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Life-history traits in the tardigrade species *Paramacrobiotus kenianus* and *Paramacrobiotus palaui*

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ABSTRACT

Although tardigrades have been studied for a long time, little is still known about their life-history traits. In the present study, two populations of the parthenogenetic African tardigrade species Paramacrobiotus kenianus, *and the parthenogenetic species* Paramacrobiotus palaui *from the pacific islands of Palau were examined and analysed related to their life-history traits under laboratory conditions. The longevity in days (mean±SD) do not vary between the* P. kenianus *population (I) (125±35) and (II) (141±54), but* P. palaui *showed a significant shorter longevity (97±31). A recorded maximum age of 212 days was reached by* P. kenianus *population (II).* P. kenianus *population (I) laid 138±71 eggs (mean±SD) and population (II) 124±78 eggs (mean±SD) during their life, whereas* P. palaui *laid only 42±54 eggs (mean±SD). While the hatching time was similar in all species, starting after 6 to 9 days, the hatching rate in* P. kenianus *population (I) was 33%, compared with 51% of population (II) and 54% of* P. palaui.

Key words: culture, life-history, longevity, population, Tardigrada.

INTRODUCTION

The number of known tardigrade species has been increasing steadily over the last decades. Today we know more than 1000 different species of tardigrades from all over the world (Degma *et al*., 2012). Tardigrades can be found in a variety of habitats, including marine, brakkish, freshwater and terrestrial ecosystems, ranging from the deep sea to the highest mountains, as well as in many extreme environments ranging from the coldest to the hottest and driest places (Marcus, 1929; Ramazzotti and Maucci, 1983; Schill *et al*., 2009; Guidetti *et al*., 2011b; Wełnicz *et al*., 2011). Such habitats frequently undergo seasonal changes that impact animals, but tardigrades are able to survive these periods of adverse conditions due to the ability to enter a cryptobiotic state. In adverse environments, terrestrial tardigrades form the *Tönnchenform* or tun state during desiccation (Baumann, 1922). Tardigrades show extraordinary tolerance to physical extremes including high pressure (Seki and Toyoshima, 1998; Horikawa *et al*., 2009), UV and high energy radiation (Jönsson *et al*., 2005; Horikawa *et al*., 2006; Jönsson *et al*., 2008; Rebecchi *et al*., 2009, 2011; Altiero *et al*., 2011; Persson *et al*., 2011), exposure to high temperatures (Doyère, 1842; Pouchet, 1859; Rahm, 1921a; Ramløv and Westh, 2001; Hengherr *et al*., 2009c) and exposure to low temperatures (Rahm, 1921b; Ramløv and Westh, 1992; Hengherr *et al*., 2009a, b; Guidetti *et al*., 2011a). Over the past few years, more and more emphasis has been placed on the genome (Schill *et al*., 2004; Förster *et al*., 2009, 2011a, 2011b; Grohme *et al*., 2011), proteome (Schokraie *et al*., 2010, 2011, 2012; Yamaguchi *et al*., 2012) and metabolome (Kunieda and Kubo, 2006; Hengherr *et al*., 2008b; Beisser *et al*., 2012; Cesari *et al*., 2012) of tardigrades for a better understanding of the survival mechanisms. However, not much is known about their life-history traits, because only few species are in culture and life-cycle observations in nature are quite rare (Altiero and Rebecchi, 2001; Suzuki, 2003; Horikawa *et al*., 2008; Altiero *et al*., 2010; Lemloh *et al*., 2011). Therefore, much information regarding longevity, sexual or parthenogenetic reproduction, and embryonic development remains missing. Therefore I studied the parthenogenetic tardigrade species *Paramacrobiotus palaui* Schill, Förster, Dandekar and Wolf, 2010 and two populations of *Paramacrobiotus kenianus* Schill, Förster, Dandekar and Wolf, 2010 to provide information about the life-history traits for further studies.

METHODS

Culture

Individuals of two populations of the tropical, parthenogenetic eutardigrade species *Paramacrobiotus kenianus* and one population of the species *Paramacrobiotus palaui* (both species belong to the order Parachela) were used to investigate the life-history traits. Population (I) of *P. kenianus* originally came from Nakuru, Kenya and population (II) from Naivasha, Kenya. The population of *P. palaui* originally came from Koror, Palau. All animals are offspring from the animals described in (Schill *et al*., 2010). Tardigrades were cultured in plastic plates on a thin layer of 3% agar (peqGOLD Universal Agarose; peqLAB, Erlangen, Germany) and a layer of 3 mm Volvic® water (Danone Waters, Wiesbaden, Germany) according to Schill *et al*. (2004) and Hengherr *et al*. (2008a). For food, rotifers of the species *Philodina citrina* Ehrenberg, 1832 were provided twice a week, which were cultured separately in Volvic™ water and fed with cultured green algae *Chlorogonium elongatum* Dangeard (Bischoff and Bold, 1963). All cultures were kept in a climate chamber at 20°C under a photoperiod of 12 h/12 h.

Life-history traits

After oviposition, 374 eggs of *P. kenianus* population (I), 244 eggs of *P. kenianus* population (II), and 250 eggs of *P. palaui* were collected using a stereo microscope (SHZ 10; Olympus, Hamburg, Germany) and kept on agar with Volvic® water for the experiments (see culture method above). We determined the hatching time (embryonic developmental time) and hatching percentages for each population/species. Subsequently, we separated 22 hatched tardigrades of each population/species and kept them individually in 32 -well plastic plates on agar with Volvic® water to determine their longevity, the first oviposition, total number of eggs, and hatching time and percentages.

A one-way ANalysis Of VAriance (ANOVA) was used to analyse the longevity and two-way ANOVA was used to analyse the effect of species/population and longevity on numbers of eggs laid.

RESULTS

Longevity

The recorded maximum age, attained by two of the 22 animals of *Paramacrobiotus kenianus* population (I), was 204 days, and in the population (II) one animal survived 212 days. *Paramacrobiotus palaui* achieved a maximum age of 187 days (Fig. 1, Tab. 1).

The mean [±standard deviation (SD)] longevity of the

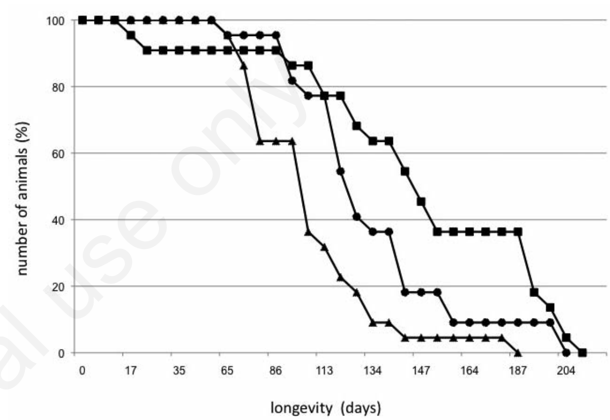

Fig. 1. Longevity of the tardigrade species *Paramacrobiotus kenianus* population (I) (circles), *Paramacrobiotus kenianus* population (II) (rectangles), and *Paramacrobiotus palaui* (triangles).

Tab. 1. Available data from tardigrade species about the longevity, number of eggs per animal, hatching success and time.

Species	Longevity (days)	Max. longevity (days)	Number of eggs/animal	Hatching success (%)	Hatching time (days)	Source
Diphascon (*Adropion*) *scoticum*	nd	263	nd	nd	5-14	Altiero and Rebecchi, 2001
Halobiotus crispae	nd	*ca.* 730	nd	nd	nd	Kristensen, 1982
Hypsibius arcticus	nd	nd	up to 84	nd	5-20	Dougherty, 1964
Hypsibius convergens	nd	159	nd	nd	5-10	Baumann, 1961
Hypsibius dujardini	nd	nd	nd	nd	5	Ammermann, 1962
*Hypsibius oberhaeuseri**	nd	nd	nd	nd	32	Marcus, 1929
Macrobiotus hufelandii	nd	84	nd	nd	26-31	Baumann, 1970
Macrobiotus joannae	nd	266	nd	nd	14-39	Altiero and Rebecchi, 2001
*Macrobiotus richtersi**	nd	nd	nd	65-100	28-87	Hohberg, 2006
Macrobiotus richtersi (clone I)*	194.9±164.4	518	37.8±29.3	83.1±12.7	41.0±9.5	Altiero *et al*., 2006
Macrobiotus richtersi(clone II)*	137.3±136.4	457	17.8±16.0	51.0±36.0	60.0±16.7	Altiero *et al.,* 2006
Macrobiotus sapiens	83.0±33.5	145	48	78	11.9±2.7	Lemloh *et al*., 2011
Milnesium tardigradum	42.7±11.8	58	41	72.2	5-16	Suzuki, 2003
Milnesium tardigradum	nd	nd	nd	nd	4	Baumann 1964
Milnesium tardigradum	82.7±2.7	107	nd	nd	nd	Hengherr *et al*., 2008a
Paramacrobiotus kenianus (population I)	125±35	204	138±71	33	7	Present study
Paramacrobiotus kenianus (population II)	141±54	212	124±78	51	8	Present study
Paramacrobiotus palaui	97±31	187	42±54	54	9	Present study
Paramacrobiotus richtersi	nd	nd	123-190	75-200	30-62	Altiero *et al.,* 2010
Paramacrobiotus tonollii	69.0±45.1	237	99	82.2	16.7±6.7	Lemloh *et al.,* 2011
Ramazzottius oberhaeuseri	nd	*ca.* 70	nd	nd	8-20	Baumann, 1966
Ramazzottius varieornatus	13-87	87	7.85	82.5	5.7±1.1	Horikawa *et al.,* 2008

nd, no data. **Species renamed.*

population (I) of *P. kenianus* was 125±35 days, the population (II) showed a mean±SD longevity of 141±54 days), whereas the population of *P. palaui* showed a mean±SD longevity of 97±31 days (Fig. 2). However, the comparison of the longevity of the *P. kenianus* population (I) and population (II) did not reveal any significant difference, although the mean longevity of population (II) is slightly higher numerically. *Paramacrobiotus palaui* has a significantly shorter longevity (P=0.002).

Ovipositions

Sexual maturity was determined based on the first laid eggs in each parthenogenetic population/species (n=22 tardigrades for each population), and a median of 10 days in all population/species was determined (data not shown). The mean (±SD) number of laid eggs counted during their longevity were 138±71 for *P. kenianus* population (I), 124±78 eggs for *P. kenianus* population (II), and 42±54 eggs for *P. palaui* (Fig. 3). The result of the two-way ANOVA analysis showed that there was no significant difference between populations and species.

Hatching time and percentages

Embryos of the two species did not vary significantly in their developmental time (Fig. 4). The first juveniles of *P. kenianus* population (I) hatched after six days, of *P. kenianus* population (II) after seven days, and of *P. palaui* after eight days at room temperature. Both *P. kenianus* populations showed a higher hatching rate after nine days, subsequently a decreased hatching rate, and an increasing hatching rate again after 13 days.

Hatching rate was estimated as 33% in *P. kenianus* population (I) (n=374 eggs), 51% in *P. kenianus* population (II) (n=224 eggs), and 54% in *P. palaui* (n=250 eggs). For the remaining eggs, no hatching was observed within 30 days after oviposition.

DISCUSSION

At the moment, less than 20 publications are available with information about the mean and maximal longevity, number of eggs per animal, hatching success, or hatching time in tardigrades (Tab. 1). However, from several studies only the hatching time or the number of eggs are available, like from the species *Hypsibius arcticus* (Murray, 1907) (Dougherty, 1964) and *Hypsibius dujardini* (Doyère, 1840) (Ammermann, 1962). Apart from the species in the present study, a mean longevity in days (±SD) is only published for the species *Macrobiotus sapiens* Binda and Pilato, 1984 (83.0±33.5; Lemloh *et al*., 2011), *Milnesium tardigradum* Doyère, 1840 (42.7±11.8; Suzuki, 2003) and (82.7±2.7; Hengherr *et al*., 2008a), *Paramacrobiotus richtersi* (Murray, 1911) clonal lineage I (194.9±164.4) and clonal lineage II (137.3±136.4; Al-

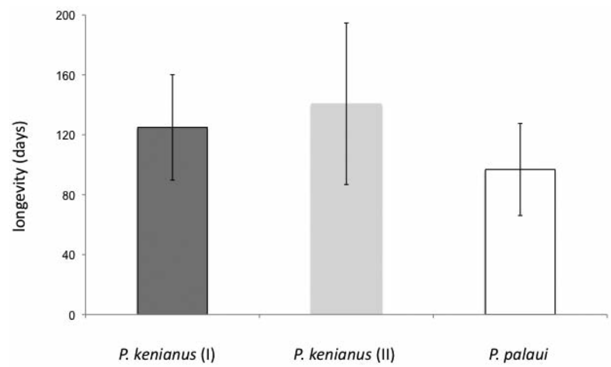

Fig. 2. Longevity in days (mean±SD) of the tardigrade species *Paramacrobiotus kenianus* population (I) (dark grey), *Paramacrobiotus kenianus* population (II) (grey) and *Paramacrobiotus palaui* (white). Whiskers represent the standard deviation.

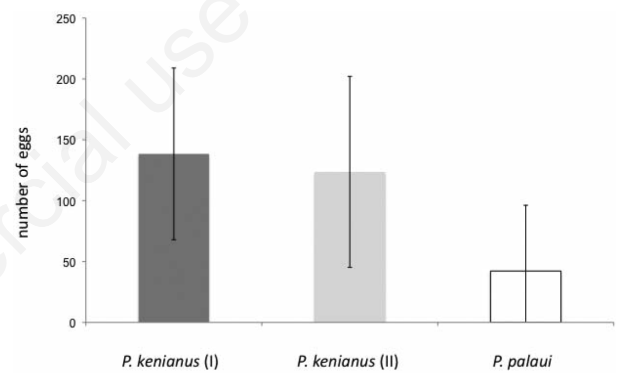

Fig. 3. Number of eggs (mean±SD) of the tardigrade species *Paramacrobiotus kenianus* population (I) (dark grey), *Paramacrobiotus kenianus* population (II) (grey) and *Paramacrobiotus palaui* (white) during their lifespan. Whiskers represent the standard deviation.

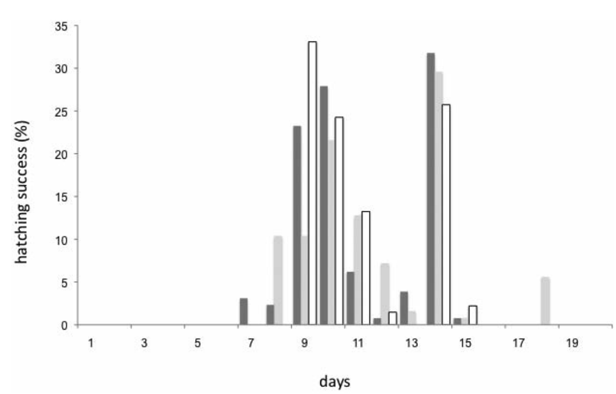

Fig. 4. Hatching success (%) of the tardigrade species *Paramacrobiotus kenianus* population (I) (dark grey), *Paramacrobiotus kenianus* population (II) (grey) and *Paramacrobiotus palaui* (white) within 30 days of observation.

tiero *et al*., 2006) and *Paramacrobiotus tonollii* (Ramazzotti, 1956) (69.0±45.1; Lemloh *et al*., 2011). The maximum longevity is known for the species *Diphascon* (*Adropion*) *scoticum* Murray, 1905 (263 days; Altiero and Rebecchi, 2001), *Halobiotus crispae* Kristensen, 1982 (*ca.* 2 years; Kristensen, 1982), *Hypsibius convergens* (Urbanowicz, 1925) (157 and 210 days; Baumann, 1961; Dougherty, 1964), *Macrobiotus hufelandi* C.A.S. Schultze, 1834 (84 days; Baumann, 1970), *Macrobiotus joannae* Pilato and Binda, 1983 (266 days; Altiero and Rebecchi, 2001), *Ramazzottius varieornatus* Bertolani and Kinchin, 1993 (87 days; Horikawa *et al*., 2008) and *Ramazzottius oberhaeuseri* (Doyère, 1840) (*ca.* 70 days; Baumann, 1966). However, for ecological studies the mean longevity (Tab. 1) seems to be much more meaningful than the maximum longevity. Furthermore, thus far only the species *M. tardigradum*, *P. richtersi*, *P. kenianus, P. palaui, P. tonollii* and *M. sapiens* have been maintained in long term cultures under controlled conditions.

With limited data it is difficult to compare the longevity of tardigrade species. Nevertheless, if one compares the two populations of *P. kenianus*, they are quite similar to the two *P. richtersi* clonal lineages studied by Altiero *et al*. (2006). Maybe this is due to the close relationship between the species. The two populations of *P. kenianus* were described earlier by molecular (18S rRNA and COI gene) and morphological studies as cryptic species within the *Paramacrobiotus richtersi group* (Guidetti *et al*., 2009). However, the minimum-evolution, maximum-parsimony (MP) and maximum-likelihood (ML) analyses gave insufficient justification to erect new species, and more supporting biochemical and biophysical data were unavailable at the time. Later it was possible to distinguish the cryptic species within the *Paramacrobiotus richtersi group* via an analysis of internal transcribed spacer 2 (ITS2) secondary structures (Schill *et al*., 2010). However, *P. palaui* showed a shorter mean longevity which is most comparable to that of *M. sapiens* (Lemloh *et al*., 2011), *M. tardigradum* (Hengherr *et al*., 2008a) and *P. tonollii* (Lemloh *et al*., 2011). Within the species *P. palaui*, there is also a large difference between the longevity of 97±31 days.

Population (I) of *P. kenianus* laid 138±71 eggs during their life, with a hatching rate of 33%. In contrast, the population (II) of the same species laid fewer eggs, but the hatching rate was much higher, 51%. Differences within two clonal lineages have been observed in *P. richtersi* (Altiero *et al*., 2006). One clone laid 37.8±29.3) with a hatching rate of 83.1±12.7%, and the other clone only 17.8±16.0%, with a hatching rate of 51.0±36.0%. Comparison of the hatching time showed no significant difference between the two populations of *P. kenianus*. In contrast, while the first juveniles of *P. kenianus* hatched after 6 to 7 days, *P. richtersi* hatched after 41-60 days and showed highly significant differences between each clone (Altiero *et al*., 2006). Another study (Altiero *et al*., 2010) studied *P. richtersi* collected in nature and reared in the lab. However, they observed significant differences between one field sample and three field and lab samples, but no differences in hatching percentage were found. This means that the number of eggs and hatching success, as well as hatching time can strongly vary within a species, population and clonal lineage. Sometimes the reasons for this also lie in abiotic factors like temperature, which can significantly influence egg development, survival rate, body growth and generation time (Hohberg, 2006). Baumann (1961) reported that sufficient food within the first 24 h was crucial for the survival of tardigrades in the species *H. convergens*. Therefore, the availability of food is also important for comparisons of life-history results, also under laboratory conditions.

CONCLUSIONS

Only from a few tardigrade species are long-term cultures available for use in meaningful life-history studies. However, the available data from lab rearing cultures and field samples showed that even populations and clonal lineages of the same species sometime have different life-history patterns, *e.g.* in the number of eggs per animal, the hatching success or the hatching time. Therefore there is an urgent need to study more tardigrade species at the same environmental conditions for a better understanding of the life-history.

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