bioRxiv preprint doi: https://doi.org/10.1101/583914; this version posted April 16, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

1 # Common biomarkers of vertebrate senescence
2 # respond to age variation in the invertebrate
3 # *Armadillidium vulgare*

4

5

6 Charlotte DEPEUX[1, 2 *], Ascel SAMBA-LOUAKA[1], Thomas BECKING[1], Christine
7 BRAQUART-VARNIER[1], Jérôme MOREAU[3], Jean-François LEMAÎTRE[2], Tiffany
8 LAVERRE[1], Hélène PAUHLAC[1], François-Xavier DECHAUME-MONCHARMONT[3], Jean-
9 Michel GAILLARD[2], Sophie BELTRAN-BECH[1]

10

11 [1] Université de Poitiers, Laboratoire Ecologie et Biologie des interactions UMR CNRS 7267, 5
12 rue Albert Turpin, TSA 51106 86073 POITIERS Cedex 9, France.
13 [2]Université de Lyon, Université Lyon 1, CNRS, Laboratoire de Biométrie et Biologie Evolutive
14 UMR 5558, F-69622 Villeurbanne, France
15 [3] Biogéosciences UMR 6282 CNRS, Université Bourgogne Franche-Comté, 6 Boulevard
16 Gabriel, 21000 Dijon, France.

17

18 * Corresponding author: charlotte.depeux@gmail.com

19

20 Declarations of interest: none

21

22 ## Abstract

23 Senescence, the decline of physiological parameters with increasing age, is a quasi-
24 ubiquitous phenomenon in the living world. However, the observed patterns of senescence can
25 markedly differ between across species and populations, between sexes and even among

bioRxiv preprint doi: https://doi.org/10.1101/583914; this version posted April 16, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

26 individuals. To identify the drivers of this variation in senescence, experimental approaches are
27 essential and involve the development of tools and new study models. In fact, current
28 knowledge of the senescence process is mostly based on studies on vertebrates and principal
29 informations about senescence in invertebrates is mostly limited to model organisms such as
30 *Caenorhabditis elegans* or *Drosophila melanogaster*. In this context, we tested whether
31 biomarkers of vertebrate aging could be used to study senescence in a very promising
32 invertebrate model of aging: the common woodlouse *Armadillidium vulgare*. More specifically,
33 we looked for the effect of age in woodlouse on three well established physiological biomarkers
34 of aging in vertebrates: immune cells (cell size, density and viability), β-galactosidase activity,
35 and Telomerase Reverse Transcriptase (TERT) (essential subunit of the telomerase protein)
36 gene expression. We found that the size of immune cells was higher in older individuals,
37 whereas their density and viability decreased, and that the β-galactosidase activity increased
38 with age, whereas the Telomerase Reverse Transcriptase (TERT) gene expression decreased.
39 These findings demonstrate that woodlouse display age-related changes in biomarkers of
40 vertebrate senescence, with different patterns depending on gender. Thus, the tools used in
41 studies of vertebrate senescence can be successfully used in studies of senescence of
42 invertebrates such as the woodlouse. The application of commonly used tools to new biological
43 models offers a promising approach to assess the diversity of senescence patterns across the
44 tree of life.

45

46 **Keywords**

47 *Armadillidium vulgare*, cellular senescence, immunosenescence, invertebrates,
48 Telomerase Reverse Transcriptase (TERT), β-galactosidase activity.

49

50 # 1. Introduction

bioRxiv preprint doi: https://doi.org/10.1101/583914; this version posted April 16, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

51 Many theories have tried to explain why senescence is a quasi-ubiquitous phenomenon
52 in the living organisms. For instance, the disposable soma theory proposed the senescence
53 process as a result of damages accumulation over time. These damages are strongly influenced
54 by the environment, leading to trade-offs between the different functions (e.g. between
55 reproduction and somatic maintenance) and shaping a high diversity of senescence patterns
56 across species and populations, among individuals, and between sexes. One current challenge
57 is to understand the selective forces and mechanisms driving this diversity of senescence
58 patterns.

59 At the cellular level, senescence corresponds to the cellular deterioration leading to stop
60 the cellular cycle (Campisi and di Fagagna, 2007). As ageing is associated with cellular
61 senescence (Herbig et al., 2006; Lawless et al., 2010; Wang et al., 2009), many biomolecular
62 parameters potentially inform about senescence and can therefore be valuable tools for studying
63 this process (de Jesus and Blasco, 2012). For example, the evolution of the integrity and
64 efficiency of immune cells is particularly relevant to study cellular senescence because a
65 diminution of the number of effective immune cells with increasing age takes place in both
66 vertebrates (e.g. Cheynel et al., 2017) and invertebrates (e.g. Park et al., 2011). Another marker
67 used to study cellular senescence is the enzymatic activity of the β-galactosidase. This enzyme
68 is a hydrolase that transforms polysaccharides in monosaccharides. The lysosomal activity of
69 this enzyme is increased when the cell enters in senescence (Dimri et al., 1995; Itahana et al.,
70 2007). This phenomenon occurs in senescent cells of many organisms ranging from humans
71 (Gary and Kindell, 2005) to honeybees (Hsieh and Hsu, 2011). Another protein linked to the
72 cellular senescence process is the telomerase, a ribonucleo protein complex composed by two
73 essential components, the telomerase reverse transcriptase (TERT) and the telomerase RNA
74 (TR) and other accessorial proteins (Podlevsky et al., 2007). Telomerase lengthens the ends of
75 telomeres (i.e. DNA sequences located at the end of chromosomes that protect chromosome

bioRxiv preprint doi: https://doi.org/10.1101/583914; this version posted April 16, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

76 integrity and shorten after each cell division). Cell senescence arises when the telomere length
77 becomes critically short (Chiu and Harley, 1997; Shay and Wright, 2005). The telomerase
78 activity depends on organism, age and also tissues (e.g. Gomes et al., 2010). For instance,
79 telomerase is active during the development before birth and after only in stem and germ cells
80 in humans (Liu et al., 2007; Morgan, 2013) while in the *Daphnia pulicaria,* the telomerase
81 activity in all tissues of the body decreases with increasing age (Schumpert et al., 2015). The
82 TERT is essential in the telomerase protein complex and has been shown to be related to cell
83 survival in humans (Cao et al., 2002). The TERT has also been detected in numerous species
84 including vertebrates, fungi, ciliates and insects (Podlevsky et al., 2007; Robertson and Gordon,
85 2006).

86 As patterns of senescence are strongly diversified within the living world, it seems
87 essential to study organisms displaying markedly different life histories strategies to understand
88 the causes and mechanisms underlying this diversity. Thus, invertebrates are increasingly used
89 in experimental studies of senescence (Ram and Costa, 2018; Stanley, 2012). In addition to
90 share similarities with vertebrates in terms of senescence, they can be manipulated
91 experimentally and they are easier to be monitored throughout their entire lifetime (Ram and
92 Costa, 2018). These advantages make them models of choice for studying senescence. Here,
93 we propose the common woodlouse *A. vulgare* as a promising new model for studying
94 senescence. Woodlouse can live beyond three years and display sex-specific senescence
95 patterns in natural populations (Paris and Pitelka, 1962). In addition, one study has already
96 reported evidence of immuno senescence in this species (Sicard et al., 2010).
97

98 In this context, we tested the suitability of β-galactosidase activity, immune cell
99 parameters and the TERT gene expression to cause age-specific responses in the common
100 woodlouse *Armadillidium vulgare.* According to the literature, we expected an increase in β-

bioRxiv preprint doi: https://doi.org/10.1101/583914; this version posted April 16, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

101 galactosidase activity, and a decrease of both TERT gene expression and immune cell viability
102 and density in *A. vulgare*. As males have higheradult survival than females (Paris and Pitelka,
103 1962), cellular senescence patterns are also expected to be sex-specific in *A. vulgare*.
104

105 2. Materials & Methods

106 *2.1. Biological model*

107 *A. vulgare* individuals used in the following experiments were derived from a wild
108 population collected in Denmark in 1982. These animals have been maintained on moistened
109 soil under the natural photoperiod of Poitiers (France 46.58°N, 0.34°E, 20°C) at 20°C fed *ad*
110 *libitum* with dried linden leaves and carrots. Crosses were monitored to control and promote
111 genetic diversity. For each clutch obtained, individuals were sexed, and brothers and sisters
112 were separated to ensure virginity. In common woodlouse, individuals molt throughout their
113 lives, with approximately one molt per month. During this process all the cells of the concerned
114 tissues are renewed at 20°C (Steel, 1980). However, the brain, the nerve cord and gonads are
115 not renewed during molting and are therefore relevant candidates for tissue-specific study of
116 senescence in this species. Males and females were tested separately to assess the impact of
117 sex.
118

119 *2.2Measure of β-galactosidase activity*

120 Animals

121 To test the impact of age on the on β-galactosidase activity,180 individuals were used:
122 90 young (i.e. 6-months-old, 45 males and 45 females) and 90 old (2-years-old, 45 males and
123 45 females) individuals.

124 Protocol

bioRxiv preprint doi: https://doi.org/10.1101/583914; this version posted April 16, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

125 Individuals were dissected separately in Ringer solution (Sodium Chloride 394 mM,
126 Potassium Chloride 2 mM, Calcium Chloride 2 mM, Sodium Bicarbonate 2 mM) and nerve
127 cord was removed. To obtain a sufficient amount of protein, we made pools of five nerve cords
128 (from five different individuals of the same age). The five nerve cords were filed in 500 µL of
129 Lyse Buffer 1X (CHAPS 5 mM, Citric acid 40 mM, Sodium Phosphate 40 mM, Benzamidine
130 0.5 mM, PMSF 0.25 mM, pH = 6) (Gary and Kindell, 2005), and then were centrifuged at
131 15000g at 4°C for 30 minutes. The supernatant was taken and kept at -80°C until its utilization.
132 The protein concentration was determined by the BCA assay (Thermofisher) and was
133 homogenized at 0.1 mg/mL. The β-galactosidase activity was measured as described by Gary
134 and Kindell (2005). Briefly, 100 µL of extracted protein at the concentration of 0.1 mg/mL were
135 added to 100 µL of reactive 4-methylumbelliferyl-D-galactopyranoside (MUG) solution in a
136 96 well-microplate. The MUG reactive, in contact to β-galactosidase, leads by hydrolysis to the
137 synthesis of 4-methylumbelliferone (4-MU), which is detectable using fluorescent
138 measurements. Measures were performed by the multimode microplate reader Mithras (LB940
139 HTS III, Berthold; excitation filter: 120 nm, emission filter 460 nm) for 120 minutes. Two
140 technical replicates were measured for each pool.

141

142 ### *2.3Measure of immune cell parameters*

143 **Animals**

144 To test the impact of age on the immune cell parameters (i.e. density, viability, and size)
145 in *A. vulgare,* 60 mature individuals were used: 30 young (i.e. 1-year-old, 15 males and 15
146 females) and 30 old (3-years-old, 15 males and 15 females) individuals.

147 **Protocol**

148 To study the impact of age on the immune parameters, a hole was bored in the middle
149 of the 6th segment and 3 µL of haemolymph were collected (per individual) with an eyedropper

bioRxiv preprint doi: https://doi.org/10.1101/583914; this version posted April 16, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

150 and deposited promptly in 15 µL of anticoagulant solution(MAS-EDTA (EDTA 9 mM,
151 Trisodium citrate 27 mM, NaCl 336 mM, Glucose 115 mM, pH 7, (Rodriguez et al., 1995))).
152 Then, 6 µL of Trypan blue at 0.4% (Invitrogen) were added to color the dead cells. Thereafter,
153 10 µL of this solution were deposed in counting slide (Invitrogen Coutness®, Thermofisher).
154 The immune cell density, the immune cell viability and the immune cell size were evaluated
155 using an automated Cell Counter (Invitrogen Countess®).
156

157 ### *2.4 Measure of TERT gene expression*

158 The identification of the Telomerase Reverse Transcriptase (TERT)gene was firstly
159 performed from the *A. vulgare* genome (Chebbi et al., 2019). In order to check whether this
160 gene was present and preserved in crustaceans, phylogenetic analyses were carried out upstream
161 (see Supplementary materials 1, 2, 3 and 4). This gene has been found in crustacean
162 transcriptomes and the topology of the TERT gene tree follows the phylogenetic relationships
163 between the involved species(Supplementary material 3), suggesting a conserved role of the
164 TERT gene.
165

166 #### *2.4.1 Gene expression*

167 **Animals**

168 We tested the effect of age on the expression of TERT gene within 4 different age
169 groups: (1) 4-months-old, (2) 1-year-old, (3) 2-years-old and (4) 3-years-old. Females and
170 males were tested separately by pools of 5 individuals in 1-, 2-, 3-years-old groups and by pools
171 of 7 individuals in 4-months-old group. All conditions require 4 replicates for each sex. 176
172 individuals were used for this experiment. For each group we tested the expression level of the
173 TERT gene in two different tissues: the nerve cord (somatic line) and gonads (germinal line).

174 **Protocol**

bioRxiv preprint doi: https://doi.org/10.1101/583914; this version posted April 16, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

175 Animals were washed by immersion for 30s in a 30% sodium hypochlorite solution
176 followed by two 30s immersion in distilled water. Tissues were dissected in Ringer solution
177 (Sodium Chloride 394 mM, Potassium Chloride 2 mM, Calcium Chloride 2 mM, Sodium
178 Bicarbonate 2 mM) and deposited by specific tissues pools of 5 on TRIzol reagent (Invitrogen)
179 to extract RNA according to the manufacturer's protocol after a cell disintegration using a Vibra
180 Cell 75,185 sonicator (amplitude of 30%). Total RNA was quantified by NanoDrop technology
181 and was stored at -80°C until use. Reverse transcriptions (RT) were made from 500ng of RNA
182 previously extracted and using the kit SuperScript™ IV Reverse Transcriptase (Thermo Fisher
183 Scientific) according to the supplier's instructions. Primers were designed using the identified
184 gene: primer TERT_F: 5'-AGGGAAAACGATGCACAACC-3' and primer TERT_R: 5'-
185 GTTCGCCAAATGTTCGCAAC- 3' (see Supplementary material 1). Quantitative RT-PCR
186 was performed using0.6 µl of each primer (10 µM), 2.4 µl of nuclease-free water and 1.5 µl of
187 cDNA template and the LightCycler LC480 system (Roche) with the following program:10 min
188 at 95 °C, 45 cycles of 10 s at 95 °C, 10 s at 60 °C, and 20 s at 72 °C. Expression levels of target
189 genes were normalized based on the expression level of two reference genes previously
190 established: the Ribosomal Protein L8 (RbL8) and the Elongation Factor 2 (EF2) (Chevalier et
191 al., 2011).
192
193 **Statistics**
194 All statistical analyses were performed using the R software (R. Core Team, 2016).The
195 β-galactosidase activity was analyzed with linear mixed effect models using the package lme4
196 (Bates et al., 2014). As two technical replicates were measured for each pool, the model
197 including the pools fitted as a random effect, age and sex and their two-way interaction as fixed
198 factors.

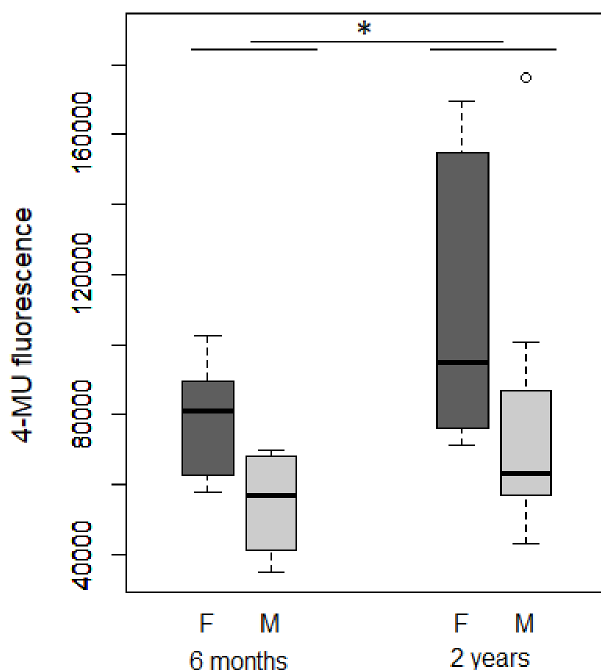
bioRxiv preprint doi: https://doi.org/10.1101/583914; this version posted April 16, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

199 Concerning the immune parameters, linear models with Gaussian distribution were
200 fitted to analyze variation in the cell size and viability. For the cell density, a linear model of
201 the cell number (log-transformed, (Ives and Freckleton Robert, 2015)) was fitted.
202 The level of TERT expression according to age in the two different tissues were
203 compared by a Kruskal–Wallis rank sum test in combination with Nemenyi's post hoc multiple
204 comparison test with the Tuckey correction using R package PMCMR.
205

206 3. Results

207 β-galactosidase activity

208 The β-galactosidase activity was higher in old (i.e. 2-years-old) than in young (i.e. 6-
209 months-old) individuals (χ^2_1=6.15, p=0.013, Figure 1). We also detected a higher β-
210 galactosidase activity in females than in males (χ^2_1=7.26, p=0.007, Figure 1).



211

212 ***Figure 1: β-galactosidase activity according to age and sex in* A. vulgare *(F=females, M=males)***

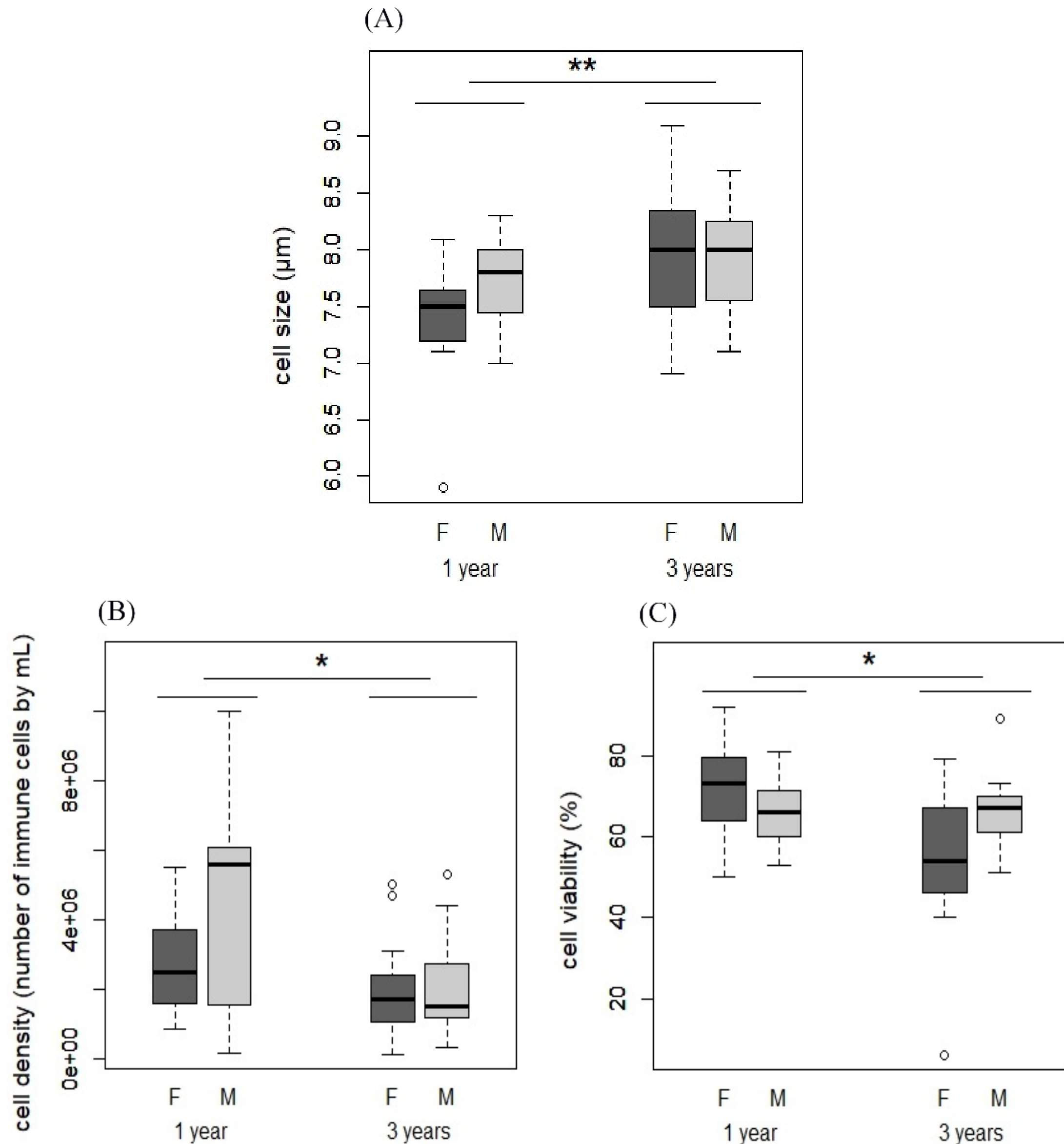
bioRxiv preprint doi: https://doi.org/10.1101/583914; this version posted April 16, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

213 The thick line depicts the median, the box the interquartile range, and the whisker are bounded to the most extreme
214 data point within 1.5 the interquartile range. The outliers outside this range are displayed as open circles. N= 24
215 pools of 5 individuals. * denotes $p<0.05$
216

217 **Immune cells parameters**

218 Cell size was larger in 3-years-old than in 1-year-old individuals ($F_{1,58}$=8.54, p=0.005, Figure
219 2A). Conversely, the cell density was higher in 1-year-old than in 3-years-old individuals ($F_{1,58}$
220 =4.33, p=0.01, Figure 2B). Concerning the immune cell viability, a statistically significant
221 interaction occurred between age and sex, with a relatively lower immune cell viability in 3-
222 years-old females ($F_{3,56}$=6.85, p=0.01, Figure 2C). No sex effect was detected on cell size
223 ($F_{2,57}$=0.76, p=0.38, Figure 2A) or cell density ($F_{2,57}$=0.32, p =0.57, Figure 2B).

bioRxiv preprint doi: https://doi.org/10.1101/583914; this version posted April 16, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.



224

225 226 ***Figure 2: Immune cell size (A), density (B) and viability (C) according to age and sex in* A. vulgare *(F=females, M=males)***

227 Thethick line depicts the median, the box the interquartile range, and the whisker are bounded to the most extreme

228 data point within 1.5 the interquartile range. The outliers outside this range are displayed as open circles.N= 60

229 individuals: 15 1-year-old females, 15 1-year-old males, 15 3-years-old females and 15 3-years-old males. *

230 denotes p<0.05 and ** denotes p<0.01

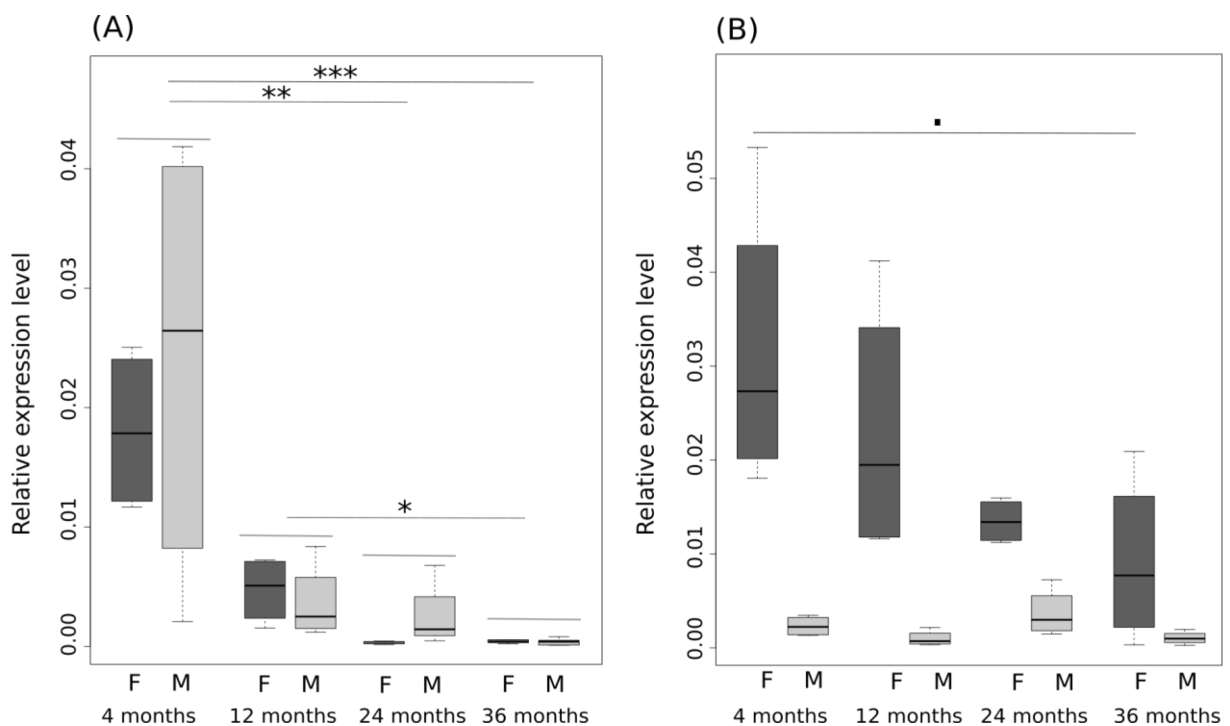
231

bioRxiv preprint doi: https://doi.org/10.1101/583914; this version posted April 16, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

232

233 **TERT gene expression**

234 The TERT gene expression decreased with increasing age in nerve cords (χ^2_3=23.30,
235 p<0.001, Figure 3A). More precisely, the TERT gene expression was higher in 4-months-old
236 individuals compared to 2-years-old and 3-years-old individuals (p=0.001 in both cases) and in
237 1-year-old individuals compared to 3-years-old individuals (p=0.038), without any detectable
238 sex effect (χ^2_1=0.14, p=0.70, Figure 3A). In gonads, the TERT gene expression was much
239 higher in females (χ^2_1=17.81, p<0.001, Figure 3B) and tended to decrease with increasing age
240 (χ^2_3=7.5, p=0.057, Figure 3B) as the TERT gene expression tended to be higher in 4-months-
241 old females compared to 3-years-old females (p=0.054). In males, a general tendency was also
242 observed (χ^2_1=7.34, p=0.061, Figure 3B), the TERT gene expression tending to be higher in 2-
243 years-old individuals compared to 1-year-old and 3-years-old individuals (p=0.14 and p=0.12,
244 respectively, Figure 3B).



245

246 ***Figure 3: Relative expression level of TERT in (A) nerve cords and (B) in gonads in A. vulgare (F=females,***
247 ***M=males).***

bioRxiv preprint doi: https://doi.org/10.1101/583914; this version posted April 16, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

248 Expression of each gene was normalized based on the expression of Ribosomal Protein L8 (RbL8) and Elongation
249 Factor 2 (EF2) as reference genes. The thick line depicts the median, the box the interquartile range, and the whisker
250 are bounded to the most extreme data point within 1.5 the interquartile range. N= 176 individuals: 284-months-old
251 females, 28 4-months-old males, 20 1-year-old females, 20 1-year-old males, 20 2-years-old females, 20 2-years-old
252 males, 20 3-years-old females, 20 3-years-old males. **.** denotes p<0.10, ** denotes p<0.01

253

254 # 4. Discussion

255 In this study, we tested several effective physiological biomarkers of vertebrate
256 senescence to assess whether they could also be used in invertebrates such as the common
257 woodlouse. Immune cells showed an increase in their size and a decrease in their density and
258 viability with increasing age. In nerve cords, the activity of the β-galactosidase enzyme
259 increased, whereas the TERT gene expression decreased with increasing age. These results
260 support the presence of increasing cellular senescence in *A. vulgare* with chronological age. In
261 contrast, in the gonads, the TERT gene expression was too low in males and was not sufficiently
262 variable between sexes to provide information on the cellular senescence status in this tissue.

263 Our study is in line with previous studies that have already revealed the possibility of
264 using vertebrate biomarkers in invertebrates(Hsieh and Hsu, 2011; Park et al., 2011; Schumpert
265 et al., 2015). By testing a set of different physiological biomarkers of vertebrate senescence,
266 often studied independently, our study supports both ideas that routinely used biomarkers in
267 vertebrates can be adapted in invertebrates and that the senescence process is quasi-ubiquitous
268 in the living world and can be expressed in a similar way in very different organisms.

269 Previous studies have shown that the probabilities to survive decrease with increasing
270 age in *A. vulgare* (Paris and Pitelka, 1962). The cellular damages accumulated during the
271 animal's life could be the cause of cell senescence and therefore the driving force behind
272 actuarial senescence. (Barja, 2000; Barja and Herrero, 2000; Finkel and Holbrook, 2000;
273 Harman, 1956). In *A. vulgare*, the 2- and 3-years-old individuals could have therefore

bioRxiv preprint doi: https://doi.org/10.1101/583914; this version posted April 16, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

274 accumulated more cellular damages during their lifetime, leading to the cellular senescence we
275 report.

276 Our study also revealed a strong difference between sexes on the response of biomarkers
277 to age changes. At a given age, females display higher β-galactosidase activity and lower
278 immune cell viability than males. Between-sex differences in lifespan have been reported in *A.*
279 *vulgare* with a longer lifespan in males than in females (Geiser, 1934; Paris and Pitelka, 1962).
280 Exact differences in actuarial senescence patterns (i.e. age-specific changes in survival
281 probabilities) remain to be quantified in *A. vulgare* but such differences are quite common both
282 in vertebrates and invertebrates (Marais et al., 2018; Tidière et al., 2015). One of the main
283 theory proposed to explain sex differences in longevity or senescence patterns relies on different
284 resource allocation strategies between sexes (Bonduriansky et al., 2008; Vinogradov,
285 1998).The shorter lifespan in females *A. vulgare,* that allocate more energy to reproduction than
286 males (Paris and Pitelka, 1962) because they carry their offspring in their marsupium during
287 one month giving nutrients and protection, supports a role of differential sex allocation.

288 Sex differences in resource allocation strategies could also be driven by environmental
289 conditions (Shertzer and Ellner, 2002). Our physiological biomarkers of vertebrate senescence
290 revealed sex differences, and as supported in Depeux et al., 2019, they could constitute useful
291 tools to identify other factors involved in variations in senescence patterns, such as
292 environmental stressors. Moreover, if these biomarkers seem to predict better the physiological
293 age than chronological age notably in terms of survival and reproduction, they could correspond
294 to biomarkers of senescence in woodlouse (Baker and Sprott, 1988; Simm et al., 2008; Sprott,
295 2010).

296 Our present study demonstrated that the physiological biomarkers of vertebrate
297 senescence respond to age changes in the common woodlouse, a new invertebrate model of
298 aging. These parameters that predict the chronological age of woodlouse individuals might offer

bioRxiv preprint doi: https://doi.org/10.1101/583914; this version posted April 16, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

299 reliable biomarkers, especially if their measurements are related to both reproductive and
300 survival prospects more than to the chronological age of individuals. In this context, and more
301 broadly in the study of senescence and of the factors involved in its diversity, the woodlouse
302 model, which has physiological similarities with other invertebrates, could be a model of choice
303 to study sex-specific actuarial and cellular senescence.

304

305 ## Acknowledgements

306 We would like to thank Sylvine Durand, Richard Cordaux, Isabelle Giraud and
307 Bouziane Moumen for our constructive discussions as well as Marius Bredon, Carine Delaunay,
308 Maryline Raimond and Alexandra Lafitte for technical assistance.

309

310 ## Funding

311 This work was supported by the 2015–2020 State-Region Planning Contract and European
312 Regional Development Fund and intramural funds from the Centre National de la Recherche
313 Scientifique and the University of Poitiers. J.F.L. and J.M.G. are supported by a grant from the
314 Agence Nationale de la Recherche (ANR-15-CE32-0002-01 to J.F.L.). This work has also
315 received funding from the "Appel à projets de recherche collaborative inter-équipes 2016-2017"
316 by the laboratory EBI.

317

318

319 ## Bibliography

320 Baker, G.T., Sprott, R.L., 1988. Biomarkers of aging. Experimental Gerontology, Special Issue
321 Biomarkers of Aging 23, 223–239. https://doi.org/10.1016/0531-5565(88)90025-3
322 Barja, G., 2000. The flux of free radical attack through mitochondrial DNA is related to aging
323 rate. Aging (Milano) 12, 342–355.
324 Barja, G., Herrero, A., 2000. Oxidative damage to mitochondrial DNA is inversely related to
325 maximum life span in the heart and brain of mammals. FASEB J. 14, 312–318.

bioRxiv preprint doi: https://doi.org/10.1101/583914; this version posted April 16, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

326 Bates, D., Mächler, M., Bolker, B., Walker, S., 2014. Fitting linear mixed-effects models using
327 lme4. arXiv preprint arXiv:1406.5823.
328 Bonduriansky, R., Maklakov, A., Zajitschek, F., Brooks, R., 2008. Sexual selection, sexual
329 conflict and the evolution of ageing and life span. Functional Ecology 22, 443–453.
330 https://doi.org/10.1111/j.1365-2435.2008.01417.x
331 Campisi, J., di Fagagna, F. d'Adda, 2007. Cellular senescence: when bad things happen to good
332 cells. Nature reviews. Molecular cell biology 8, 729.
333 Cao, Y., Li, H., Deb, S., Liu, J.-P., 2002. TERT regulates cell survival independent of
334 telomerase enzymatic activity. Oncogene 21, 3130–3138.
335 https://doi.org/10.1038/sj.onc.1205419
336 Chebbi, M.A., Becking, T., Moumen, B., Giraud, I., Gilbert, C., Peccoud, J., Cordaux, R., 2019.
337 The Genome of *Armadillidium vulgare* (Crustacea, Isopoda) Provides Insights into Sex
338 Chromosome Evolution in the Context of Cytoplasmic Sex Determination. Molecular
339 Biology and Evolution. https://doi.org/10.1093/molbev/msz010
340 Chevalier, F., Herbinière-Gaboreau, J., Bertaux, J., Raimond, M., Morel, F., Bouchon, D.,
341 Grève, P., Braquart-Varnier, C., 2011. The Immune Cellular Effectors of Terrestrial
342 Isopod *Armadillidium vulgare*: Meeting with Their Invaders, Wolbachia. PLOS ONE
343 6, e18531. https://doi.org/10.1371/journal.pone.0018531
344 Cheynel, L., Lemaître, J.-F., Gaillard, J.-M., Rey, B., Bourgoin, G., Ferté, H., Jégo, M., Débias,
345 F., Pellerin, M., Jacob, L., Gilot-Fromont, E., 2017. Immunosenescence patterns differ
346 between populations but not between sexes in a long-lived mammal. Scientific Reports
347 7. https://doi.org/10.1038/s41598-017-13686-5
348 Chiu, C.-P., Harley, C.B., 1997. Replicative Senescence and Cell Immortality: The Role of
349 Telomeres and Telomerase. Experimental Biology and Medicine 214, 99–106.
350 https://doi.org/10.3181/00379727-214-44075
351 de Jesus, B.B., Blasco, M.A., 2012. Assessing cell and organ senescence biomarkers.
352 Circulation research 111, 97–109.
353 Depeux, C., Samba-Louaka, A., Braquart-Varnier, C., Moreau, J., Lemaître, J.-F., Laverre, T.,
354 Pauhlac, H., Dechaume-Moncharmont, F.-X., Gaillard, J.-M., Beltran-Bech, S., 2019.
355 Impact of temperature and photoperiod impact on survival and biomarkers of
356 senescence in common woodlouse. bioRxiv. https://doi.org/10.1101/433011
357 Dimri, G.P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E.E., Linskens,
358 M., Rubelj, I., Pereira-Smith, O., 1995. A biomarker that identifies senescent human
359 cells in culture and in aging skin in vivo. PNAS 92, 9363–9367.
360 Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. Nature
361 408, 239–247.
362 Gary, R.K., Kindell, S.M., 2005. Quantitative assay of senescence-associated β-galactosidase
363 activity in mammalian cell extracts. Analytical biochemistry 343, 329–334.
364 Geiser, S.W., 1934. Further observations on the sex-ratios of terrestrial isopods. Field Lab 3,
365 7–10.
366 Gomes, N.M.V., Shay, J.W., Wright, W.E., 2010. Telomere biology in Metazoa. FEBS Letters
367 584, 3741–3751. https://doi.org/10.1016/j.febslet.2010.07.031
368 Harman, D., 1956. Aging: a theory based on free radical and radiation chemistry. Journal of
369 gerontology 11, 298–300.
370 Herbig, U., Ferreira, M., Condel, L., Carey, D., Sedivy, J.M., 2006. Cellular senescence in aging
371 primates. Science 311, 1257. https://doi.org/10.1126/science.1122446
372 Hsieh, Y.-S., Hsu, C.-Y., 2011. Honeybee trophocytes and fat cells as target cells for cellular
373 senescence studies. Experimental Gerontology 46, 233–240.
374 https://doi.org/10.1016/j.exger.2010.10.007

bioRxiv preprint doi: https://doi.org/10.1101/583914; this version posted April 16, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

375 Itahana, K., Campisi, J., Dimri, G.P., 2007. Methods to detect biomarkers of cellular
376 senescence: the senescence-associated β-galactosidase assay. Biological Aging:
377 Methods and Protocols 21–31.
378 Ives, A.R., Freckleton Robert, 2015. For testing the significance of regression coefficients, go
379 ahead and log-transform count data. Methods in Ecology and Evolution 6, 828–835.
380 https://doi.org/10.1111/2041-210X.12386
381 Lawless, C., Wang, C., Jurk, D., Merz, A., Zglinicki, T. von, Passos, J.F., 2010. Quantitative
382 assessment of markers for cell senescence. Exp. Gerontol. 45, 772–778.
383 https://doi.org/10.1016/j.exger.2010.01.018
384 Liu, L., Bailey, S.M., Okuka, M., Muñoz, P., Li, C., Zhou, L., Wu, C., Czerwiec, E., Sandler,
385 L., Seyfang, A., Blasco, M.A., Keefe, D.L., 2007. Telomere lengthening early in
386 development. Nature Cell Biology 9, 1436–1441. https://doi.org/10.1038/ncb1664
387 Marais, G.A.B., Gaillard, J.-M., Vieira, C., Plotton, I., Sanlaville, D., Gueyffier, F., Lemaitre,
388 J.-F., 2018. Sex gap in aging and longevity: can sex chromosomes play a role? Biology
389 of Sex Differences 9. https://doi.org/10.1186/s13293-018-0181-y
390 Morgan, G., 2013. Telomerase regulation and the intimate relationship with aging. Research
391 and Reports in Biochemistry 71. https://doi.org/10.2147/RRBC.S28603
392 Paris, O.H., Pitelka, F.A., 1962. Population characteristics of the terrestrial isopod
393 *Armadillidium vulgare* in California grassland. Ecology 43, 229–248.
394 Park, Y., Kim, Y., Stanley, D., 2011. Cellular immunosenescence in adult male crickets, Gryllus
395 assimilis. Archives of Insect Biochemistry and Physiology 76, 185–194.
396 https://doi.org/10.1002/arch.20394
397 Podlevsky, J.D., Bley, C.J., Omana, R.V., Qi, X., Chen, J.J.-L., 2007. The Telomerase
398 Database. Nucleic Acids Research 36, D339–D343.
399 https://doi.org/10.1093/nar/gkm700
400 R. Core Team, 2016. R: A language and environment for statistical computing. Vienna: R
401 Foundation for Statistical Computing; 2014.
402 Ram, J.L., Costa, A.J., 2018. Invertebrates as Model Organisms for Research on Aging Biology
403 *, in: Conn's Handbook of Models for Human Aging. Elsevier, pp. 445–452.
404 https://doi.org/10.1016/B978-0-12-811353-0.00032-4
405 Robertson, H.M., Gordon, K.H.J., 2006. Canonical TTAGG-repeat telomeres and telomerase
406 in the honey bee, Apis mellifera. Genome Research 16, 1345–1351.
407 https://doi.org/10.1101/gr.5085606
408 Rodriguez, J., Boulo, V., Mialhe, E., Bachere, E., 1995. Characterisation of shrimp haemocytes
409 and plasma components by monoclonal antibodies. Journal of Cell Science 108, 1043–
410 1050.
411 Schumpert, C., Nelson, J., Kim, E., Dudycha, J.L., Patel, R.C., 2015. Telomerase Activity and
412 Telomere Length in Daphnia. PLOS ONE 10, e0127196.
413 https://doi.org/10.1371/journal.pone.0127196
414 Shay, J.W., Wright, W.E., 2005. Senescence and immortalization: role of telomeres and
415 telomerase. Carcinogenesis 26, 867–874. https://doi.org/10.1093/carcin/bgh296
416 Shertzer, K.W., Ellner, S.P., 2002. State-dependent energy allocation in variable environments:
417 life history evolution of a rotifer. Ecology 83, 2181–2193. https://doi.org/10.1890/0012-
418 9658(2002)083[2181:SDEAIV]2.0.CO;2
419 Sicard, M., Chevalier, F., Vlechouver, M.D., Bouchon, D., Grève, P., Braquart-Varnier, C.,
420 2010. Variations of immune parameters in terrestrial isopods: a matter of gender, aging
421 and Wolbachia. Naturwissenschaften 97, 819–826. https://doi.org/10.1007/s00114-010-
422 0699-2

bioRxiv preprint doi: https://doi.org/10.1101/583914; this version posted April 16, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

423 Simm, A., Nass, N., Bartling, B., Hofmann, B., Silber, R.-E., Navarrete Santos, A., 2008.
424 Potential biomarkers of ageing. Biological Chemistry 389.
425 https://doi.org/10.1515/BC.2008.034
426 Sprott, R.L., 2010. Biomarkers of aging and disease: Introduction and definitions. Experimental
427 Gerontology 45, 2–4. https://doi.org/10.1016/j.exger.2009.07.008
428 Stanley, D., 2012. Aging and immunosenescence in invertebrates. Invertebrate Survival Journal
429 9, 102–109.
430 Steel, C.G.H., 1980. Mechanisms of coordination between moulting and reproduction in
431 terrestrial isopod crustacea. The Biological Bulletin 159, 206–218.
432 https://doi.org/10.2307/1541019
433 Tidière, M., Gaillard, J.-M., Müller, D.W.H., Lackey, L.B., Gimenez, O., Clauss, M., Lemaître,
434 J.-F., 2015. Does sexual selection shape sex differences in longevity and senescence
435 patterns across vertebrates? A review and new insights from captive ruminants:
436 SEXUAL SELECTION AND SENESCENCE. Evolution 69, 3123–3140.
437 https://doi.org/10.1111/evo.12801
438 Vinogradov, A.E., 1998. Male Reproductive Strategy and Decreased Longevity. Acta
439 Biotheoretica 46, 157–160. https://doi.org/10.1023/A:1001181921303
440 Wang, C., Jurk, D., Maddick, M., Nelson, G., Martin-Ruiz, C., von Zglinicki, T., 2009. DNA
441 damage response and cellular senescence in tissues of aging mice. Aging Cell 8, 311–
442 323. https://doi.org/10.1111/j.1474-9726.2009.00481.x
443

444