

Otoconia From Four New Zealand Chimaeriformes

K. P. Mulligan, R. W. Gauldie, and R. Thomson

ABSTRACT: A scanning electron microscopy and x-ray diffraction investigation of chimaeriform otoliths shows densely packed and strongly bound aggregations of otoconia in the form of aragonitic spherulites. Characteristic sizes, shapes, and surface features are described for each of the four species investigated. Otoconial diameters differ among species, but the chimaeras are nonetheless a uniform group in terms of otoconia type and otolith shape.

Members of the Class Chondrichthyes (elasmobranchs and Chimaeriformes) are known to have otoliths composed of otoconia bound in a protein matrix (Stewart 1903; Iseltöger 1941; Carlström 1963; Barber and Emerson 1980). The use of the term otolith in reference to chondrichthyan fish requires some clarification. There are difficulties in establishing chemical or crystalline homologies between aggregated otoconia and otoliths proper as they occur in teleosts. However, they are similar in being semirigid or rigid structures supported on a hair cell pad and involved in sound transduction in both elasmobranchs and teleosts (Popper and Fay 1977; Fay 1983). When homologies are established, a general term for a crystalline, calcium carbonate structure associated with sound transduction may appear, but until then we will persist in using the term otolith in a more general sense than its derivation implies.

Studies on the otolith of the chimaera *Callorhynchus milii* (Callorhynchidae), which is found in inshore waters of New Zealand, showed that it was composed of fused spherulitic otoconia (Gauldie et al. 1987). Spherulite otoconia were one of three differing forms of otoconia, which were found in the Australian lungfish *Neoceratodus forsteri* (Gauldie et al. 1986a), and were sim-

ilar to the type of free otoconia found in conjunction with the otoliths of some teleosts (Dale 1976; Gauldie et al. 1986b) and described in some shark species (Carlström 1963).

Spherulitic otoconia fused into otoliths have also been described in humans, occurring in association with hereditary deafness (Johnsson et al. 1981). Humans normally have only calcitic otoconia similar to those occurring in the lungfish, but apparently there are still human genes that will code for the spherulitic otoconia. It is tempting to see the widespread distribution of spherulitic otoconia as an indication that they are the most primitive kind of crystalline calcium carbonate secreted by the vertebrate ear. There are strong similarities between the shapes and anatomical arrangement of the otoliths in the chimaera *C. milii* and the lungfish *N. forsteri*, as well as in some of their constituent otoconia. These observations support the suggestion (Patterson 1965; Romer 1968) that the modern chimaeras might be descended from ptyctodont placoderms. Thus, apart from the issue of chimaera affinities within the elasmobranchs (Zangerl 1973), the spherulitic otoconia of the chimaeras may hold some clues to the fundamental mechanism of otolith deposition.

Several species of deep-water chimaeras occur in New Zealand's waters. Otolith form and structure from the two families Rhinochimaeridae (*Harriotta raleighana* and *Rhinochimaera* sp.) and Chimaeridae (*Hydrolagus novaezealandiae* and *Chimaera* sp.) are investigated in this paper.

MATERIALS AND METHODS

Three specimens of each species—*H. raleighana*, *Rhinochimaera* sp., *H. novaezealandiae*, and *Chimaera* sp.—were caught in a deep-water trawl survey of the Chatham Rise area, December 1985. Heads were placed in a 10% neutral buffered formalin solution for 24 hours and transferred to 70% isopropanol until dissection. The preservative solutions remained alkaline during storage time, but after more

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lengthy periods (more than 6 months), 70% isopropanol became weakly acidic with a pH of 6.5–6.7. All otic complexes were removed from heads within three weeks of initial fixation. Samples were then photographed with a Wild M400¹ photomicroscope before their otoliths were removed in preparation for scanning electron microscopy (SEM). Selected whole and fractured portions were then dehydrated in an ethanol series, mounted on stubs with a metallic cement, and sputter-coated with gold under five atmospheres of pressure before being viewed on a Philips 505 SEM.

Analyses of samples by x-ray diffraction were carried out on a Philips PW1279/PW1710 x-ray diffractometer. Operating conditions for the diffractometer were the following: 45 kV for the Cu tube; 40 mA for the automatic divergence slit; 0.1 mm for the receiving slit; 1° for the scatter slit; and a Ni filter. Goniometer speed was set at 3°2θ/min, and chart speed was set at 10 min/°2θ from 5 to 55°2θ. The error of estimated proportion of calcium carbonate morph (calcite, aragonite, and vaterite) was ±5%. Otoconial di-

ameters were directly measured from SEM photographs.

RESULTS

The general shape and organization of the four chimaeriform otic complexes (including semi-circular canals and otoliths) resembled the shape and organization of *C. milii* (Gauldie et al. 1987). Two otoliths occurred within a single sac, oriented at more or less right angles to each other, and presumably corresponded with the sagitta and astericus of teleosts. All of the otoliths described in this study were the sagittae.

The otoliths of the Chimaeriformes were solid masses of aggregated otoconia (Fig. 1A, B, C). Otoliths of *H. novaezelandiae* had densely packed central areas with looser aggregations of otoconia on the exterior surface (Fig. 1B). The matrix was less apparent in *H. raleighana*, and the otolith was characterized by many small otoconia (Fig. 1C), giving a loose granular appearance, while *Rhinochimaera* sp. otoliths (Fig. 2A) had closely packed arrays of otoconia. *Chimaera* sp. otoliths (Fig. 2B) were solidly packed with less cohesive matrix material visible.

¹Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

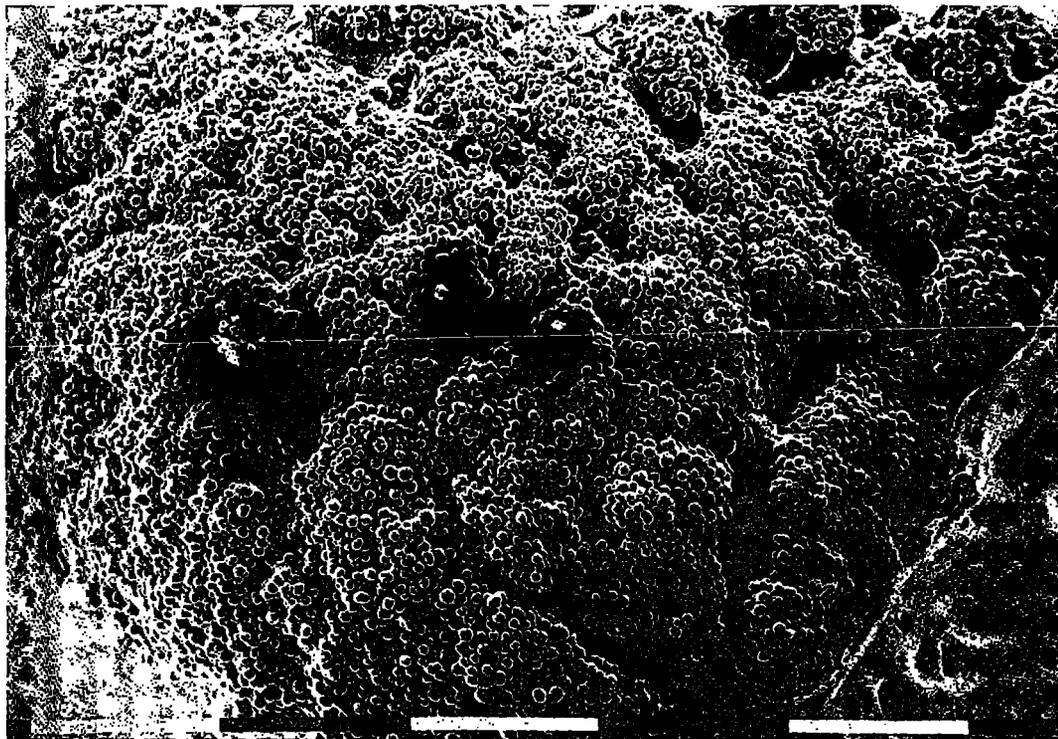


FIGURE 1A.—*Rhinochimaera* sp. otolith. Bar = 1 mm.

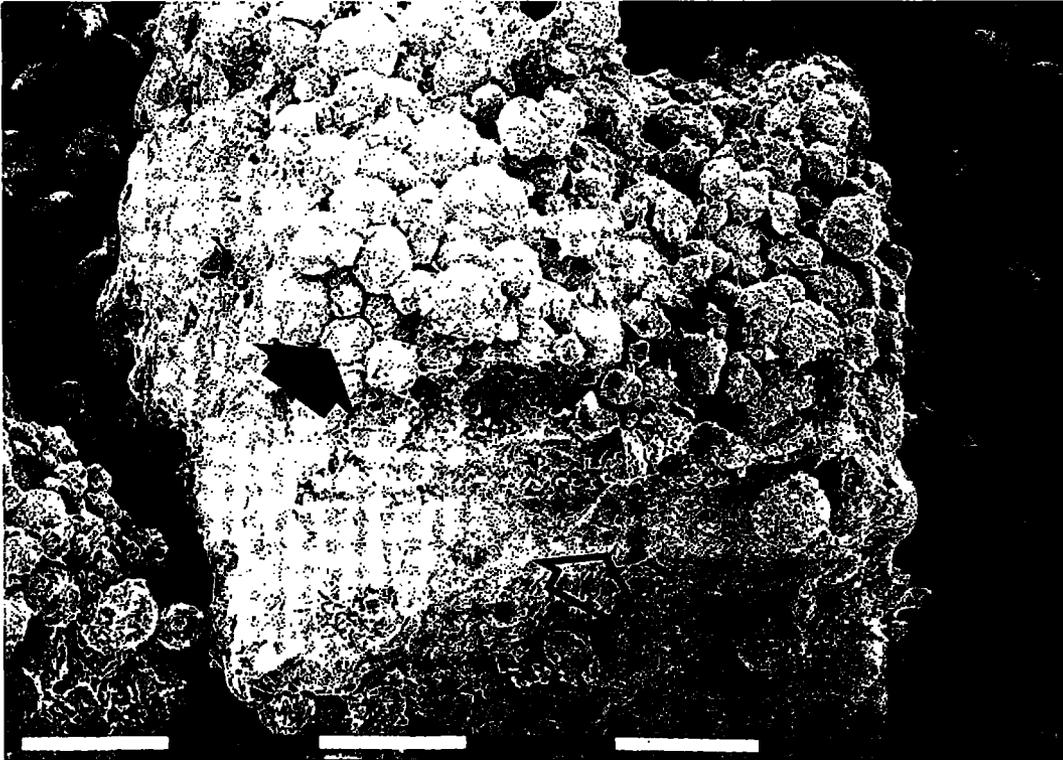


FIGURE 1B.—Broken portion of central mass of *Hydrolagus* otolith. Closed arrow indicates pockmarked features on the surface of the otoconium. The internal morphology of otolith consists of otoconia fused together (open arrow). Bar = 0.1 mm.

FIGURE 1C.—Mass of otoconia from *Harriotta*. Bar = 1 mm.



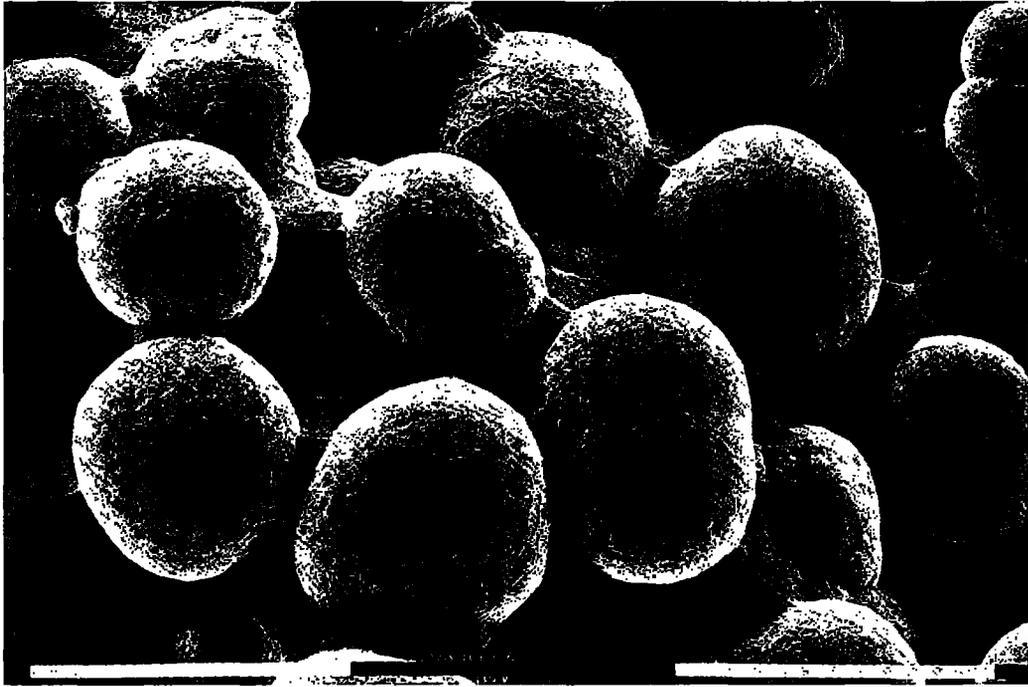


FIGURE 2A.—*Rhinochimaera* sp. otolith showing the encapsulation of otoconia by the matrix. Bar = 0.1 mm.

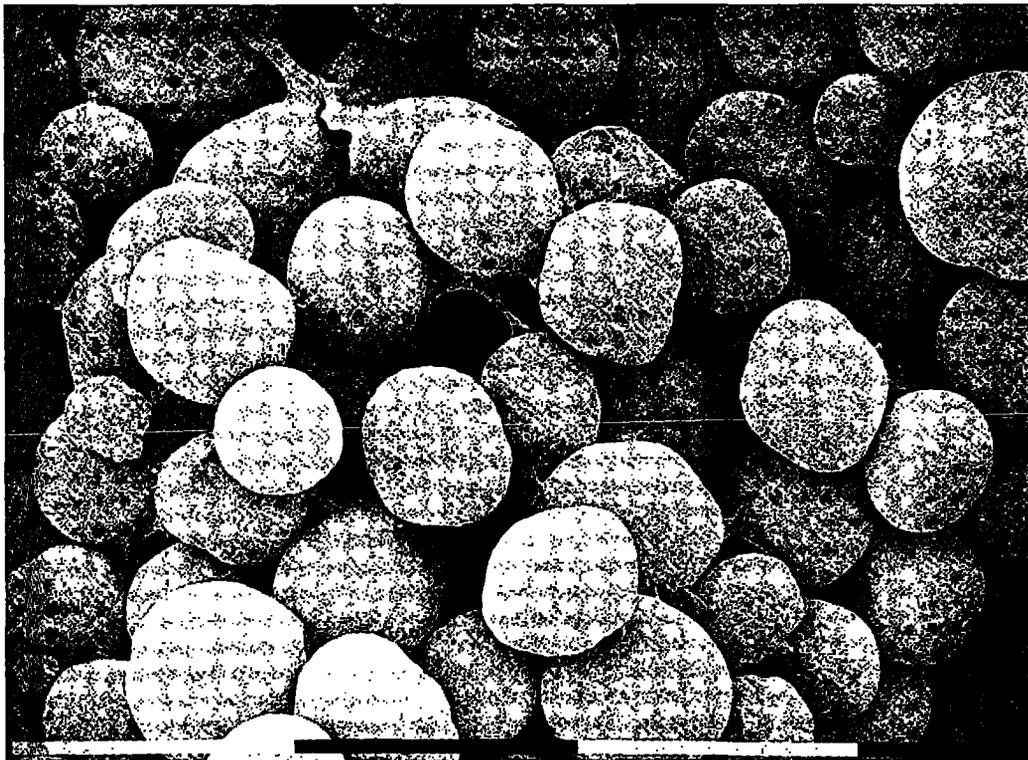


FIGURE 2B.—Otoconia of *Chimaera* sp. have little encapsulating matrix. Bar = 0.1 mm.

A dense aggregation of otoconia occurred within the otoliths of *Rhinochimaera* sp., each otoconia having a distinctive pockmarked surface topography (Fig. 3A). Surface otoconia (see Figure 2A) lacked these pockmarked features. At high magnifications, the pockmarked surface could be seen more clearly (Fig. 3B). In some cases the pockmarks resulted in enough material being removed from the surface to show that otoconia were deposited as layers (Fig. 3C). A few otoconia showed small ($\approx 1 \mu\text{m}$) spherules on their surface and some indications of fusion of the spherules into a distinct layer (Fig. 3B, D). Broken otoconia showed the expected epitaxial growth of crystals from a central point. The growth of individual otoconia appeared to be parallel to that of the otolith: small spherules ($\approx 1 \mu\text{m}$) continued to grow ($1 \mu\text{m}$) and progressively fuse into larger otoconia, which in turn fused to the form of the otolith itself (Fig. 3E).

The otoconia from *H. novaezelandiae* had a greater size range than those of *Rhinochimaera* sp. and *H. raleighana* and were more oval in shape. Although small ($\approx 1 \mu\text{m}$) spherules occurred on the surface of otoconia, they did not assume the more coordinated, layered appearance

seen on the otoconia from *Rhinochimaera* sp. and may have represented recrystallization.

At similar magnifications to *Rhinochimaera* sp. and *H. novaezelandiae*, individual otoconia of *H. raleighana* showed a smooth and uniform surface. However, numbers of small crystals with variable shapes were commonly found in the otoconial mass of *H. raleighana*. Among these crystals were many rod-shaped and twinned rod-shaped crystals, some of which had small, surface recrystallizations on them (Fig. 4A). Crystalline aggregations occurred amongst the *H. raleighana* otoconia, which had the appearance of being part spindle-shaped aragonite crystal, part aragonite spherule, and part fused rod-shaped crystal (Fig. 4B).

The majority of otoconia observed from *Chimaera* sp. were regular spheres. On closer examination, the surfaces of *Chimaera* sp. otoconia varied from smooth to crystalline, and some otoconia surfaces exhibited a highly textured, irregularly crenellated surface (Fig. 5A). Deposits of irregularly formed otoconia occurred in *Chimaera* sp.; they were marked by the absence of any readily observable matrix binding and by the appearance of partially eroded

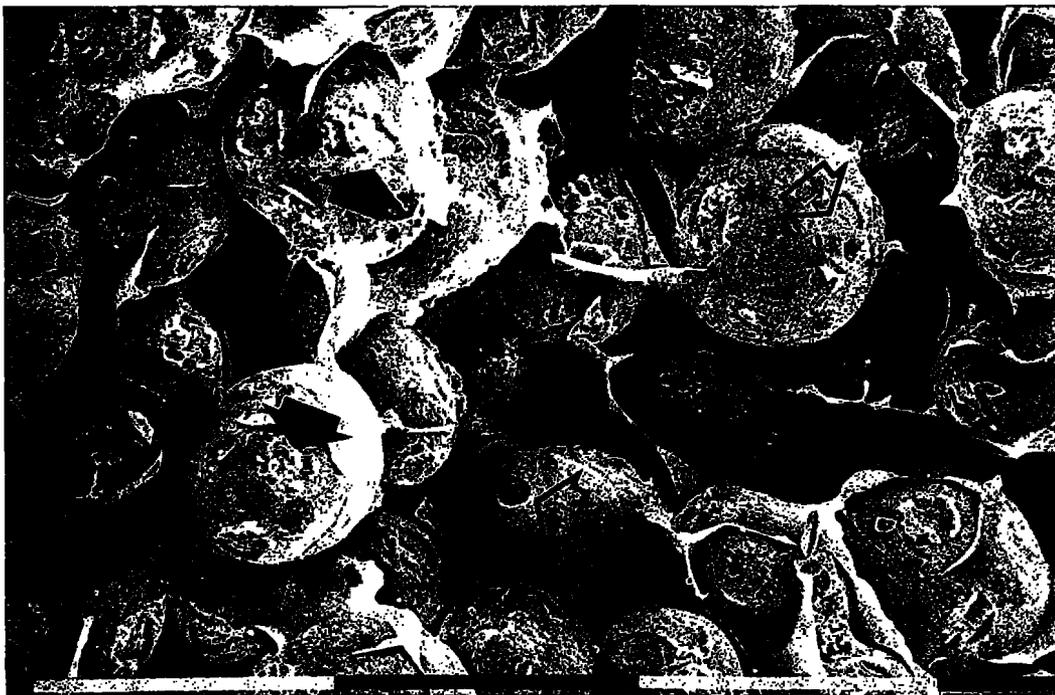


FIGURE 3A.—Otoconia from *Rhinochimaera* sp. have a distinctive pockmarked appearance, that shows possible points of adhesion (closed arrows) and other occurrences on the surface of the otoconia (open arrows) Bar = 0.1 mm.

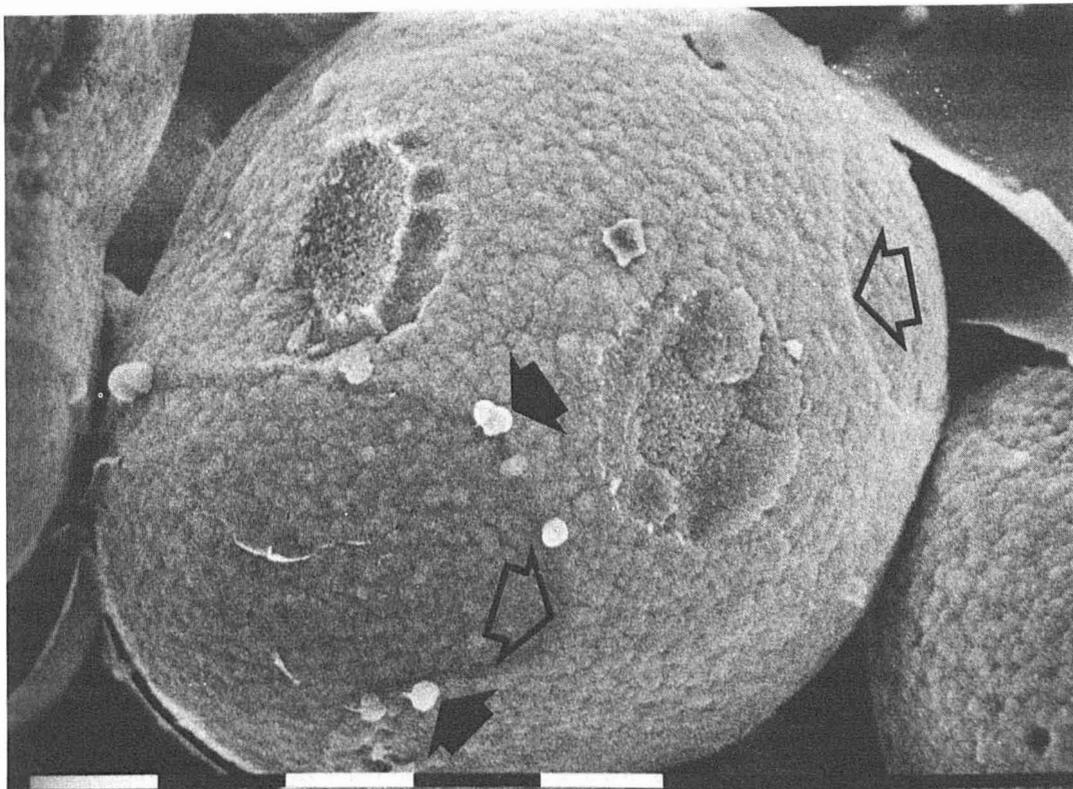


FIGURE 3B.—Small spherules (arrows) appear on the surface of the otoconia from *Rhinochimaera* sp. The body of the otoconia itself appears to be formed by the fusion of such small spherules, which may account for the smoothly bounded depressions (open arrows) on the surface as well as for the more obvious pockmarks. Bar = 10 μ m.

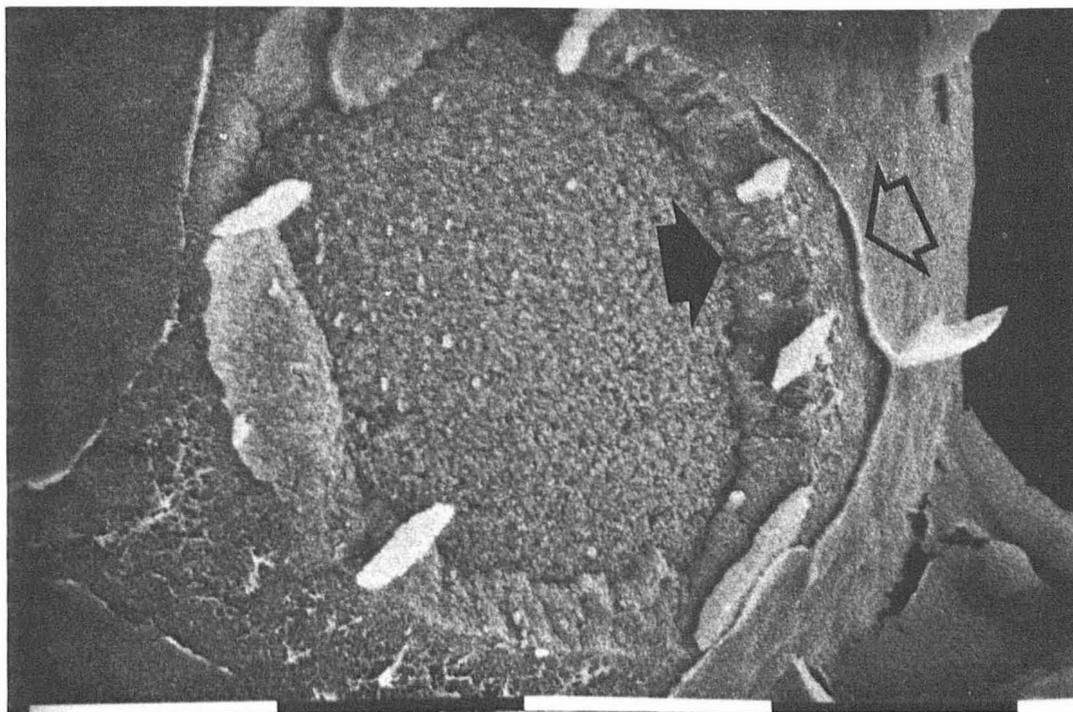


FIGURE 3C.—Small *Rhinochimaera* sp. otoconia show an apparently layered surface (closed arrow) and an enveloping matrix (open arrow). Bar = 10 μ m.

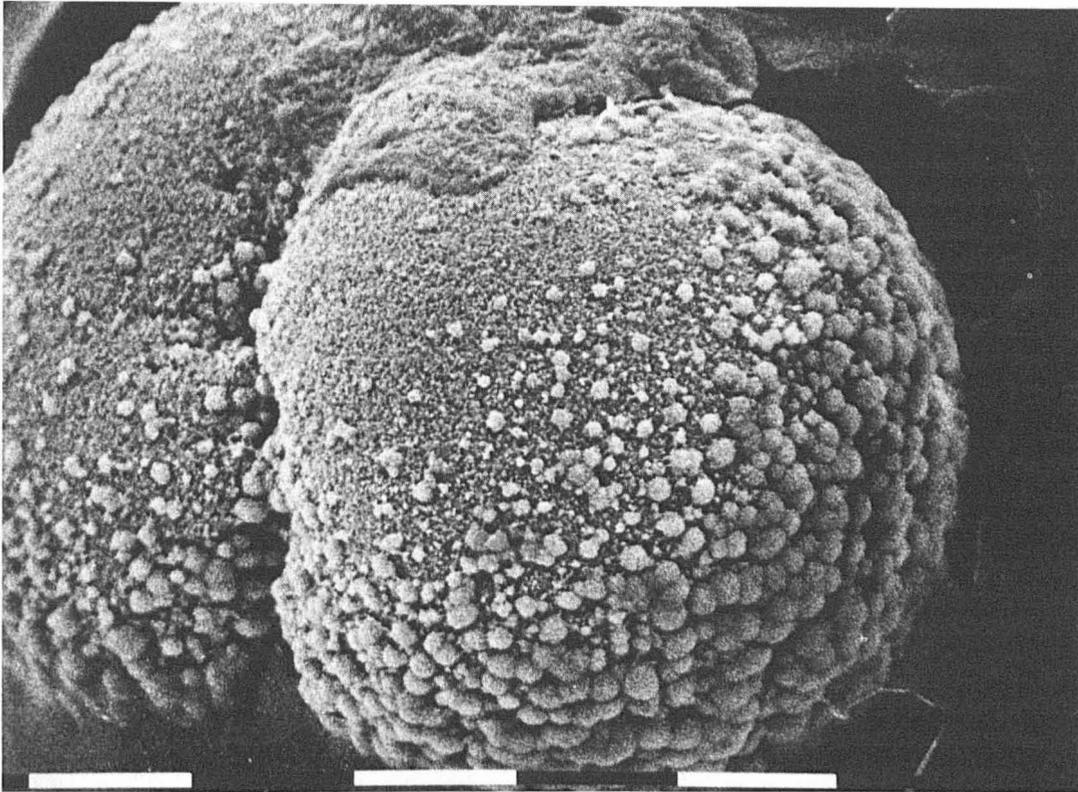


FIGURE 3D.—Some otoconia from *Rhinochimaera* sp. have surfaces formed of many small ($\approx 1 \mu\text{m}$) spherules which have yet to fuse to form the next smooth layer of surface. Bar = $10 \mu\text{m}$.

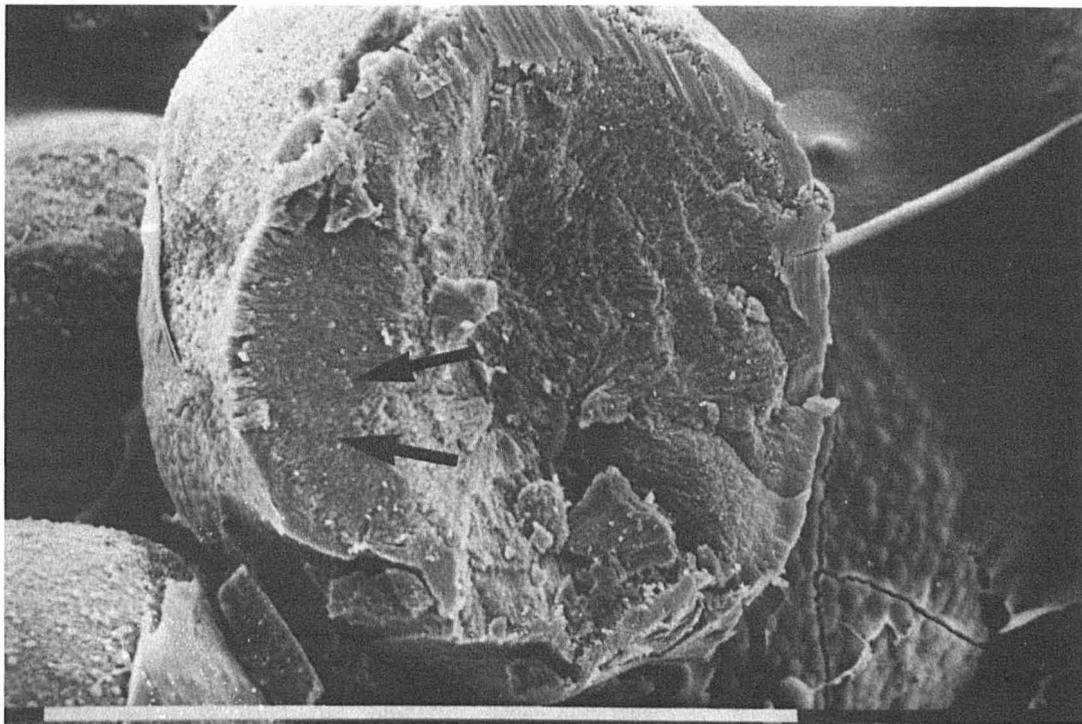


FIGURE 3E.—Broken otoconia from *Rhinochimaera* sp. show a radiating crystal structure with faint traces of small spherules (arrows). Bar = 0.1 mm .

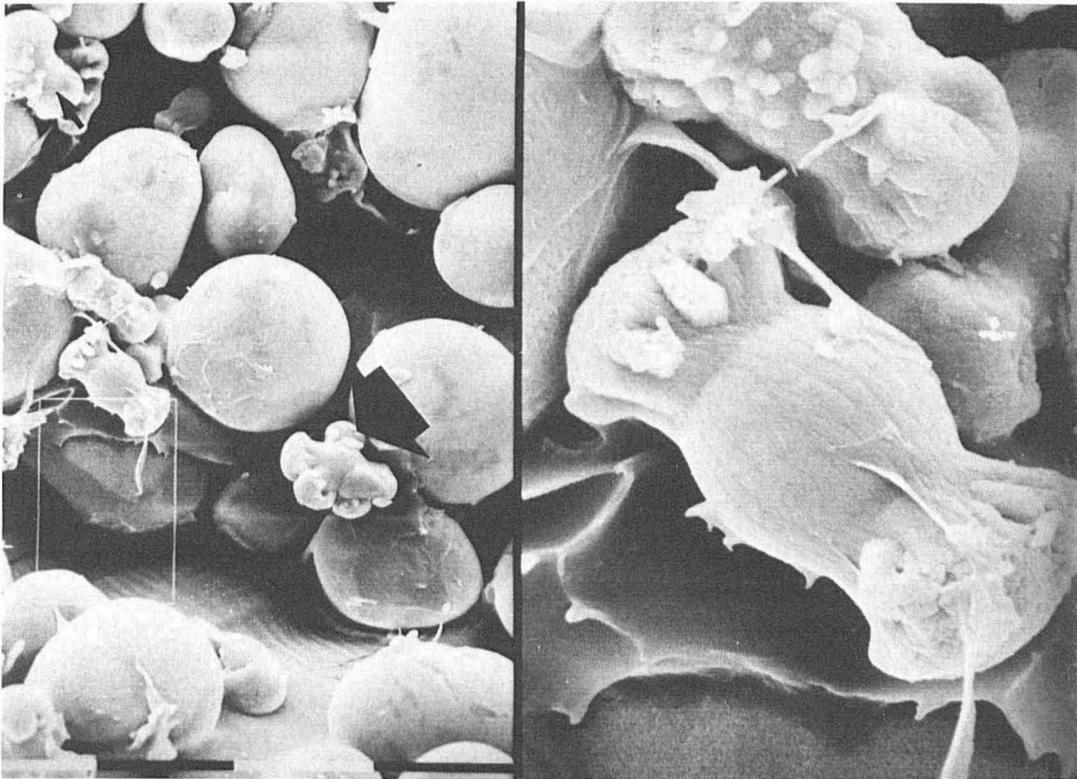


FIGURE 4A.—The round otoconia of *Harriotta raleighana* are very smooth compared with the otoconia of other chimaeras. An unusual rod-shaped crystal occurs in association with the otoconia which has a single (small closed arrow) and twinned (large closed arrow) form. In the higher magnification section the rod shaped crystals show further recrystallization on their surfaces. Bar = 10 μ m.

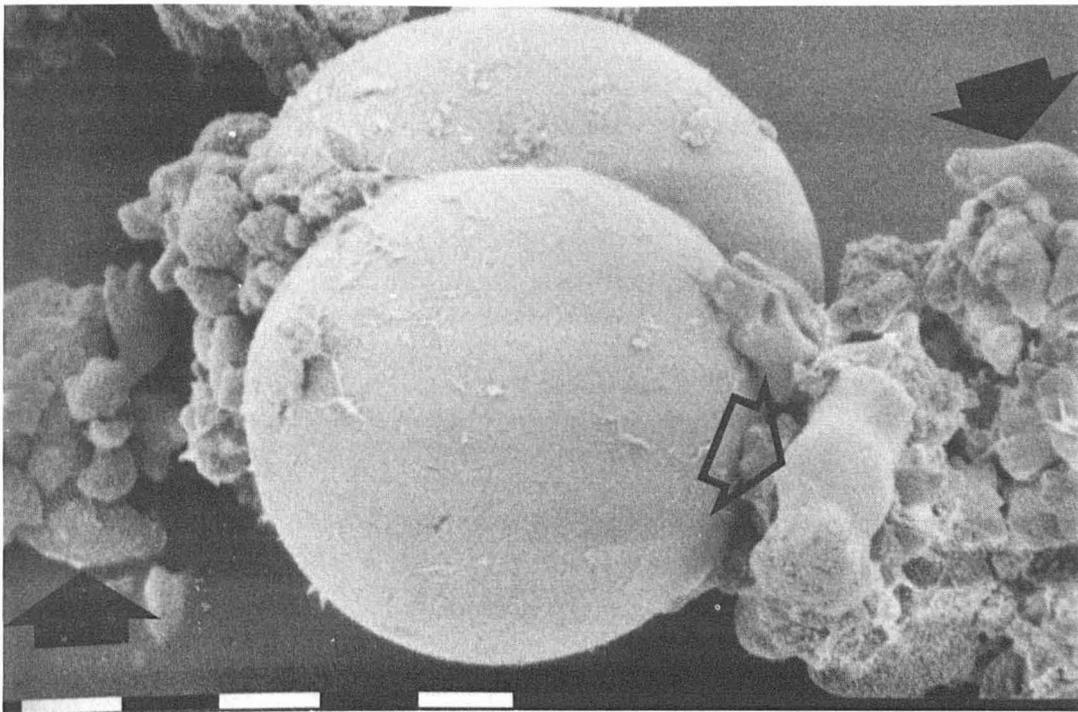


FIGURE 4B.—Crystalline aggregates of the rod-shaped crystal (open arrow) and apparent aragonite spindles (closed arrows) are found in association with the typical aragonite spherule type crystals. Bar = 50 μ m.

spindle-shaped aragonite crystals (Fig. 5B). X-ray diffraction studies of the chimaeriform otoconia examined, including *Chimaera* sp., in-

dicated that all of the calcium carbonate present was in the aragonite form.

Mean otoconial diameters of all four species of

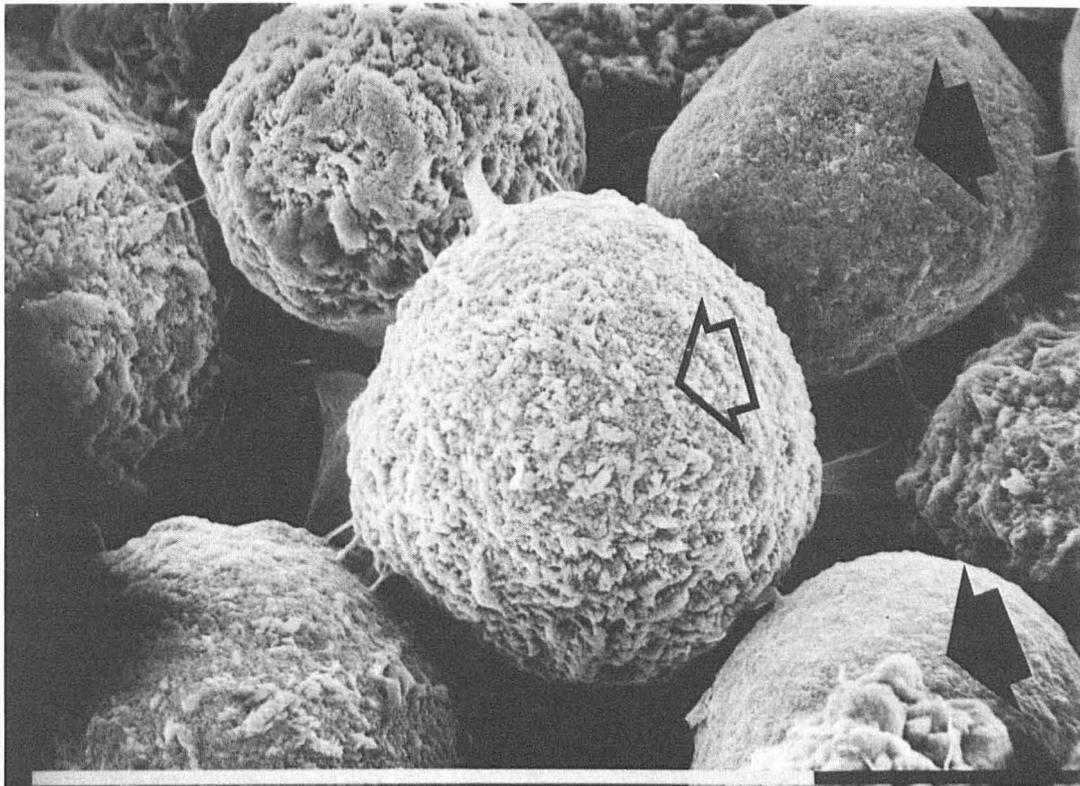


FIGURE 5A.—Otoconia from *Chimaera* sp. include both smooth (closed arrows) and highly textured (open arrow) otoconia. Bar = 0.1 mm.

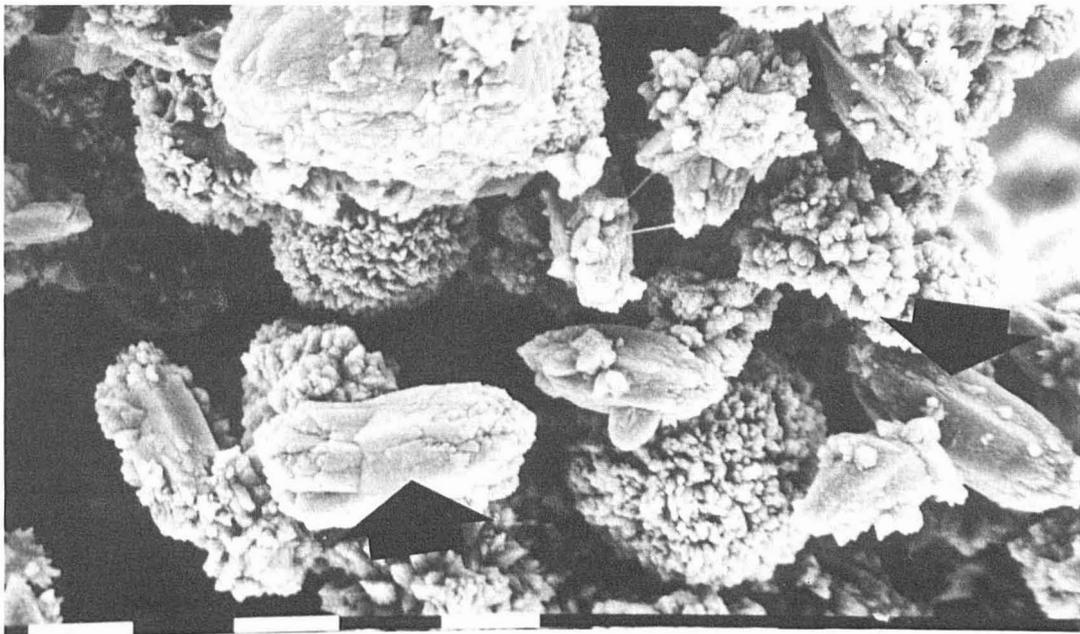


FIGURE 5B.—Parts of the otolith of *Chimaera* sp. are composed of small otoconia of the highly textured type as well as what appear to be partially eroded free aragonite crystals (arrows). Bar = 10 μ m.

Chimaeriformes are listed in Table 1. A *t*-test weighted for unequal variances (Sokal and Rohlf 1969) shows statistically significant differences in the diameters of otoconia from different species ($P \leq 0.05$).

been described in the shark *Somniosus pacificus* (Lowenstam 1980), albeit with a distinctive polycrystalline appearance at the SEM level that is distinctively different from the aragonite spherule otoconia of chimaeras.

TABLE 1.—Mean otoconial diameters (μm) of all four species of Chimaeriform fish. n = sample size, \bar{x} = mean otoconial diameter, SD = standard deviation.

Species	n	\bar{x}	SD	t	$t(.05,49)$
<i>Rhinochimaera</i> sp.	50	66.72	4.91		
				→3.6756	
<i>Harriotta raleighana</i>	50	62.19	7.20		2.0094
				→9.1056	
<i>Chimaera</i> sp.	50	50.26	5.83		
				→1.4738	
<i>Hydrolagus novaezelandiae</i>	50	48.19	8.04		

DISCUSSION

Carlström (1963) described the otoconia of *Chimaera monstrosa* as "almost perfect aragonite spheres". This study at the SEM level confirmed the generally spherical nature of chimaeriform otoconia that was observed at the light microscope level, and revealed considerable detail in variation in size, shape, and crystallinity of otoconia from four species of chimaeriforms.

There was a statistically significant variation in the diameters of otoconia amongst the species examined. In part, this variation reflected the relative amounts of small and large otoconia in the different species, as well as the maximum size of otoconia. Size variation in otoconia may represent physiological differences between individuals of the same species, interspecific differences, and perhaps age differences. Testing these alternatives was not possible with the samples at our disposal.

The predominant type of otoconia among the samples examined was more or less spherical, 40–70 μm in diameter. It was similar in size and in shape to otoconia described in the lungfish (Gauldie et al. 1986a), in the chimaera *C. milii* (Gauldie et al. 1987), and in a number of teleost species (Dale 1976; Gauldie et al. 1986b). X-ray diffraction studies showed that the spherule otoconia were formed from aragonite. Aragonitic spherule otoconia occurred in the chimaera *C. milii* (Gauldie et al. 1987) and in the lungfish *N. forsteri* (Gauldie et al. 1986a). However, spherule shaped otoconia composed of vaterite have

The rod-shaped crystals found in the otoconial mass of *H. raleighana* have not been described in the literature. It is possible that they were bacteria or some other organism. However, their crystalline appearance, which included twinning and surface recrystallization (Fig. 4B), as well as their apparent fusion with the spindle and spherule forms of aragonite, strongly suggest that they are some form of crystal.

There was considerable variation in the surface texture of the otoconia amongst the species described here and those described elsewhere. The otoconia of *H. raleighana* had the smoothest surface texture, but were among the larger otoconia. Therefore, one could reasonably conclude that variation in surface texture may not be due to the rate of crystal growth. In addition, otoliths of *Chimaera* sp. consisted of otoconia of about the same size, but with greatly differing surface texture. We have assumed that during storage the fluids of the endolymphatic sac were alkaline, but it is possible that in the stress of trawling the endolymph may have become acidic. Thus, variation in surface texture may be a preservation artifact. However, the similarity of the appearance of the surface texture of otoconia to those described from other studies, using different preservation techniques, suggests that erosion and recrystallization had not occurred. We conclude that the texture of the otoconia surface does not reveal any useful information about the growth rate of otoconia, but that it may provide clues to probable growth mechanisms.

For example, the layered appearance of some

otoconia may have been due to incremental growth. It was similar to the layered appearance of the lungfish otoconia (Gauldie et al. 1987) and to the layered appearance of statoconia in some mollusc species (Geuze 1968). If there was incremental growth of the otoconia, it indicates that, even at the most primitive level, the calcium metabolism of the inner ear has a definite periodicity. However, there is a major difference between the growth process of otoconia in the chimaera and that of statoconia in the mollusc (Kuzirian et al. 1981): the aragonitic otoconia of the chimaera obviously grows (and fuses into an otolith) in situ. The otoconia of the chimaera otolith often showed small surface crystals that apparently fuse together, implying continuous growth in situ, which is characteristic of teleost otoliths.

It can be assumed that a rigid otolith is required if the otolith functions as a sound transducer (Fay 1983) and that, in contrast, a loose aggregation of otoconia serves as a tilt, or angular momentum detection mechanism (Marmo 1983). The otoliths of the four species described here are rigid in comparison to those of *C. milii* (Gauldie et al. 1987), less rigid than the lungfish otoliths (Gauldie et al. 1986), and much more rigid than the loose and friable aggregations of otoconia that occur in most sharks and rays (Mulligan and Gauldie 1989). One might therefore conclude from our observations that the otoliths of chimaerids were functionally at a stage between a rigid sound transducer of the teleost type (which is found also in the lungfish) and an angular momentum detection mechanism.

The presence of spindle-shaped aragonite crystals in the otolith of *Chimaera* sp. is a particularly interesting observation because both spindle-shaped and spherule-shaped aragonite otoconia occur together with calcite crystals in the lungfish otolith. "Aragonite spherule" otoconia have also been described for the primitive shark *Heptanchus cinereus* (Nishio 1926) and have been observed (Mulligan and Gauldie 1989) in the related *Heptanchias perlo* of New Zealand waters. Furthermore, aggregated spherulitic otoconia have been observed in humans with congenital hearing disorders (Johnsson et al. 1981) and are difficult to distinguish visually from otoconial aggregations in chimaeras and some teleosts (Gauldie et al. 1986b).

It is tempting to see parallels between the otoconia of chimaeras, lungfish, and primitive sharks. However, the nature of evolutionary

processes (particularly convergence processes) does not allow simple extrapolation of "primitive" features, and hence phylogenetic reconstruction, from modern species (Cain 1983). This is particularly true when the physiological effects of otoconia types on inner ear function are completely unknown. However, it is clear that the spherulitic otoconia and otic organization of chimaeriform fish have chemical and anatomical parallels with the inner ear of teleost fish in the orders Cheilodactylidae, Moridae, Gadidae, Balistidae, and Gempylidae; with some sharks; and, rarely, with humans. The occurrence of spindle shaped aragonite otoconia in conjunction with spherule otoconia has a parallel in the otoconia of the lungfish. Such similarities makes it very difficult to assign any taxonomic value to otoconia types. Perhaps spherulitic otoconia represent one of very few successful calcium carbonate matrix/mineralization systems which converge in so many vertebrates simply because there are so few alternatives.

ACKNOWLEDGMENTS

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