

Tardigrades

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Tardigrades are everywhere. They're tiny – usually under a millimeter long – and they're mostly transparent, so they're easy to miss. But you probably walk by them every day. We've been grooming them as emerging models for studying how body forms evolve and how biological materials can survive extremes.

Tardigrades are one of nature's superheroes. Dry them out, and they can remain in lifeless, suspended animation for decades until they're given water again. This ability is common in plant seeds and fungal spores, but very few animals can survive desiccation^{1,2}. Once dried, tardigrades can survive remarkable extremes. Some tardigrade species can withstand temperatures from nearly absolute zero to above boiling temperatures and a range of exceptionally low or high pressures. Some have survived a trip on a rocket exposed to the vacuum of space for days. And whether wet or dry, they can survive radiation doses about 1000 times greater than we humans can.

I first encountered tardigrades in a college course on animal diversity, and I developed an obsession for them later, starting in 1997. That's when a paper by Anna Maria Aguinaldo and colleagues demonstrated that two of biology's best model organisms – *C. elegans* and *Drosophila* – are much more closely related to each other than scientists had thought³. I figured that among the other animals in this newly recognized grouping, it might be possible to develop some great future models for answering questions in comparative biology.

Six phyla of animals are close relatives of the phyla that contain *C. elegans* and *Drosophila*, including tardigrades (Figure 1), but no animals in these six phyla had been widely used in labs. So tools for modern molecular study were meager. I chose a tardigrade species that appeared well suited for microscopy – it has small, clear embryos and is transparent throughout its life cycle – and that was likely to have a compact genome⁴. An amateur scientist in England had already developed reliable methods to culture the animals, as he had been growing them in his shed for over a decade, and he generously shared his culture methods⁵. Only five genes were represented in Genbank at the time for the entire tardigrade phylum, but another lab was beginning to collect thousands of sequences^{5,6}. We began to describe the animals' development. And we established, for the first time for the phylum, some needed protocols – protocols for immunostaining, *in situ* hybridization, live microscopy by DIC and fluorescence imaging, microinjection of animals, RNA interference, and methods for identifying protectants

that enable the animals' remarkable desiccation tolerance⁷. The species we chose was recognized recently as a previously unnamed species in the genus *Hypsibius*, and hence was dubbed *Hypsibius exemplaris*, for "exemplar" or "model" species⁸ (Figure 2).

Over a thousand species of tardigrades have been described to date⁹, and they all share a similar body plan: a head bearing an endearing pair of dark eyespots, and four pairs of legs, on a body that's typically less than a millimeter long. Like many animals, tardigrades have a tube-within-a-tube organization, i.e. with a digestive tube running through the middle of an epidermal tube. Between these two tubes is a fluid-filled region that has their brain and ganglia, a gonad, hundreds of free-floating cells, and muscle cells that criss-cross the body and enable the eight-legged water-balloons to walk.

To understand how this stubby body form arose, we've examined expression patterns for genes that can help inform what parts of modern animals' bodies derived from common parts of their ancestors' bodies¹⁰. We found Hox gene expression patterns that suggested that the tardigrade body plan likely originated when a mutation in an ancestral animal caused the deletion of a large part of the animal's body: the expression patterns suggested that almost the entire body of a tardigrade – its head and its first three pairs of legs – is homologous to only the head of arthropods like *Drosophila*. And the remainder of the body, where the last pair of legs form, shares homology with the very posterior end of a *Drosophila* abdomen. The parts that would correspond to *Drosophila*'s entire thorax and nearly their entire abdomen, along with the Hox genes that would specify that large region, have been lost in tardigrades.

It's possible that this compact body plan helps tardigrades telescope down to an even-more compact form during desiccation, a form known as a "tun". And their ability to survive desiccation might have predisposed the animals to survive other extreme conditions. The extreme conditions that tardigrades can survive include some conditions that would kill most animals. Indeed, some of these conditions should destroy even the things that animals are made of – for example cells, membranes, DNA, RNA, and proteins. This suggested to us that tardigrades must make protectants, and probably lots of different kinds of protectants for different kinds of molecules and for different compartments of cells. Tardigrades might also harbor some exceptional repair mechanisms. Such protectants and repair mechanisms would be fascinating to understand, and they might additionally suggest valuable, new routes toward protecting fragile biomedical products like medicines and vaccines. If these fragile materials could be made to survive desiccation at room temperature as well as tardigrades can, this could dramatically improve how effectively such medicines and vaccines could be distributed and used.

Recent work on tardigrades has identified several protectants that are beginning to explain some tardigrade superpowers. For example, research by other labs on the tardigrade species *Ramazzottius varieornatus* has identified a tardigrade protein that coats DNA and protects it from ionizing radiation¹¹. When this protein was expressed in human cultured cells, the cells were protected from damaging X-ray irradiation. Our lab has found that genes encoding intrinsically disordered proteins in *Hypsibius exemplaris* are upregulated in response to desiccation and are important for the tardigrades to survive desiccation^{11,12}. Expressing some of

these genes in bacterial or yeast cells can enhance desiccation tolerance in these cells. How the identified protectants function is the subject of ongoing work^{11–14}.

Tardigrades are well poised to continue informing interesting questions about how body forms evolve, taking advantage of *C. elegans* and *Drosophila* as comparative models, and about how living materials can be made to survive some remarkable extremes. Although the experimental tools and resources for tardigrades have grown in recent years, some important ones have yet to be developed. Genome sequences for *Hypsibius exemplaris* and *Ramazzottius varieornatus* are fairly complete¹⁵ but not yet as well assembled as are *C. elegans* or *Drosophila* genomes. And RNA interference works for disrupting gene functions¹⁶, but there are not yet methods for making transgenic or CRISPR-modified tardigrades. As a result, some of the inferences about tardigrade gene functions to date have relied on heterologous expression of genes rather than disrupting *in vivo* functions. Still, the use of existing tools and resources has enabled a tardigrade experimental community to grow, which bodes well for expanding the methods available for answering questions of interest. The Kuneida lab recently reported the first success using CRISPR in tardigrades, to delete sequences from somatic cells¹⁷.

Tardigrades are one example among a growing roster of emerging model organisms that have flourished in labs in recent years¹⁸. Modern techniques that work in almost any organism, like transcriptomics for example, have allowed scientists to answer questions that could not be answered using traditional model organisms – or indeed questions that were largely forgotten to be interesting or broadly relevant.

Statement of competing financial interest

The author declares that they have no competing interests.

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Figures

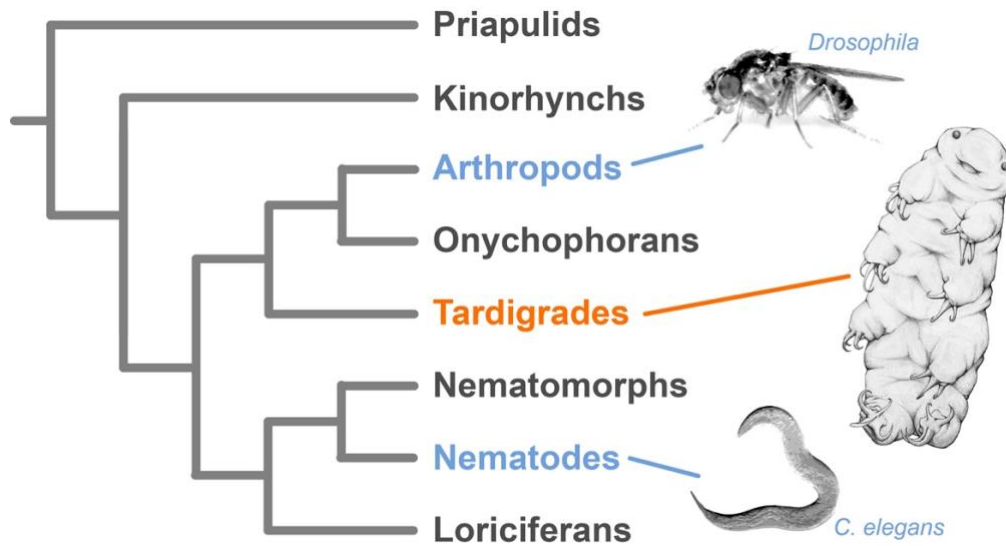


Figure 1: Evolutionary relationships among the ecdysozoan phyla. Based on Laumer et al. 2019¹⁹ but with Nematomorphs added in their usual position. *Drosophila* image adapted with permission from Hannah Davis, 2019 CCBY-SA 4.0 (https://commons.wikimedia.org/wiki/File:Standing_female_Drosophila_melanogaster.jpg); tardigrade illustration by Anya Broverman-Wray, 2013.



Figure 2: The tardigrade *Hypsibius exemplaris*. The animal is about 200 μm long. It is shown next to algae and with ingested algae inside its stomach.

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