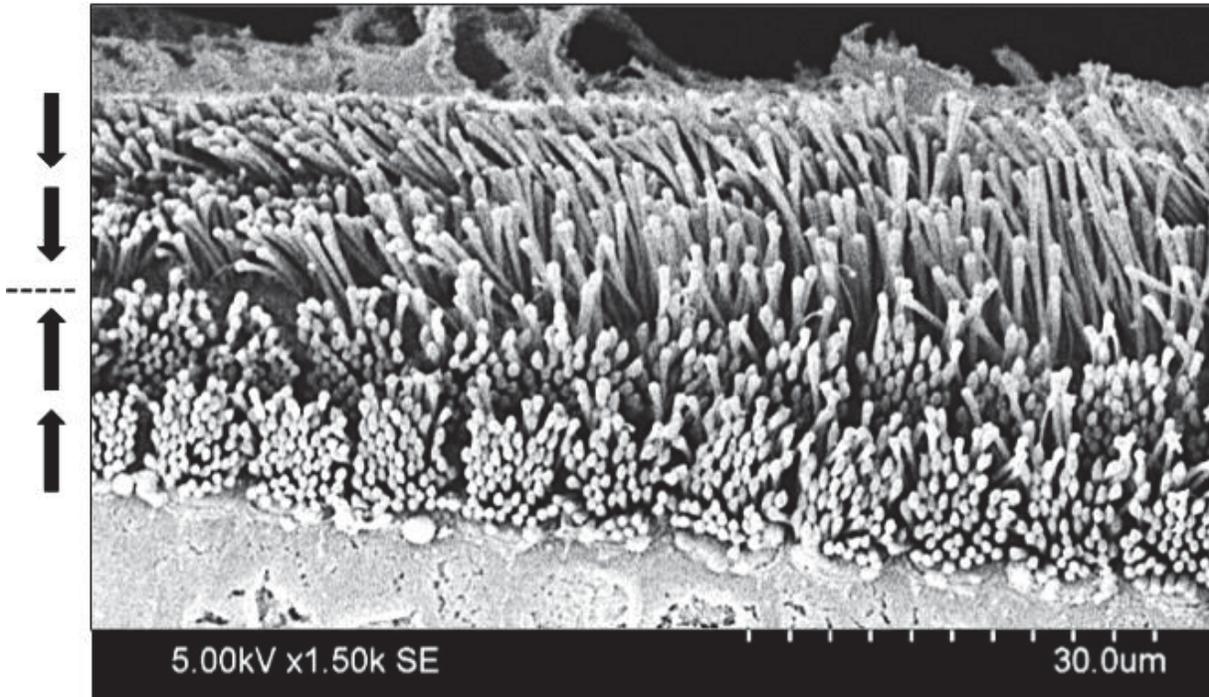


Figure 5: Stereocilia orientation of four rows of haircells along the dorsal length of the basilar papilla.

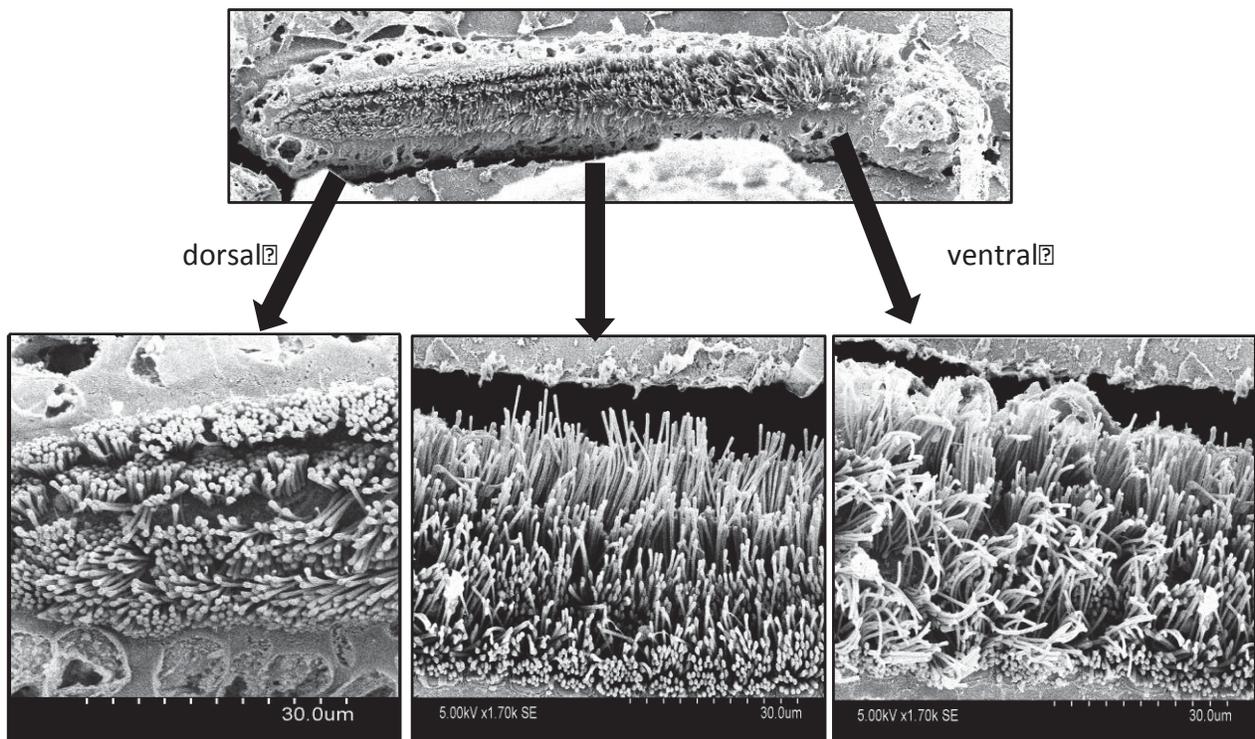
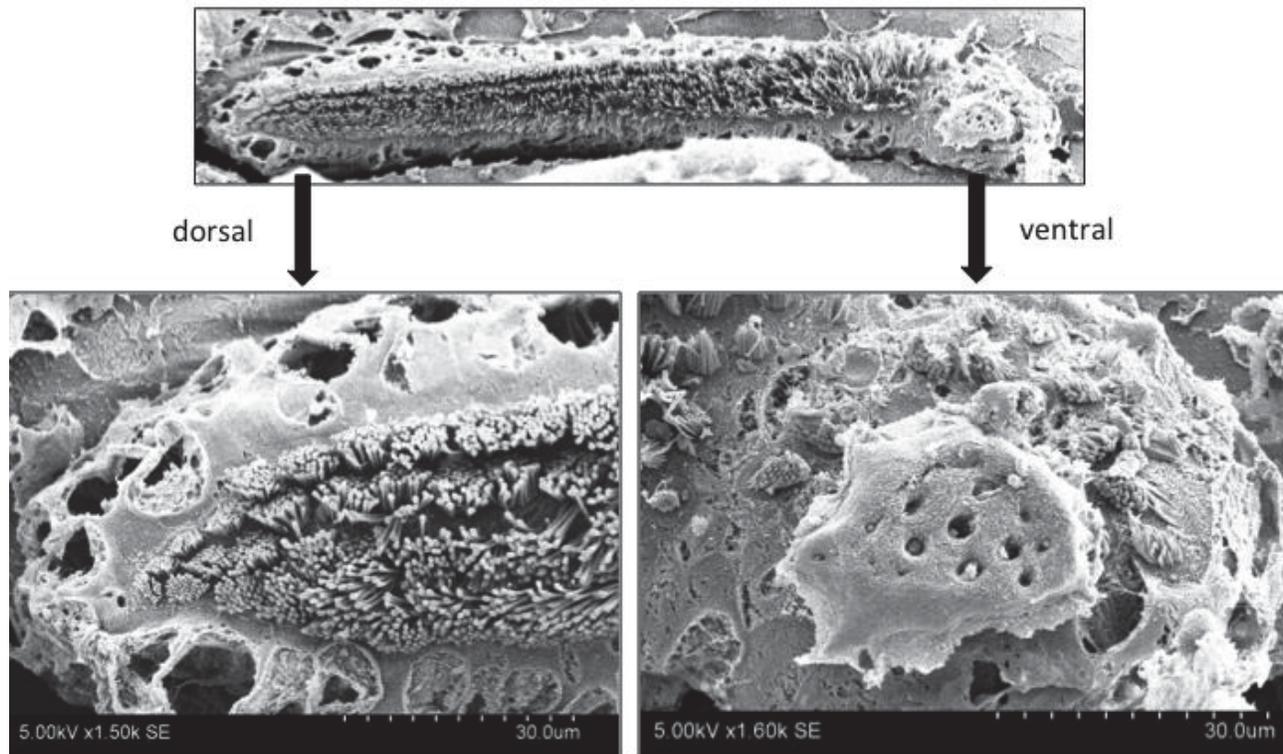


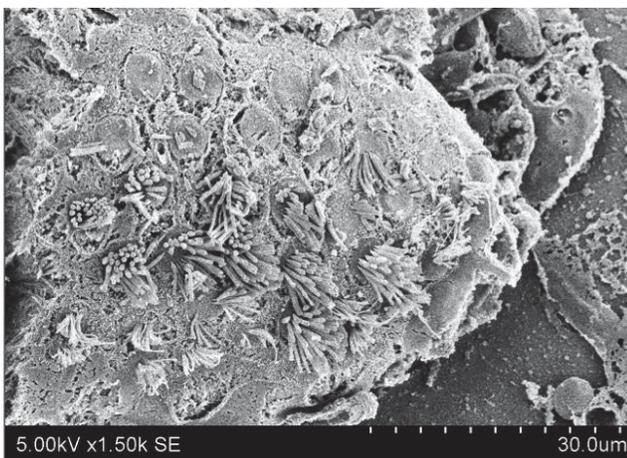
Figure 6: Changes in haircell stereocilia length according to position along the basilar papilla.



**Figure 7:** Haircells with short stereocilia at both extremes of the basilar papilla. At the dorsal tip (left image) small rudimentary haircells appear to be regenerating. At the ventral end (right image) remnants of a tectorial plate or sallet partially covers the short stereocilia haircells.

#### 4 Conclusion

We have provided here a complete description of methods to prepare inner ear specimens from the lizard species *Anolis carolinensis* for scanning electron microscopy. We have made a detailed description of the auditory end-organ, the basilar papilla, as revealed using SEM.



**Figure 8:** The ventral region of the basilar papilla showing short stereocilia haircells after removal of an overlying tectorial plate or sallet.

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