# A Feulgen glimpse into genome evolution during range expansion: a case study of the subterranean amphipod *Niphargus schellenbergi*

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#### **Abstract**

In 1923, Robert Feulgen designed a quantitative approach to measure the relative DNA content of a species (its so-called "C-value", which is defined as half of the DNA content of a somatic cell and is expressed in pg). The Feulgen approach has several advantages: it can be used on ethanol-preserved samples, it is relatively inexpensive, and it does not require sophisticated equipment (Hardie et al. 2002, Jeffery and Gregory 2014, Kasten 2003). Gathering DNA content information is a key preliminary step for whole-genome sequencing of non-model species and also yield important information for ecological and evolutionary studies as well as for conservation (Jeffery et al. 2013), since genome size has been shown to be strongly correlated to extinction risk in animals and plants (Vinogradov 2004, Vinogradov 2003).

Among invertebrates, Niphargidae is among the largest families of freshwater subterranean amphipods in the world, and more than 450 niphargid species have been described (Horton et al. 2021). Thus, it is considered an ideal model for evolutionary and ecological studies. Using a new, improved Feulgen protocol, we generated novel genome size estimates for 26 samples of *Niphargus schellenbergi* collected from central and western European niphargids. The obtained C-values ranged from 2.75 to 5.25 pg. Such a two-fold intraspecific variation is unusual but not unheard of: it occurs also in the monogonont rotifer *Brachionus asplanchnoidis* (Stelzer et al. 2021). Further research will be required to find out the mechanisms responsible for this variation, which may result from copy-number variation, polyploidy and/or various amounts of transposable elements.

# **Keywords**

Feulgen image analysis densitometry, genome size, C-value, niphargids.

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#### Conflicts of interest

None.

## References

- Hardie D, Gregory TR, Hebert PN (2002) From pixels to picograms: A beginners' guide to genome quantification by Feulgen image analysis densitometry. Journal of Histochemistry & Cytochemistry 50 (6): 735-749. <a href="https://doi.org/">https://doi.org/</a>
   10.1177/002215540205000601
- Horton T, Lowry J, De Broyer C (2021) World Amphipoda Database. <a href="https://www.marinespecies.org/amphipoda./index.php">https://www.marinespecies.org/amphipoda./index.php</a>. Accessed on: 2022-6-01.
- Jeffery N, Jardine C, Gregory TR (2013) A first exploration of genome size diversity in sponges. Genome 56 (8): 451-456. https://doi.org/10.1139/gen-2012-0122
- Jeffery N, Gregory TR (2014) Genome size estimates for crustaceans using Feulgen image analysis densitometry of ethanol-preserved tissues. Cytometry Part A 85 (10): 862-868. https://doi.org/10.1002/cyto.a.22516
- Kasten F (2003) Robert Feulgen and his histochemical reaction for DNA. Biotechnic & Histochemistry 78 (1): 45-49. https://doi.org/10.1080/10520290312120009
- Stelzer CP, Blommaert J, Waldvogel AM, Pichler M, Hecox-Lea B, Mark Welch DB
   (2021) Comparative analysis reveals within-population genome size variation in a rotifer
   is driven by large genomic elements with highly abundant satellite DNA repeat
   elements. BMC Biology 19 (1). https://doi.org/10.1186/s12915-021-01134-w
- Vinogradov A (2004) Genome size and extinction risk in vertebrates. Proceedings of the Royal Society of London. Series B: Biological Sciences 271 (1549): 1701-1705. <a href="https://doi.org/10.1098/rspb.2004.2776">https://doi.org/10.1098/rspb.2004.2776</a>
- Vinogradov AE (2003) Selfish DNA is maladaptive: evidence from the plant Red List.
  Trends in Genetics 19 (11): 609-614. https://doi.org/10.1016/j.tig.2003.09.010