Phylogenetic analysis of tardigrade tubulins
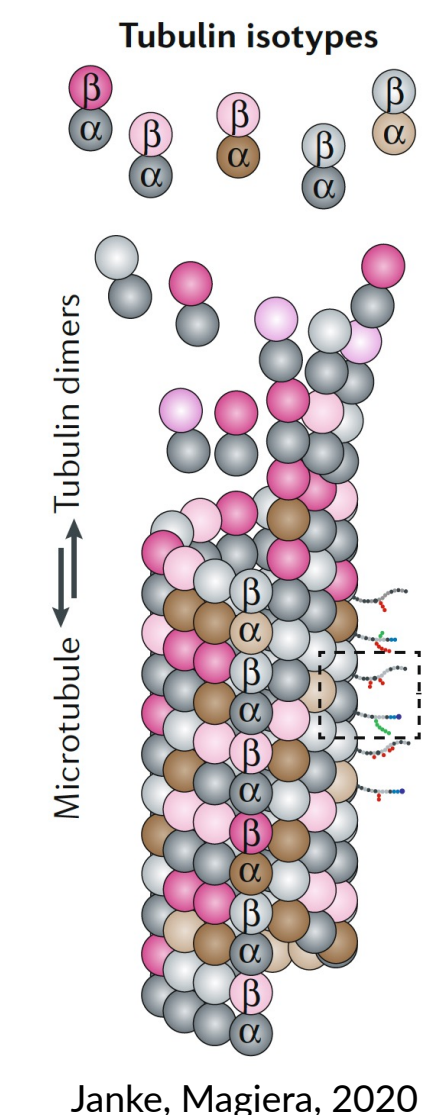
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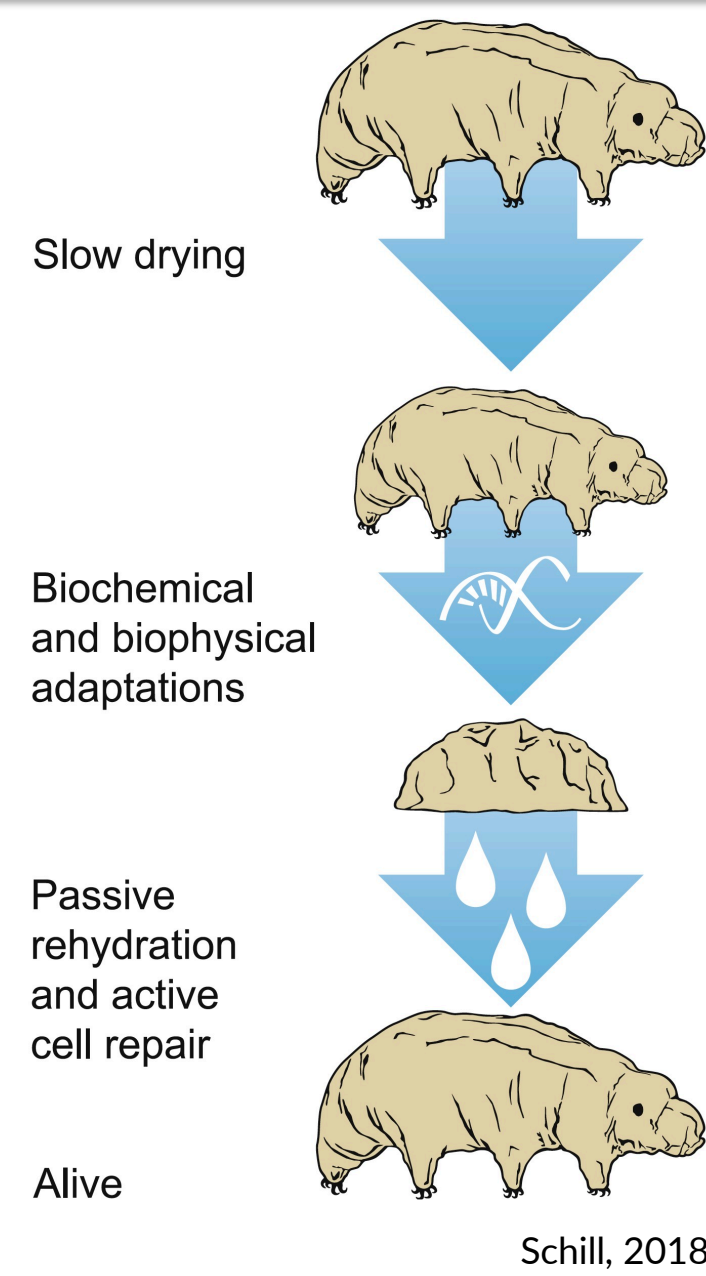[2]Comparative Bioinformatics, Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Dr. Aiguader 88, 08003 Barcelona, Spain
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Introduction

Janke, Magiera, 2020

Tardigrades are known for their remarkable ability to withstand extreme environmental challenges including draught, intense radiation, vacuum, low temperatures and high osmolarity. Upon unfavourable change of the environment, some tardigrade species can morphologically transform to so called tuns or cysts. They reduce their volume, lose most of their water content and basically cease to metabolize. After the external conditions return to normal, they can come back to life. However, underlying molecular mechanisms are mostly unknown.

Schill, 2018

Microtubule cytoskeleton is critical for many cellular processes. Nevertheless, any specific knowledge about tubulins in tardigrades is lacking. We hypothesize that microtubules play an important role in tardigrade physiology including the cryptobiosis. Therefore, we decided to analyze tardigrade tubulins.
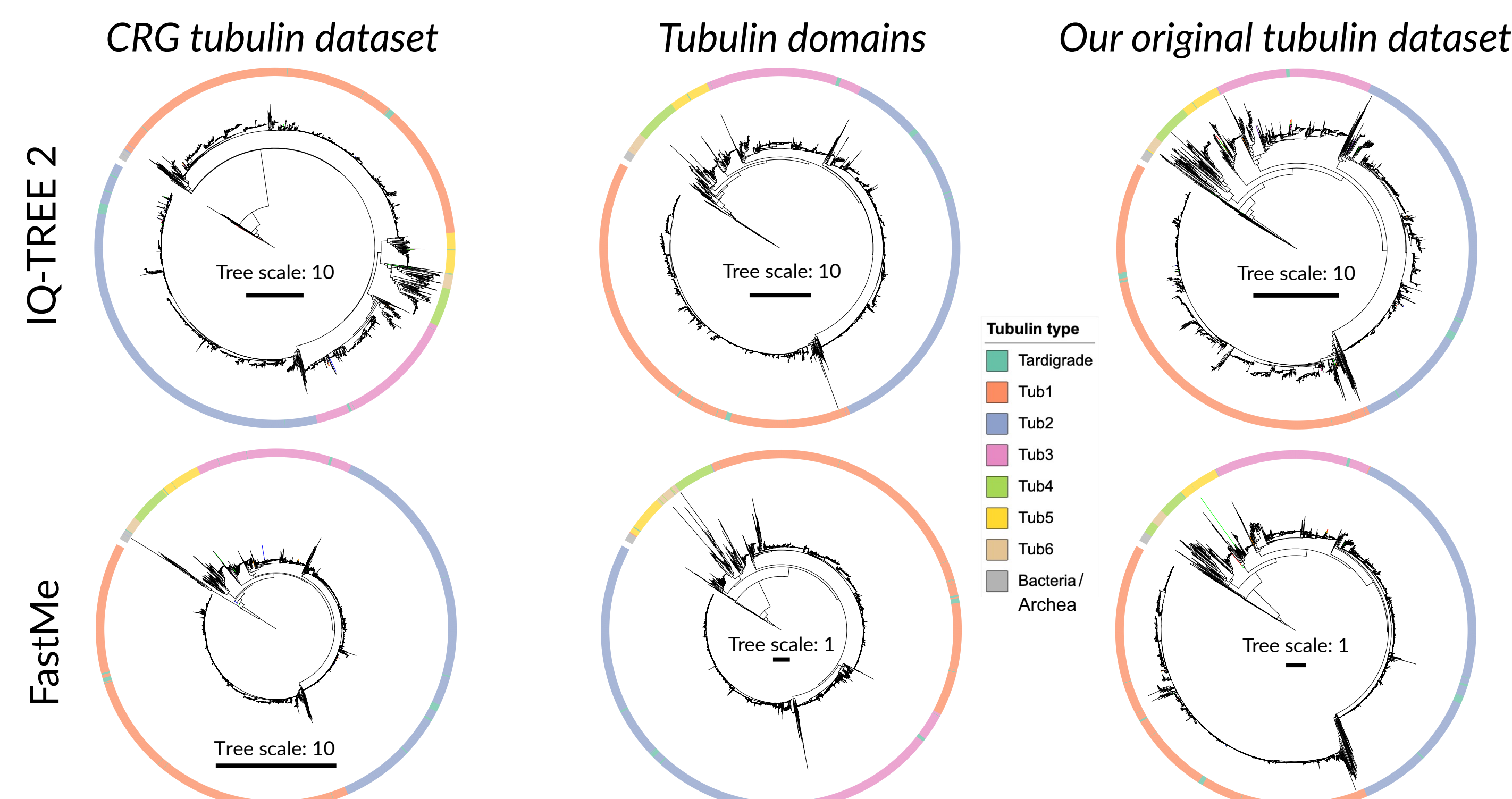
Data mining

A local BLAST database prepared from available genomes and transcriptomes of two heterotardigrade and six eutardigrade species.

tEs *Echiniscoides* cf. *sigismundi* (from Kamilari, 2019) – transcriptome
tEt *Echiniscus testudo* (from Mapalo, 2020) - transcriptome
tRc *Richtersius* cf. *coronifer* (from Kamilari, 2019) - transcriptome
tMp *Mesobiotus philippinicus* (from Mapalo, 2020) - transcriptome
tMt *Milnesium tardigradum* (GFGZ00000000.1) - transcriptome
tPr *Paramacrobiotus richtersii* (GFGY00000000.1) - transcriptome
tHe *Hypsibius dujardini* (nHd.3.1.5, from tardigrades.org) - CDS
tRv *Ramazzottius varieornatus* (Rv101, from tardigrades.org) - CDS

- **Tblastn** search using annotated tubulin protein sequences from *Homo sapiens*, *Drosophila melanogaster*, *Caenorhabditis elegans* and *Mus musculus*.
- Filtering out multiplicates based on unique sequence IDs, translation of unique hits in Benchling.
- Manual curation of the resulting dataset based on protein sequence identity using Clustal Omega, T-coffee and Jalview.
- Blast search and dataset curation were independently reproduced in collaboration with CRG in Barcelona resulting in a CRG dataset. In addition, tubulin domains were extracted from our dataset using HMMER and PFAM, Pfam family (PF00091.25).

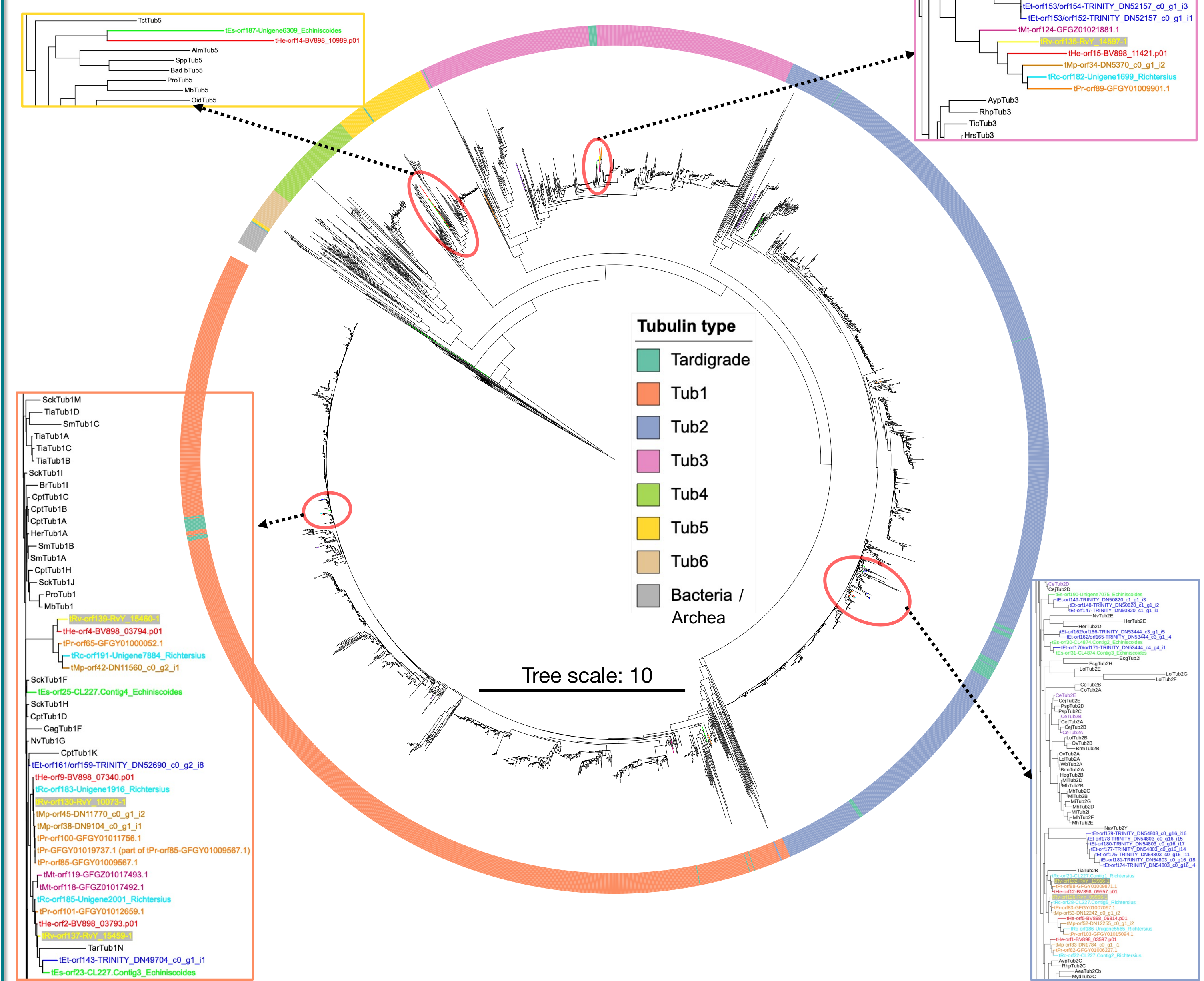## Multiple sequence alignments and construction of phylogenetic trees

- Tardigrade tubulin datasets were aligned with 3200 eukaryotic tubulin sequences from more than 500 species (Findeisen, 2014) using regressive mode of T-coffee.
- The resulting alignments served for phylogenetic inference based on both maximum likelihood (IQ-TREE 2) and minimum evolution (FastME) methods allowing us to assign the newly found tubulins to individual isotypes.
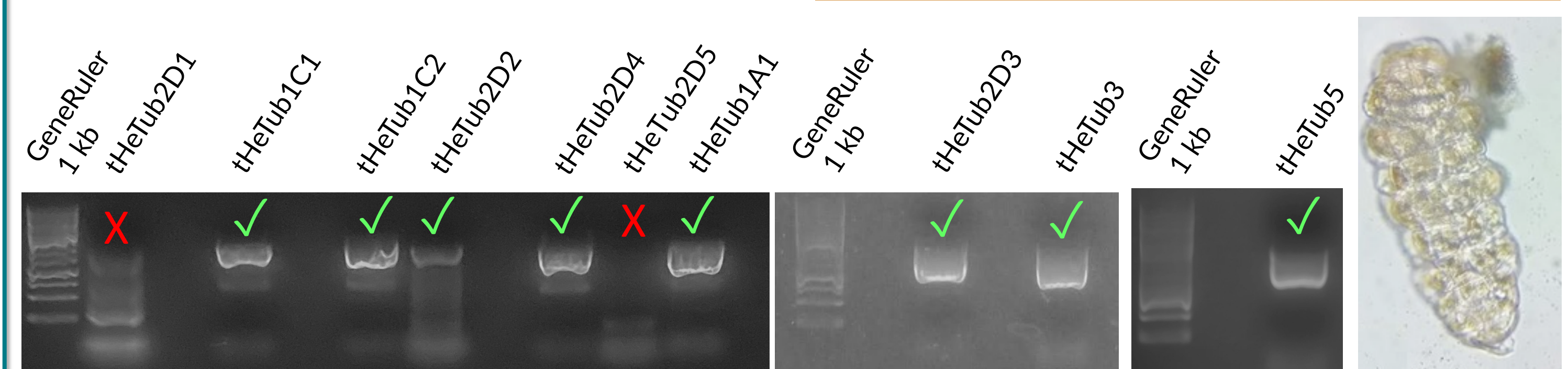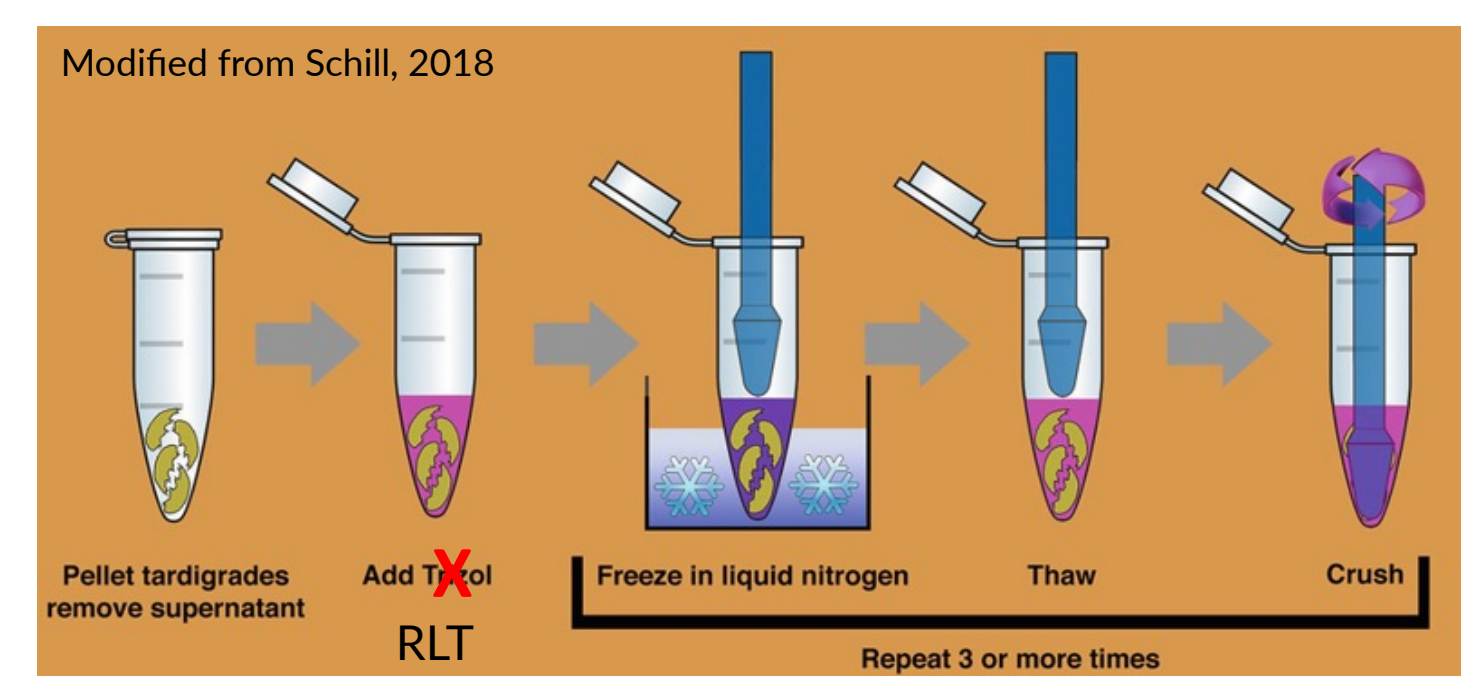

CRG tubulin dataset   Tubulin domains   Our original tubulin dataset

Tubulin type:
- Tardigrade
- Tub1
- Tub2
- Tub3
- Tub4
- Tub5
- Tub6
- Bacteria / Archea

Found tardigrade tubulin sequences

Classes	Species	Complete	Partial	Tub1 (α-tubulin)	Tub2 (β-tubulin)	Tub3 (γ-tubulin)	Tub5 (ε-tubulin)
Eutardigrada	*H. exemplaris*	10	-	3	5	1	1
	M. philippinicus	5	3	3	4	1	-
	M. tardigradum	2	5	3	3	1	-
	P. richtersi	7	7	5	7	2	-
	R. varieornatus	2	7	3	5	1	-
	R. coronifer	7	-	3	3	1	-
Heterotardigrada	*E. testudo*	2	23	5	15	4	1
	E. sigismundi	6	4	3	4	1	1

A detailed phylogenetic tree computed using maximum likelihood method (IQ-TREE 2)

Tubulin type:
- Tardigrade
- Tub1
- Tub2
- Tub3
- Tub4
- Tub5
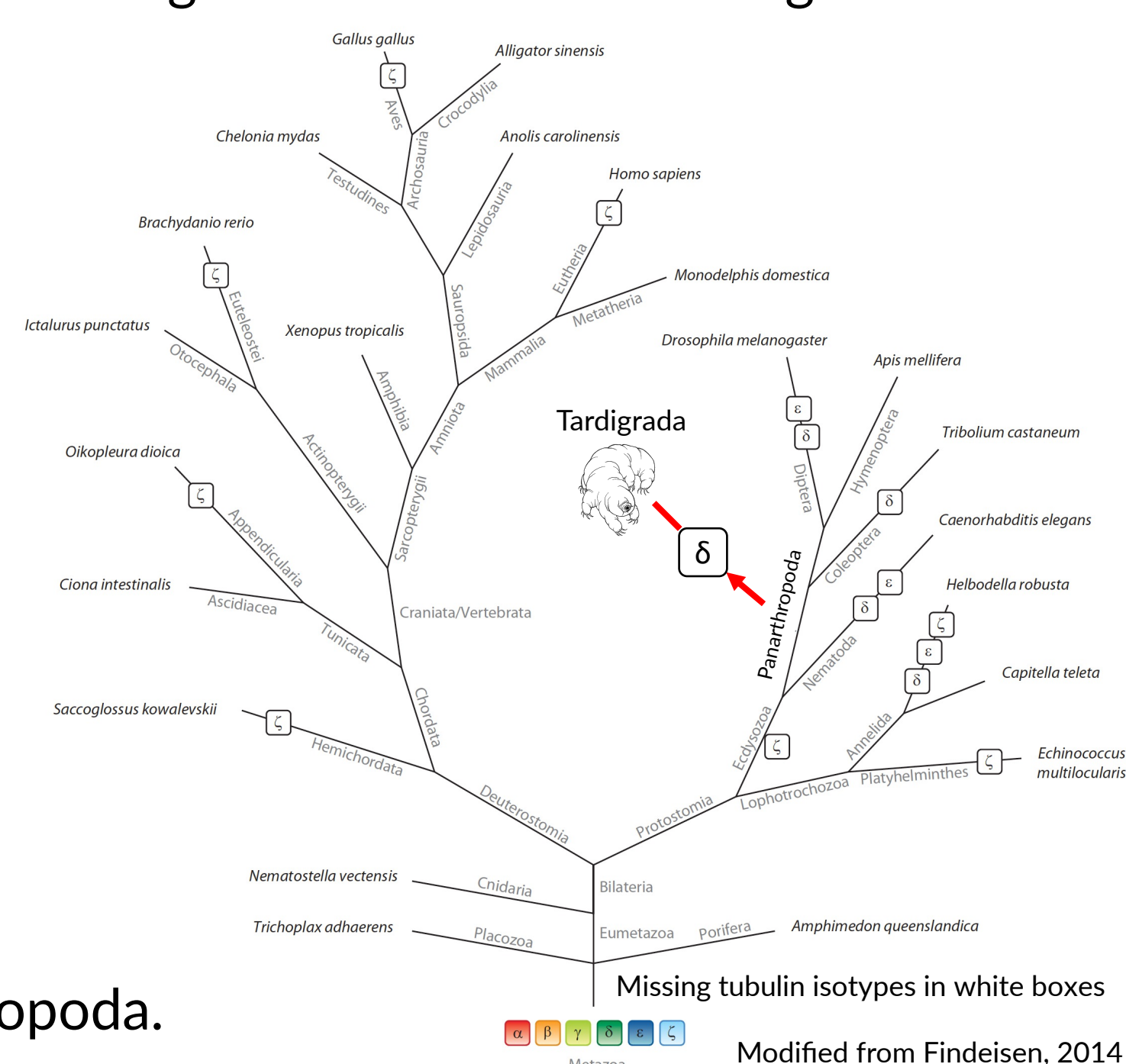- Tub6
- Bacteria / Archea

Tree scale: 10

Amplification of tubulin coding sequences from adults of *H. exemplaris*

- The best method for RNA isolation was sample disruption in liquid nitrogen in the presence of RLT buffer and subsequent column purification (RNeasy, Qiagen).

Modified from Schill, 2018

Conclusions

- We developed tools for gene analysis in Tardigrades and identified 90 unique tardigrade tubulin sequences.
- We performed many independent MSAs and constructed phylogenetic trees using various methods. Based on these results we assigned found tardigrade tubulins to individual tubulin isotypes. The minority of assignments that were ambiguous were resolved by manual sequence analysis.
- We were able to amplify 8 out of 10 predicted coding sequences from *Hypsibius exemplaris* adult specimens.
- The phylogenetic position of tardigrades within the Ecdysozoa is still controversial and debated. Although Nematoda lost their δ- and ε-tubulin, some groups of Arthropoda like order Hymenoptera still possess them.
- We found 3 epsilon tubulins, two in heterotardigrades, one in an eutardigrade. Thus, our current data support the placement of tardigrades to Panarthropoda.

Missing tubulin isotypes in white boxes
Modified from Findeisen, 2014

References

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