Cryobiology xxx (2015) 1–4

Contents lists available at ScienceDirect

Cryobiology

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

Recovery and reproduction of an Antarctic tardigrade retrieved from a moss sample frozen for over 30 years

Megumu Tsujimoto [a, *], Satoshi Imura [a, b], Hiroshi Kanda [a]

[a] National Institute of Polar Research (NIPR), 10-3 Midori-cho, Tachikawa-shi, Tokyo 190-8518, Japan
[b] SOKENDAI (The Graduate University for Advanced Studies), Tokyo, Japan

ARTICLE INFO

Article history:
Received 9 November 2015
Received in revised form
22 December 2015
Accepted 22 December 2015
Available online xxx

Keywords:
Long-term survival
Cryptobiosis
Cryobiosis
Freezing
Acutuncus antarcticus

ABSTRACT

Long-term survival has been one of the most studied of the extraordinary physiological characteristics of cryptobiosis in micrometazoans such as nematodes, tardigrades and rotifers. In the available studies of long-term survival of micrometazoans, instances of survival have been the primary observation, and recovery conditions of animals or subsequent reproduction are generally not reported. We therefore documented recovery conditions and reproduction immediately following revival of tardigrades retrieved from a frozen moss sample collected in Antarctica in 1983 and stored at −20 °C for 30.5 years. We recorded recovery of two individuals and development of a separate egg of the Antarctic tardigrade, *Acutuncus antarcticus*, providing the longest records of survival for tardigrades as animals or eggs. One of the two resuscitated individuals and the hatchling successfully reproduced repeatedly after their recovery from long-term cryptobiosis. This considerable extension of the known length of long-term survival of tardigrades recorded in our study is interpreted as being associated with the minimum oxidative damage likely to have resulted from storage under stable frozen conditions. The long recovery times of the revived tardigrades observed is suggestive of the requirement for repair of damage accrued over 30 years of cryptobiosis. Further more detailed studies will improve understanding of mechanisms and conditions underlying the long-term survival of cryptobiotic organisms.

© 2015 Published by Elsevier Inc.

Keilin [12] introduced the term 'cryptobiosis' as "the state of an organism when it shows no visible signs of life and when its metabolic activity becomes hardly measurable, or comes reversibly to a standstill". Several strategies of cryptobiosis are known, induced by different physiological stimuli including desiccation (anhydrobiosis), freezing (cryobiosis), osmotic pressure (osmobiosis), and oxygen deficiency (anoxybiosis) [12]. Long-term survival, mainly in an anhydrobiotic state, has been one of the most studied of the extraordinary physiological characteristics of cryptobiosis in micrometazoans such as nematodes, tardigrades and rotifers e.g. Refs. [1,2,6–9,13,16,20].

The documented record of long-term survival of a micrometazoan under anhydrobiosis belongs to a plant-parasitic nematode, with five individuals of *Tylenchus polyhypnus*, two young females and three larvae, being revived after almost 39 years [20]. Many second-stage larvae of the nematode *Anguina tritici* in wheat galls revived after 32 years of storage either under low constant humidity or refrigeration at about 5 °C [13]. Fielding [6] reported survival of larvae of the same species in dry galls after 28 years in storage.

Limber [13] reported the ability of revived nematodes to invade wheat seedlings, with maximum survival of 408 days in tap water after revival, but subsequent reproduction was not considered in any detail. Reproduction by revived animals after long-term cryptobiosis was reported in a free-living soil nematode, *Panagrolaimus* sp [1]. Four juveniles (two males and two females) recovered from anhydrobiosis in a dried soil sample stored at room temperature for 8.7 years, and the females laid many eggs that hatched and developed into fertile adults.

There are also some reports of long-term anhydrobiotic survival in rotifers and tardigrades. The longest published records for both groups are 9 years, with the rotifer *Mniobia* sp. being found alive and eggs of the tardigrade *Ramazzottius oberhaeuseri* hatched from samples of dried lichen and moss [9]. The juveniles of *R. oberhaeuseri* survived in distilled water without any food source for a maximum of 40 days [9]. Survival of three Antarctic tardigrade

* Corresponding author. National Institute of Polar Research, 10-3 Midori-cho, Tachikawa-shi, Tokyo 190-8518, Japan.
E-mail address: tsujimoto@nipr.ac.jp (M. Tsujimoto).

http://dx.doi.org/10.1016/j.cryobiol.2015.12.003
0011-2240/© 2015 Published by Elsevier Inc.

Please cite this article in press as: M. Tsujimoto, et al., Recovery and reproduction of an Antarctic tardigrade retrieved from a moss sample frozen for over 30 years, Cryobiology (2015), http://dx.doi.org/10.1016/j.cryobiol.2015.12.003

species, *Echiniscus jenningsi*, *Macrobiotus furciger* and *Diphascon chilenense*, was confirmed after 8.3 years in anhydrobiosis under frozen conditions [19].

Fewer studies of long-term survival under cryobiotic conditions are available. Live individuals of the Antarctic nematode *Plectus murrayi* were recovered from a non-desiccated Antarctic moss sample after 25.5 years of storage under frozen conditions, and cultured for use in further studies on freezing and desiccation tolerance [11]. Various Antarctic micrometazoans including nematodes, tardigrades and rotifers were revived from a liverwort sample stored frozen at −80 °C for 6 years [14]. Similarly, live individuals of the Antarctic tardigrade *Acutuncus antarcticus* were retrieved from frozen phytobenthos samples collected in Antarctica and stored at −70 °C for 5 years [21], again being cultured for further studies of life history traits [22].

In the available studies of long-term survival of micrometazoans, survival has been the primary observation reported and discussed [9,10], with recovery conditions of animals or subsequent reproduction (i.e. indicating long term viability) generally not being reported. Detailed descriptions of the recovery process and reproduction following resuscitation are essential to develop further understanding of the mechanisms underlying the long-term survival of these animals in cryptobiosis. In the current study we therefore documented recovery conditions and reproduction immediately following revival of tardigrades collected from an Antarctic moss sample frozen for over 30 years. We focused on the Antarctic tardigrade, *A. antarcticus*, as a culturing protocol for this species is already established [22].

Moss samples were collected by Hiroshi Kanda in Yukidori Valley, Langhovde, Sôya coast, Dronning Maud Land (East Antarctica; 69°14′30″S, 39°46′00″E) on 6 November 1983 during the 24th Japanese Antarctic Research Expedition (JARE) winter operation. Samples were wrapped individually in paper, enclosed in separate plastic bags and stored at −20 °C after collection. A frozen moss sample, F01096 (NIPR), of *Bryum argenteum* (1.0 cm^3) was thawed on 7 May 2014 at 3 °C for 24 h. The thawed sample was placed into a Petri dish where water was added and it was soaked for a further 24h. The moss sample was teased apart with tweezers in the dish, and individual tardigrades were retrieved using a pipette under a dissecting microscope.

Amongst the tardigrades extracted from sample F01096, there were two individuals whose bodies were not fully-extended. Since body extension is typical of a dead tardigrade, these two individuals (named Sleeping Beauty (SB)-1 and SB-2 respectively) were placed into individual wells on a culture plate for further observation. At the same time, one egg extracted from the same sample was placed into another well of the same culture plate (SB-3). The TPP® tissue culture plate (12 wells, flat bottom) was prepared with a layer of 300 μl of 1.5% agar gel on the bottom of each well, to which was added 600 μl of Volvic® water and 1.8 μl of a suspension of *Chlorella* sp. (Chlorella Industry Co., Japan) to provide a food source (see Ref. [22]). The culture dishes were stored in the dark at 15 °C.

The individual tardigrades and the egg were inspected daily and their survival and egg production monitored (reproduction was recorded only for SB-1 and SB-3 since SB-2 did not produce any eggs before dying). Animals were transferred to new culture dishes every week (following [22]). Eggs from new clutches were isolated on the day of oviposition, separated and transferred individually to wells on new culture plates. Subsequent hatching of the isolated eggs was monitored daily until 30 days after oviposition. Data on timing and clutch size of each oviposition event, egg development time to hatching (hatching time), and hatching success were recorded. Due to accidental drying during the transfer of SB-1 after its 5th oviposition, no records were taken after this event for this individual. Observations were made using a dissecting microscope (Olympus SZX7) at 56x magnification.

Digital images of movement and conditions of SB-1 and SB-2 were recorded using a digital camera system (Olympus DP70) mounted on a dissecting microscope (Nikon SMZ1500) at 112.5x magnification. Body length, from the tip of the head to the junction of the 4th pair of legs, was calculated using still images of the most extended length. The body length can be variable depending on the mode of walking. The body postures of SB-2 was curved both while inactive and during walking. Thus, the body length of SB-2 was measured only when its body was temporally extended on day 17 whilst the condition was declining close to its death. Conditions and body length of SB-3 subsequent to hatching were not monitored in detail since there were no obvious abnormalities recognized in its movement or growth.

After the successful reproduction of one individual (SB-1) and hatching of the egg SB-3, offspring of both individuals were incubated for several generations to establish isogenic lines of each parthenogenetic strain (Fig. 1). For morphological identification, sub-samples of each strain were mounted on slides in Faure's solution, and animals were identified under a phase-contrast microscope (Olympus BX53, 100x).

Two individuals, SB-1 and SB-2, retrieved from the moss sample frozen for 30.5 years revived after rehydration. SB-1 proceeded to reproduction while SB-2 died without ovipositing. Another individual, SB-3, hatched from an egg retrieved from the frozen moss went on to reproduce successfully. Both SB-1 and SB-3 were morphologically identified as *A. antarcticus* (the identity of SB-2 was not confirmed). The recovery and subsequent reproduction of SB-1, recovery and survival of SB-2, and hatching and subsequent reproduction of SB-3 are described below.

SB-1 first showed slight movement in its 4th pair of legs on the first day after rehydration (Table 1). This progressed to twisting of the body from day 5 along with movement in its 1st and 2nd pairs of legs, but the movements remained slow. After starting to attempt to lift itself on day 6, SB-1 started to slowly crawl on the agar surface of the culture well on day 9, and started to eat the algal food provided (*Chlorella* sp.) in the culture plate on day 13. Development

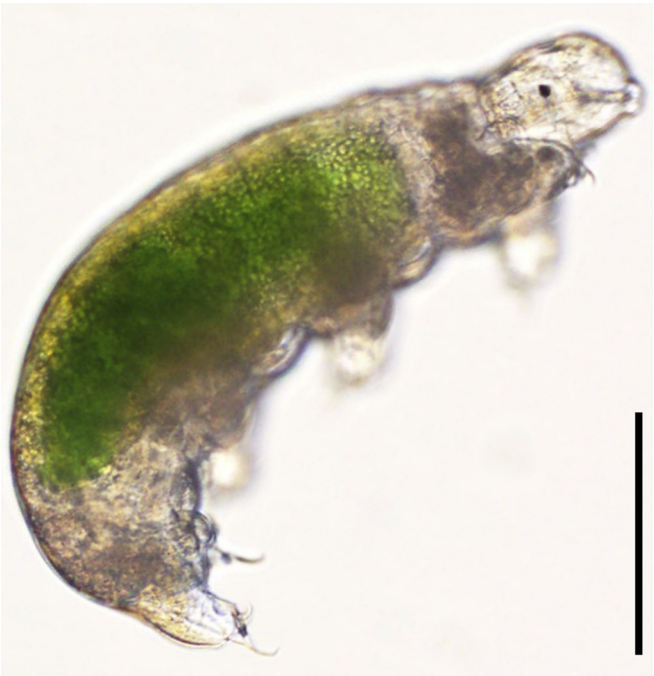

Fig. 1. *Acutuncus antarcticus*, an individual representing the SB-3 strain, showing *Chlorella* sp. inside its stomach. Scale bar, 100 μm.

Please cite this article in press as: M. Tsujimoto, et al., Recovery and reproduction of an Antarctic tardigrade retrieved from a moss sample frozen for over 30 years, Cryobiology (2015), http://dx.doi.org/10.1016/j.cryobiol.2015.12.003

Download English Version:

https://daneshyari.com/en/article/10927643

Download Persian Version:

https://daneshyari.com/article/10927643

Daneshyari.com