

How to Kill a Tardigrade – Without Even Trying

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Why Tardigrades?

Tardigrades are small animals that are known for their ability to tolerate extreme desiccation as well as ionizing radiation. The molecular mechanisms underlying such radiation resistance has not been fully characterized, though previous research suggests that tardigrades possess species-specific DNA protection proteins that confer enhanced DNA protection as opposed to enhanced DNA repair. Understanding the molecular basis behind such radiotolerance is critical for space travel beyond the Van Allen Belt, where radiation levels are beyond that which humans can feasibly survive in.

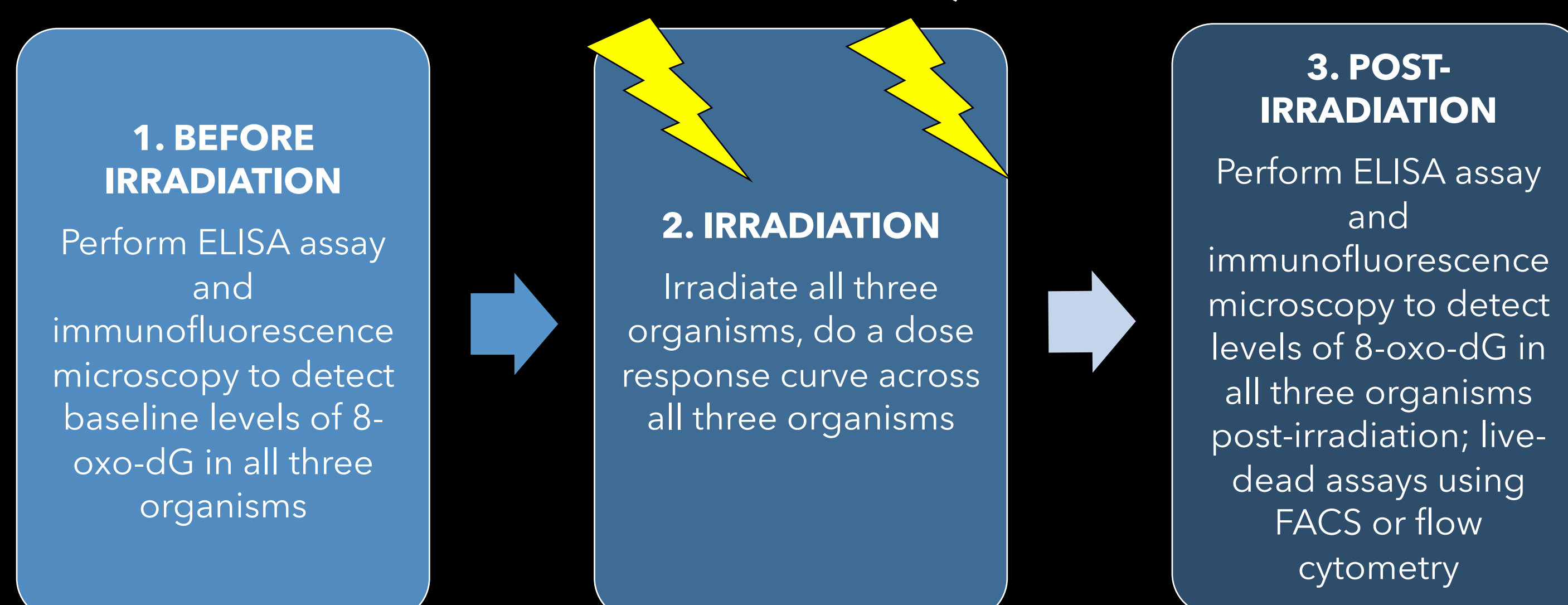


SEM image of a hydrated tardigrade, *Hypsibius dujardini*. Scale bar shows 100 μm increments. Credit: John Varelas

Experimental Design and Purpose

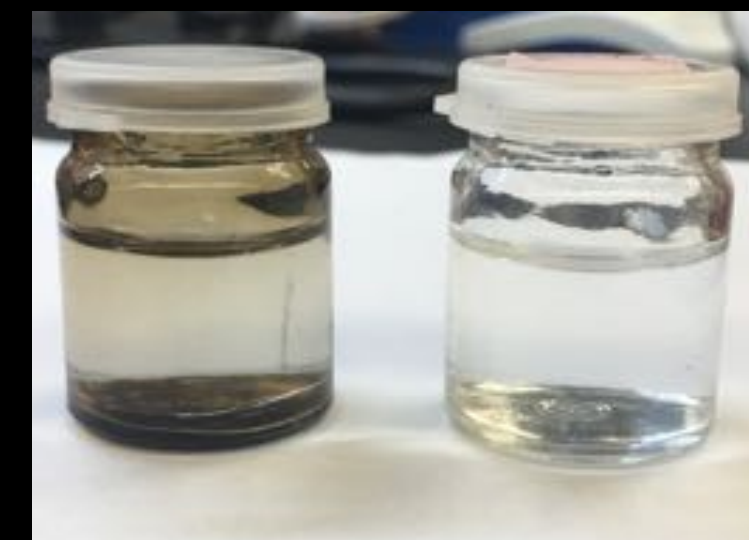
- Quantify the relative amount of DNA damage via detection of 8-oxo-dG per unit of DNA after X-ray irradiation and further elucidate the mechanisms of radiation tolerance in *H. dujardini*
- Do a comparative analysis of DNA damage across three different species
 - Hypsibius dujardini*
 - Deinococcus phoenicis*
 - Saccharomyces cerevisiae*
- Question: Do the nuclei of tardigrades demonstrate the same amount of DNA damage per unit of DNA as *Deinococcus phoenicis* and *Saccharomyces cerevisiae*?

HYPOTHESIS: *H. dujardini* possess species-specific DNA protection proteins that prevent DNA damage from occurring, thus conferring tolerance to extreme levels of radiation (as opposed to DNA repair mechanisms).



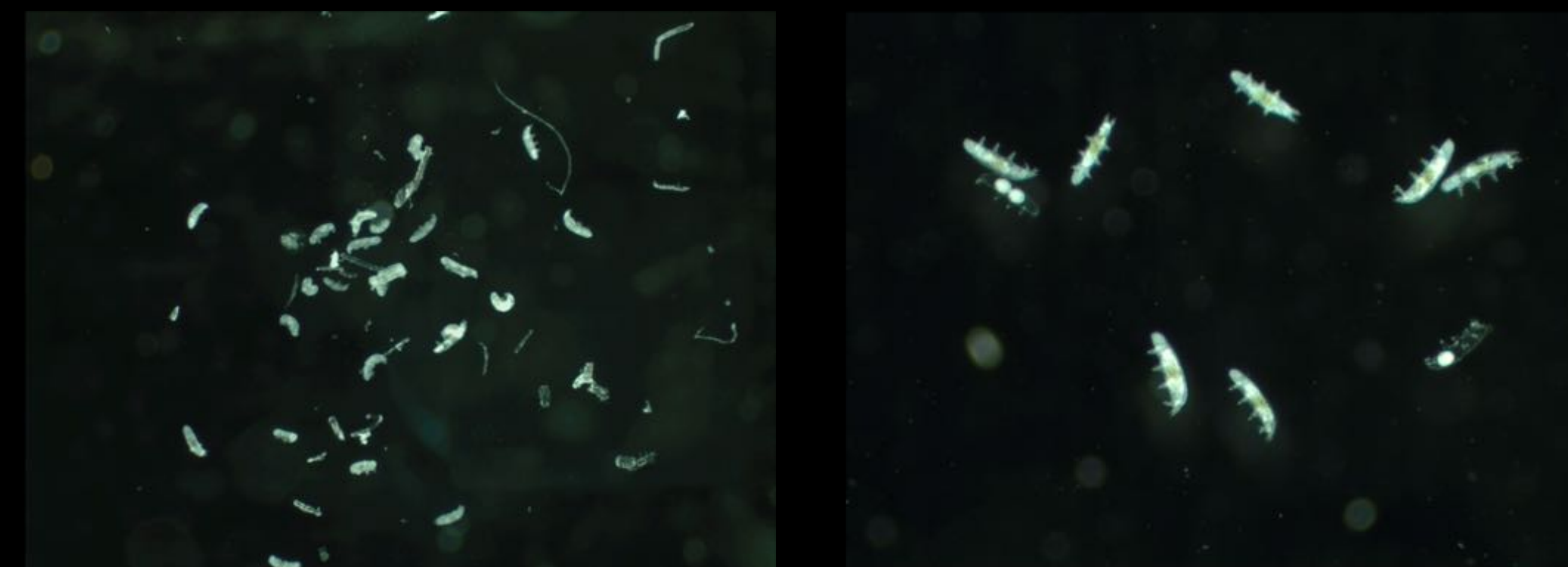
Materials Consideration

Containers in which organisms are irradiated should not shield any radiation or cause organisms to adhere to the surface post-irradiation. For this reason, many containers were tested at 1000 Gy for color change and tardigrade adherence.



Comparative image of an irradiated vessel (left) and a control vessel (right). Concerns of shielding led way to a materials consideration across a variety of different vessel types, as shown in the table.

Container	Active	Tuns	Dead	Color Change	Sticky
Small Tissue Culture Dish	15	5	2	No	No
Small Glass Pyrex Petri Dish	18	3	0	Yes	No
Teflon Cup	All were dead ☹			No	Yes
Plastic Vial (suspect polycarbonate)	22	7	8	No	Yes
Scintillon Glass	26	5 (mainly smaller ones)		Yes	No

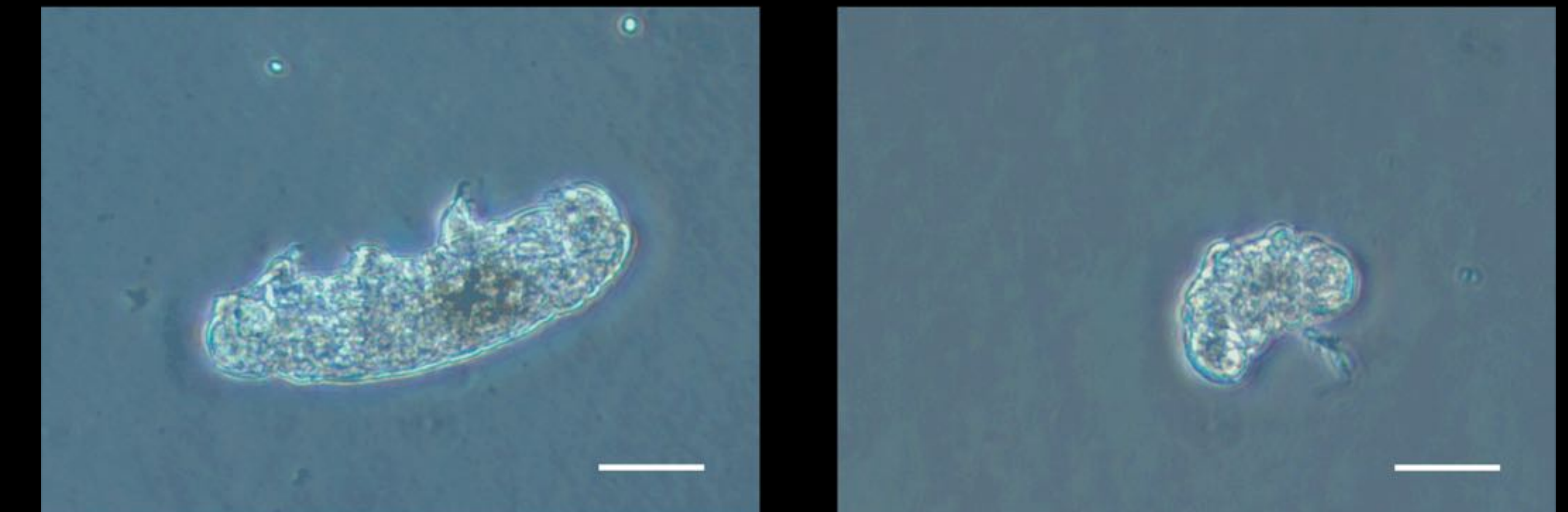


Representative images of tardigrades post-irradiation in the small tissue culture dish (left) and the Teflon cup (right), showing a preference for the small tissue culture dish based on tardigrade mortality, dish color change, and adhesiveness.

Comparative Analysis Across Species

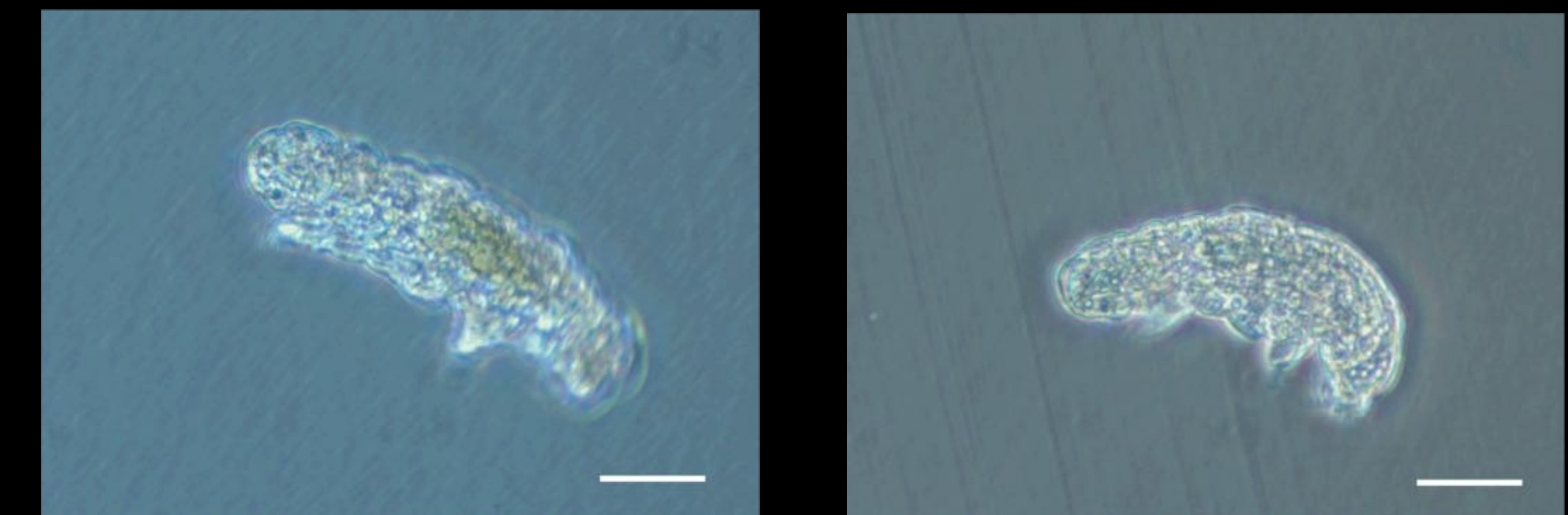
<i>Hypsibius dujardini</i>	<i>Deinococcus radiodurans</i>	<i>Saccharomyces cerevisiae</i>
<ul style="list-style-type: none">Genome size: 75 MbLD₅₀ = 4180 GyWithin the same class as the species of tardigrades that were sent up to space	<ul style="list-style-type: none">Genome size: 3MbCan survive up to 15k Gy of radiationDosed with 5000 Gy with no decrease in viabilityExperiments will use <i>D. phoenicis</i>	<ul style="list-style-type: none">Genome size: 12MbLittle radioresistanceWill not survive more than 150 GyLD₅₀ = 16.6 Gy

4200 Gy Dose



Representative images of tardigrades post-irradiation at 4200 Gy (LD₅₀ for *Hypsibius dujardini*). Irradiated tardigrade (left), control (right). Out of the 100 tardigrades irradiated, only 2 were moving slowly post-irradiation. In the control dish, about 89/100 were moving vigorously. Scale bar shows 100 μm. Max dose rate with parameters: 4.6 Gy/min.

1000 Gy Dose



Representative images of tardigrades post-irradiation at 1000 Gy, the about 250 times what humans can tolerate. Irradiated tardigrade (left), control (right). Out of the 100 tardigrades irradiated, about 90 were moving rapidly post-irradiation. In the control dish, about 73/100 were moving vigorously. Scale bar shows 100 μm. Irradiated glass vial turned brown post-irradiation. Max dose rate with parameters: 4.6 Gy/min.

Rapid Depressurization



About 100 adult *Hypsibius dujardini* were went to a maximum altitude of about 67,000 feet in a HAB in an OptiCell system with algae and fresh media to see how they fared in a variety of extreme conditions in the metabolically active state. Analysis showed that the tardigrades succumbed to the “bends,” causing them to lose various limbs and look deformed. Only about 7 tardigrades were completely intact post-flight; all were found dead. Left shows a normal rounded and dead tardigrade; middle shows a slightly deformed tardigrades that looks squished with its pharynx out of position; right shows a headless tardigrade.

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