A Reassessment of *Astraspis desiderata*, the Oldest North American Vertebrate

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The fossil evidence for the early evolution of vertebrates consists of the remains of agnathans, jawless vertebrates, from the Ordovician of Australia, North America, and Bolivia. Because of the fragmentary nature of the material, it has not been possible to reconstruct these animals sufficiently well to understand their relation to each other and to later vertebrates. Though two genera have been known from the Harding Sandstone of North America since 1892, there are still only three articulated specimens known, two of *Astraspis desiderata* and one of *Eriptychius americanus*. The most recently found specimen of *Astraspis* was reexamined and found to show the orbit, a series of eight branchial openings and a complete tail, structures hitherto undescribed in any Ordovician vertebrate. A reconstruction of *Astraspis* shows, on the basis of the series of branchial openings, that it is a primitive craniate and not a heterostracan as previously thought.

SINCE THE DISCOVERY OF VERTEBRATES in the Ordovician (470 million years ago) Harding Sandstone of Colorado in 1892 (*1*), only two other horizons worldwide have yielded unequivocal vertebrates of similar antiquity. Slightly older vertebrates occur in sediments from the Amadeus Basin of central Australia (*2*) and slightly younger vertebrates have recently been reported from Bolivia (*3*). Though phosphatic fragments from the Upper Cambrian and Lower Ordovician have been identified as vertebrate (*4*), their status is uncertain at present.

These vertebrates have all been referred to a single group of jawless fish, the Heterostraci, which were particularly abundant during Upper Silurian and Devonian times. The external bony armor of these fishes is common at some localities, but is normally disarticulated and fragmentary. No complete individuals are known from any Ordovician locality and only three articulated shield fragments represent the two genera from the Harding Sandstone. The one specimen of *Eriptychius americanus* (*5*) is too poorly preserved to allow a reconstruction to be made. Two specimens of *Astraspis desiderata* exist. The original (*1*) consists of a natural external mold of a partial dorsal headshield and shows that the external armor consisted of closely fitting, polygonal, bony tesserae or plates. The second specimen was discovered in 1968 and was reported (*6*) as a poorly preserved mass of scales and plates in which the head and tail were missing. I was able to study this specimen recently and discovered that its major features had been incorrectly identified. The area identified as a tail is in fact part of the headshield, and the area described as a partial headshield is in fact a complete tail. This is, therefore, the most complete Ordovician vertebrate known and provides important new information about the morphology of early vertebrates.

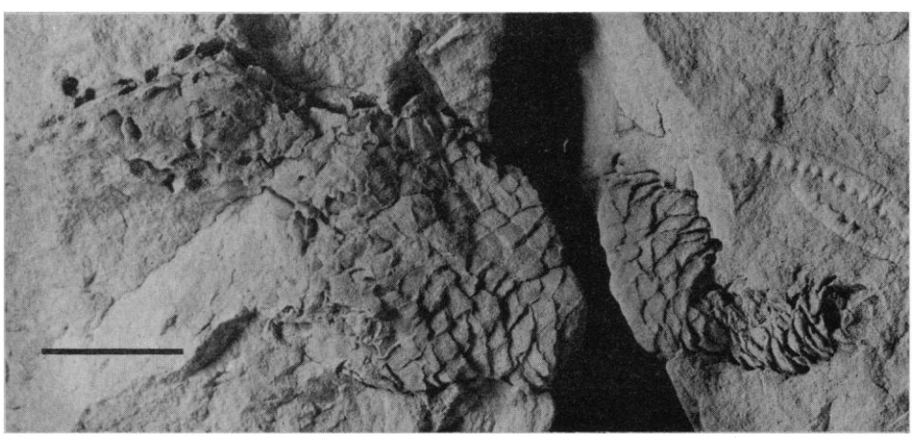

Fig. 1. Articulated specimen of *Astraspis desiderata* (PF 5733) preserved as natural internal mold. Anterior is to the left. The lateral series of dorsal shield plates forms the upper margin of the larger fragment. The smaller fragment contains the articulated tail and caudal fin. The elongated and toothed objects above the caudal fin are specimens of *Dictyorhabdus priscus*, an organism of unknown affinity. Scale bar, 20 mm.

The specimen (Fig. 1) is preserved as an internal mold in typical reddish Harding Sandstone. It consists of two pieces that do not fit together, thus there is an unknown amount of material missing from the middle of the specimen. The direction of imbrication of the scales makes it quite clear that the larger piece contains the head and anterior part of the tail while the smaller piece contains the posterior part of the tail and the caudal fin. Much of the left side of the dorsal shield is missing as is the entire ventral shield. However, one of the most important aspects of this specimen is that the right lateral margin of the shield is present and the individual plates are still in an articulated position.

By removing the last fragments of weathered bone and then making a latex peel of the surface, I was able to produce a replica of the ventral surface of the dorsal shield that includes the lateral margin along almost its entire length (Fig. 2). A regular series of plates occupies a ventrolateral position along the edge of the shield and attaches dorsally to a series of large marginal plates. The position of the orbit can be clearly seen from the presence of a large supraorbital plate, and a small antorbital plate that is slightly out of position shows that the orbit was surrounded by a series of tesserae in life. A short section of the infraorbital sensory canal is present on the small postorbital plate and corroborates the position of the orbit suggested from analysis of the type dorsal shield (*7*). As the anterior part of the headshield is missing in both specimens, the position of the orbits in relation to the front of the shield is unknown.

Behind the orbit are a series of nine roughly pentagonal plates (Fig. 2) bearing tubercular ornamentation on their outer surfaces that is typical of *Astraspis*. Each plate is attached to the dorsolateral series by its longest edge and to the adjacent plates by two short surfaces. The remaining two edges form a ventrally directed point such that a triangular space is enclosed between each pair of plates. The edges of the triangular spaces are tapered and are offset from each other, the posterior edge being more adaxially placed. As sutured plates do not have tapered edges, it appears that the spaces were not filled by other plates, but, in fact, formed a series of short, posteriorly directed ducts. Eight openings can be identified (Fig. 2, 1 through 8); however, as the lateral series appears to be incomplete posteriorly, and as there is no clear demarcation of the posterior margin of the dorsal shield, it may be that additional openings were present.

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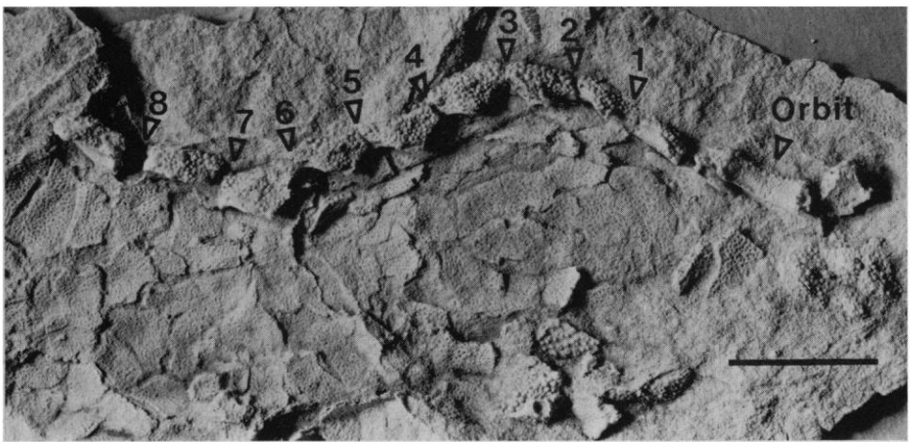

Fig. 2. Latex peel of the lateral margin of the dorsal shield of *Astraspis desiderata* showing the orbit, a series of branchial plates, and branchial openings (1 through 8). Anterior is to the right. Scale bar, 10 mm.

The tesserae of the dorsal shield are sutured marginally, but posteriorly they become imbricated and form polygonal scales (Fig. 1). The body behind the dorsal shield is oval in cross section, though this may be due in part to dorsoventral crushing during preservation. The posterior part of the tail is also oval in cross section, but tapers rapidly to its termination as a stubby caudal fin. The scales in the posterior part of the tail show considerable overlap, which may indicate a greater degree of flexibility in this area. However, as the tail and caudal fin are visible in lateral view, they must have been rotated, which would disrupt the scale pattern. The caudal fin consists of a clump of large triangular scales and does not appear to be deeper than the more anterior part of the tail though this similarity in proportions may also be due to crushing. The termination is clearly symmetrical, however, and not reverse heterocercal (with the notochord extending into the ventral lobe) as stated in the original description of the specimen (*6*).

This is the first description of the position and shape of the orbit, the position and number of the branchial openings, and the shape of the tail and caudal fin for an Ordovician vertebrate. A reconstruction of *Astraspis desiderata* based on the new information shows the animal to have been about 130 mm long and somewhat dorsoventrally compressed over much of its body (Fig. 3). An exoskeleton of polygonal bony tesserae that are peripherally sutured covers the anterior part of the body and may become fused to form a continuous shield dorsally. Posteriorly, the tesserae become imbricated and form polygonal scales. The rostral area is poorly sutured and is not present in either specimen of *Astraspis*. The orbit is lateral, circular, and surrounded by a series of orbital plates. Behind the orbit a series of branchial plates enclose at least eight branchial openings. Though a section of the tail is missing in the new specimen, it has been restored in Fig. 3 at about the same length as the dorsal shield, as these are common proportions in Silurian and Devonian Heterostraci. The caudal fin was small relative to the rest of the animal, which suggests that it was not an active swimmer. The Harding Sandstone was deposited in restricted marine conditions with fluctuating salinity, possibly on a widespread tidal flat (*8*), suggesting that *Astraspis* may have been euryhaline. Though they were probably benthonic organisms, there is no evidence to support the view that they may have lived just below the surface of the sediment (*9*).

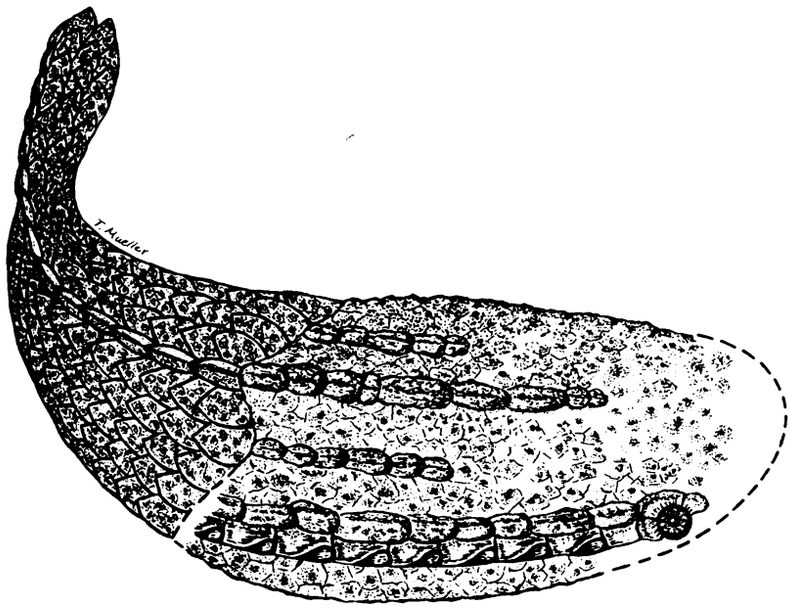

Fig. 3. Reconstruction of *Astraspis desiderata* based on PF 5733 and with additional information from the type specimen USNM 8121. Length of specimen, 130 mm.

The Heterostraci were originally defined on the basis of a common external branchial opening and large dermal plates covering the branchiocephalic region (*10*). Many other poorly known taxa were added later, however, on the basis of the acellular nature of their exoskeleton. This is true of the known Ordovician vertebrates (*1–3*) for none of which was the structure or placement of the branchial openings known. The demonstrated presence of a series of branchial openings in *Astraspis* shows that this genus can no longer be considered a heterostracan. Whether the other Ordovician vertebrates can still be considered heterostracans cannot be resolved at present, although platelets in the branchial areas of the Australian and Bolivian genera (*2, 3*), and an isolated perforated plate of *Eriptychius* from the Harding Sandstone (*11*) suggest that these forms may also have had a series of branchial openings.

It appears, therefore, that the Ordovician vertebrates may constitute a group of primitive craniates possessing aspidin (acellular bone) as do the heterostracans, but differing from them in the presence of numerous branchial openings. Unless aspidin is considered to be a derived character, which is unlikely, it seems clear that the presence of numerous branchial openings is a primitive character state and that the Heterostraci represent a derived condition.

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scaled ornamentation have been described from Upper Cambrian to Middle Ordovician rocks, it has yet to be conclusively shown that any of them are vertebrate.
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Mapping Patterns of c-*fos* Expression in the Central Nervous System After Seizure

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A dramatic and specific induction of c-*fos* was observed in identifiable neuronal populations in vivo after administration of the convulsant Metrazole. This effect was time- and dose-dependent and was abolished by prior treatment with the anticonvulsant drugs diazepam or pentobarbital. About 60 minutes after administration of Metrazole, c-*fos* messenger RNA reached a maximum and declined to basal levels after 180 minutes. A further decrease below that in normal brain was observed before a return to basal levels after 16 hours. While Metrazole still elicited seizures during this period, reinduction of c-*fos* was largely refractory. At 90 minutes, c-*fos* protein was observed in the nuclei of neurons in the dentate gyrus, and in the pyriform and cingulate cortices. Subsequently, c-*fos* protein appeared throughout the cortex, hippocampus, and limbic system. Thus, seizure activity results in increased c-*fos* gene expression in particular subsets of neurons.

THE C-*fos* GENE IS THE CELLULAR homolog of the oncogene (v-*fos*) carried by the FBJ and FBR murine osteogenic sarcoma viruses (*1*). It encodes a nuclear protein (Fos) that is associated with chromatin and exhibits a DNA-binding activity in vitro (*2, 3*). In most cell types, the basal level of c-*fos* expression is relatively low; however, it can be induced rapidly and transiently by a diverse range of extracellular stimuli (*4*). Although initial observations suggested that Fos was associated with mitogenesis, this viewpoint has been challenged, since induction of c-*fos* has been observed where promotion of differentiation rather than stimulation of cell division occurs. Furthermore, in studying the mechanisms that couple membrane events to c-*fos* activation in PC12 cells, we found that agents or conditions that provoke a voltage-dependent calcium influx were potent inducers of c-*fos* (*5*). Similarly, others have found that occupation of the nicotinic acetylcholine receptor on PC12 cells also elicited c-*fos* induction (*6*). These studies led us to speculate that c-*fos* expression might be regulated by neuromodulators in vivo. Therefore, we studied c-*fos* expression in brains of mice that had been treated with a convulsant. The c-*fos* messenger RNA (mRNA) was monitored by Northern transfer and hybridization, while the subcellular and brain regional localization of the c-*fos* protein were followed by immunocytochemistry.

We have used a procedure, Metrazole

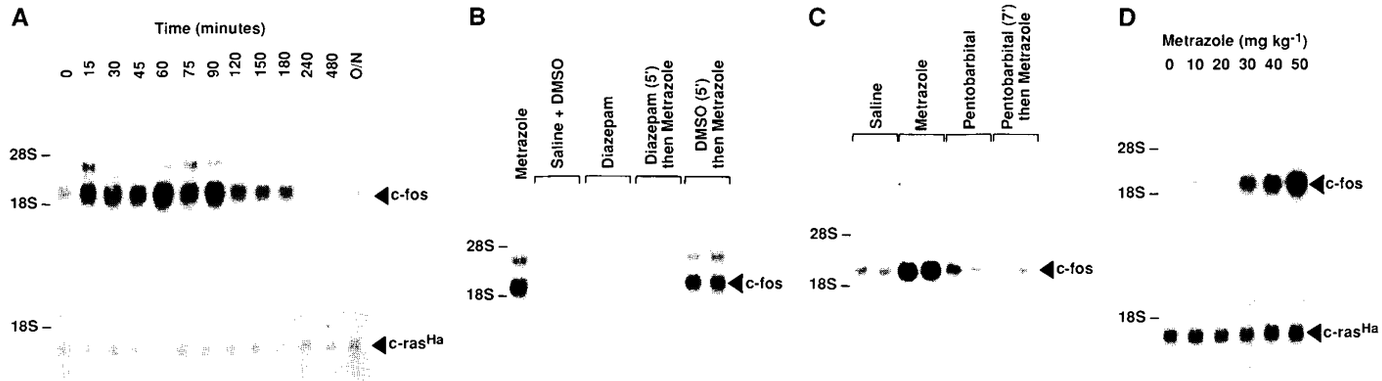

Fig. 1. RNA analysis of Metrazole-induced c-*fos* expression. BALB/c mice were injected intraperitoneally with Metrazole [50 mg/kg in (A) and (B) at the doses indicated in (C)]; the animals were killed by decapitation [at times given in (A) and (B), or at 60 minutes in (C)]. The whole brain was removed and immediately frozen in liquid nitrogen, and frozen tissues were homogenized in a 3*M* LiCl plus 6*M* urea solution. The homogenates were held overnight on ice, and RNA was then extracted by a modification of the LiCl and urea procedure (*20, 21*). After gel electrophoresis and transfer onto nitrocellulose filters, RNA's were hybridized with *fos* and *ras*^{Ha} probes (*22*). (**A**) Time course of c-*fos* expression. Each animal was injected intraperitoneally with Metrazole (50 mg/kg) and killed at the time indicated following treatment. Identical filters were probed either for c-*fos* or for c-*ras*^{Ha} mRNA. The position of 28*S* and 18*S* RNA's are indicated (c-*fos* filter exposure 4 hours, c-*ras*^{Ha} filter exposure 17 hours). (**B**) Effect of diazepam on Metrazole-induced c-*fos* expression. Pairs of animals were injected with Metrazole alone (in saline) (50 mg/kg), diazepam alone [in dimethyl sulfoxide (DMSO)] (10 mg/kg), or diazepam (10 mg/kg) 5 minutes before administration of Metrazole (50 mg/kg). Pairs of control animals injected with either a saline plus DMSO solution or with DMSO alone 5 minutes before administration of Metrazole (50 mg/kg) were treated in parallel. Animals were killed 60 minutes after treatment and total RNA was isolated from whole brain (see A). Northern analyses for the presence of c-*fos* mRNA was as described for (A) (4-hour film exposure). (**C**) Effect of pentobarbital on Metrazole-induced c-*fos* expression. Pairs of BALB/c mice were injected with saline, Metrazole (50 mg/kg), sodium pentobarbital (80 mg/kg), or sodium pentobarbital (80 mg/kg) 7 minutes prior to Metrazole (50 mg/kg). Animals were killed 60 minutes after treatment with Metrazole, and total RNA was isolated from whole brain as described in (A). Northern analyses for the presence of c-*fos* mRNA was performed as in (A). (**D**) Dose response of Metrazole-induced c-*fos* expression. Animals were injected with Metrazole at 0, 10, 20, 30, 40, or 50 mg/kg and killed after 60 minutes. Animals given higher doses did not survive the treatment. Identical Northern filters were probed either for c-*fos* or for c-*ras*^{Ha}, filter was exposed for 17 hours.