antibodies against both native AChR or SDS-treated AChR, whereas denatured purified subunits react only with antibodies against SDS-treated AChR (*20, 21*). Our results indicate that the putative AChR peptides synthesized in this system are immunoprecipitated by both antiserums. This would indicate that the conformation of the cell-free synthesized peptides is similar in terms of antigenic determinants to those present in the native AChR. However, experiments with ^{125}I-labeled α-bungarotoxin have failed to show binding of the toxin to the in vitro synthesized peptides.

The above system constitutes a simple assay for the detection of mRNA coding for AChR peptides and provides a method for the study of the mechanisms of AChR biosynthesis. It may eventually allow the purification of any AChR mRNA's that would made the synthesis of complementary corresponding DNA's feasible; the latter can be used as (i) genetic probes in studies of AChR genes, transcription, and mRNA processing and degradation and (ii) for detailed analysis of the primary structure of these peptides by DNA sequencing.

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Viviparity and Intrauterine Feeding in a New Holocephalan Fish from the Lower Carboniferous of Montana

Abstract. *A new species of Lower Carboniferous holocephalan chondrichthyan,* Delphyodontos dacriformes, *is described from two fetal specimens. The well-developed slashing and piercing dentition, enlargement of the abdominal region, and fecal material indicate the probable evolution of intrauterine oophagy and viviparity in Paleozoic Chondrichthyes.*

Viviparity has been extensively documented among members of the chondrichthyan Elasmobranchii (*1*), osteichthyan Teleostei (*2*), modern Coelacanthiformes (*3*), and urodele and caecilian amphibians (*4*), but all known modern chondrichthyan holocephalan fish are reported to be oviparous (*1*). A new fetal holocephalan from the Lower Carboniferous of Montana has slashing and piercing dentition and evidence of a greatly enlarged, functioning gut, indicating that viviparity was a significant adaptive feature among the Paleozoic chondrichthyans, irrespective of their phylogenetic status.

The Bear Gulch Limestone assemblage contains 69 known species of fish, of which 35 species are chondrichthyans. Preservation of whole bodies is excellent in the fine-grained matrix, and growth series are known for several chondrichthyan species (*5*). Two fetal specimens of a chondrichthyan from this assemblage are provisionally assigned to the Holocephali on the basis of cranial morphology, tooth plate numbers, body form, and incipient cranial ornamentation. The large size of the fetuses, specialized dentition, and lack of developed fins show that they are unrelated to any of the other five holocephalans in the Bear Gulch assemblage with similar features.

Class: Chondrichthyes

Subclass: Bradyodonti

Infraclass: Holocephali

Delphyodontos, n. gen.

Type species: Delphyodontos dacriformes, n. sp.

Diagnosis: A holocephalan with a blunt, large head, a small, ventroterminal mouth, and a body with a horizontal ventral outline and a dorsal outline sloping evenly down from its highest point immediately behind the head. The squamation is complete and of placoid denticles. There are two tooth plates in each upper jaw and one tooth plate in each lower jaw. Upper tooth plates are sharply bladed and composed of high, coarsely serrated cusps; lower tooth plates each contain three compressed, simple recurved cusps on a convex basal lamina. Cusps on the posterior upper and lower plates increase in size in an anteroposterior direction corresponding to the order of their addition to the plates. Enlarged denticles are present along the postorbital arcade, and there is evidence of incipient paired occipital and mandibular spines. Differentiation of hypochordal caudal radials is seen, but no other fins are differentiated. Size at birth is believed to exceed 35 mm.

Delphyodontos dacriformes, n. sp.

Type specimen: University of Montana 6148.

Referred specimen: Carnegie Museum of Natural History 35455.

Horizon and locality: Bear Gulch Limestone, Heath Formation, Namurian E_2B, south of Becket, Fergus County (University of Montana vertebrate locality 7106).

Diagnosis: The same as for the genus.

Derivation of name: Delphyodontos (womb tooth) *dacriformes* (teardrop-shaped body).

Morphology: The holotype is 35 mm long, preserved outstretched in lateral view, and shows only a slight ventral expansion in the abdominal region (Fig. 1). Outlines of calcified cartilage on the head indicate a short rostrum, large, dorsally unrestricted orbits, and a mouth of relatively short gape and holostylic suspension (Fig. 2), as in the Chimaeriformes. The postorbital region of the braincase is relatively longer than that of the Chimaeriformes (*5*); chimaeroids are also not known to possess paired occipital, postorbital, or mandibular spines, although these are known in related extinct groups (*5, 6*).

The second specimen is 29 mm long, fully squamated, and preserved in lateral view curved around a fully squamated, rounded protrusion of the abdominal region resembling a yolk sac (Figs. 3 and 4). Eye and branchial pigments are preserved, as is a bolus of material, at the rear of the abdominal region, that

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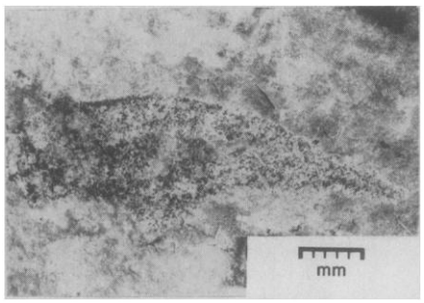

Fig. 1. *Delphyodontos dacriformes* holotype (University of Montana specimen 6148).

strongly resembles the amorphous calcium phosphate typical of coprolite (Fig. 4).

The dentition is identical in both specimens. Anterior upper plates are smaller than the posterior plates and bear cusps that are subequal in size and free from each other for about half the height of the plates. Each posterior upper plate consists of a highly compressed blade with only the coarsely serrated tips free from each other. Lateral flutings mark the positions at which new cusps were added posteriorly. There is a noticeable increase in the height of the posterior upper plate at midlength (Figs. 2 and 3). The teeth of the lower plate are tall, thin, recurved, and free of each other for most of their length (Figs. 1 and 4). The dentition as a whole forms a sharp-edged tearing and piercing structure, totally without precedence among recent or fossil holocephalans.

The abdominal swellings, body shape and curvature, and undifferentiated fins indicate that the *Delphyodontos* specimens are fetuses but do not give any indication of the size at birth. The abdominal enlargement of *Delphyodontos* is internal and cannot be a yolk sac, as shown by the complete squamation and lack of an umbilicus. Furthermore, the large fecal bolus in the smaller specimen implies a considerable amount of feeding. The greatly expanded foregut of the modern intrauterine-feeding shark *Lamna* is essentially the same as in these fetuses (*7*). Modern intrauterine-feeding sharks and amphibians absorb the relatively limited amount of yolk at an early stage; subsequent growth of the fetus is supported by oophagy or, in the more complex amphibians, by uterine secretions (*1*, *4*). Most modern viviparous sharks are supported fetally by trophonemic structures; in two families, nutrition is supplied through yolk sac placentation. Modern holocephalans ingest their own yolk (*1*).

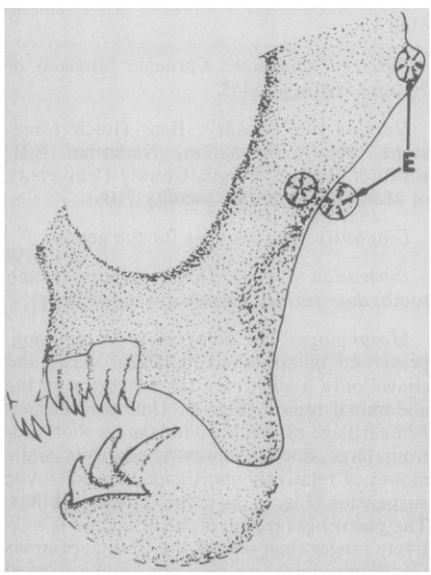

Fig. 2. Diagram of *Delphyodontos dacriformes* head showing dentition of the left side and calcified areas of cartilage (shaded); *E*, enlarged denticles (University of Montana specimen 6148).

Large, well-developed slashing and piercing dentition is unusual in a fetus and even more unusual in a holocephalan. Modern holocephalans are oviparous, and there are no known dentitional differences between adults and the young. The dentition of modern and known fossil Holocephali varies from plucking and shearing plates to durophagous plates. While the dentition of *Delphyodontos* could be interpreted as that of a predator, the dacriform body shape and fetal condition all but prohibit a predatory life-style. The dentition is thus interpreted as specialized for opening egg capsules during intrauterine feeding. Functioning dentition in intrauterine feeders among both sharks and amphibians has been reported to differ from that of the adults (*4*, *7*, *8*).

There are five other Bear Gulch holocephalans with similar body forms, only one of which has been described (*5*). The minimum body size of well-developed, apparently free-living young among these species is about 16 mm; the maximum body size of sexually mature adult males is 150 mm (*5*). The fetal *Delphyodontos* is unusually large for members of its taxonomic group in the assemblage, and the cranial calcifications indicate an unusually high fetus/adult size ratio, characteristic of oophagous forms.

In view of the well-developed fetal dentition of *Delphyodontos*, the evidence of feeding in utero, the abdominal distention strongly suggestive of a specialized foregut for oophagy, and the unusually large fetal size, it must be concluded that *Delphyodontos* is viviparous. Thus it constitutes the first direct evidence of viviparity in the Holocephali and in the Paleozoic Chondrichthyes.

Viviparity is an adaptation in which fewer young are born relatively large; maternal investment per fetus is very high. Internal fertilization is a necessary precondition for viviparity. The energetics of development indicate that, regardless of yolk size, around 25 percent of the organic content of the egg must be consumed for energy (*1*). Fetal maintenance beyond the yolk-absorption stage can only be accomplished through the provision of other sources of nutrition on demand, such as the development of trophonemata or a placenta, maternal secretions from the oviductal wall, or maintenance of the actively feeding young in the oviduct on eggs or perhaps younger siblings. Such complex maternal adaptations as trophonemata are common among modern Chondrichthyes and are more advanced than the process of retaining young with an already long incubation period within the oviduct and producing eggs for their nutrition. Yolk ingestion, as seen in the modern chimaeroids, is more specialized than simple oviparity, but may have served as a necessary preadaptation to the oophagy of *Delphyodontos*.

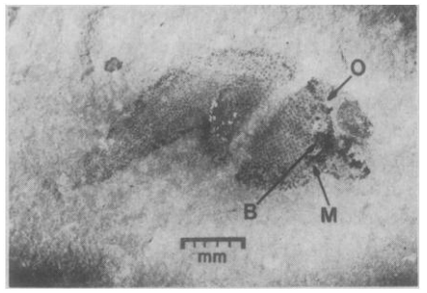

Fig. 3. *Delphyodontos dacriformes*, left side; *B*, presumed branchial pigmentation; *M*, mandibular denticle aggregation; *O*, occipital denticle aggregation. Upper dentition is visible (Carnegie Museum specimen 35455).

Wourms (*1*) discussed factors that have been advanced as significant in the evolution of viviparity in sharks. The implication of the first factor, phylogenetic position, is that holocephalans, being more "primitive," are oviparous. Neither part of the statement is acceptable. Also, Wourms could find no correlation

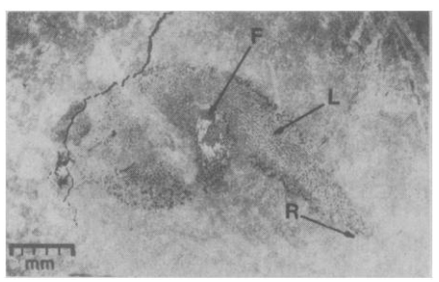

Fig. 4. *Delphyodontos dacriformes*, right side; *F*, fecal bolus; *L*, lateral line; *R*, caudal radials. Lower dentition is visible (Carnegie Museum specimen 35455).

between viviparity in recent sharks and distribution geographically (tropical versus temperate) or by depth of habitat (benthic versus pelagic). Finally, Wourms justifiably dismissed purported osmoregulatory considerations. Little is known about the food habits or feeding ecology of sharks during ontogeny except that the young are frequently found in different places than the more migratory adults. The suggestion that oophagy produces a predator trained before birth is not acceptable for holocephalans, among which no known members can be considered predacious. However, there is a strong correlation of viviparity with large adult size, which in turn has implications for feeding ecology. Viviparous species produce not only larger offspring but also fewer of them. Large size is an absolute advantage in reducing the number of potential predators. Fewness in the number of young is an absolute advantage in reducing competition for food among them. Large size and low numbers may have been a particularly advantageous combination in a community such as Bear Gulch, where the extreme diversity of the chondrichthyans indicates very fine-grained partitioning of resources based in part on size and age segregation (*5*).

Chondrichthyan viviparity has evolved independently many times, and must be seen as of considerable selective advantage by virtue of, rather than in spite of, the production of fewer, larger young. The probable evolution of viviparity with dental specializations for intrauterine feeding in a Paleozoic holocephalan reinforces the suggestion that this is evolutionarily the simplest method of viviparity, requiring no specialized maternal structures and few fetal modifications.

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Sodium-Calcium Exchange in Rabbit Heart Muscle Cells: Direct Measurement of Sarcoplasmic Ca^{2+} Activity

Abstract. *Calcium ion-selective microelectrodes made with Simon's neutral carrier were used to measure simultaneously sarcoplasmic Ca^{2+} activity (a^i_{Ca}) and resting tension (T_r) of rabbit ventricular muscle during reduction and restoration of external sodium ion concentration, $[Na]_o$. Under the same experimental conditions the change in contractile tension (T_a) also was measured. In resting muscle the a^i_{Ca} was 38 ± 17 nanomolar (mean ± standard deviation; N = 10). The reduction of $[Na]_o$ from 153 to 20 millimolar led to about a threefold increase in a^i_{Ca} with parallel increases in T_r and T_a. The time course of the change in a^i_{Ca} was similar to that of the changes in T_r and T_a. The results are consistent with an important role of the sodium-calcium exchange system for regulating sarcoplasmic Ca^{2+} activity.*

The concentration of calcium ions in the sarcoplasm is critical in the regulation of muscle contraction. Although sarcoplasmic Ca^{2+} concentrations of resting heart muscle appear low, they have not been measured directly. In this study, we measured the sarcoplasmic Ca^{2+} activity of rabbit heart muscle with Ca^{2+}-selective microelectrodes. To test the occurrence of a sodium-calcium exchange across the sarcolemma, we measured simultaneously the changes in sarcoplasmic Ca^{2+} activity and resting tension of the heart muscle during reduction and restoration of external Na^+ concentration. This maneuver is thought to diminish Na^+ influx and hence Ca^{2+} efflux. Thus, sarcoplasmic Ca^{2+} activity is expected to increase.

The Ca^{2+}-selective microelectrodes were made with a Ca^{2+}-selective liquid provided by Simon (*1*). Glass micropipettes with tip diameters of about 1 μm or less were made from borosilicate glass capillaries (Corning, code 774). The surface of the micropipettes was silanized by exposure to dichlorodimethylsilane gas. The micropipettes were filled with 100 m*M* $CaCl_2$ solution, and then by suction they were filled with the Ca^{2+}-selective liquid up to 400 to 800 μm from the tip. The right panel of Fig. 1 shows the potential recordings measured at the Ca^{2+} concentrations of 10^{-8} to 10^{-3}*M*; the left panel shows the measured potentials plotted against Ca^{2+} activities. We presented the results as Ca^{2+} activities rather than Ca^{2+} concentrations because

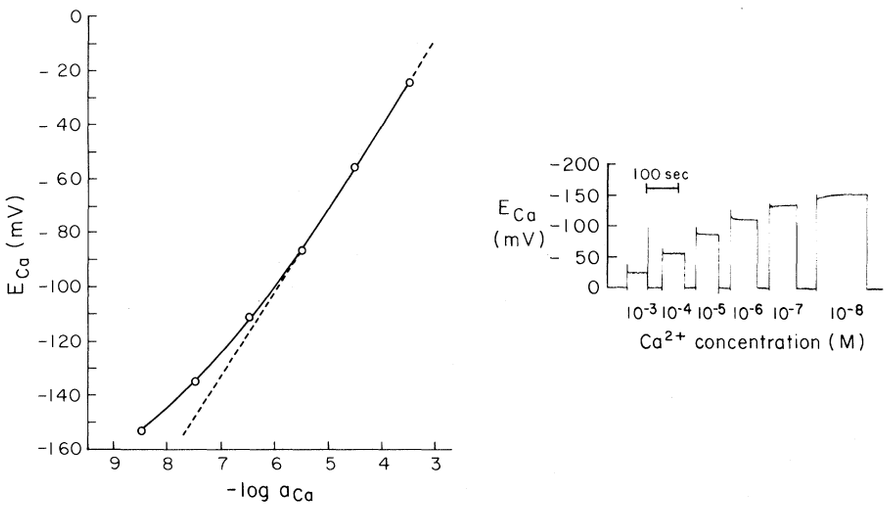

Fig. 1. Calibration of a Ca^{2+}-selective microelectrode. For the microelectrode calibration, solutions of 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3}*M* Ca^{2+} concentration were used. Those containing 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5}*M* Ca^{2+} were made from a Ca^{2+}-buffer containing EGTA. The Ca^{2+} concentrations of the solutions were calculated with the apparent stability constant of $5.76 \times 10^6 M^{-1}$ for Ca-EGTA at *p*H 7.0. The solutions containing 10^{-4} and 10^{-3}*M* Ca^{2+} were made by dilution of 0.1*M* $CaCl_2$ solution. All the solutions contained 150 m*M* K^+ and 1 m*M* Mg^{2+}, and had a *p*H of 7.0. The solutions have similar ionic strength; therefore, the calibration solutions have similar activity coefficients of Ca^{2+}. Bates and Alfenaar (*7*) have described the conventions for determining single ion activity coefficients for various ions including those of calcium. In the mixture containing $CaCl_2$ and KCl or NaCl, the activity coefficients of Ca^{2+} at various ionic strengths have been reported (*8*) and can be calculated by the extended Debye-Huckel equation (*9*). The experimental values agree with the calculated values. At the ionic strength of 0.15, the activity coefficient of Ca^{2+} (calculated) was 0.32. The microelectrodes were calibrated before and after each experiment. The properties of Ca^{2+}-selective ligands including selectivities have been reported (*10*).

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