



# **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)**

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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)

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# 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

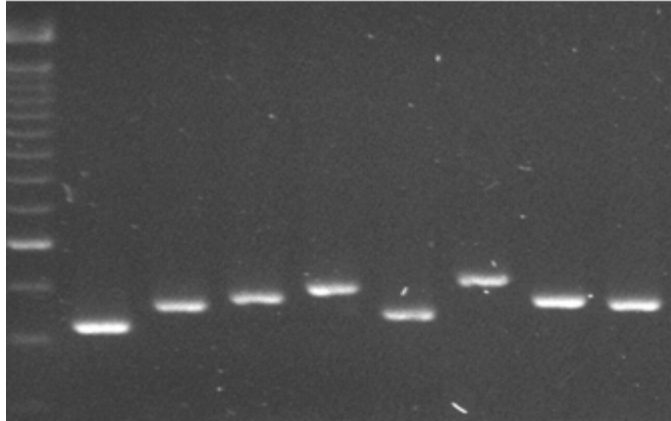
# Methods

- 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

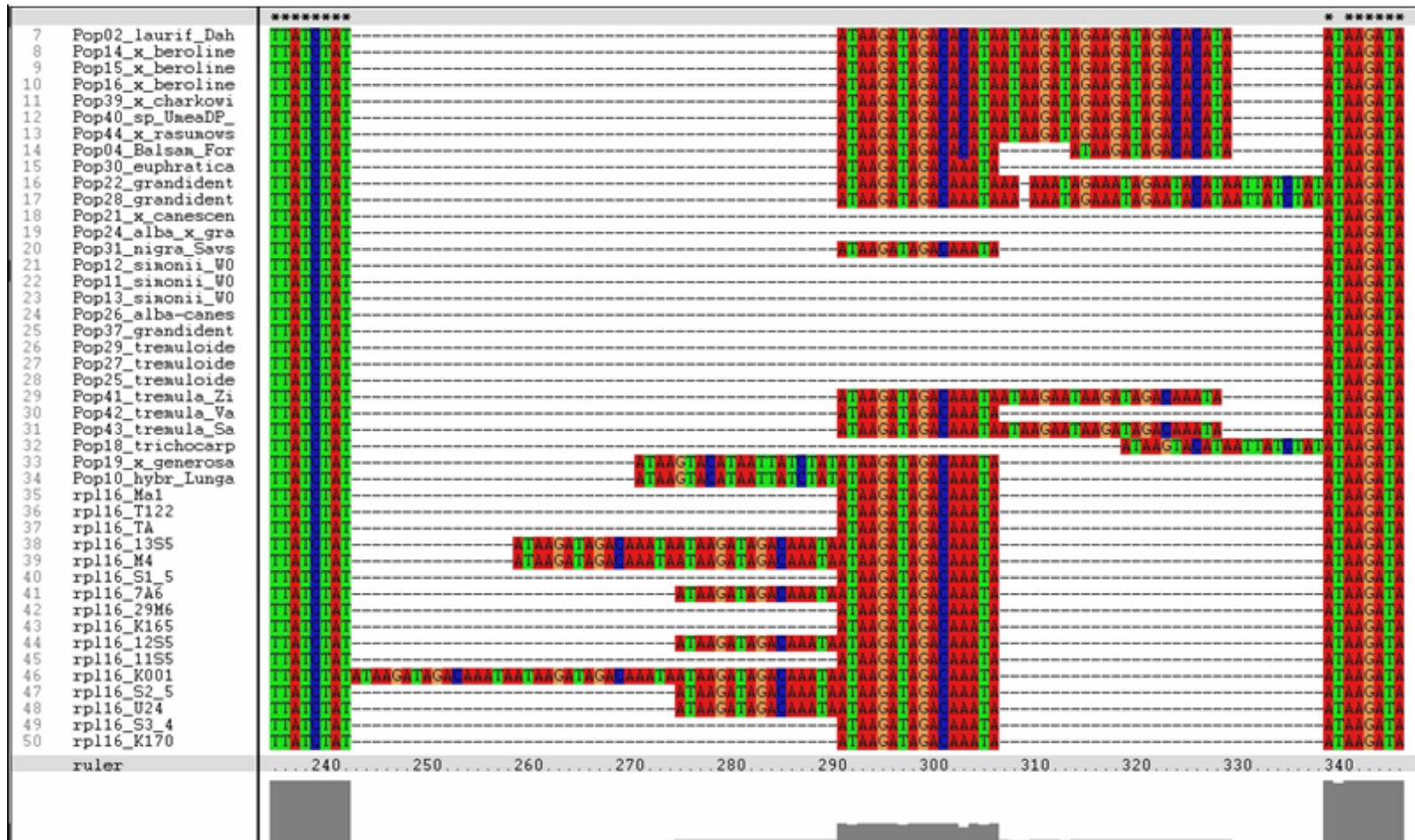
- Sequences were aligned using the software MUSCLE and ClustalX
- The repeat structure was visualized using the software Tandem Repeat Finder
- Gap/indel coding using SeqState and subsequent phylogenetic analysis were performed in PAUP.

# Tandem repeat structure

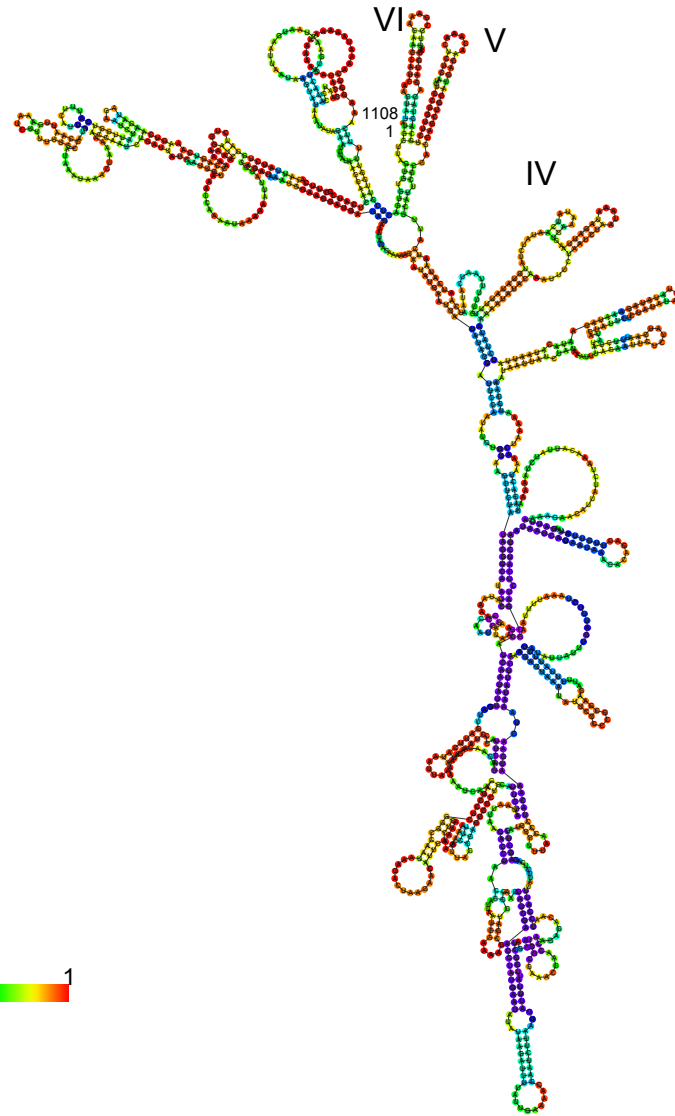


Stepwise length variation of 16bp in *P. alba* and *P. tremula* on Agarose gels

# Sequencing revealed tandem repeats in 5 locations



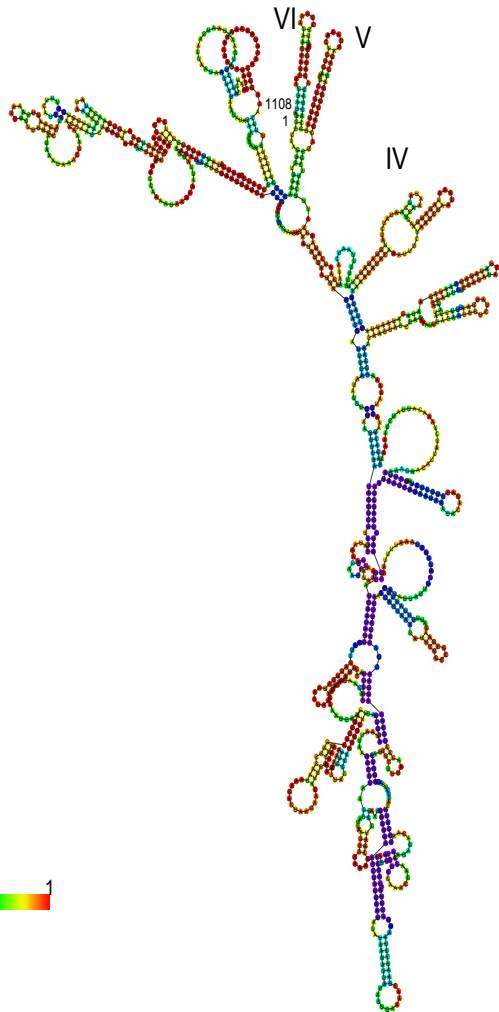
# Secondary structure of rpl16 group II intron in *P. alba*



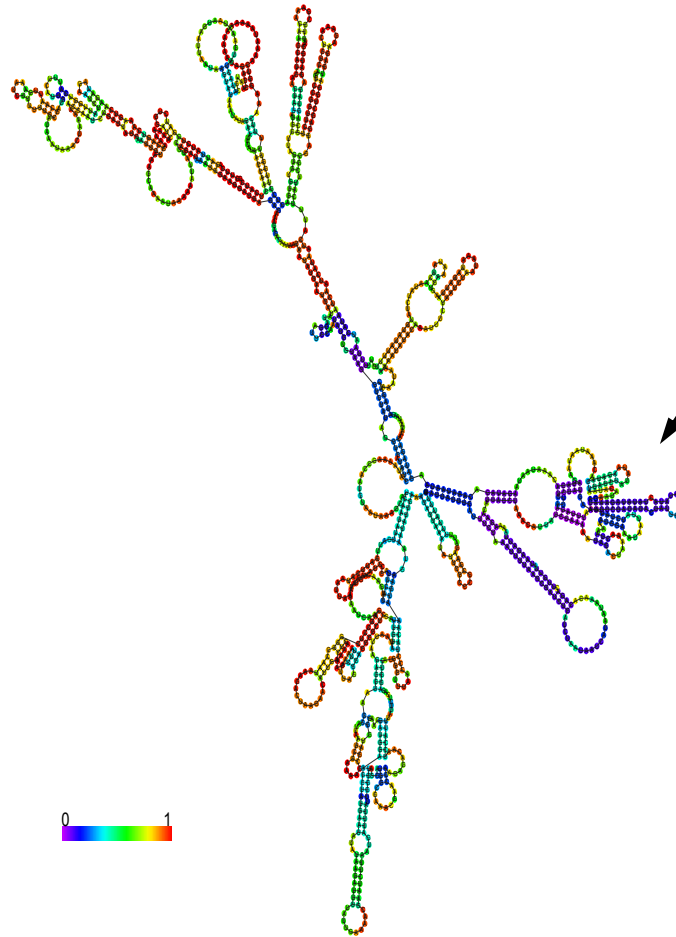
- 16bp repeats
- differences in domain IV
- Folding without 16bp repeat



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



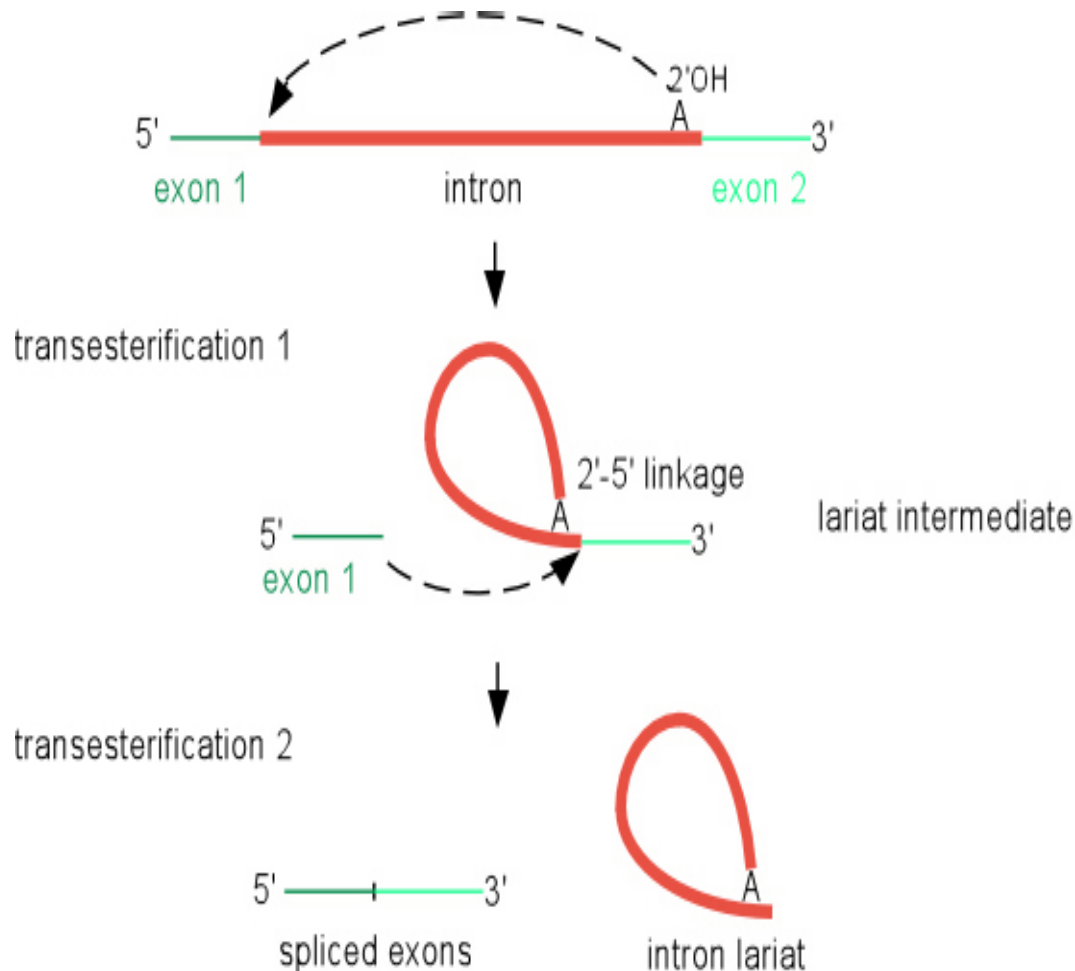
Folding without 16bp repeat



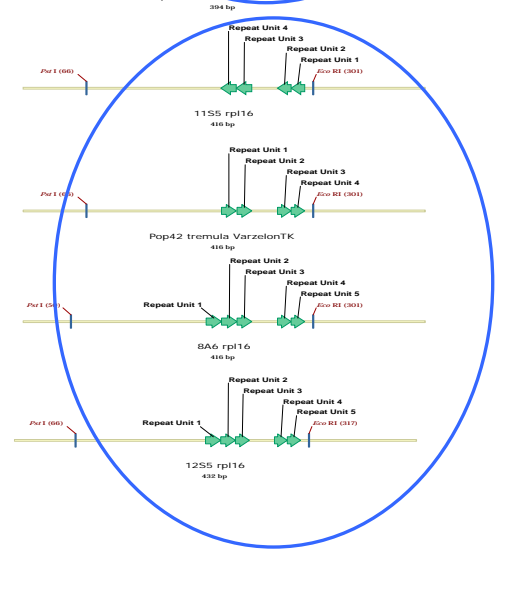
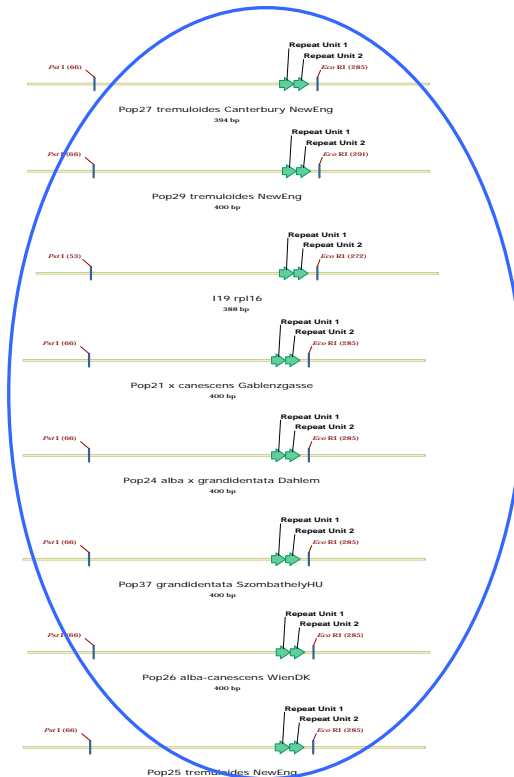
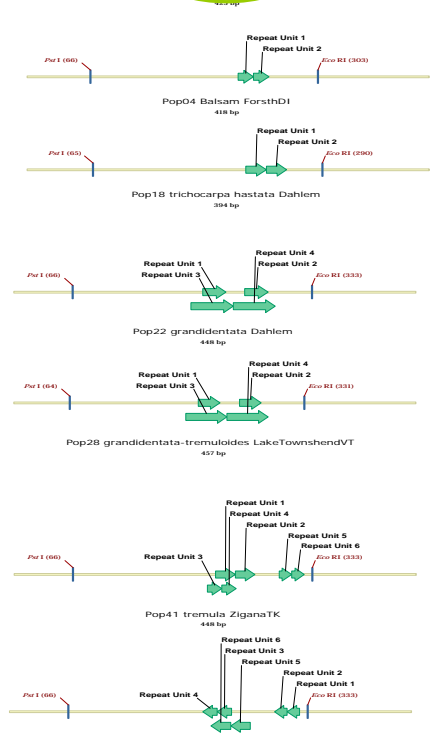
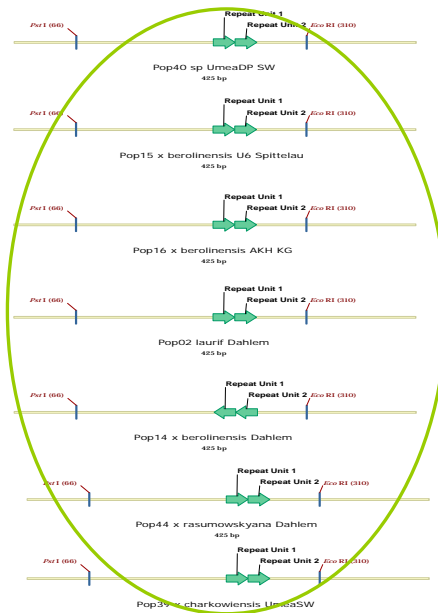
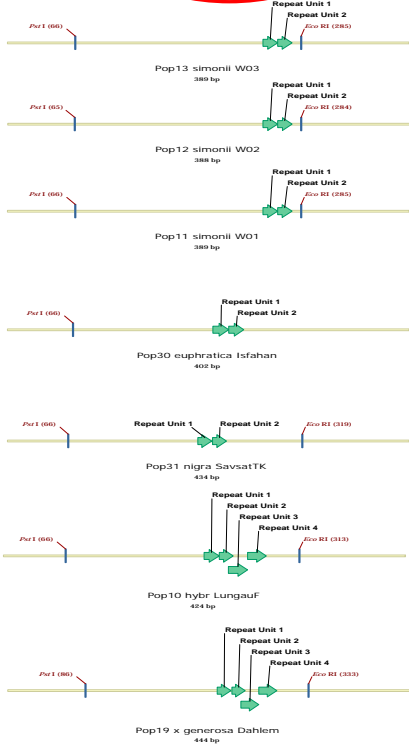
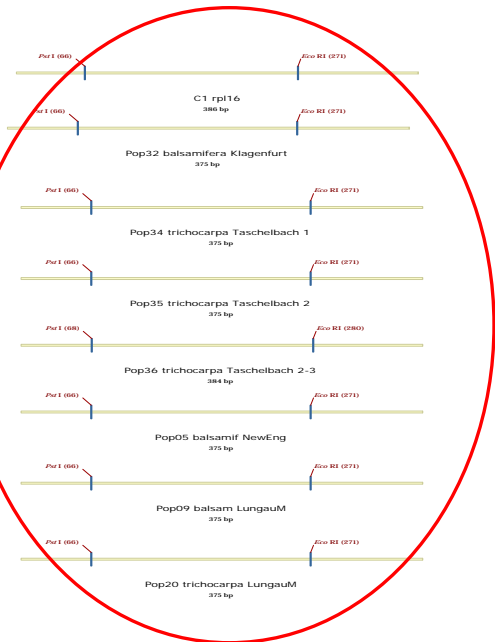
Folding contains 6 tandem repeats of 16bp indicated by the arrow

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

# Self-splicing introns

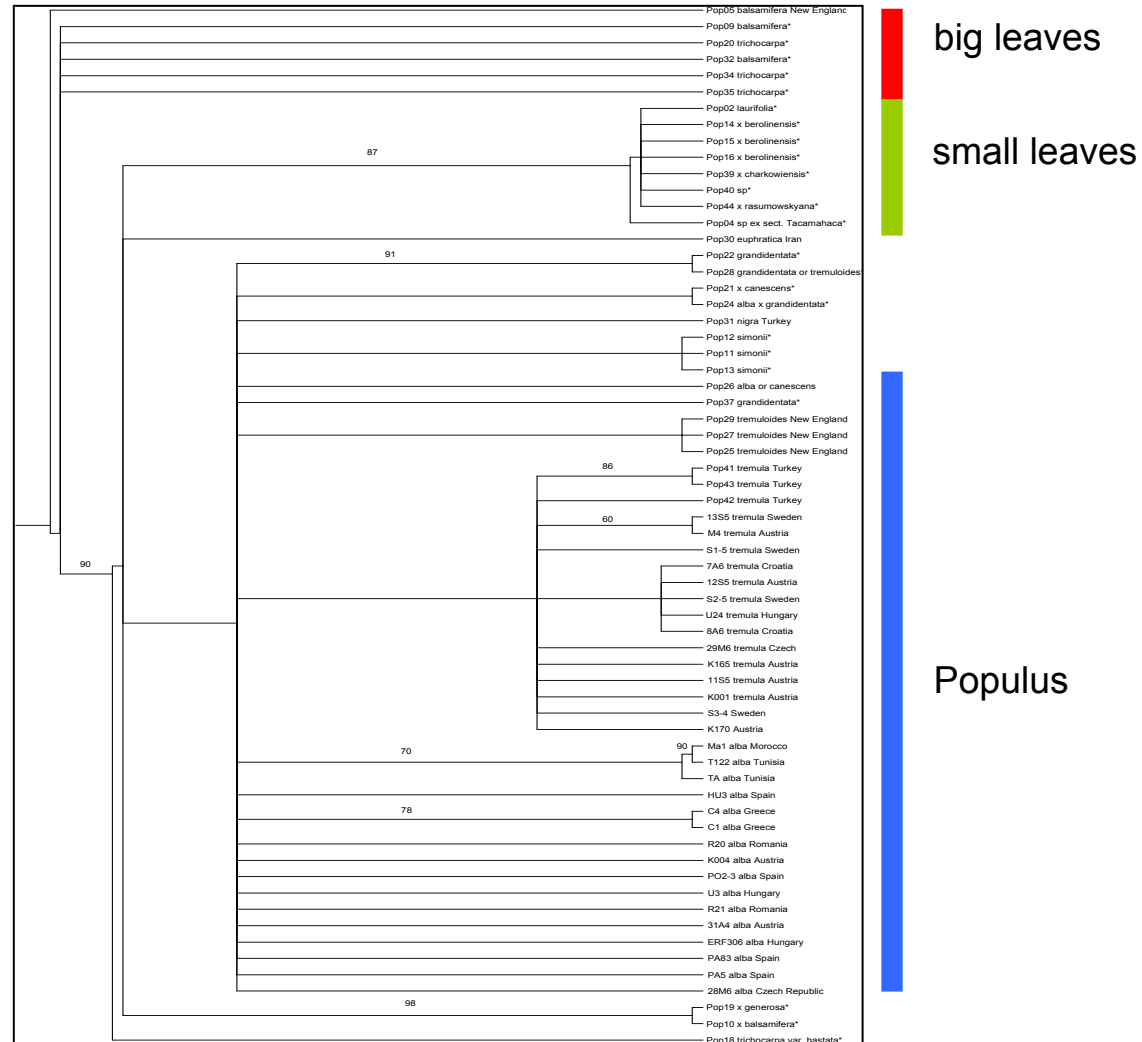


- selfsplicing introns require help of an additional protein
- domain IV can be an ORF for the maturase
- domainIV can interact with additional splicing factors

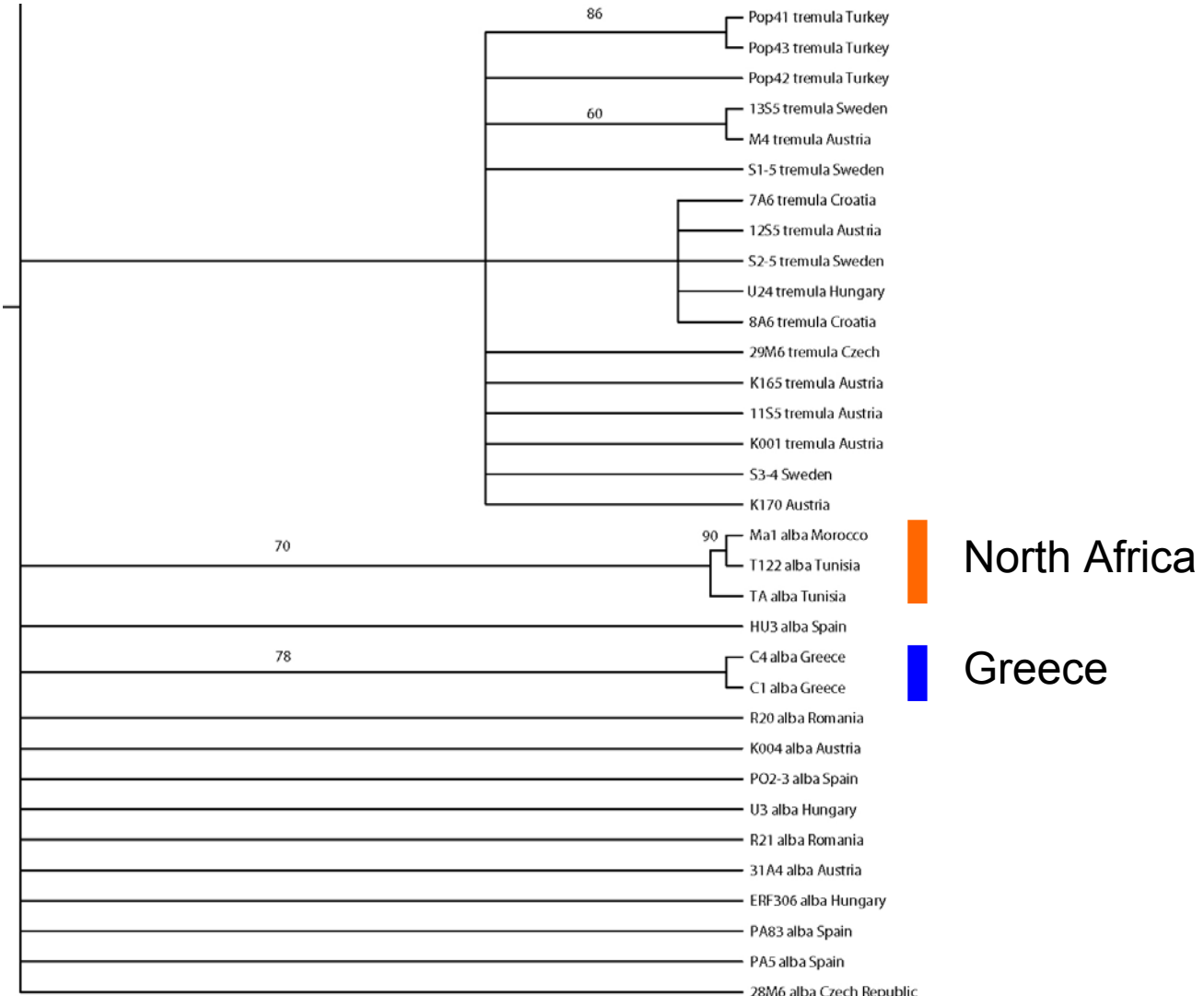


# Phylogenetic relationship of 64 accessions of the genus *Populus*

- 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*