



**Tandem repeats in a group II intron
provide resolution in phylogenetic and
phylogeographic studies
of the genus *Populus*.**

Barbara Fussi

**Federal Research Centre for Forests,
Vienna (AUSTRIA)**

- Thanks to all colleagues who provided us with samples, or supported our own collection trips:

Nica M.S (ICAS Craiova, RO)

Ipati A. (ICAS Tulcea, RO)

Cvrčková H., Máchová P. (FGMRI, CZ)

Bartha D. (Sopron, HU)

Benke A. (ERTI Sárvár, HU)

Kaijba D., Bogdan S. (Faculty of Forestry, Zagreb, CRO)

Castiglione S. (Salerno, I)

Gonzales-Martinez S. (Madrid, E)

- Supported by the AUSTRIAN ACADEMY OF SCIENCE (ÖAW)

The idea behind

- To study the structure of a chloroplast group II intron (rpl16) in *Populus*
- To find out how different numbers of tandem repeats affect the folding of the intron
- To help us understand how different foldings influence the functioning of the intron during the splicing process
- To resolve phylogenetic relationships based on tandem repeats

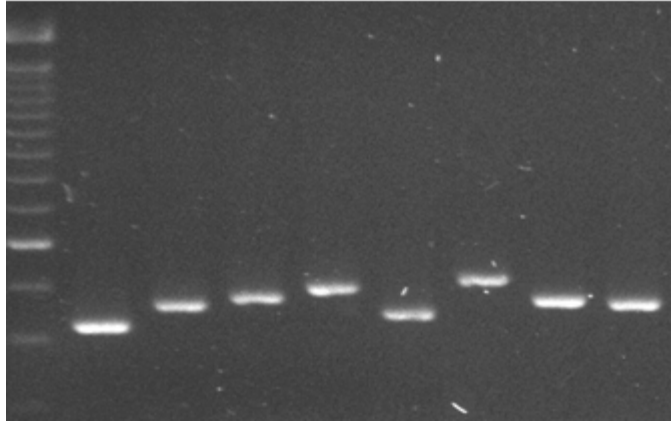
Methods

- DNA was extracted from dry leaves of 15 poplar species and hybrids
- Sequencing was performed directly on PCR products on an automated sequencer
- Obtained sequences were edited using Sequence Investigator and VectorNTI (Invitrogen)
- For determining the secondary structures we used the Vienna RNA package (thermodynamic structure prediction and phylogenetic comparison)

Methods

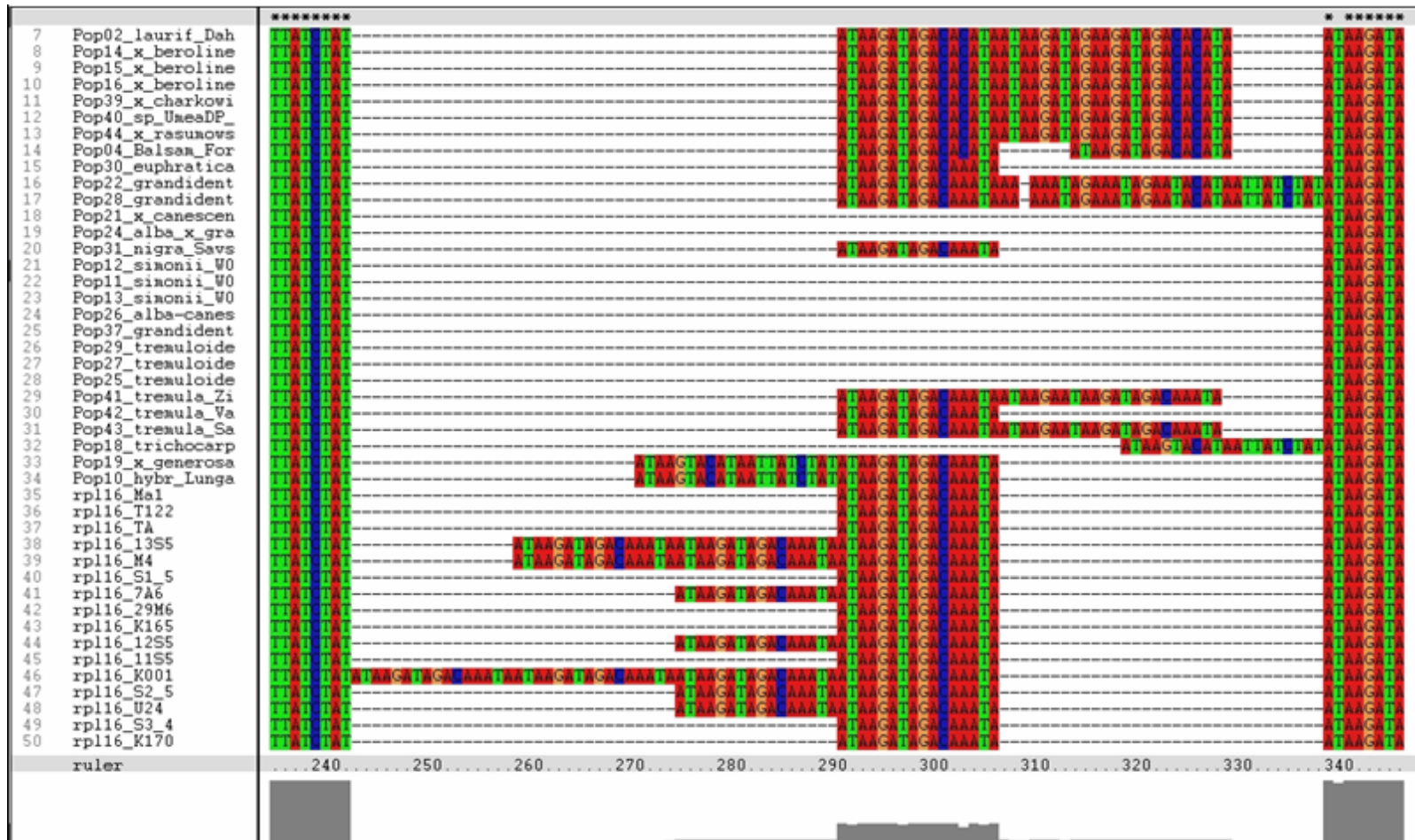
- Sequences were aligned using the software MUSCLE and ClustalX
- The repeat structure was visualized using the software Tandem Repeat Finder
- Gap/indel coding using SeqState and subsequent phylogenetic analysis were performed in PAUP.

Tandem repeat structure

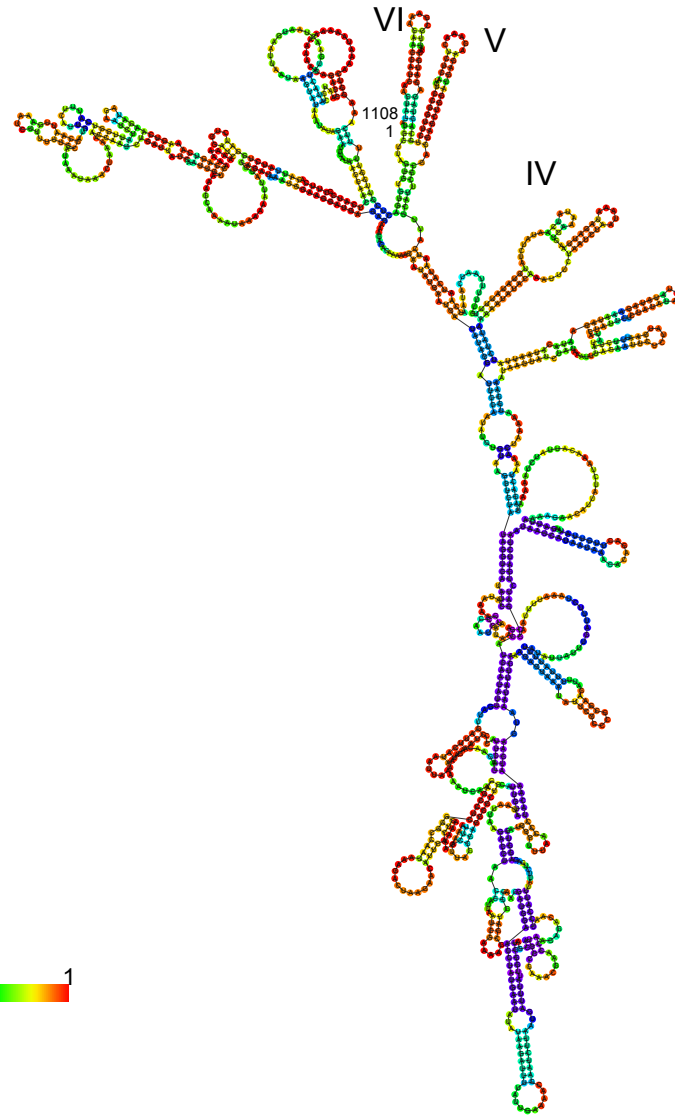


Stepwise length
variation of 16bp in
P. alba and *P.*
tremula on Agarose
gels

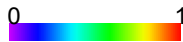
Sequencing revealed tandem repeats in 5 locations



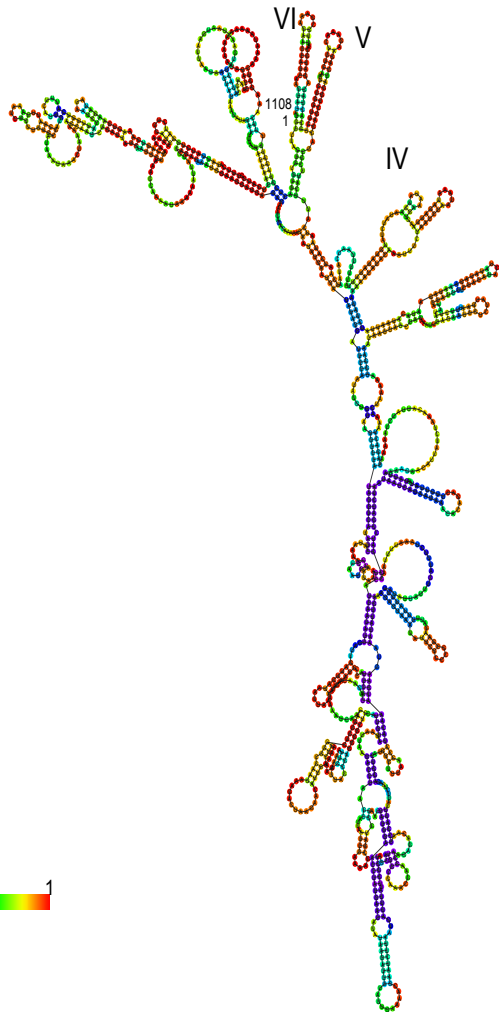
Secondary structure of rpl16 group II intron in *P. alba*



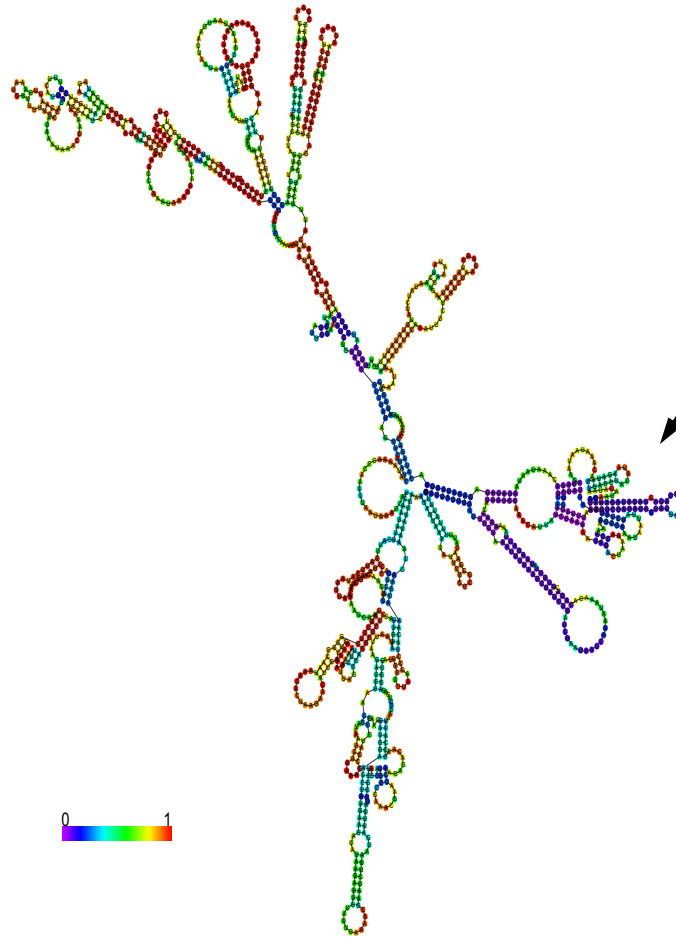
- 16bp repeats
- differences in domain IV
- Folding without 16bp repeat



Secondary structure of rpl16 group II intron in *P. alba*



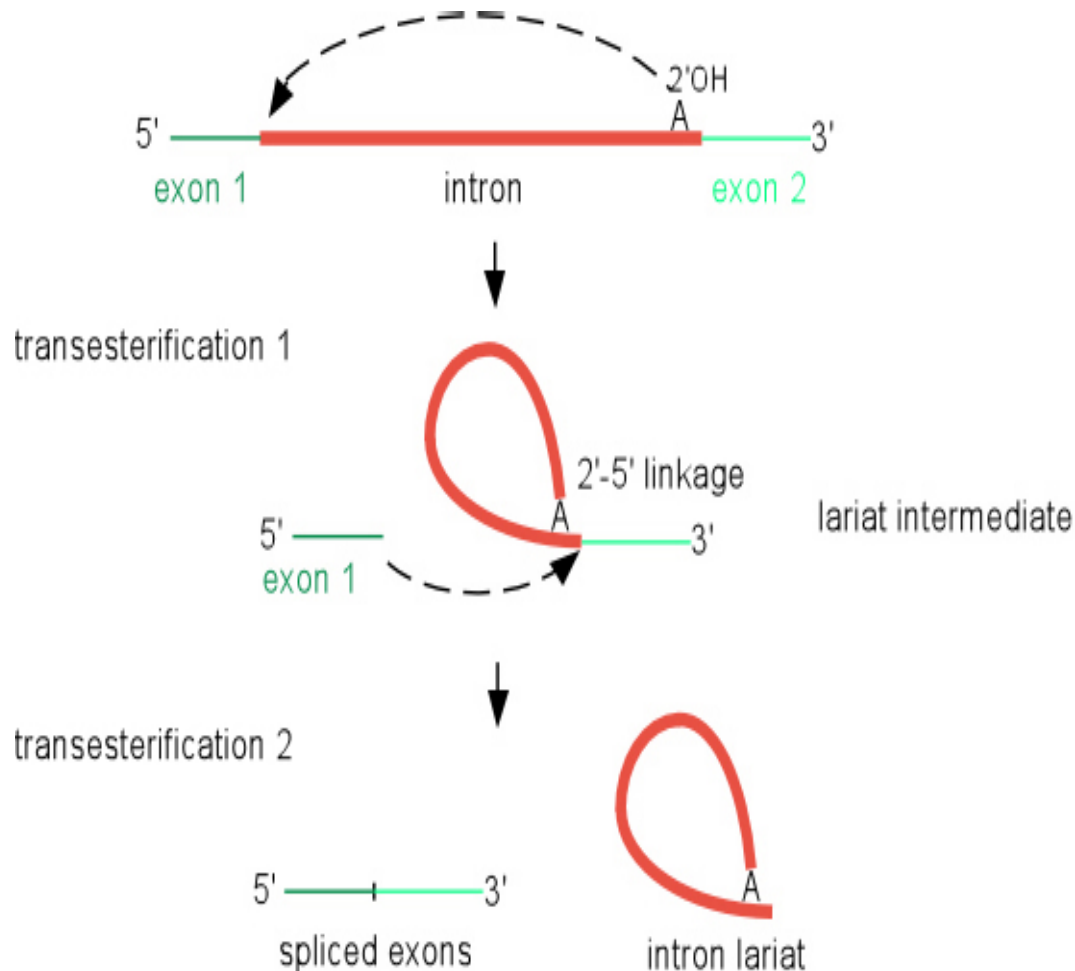
Folding without 16bp repeat



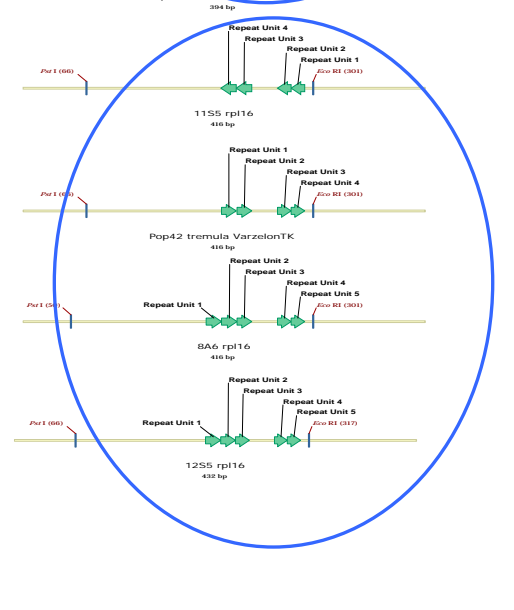
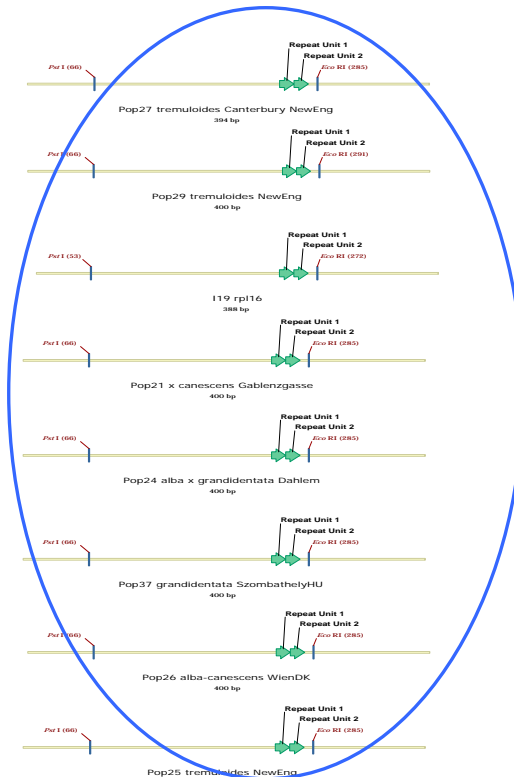
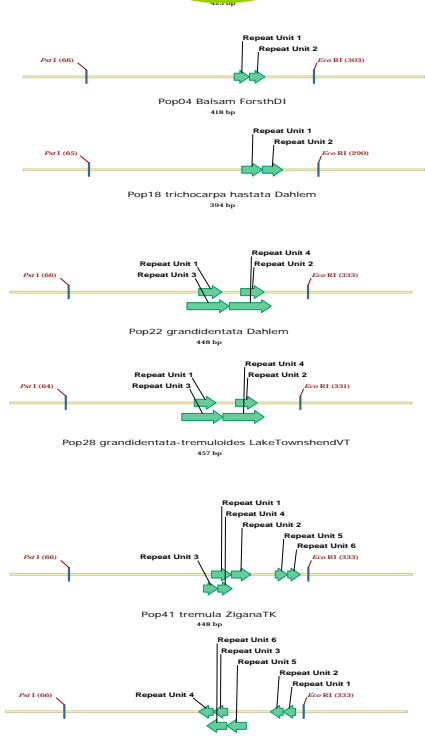
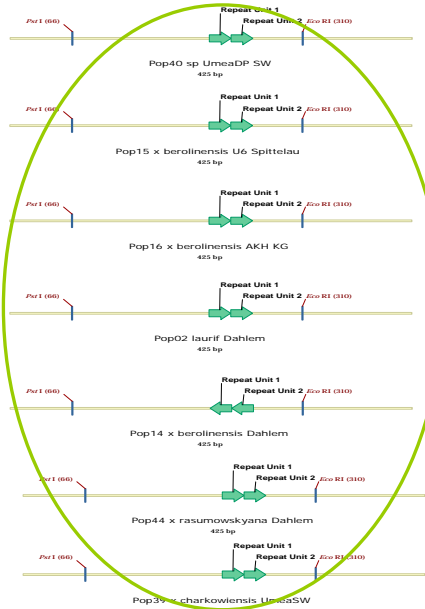
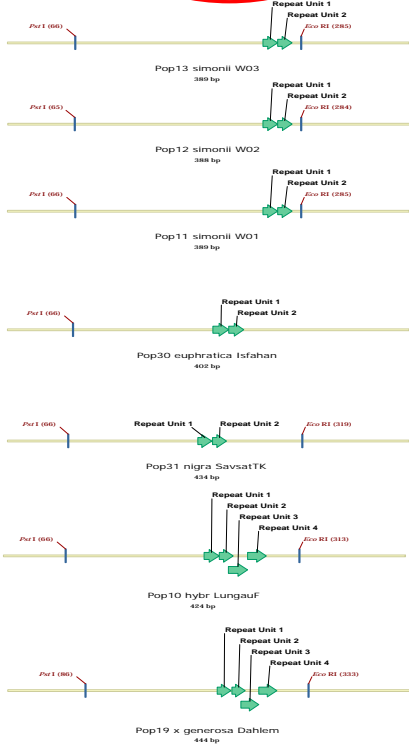
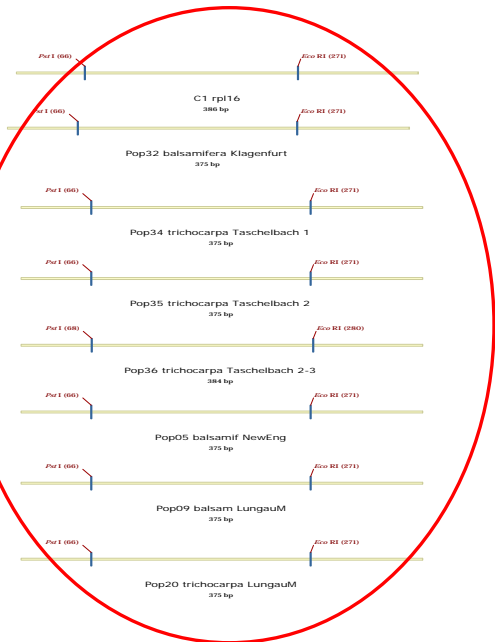
Folding contains 6 tandem repeats of 16bp indicated by the arrow

- 16bp repeats
- differences in domain IV
- one hairpin is missing, if 16bp sequence is repeated

Self-splicing introns

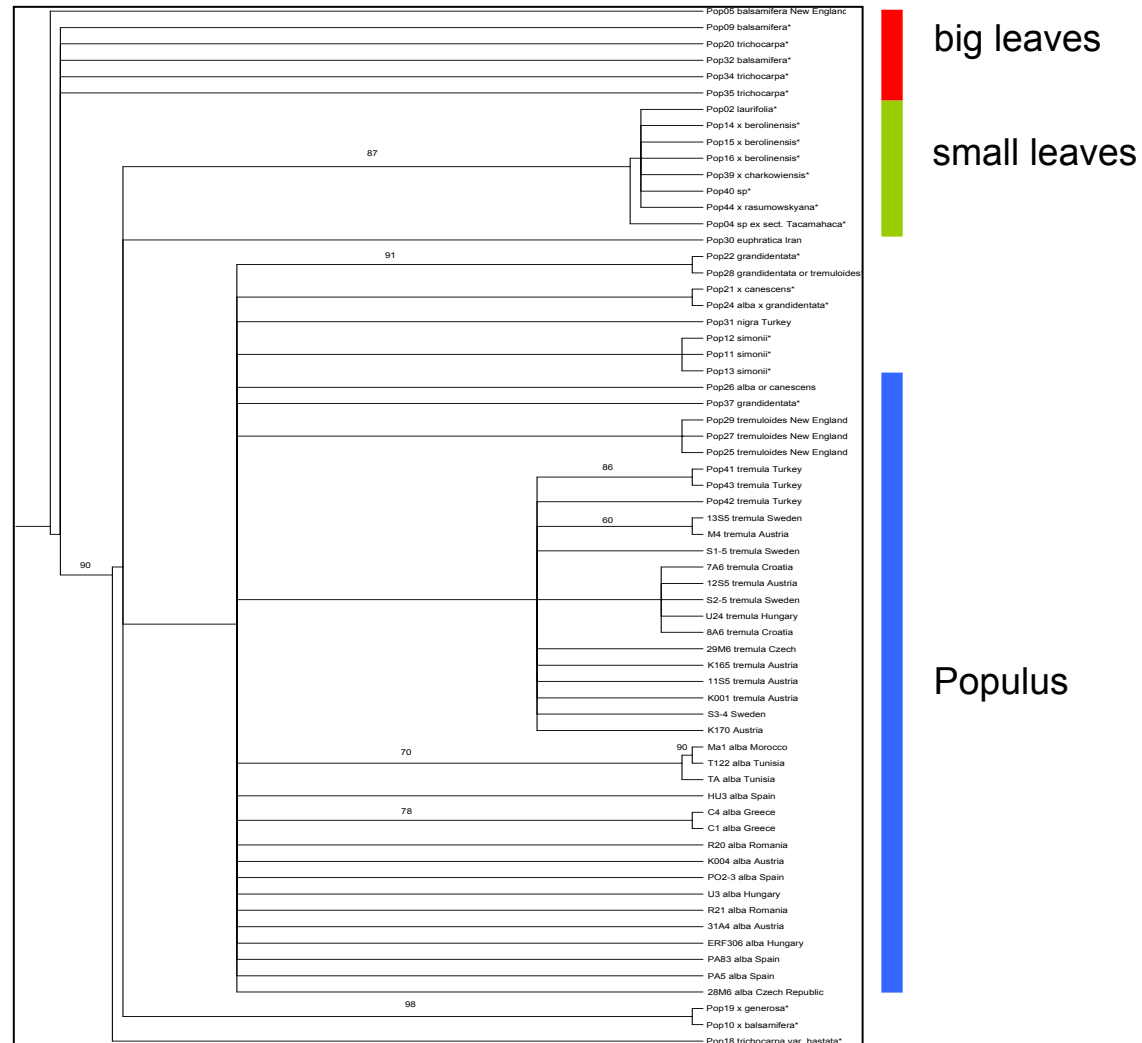


- selfsplicing introns require help of an additional protein
- domain IV can be an ORF for the maturase
- domainIV can interact with additional splicing factors

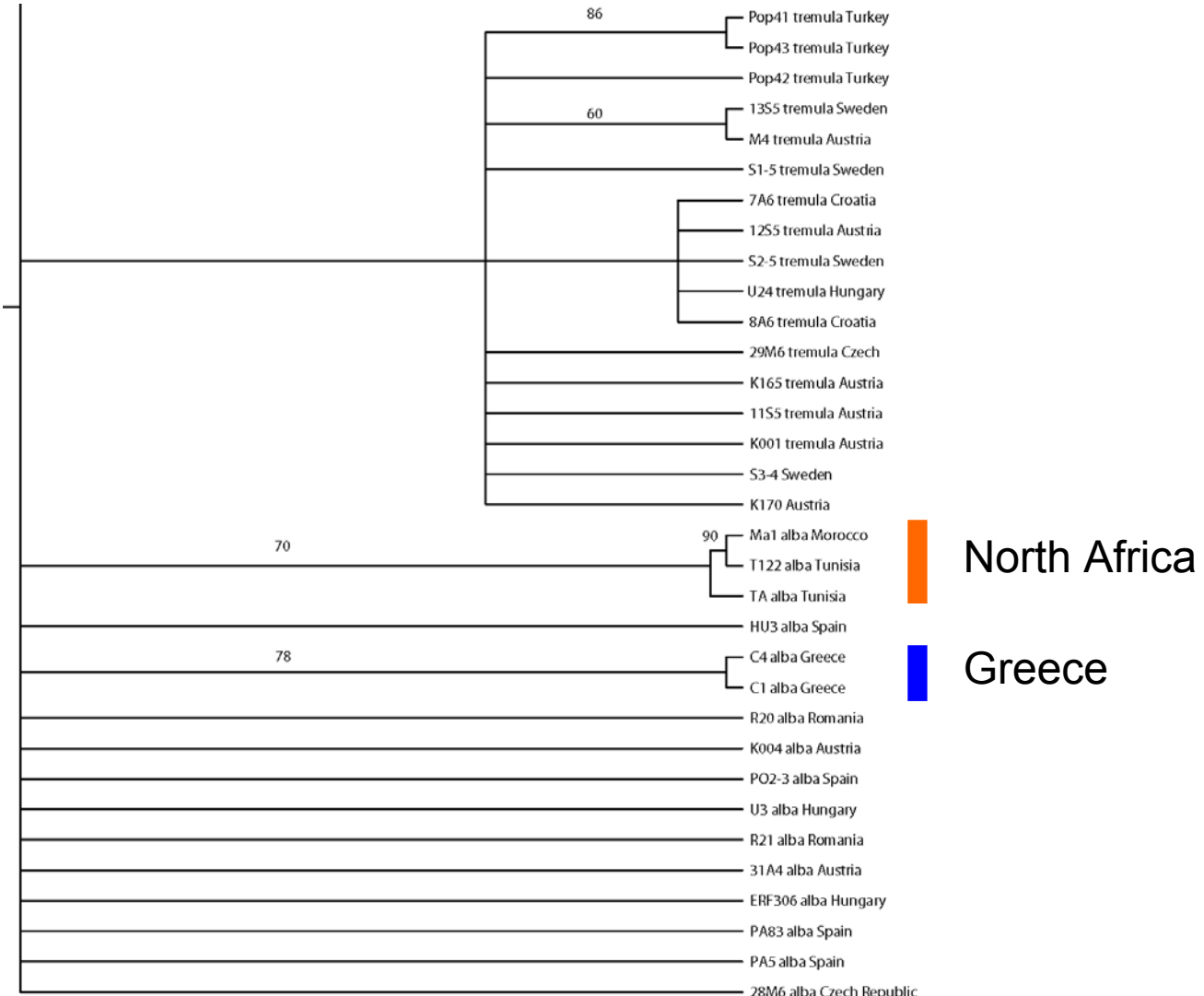


Phylogenetic relationship of 64 accessions of the genus *Populus*

- Two groups within Section Tacamahaca
- *Populus* as a separate section (with interference of *P. nigra* and *P. simonii*)
- Bootstrap values of more than 50 are given above branches
- * cultivated, hybrids and ornamentals



Phylogeographic relationship of 30 accessions of the alba-tremula complex



Summary

- High variable tandem repeats are located in the domain IV of the intron
- Maturase aided splicing in the chloroplast: require an ORF in the intron or a nuclear encoded maturase
- Within rpl16: domain IV has lost its maturase function thus evolves fast
- Different numbers of repeats of a 16bp fragment only influenced the domain IV - other domains appeared stable
- The formation of certain hairpin structures with high base pair probabilities within the domain IV may indicate the interaction with additional splicing factors
- Phylogenetic analysis revealed deeper resolution when modified complex indel coding (MCIC) was applied
- A 50% majority rule consensus tree using the MCIC supported 3 main groups: Populus and 2 groups within section Tacamahaca

Conclusions

- Tandem repeats influence the folding, but may not influence the function of the intron
- Introns are useful for phylogenetic studies
- Indels should not be excluded in the analysis
- Future plans: adding more species and basepairs for a phylogeny in *Populus*