Planetary and Space Science 74 (2012) 97–102

Contents lists available at SciVerse ScienceDirect

Planetary and Space Science

journal homepage: www.elsevier.com/locate/pss

What can we learn from the toughest animals of the Earth? Water bears (tardigrades) as multicellular model organisms in order to perform scientific preparations for lunar exploration

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ARTICLE INFO

Article history:
Received 29 February 2012
Received in revised form
9 May 2012
Accepted 29 May 2012
Available online 7 June 2012

Keywords:
Tardigrada
Lunar exploration
Space biology
Environmental extremes
Anhydrobiosis

ABSTRACT

Space missions of long duration required a series of preliminary experiments on living organisms, validated by a substantial phase of ground simulation experiments, in the field of micro- and inter-mediate gravities, radiobiology, and, for planetary explorations, related to risks deriving from regolith and dust exposure. In this review, we present the tardigrades, whose characteristics that recommend them as an emerging model for space biology. They are microscopic animals but are characterized by a complex structural organization similar to that of larger animals; they can be cultured in lab in small facilities, having small size; they are able to produce clonal lineages by means of parthenogenesis; they can completely suspend their metabolism when entering in dormant states (anhydrobiosis induced by dehydration and cryobiosis induced by freezing); desiccated anhydrobiotic tardigrades are able to withstand chemical and physical extremes, but a large tolerance is showed also by active animals; they can be stored in dry state for many years without loss of viability. Tardigrades have already been exposed to space stressors on Low Earth Orbit several times. The relevance of ground-based and space studies on tardigrades rests on the presumption that results could suggest strategies to protect organisms, also humans, when exposed to the space and lunar environments.

1. Introduction

One of the major interests of space agencies is to test the response of biological systems to stressful conditions characterizing space flights and extraterrestrial environments. This is particularly relevant in predicting the future effects of long-term human space flights, such as permanent lunar bases, or planetary explorations. As the interest in space exploration grows, and lunar exploration becomes an issue in the space agency calendars, laboratory exposures to outer Earth conditions are of great importance in order to predict the response of multicellular organisms, including humans, to unfavorable conditions, and to elaborate the opportune countermeasures to avoid the risks imposed by space and lunar environments.

Space missions of long duration required a series of preliminary experiments on living organisms, validated by a substantial phase of ground simulation experiments, in the field of micro- and inter-mediate gravities, radiobiology, and, for planetary explorations, related to risks deriving from regolith and dust expositions. To date, many experimental organisms for space investigation were unicellular organisms and cultivated cells of multicellular organisms. Although the experiments on cell cultures are useful for understanding some mechanisms involved in stress response, it is equally clear that cell cultures represent only the first level of life organization. They cannot be compared to an entire living organism, even when individual isolated cells are derived from a multicellular organism. The use of multicellular heterotrophic organisms allows the conduction of experiments with animals characterized by a high level of hierarchical biological complexity and by complete physiological processes, comparable to those of humans. The use of animals permits the study of complex interaction of the biological systems and the application of a system biology approach. This integrated approach proves very informative, integrating diverse sources of data acquired from diversified quantitative and qualitative techniques.

Even though multicellular organisms are useful models in space research, their use is often limited by the fact that many of them need large volumes, specific rearing bioreactors, and may need time consuming activities by astronauts (Horn, 2006;

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0032-0633/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.pss.2012.05.021

Marthy, 2002). Because of special requirements, particularly in unmanned missions, and in view of lunar exploration, some animal models are more suitable than others for performing experiments both in space and on ground. We propose the cryptobiotic species of the phylum Tardigrada (also called water bears) as a convenient model.

2. What is a tardigrade?

Tardigrades are microscopic animals (body length from 200 μm to 1 mm; Fig. 1a,b) related to arthropods (Campbell et al., 2011), living in the sea from littoral to abyssal depth, and in freshwater and terrestrial habitats at all latitudes, longitudes, and altitudes. They show an elongated cylindrical body coated by a chitinous cuticle, which must be periodically molted to allow organism growth, and consisting of a head followed by four trunk segments each with a pair of legs bearing claws at their extremities (Figs. 1b, 2a). In spite of their miniaturized size, they have a complex internal organization similar to that of larger animals (Nelson et al., 2010; Figs. 1a, 2b). They have a large dorsal brain, often with small eye-spots, and a ventral chain of nerve ganglia. The complete digestive system is characterized by perforating mouth parts and a sucking pharynx that are used to suck the cell content of a range of items including plants, fungi, microalgae, protozoans, and small invertebrates as nematodes and rotifers.

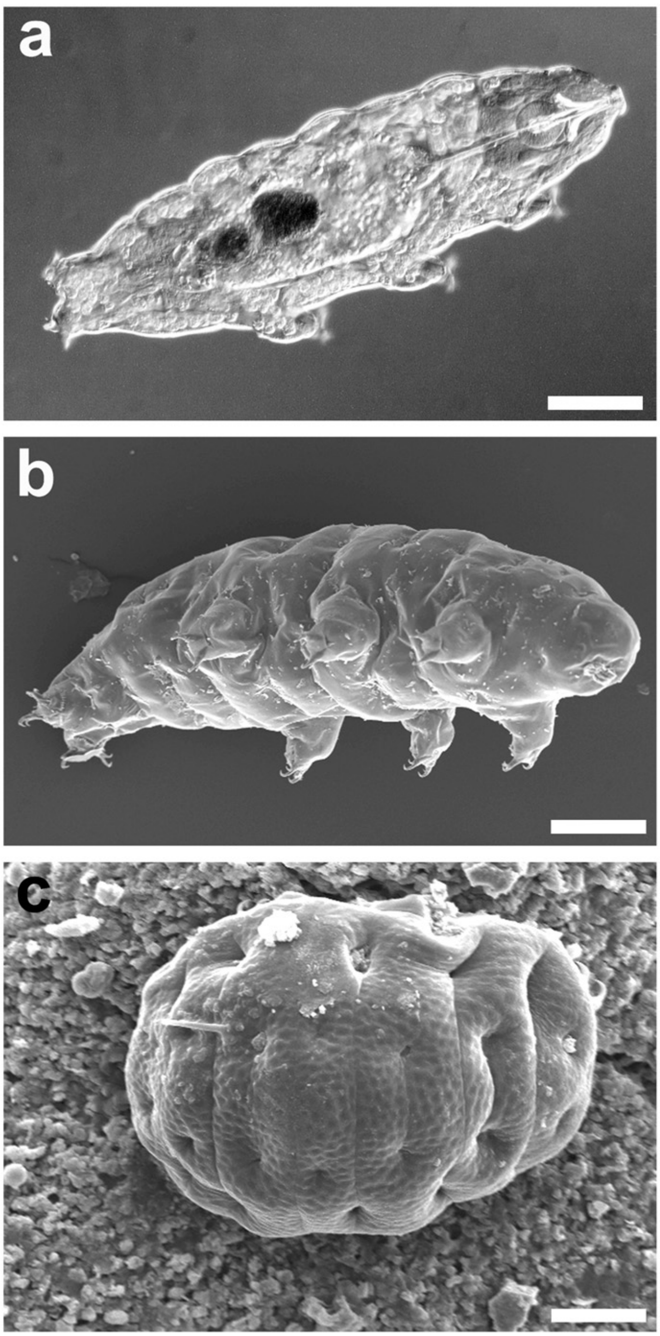

Fig. 1. Tardigrades in active and anhydrobiotic state. (a) Living specimens of *Macrobiotus sandrae* in active state (DIC). (b) Specimen of *Richtersius coronifer* in the shape of an active animal (SEM). (b) Dry specimen of *Ramazzottius oberhaeuseri* on lichen (SEM). Scale bars: a, b = 50 μm, c = 25 μm.

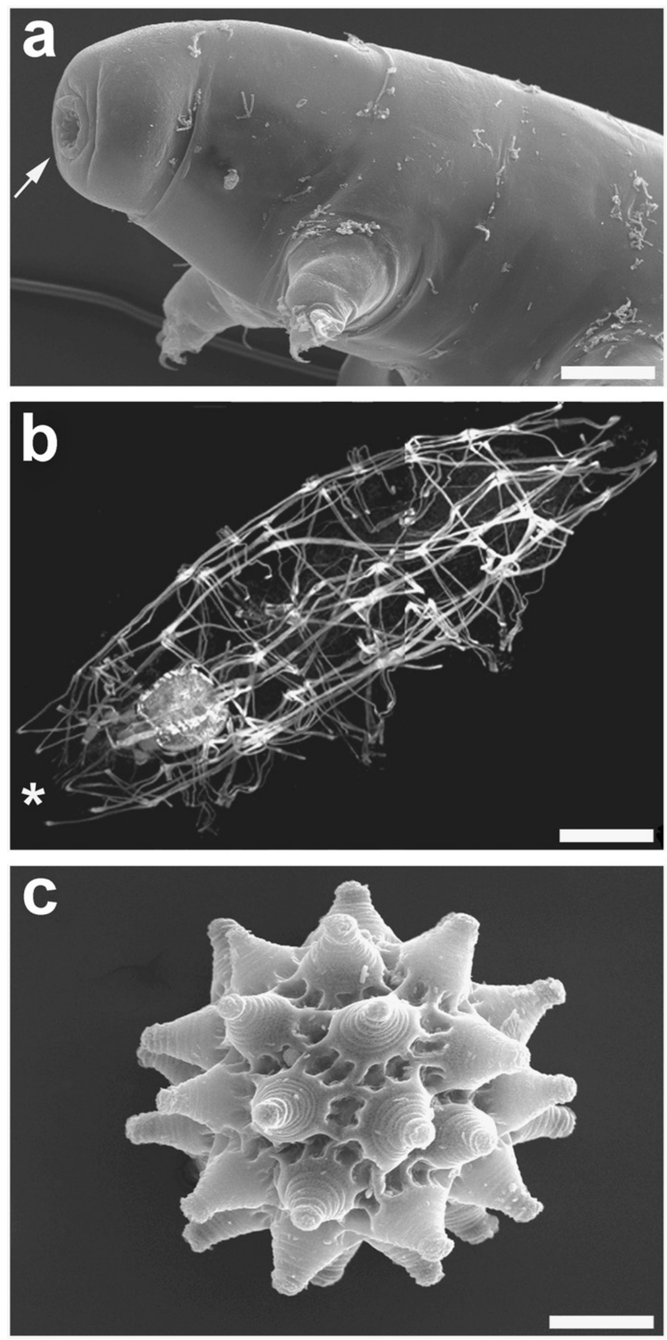

Fig. 2. (a) Anterior portion of a specimen of *Paramacrobiotus richtersi* (arrow = mouth; SEM). (b) Muscular system of *P. richtersi* (* = anterior end; CLSM). (c) Egg of *P. richtersi* (SEM). Scale bars: a, c = 25 μm, b = 50 μm.