First Fossil Hagfish (Myxinoidea): A Record from the Pennsylvanian of Illinois

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A fossil hagfish (Myxinoidea), a new genus from the Pennsylvanian, shows tentacles, structures of the head skeleton and internal organs. No other fossils of this group have been reported. Although this new hagfish differs from living forms in position of the gills, feeding apparatus, and relatively well developed eyes, it is quite similar to its recent relatives. Thus, hagfishes have a long, conservative geological history. Cladograms showing myxinoids as a sister group to the vertebrates are supported.

THE JAWLESS FISHES HAVE A DIVERSE and abundant fossil record in the Paleozoic but living forms are limited to two families, the Petromyzontidae (lampreys) and the Myxinidae (hagfishes). These families are easily distinguished from each other and from other fossil agnathans (*1*). Despite many attempts to relate hagfishes with specific fossil groups (*2*), it is clear that they represent a distinct lineage (*1*). The earliest fossil representatives of lampreys were reported in 1968 (*3*) from the Pennsylvanian (Westphalian D) of Illinois and later (*4*) from the Upper Mississippian (Namurian A) of Montana. Here the first fossil record of a hagfish is reported (*4a*) (Fig. 1).

The new fossil comes from the Pennsylvanian (approximately 300 million years ago) of northeastern Illinois which has yielded an extensive fauna of marine aspect (*5*). These fossils are preserved in ironstone concretions. The hagfish appears as light and dark stains representing cartilaginous and soft tissues on the bedding plane of the concretion. Such structures as eyes, tentacles, blood vessels, and cranial skeletal elements can be distinguished (Fig. 2). After emphasizing these markings on photographic enlargements, a pattern arises (Fig. 3) that can be related to the structures of modern hagfishes such as *Myxine* or *Eptatretus* (*6, 7*) (Fig. 4).

The new genus, *Myxinikela* (Subphylum craniata; Order Myxiniformes), is characterized by a tubular body that is expanded dorsoventrally by elongate and confluent dorsal, caudal, and anal fins. The eyes are prominent and anteroposteriorly elongated. This length is contained about four and one-half times in the distance between the front of the head and the eye. Gill pouches lie closer to the head skeleton than in living myxines.

The single species, *Myxinikela siroka*, of Pennsylvanian age, comes from the Francis Creek Shale, Carbondale Formation of Will County, Illinois. The generic name is derived from myxine and ikelos (Greek for myxine-like).

Description. The specimen measures 7.2 cm in total length and has a maximum body depth of 1.2 cm. The forward third of the body is bent slightly downward at about the point where a large, dark globular stain representing digestive organs (Fig. 4), primarily the liver, is located. The head end is twisted somewhat so that it is viewed dorsolaterally. A continuous median fin arises dorsally behind the area of the digestive organs, extends around the caudal end of the body, and continues anteriorly along the ventral margin almost as far forward as the digestive organs. There is no evidence of skeletal support for the fins. An elongate series of dark stains marks the junction between body and fins. The dorsal component of the fin rises gradually to a point where it reaches about one-half the body thickness approximately four-fifths of the distance from its origin to the posterior end of the body. The ventral component of the fin, about half the maximum height of the dorsal, is divided at the position of the cloaca, which is marked by a set of stains. No mucous gland pores are noted but the position of preservation may make it difficult to see these.

The mouth opening lies at an oblique angle to the body axis. Somewhat behind the mouth there is a pair of short labial tentacles (Fig. 4) and before these a pair of more elongate oral tentacles. These tentacles appear to lie somewhat more posterior to the mouth than in living hagfishes. Dorsally, two pairs of nasal tentacles surround the opening of the nasal tube. The upper nasal tentacles emerge from an elongate subnasal cartilage. The other, and topographically lower nasal tentacles, are continuous with the cartilage supporting the oral tentacles. The subnasal cartilage can be traced posteriorly along the midline of the head as a slender dark band. Above this cartilage, remnants of the nasal tube cartilages are recognizable in an irregular series of transverse bands. In living forms these are ring-shaped but in the compressed fossil they are flattened. Anteriorly the nasal tube is rhomboidal in outline with several perforations. This is not significantly different from its appearance in *Eptatretus* (*7*). More posteriorly and just anterior to the eyes a broad stain representing the nasal capsule marks the posterior end of the nasal tube canal. As in living hagfishes, the nasal capsule appears to have a few longitudinal openings indicated by clearer areas in the ironstone stains representing the cartilage of the fossil. A small, elongate stain, possibly a remnant of the palatine cartilage, lies below and anterior to the nasal capsule.

Other principal skeletal structures in the forward part of the head are represented by remains of two massive cartilages beginning just behind the mouth and extending posteriorly almost to the eyes. These are parts of the dentigerous cartilage and anterior lingual cartilage. In modern hagfishes there are teeth on the dentigerous cartilage. Several discrete, particularly dark stains on the fossil structure suggest their presence. In contrast to living myxiniforms (*6*), these cartilages occupy a more anterior position. Perhaps they may have been drawn forward after death but more likely in this extinct form may have been situated more anteriorly due possibly to less development of the lingual cartilage complex in comparison to living hagfishes.

Behind the nasal structures and feeding apparatus a pair of elongate eyes is situated above and slightly to the side of fragmentary elements of the cranial skeleton. The dark stains denoting the eyes represent preserved retinal pigments. In *Myxine* eyes are not discernible in external view but may be dimly noted below unpigmented skin in *Eptatretus* (*8*). A small white spot could represent the site of a lens, another structure seen in *Eptatretus*. Living hagfishes differ in occular development which is correlated with their differing habitats and behavior (*9*). Slightly posterior to the eyes and attached to the cranial skeleton, a pair of smaller, circular dark areas represent the otic capsules.

Behind and slightly below the otic cap-

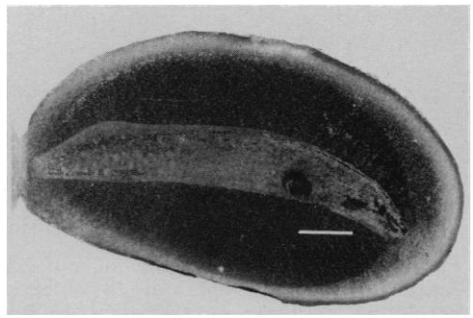

Fig. 1. *Myxinikela siroka* (NEIU MCP 126), whole specimen. Scale bar, 1 cm.

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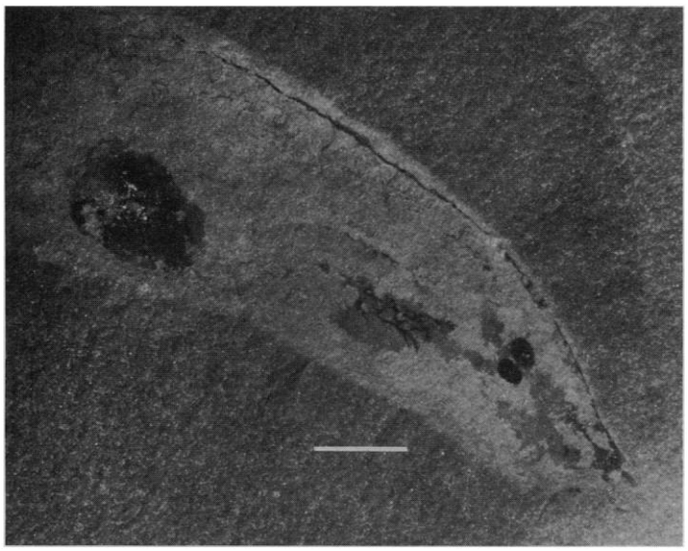

Fig. 2. *Myxinikela siroka* NEIU MCP 126, enlarged head. Scale bar = 0.5 cm.

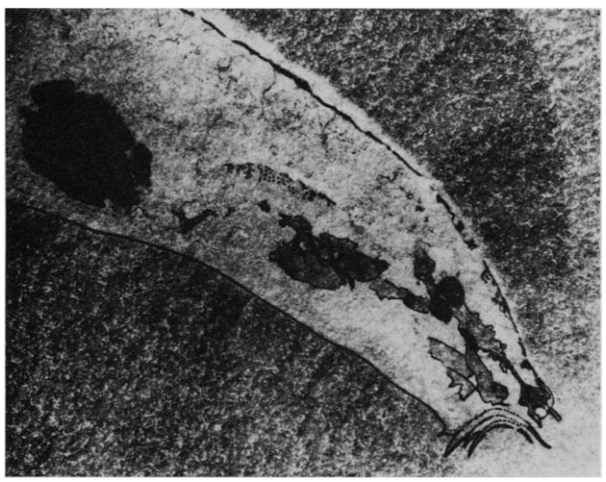

Fig. 3. *Myxinikela siroka* NEIU MCP 126, enlarged head with preserved structures emphasized.

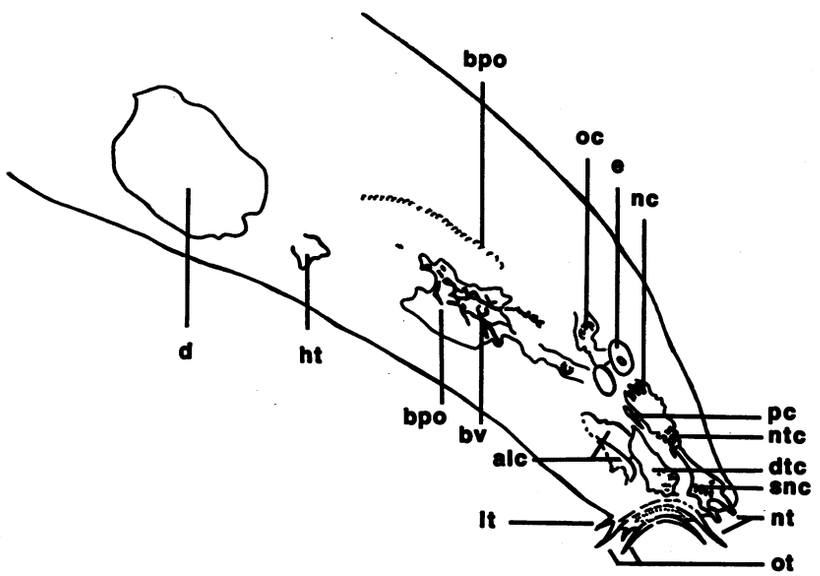

Fig. 4. *Myxinikela siroka*, restoration of head skeleton and associated structures. Abbreviations: alc, anterior lingual cartilage; bpo, branchial pouches and outline; bv, branchial blood vessels; d, digestive organs; dtc, dentigerous cartilage; e, eye; ht, heart; lt, labial tentacles; nc, nasal capsule; nt, nasal tentacles; ntc, nasal tube cartilage; oc, otic capsule; ot, oral tentacles; pc, palatine cartilage; and snc, subnasal cartilage.

sules, the specimen shows a pair of broadly elongate, somewhat swollen structures. Their length is about equal to the distance from the mouth to the otic capsule. Included in this mass are the branchial elements, the musculature that surrounds them, and the blood vessels that supply them. Individual branchial pouches are somewhat difficult to resolve from the light, rather uniformly colored stains in this area. Their presence is suggested in elliptical areas outlined by the branchial blood vessels. The latter are a dark, elongate, slender complex of linear, circular, and triangular bands. It is unlikely that these structures represent branchial skeleton although their coloration is similar to skeletal elements anterior to the eyes. In living myxines, the head skeleton terminates far anterior to the branchial region and the branchial skeleton is much reduced (*6*). During ontogeny of living hagfishes, the gill pouches move posteriorly, separating the gills from branchial arch structures (*10*). Although this process has not gone as far in *Myxinikela* as in *Myxine*, the dark stains among which the branchial pouches lie are readily matched to the complex branchial circulation pattern that emerges from each gill pouch, divides around the afferent branchial duct, and continues laterally as the lateral aorta with a branch leading to the median or dorsal aorta. Just behind the branchial pouches, a thin, slightly darkened area represents the position of the heart. The large mass just behind it is the liver.

Myxinoids are a sister group to all other craniates (*1*). The new fossil specifically shows that a basically modern hagfish was in existence at least by mid-Pennsylvanian. Further, the close similarity between *Myxinikela* and living hagfishes shows that the group has a long conservative history and surely originated well before its Pennsylvanian record. Cladograms based on living myxiniforms (*1*), which place them as the sister group to all vertebrates, are supported by this fossil form. No trace of features of lampreys or other agnathans are seen in *Myxinikela*. Differences between *Myxinikela* and living hagfishes, particularly in the position of the branchial pouches, feeding apparatus, location of the oral tentacles, and minimal development of skeletal elements suggest that this specimen represents a juvenile individual. Adults of *Myxinikela* might be more similar to living hagfishes in these features. But lampreys from the Mazon Creek fossil deposits also show several juvenile features (*3*), and young individuals characterize much of the Mazon Creek biota (*11*). Perhaps these hagfishes and lampreys matured into adults not structurally different from modern forms when they moved outside of the

deltaic environment of the Mazon Creek marine-brackish assemblage. Alternatively, *Myxinikela* may be an adult and characteristics of the living myxines might have evolved phylogenetically later. Thus *Myxinikela* is primitive relative to living myxiniforms in the above features as well as the larger eyes. Finally, in the last 30 years of intense collecting efforts, only one hagfish but in excess of 40 lampreys have been found. Perhaps hagfish were rare entrants to the Mazon Creek habitat.

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Calcium Gradients Underlying Polarization and Chemotaxis of Eosinophils

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The concentration of intracellular free calcium ($[Ca^{2+}]_i$) in polarized eosinophils was imaged during chemotaxis by monitoring fluorescence of the calcium-sensitive dye Fura-2 with a modified digital imaging microscope. Chemotactic stimuli caused $[Ca^{2+}]_i$ to increase in a nonuniform manner that was related to cell activity. In cells moving persistently in one direction, $[Ca^{2+}]_i$ was highest at the rear and lowest at the front of the cell. Before cells turned, $[Ca^{2+}]_i$ transiently increased. The region of the cell that became the new leading edge had the lowest $[Ca^{2+}]_i$. These changes in $[Ca^{2+}]_i$ provide a basis for understanding the organization and local activity of cytoskeletal proteins thought to underlie the directed migration of many cells.

THE ABILITY OF CELLS TO MIGRATE toward a directional stimulus is a basic property of virtually all cells at some stage in their development (*1*) Directed migration results from the development of a polarized cell morphology (*2*). The region of the cell closest to the attractant forms a broad lamellipod whose leading edge then moves toward the stimulus. The posterior contents of the cell flow forward into this lamellipod and the rearmost regions become constricted. These local differences must reflect local differences in cell chemistry, but these chemical processes are not well understood. Many stimuli that induce polarization and chemotaxis also cause $[Ca^{2+}]_i$ to rise, and where a rise in $[Ca^{2+}]_i$ has been noted (*3*), it has often (*4*), but not always (*5*), been spatially nonuniform in nature. The relation between the $[Ca^{2+}]_i$ signal and local changes in cell behavior has been difficult to understand, and the spatial patterns observed have been variable (*6*) perhaps because of local differences in indicator distribution in the cytoplasm or into cellular compartments (*7*). Furthermore, the optical conditions required for assessing $[Ca^{2+}]_i$ often did not allow for simultaneous assessment of cell morphology and behavior. We have now observed changes in $[Ca^{2+}]_i$ during polarization and chemotaxis by simultaneously imaging both $[Ca^{2+}]_i$ and cell morphology under conditions obviating many of these problems. Single eosinophils from the newt *Taricha granulosa* were studied because of their large size and rapid responsiveness to chemotactic stimuli (*8*).

Experiments were carried out to determine if treatments with agents that interfere with Ca^{2+} signaling altered the ability of eosinophils to polarize and move in response to newt serum. Exposure to newt serum (10%) induces polarization, chemokinesis, and chemotaxis in these cells (*8*). Treatment with agents that block Ca^{2+} entry into cells (EGTA, a chelator of extracellular Ca^{2+}; verapamil, an organic Ca^{2+} channel antagonist; and Co^{2+}, an inorganic Ca^{2+} channel blocker), caused the cells to round up and inhibited their movement (*9*). Caffeine, which releases Ca^{2+} from internal stores and prevents regulation of cytoplasmic Ca^{2+}, had similar effects as did the Ca^{2+}-specific ionophore ionomycin (*9*). Ionomycin exerted these inhibitory effects at both normal (1.8 mM) and reduced (1 μM) extracellular $[Ca^{2+}]$. By contrast, the K^+-specific ionophore, valinomycin (0.6 μM) had no effect on cell movement or shape at both normal (3 mM) and elevated (120 mM) concentrations of extracellular K^+ (*10*). This suggests that the membrane potential of the cell is unimportant for polarization and locomotion in response to serum and that the effects of ionomycin are specific to its ability to act as a Ca^{2+} ionophore. These treatments had the expected effect on $[Ca^{2+}]_i$; for example, treatment of cells with verapamil lowered $[Ca^{2+}]_i$ by 24.7 ± 11.1% in three cells, ionomycin increased $[Ca^{2+}]_i$ by 801 ± 233% in five cells, and caffeine reduced $[Ca^{2+}]$ in regions believed to reflect Ca^{2+} in internal stores (*10*). Thus, agents expected to lower $[Ca^{2+}]_i$ diminished polarity and locomotion as did agents that raised $[Ca^{2+}]_i$. Regulation of $[Ca^{2+}]_i$ therefore appeared to be necessary for these processes, suggesting that Ca^{2+} functions in chemoattractant signal transduction.

We therefore investigated the relation between cell shape, cell movement, and $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ was measured with the Ca^{2+}-sensitive fluorescent probe Fura-2, which was microinjected into the cells in the free acid form to obviate problems from incomplete de-esterification or intracellular compartmentation that may occur when cells are incubated with the acetoxymethyl ester of this dye (*11*). Pairs of fluorescent images at 340 and 380 nm excitation were taken in rapid succession with a high efficiency, low noise, cooled, charge-coupled device (CCD camera) at intervals of 10 to 30 s, while phase contrast images were recorded at more frequent intervals at 680 nm (Fig. 1A).

Eosinophils stimulated with serum developed a polarized motile morphology (Fig. 1B) and increased their overall $[Ca^{2+}]_i$. The average $[Ca^{2+}]_i$ in resting

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