Genome and karyotype evolution after whole-genome duplication in free-living flatworms of the genus *Macrostomum*

Kira Zadesenets[1] , Nikita Ershov[1] , Dmitry Oshchepkov[1] , Eugene Berezikov[1,2] , Lukas Schärer[3] , Nikolay B. Rubtsov[1]

[1] ICG SB RAS, Novosibirsk, Russia; [2]ERIBA, Groningen, The Netherlands; [3]University of Basel, Basel, Switzerland

Whole genome duplication (WGD) is a large-scale evolutionary transformation that occurred in genome evolution in many taxa of existing animal species. However, the mechanisms underlying the early stages of genome evolution after a WGD event in animals has remained unclear. The study of genome organization of neopolyploid species may shed light on the processes of genome reorganization leading to its re-diploidization after a recent WGD event.

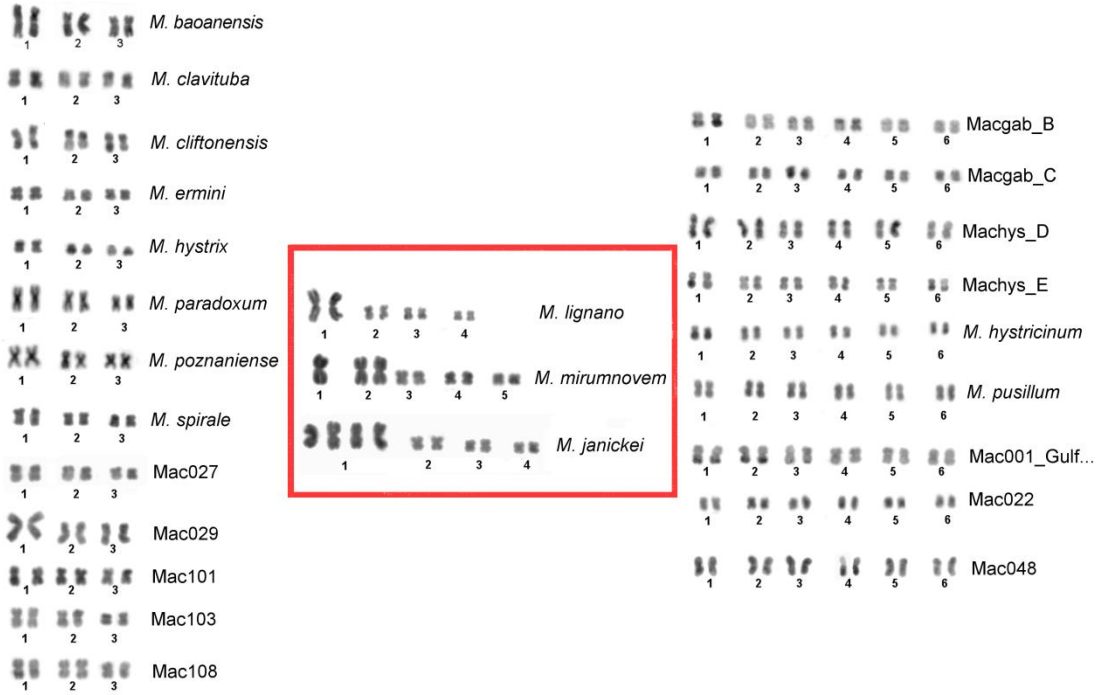

Fig. 1. The karyotypes of *Macrostomum* species.

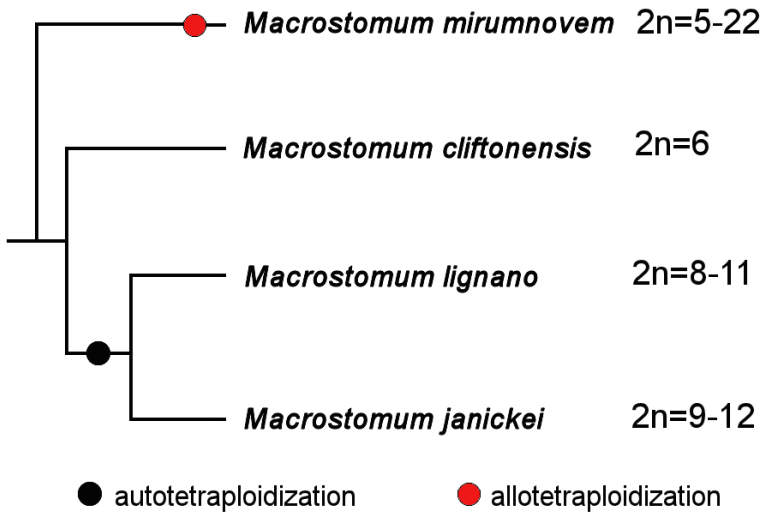

Fig. 2. The uncovered WGD events in the genus *Macrostomum.*

Earlier we uncovered a group of free-living marine flatworms in which genomes have likely undergone a recent WGD (Fig. 1, 2). We found out that karyotype instability was linked to hidden polyploidy in both species *M. lignano* (2n = 8) and its sibling species *M. janickei* (2n = 10) . Additionally we studied other species of the genus *Macrostomum* and revealed a new species (further called *M. mirumnovem*) with a highly unstable karyotype. We explored the peculiarities of karyotype and genome organization in three *Macrostomum* species.

This study is supported by grant of the Russian Science Foundation (RSF) (project No. 19-14-00211).

Scenario 1: formation of the doubled genome via autotetraploidization (*M. lignano* and *M. janickei*)

The detailed cytogenetic analyses using a set of different DNA probes (microdissected region- and chromosome specific DNA probes, DNA repeats, unique DNA fragments) revealed the karyotype and genome organization in *M. lignano* and *M. janickei*. In both, a recent WGD round accompanied with chromosome fusions of one ancestral chromosome set leading to the formation of large metacentric chromosome. The emergence of this chromosome provided the unusual case of hidden polyploidy in the genomes of both species (Fig. 3).

The combined approach allowed us to explore the peculiarity of the *M. lignano* genome organization, the strategy included bioinformatics analysis of the existed genome assemblies of *M. lignano* and NGS data for its separate chromosomes. Based on the results, we explored the presence of three subgenomes (Fig. 4a) and low-level divergence between them (Fig. 4b).

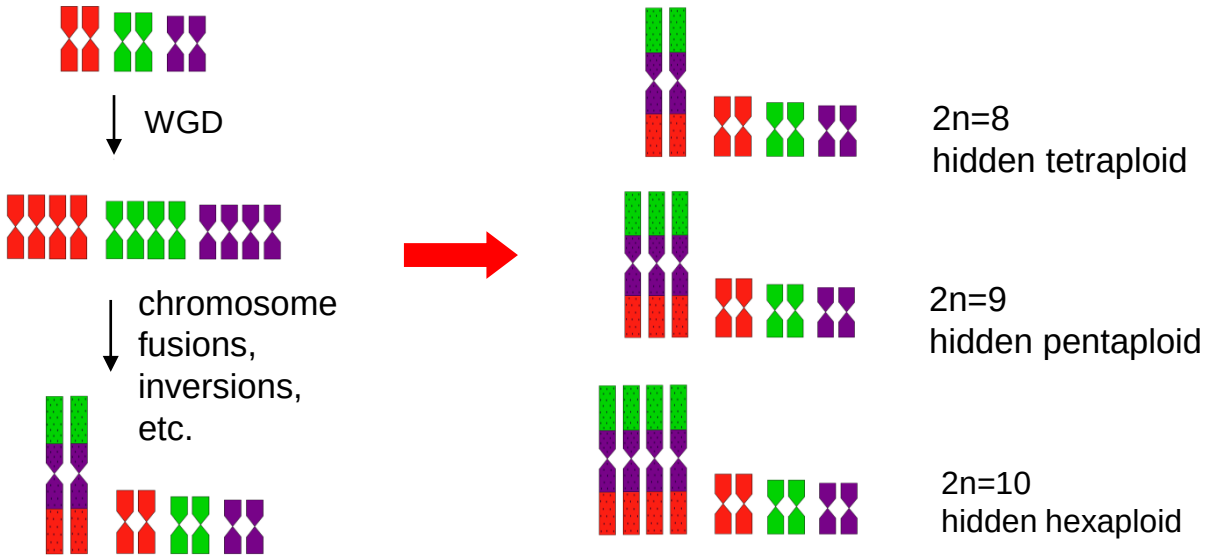

Fig. 3. The evolutionary scenario for the genome formation in *M. lignano* and *M. janickei.*

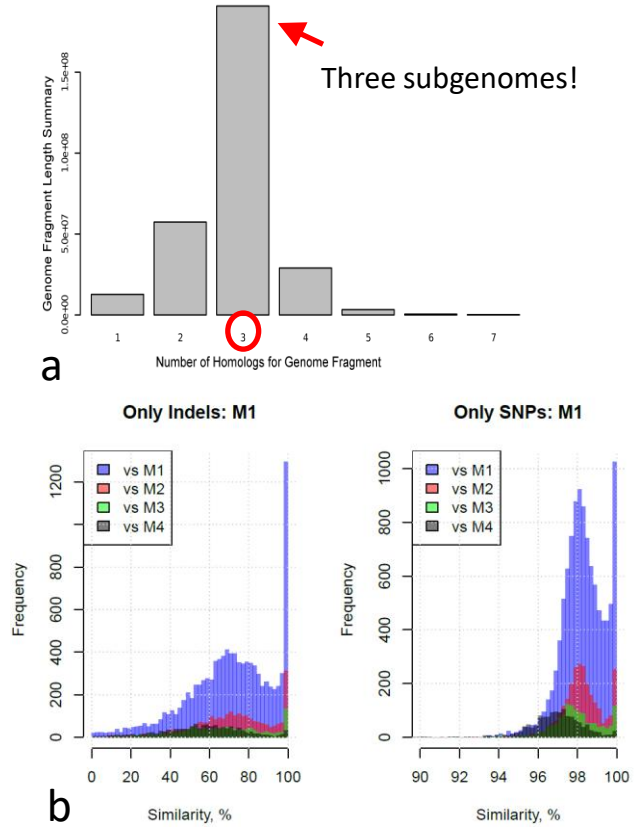

Fig. 4. The bioinformatics analyses based of the NGS data for chromosomes of *M. lignano* and its genome assemblies.

•coverage of entire genome assembly by NGS reads from MLI1;
• the presence of chimeric contigs/scaffolds in the existing genome assemblies of *M. lignano*;
• low level divergence between subgenomes (identity about 70%, similarity >90%);
• development of algorithm for chromosome assignment of contigs/scaffolds to chromosomes

Scenario 2: formation of the doubled genome through allotetraploidization (*M. mirumnovem*)

Another mechanism of genome doubling has been suggested for *M. mirumnovem* (Fig. 5). Similar to *M. lignano* and *M. janickei*, this species contains large metacentric chromosomes (2 instead of 1) arisen due to chromosome fusions after WGD. The karyotype showed a prominent instability mainly associated with chromosome number variation (2n=5-22) (Fig. 6). The latter was mostly linked with a copy number of small chromosomes, and we revealed that the additional small chromosomes are B chromosomes. The formation of Bs *de novo* can serve as indirect evidence of the hybrid origin of the *M. mirumnovem* genome.

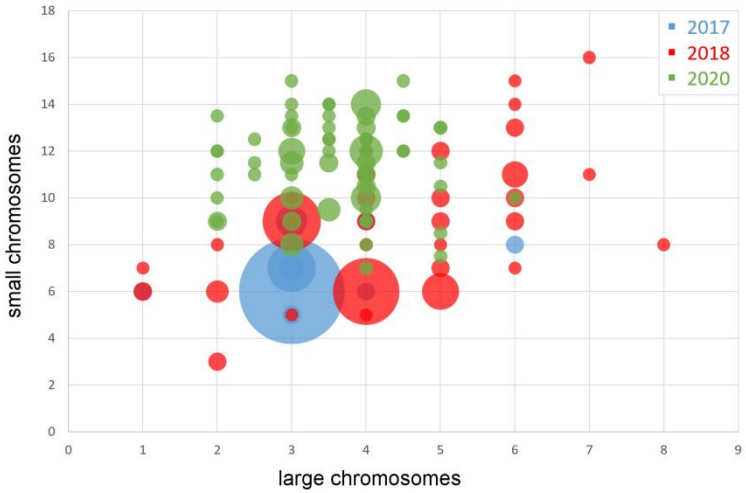

Fig. 6. Karyotype diversity of *M. mirumnovem*.

Activation of transposable elements (TEs) belonging to both parental subgenomes triggered genome reshuffling and its destabilization. The intense expansion and/or amplification of DNA repeats in large chromosomes of *M. mirumnovem* led to their differentiation (Fig. 7). The studies of repeatome of *M. mirumnovem* are in progress. Given the low homology level between large and small chromosomes, prominent karyotype instability, the expansion and amplification of DNA repeats, *M. mirumnovem* was formed through allopolyploidization.

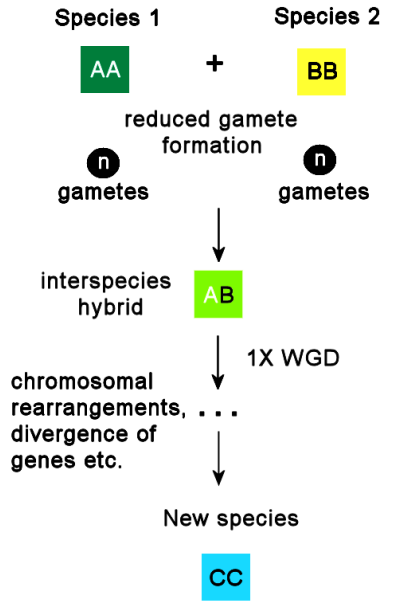

Fig. 5. The formation of the duplicated genome through allotetraploidization.

In conclusion, we would like to note that the post-WGD *Macrostomum* species (*M. lignano*, *M. janickei*, and *M. mirumnovem*) analyzed in this study represent a very promising model system for studying the mechanisms and regularities of karyotype and genome evolution after a recent WGD in animals.

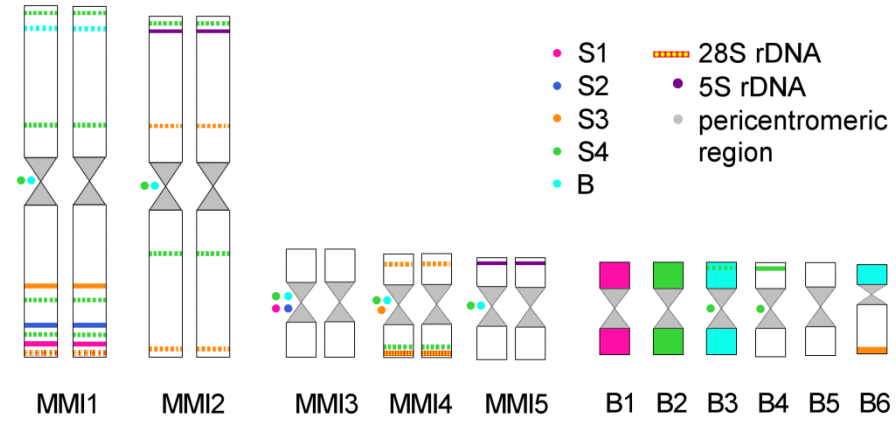

Fig. 7. The expansion and amplification of DNA repeats in the *M. mirumnovem*.