2001

Morphology of the Sacculle and Cochlear duct of amphisbaenians

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**MORPHOLOGY OF THE SACCULE AND COCHLEAR DUCT
OF AMPHISBAENIANS**

A Thesis

Presented to

The Faculty of the Department of Biological Sciences

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Mary Elizabeth Dawson

May 2001

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ABSTRACT

MORPHOLOGY OF THE SACCULE AND COCHLEAR DUCT OF AMPHISBAENIANS

by Mary Elizabeth Dawson

The evolutionary relationships (phylogeny) among squamate reptiles – lizards, snakes, and amphisbaenians – are not clear. Inner ear morphology has proven to be a useful phylogenetic tool, in part because the morphology of the basilar papilla varies greatly between families of lizards but varies little among snakes. The inner ear of the amphisbaenians has not been well studied. This research uses scanning electron microscopy to describe the ultrastructure of the sensory epithelia in the saccule and cochlear duct of four species of *Amphisbaena*. The basilar papilla in the cochlear duct most closely resembles that of snakes. The amphisbaenian basilar papilla is small and has a low density of hair cells with unidirectionally-oriented stereovillar bundles. The saccular macula and its associated otoconial mass suggest an ability to detect ground-borne acoustical signals. These data are compared to those known in lizards and snakes, and discussed with reference to the evolution of squamates.

ACKNOWLEDGEMENTS

Dr. Michael Sneary, who was creative enough to suggest the topic for this thesis, was an inspiration throughout my graduate career at San Jose State University; the fruition of this research is due in large part to his vision and unwavering optimism.

I wish to especially thank Dr. Richard Thomas of the University of Puerto Rico, who generously shared his passion for field work and his appetite for anchovy pizza. Dr. Wayne Savage, Dr. John Matson, and Dr. John Boothby helped shape these ideas and always challenged me to go further. The friendly and knowledgeable staff at the Microscope and Graphics Imaging Center at California State University, Hayward, contributed expertise at all stages of the electron microscopy.

All of the students, professors, and staff of San Jose State provided a stimulating and supportive environment in which to train as a scientist. Special thanks to Josephine Jose who always had the best lab tricks and the biggest smile.

My husband, Greg, and my brother, Wynne, who are now experts in the field of reptile ears, deserve many thanks for their encouragement, patience and love.

Dedicated to my father, with love.

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INTRODUCTION

The order Squamata (class Reptilia) is divided into three suborders: lizards, snakes, and amphisbaenia. Amphisbaenians comprise more than 150 species in four families and are found in tropical regions of the Americas, Africa, Spain, and the Middle East (Gans, 1978). Amphisbaenians are fossorial, burrowing temporary tunnels through soft soil in search of invertebrate prey. All are limbless except for the three species of the Mexican genus, *Bipes*.

The evolutionary history of squamates is not well known (Figure 1). According to currently accepted theories, all modern reptiles share an ancestry with an original stem reptile from the Paleozoic era. About 300 million years ago, this lineage branched into Lepidosauria (scaled reptiles) and Archosauria (ruling reptiles). During a rapid radiation about 100 million years ago, the Lepidosauria diverged into current-day squamates and *Sphenodon* (the tuatara of New Zealand) but how and when the three suborders of squamates separated is unclear. One of the most complete phylogenies of squamates that considers soft tissue characters as well as osteological characters (Estes et al., 1988) places three taxa – amphisbaenians, dibamid lizards, and snakes – *incertae sedis*, and suggests that they diverged after geckos and pygopods. A phylogenetic study built solely on skeletal material (Lee, 1998) suggests that amphisbaenians share a clade with dibamids, and that both share ancestry with geckos and pygopods. A novel phylogeny based solely on the morphology of tongue and lingual characters shows a close relationship between amphisbaenians, teiids and gymnophthalmids (Schwenk, 1988).

As early as the 1950's, researchers found that the inner ear anatomy was a useful

tool for characterizing squamates (Shute and Bellairs, 1953). Subsequent research has described many of the details of inner ear anatomy and auditory function in a wide range of lizards and snakes (Schmidt, 1964; Miller, 1966, 1968; Wever, 1978). In particular, Miller (1966, 1968) demonstrated that the auditory sensory epithelium, the basilar papilla, varies at the family level in lizards but little at all in the families of snakes. A case in point are the structure parameters of the stereovillar bundles, located at the apical surface of the sensory cells, the hair cells. Each bundle consists of an array of microvilli (stereovilli) arranged in increasing height, the tallest members of which are adjacent to a true cilium (kinocilium). The stereovilli of these hair cell bundles are all oriented in the same direction in snakes, turtles, crocodilia, and *Sphenodon*, but have complex patterns of orientation in lizards.

The inner ear of reptiles is a labyrinth of spaces enclosed in an otic capsule comprised of prootic, opisthotic, and exoccipital bones (Wever, 1978). This labyrinth consists of two parts. The inner endolymphatic labyrinth is a complex of interconnected, fluid-filled chambers containing a number of sensory organs. An outer perilymphatic labyrinth is defined by the surrounding bone and is also filled with fluid. The largest chamber of the endolymphatic labyrinth, the saccule, contains a sensory macula (epithelial patch) in the medial and ventral walls. The saccular macula is a specialized population of hair cells typically covered by a large otoconial membrane which encloses a mass of crystalized biomineral otoconia. Throughout the vertebrates, the saccular macula has been shown to be involved in transducing acoustic signals – both airborne auditory sounds and ground-borne vibrations (seismic signals) (Koyama et al., 1982).

Joined to the saccule at the caudal end is a cochlear duct that includes the basilar papilla and lagenar macula sensory organs. The cochlear duct can be constricted as to almost divide the space into two separate sacs or may have little constriction at all. The sensory lagenar macula is situated along the distal end of the cochlear duct. A raised basilar papilla is in the more proximal region of the cochlear duct. The shape of the papilla and the relative sizes of the basilar and lagenar regions of the cochlear duct vary among suborders of squamata and families of lizards. The basilar papilla is the primary auditory sensor while the function of the lagena is less well established.

Surrounding these endolymphatic chambers are the tubular semicircular canals. These three canals connect into a small sac known as the utricle. Each canal has an expanded ampulla with hair cell population, the cristae, known to respond to angular acceleration of the head in space. The utricle has its own macula of unknown function.

All of the sensory epithelia in the inner ear are innervated by the acoustic (eighth) cranial nerve.

The morphology of the inner ear organs of the least-known suborder, amphisbaenia, is not well described. Indeed, there is little information concerning the auditory system of this squamate (Miller, 1966; Gans and Wever, 1972, 1975; Wever and Gans, 1972, 1973; Wever, 1978). A few studies have described the middle and inner ear anatomy and auditory function. Particularly interesting are the adaptations of the middle ear, including a novel extracolumella that attaches the dermal pad of the facial region to the columella and to the stapedial footplate of the inner ear. This extracolumellar structure aids in the conduction of air-borne sounds and ground-borne vibrations to the

sensory organs of the inner ear. Cochlear microphonic responses showed peak sensitivity to air-borne sounds in the range from 300 to 700 Hz, and substrate-borne vibrations in the range of 500 to 1500 Hz, although sensitivity to sounds and vibrations below 100 Hz were not studied (Gans and Wever, 1972, 1975; Wever and Gans, 1972). The ability to detect airborne sounds was severely impaired when the extracolumella was disconnected; sensitivity to vibrations was less affected by the absence of the extracolumella. Anatomical details of the middle and inner ear structures were studied with light microscopy (Miller, 1966; Gans and Wever, 1972, 1975; Wever and Gans, 1972, 1973; Wever, 1978). The 20 μm sections prepared for these studies were not sufficient to provide details concerning the morphology and orientation of the hair cell bundles.

This study uses scanning electron microscopy to reveal the ultrastructure of the saccule and cochlear duct in four species of the genus, *Amphisbaena*. In particular, the surface features of the saccular macula and basilar papilla are described and compared with those of lizards and snakes. A possible relationship between structure and function of these organs is discussed, with comments on the evolution of the inner ear morphology in burrowing squamate reptiles. The value of inner ear morphology as a phylogenetic tool is presented, with a focus on needs for future research in this area.

MATERIALS AND METHODS

Adults of four species of the genus *Amphisbaena* were collected in Puerto Rico: *Amphisbaena bakeri*, *A. caeca*, *A. schmidtii*, and *A. xera*. Weights of the specimens ranged from 3 to 9.5 grams, with snout-vent lengths from 15 to 18.5 cm. After the

animals were sacrificed (IACUC protocol #650), the temporal muscles were removed to expose the bony otic capsule. This capsule was opened with a pair of fine Rongeur forceps, and the membranous labyrinth of the inner ear flushed with fixative (3% glutaraldehyde in sodium cacodylate buffer). Initial specimens were fixed in 0.1M buffer, which produced artifactual extrusions near the stereovillar bundles of the hair cells. Later specimens prepared in 0.2M buffer did not have the extrusions. The head was then placed in fresh fixative and stored at 4°C for 2-4 hours. Further dissection exposed the inner ear tissue which was removed to fresh fixative and placed in 4°C for 2-4 hours to complete the process of chemical fixation. Specimen damage was minimized by placing tissue in microcontainers.

When fixation was completed, the tissue was dehydrated in a graded series of ethanol solutions, immersed for two minutes each in 70%, 80%, 90%, and twice for two minutes each in 95% and 100% ethanol. The ears were then transferred to a DCP-1 (Denton Vacuum, Inc.) critical point dryer and dessicated using carbon dioxide. The dried tissue was mounted on aluminum stubs and further dissected to expose the saccular macula and basilar papilla. After sputter coating with gold-palladium using a Hummer II (Technics, Inc.) to a depth of 250-300 Ångstroms, the specimens were examined in a Philips XL 40 scanning electron microscope at the Microscope and Graphics Imaging Center, California State University, Hayward. Digital images were analyzed using Photoshop® 6.0 (Adobe, Inc.).

Hair cell numbers on the basilar papilla were obtained by counting the number of distinct, visible bundles. In the saccular macula, hair cell bundle numbers were obtained

by counting the number of bundles and the holes in the overlying otoconial membrane, assuming that each hole in the membrane corresponds to a single bundle. All measurements are given for the dessicated tissue. A 20% shrinkage of tissue from the chemical and mechanical preparations is assumed.

RESULTS

Inner ear anatomy

The dorsal portion of the inner ear of amphisbaenians is comprised of a large saccular space with a specialized macular epithelium that lies in a mostly horizontal, dorsal-facing orientation. The entire sacculus is approximately 1.0 mm x 0.6 mm in size. The cochlear duct is connected to and lies ventral to the sacculus and is an ovoid chamber approximately 500 to 750 μm rostral-caudal by 350 μm dorsal-ventral. The basilar papilla (BP) sits at the caudal end of the cochlear duct, ventral to the sacculo-cochlear duct (Figures 2, 3). A lagenar macula is located at the rostral region of the cochlear duct. Results for three specimens are summarized in Table 1.

Basilar papilla ultrastructure

The BP is a small, cylindrical epithelium of uniform width, ranging from a smallest specimen of 50 by 90 μm to a largest of 95 by 125 μm . The height of the papilla, including supporting cells and hair cells, is approximately 50-60 μm from the basilar membrane, which closely surrounds the base of the papilla (Figure 4). Along the length of the papilla, hair cells are loosely organized into rows and are surrounded by a

substantial area of the apical surface of the supporting cells. The number of hair cells ranges from a low of 80 in the smallest animal, *A. xera*, to a high of 125 in the largest specimen, *A. caeca*. The density of hair cells ranges from a low of 105 to a high of 178 hair cells per 10,000 μm^2 . A considerable space between each hair cell apical surface is comprised of supporting cell surface. A tectorial plate attaches to the neural side of the midregion of the BP. This substantial structure appears to cover most of the hair cells, with the possible exception of a few bundles at the lagenar and saccular ends of the papilla. The structure is uniform in appearance over the surface of the BP.

Hair cell bundles

The orientation of the stereovillar bundles (Figures 5,6) in the BP is predominantly unidirectional, toward the abneural side of the papilla. A few outlying bundles on the lagenar and saccular ends of the papilla comprise small, roughly bidirectional populations oriented toward the midpapillary axis. The number of stereovilli in each bundle ranges from 80 to 110 in seven bundles observed. The stereovilli are arranged in staggered rows, with a kinocilium adjoining the villi of the tallest row.

Saccule

The L-shaped sensory epithelium of the saccule is 400-500 μm long by 75-90 μm wide, and is located on the ventral face of the saccule. Unlike the basilar papilla, the saccular epithelium is embedded in the surrounding tissue and not raised above it. The apices of the hair cells and supporting cells are contiguous with the epithelial cells of the

surrounding cochlear duct wall. This saccular macula is covered by an overlying otoconial membrane enclosing a large otoconial mass, approximately 1.0 to 1.5 mm in diameter, that fills the saccular space (Figure 7). This mass is composed of individual otoconia suspended in a fluid medium. The stereovillar bundles project into holes in the overlying otoconial membrane (Figure 8). The saccular macula consists of 650-700 hair cells, giving this epithelium a hair cell density of 155-220 hair cells per 10,000 μm^2 . The hair cell stereovilli at the edge of macula have kinocilia much longer than the tallest stereovilli and with pronounced end bulbs (Figure 9). Hair cells at the center of macula have shorter kinocilia of height equal to that of the tallest stereovilli (Figure 10). The otoconial mass fills the entire saccular space. The two saccular spaces are so large that the surrounding otic capsule significantly reduces the volume of the midline brain cavity.

DISCUSSION

The inner ear gross anatomy observed in these specimens of amphisbaenians resembles that described (Miller, 1966; Gans and Wever, 1972, 1975; Wever and Gans, 1972, 1973). The location of the organs and sizes of the sensory structures are within the ranges reported earlier. New information presented here includes description of the bundle morphology and a clarification of the hair cell orientation patterns. In the specimens studied here and those previously reported, there is little difference in the morphology of the inner ear structures among amphisbaenians.

The basilar papilla of amphisbaenians is most similar to that of snakes. The papilla is a uniform shape with predominantly unidirectional hair cell bundles. Some

bundles at the lagenar and saccular ends of the papilla are oriented toward the midpapillary axis, a pattern also seen in primitive snakes, the boids (Miller, 1968).

The unidirectional orientation of the hair cell bundles is also similar to the morphology of the region of lizard basilar papillae known to be sensitive to low frequencies (Köppl and Manley, 1992). In addition, the papilla is relatively small (100-300 μm in length) and does not have anatomical gradients such as changes in the width or shape along its length, morphologies also seen in the low frequency area of lacertilian basilar papillae.

Miller (1980) has described certain BP characteristics as “primitive” or little changed from the ancestral reptilian form. Of the seven of these features visible by SEM, five are of the “primitive” state in amphisbaenians: a low density of hair cells, unidirectional populations of hair cells, a large number of stereovilli per hair cell bundle, an unspecialized tectorial membrane, and no arrangement of hair cell bundles into distinct rows along the length of the papilla. The presence of a unidirectional hair cell population in the basilar papillae of turtles, *Sphenodon*, and crocodilia suggests that this orientation does represent the ancestral form in reptiles (Figure 11). The density of hair cells in the basilar papilla is one of the lowest reported for any reptile. With a calculated density of 105-178 hair cells for 10,000 μm^2 , amphisbaenians have a lower density than the “low” value for snakes of 250 hair cells per 10,000 μm^2 but a comparable value to the 150-250 hair cells per 10,000 μm^2 for turtles (Miller, 1980). By contrast, lizards have higher density, 300-400 hair cells per 10,000 μm^2 (Miller, 1980).

Nevertheless, two features ascribed to the primitive form are not seen in

amphisbaenians. Specifically, there is no noticeable separation of the lagena and BP in the cochlear duct and the basilar membrane is not significantly wider than the BP.

Although the morphology of amphisbaenian basilar papillae described here may represent the primitive reptile form, these characteristics may in fact be due to the selective processes of a burrowing lifestyle and the selective forces acting on auditory and seismic sensors in a subterranean environment. The amphisbaenian inner ear may demonstrate a divergence that suggests specialization for subterranean habitats.

Burrowing snakes such as Typhlops typically have larger and longer papillae (Miller, 1980), but this may be a relic from their recent terrestrial ancestors. Indeed, the selective pressures for obligate burrowers that create their own tunnels using reinforced skeletal and musculature structures of the head may compromise hearing (Wever and Gans, 1972). Further study of a broad range of squamate burrowers, especially typhlopids, boids, dibamid lizards, and annelids (legless lizards) could help elucidate the relationships between structure and function of acoustic sensors in fossorial reptiles.

Two features of the amphisbaenian saccule are interesting and suggest a particular sensitivity. First, there is a tight connection between the hair cell bundles and the overlying otoconial membrane. Previous reports discuss the importance of this morphology in networking the population of hair cells in a way necessary for auditory and seismic sensitivity (Lewis, 1987). The particularly large otoconial mass is also indicative of seismic sensors (Lewis, 1987).

Continued research on the auditory and seismic physiology of amphisbaenians is needed to clearly describe the function of the inner ear end organs. In particular,

electrophysiological research could characterize the possible seismic sensitivity of the amphisbaenian inner ear. Such data has already been presented for frogs (see Koyama et al., 1982; Narins and Lewis, 1984; Yu et al., 1991). In addition, a comprehensive review of the morphology and physiology of these organs in fossorial reptiles would be useful in determining the evolution of auditory and seismic function in squamate reptiles.

Additional research could also focus on further clarifying the morphology of inner ear sensory epithelia in specimens from all four families of amphisbaenians. Earlier research (Gans and Wever, 1972, 1975; Wever and Gans, 1972, 1973) suggests that there are no relevant differences among families of amphisbaenians. More complete studies of amphisbaenians and other squamates might prove that sensory systems are a valuable data set for phylogeny, as has been the case in lizards (Miller, 1966).

In summary, the cochlear duct and basilar papilla of amphisbaenians most closely resemble those of snakes. The structure of the saccular macula and its otoconial mass suggest seismic function. The morphological data presented here provides a basis for further electrophysiological studies on a wider range of burrowing squamates. This research could provide important evidence to the relationship of structure and function of acoustic sensors in fossorial reptiles.

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APPENDIX: TABLES AND FIGURES

Figure 1. Evolution of squamates. Along with *Sphenodon*, the three current-day suborders of Squamata evolved from Lepidosauria sometime during the Mesozoic era. About 100 million years ago, a rapid adaptive radiation of squamates diverged into the contemporary suborders: lizards, snakes, and amphisbaenians. However, the exact nature of the radiation of Squamates is not understood.

Figure 2. The head and inner ear of amphisbaenians. This representation of the amphisbaenian head shows the position of the inner ear. The L-shaped saccular macula is visible in the saccule, while the basilar papilla lies in the cochlear duct, just ventral to the sacculo-cochlear duct. The asterisk indicates the location of the lagenar macula, although the shape of this structure is uncertain. A ventrocaudal reunient duct connects the saccular space with the cochlear duct. Drawing by A. Kappl and B. Dawson.

Figure 3. The saccular space and cochlear duct of *Amphisbaena caeca*. In this figure, the saccular space is positioned above and slightly to the right of the cochlear duct (large outline). The basilar papilla (small outline) is just visible as an ovoid shape on the lower left edge of the cochlear duct, at a caudal position. The lagenar macula is located on the lower right region of the cochlear duct.

Species	Wt (g)	Snout-Vent Length (cm)	Saccule (mm)	Saccular Macula Area (μm)	No. of Hair Cells, Saccular Macula	Oto-conial Mass (mm)	Coch-lear Duct (μm)	Basilar Papilla (μm)	No. of Hair Cells, Basilar Papilla
<i>A. bakeri</i>	3.5	17		400 x 75	650-700	1.5 x 1.5	500 x 350	115 x 55	
<i>A. caeca</i>	8.3	18.3	1.0 x 0.6				800 x 350	95 x 125	125
<i>A. xera</i>	3.8	16.5				1.5 x 1.0		60 x 95	80

Table 1. Summary of data. These data were acquired from three specimens, one each of three of the species studied. Not all measurements were possible from each specimen due to dissection and mounting techniques.

Figure 4. Basilar papilla of *A. caeca*. The basilar papilla (BP) is raised, with a relatively low density of hair cells. The hair cell bundles are mostly unidirectional toward the abneural side of the papilla (direction indicated by the arrow), although some bundles at the lagenar and saccular ends of the BP are oriented toward the midline of the papilla. The round protrusions at the base of the stereovillar bundles are artifacts of the early fixation process.

Figure 5. Stereovillar bundle of the basilar papilla of *A. xera*. A stereovillar bundle from the basilar papilla shows the staggered arrangement of the villi. A kinocilial bulb is visible behind the tallest row of stereovilli.

Figure 6. Stereovillar bundle of the basilar papilla of *A. bakeri* . Lines indicate the staggered row arrangement of the stereovilli. The ovoid kinocilial bulb (*) is visible on the left side of the stereovillar bundle.

Figure 7. Saccular macula of *A. schmidtii*. The saccular macula is covered by an otoconial membrane, which extends upward to surround the overlying mass of otoconia. There are approximately 650-700 hair cells in this end organ.

Figure 8. Saccular bundles and otoconial membrane of *A. caeca*. The overlying membrane in the saccular macula fits closely over the stereovilli of the hair cells.

Figure 9. Stereovillar bundles in the saccular macula of *A. schmidti*. The stereovillar bundles at the edge of the saccular macula have long kinocilia that extend well above the tallest stereovilli. The extrusion at the base of the stereovilli is artifactual.

Figure 10. Stereovillar bundles in the saccular macula of *A. caeca*. The stereovillar bundles near the middle of the saccular macula do not have pronounced kinocilia.

Figure 11. Directionality of hair cell bundles in non-lacertilian reptiles and a mammal. All reptiles have unidirectional hair cell bundles, except for lizards which have hair cell bundles that show complex patterns of orientation. Amphisbaenians have relatively small basilar papillae, with almost entirely unidirectional hair cell bundle orientation. Mammals have coiled cochlea with unidirectional hair cell bundles. Not to scale. (Adapted from Miller, 1980, with permission).

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Date Published: 1980

Figure: 6-4 (a), page 173

Dr. Miller's figure shows the shape of the basilar papilla and orientation of hair cell bundles in four of the six orders or suborders of the class Reptilia: sphenodon, turtle, snake and crocodilia as represented by a caiman. A fifth suborder, the lizards, are described in Figure 6-4 (b). The sixth, a little-known squamate reptile - amphisbaenia - was not included because the shape and orientation were not known for this animal.

I have recently completed my master's research on the morphology of the cochlear duct and basilar papilla of four species of amphisbaenians. Using scanning electron microscopy, I describe the shape of the basilar papilla and the orientation of the hair cell bundles on its surface. The results show that the amphisbaenian basilar papilla resembles that of snakes.

This research has been done in the neurophysiology laboratory of San Jose State University, under the guidance of Dr. Michael Sneary. Dr. Sneary completed his Ph.D. at University of California, San Francisco, with Dr. Miller.

I would like to ask permission to use Dr. Miller's Figure 6-4 (a) to create a new figure that includes the amphisbaenian basilar papilla. This figure would be published in my master's thesis, published by California State University, and would be submitted for publication in the journal Hearing Research, published by Elsevier Science.

Sincerely,

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March 12, 2001

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