Bowfin genome reveals old dogfish can teach researchers new tricks

August 30 2021

Freshly deposited bowfin eggs attached to nest material. Male bowfin build nests in which females lay eggs. After the male fertilizes the eggs, it will remain with the nest to guard the young. Credit: M. Brent Hawkins

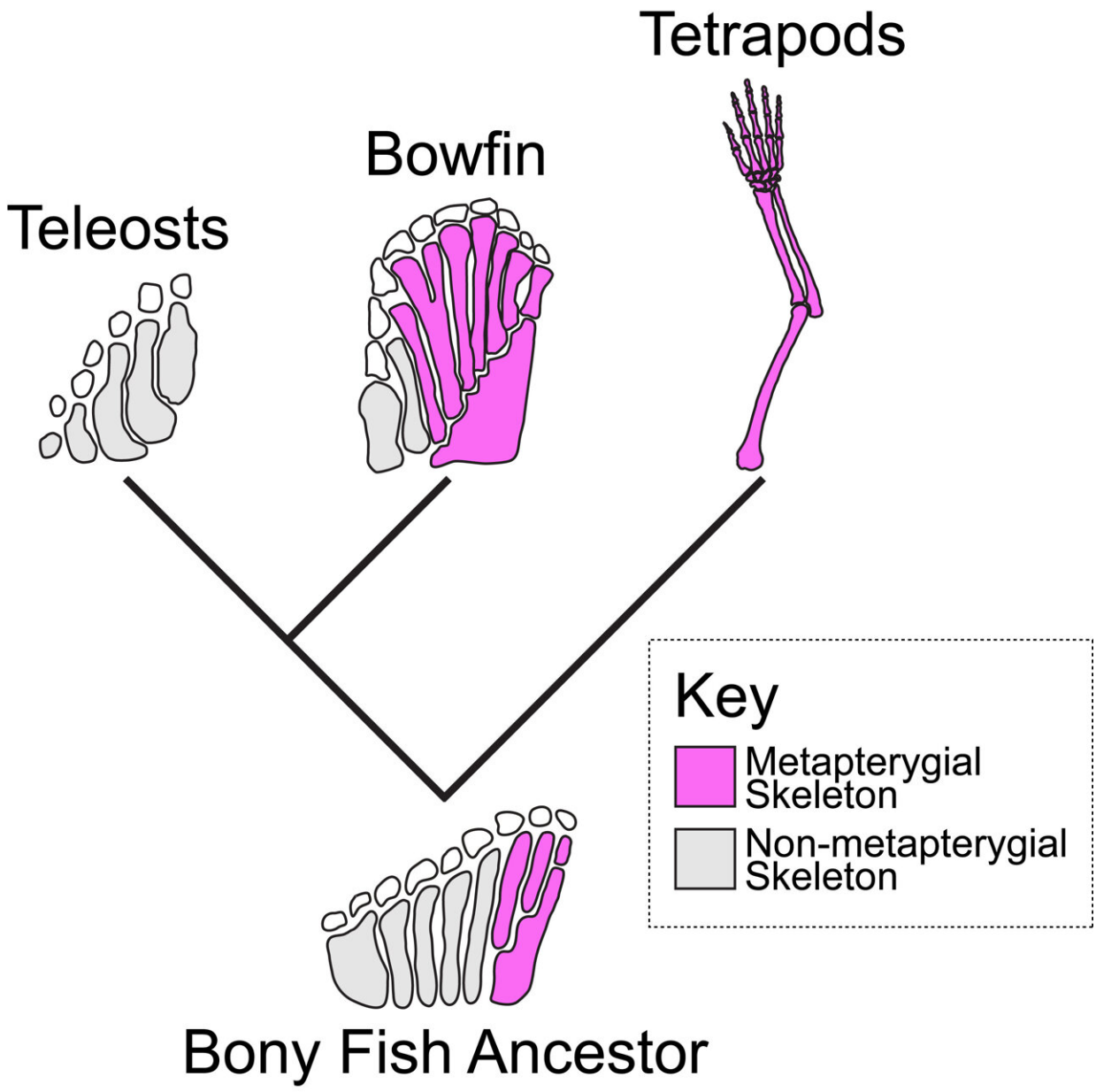
The fish species *Amia calva* goes by many names including bowfin, freshwater dogfish, grinnel, and mud pike. No matter what you call it,

this species is an evolutionary enigma because it embodies a unique combination of ancestral and advanced fish features.

In a paper published August 30 in *Nature Genetics* an international and collaborative team of researchers, headed by Ingo Braasch and Andrew Thompson of Michigan State University, have begun to unravel the enigma by sequencing the genome of the bowfin fish. Their collaborative analysis yielded unexpected insights into diverse aspects of the biology of this mysterious, ancient lineage.

The bowfin is a bony fish endemic to eastern North America and is the sole surviving member of a once large lineage of many species that are now known only from fossils. Scientists have long been fascinated with the bowfin because it bears a combination of ancestral features, such as lung-like air breathing and a robust fin skeleton, and derived features like simplified scales and a reduced tail. The bowfin also occupies a key position in the fish family tree, where it sits between the teleosts, a large and diverse group that arose recently, and more ancient branches that include sturgeons, paddlefish, and bichirs.

Due to this special position in the fish family tree, the bowfin can help scientists understand how aspects of modern fishes evolved from their ancient antecedents. By examining the bowfin genome, scientists can investigate the genetic basis of the unique set of old and new features of the bowfin. They can also use this genomic information as a framework to better understand the origin of the teleosts, which have duplicated and extensively modified their genomes since separating from the bowfin lineage and emerging as the dominant lineage in most aquatic habitats.



Schematics show the arrangement of bones in fins and limbs. Elements that are derived from the ancestral metapterygium are shown in magenta. The tetrapod limb and a portion of the bowfin fin arose from the metapterygium, while teleosts have lost the metapterygial components. Credit: M. Brent Hawkins

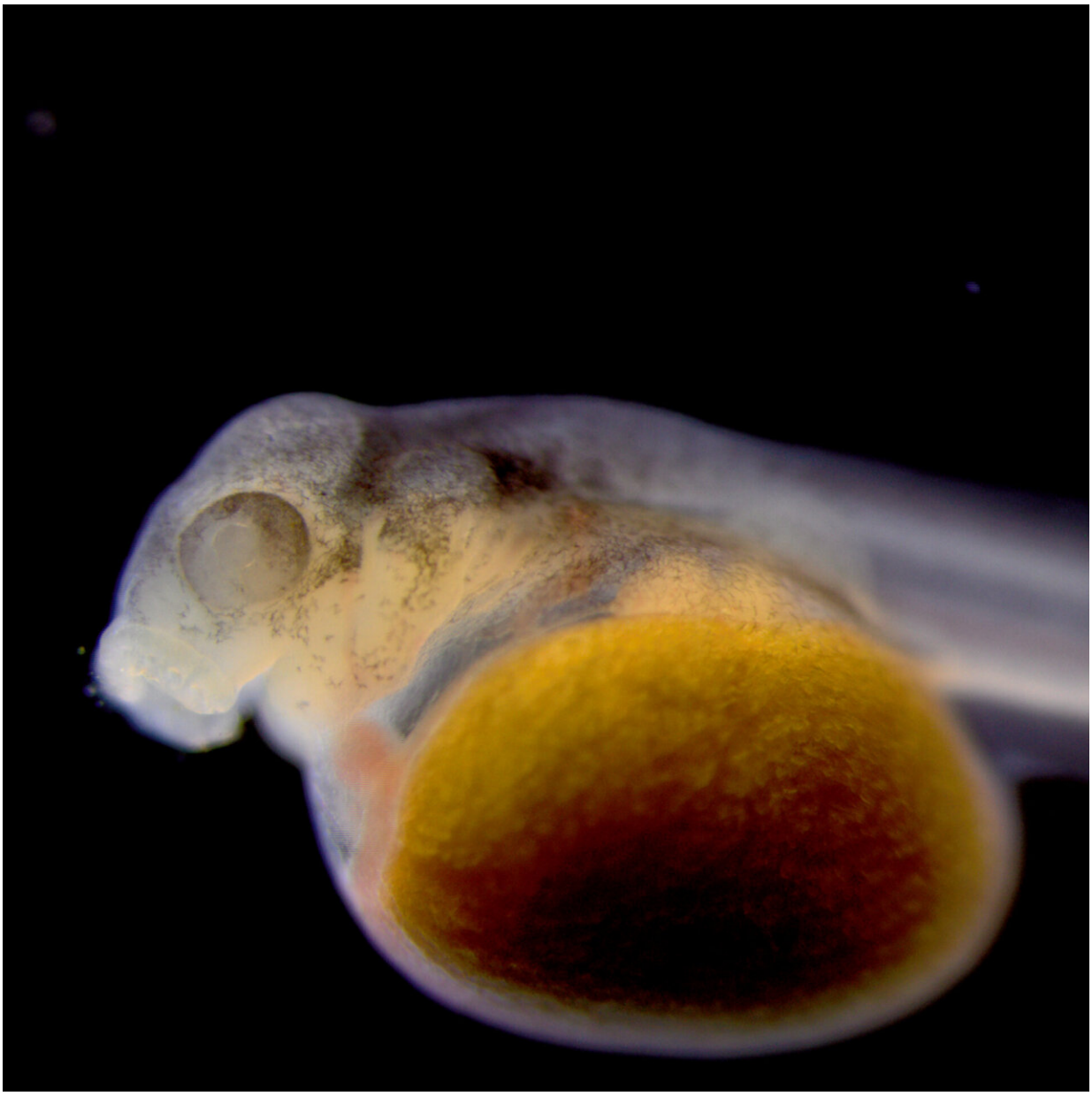
As a doctoral candidate in the Department of Organismic and

Evolutionary Biology at Harvard University, study co-author M. Brent Hawkins (Ph.D. '20) examined the evolution and development of the bowfin pectoral fin. Hawkins' doctoral thesis, conducted with Professor Matthew P. Harris, Harvard Medical School and Boston Children's Hospital, and Professor James Hanken, Department of Organismic and Evolutionary Biology at Harvard university, contributed some of the study's most surprising findings.

Hawkins focused on the pectoral fin of the bowfin because of its ancestral configuration of the skeleton. The bowfin retains the metapterygium, which is a portion of the fin skeleton that is homologous to the limb bones of tetrapods. Model organisms such as the widely used zebrafish and medaka have lost the metapterygium, which makes comparisons between the fin and the limb difficult. By studying the bowfin fin, scientists can use knowledge of bowfin development as a steppingstone to bridge teleost fin development to tetrapod limb development and help explain the evolution of the fin-to-limb transition.

With co-authors Emily Funk and Amy McCune, both at Cornell University, Hawkins collected young bowfin embryos from nests in the wild in upstate New York. Hawkins raised the embryos, collecting pectoral fin samples as they developed. He extracted mRNA from the samples and performed Transcriptome Sequencing with the help of the Harvard University Bauer Core to determine which genes are turned on in the developing fin by parsing the transcriptome data using the genomic reference sequence. Once identified, he used in situ hybridization to visualize where these genes are activated during fin outgrowth. Initially, Hawkins expected the bowfin gene data to look very similar to other fins and limbs. "As a field, we have characterized many of the genes involved in appendage patterning. We have a good idea of what the essential fin and limb genes are and where they should be turned on," said Hawkins. However, when he analyzed the fin data he was shocked by the results.

While the bowfin pectoral fins did express many of the expected appendage growth genes, some of the most critical of these genes were in fact entirely absent. One such gene called fibroblast growth factor 8 (Fgf8) is turned on at the far tip of developing fins and limbs and is required for the outgrowth of these appendages. When Fgf8 is lost appendage outgrowth is impaired, and if extra Fgf8 is applied to an embryo, it can cause a new limb to form. "Every other fin and limb we know of expresses Fgf8 during development," Hawkins said. "Discovering that bowfin fins don't express Fgf8 is like finding a car that runs without a gas pedal. That the bowfin has accomplished this rewiring indicates unexpected flexibility in the fin development program. With the genome in hand, we can now unlock how this flexibility evolved."



A recently hatched bowfin larva facing to the left as seen through a microscope. Credit: M. Brent Hawkins

While some genes like Fgf8 were mysteriously absent from the bowfin fin, other genes were unexpectedly activated in the fins. The HoxD14 gene is expressed in the fins of fishes from the deeper branches of the

fish family tree, such as paddlefish, but this gene was lost in more recent branches including the teleosts. When the authors found this gene in the bowfin genome data, they thought it must not be expressed because the DNA sequence did not encode a functional protein. Surprisingly, Hawkins and colleagues found that bowfin fins made HoxD14 gene transcripts at high levels, even though it did not code for a protein. "The fact that the HoxD14 gene can no longer make a protein, but it still transcribed into mRNA at such high levels suggests that there might be another function that we do not yet understand. We might be seeing a new level of Hox gene regulation at play in the bowfin," said Hawkins.

Taken together the Fgf8 and HoxD14 results indicate that genetic programs, even those that guide the formation of important structures such as fins and limbs, are not as invariable as previously thought. "By studying more species, we learn which rules are hard and fast and which ones evolution can tinker with. Our study shows the importance of sampling a broader swath of natural diversity. We might just find important exceptions to established rules," said Hawkins.

Hawkins also suggests that the results of the bowfin study serve as a warning against treating members of deeper branches of the tree of life as stand-ins for actual ancestors. "Some people might describe species like the bowfin as a 'living fossil' that reliably represents the ancestral condition of a lineage. In reality, these deeper branches have been evolving past that ancestor for just as long as the more recent branches, doing their own thing and changing in their own ways. In evolution, old dogs do learn new tricks."

Hawkins is currently a postdoctoral researcher in the lab of Matthew P. Harris at Harvard Medical School and Boston Children's Hospital.

More information: The bowfin genome illuminates the developmental evolution of ray-finned fishes, *Nature Genetics* (2021). DOI:

10.1038/s41588-021-00914-y ,
www.nature.com/articles/s41588-021-00914-y

Provided by Harvard University