Discovery of 500-million-year-old fossil reveals astonishing secrets of tunicate origins

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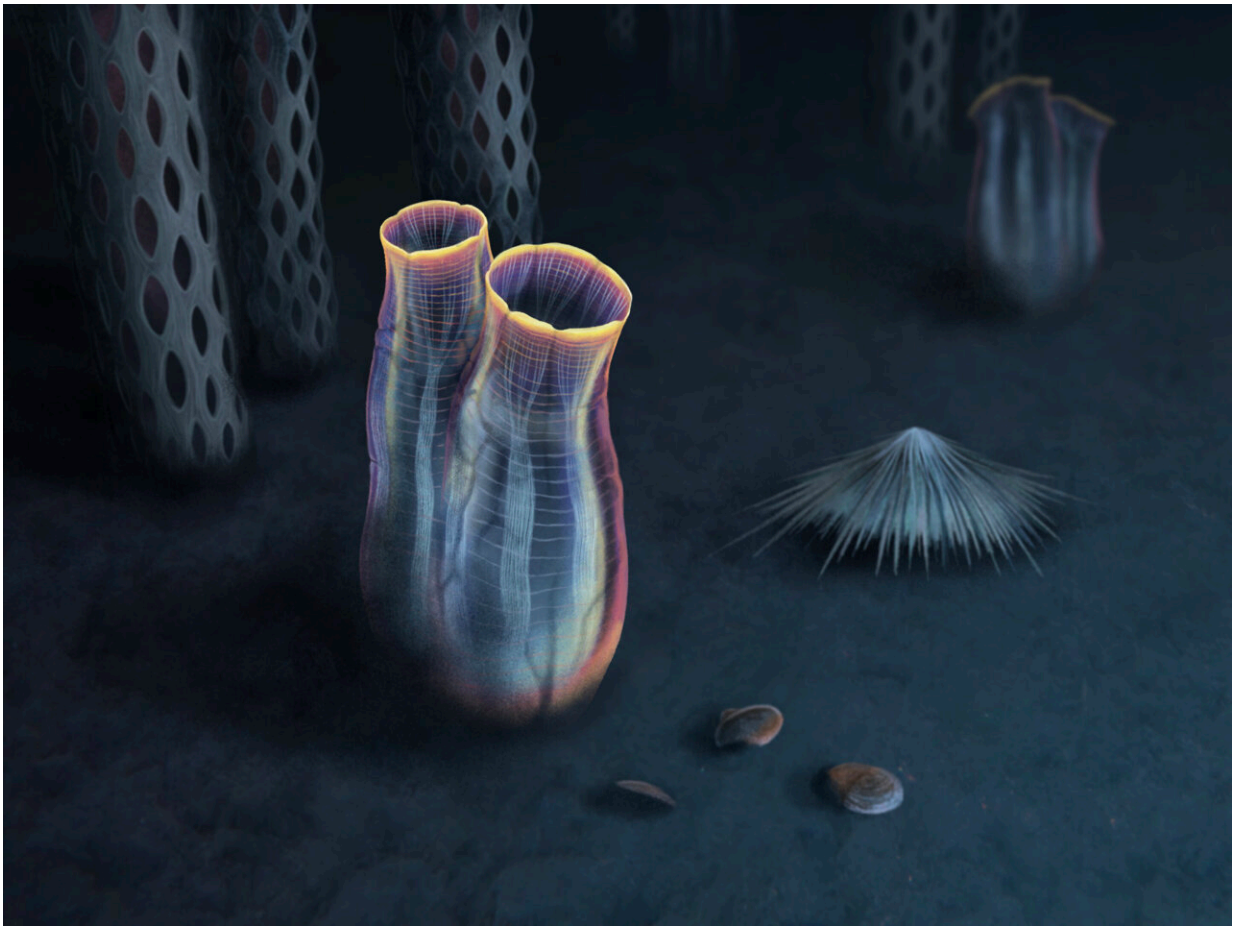


Artistic reconstruction of Megasiphon thylakos, a benthic organism that lived directly on the seafloor. M. thylakos was also sessile (non-moving) and spent its time filter feeding using its prominent siphons. Also reconstructed in the vicinity are other species commonly found in the Marjum Formation, the site from which M. thyalkos was discovered. Nearby brachiopods (bottom center) and the spiny sponge Choia (center middle) are common in many Cambrian

environments. In the background is the hemichordate Oesia, which lived in perforated tubes. Credit: Original artwork by Franz Anthony

Karma Nanglu says his favorite animal is whichever one he's working on. But his latest subject may hold first place status for a while: a 500-million-year-old fossil from the wonderfully weird group of marine invertebrates, the tunicates.

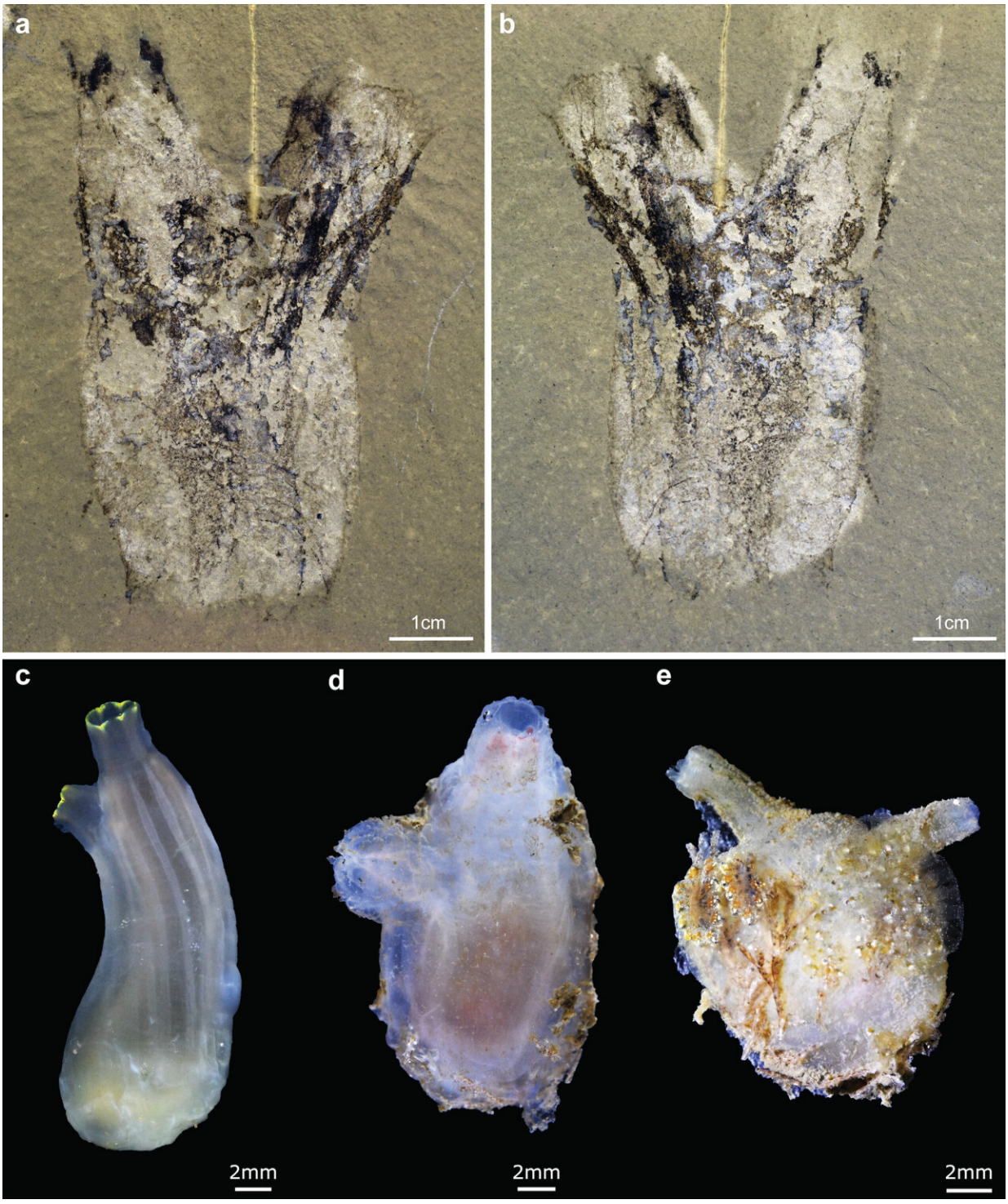
"This animal is as exciting a discovery as some of the stuff I found when hanging off a cliffside of a mountain, or jumping out of a helicopter. It's just as cool," said Nanglu, postdoctoral researcher in the Department of Organismic and Evolutionary Biology at Harvard University.

In a new study in *Nature Communications*, Nanglu and co-authors describe the new fossil, named Megasiphon thylakos, revealing that ancestral tunicates lived as stationary, filter-feeding adults and likely underwent metamorphosis from a tadpole-like larva.

Tunicates are truly strange creatures that come in all shapes and sizes with a wide variety of lifestyles. An adult tunicate's basic shape is typically barrel-like with two siphons projecting from its body. One of the siphons draws in water with food particles through suction, allowing the animal to feed using an internal basket-like filter device. After the animal feeds, the other siphon expels the water.

There are two main tunicate lineages, ascidiaceans (often called "sea squirts") and appendicularias. Most ascidiaceans begin their lives looking like a tadpole and mobile, then metamorph into a barrel shaped adult with two siphons. They live their adult life attached to the seafloor. In contrast, appendicularians retain the look of a tadpole as they grow to adults and swim freely in the upper waters.

"This idea that they begin as tadpole-looking larva that, when ready to develop, basically headbutts a rock, sticks to it, and begins to metamorphosis by reabsorbing its own tail to transform into this being with two siphons is just awe-inspiring," sais Nanglu.



Comparisons between the new Cambrian tunicate Megasiphon thylakos (a,b) with some modern tunicates (c,d,e). In particular, M. thylakos shares the rounded vase or barrel-like body and prominent pair of siphons of the modern ascidiacean tunicates. Given the fact that M. thylakos is half-a-billion years old,

this suggests that ancestrally, tunicates lived much like modern ascidiaceans: they had a non-moving adult form with siphons for filter feeding, a body plan that was arrived at after metamorphosing from a tadpole-like juvenile. The modern species represented are c: Ciona, d: Ascidiella, e: Molgula. Credit: Rudy Lerosey-Aubril (a,b) and Karma Nanglu (c,d,e)

Interestingly, tunicates are the closest relatives of vertebrates, which includes fish, mammals, and even humans. How this odd-looking creature could be related to vertebrates is hard to imagine were it not for that tadpole beginning. Tunicate's close relationship to vertebrates makes studying them critical for understanding our own evolutionary origins. Unfortunately, it's not easy to do as tunicates are almost completely absent from the entire fossil record, with only a handful of fossils appearing convincingly as members of the group.

With so few fossils, scientists relied mainly on what could be learned from modern tunicate species. Because no one knew the morphology and ecology of the last common ancestor of the tunicates, scientists could only hypothesize that it was either a benthic animal with two siphons, like the ascidiaceans, or a free-swimming animal like the appendicularians.

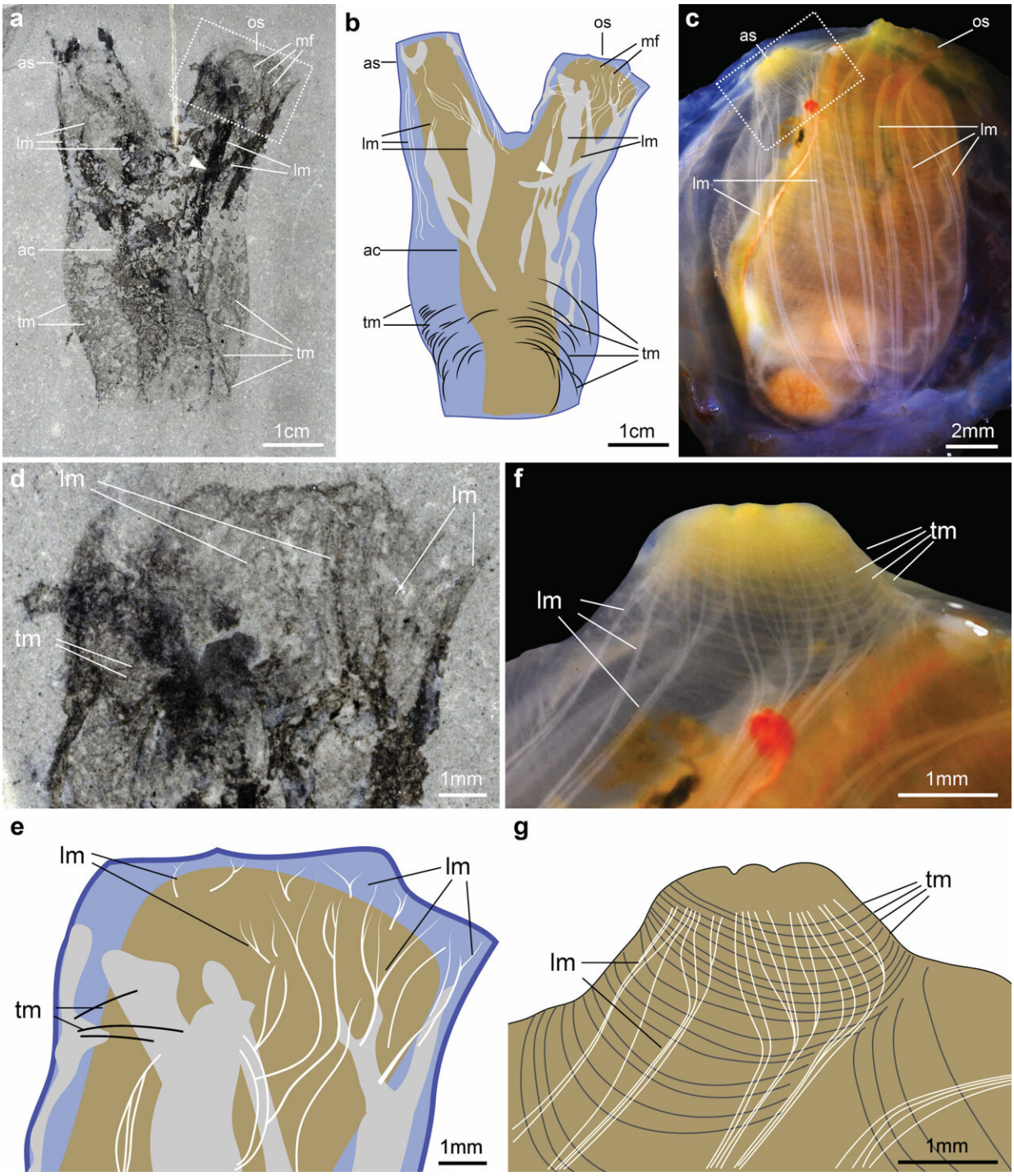
M. thylakos had all the basic hallmarks of an ascidiacean tunicate, a barrel-shaped body and two prominent siphon-like growths. But the feature that stood out to the team was the dark bands running up and down the fossil's body.

High powered images of M. thylakos allowed the researchers to conduct a side-by-side comparison to a modern ascidiacean. The researchers used dissected sections of the modern tunicate Ciona to identify the nature of Megasiphon's dark bands. The comparisons revealed remarkable

similarities between Ciona's muscles, which allow the tunicate to open and close its siphons, and the dark bands observed in the 500-million-year-old fossil.

"Megasiphon's morphology suggests to us that the ancestral lifestyle of tunicates involved a non-moving adult that filter fed with its large siphons," said Nanglu. "It's so rare to find not just a tunicate fossil, but one that provides a unique and unparalleled view into the early evolutionary origins of this enigmatic group."

M. thylakos is the only definitive tunicate fossil with soft tissue preservation that has been discovered to date. It is the oldest of its kind originating from the middle Cambrian Marjum Formation in Utah. The fossil was recognized as a tunicate by co-authors research associate, Rudy Lerosey-Aubril, and Professor Javier Ortega-Hernández (both in the Department of Organismic and Evolutionary Biology) while visiting the Utah Museum of Natural History (UMNH) in 2019.



Details of the anatomy of Megasiphon thylakos. M. thylakos had two prominent siphons and a barrel shaped body. It also had prominent longitudinal muscles running from the tips of the siphons to the base of the body. These are comparable with modern tunicates, including Ciona intestinalis, which is dissected in c and f. Even the micrometer sized individual muscle fibers can be

compared between this 500-million year old fossil and modern tunicates. Credit: James Wheeler (a,d) and Karma Nanglu (b,c,e,f,g)

"The fossil immediately caught our attention," said Ortega-Hernández, "although we mostly work on Cambrian arthropods, such as trilobites and their soft bodied relatives, the close morphological similarity of Megasiphon with modern tunicates was simply too striking to overlook, and we immediately knew that the fossil would have an interesting story to tell."

Fossils from the Marjum Formation date from shortly after the Cambrian Explosion, one of the most significant evolutionary events in Earth's history which occurred approximately 538 million years ago. During this time the most major animal groups appeared in the fossil record for the first time radically changing marine ecosystems. Tunicates, however, are noticeably absent in Cambrian rocks even though they are diverse and abundant in modern oceans.

There are many Cambrian fossil sites with exceptional preservation in the United States, but these are often overlooked compared to those from the Burgess Shale in Canada and Chengjiang in China. "The discovery of Megasiphon perfectly illustrates why Javier and I have been conducting fieldwork in Utah for the last ten years," said Lerosey-Aubril. "The Marjum strata has all of our attention right now as we know that it preserves fossils of animal groups, such as tunicates or comb jellies, that are almost entirely absent from the Cambrian fossil record."

Molecular clock estimates suggest that ascidiaceans originated 450 million years ago. However, at 500 million years old, M. thylakos provides the clearest view into the anatomy of ancient tunicates and their earliest evolutionary history. Significantly, M. thylakos provides

evidence that most of the modern body plan of tunicates was already established soon after the Cambrian Explosion.

"Given the exceptional quality of preservation and the age of the fossil, we can actually say quite a bit about the evolutionary history of the tunicates," said Nanglu. "This is an incredible find as we had virtually no conclusive evidence for the ancestral modes of life for this group before this."

After collecting hundreds of new fossils again this spring, the researchers are convinced the Marjum Formation has only started to reveal its secrets.

More information: A mid-Cambrian tunicate and the deep origin of the ascidiacean body plan, *Nature Communications* (2023). DOI: 10.1038/s41467-023-39012-4

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