# 3D preservation of trilobite soft tissues sheds light on convergent evolution of defensive enrollment

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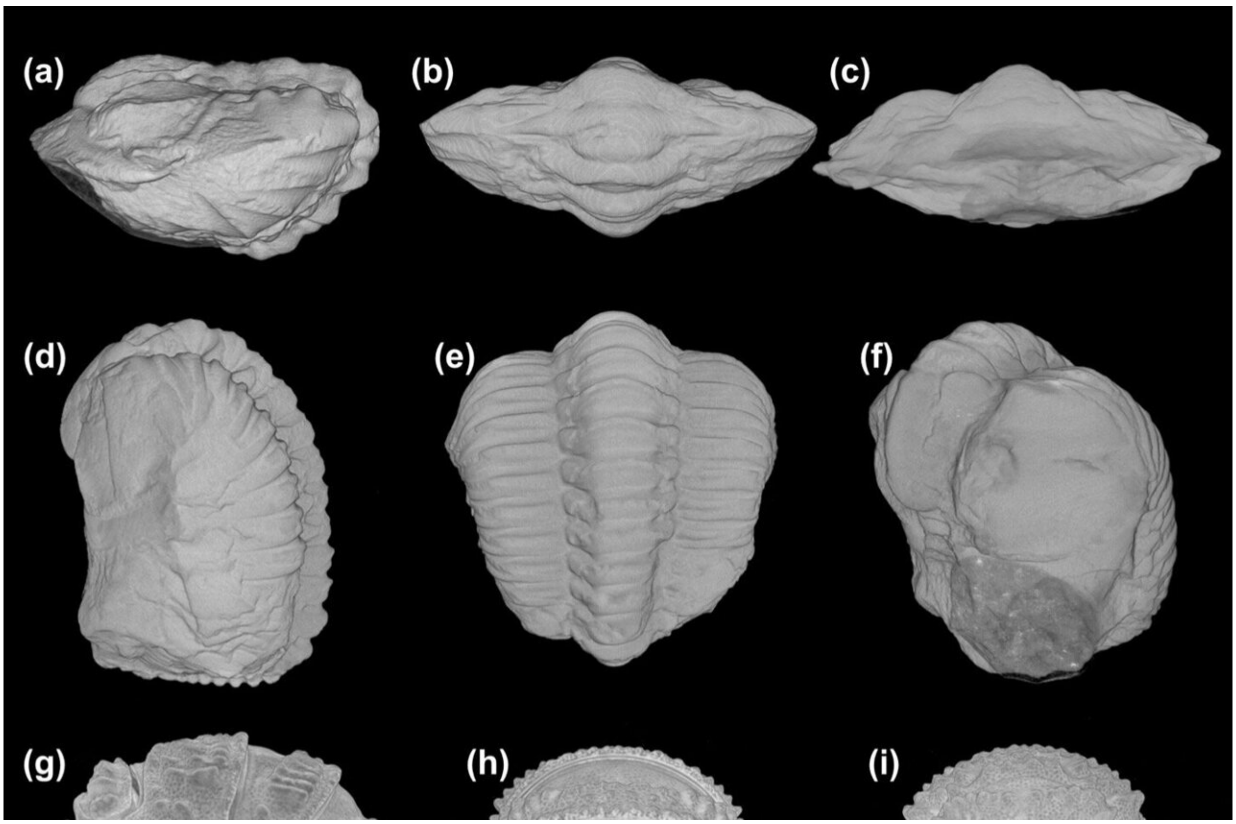


Enrollment in arthropods is an important defensive strategy that provides protection against predation. A—C, Enrolled Ceraurus from the Walcott-Rust Quarry. D—F, Enrolled Flexicalymene from the Walcott-Rust Quarry. G—I, Enrolled isopod. J—L, Enrolled glomerid millipede. Credit: Sarah R. Losso

They'd been in the collections of the Harvard Museum of Comparative Zoology (MCZ) since the 1870s when they were first discovered. Nestled in among the largest collection of trilobites, the unique fossils rested in drawers until 145 years later when Sarah Losso, Ph.D. candidate in the Department of Organismic and Evolutionary Biology (OEB) at Harvard, began combing through the collection of trilobites as part of her dissertation.

"I started my Ph.D. going through all of these thin sections of trilobites, imaging them, and trying to figure what we can actually see," Losso said. "And then I came across something we never see in trilobite fossils."

In a new study, published in *Proceedings of the Royal Society B*, lead author Losso describes the unusual three-dimensional trilobite fossils prepared as thin sections showing the 3D soft tissues during enrollment. The study reveals the soft undersides of enrolled trilobites and the evolutionary mechanism that allows arthropods to enroll their bodies for protection from predators and adverse environmental conditions.

Trilobites are early arthropods from the Paleozoic Era. They were numerous and super diverse until they were wiped out in the End Permian mass extinction. Trilobites are named for their three-lobed body, which is covered by a durable exoskeleton enriched in calcite that is easily preserved; making trilobites an iconic part of the Paleozoic fossil record. Their segmented bodies have numerous limb pairs that include a walking leg and a gill for respiration. Unlike their durable exoskeleton, their undersides, including the legs, are much softer so are rarely fossilized unless the perfect conditions are met. Trilobites have no close relatives, despite their resemblance to horseshoe crabs. However, horseshoe crabs can serve as a useful comparison because of their similar lifestyle.

The challenges associated with fossilizing soft tissues make the trilobites

Losso studied even more special. The fossils are from the Mohawkian Stage of the Ordovician Period (462-451 million years ago). They were discovered in the Walcott-Rust Quarry located in upstate New York near Trenton Falls; a region originally inhabited by the Iroquois tribe. The quarry is named in part after the scientist Charles D. Walcott, who discovered the enrolled trilobites there in his youth, before going on to famously discover the Burgess Shale while Director of the Smithsonian Institution.

The fossils, which Walcott sold to the MCZ and the Smithsonian in the 1870s, were trapped in a sediment slurry that quickly moved downslope and entombed the trilobites, leading to the preservation of delicate tissues before decay destroyed them. They are unusual in that the soft tissues, such as legs and antennae, are preserved in 3D. Walcott studied the fossils by cutting them into sections of paper-thin slices of rock and attaching them to glass slides using balsam sap. Though doing the best with what was available at the time, Walcott's method of preparation makes the fossils difficult to study, because 3D structures are being seen as a 2D plane.

"These were the first known complete trilobite appendages," said Losso, "before their discovery in the late 1800s, scientists knew of the walking leg, but not what the gill branches looked like." Because of the environmental disturbance, the trilobites enrolled to protect their more delicate appendages. Sediment then surrounded the legs of the partially enrolled trilobites, creating a mold of the external shape even while the tissues decayed.

Enrollment occurs in many different organisms. It is a defense strategy for animals with hard exoskeletons and softer tissues on their underside. We see enrollment in modern animals including pill bugs (isopods), pill millipedes (millipedes), and even armadillos. By enrolling their bodies, these animals can protect their vulnerable soft tissues with their hard exoskeletons from predators. In modern terrestrial arthropods, it can also protect against desiccation and loss of moisture.

While the mechanics of trilobite enrollment are well studied, these observations have only been made by examining their exoskeletons due to a lack of enrolled fossils with soft tissue preservation. Of the 20,000 species of trilobites, fewer than 40 have soft tissue preservation. And of those 40, most only preserve parts of a leg or antenna. Only a dozen species have known complete appendages, yet most of those are preserved as highly compressed, flat fossils, as is seen in the Burgess Shale from British Columbia.

"These fossils give us the first clear view of the three-dimensional organization of trilobite soft tissues, as well as the first molds of trilobites in different stages of enrollment, which allowed us to actually see how they moved their appendages and other structures in order to enroll," Losso said.

Trilobites and other arthropods have rows of dorsal exoskeletal plates on their back and undersides. The plates on the back, called tergites, are reinforced and much larger than the plates on the undersides. The sternites are a row of rigid plates along the underside and are softer and more prone to decay, so are rarely seen in the fossil record. The Walcott-Rust fossils, however, had preserved ventral structures including the sternites and limbs.

Though on the softer side, the sternites are too long and rigid for the animal to enroll if they cannot flex or articulate. To get around this, the sternites actually slide past each other in a dipping motion, similar to window blinds, which allows their body to scrunch up into a ball. Trilobite legs have also adapted to allow for this movement by evolving into a wedge shape that can fit together like pizza slices inside the enrolled ball.

"Because the part of the leg that attaches to the body is rarely seen, and certainly not in 3D, people frequently represented it as oval or squarish in cross-section," Losso said, "but oval or square legs would not allow for the flexibility needed for full enrollment."

Losso compared the Walcott-Rust fossils to CT scans of modern arthropods of millipedes, isopods, and horseshoe crabs, which were also housed in the MCZ collections. Losso found that modern arthropods used the same movement of sternites as we see in trilobites to enroll their bodies. Trilobites have been found throughout the Paleozoic Era. The adaptation to enroll allowed them to thrive, and they evolved structures to aid in enrollment such as the correct proportions and number of segments in the ventral to keep the body enrolled and wedge-shaped legs.

"The fossils have been known for a long time, but no one had put it together that we could study the ventral adaptations for enrollment using these really great fossils," Losso said. "These fossils allowed us to compare trilobites with modern arthropods and see that there is really only one way to accomplish enrollment given the arthropod body plan. It's a great example of convergent evolution amongst all these different lineages, and across a huge swath of time because we're seeing this in the Ordovician and today. It's an important strategy for survival that thrives today."

"Sarah's work has greatly improved our understanding of a key behavioral strategy that made trilobites incredibly successful for over 200 million years, and also brings new attention to the historical collections of Walcott-Rust fossils at the MCZ that went unstudied over 100 years," said senior author Javier Ortega-Hernández, Assistant Professor in OEB and Curator of Invertebrate Paleontology at the MCZ. "The new data on the three-dimensional morphology of trilobites during enrollment will allow us to accurately model this complex strategy for the first time, and represent a beautiful example of convergent evolution in action across distantly related species."

**More information:** Sarah R. Losso et al, Convergent evolution of ventral adaptations for enrolment in trilobites and extant euarthropods, *Proceedings of the Royal Society B: Biological Sciences* (2023). DOI: 10.1098/rspb.2023.2212

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