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Microscopic Structure and Digital Morphometric Analysis of the Statoconia of Hagfish, *Paramyxine nelsoni* (Myxiniformes)

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Yi-Hsin Lee, Hung-Tu Huang, and Hin-Kiu Mok (2007) Microscopic structure and digital morphometric analysis of the statoconia of hagfish, *Paramyxine nelsoni* (Myxiniformes). *Zoological Studies* **46**(1): 1-5. The structure and parameters of the statoconia (including the size, shape, number, and setting) in the macula communis in glycol methacrylate sections of the ventromedial side of the labyrinth of the hagfish, *Paramyxine nelsoni* (Myxinidae), are reported. The objective of this study was to ascertain if a relationship exists between body length, a parameter relating to age, and traits of the statoconia. Variations in these parameters with body size were investigated. The number of statoconia increased with body length. Individual hagfish contained statoconia of various sizes ranging from 1 to 26 μm. Small statoconia were present even in adults that had already reached a maximum body size, while very large statoconia were found in smaller individuals. Statoconia have a clear core and a few concentrically arranged alternating dense and clear layers. Statoconia are extracellular concretions that probably originate from vacuolated epithelial cells in the macula communis, and large statoconia are the result of the fusion of smaller statoconia. We concluded that these parameters of the hagfish's statoconia contain no information which may be useful in estimating hagfish age.
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Key words: Hagfish, Statoconia, *Paramyxine nelsoni*.

In the inner ear of craniates, the sizes of the statoliths greatly vary. Small (with a diameter of < 50 μm) and large (> 50 μm) statoliths are respectively termed statoconia and statoliths (Maisey 1987). The statoconia of hagfish and lamprey are aggregates of apatite which is composed of calcium phosphate and carbonate. Their rather-high carbonate contents prevent crystallization of the apatite (Carlstrom 1963). Consequently, the statoconia of *Myxine* and *Petromyzon* consist of poorly crystallized and noncrystalline phases, respectively (Maisey 1987). Unlike cyclostomes, gnathostome statoconia are made of vaterite which is characterized by its unstable calcium carbonate polymorph (Maisey 1987). Dense bodies of mineralogical calcium carbonate form into polycrystalline aggregates and even single crystals (Maisey 1987). The nonhomogenous crystallization pattern in the statoliths of a gnathostome show a certain correlation between the concentric rings of the statolith and the age of the fish. Consequently, statoliths have been used as a tool for age determination in gnathostomes (e.g., Beckman and Wilson 1995).

The noncrystalline statoconia of *Lampetra* (with diameters ranging from 2 to 25 μm) appear as transparent spheres and exhibit concentric layering (Carlstrom 1963). The largest one recorded was 250 μm, had layered structures (i.e., displayed a pattern of alternating dark and opaque bands), and was not considered to have been formed by the fusion of smaller statoconia (Carlstrom 1963). Volk (1986) and Beamish and Medland (1988) described a positive relationship

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between the number of dark bands in a statolith and the total body length in larval sea lampreys, *Petromyzon marinus*, and American brook lampreys, *Lampetra appendix*. They found that 1 narrow black band and 1 broad opaque band were respectively produced each winter and summer.

In the hagfish's otic capsule, the macula communis is covered by numerous globular statoconia, which rest on a thin gelatinous layer connected to the supporting cells of the sensory epithelium. Statoconia at various stages of formation can be found inside or outside the macula communis (Jørgensen et al. 1998). However the actual process through which statoconia form remains unclear.

Due to the poor crystallization of hagfish's statoconia and the absence of other bony structures (such as scales and vertebrae), it remains difficult to determine the age of hagfish. One of the few studies on the statoconia of hagfishes focused on *Myxine glutinosa* (Carlstrom 1963). Concentric rings were reported in its statoconia (Jørgensen et al. 1998). However, Jorgensen and coworkers did not discuss the possible relations of the number of rings and the size of the statoconia with the age (or body length) of the hagfish. The objective of this study was to investigate whether a relationship exists between body length, a parameter related to the age of a hagfish, and traits of the statoconia (e.g., the number, size, and shape).

Paramyxine nelsoni, which was selected for this study, is quite common in the southwestern coastal waters of Taiwan (Mok and Chen 2001). Due to its small body size (with a maximum total body length of about 30 cm; Kuo and Mok 1999), it is of no commercial value. Little information on its general biology is available in the literature (Huang et al. 1994, Kuo and Mok 1999).

MATERIALS AND METHODS

Twenty-four specimens of *P. nelsoni* were captured using shrimp traps, and 30 specimens were obtained from the by-catches of commercial trawlers. Identification was based on the diagnostic characters given by Kuo and Mok (1999). The body length of each fish was measured.

The left otic capsule containing a single semicircular duct was removed from 54 fresh specimens and immersion-fixed in 0.1 M phosphate buffer containing 5% glutaraldehyde and 4% paraformaldehyde (pH 7.2) at 4°C for several days. They were then transferred to 0.1 M phosphate buffer for the advanced process as described next.

In order to observe the detailed external morphology of the macula communis and statonia, otic capsules from 5 specimens were dissected to expose the irregularly ring-shaped membranous inner ear (also called the labyrinth). Along the anterior and posterior sides, it has an ampulla-like swelling (Jørgensen et al 1998). The single macula communis lies along the ventromedial side of the labyrinth (Fig. 1A). The space of the inner ear where the macular communis is located may be the homologue of the utriculus of vertebrates. Dissected tissue specimens with the macula communis were then post-fixed in 1% OsO_4 in 0.1 M phosphate buffer, dehydrated in a graded series of alcohol (from 70% to 100%), dried in a critical point dryer, and coated with an ion-sputter coater. The coated samples were examined under a scanning electron microscope (SEM, Hitachi-s2400).

As the SEM can only provide information on the top layer of the statoconia, histological sectioning is required to gain information concerning the entire mass of the statoconia through which the numbers of statoconia can be estimated. Therefore, semi-thin sections of the otic capsules from the remaining 49 specimens were prepared. They were washed with 0.1 M phosphate buffer for 24 h, dehydrated in a graded series of acetone (from 70% to 100%), and infiltrated and embedded with glycol methacrylate. Cross-sections, 2 μm thick, were cut with a glass knife on a Supercut (Leica, Jung Rm2065) and stained with toluidine blue. The mounted slides were observed under a light microscope. One cross-section of each macula communis was selected for each of the 49 otic capsules of the examined specimens. Statoconia on each section were counted. The regression coefficient was calculated as an index of the relationship between the number of statoconia and body length.

In order to describe the shape of the statoconia, the long and short diameters of each statoconia in the plastic sections were measured using the software Photoshop. A statoconium was said to be globular when no long and short diameters could be differentiated. A frequency distribution of the long diameter versus the body-length classes (10.2-29.5 cm) was prepared. Correlations of the number, shape, and size of the statoconia with body length were estimated.

RESULTS

Location of statoconia

The macula communis occupies the ventromedial floor of the ventromedial side of the labyrinth (Fig. 1A). The macula communis consists of pseudostratified columnar epithelium and a mass of statoconia over the epithelium (Fig. 1B, C). The pseudostratified columnar epithelium is composed of hair cells and supporting cells. Many cells contained vacuoles with diameters of around 7 μm (Fig. 1C). Large statoconia were only present on the top layer of the mass, while smaller ones were close to the cells of the macula communis (Fig. 1C).

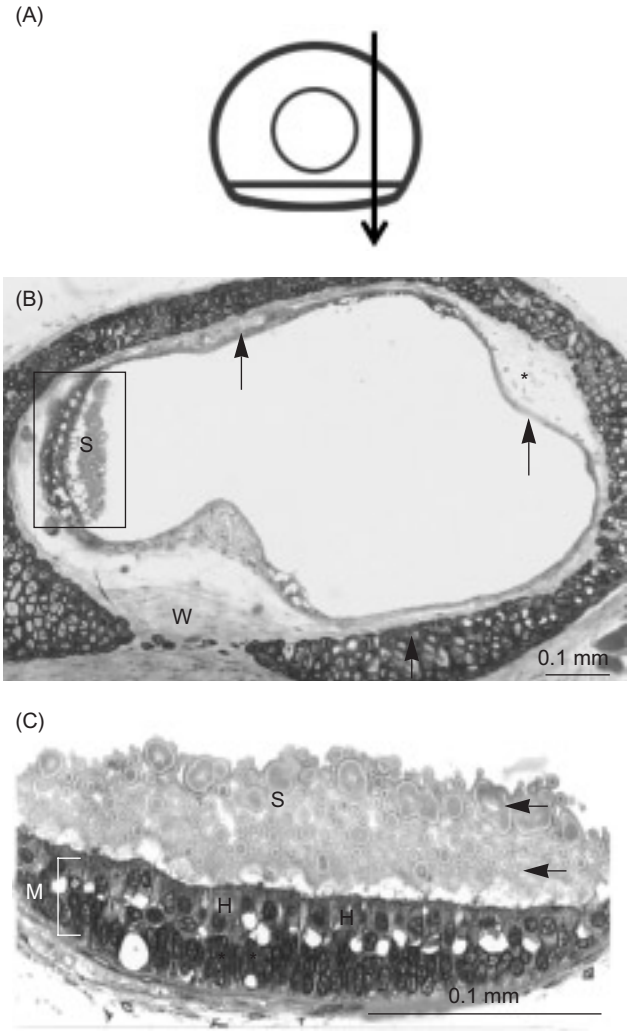

Fig. 1. Histological structure of the inner ear of *Paramyxine nelsoni*. (A) Schematic diagram showing the direction of the section (arrow) passing through the lumen of the semicircular duct which is vertical to the macula. (B) Photomicrograph of a sectional profile of the hagfish semicircular canal. The membranous duct (arrows) is separated by a connective tissue space (*) from the cartilaginous capsule (arrowhead). A membranous window structure (W) is on the medial side of the cartilage capsule. The mass of the statoconia (S) is located on the surface of the macular epithelium. (C) The epithelium of the macula (M) is of a pseudostratified columnar type wihch consists of hair cells (H) and supporting cells. Large statoconia (arrow) were only present on the top of the mass, while the smaller ones (arrohead) were close to the macula epithelial cells.

Number of statoconia

Totals of 72-1055 statoconia were recorded in the sections of the macula communis. The number of statoconia (*Y*) in each section increased linearly with body length (*X*) ($Y = 20.72X$, $p < 0.01$, $n = 49$; Fig. 2). However, the number of statoconia with a diameter of > 6 μm did not increase with body length (also see below).

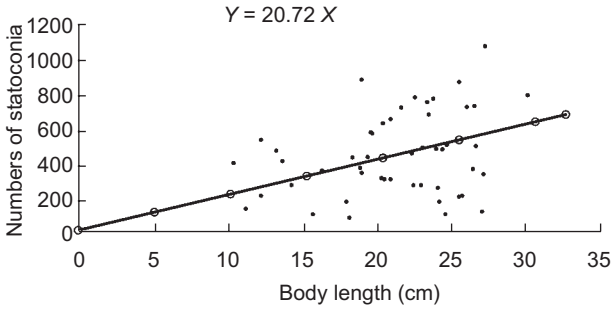

Fig. 2. The number of statoconia (*Y*) significantly related to the body length (*X*) of *Paramyxine nelsoni* according to the equation $Y = 20.72X$.

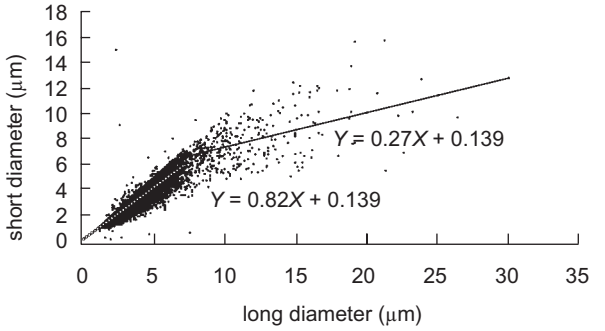

Fig. 3. Linear regression of short and long diameters of statoconia of *Paramyxine nelsoni*. The ratio of the short to the long diameter was smaller (more oval in shape) in larger statoconia (with a long diameter of > 6 μm) than in smaller statoconia.

Shape of the statoconia

Small statoconia were globular, and the shape seemed to change with size (becoming elliptical). The short and long diameters were positively correlated, with an obvious change in the slope of the correlation lines at 6 μm (Fig. 3). The ratio of the short and long diameters was smaller (more oval in shape) in larger statoconia (with the long diameter of > 6 μm) than in smaller statoconia (Student's *t*-test; df = 1046, $p < 0.001$; Fig. 3).

Size of the statoconia

The long diameters ranged from 1 to 26 μm with most of the statoconia between 4 and 8 μm. Statoconia with a 5 μm diameter were the dominant size group. Statoconia < 4 μm in the long diameter were observed in specimens of all sizes.

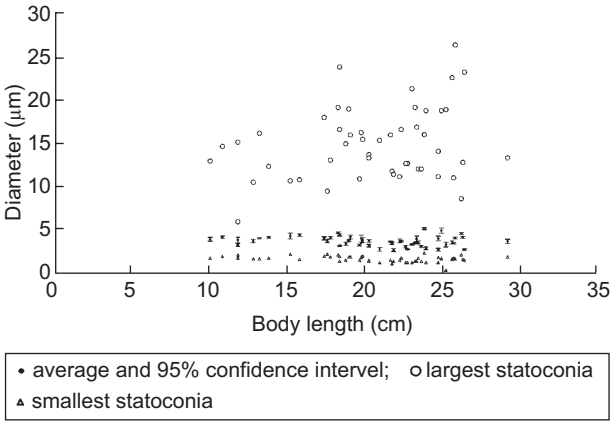

Fig. 4. Average diameters of statoconia and the 95% confidence interval of each specimen. The diameters of the statoconia did not increase with body length. The smallest statoconia of each specimen were about the same size (2 μm), and the largest statoconia exceeded 10 μm in diameter.

Statoconia > 8 μm were uncommon, but they were also found in some of the smallest individuals examined (Fig. 4).

Structure of the statoconia

Sectional profiles of the statoconia were not evenly stained by toluidine blue. They had concentrically arranged alternate dense and clear layers (Fig. 1C). Larger statoconia had a lightly stained central core (Fig. 1C). SEM also showed the nature of the multilayered structure (Fig. 5A, B). The number of these layers did not change with the size of the statoconia. No statoconia contained more than 1 core.

DISCUSSION

As larger statoconia were found at the top of the statoconia mass and smaller ones were found in the lower layer, an extracellular size increment is believed to have occurred in the smaller ones. The alternate arrangement of dense and clear layers around the central clear core of a statoconium suggests evidence of a concentric accumulation of material leading to an increase in size. Although no statoconia had more than 1 core, the larger statoconia are believed to be aggregates of 2 or more smaller statoconia.

Hagfish statoconia are structurally similar to extracellular concretions in the gerbil pineal gland (Welsh and Reiter 1978, Welsh 1985). Occurrence of extracellular pineal concretions is closely related to vacuolated pinealocytes. These come from the breakdown of vacuolated pinealocytes. Furthermore, the number of gerbil pineal concretions is dependent on the age (Welsh and Reiter

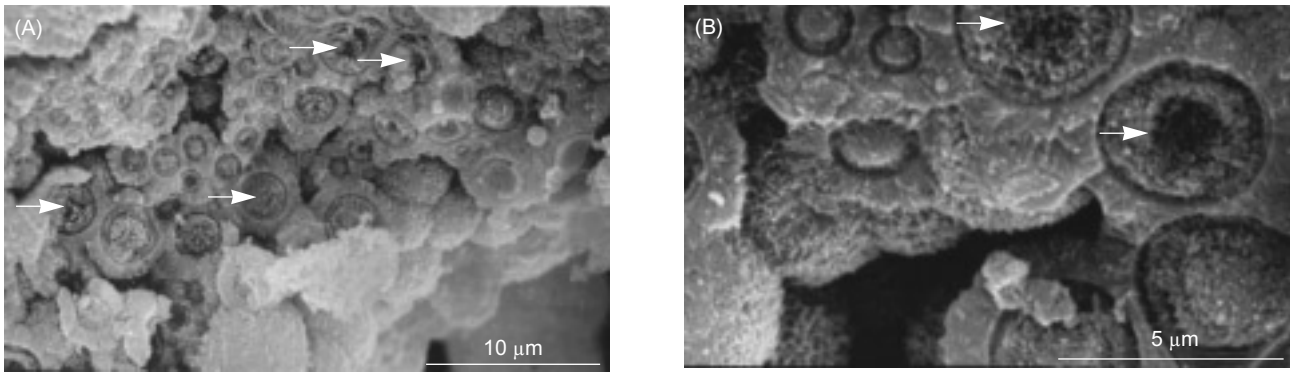

Fig. 5. Scanning electron micrographs showing the internal structure of the statoconia. (A) Layered structure (arrow) of a statoconium. (B) Higher magnification of a statoconium showing the dense layer (arrow) surrounding the central core.

1978, Welsh 1985). In the present study, it was interesting to discover that the number of statoconia in the hagfish macula communis increased with body length. There were many vacuolated epithelial cells in the macula communis. The statoconia probably originate from vacuolated cells.

The appearance of the smallest size-class of statoconia in the maximum body-size class suggests that statoconia are produced throughout the life span of *P. nelsoni*. An informative character for age should exhibit feature(s) that correspond to a certain period of time or some periodic environmental phenomenon (Helfman et al. 1997). The appearance of opaque and light bands in a fish otolith or scale in which such bands are numerous has been suggested to be an indication of a temporal growth cycle. Unfortunately, even though hagfish statoconia display bands, the total numbers of these bands were too few to be a possible clue for the age of the fish. We show that hagfish statoconia are not likely to be a structure suitable for age determination as (1) the size range of the statoconia in each individual was large, (2) the layered structure cannot easily be observed so that the number of the layers is not readily available, and (3) this number was low so it is not likely to have been a result of a rather rapid and periodic accumulation process.

Paramyxine nelsoni is a rather small species with a maximum body size of < 30 cm (Kuo and Mok 1999). If a hagfish stops increasing its body length after reaching the maximum size for the species, then a high variation in the statoconia parameters in specimens with the maximum body length is expected. A study along these lines should be carried out. Small statoconia were observed in specimens of all size classes suggesting that statoconia continue to be produced during growth.

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