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Age Dynamics of Telomere Length of Baikal Gastropods is Sex-Specific and Multidirectional

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The age dynamics of telomeres are affected by various internal and external factors. We examined the age dynamics of relative telomere length (RTL) and telomerase activity in freshwater dioecious gastropod species from Lake Baikal (*Benedictia fragilis*, *B. baicalensis* and *Kobeltocochlea martensiana*). They differ in habitats, growth rate, size of mature specimens, reproductive strategy and degree of phylogenetic relatedness, but do not possess sexual dimorphism and, irrespective of sex, they have a similar lifespan (~8 years) and time to maturity (~4 years). Sex specificity in changes of telomere with age was observed in each species of benedectiids. Immature specimens do not differ in telomere size irrespective of sex. However, when they become mature, we recorded differences and similarities in telomere length between females and males. Telomeres are longer in 4-6 year-old adult females than in males. At the age of 7-8 years, sex differences in telomere sizes are leveled in *B. fragilis* and *K. martensiana*, while in *B. baicalensis* the difference remains. Sex differences in the telomere age dynamics of *B. baicalensis* have also been revealed between the embryonic and mature stages of development. Telomeres in mature males are shorter than in embryos and mature females. In females after the embryonic stage, telomeres shorten rapidly, and then at the age of 4-8 years telomere length is restored up to the embryonic level. Active telomerase was revealed in all species irrespective of sex and developmental stage. The multidirectionality of sex-dependent age dynamics of telomere size may be attributed to the combined effect of internal and external factors on telomeres during ontogenesis.

Key words: Telomere length, telomerase activity, age dynamics, dioecious gastropods, Baikal.

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Telomeres have been extensively investigated over the last few decades. There is an increasing interest in telomeres as biomarkers of cellular aging, stress and life-histories (DANTZER & FLETCHER 2015; INGLES & DEAKIN 2016). Telomeres are DNA-capping structures that protect chromosome ends from recombination and fusion, maintaining genomic stability. Their sizes can change with age. The age dynamics of telomeres have been described for a large number of mammals and birds as well as for some reptiles, fish, and invertebrates (OVENDEN & GODWIN 2011; DANTZER & FLETCHER 2015; INGLES & DEAKIN 2016). The age dynamics of telomere lengths may be attributed to various internal and external factors. The main role in the regulation of telomere length belongs to the enzyme telomerase, the activity of which is regulated by specific mechanisms, promoting telomeric repair and reducing telomere erosion (CIFUENTES-ROJAS & SHIPPEN 2012). Telomerase is inactive in most somatic cells of some animals, and throughout life telomeres shorten with each cell division (e.g., ALLSOPP *et al.* 1992; GREIDER 1996). During the activity period of telomerase, in some animals the telomere size remains unchanged or increases with age (FRANCIS *et al*. 2006; GOMES *et al*. 2010; CHANG 2012; GRUBER *et al.* 2014), whereas in other ani-

mals, despite telomerase activity telomere lengths shorten (HATAKEYAMA *et al.* 2008; ANCHELIN *et al.* 2011). Age dynamics of telomeres in animals may be specific depending on lifespan (HAUSSMANN *et al.* 2003, 2007), stage of development (HATAKEYAMA *et al.* 2016; CERCHIARA *et al.* 2017), growth rate (MCLENNAN *et al.* 2016) and sex (BARRETT & RICHARDSON 2011; NOGUERA *et al.* 2015; TAFF & FREEMAN-GALLANT 2017; WATSON *et al.* 2017). During ontogenesis, the character of telomere length change also depends on specific features of animal habitat, ecological preferences, changes in the environment and adaptations acquired during evolution (MIZUTANI *et al.* 2013; NUSSEY *et al.* 2014; DEBES *et al.* 2016; MCLENNAN *et al.* 2016; WILBOURN *et al.* 2017).

At the same time, in natural populations without experimental data it is sometimes difficult to definitely ascertain the effect of direct, indirect or random factors. It is possible to overcome these difficulties by studying endemic groups of organisms. The composition of such groups includes both closely related species with distinct biological and ecological characteristics and distantly related species with similar characteristics. This allows the exclusion of factors of close relation or similar habitats and degree of adaptation in the species under study.

Lake Baikal, the world's deepest and oldest lake, is inhabited by a great number of endemic groups of species. It houses over 2500 species and subspecies of animals, mostly represented by invertebrates (~98%), of them 56% being endemic (TIMOSHKIN 2001). Underwater landscapes differing in physico-geographic characteristics and morphological structure of sediments affect habitat conditions in Lake Baikal (KARABANOV 1990). The main environmental factors in the landscapes of the shallow-water terrace (at depths of 0 and 5-20 m) are as follows: an abundance of light, water enrichment with oxygen, significant fluctuations of water temperature, active hydrodynamic regime and specific substrate mobility (boulder-pebble or pebble-sand). These factors weaken with depth and landscape transformation to the underwater slope (5-20 m up to 600-1500 m); solid substrate changes to soft, hydrostatic pressure increases and the density of the surface sediments layer and feeding conditions change (KARABANOV 1990; KOZHOVA & IZMESTEVA 1998). Many macrozoobenthic communities of the shallow-water terrace are dominated by abundant gastropods (KRAVTSOVA *et al.* 2004, 2009) with their highest species diversity at a depth of 5-20 m (SITNIKOVA 2004). In the communities of the underwater slope, gastropod number reduces with depth reaching their minimal values at a depth of about 1400 m (KOZHOVA & IZMESTEVA 1998; SITNIKOVA 2004). According to recent estimates, Lake Baikal is inhabited by 148 gastropod species of which 79% are endemic, including 13% belonging to the Benedictiidae family (SITNIKOVA *et al*. 2004). The benedictiids in the lake are highly diversified; they occupy almost all biotopes of the lake from the inshore zone of destructive wave activity to the areas of natural oil and gas seepages in the deep zone (SITNIKOVA 2004; ZEMSKAYA *et al.* 2012). Under different environmental conditions, benedictiids have acquired specific adaptations and survival strategies (SITNIKOVA & SHIMARAEV 2001; SITNIKOVA 2004).

In this study, we analyzed age dynamics of telomeres in three endemic species of benedictiids: *Benedictia fragilis*, *B. baicalensis,* and *Kobeltocochlea martensiana*. They differ in habitats, growth rate, size of mature specimens, reproductive strategy (Fig. 1) and degree of phylogenetic relatedness. Members of the genus *Benedictia* are sister species, whereas *K. martensiana* is significantly distant from them (unpublished data). These three species are dioecious and oviparous; they have an XX-XY-type of sex determination (POBEREZHNY 1989). They do not possess sex dimorphism and, irrespective of sex, they have a similar lifespan (~8 years) and time to maturity (~4 years). Previously, we have reported that the telomeres of these benedictiids are constituted by a $(TTAGGG)_n$ repeat motif (KOROLEVA *et al.* 2015). Absolute telomere lengths in the largest of the three species, *B. fragilis*, and middle-sized *B. baicalensis*, were significantly longer than those in *K. martensiana*, with a relatively small shell (Fig. 1). Among mollusks, age dynamics of telomeres have been described only in several species (ESTABROOKS 2007; OVENDEN & GODWIN 2011; GODWIN *et al.* 2012; GRUBER *et al.* 2014). Despite scarce data, this group of animals demonstrates species-specificity and population variability in telomere changes with age.

The main aims of this study were (i) to assess age dynamics of telomeres in three gastropod species, (ii) to analyze differences in telomerase activity in each species, and (iii) to study age changes of telomeres in comparison with the known biological and environmental parameters of these species.

Material and Methods

Sampling procedure

B. fragilis individuals were collected using trawl nets; *B. baicalensis* and *K. martensiana* were collected by SCUBA divers (Table 1).

Relative telomere length (RTL) in *B. baicalensis* was examined at various stages, from embryos obtained *in vitro* to mature specimens. It was impos-

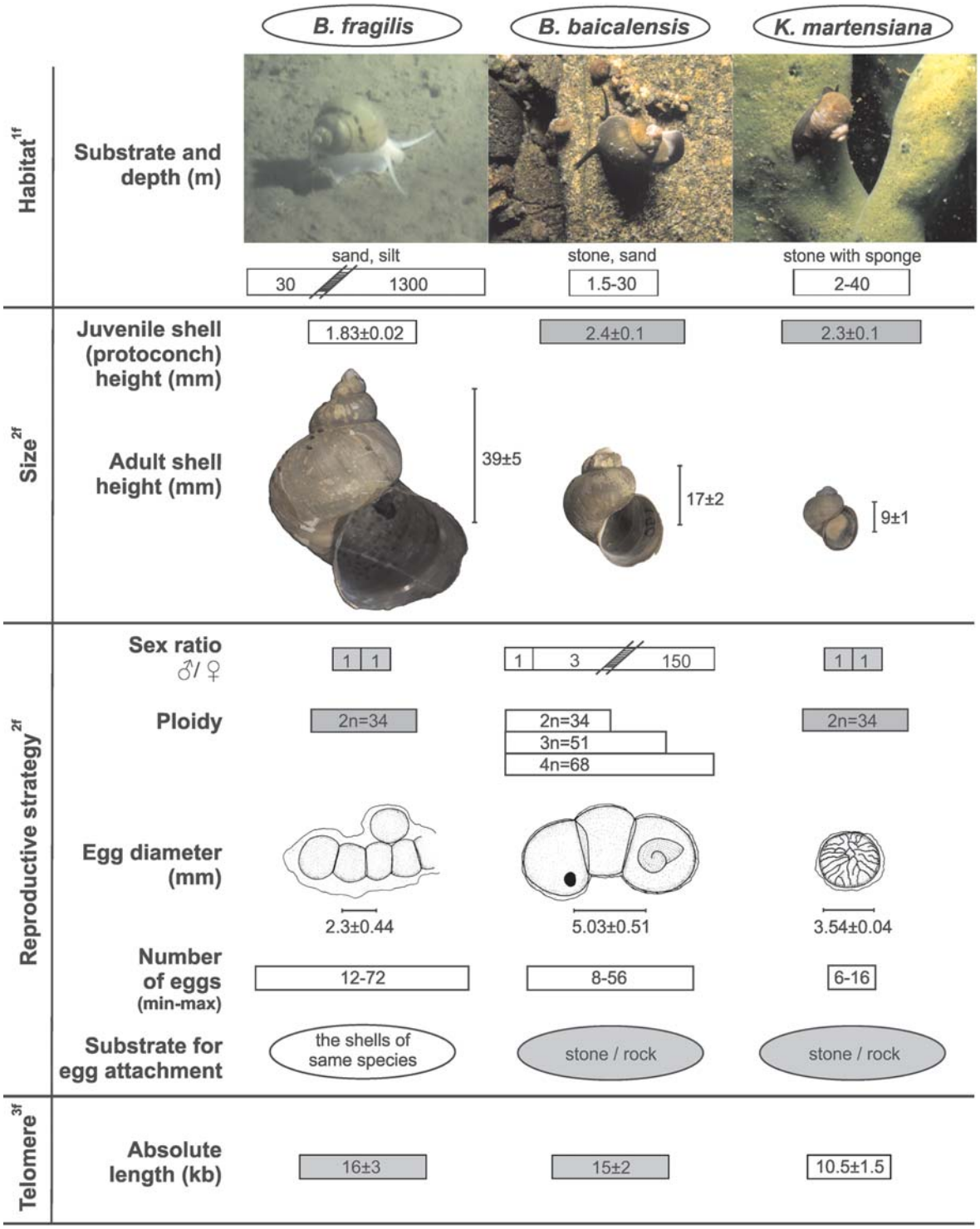

Fig. 1. Comparison of the ecological and biological properties of the three gastropod species. Indistinguishable characteristics are marked in gray. Footnote – Refs. 1f – SITNIKOVA (2004), 2f – SITNIKOVA *et al*. (2004) and unpublished data, 3f – KOROLEVA *et al*. (2015).

sible to trace embryonic development in other species under laboratory conditions. To obtain *B. baicalensis* embryos, adult specimens of both sexes were placed in tanks filled with Baikal water. Embryonic development of *B. baicalensis* lasts from 3 months to 1 year, depending on reproduction time (early or late summer) (SITNIKOVA 2004). The tanks were kept at 6-10°C under natural light. The water was constantly aerated. Every month, the appearance of eggs and embryonic development were monitored. Embryos at final stage ready to hatch were used for the analysis.

Table 1

Sampling areas, collection dates, and number of individuals included in the analysis (qPCR – quantitative PCR; TRAP – telomerase activity)

Species	Location	Depth (m)	Date	Analysis			
				qPCR		TRAP	
				♀	♂	♀	♂
B. fragilis	Selenga Shoal 52°20′ N 106°05′ E Chivyrkuy Bay 53°49′ N 109°09′ E	40-60 100-300	July 2010, August 2012, July 2013	50	45	11	7
B. baicalensis	Listvennichny Bay 51°51′ N 104°50′ E Bolshie Koty Bay 51°54′ N 105°05′ E	3-20	April 2010, March 2011, December 2012, February 2013	93	28	12	4
K. martensiana	Bolshie Koty Bay 51°54′ N 105°05′ E	10-40	March 2011, February 2013	50	61	5	4

Morphological analysis

The height and width of each mollusk shell were measured, and age was determined based on the annual growth interruption lines on the shell. To confirm the validity of this method for determining the age of individual benedictiids, the internal shell structure of *B. baicalensis* was examined using shell cuts made according to KOZMINSKY (2003). Cuts of eight shells were analyzed. A characteristic wedge-shaped structure described for gastropods was seen on the annual growth interruption line (KOZMINSKY 2003). The individual age of a mollusk was estimated twice, with an interval of several days. Values were considered doubtful if the two estimates did not coincide. These values were excluded from the analysis.

To identify sex and maturity, all mollusks were dissected. The evidence of female maturity was the presence of sperm in seminal receptacles, male maturity – the presence of sperm in seminal vesicles (KOENE 2017). It should be noted that it was difficult to define the sex in *B. baicalensis* embryos and recently hatched young specimens based on anatomical characters. Therefore, they were compared to specimens of both sexes.

Quantitative PCR (qPCR)

DNA was isolated from foot tissues by phenol-chloroform extraction (SAMBROOK *et al.* 1989). Then, the DNA was further cleaned using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The DNA concentration was measured using a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA).

The beta actin gene was chosen as reference gene. The fragment of beta actin gene of benedictiids was sequenced and primers were designed using Integrated DNA Technologies online tool (http://eu.idtdna.com/Primerquest/Home/Index). The forward and revers primer were 5'-GTGTGACGTGGACATCCGTA-3' and 5'-GATCCATGCAGGGTATTTGC-3' respectively, with an amplicon length of 110 bp. Quantitative PCR was performed using a Rotor-Gene Q 6000 according to the methods of CAWTHON (2002). The reaction was performed in a 10 µl reaction mixture, with 1× *Snp*-buffer (Evrogen, Moscow, Russia), 3.5 mM $MgCl_2$, 0.25 mM each dNTP, 0.5 pmol of primers for telomere fragments and the actin gene (in different tubes), 0.2 U *Snp*-polymerase (Evrogen, Moscow, Russia), 0.4-1.2 ng of total DNA, and 0.3× SYBR Green (Lumiprobe, Moscow, Russia).

The amplification procedure was as follows: 95°C for 3 min, followed by five cycles of 94°C for 10 s, 54°C for 15 s, and 74°C for 10 s, followed by one cycle of 10 s at 94°C, 15 s at 57°C, and 10 s at 74°C, repeated 35 times.

A standard curve was obtained for each plate by serial dilutions of a reference sample ranging from 0.16 to 4.0 ng. The Ct threshold for each reaction was determined from the reference sample. A negative control was also included on each plate. All samples were run in triplicate. Relative telomere length (RTL) was calculated as the ratio between the number of telomere repeat copies to the

number of copies of the beta-actin gene (PFAFFL 2001).

Telomerase activity (TRAP analysis)

The extract containing protein and RNA was isolated from foot tissues according to the telomeric repeat amplification protocol (TRAP; Chemicon, Billerica, MA, USA). Protein concentration was measured using a standard dilution of albumin (5-25 μg/ml) and Coomassie dye G-250 (BRADFORD 1976).

For the TRAP analysis, TS and ACX primers developed by KIM and WU (1997) were used. Protein extract (PE) of human cancer cells taken from the TRAPeze RT Kit served as a positive control. All PEs inactivated at 95°C for 30 min and the reaction mixture without PE were used as negative controls. The reaction was performed using 10-15 μl, with 1× buffer, 300 ng of native or inactivated PE, 0.2 mM each dNTP, 1 pmol of TS primer, 0.5 pmol of ACX, and 1× Encyclo-polymerase (Evrogen, Moscow, Russia).

The reaction mixtures with PE from cancer cells and benedictiid samples were kept at room temperature or 0±4°C for 30 min, respectively. Then, they were heated at 94°C for 3 min, 94°C for 30 s, 58°C for 30 s, and 72°C for 1 min, repeated 30 times.

The PCR products (1 to 25 μl) were separated on a 12.5% non-denaturing polyacrylamide gel (thickness, 1.5 mm; length, 17 cm; and width, 12.5 cm) for 2.5 h at 130-150 V and 36 mA in 0.5×TBE. The gel was stained according to the method of DALLA TORRE *et al.* (2002).

Data analysis

Regression analysis with generalized linear models was used for estimation of dependence between age (Yr) and RTL (DOBSON 1990). Linear, quadratic, cubic and quartic functions were chosen as regression models. The most optimal regression model was chosen on the basis of the Akaike Information Criterion (AIC) with the lowest value. The rate of RTL changes was determined as a derivative of the regression model function. R programming was used for regression analysis.

Differences among age groups depending on sex were identified with the non-parametric Mann-Whitney test (Statistica-10).

To compare RTL age changes with biological and ecological characteristics of the species, Mantel tests (SMOUSE *et al.* 1986) were performed between Euclidean distances of absolute RTL distinction among species and the following qualitative characteristics: species, sex, developmental stage of the sex system (immature/mature), sex ratio, absolute length of telomeres. The metric of binary distances was used for qualitative features (factors). The intensity of the interrelation of factors with RTL age changes was estimated from the value of the square of the correlation coefficient r^2. The vegan package (DIXON 2003) in R was used for statistic calculations. The correlation matrix was clustered and visualized as a heat map with the help of the "gplot" package (WARNES *et al.* 2015) for R .

Results

Telomere dynamics

Variability of telomere length was recorded in each of the studied species irrespective of sex, age and development stage (Fig. 2). Fitted regression models with the lowest AIC value (Table 2) show interspecific and sex differences in age dynamics of telomeres. Cubic and quadratic functions are the most optimal for *B. fragilis* females and males, respectively, whereas a quartic function for both *B. baicalensis* sexes and a linear function for both *K. martensiana* sexes (Fig. 2A). According to the optimal model for telomere dynamics, the rate of telomere change differs depending on species and sex (Fig. 2B).

The comparison of RTL in specimens of the same sex from different age groups shows (Fig. 2C) that no significant changes of telomere length with age occur in *B. fragilis* females, whereas telomeres in males shorten at the age of 4-6 years. Telomere length in *B. baicalensis* females shortens significantly after birth at the age of half a year and a year, and then it lengthens at the age of 2-3 years reaching the RTL of embryos at the age of 4-8 year-old. In males of this species, telomere length does not change with age. However, in comparison with the embryonal stage, their RTL is significantly shorter. In both *K. martensiana* sexes, we observed significant enlargement of telomeres with age.

Immature males and females of all species do not differ in RTL at different age telomere dynamics (Fig. 2C). Among mature 4-6 year-old specimens, telomeres in females are much longer than in males. Mature 7-8 year-old *B. fragilis* and *K. martensiana* do not have sex differences in RTL, whereas telomeres in *B. baicalensis* females are longer.

Telomerase activity

Based on a TRAP analysis, we determined that telomerase was active in the foot tissues of the three benedictiid species (Fig. 3), in both young and mature specimens of both sexes.

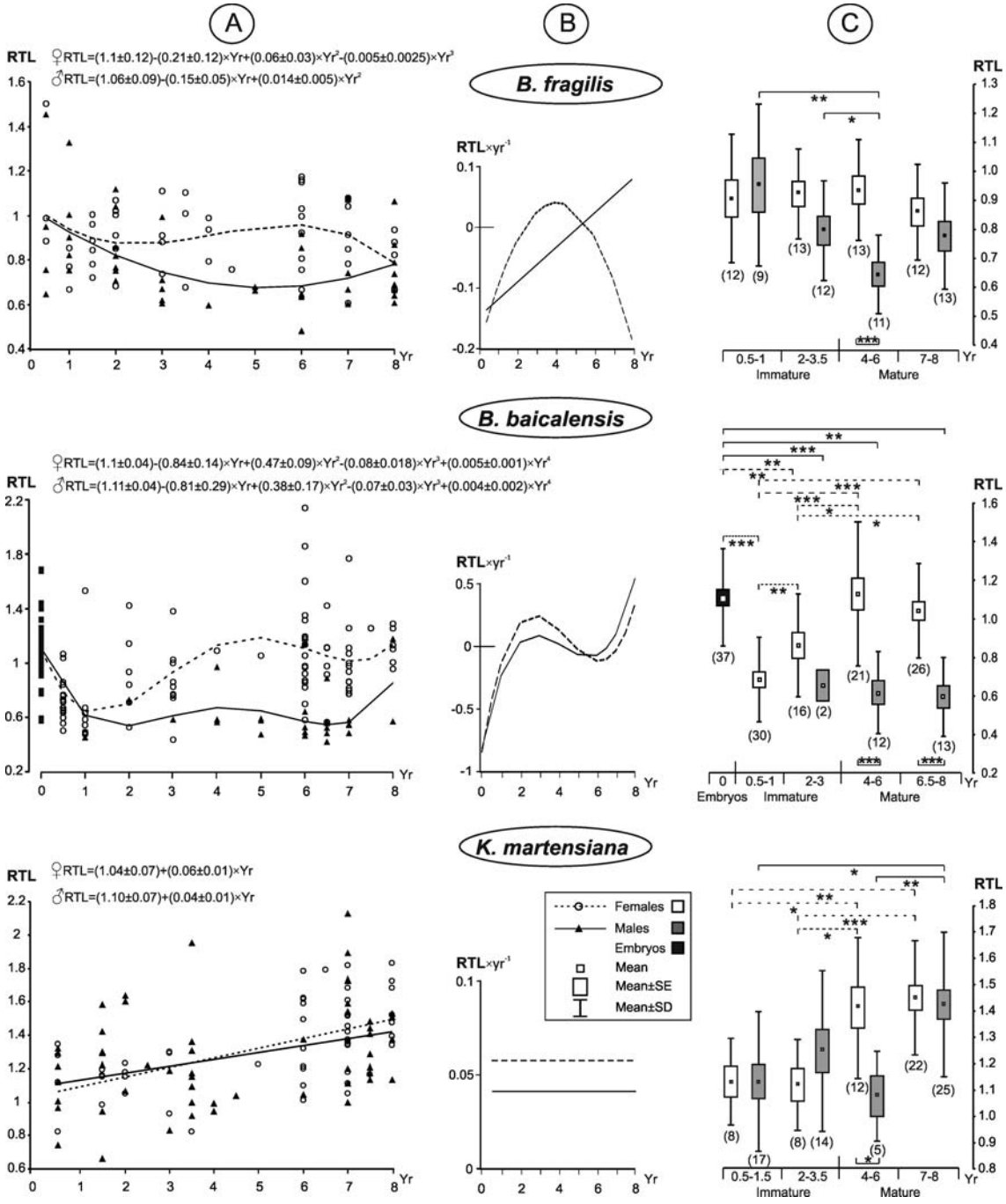

Fig. 2. RTL in gastropods with respect to age and sex. A – Scatterplots of the age dynamics and fitted regression models. B – The rate of telomere change. C – Comparison of the age groups. Significant differences among groups: * – P<0.05; ** – P<0.005; *** – P<0.0005. The number of analyzed specimens is shown in brackets.

Table 2

Model selection for telomere dynamics in three benedictiid species

Model	AIC					
	B. fragilis		*B. baicalensis*		*K. martensiana*	
	♀	♂	♀	♂	♀	♂
Linear function	-26.85	-11.55	70.78	8.29	-8.54	21.40
Quadratic function	-25.08	-15.52	66.12	1.51	-7.78	23.34
Cubic function	-27.36	-13.53	53.27	3.45	-6.11	25.22
Quartic function	-25.81	-14.17	38.51	0.53	-4.11	23.73

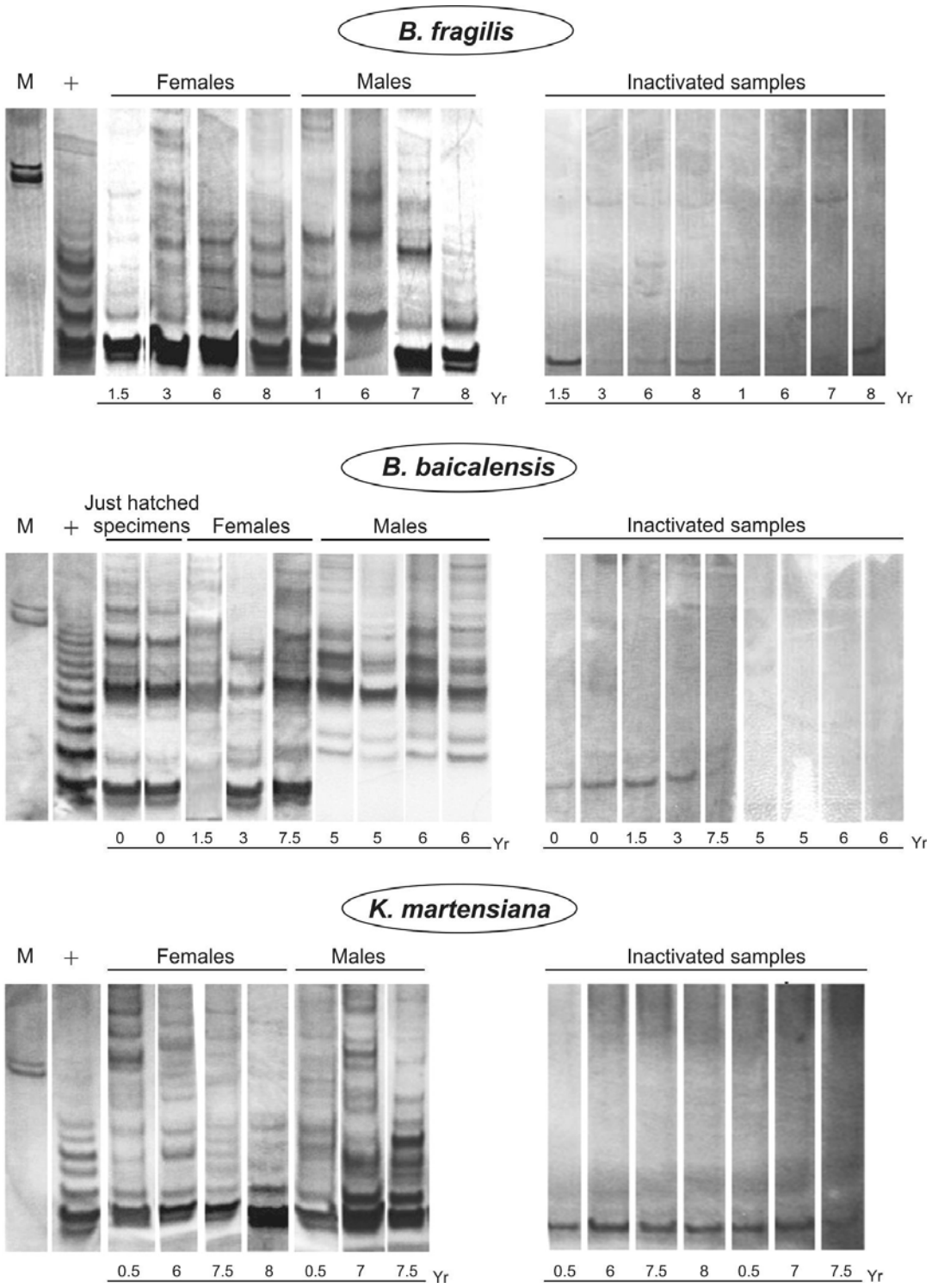

Fig. 3. Telomerase activity in foot tissues of gastropods. M is a marker of molecular weight (103 and 107 bp); + is a positive control, i.e., a telomerase of cancer cells; inactivated samples are the same samples, but inactivated at 95°C for 30 min.

Relationship between telomere length and biological and ecological features of the species

In all species, intraspecific differences in age telomere dynamics correlate with sex (Fig. 4), whereas in *K. martensiana* and *B. fragilis* they also correlate with the developmental stage (immature/mature). The comparison of the total set of RTL values with different characteristics of the studied species shows (Fig. 4) that the age changes of telomeres depend, to a large extent, on species affiliation and absolute telomere length.

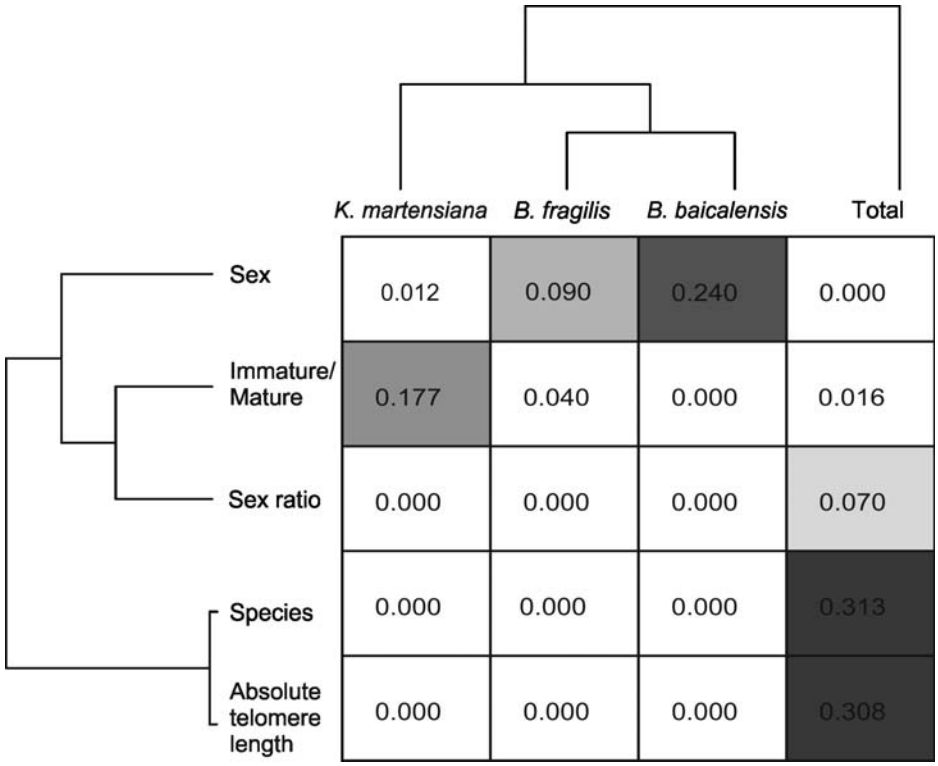

Fig. 4. Heat map of relationship between RTL and biological and ecological features of the species.

Discussion

Age dynamics of telomeres in mollusks may differ among species and populations that have both various and similar biological characteristics and habitats (OVENDEN & GODWIN 2011; GODWIN *et al.* 2012; GRUBER *et al.* 2014). For example, the shallow-water and short-lived *Argopecten irradians* has rather short telomeres which shorten with age faster compared to the deep-water *A. purpuratus* with longer telomeres and longer lifespan (ESTABROOKS 2007). We revealed differences in age dynamics of telomeres between sister species (*B. fragilis* and *B. baicalensis)* with the same telomere length inhabiting different biotopes of Lake Baikal and phylogenetically distant species (*K. martensiana* and *B. baicalensis*) with different telomere length living in the shallow-water terrace and subjected to the same environmental factors. *B. fragilis* is a eurybathic species inhabiting soft substrates within a wide depth range of over 1 km (Fig. 1) at constantly low water temperatures (+4°C). *B. baicalensis* inhabits a limited depth range, but a wide spectrum of substrates, whereas *K. martensiana* lives under specific conditions, i.e., on solid substrates with fouling sponges at a depth of up to 40 m. Both *B. baicalensis* and *K. martensiana* can withstand short-term temperature increases from +4°C to +15°C at a depth of 2-5 m. In different habitats during the same period of growth (i.e., 4 years) before maturation, the shell of each species grows at a different rate, requiring different energetic resources. *B. fragilis* has the smallest protoconch (Fig. 1), which is more than 20 times smaller than the shell of an adult snail. The shell of *B. baicalensis* becomes seven times larger during growth, and the shell of *K. martensiana* increases four-fold. The fastest rates of *B. fragilis* growth can affect the age telomere dynamics which is directly associated with cell division. Among the examined benedectiids, in *B. fragilis* the telomere length does not change in females and in males it shortens during growth from 0.5-1 year to 4-6 years (Fig. 2C).

Sex specificity in changes of telomere with age was observed in each benedectiid species (Fig. 2). Immature specimens do not differ in telomere size irrespective of sex (Fig. 2C). We recorded differences and similarities in telomere length between females and males at maturity. Specific characteristics of telomere length revealed in the species may be incomplete because of an extremely low number of samples of some age groups (e.g., in *B. baicalensis* because of uneven distribution of sex structure (Fig. 1)). At the same time, in samples of different size, all benedectiid species demonstrate sex specificity of telomere length among mature species (Fig. 2C). Telomeres are longer in 4-6 year-old adult females than in males. At the age of 7-8 years, sex differences in telomere sizes

are leveled in *B. fragilis* and *K. martensiana*, while in *B. baicalensis* the difference remains. The sex-specificity in telomere length in the 4-6 year-old individuals are likely associated with the period of active reproduction and might be triggered by differential reproductive investment between males and females (BARRETT & RICHARDSON 2011; GAO & MUNCH 2015). The maintenance of different telomere length in *B. baicalensis* males and females up to 7-8 years may be attributed to low reproductive significance of males in this species. For mature *B. fragilis* and *K. martensiana*, sex ratios are equal, whereas in *B. baicalensis* there is a considerable lack of males, and polyploids have been observed in both sexes (Fig. 1). Moreover, polyploidy among *B. baicalensis* females is 50% or more and among males the frequency of polyploidy varies from 5 to 54% (SITNIKOVA 2004). Polyploidy in gastropod females is accompanied by asexual reproduction, reducing the cost of sex owing to males (GIBSON *et al.* 2017). In snails, the investment in male and/or female reproduction can be influenced by mating history (e.g., traits involved in pre- and post-copulatory processes, reproductive behavior) (KOENE 2017). For males the costs are allocated towards the performance of courtship and copulation, and the energetic value of sperm; in females costs involve investment in egg laying behavior and egg mass. Data on benedectiid reproduction (Fig. 1) are limited mainly to reproductive characteristics of females, which have significant species differences and likely affect their telomere dynamics. Constant telomere length during the life cycle has been recorded in *B. fragilis* females that oviposit the largest number of eggs of smallest diameter. In *B. baicalensis* and *K. martensiana* females, telomeres increase with age, whereas the first species oviposits an average number of large eggs in comparison with other benedectiids, and the second species lays the lowest number of singular eggs of average diameter. The ratio of telomere size and fecundity of females may be species-specific. For example, among birds and mammals there are species with shorter telomeres characteristic of females with a high level of fecundity (KOTRSCHAL *et al.* 2007; BAUCH *et al.* 2012; GRAY *et al.* 2014) and species without any dependence between these parameters (BEAULIEU *et al.* 2011; BOUDREAU *et al.* 2014; CERCHIARA *et al.* 2017).

Sex differences in the telomere age dynamics of *B. baicalensis* have also been revealed between the embryonal and mature stages of development (Fig. 2C). Telomeres in mature males are shorter than in embryos and mature females. In females after the embryonic stage, telomeres shorten rapidly, and then at the age of 4-8 years telomere length is restored up to the embryonic level. Restoration of telomere length up to the level of hatched specimens has been revealed in the medaka fish during adolescence (age 7 months – 1 year), in which telomeres shorten with age (HATAKEYAMA *et al.* 2016). For a shorter period after birth (age 30 days), similar restoration of telomeres was observed in a long-lived magellanic penguin whose telomere length is constant during its life cycle (CERCHIARA *et al.* 2017).

The reasons for sex specificity in telomere biology are unknown; yet, relationships between telomere length and differential telomerase expression and effects of hormones on telomeres have been detected (BARRETT & RICHARDSON 2011; PETERSON *et al.* 2015; INGLES & DEAKIN 2016; HATAKEYAMA *et al.* 2016). Different age dynamics of telomeres in females and males of the benedictiids are likely associated with changes in telomerase activity. Telomerase activity was not assessed quantitatively within the framework of this study. Nevertheless, active telomerase was revealed in all species irrespective of sex and developmental stage (Fig. 3). Factors of hormonal origin can regulate telomerase activity leading, in our case, to sex differentiation of telomere length. Estrogens in mammals possess not only antioxidant characteristics making females more resistant to stress, but also regulate gene expression of telomerase (KYO *et al*. 1999; MISITI *et al*. 2000). In medaka fish females, the level of estrogens correlates positively with telomerase activity and telomere dynamics (GOPALAKRISHNAN *et al.* 2013): telomeres during maturation become longer in females with maximal values of estrogens and telomerase activity. Further, these values are reduced with age and telomere length with both sexes becoming equal.

Our data suggest that the age dynamics of telomere length in Baikal gastropods is sex-specific and multidirectional. The relationship between sex and telomere is not ubiquitous for animals with different levels of phylogenetic relatedness from insects to mammals (BARRETT & RICHARDSON 2011; MIZUTANI *et al.* 2013; INGLES & DEAKIN 2016). As in our case, sex differentiation of telomere length has been recorded mainly in adult animals. However, among well-studied groups of animals (fish, birds, reptiles and mammals) there are species without sex differentiation in telomere length and species in which telomeres are longer in females or in males (BARRETT & RICHARDSON 2011; ALMROTH *et al.* 2012; GOPALAKRISHNAN *et al*. 2013; YOUNG *et al.* 2013; SUDYKA *et al.* 2014; IZZO *et al.* 2014; GAO & MUNCH 2015; BELMAKER 2016; DANTZER & GARRATT 2017; WATSON *et al.* 2017). The multidirectionality of sex-dependent age dynamics of telomere size may be attributed to the combined effect of internal and external factors on telomeres during ontogenesis.

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