# BULGARIAN ANTARCTIC RESEARCH

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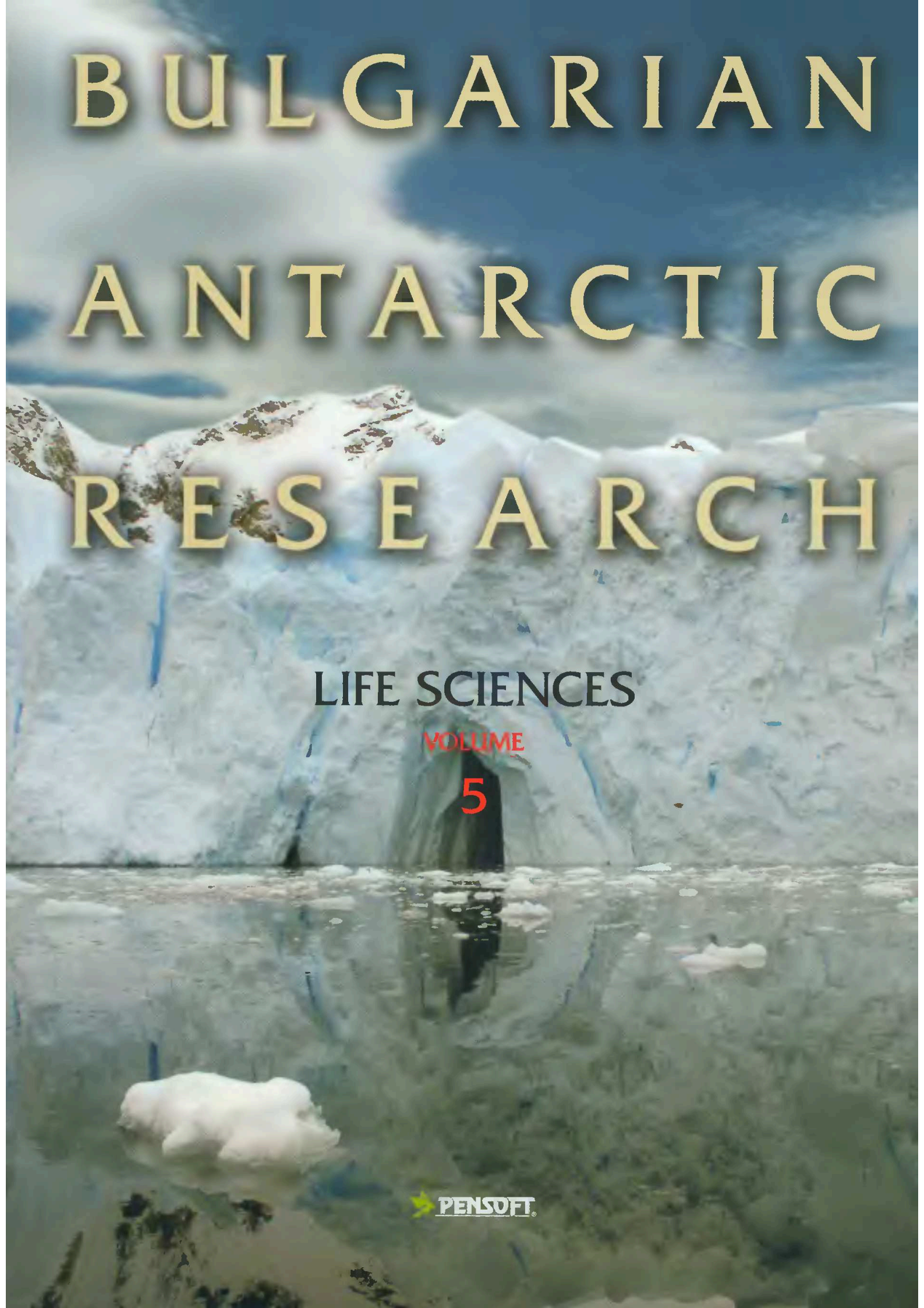

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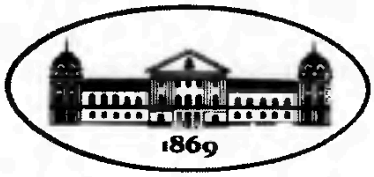


**Bulgarian Antarctic Institute**

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# Studying Telomeres Length of Gentoo Penguins on the Antarctic Peninsula

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## ABSTRACT

In this report we present the first results of telomere length measuring in known-aged family individuals of Penguins Gentoo (Pygoscelis papua) to determine whether telomeres from the nucleated blood cells (mainly erythrocytes) of these birds shorten during aging and how the rate of shortening varies with maximum lifespan.

We determined the telomere restriction fragment length in erithrocyte DNA. A special telomeric probe was constructed by ligating telomeric repeats with the following cloning in pUC19. It was labeled by $P^{32}$dCTP and used for hybridization. The obtained results are discussed in connection with the data on other species.

## KEY WORDS

Penguins, Gentoo, telomeres, pUC19.

## INTRODUCTION

Ukrainian Antarctic station "Academic Vernandsky" is located on the Antarctic Peninsula [1,2]. Five species of penguins live there and three of them nest (Adelie, Gentoo and Chinstrap). Gentoo penguins are a very important part of the Antarctic ecosystem. They are different from other taxonomic groups and provide possible surviving and adaptation in Antarctica. The research of Gentoo penguins (Pygoscelis papua) is carried out with the support of the international INTAS project and the Ukrainian Antarctic

Centre. Telomeres are short tandem repeated sequences of DNA found at the ends of eukaryotic chromosomes [3]. The repeats consist of a short G-rich sequence; in vertebrates, the telomeric repeat (TTAGGG)n is conserved. The function of the repeat is in stabilizing chromosomal end integrity [4]. Telomeric repeats are lost during each cell cycle because DNA polymerase is unable to replicate the 3' end completely [5]. In vivo studies of somatic tissue of mammals and birds have shown a correlation between telomere length and organismal age within species, and correlations between telomere shortening rate and lifespan among species. Maximum telomere length and the telomere length rate of change differ among species, as does maximum lifespan among species. Haussmann and colleagues found [6, 7] that telomere length at a given life stage did not correlate with lifespan but telomere length rate of change correlated with lifespan in birds and mammals. Telomere length and telomere length rate of change vary among individuals of the same species and among tissues from an individual. Inter-species, interindividual and intertissue differences are caused mainly by factors such as different rates of cell replication, levels of telomerase activity and levels of oxidative stress [8]. Thus telomere length could be used to provide the much needed information on age, ageing and survival in natural populations where such studies are lacking.

The aim of the present work was to evaluate the possibility of telomere length measuring by means of a special telomeric probe labeled by $P^{32}$dCTP in known-aged family individuals for Gentoo penguins' population research.

## MATERIAL AND METHODS

### 2.1. Source of genomic DNA

Blood samples of penguins were collected at Pitermann Island by the Ukrainian team during the 8 Antarctic expeditions in the summers of 2002-2004. The blood samples with heparin were kept at -20°C. Genomic DNA was isolated by the modified salt extraction method [9]. Concentration of DNA probes was determined using a spectrophotometer based on absorbance at 260 and 280 nm, respectively, and was defined more accurately by electrophoresis in 0,8% agarose gel.

### 2.2. Labeling of the telomere probe

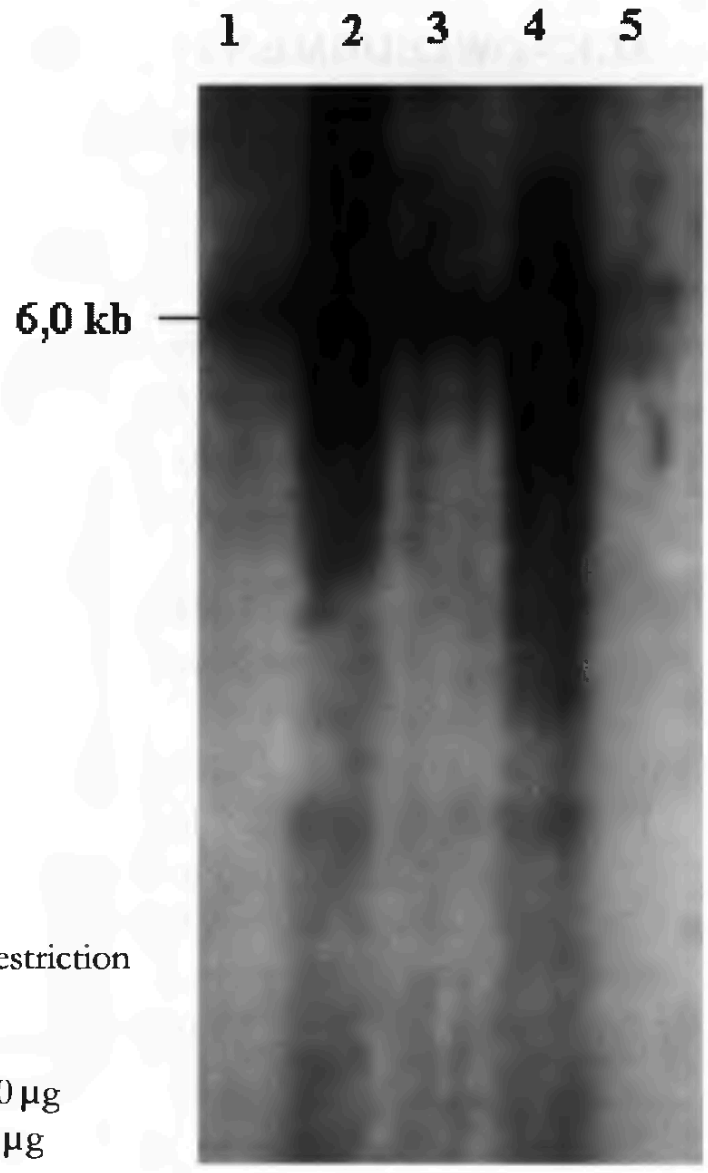
For obtaining probes with $(TTAGGG)_n$ repeat the corresponding oligonucleotide $(TTAGGG)_3$ and complementary them oligonucleotide $(TAACCC)_3$ were synthesized. The primers were prepared for anneling and then ligated into a pUC19 plasmid vector. The cloned fragment (450 bp.) was labeled with $P^{32}$dCTP using forward and reverse standard primers in a polymerase chain reaction.

### 2.3. AGAROSE GEL ELECTROPHORESIS AND VISUALIZATION OF PATTERNS

After obtaining by standard salt-extraction alcohol-precipitation, DNA probes were digested by Hinf I for 16 h, separated on agarose gel and blotted on nylon. Approximately 3 and 10 μg of digested DNA were analyzed by electrophoresis in 2-2,5% agarose gel and detected by staining with ethidium bromide and transferred to nylon filters [10]. For hybridization we used PCR product labeled with $P^{32}$dCTP (see above). Detection was performed by X-ray film and exposition was carried out during 5-7 days.

## RESULTS AND DISCUSSION

There are a number of techniques available for measuring telomere length. The telomere (terminal) restriction fragment (TRF) analysis and related methods such as the telomere amount and length assay (TALA) are relatively easy methods and are probably the most widely used in telomere research. In these methods, average lengths of TRFs (created by particular restriction enzymes and hybridized with a radioactive oligonucleotide) are measured [11].




**Picture 1.** Southern blotting of telomere restriction fragments of blood from Gentoo penguins.
1 – Plasmid marker with telomere repeats;
2, 4 – Samples with concentration of DNA 10 μg
3, 5 – Samples with concentration of DNA 3 μg

To study telomere length in Gentoo penguin's populations, a special telomeric probe was constructed by ligating telomeric repeats with the following cloning in pUC19. The probe was labeled by $P^{32}$dCTP and used for hybridization. The average observed telomere length of Gentoo penguins was approximately 8000 bp in our research (Picture 1). Our preliminary results can be used only with data obtained on penguins Adelie (Haussmann et al. 2003; Nakagawa et al. 2004) where the maximum observed telomere length was 9500 bp. Thus we intend to use our data for further research on Gentoo penguins populations with regard to penguins' age and distribution.

## CONCLUSIONS

Thus, the self-descriptiveness of determination of telomere restriction fragment length in Gentoo penguins erithrocyte DNA by a special telomeric probe constructed by means of ligating telomeric repeats with the following cloning in pUC19 was approved. The results obtained are discussed in connection with the data on other species.

## ACKNOWLEDGMENTS

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