Natural diversity of telomeric DNA sequences in bryophytes

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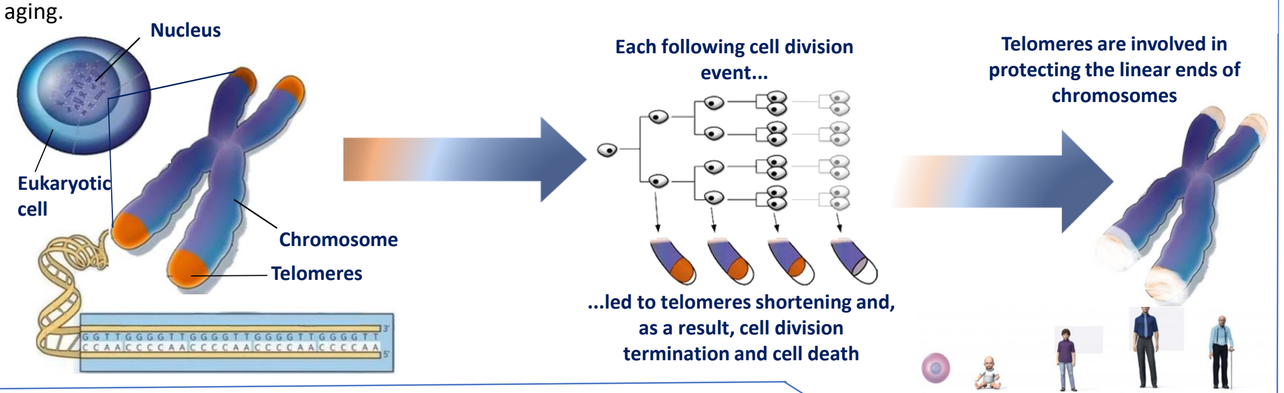
Introduction

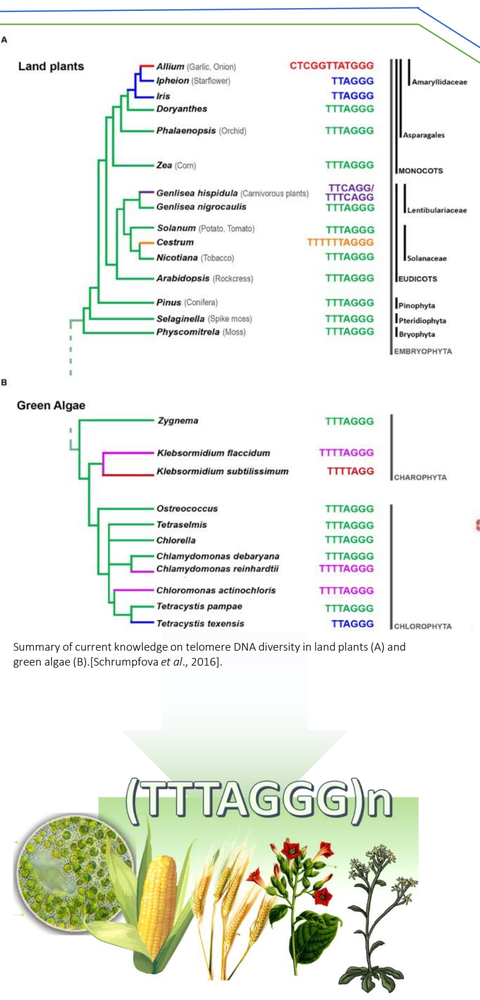
Telomeres are specialized nucleo-protein structures at the physical ends of linear chromosomes of eukaryotic organisms. Telomeres play a crucial role in chromosome's integrity protection from the exonucleases activity and the action of cell repair systems, as well as chromosome separation during cell division process. Thus, telomeres shortening results in the loss of their protective functions. Moreover, telomeres gradually shorten with every subsequent cell division, which correlates with the induction of cellular aging (Hayflick limit). Therefore, telomeres have come to be consider as the "molecular clock" of cell aging.



The structure of telomeres is highly conserved in all eukaryotes. But at the same time the composition of the telomeric nucleotide sequence varies in different organisms, that indicates possible natural variations in the regulatory mechanisms of telomere maintenance.

The telomeric DNA of most plants, including algae, angiosperms and gymnosperms, as well as bryophytes, consists of a palindromic motif (TTTAGGG)n.

The length of telomeres is the main functional characteristic of these structures. In plants telomere length is a species-specific feature that varies in a certain range in populations, in individual organisms, as well as in various tissues and even on different arms of chromosomes. In addition, the length of telomeres can vary not only from species to species, but even within different populations of the same species. Therefore, study of plant telomeres could provide us a new knowledge of telomere length regulation, plant viability and longevity.



Summary of current knowledge on telomere DNA diversity in land plants (A) and green algae (B).[Schrumpfova *et al*., 2016].

Object

Moss species and ecotypes used in the study

Physcomitrium patens

Ecotypes

- Gransden (Great Britain)
- Reute (Germany)
- Villersexel (France)
- Kaskaskia (USA)

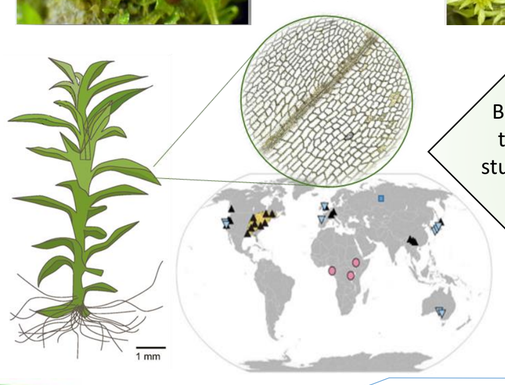
Sphagnum

Ecotypes

- *S. girgensohnii* (Russia)
- *S. fallax* (USA)
- *Sphagnum sp.* (Russia)

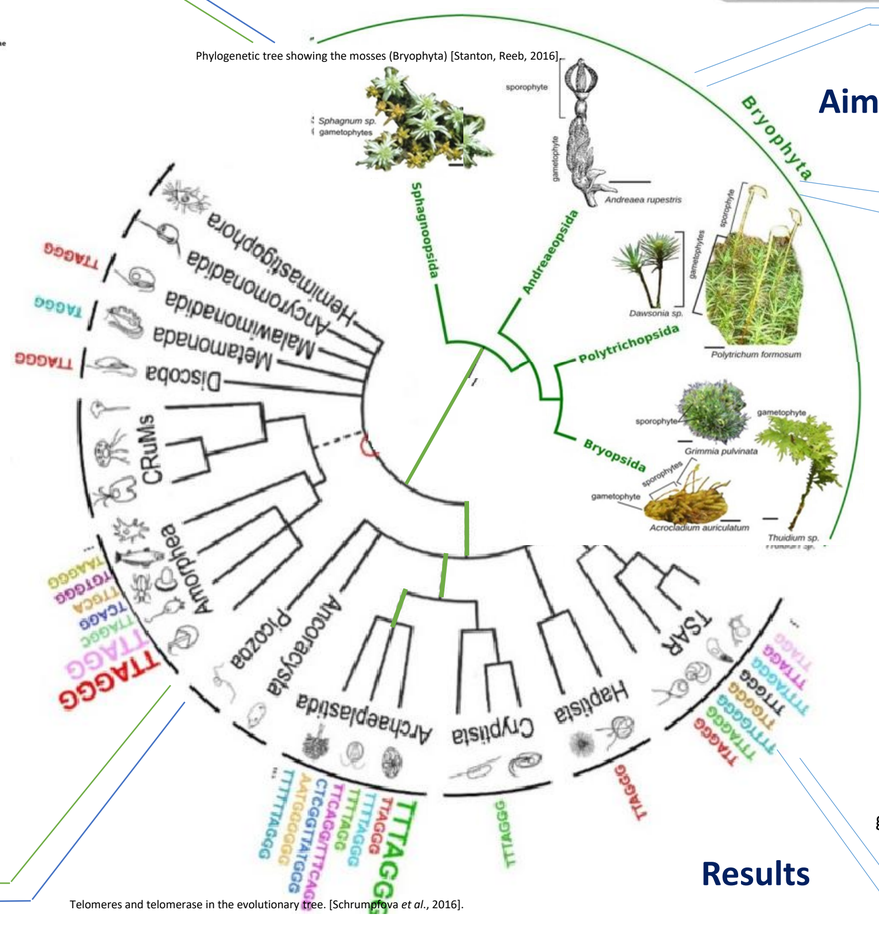
Ceratodon purpureus

- Male (R40)
- Female (GG1)



Bryophytes have **advantages** that make them convenient model organisms for studies of complex biological processes in plants.

- **Cosmopolitan plants**
- **Simple building plan**
- **Fast tissue regeneration**
- **Evolutionary significance**
- **Simplicity of cultivation in the laboratory**



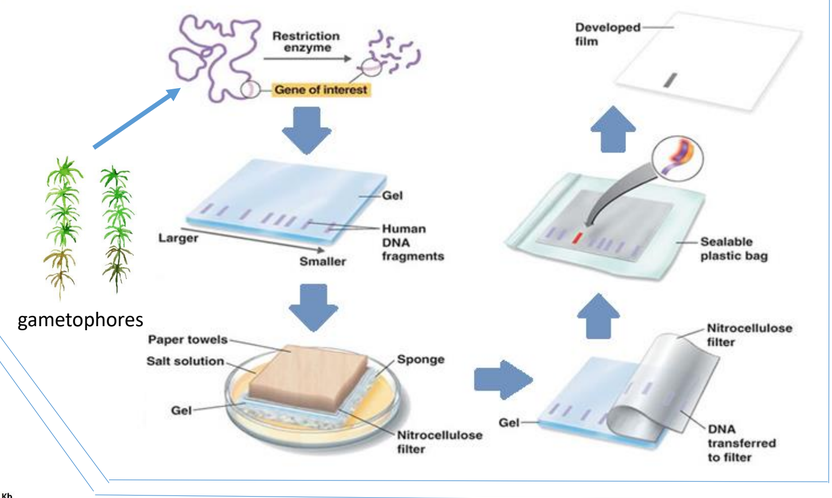
Aim

The aim of the work was to conduct a comparative analysis of telomere length in various Bryophytes: *Physcomitrium patens*, *Ceratodon purpureus* and mosses of the genus *Sphagnum*

Materials and methods

To analyze the telomere length, DNA isolated from the tissues of 14-day-old protonema of *P. patens*, *C. purpureus*, and sphagnum gametophores was used. Proton was obtained from homogenized moss tissues. Telomere length analysis was performed by **TRF** (Terminal Restriction Fragment analysis) together with Southern Blot analysis.

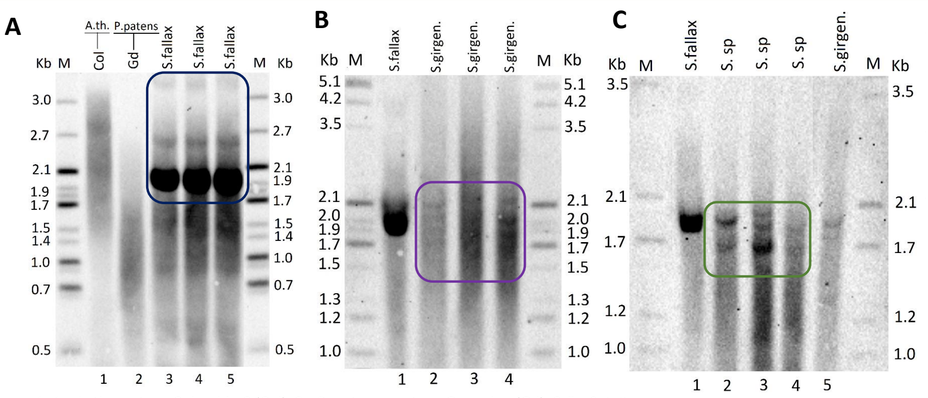
Southern Blot Analysis



Results

Analysis of the telomere length of *Sphagnum* plants

The mean length of *Sphagnum* species telomeres were 1.1-1.5 kb, 2 kb, and 1.86-2.1 kb. Whereas the mean telomere length of model angiosperm plant *A. thaliana* Columbia ecotype (Col-0) is 2.5 Kb

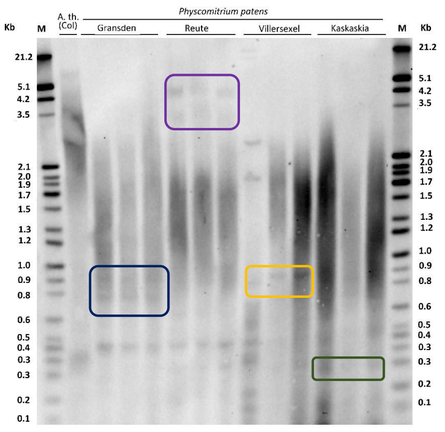


(A)The mean telomere length in *S. fallax* ranged from 1.86 to 2.1 Kb

(B) *S. girgensohnii* the mean telomere length was ~ 2 Kb.

(C) The telomeres of *Sphagnum sp*. turned out to be somewhat shorter, the mean length of telomeres of which ranged from 1.1 to 1.50 Kb.

A. 1 - *A. th.* ecotype Columbia-0 (Col), 2 - *P. patens*, ecotype Gransden (Gd); 3-5 - *S. fallax*.
B. 1 - *S. fallax*; 2 - 4 - *S. girgensohnii* (S. girgen.).
C. 1 - *S. fallax;* 2 - 4 - *Sphagnum sp*.; 5 - *S. girgensohnii* (S. girgen.).
On the left and right are the values of DNA markers of molecular weight Kb.



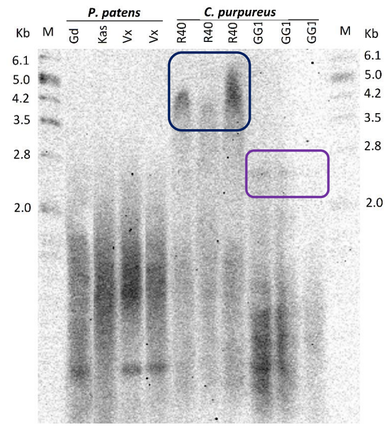
The values of DNA markers of molecular weight are indicated on the left and right (Kb).
(Col) –A. th. - *Arabidopsis thaliana* ecotype Columbia-0.

Analysis of the telomere length of P. *patens* plants

We have shown that different ecotypes of *P. patens* plants have telomere lengths ranging from 1.1 to 1.5 Kb, which is 1.5-3 times shorter than telomeres of the model angiosperm *Arabidopsis thaliana*.

- The mean telomere length of the Gransden, Reute and Villersexel ecotypes was ~ 1.3 Kb
- The mean telomere length in the Kaskaskia ecotype was slightly less (~ 1.2 Kb).

In addition, we have shown that all the studied ecotypes have specific telomeric sequences, presumably of intra-chromosomal localization, and they do not coincide in all ecotypes.



Analysis of the telomere length of *C. purpureus* plants

The length of telomeres in the male and female line differs by 2 times, which, presumably, may be due to the presence of genetic factors regulating the length of telomeres specific to sexual U or V chromosomes.

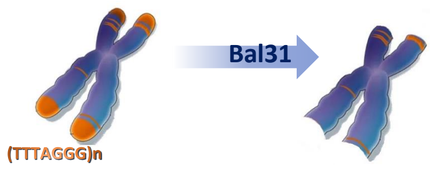
- The telomere length in a female plant (GG1) mean from 0.48 Kb. to 0.5 Kb.
- In male plants (R40) from 1.3 Kb. to 2.6 Kb.

Also, we have shown that the male line of *C. purpureus*, unlike the female line, has telomeric DNA ~ 4.5 Kb. long, which can be intra-chromosomal DNA. Also, brands at the level of ~ 2.5 Kb were found in the female line, which may also be interstitial telomeric DNA.

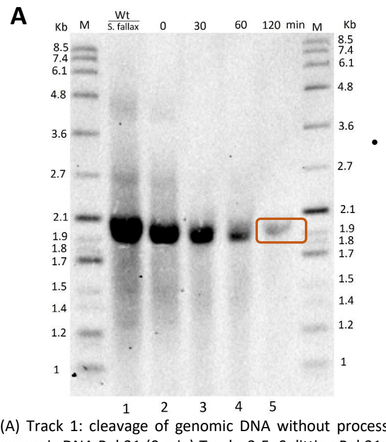
R40 (male plants), GG1 (female plant).The values of DNA markers of molecular weight are indicated on the left and right (Kb).
Gd – ecotype Gransden, Vx – ecotype Villersexel, Kas – ecotype Kaskaskia

Identification of intracromosomal telomeric repeats in *Sphagnum fallax* and *Sphagnum sp.*

To make sure that the sequences detected by TRF analysis correspond to the end sections of chromosomes, genomic DNA was pre-incubated with Bal 31 nuclease. Exonuclease Bal31 gradually shortens DNA fragments at both ends of the chromosome.

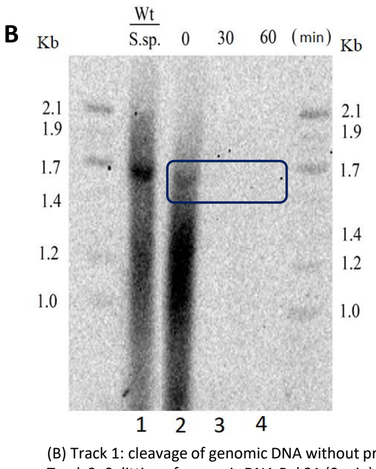


The true telomeric signal disappears over time, while the interstitial region is resistant to enzyme treatment. With the exception of a single interstitial bend (indicated in red), the signal of mass hybridization began to be lost from 30 minutes and was completely lost by 120 minutes by processing Bal 31 in *S. fallax*. (A). However, similar interstitial telomeric DNA has not been found in *Sphagnum sp.*(B).



- In *S. fallax,* intra-chromosomal telomeric sequences with a length of ~ 2.0 Kb. have been identified.

(A) Track 1: cleavage of genomic DNA without processing Bal 31. Track 2: Splitting of genomic DNA Bal 31 (0 min) Tracks 3-5: Splitting Bal 31 for 30, 60 and 120 minutes. Red indicates of interstitial telomeric DNA that is not sensitive to Bal 31 cleavage up to 120 min.



Similar interstitial telomeric DNA has not been found in *Sphagnum sp*.

(B) Track 1: cleavage of genomic DNA without processing Bal 31.
Track 2: Splitting of genomic DNA Bal 31 (0 min) Tracks 3-4: Splitting Bal 31 for 30 and 60 minutes.

Conclusion

- Therefore, we have shown that the telomere length of bryophytes can vary both between species and within one species.
- The telomere length in *P. patens* did not vary within the species.
- The telomere length of *C. purpureus* differs between male (R40) and female(GG1) plants. The male plant has longer telomeres than the female plant.
- Telomere length in various *Sphagnum* species ranged from 1.1 to 2.0 Kb. However, we found differences in the localization of telomeric DNA repeats in *Sphagnum* species.
- Bryophytes have shorter telomeres than angiosperms.
- Mosses have a significant diversity in the distribution of telomeric DNA, which presumably may indicate the presence of intra-chromosomal telomeric sequences, or variability in the regulation of telomere length during the life cycle.

Acknowledgements

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