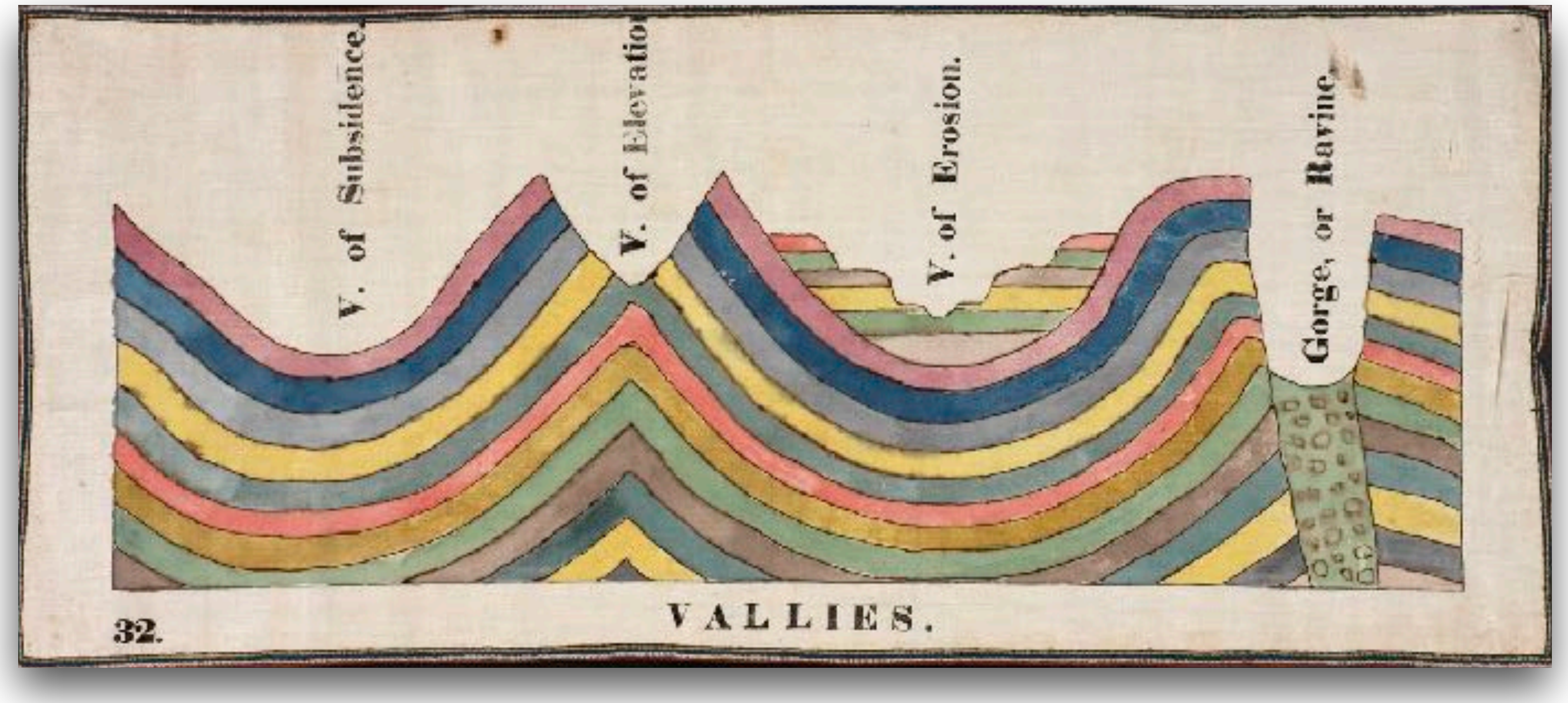
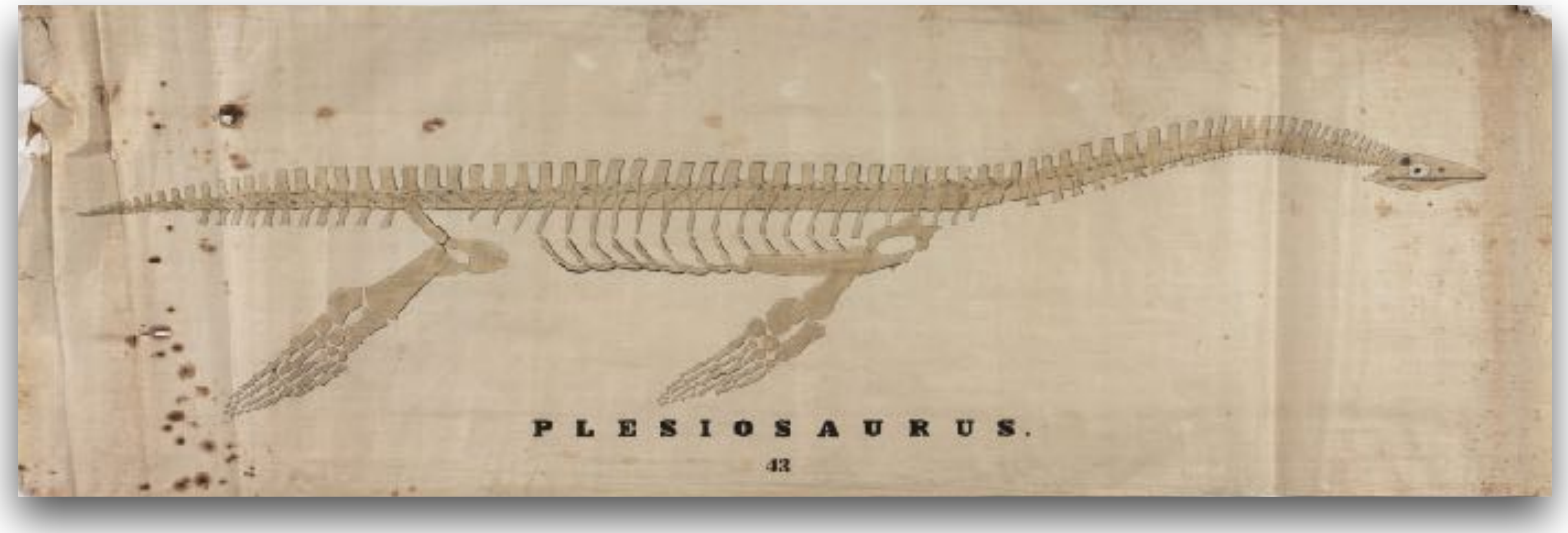
Support Metrics





Phylogenetic trees are just …

Phylogenetic trees are just … hypotheses!

It is important to know how **confident** we can be in our findings…

These are the metrics we are going to explore:

- nonparametric & parametric bootstrap (**BPs**)
- Transfer Bootstrap Expectation (**TBE**)
- parametric & nonparametric jackknife
- approximate Likelihood Ratio Test (**aLRT**)
- genes & sites Concordance Factors (**gCFs** & **sCFs**)
- Posterior Probabilities (**PP**)
- Quartet Support (**QS**) … will see that in **lesson 13**!

non-parametric bootstrapping

Is a general statistical method for estimating the parameters of an unknown probability distribution by resampling from a sample that was drawn from this distribution.

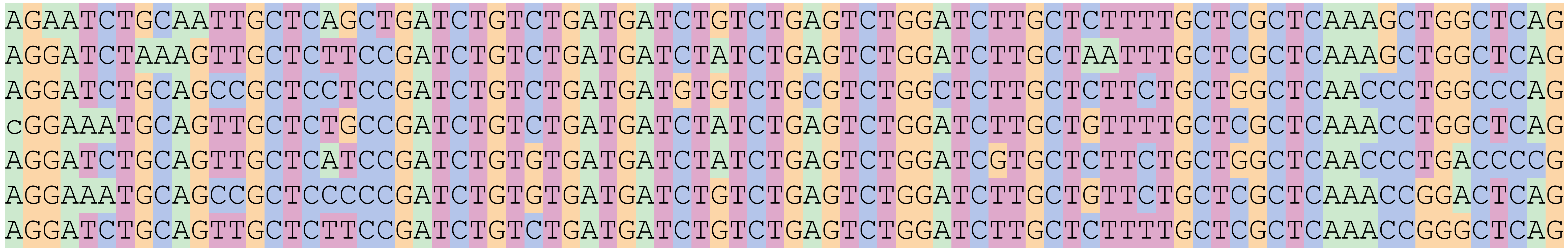
Transferred to phylogenetics by **Felsenstein** in this paper from 1985.

This is the process step-by-step:

- start with an alignment of length **n**
- generate a bootstrapped alignment:
  - randomly sample **n** columns with replacement
  - arrange the new columns into a bootstrapped alignment of length **n**
- build a tree from the bootstrapped alignment with the same method as for the original alignment
- repeat the process many times and store all resulting bootstrap trees
- the **bootstrap proportion (BPs)** is the percentage of trees containing a given bipartition

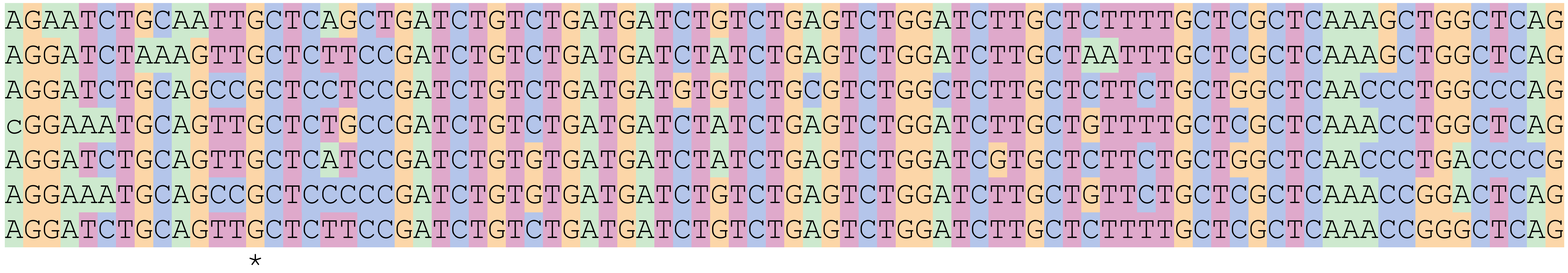
It reflects the robustness of the signal, not a direct probability that the branch is correct.

BPs are typically shown on the “*best tree*” or the majority-rule consensus of bootstrap trees.



```
species 1   AGAATCTGCAATTGCTCAGCTGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAAGCTGGCTCAG
species 2   AGGATCTAAAGTTGCTCTTCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTAATTTGCTCGCTCAAAGCTGGCTCAG
species 3   AGGATCTGCAGCCGCTCCTCCGATCTGTCTGATGATGTGTCTGCGTCTGGCTCTTGCTCTTCTGCTGGCTCAACCCTGGCCCAG
species 4   cGGAAATGCAGTTGCTCTGCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTGTTTTGCTCGCTCAAACCTGGCTCAG
species 5   AGGATCTGCAGTTGCTCATCCGATCTGTGTGATGATCTATCTGAGTCTGGATCGTGCTCTTCTGCTGGCTCAACCCTGACCCCG
species 6   AGGAAATGCAGCCGCTCCCCCGATCTGTGTGATGATCTGTCTGAGTCTGGATCTTGCTGTTCTGCTCGCTCAAACCGGACTCAG
species 7   AGGATCTGCAGTTGCTCTTCCGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAACCGGGCTCAG
```

species 1
species 2
species 3
species 4
species 5
species 6
species 7



```
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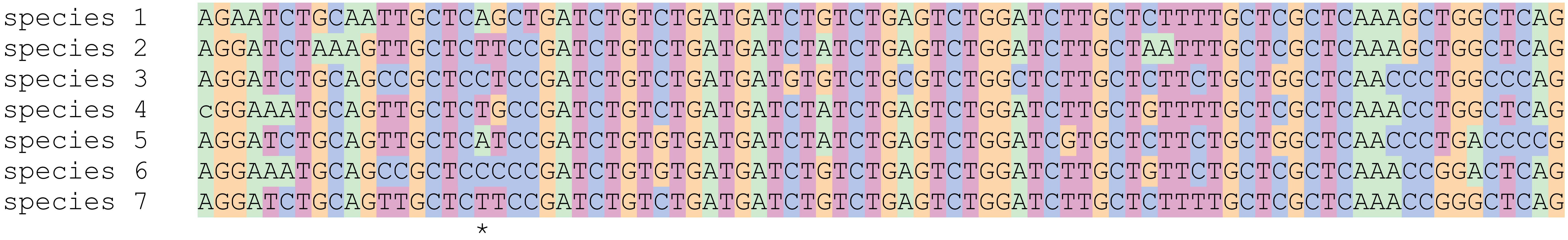
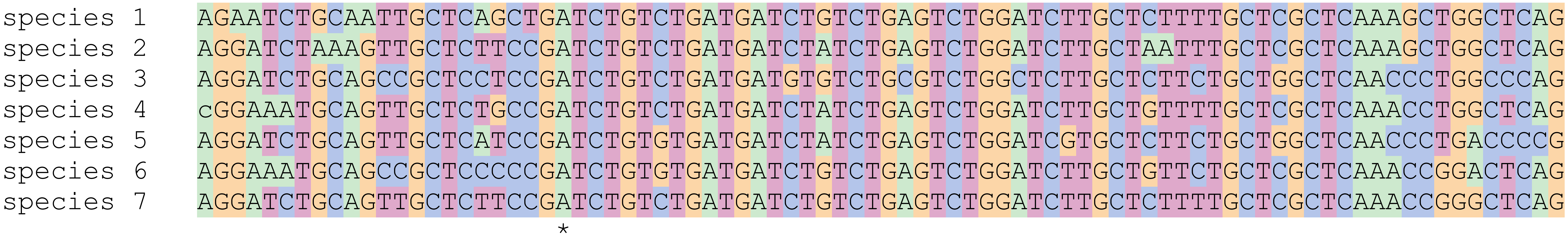
species 2   AGGATCTAAAGTTGCTCTTCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTAATTTGCTCGCTCAAAGCTGGCTCAG
species 3   AGGATCTGCAGCCGCTCCTCCGATCTGTCTGATGATGTGTCTGCGTCTGGCTCTTGCTCTTCTGCTGGCTCAACCCTGGCCCAG
species 4   cGGAAATGCAGTTGCTCTGCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTGTTTTGCTCGCTCAAACCTGGCTCAG
species 5   AGGATCTGCAGTTGCTCATCCGATCTGTGTGATGATCTATCTGAGTCTGGATCGTGCTCTTCTGCTGGCTCAACCCTGACCCCG
species 6   AGGAAATGCAGCCGCTCCCCCGATCTGTGTGATGATCTGTCTGAGTCTGGATCTTGCTGTTCTGCTCGCTCAAACCGGACTCAG
species 7   AGGATCTGCAGTTGCTCTTCCGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAACCGGGCTCAG
                         *
```

```
species 1   G
species 2   G
species 3   G
species 4   G
species 5   G
species 6   G
species 7   G
```



```
species 1   AGAATCTGCAATTGCTCAGCTGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAAGCTGGCTCAG
species 2   AGGATCTAAAGTTGCTCTTCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTAATTTGCTCGCTCAAAGCTGGCTCAG
species 3   AGGATCTGCAGCCGCTCCTCCGATCTGTCTGATGATGTGTCTGCGTCTGGCTCTTGCTCTTCTGCTGGCTCAACCCTGGCCCAG
species 4   cGGAAATGCAGTTGCTCTGCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTGTTTTGCTCGCTCAAACCTGGCTCAG
species 5   AGGATCTGCAGTTGCTCATCCGATCTGTGTGATGATCTATCTGAGTCTGGATCGTGCTCTTCTGCTGGCTCAACCCTGACCCCG
species 6   AGGAAATGCAGCCGCTCCCCCGATCTGTGTGATGATCTGTCTGAGTCTGGATCTTGCTGTTCTGCTCGCTCAAACCGGACTCAG
species 7   AGGATCTGCAGTTGCTCTTCCGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAACCGGGCTCAG
                             *
```

```
species 1   GA
species 2   GT
species 3   GC
species 4   GT
species 5   GA
species 6   GC
species 7   GT
```



```
species 1   AGAATCTGCAATTGCTCAGCTGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAAGCTGGCTCAG
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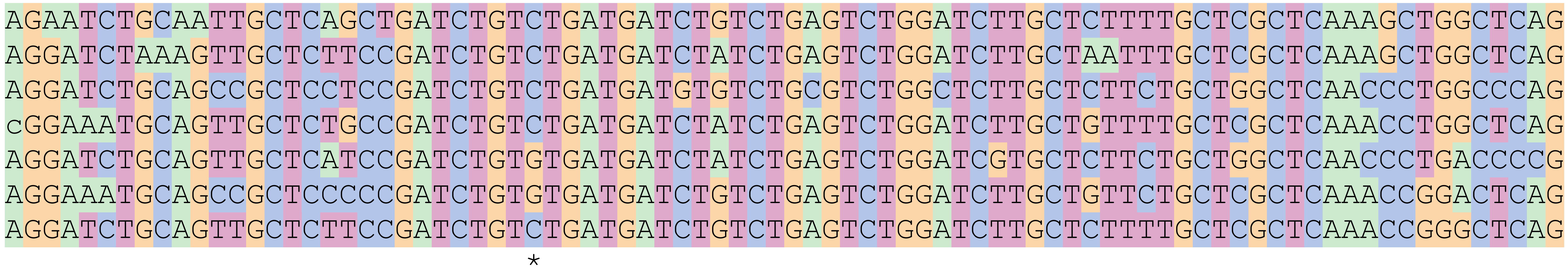
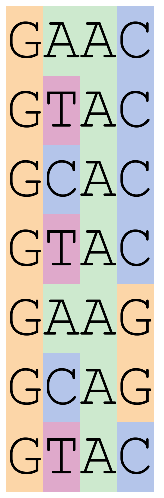
species 3   AGGATCTGCAGCCGCTCCTCCGATCTGTCTGATGATGTGTCTGCGTCTGGCTCTTGCTCTTCTGCTGGCTCAACCCTGGCCCAG
species 4   cGGAAATGCAGTTGCTCTGCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTGTTTTGCTCGCTCAAACCTGGCTCAG
species 5   AGGATCTGCAGTTGCTCATCCGATCTGTGTGATGATCTATCTGAGTCTGGATCGTGCTCTTCTGCTGGCTCAACCCTGACCCCG
species 6   AGGAAATGCAGCCGCTCCCCCGATCTGTGTGATGATCTGTCTGAGTCTGGATCTTGCTGTTCTGCTCGCTCAAACCGGACTCAG
species 7   AGGATCTGCAGTTGCTCTTCCGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAACCGGGCTCAG
                                  *
```

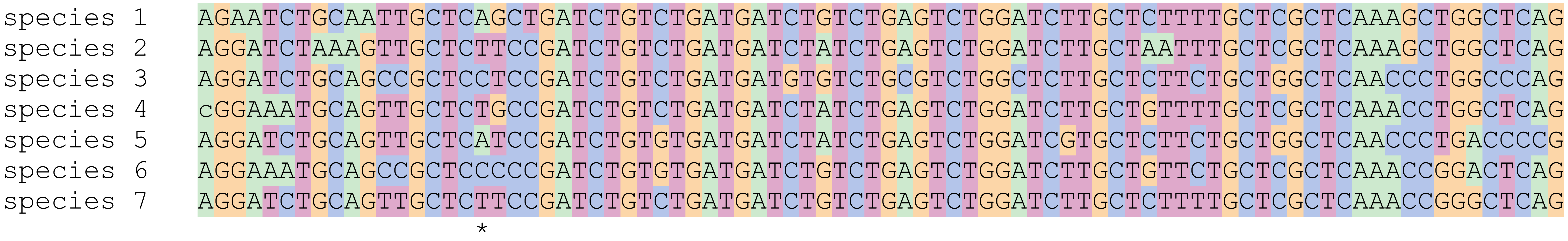
```
species 1   GAA
species 2   GTA
species 3   GCA
species 4   GTA
species 5   GAA
species 6   GCA
species 7   GTA
```



```
species 1   AGAATCTGCAATTGCTCAGCTGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAAGCTGGCTCAG
species 2   AGGATCTAAAGTTGCTCTTCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTAATTTGCTCGCTCAAAGCTGGCTCAG
species 3   AGGATCTGCAGCCGCTCCTCCGATCTGTCTGATGATGTGTCTGCGTCTGGCTCTTGCTCTTCTGCTGGCTCAACCCTGGCCCAG
species 4   cGGAAATGCAGTTGCTCTGCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTGTTTTGCTCGCTCAAACCTGGCTCAG
species 5   AGGATCTGCAGTTGCTCATCCGATCTGTGTGATGATCTATCTGAGTCTGGATCGTGCTCTTCTGCTGGCTCAACCCTGACCCCG
species 6   AGGAAATGCAGCCGCTCCCCCGATCTGTGTGATGATCTGTCTGAGTCTGGATCTTGCTGTTCTGCTCGCTCAAACCGGACTCAG
species 7   AGGATCTGCAGTTGCTCTTCCGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAACCGGGCTCAG
                                        *
```

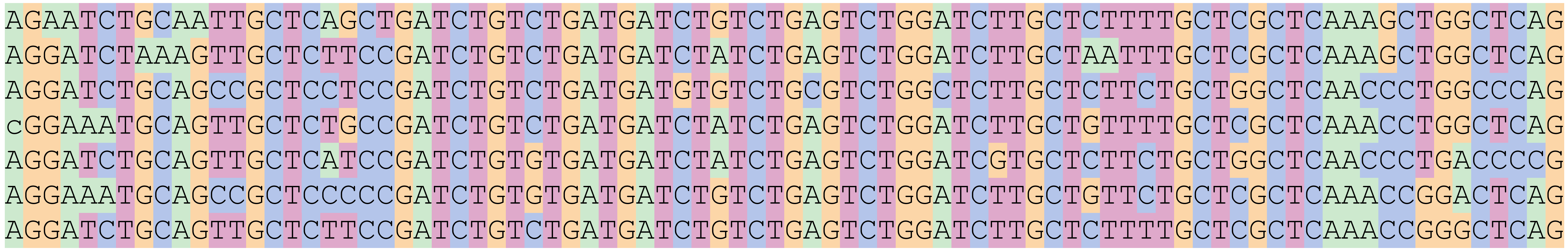


```
species 1   GAAC
species 2   GTAC
species 3   GCAC
species 4   GTAC
species 5   GAAG
species 6   GCAG
species 7   GTAC
```

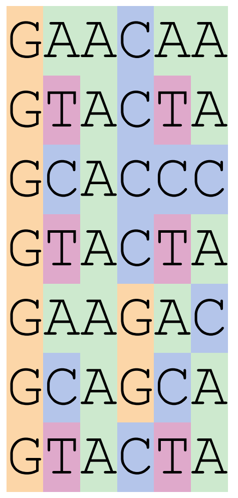


```
species 1   AGAATCTGCAATTGCTCAGCTGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAAGCTGGCTCAG
species 2   AGGATCTAAAGTTGCTCTTCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTAATTTGCTCGCTCAAAGCTGGCTCAG
species 3   AGGATCTGCAGCCGCTCCTCCGATCTGTCTGATGATGTGTCTGCGTCTGGCTCTTGCTCTTCTGCTGGCTCAACCCTGGCCCAG
species 4   cGGAAATGCAGTTGCTCTGCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTGTTTTGCTCGCTCAAACCTGGCTCAG
species 5   AGGATCTGCAGTTGCTCATCCGATCTGTGTGATGATCTATCTGAGTCTGGATCGTGCTCTTCTGCTGGCTCAACCCTGACCCCG
species 6   AGGAAATGCAGCCGCTCCCCCGATCTGTGTGATGATCTGTCTGAGTCTGGATCTTGCTGTTCTGCTCGCTCAAACCGGACTCAG
species 7   AGGATCTGCAGTTGCTCTTCCGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAACCGGGCTCAG
                             *
```

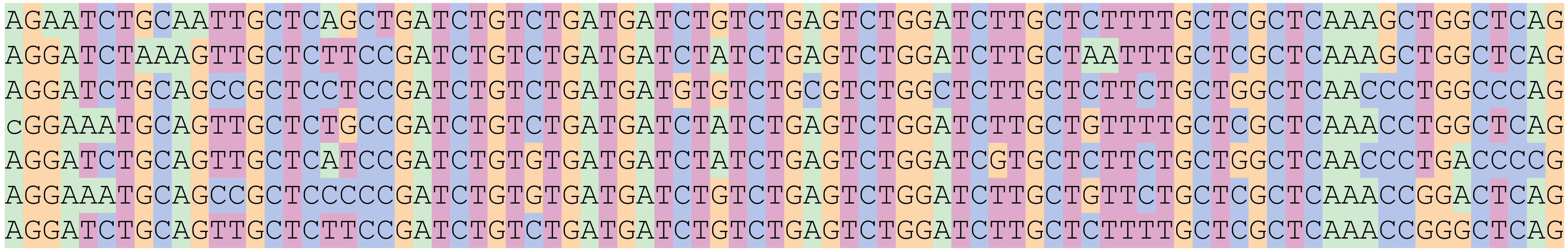
```
species 1   GAACA
species 2   GTACT
species 3   GCACC
species 4   GTACT
species 5   GAAGA
species 6   GCAGC
species 7   GTACT
```



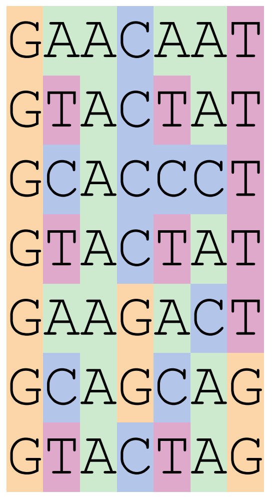
```
species 1    AGAATCTGCAATTGCTCAGCTGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAAGCTGGCTCAG
species 2    AGGATCTAAAGTTGCTCTTCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTAATTTGCTCGCTCAAAGCTGGCTCAG
species 3    AGGATCTGCAGCCGCTCCTCCGATCTGTCTGATGATGTGTCTGCGTCTGGCTCTTGCTCTTCTGCTGGCTCAACCCTGGCCCAG
species 4    cGGAAATGCAGTTGCTCTGCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTGTTTTGCTCGCTCAAACCTGGCTCAG
species 5    AGGATCTGCAGTTGCTCATCCGATCTGTGTGATGATCTATCTGAGTCTGGATCGTGCTCTTCTGCTGGCTCAACCCTGACCCCG
species 6    AGGAAATGCAGCCGCTCCCCCGATCTGTGTGATGATCTGTCTGAGTCTGGATCTTGCTGTTCTGCTCGCTCAAACCGGACTCAG
species 7    AGGATCTGCAGTTGCTCTTCCGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAACCGGGCTCAG
                                                                                      *
```



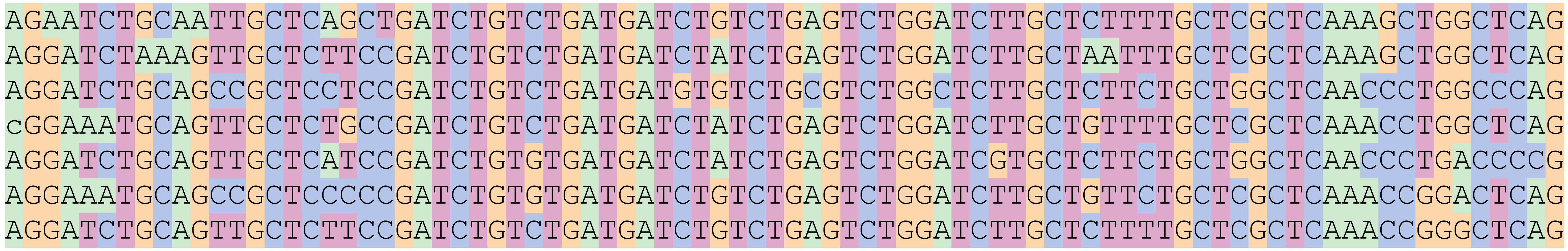
```
species 1    GAACAA
species 2    GTACTA
species 3    GCACCC
species 4    GTACTA
species 5    GAAGAC
species 6    GCAGCA
species 7    GTACTA
```



```
species 1    AGAATCTGCAATTGCTCAGCTGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAAGCTGGCTCAG
species 2    AGGATCTAAAGTTGCTCTTCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTAATTTGCTCGCTCAAAGCTGGCTCAG
species 3    AGGATCTGCAGCCGCTCCTCCGATCTGTCTGATGATGTGTCTGCGTCTGGCTCTTGCTCTTCTGCTGGCTCAACCCTGGCCCAG
species 4    cGGAAATGCAGTTGCTCTGCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTGTTTTGCTCGCTCAAACCTGGCTCAG
species 5    AGGATCTGCAGTTGCTCATCCGATCTGTGTGATGATCTATCTGAGTCTGGATCGTGCTCTTCTGCTGGCTCAACCCTGACCCCG
species 6    AGGAAATGCAGCCGCTCCCCCGATCTGTGTGATGATCTGTCTGAGTCTGGATCTTGCTGTTCTGCTCGCTCAAACCGGACTCAG
species 7    AGGATCTGCAGTTGCTCTTCCGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAACCGGGCTCAG
                                                                                         *
```



```
species 1    GAACAAT
species 2    GTACTAT
species 3    GCACCCT
species 4    GTACTAT
species 5    GAAGACT
species 6    GCAGCAG
species 7    GTACTAG
```

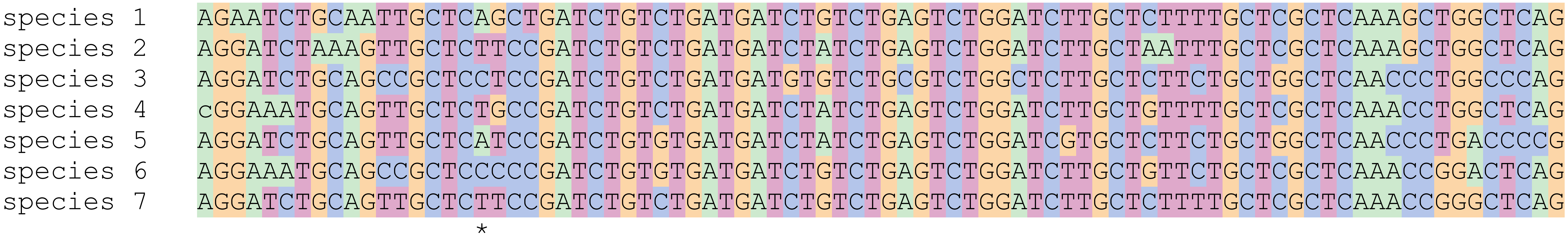


```
species 1  AGAATCTGCAATTGCTCAGCTGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAAGCTGGCTCAG
species 2  AGGATCTAAAGTTGCTCTTCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTAATTTGCTCGCTCAAAGCTGGCTCAG
species 3  AGGATCTGCAGCCGCTCCTCCGATCTGTCTGATGATGTGTCTGCGTCTGGCTCTTGCTCTTCTGCTGGCTCAACCCTGGCCCAG
species 4  cGGAAATGCAGTTGCTCTGCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTGTTTTGCTCGCTCAAACCTGGCTCAG
species 5  AGGATCTGCAGTTGCTCATCCGATCTGTGTGATGATCTATCTGAGTCTGGATCGTGCTCTTCTGCTGGCTCAACCCTGACCCCG
species 6  AGGAAATGCAGCCGCTCCCCCGATCTGTGTGATGATCTGTCTGAGTCTGGATCTTGCTGTTCTGCTCGCTCAAACCGGACTCAG
species 7  AGGATCTGCAGTTGCTCTTCCGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAACCGGGCTCAG
                                         *
```

```
species 1  GAACAATG
species 2  GTACTATG
species 3  GCACCCTG
species 4  GTACTATG
species 5  GAAGACTG
species 6  GCAGCAGG
species 7  GTACTAGG
```

```
species 1    AGAATCTGCAATTGCTCAGCTGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAAGCTGGCTCAG
species 2    AGGATCTAAAGTTGCTCTTCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTAATTTGCTCGCTCAAAGCTGGCTCAG
species 3    AGGATCTGCAGCCGCTCCTCCGATCTGTCTGATGATGTGTCTGCGTCTGGCTCTTGCTCTTCTGCTGGCTCAACCCTGGCCCAG
species 4    cGGAAATGCAGTTGCTCTGCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTGTTTTGCTCGCTCAAACCTGGCTCAG
species 5    AGGATCTGCAGTTGCTCATCCGATCTGTGTGATGATCTATCTGAGTCTGGATCGTGCTCTTCTGCTGGCTCAACCCTGACCCCG
species 6    AGGAAATGCAGCCGCTCCCCCGATCTGTGTGATGATCTGTCTGAGTCTGGATCTTGCTGTTCTGCTCGCTCAAACCGGACTCAG
species 7    AGGATCTGCAGTTGCTCTTCCGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAACCGGGCTCAG
                                                                  *
```

```
species 1    GAACAATGGT
species 2    GTACTATGTT
species 3    GCACCCTGTT
species 4    GTACTATGGT
species 5    GAAGACTGTG
species 6    GCAGCAGGCT
species 7    GTACTAGGTT
```



```
species 1   AGAATCTGCAATTGCTCAGCTGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAAGCTGGCTCAG
species 2   AGGATCTAAAGTTGCTCTTCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTAATTTGCTCGCTCAAAGCTGGCTCAG
species 3   AGGATCTGCAGCCGCTCCTCCGATCTGTCTGATGATGTGTCTGCGTCTGGCTCTTGCTCTTCTGCTGGCTCAACCCTGGCCCAG
species 4   cGGAAATGCAGTTGCTCTGCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTGTTTTGCTCGCTCAAACCTGGCTCAG
species 5   AGGATCTGCAGTTGCTCATCCGATCTGTGTGATGATCTATCTGAGTCTGGATCGTGCTCTTCTGCTGGCTCAACCCTGACCCCG
species 6   AGGAAATGCAGCCGCTCCCCCGATCTGTGTGATGATCTGTCTGAGTCTGGATCTTGCTGTTCTGCTCGCTCAAACCGGACTCAG
species 7   AGGATCTGCAGTTGCTCTTCCGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAACCGGGCTCAG
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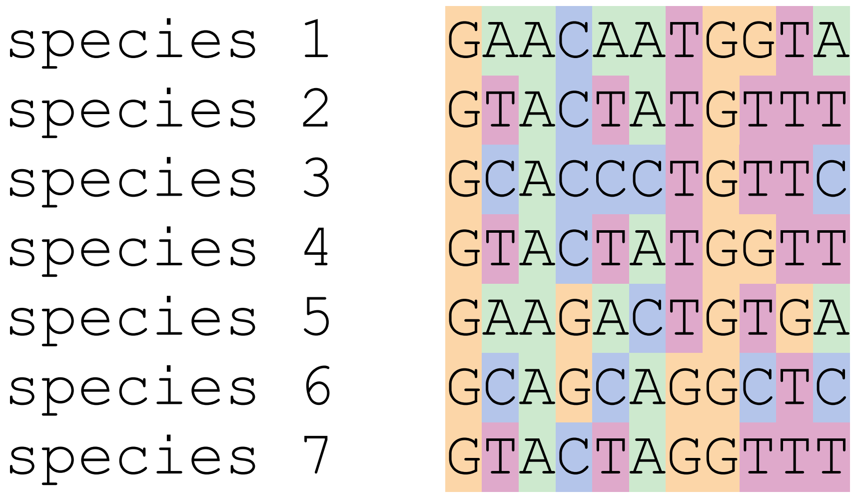
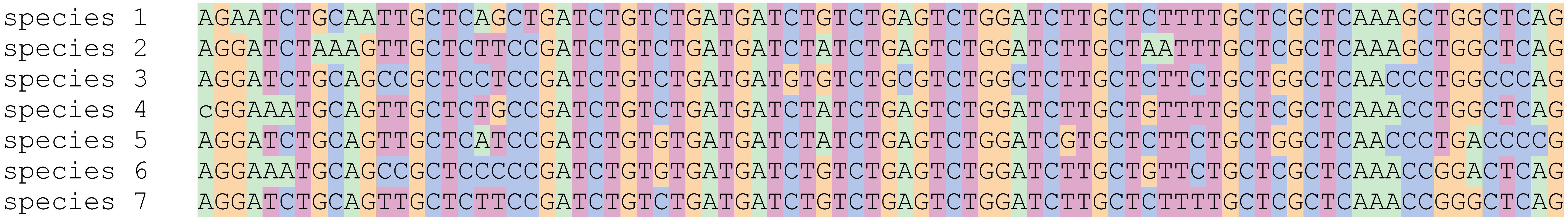


```
species 1   GAACAATGGTA
species 2   GTACTATGTTT
species 3   GCACCCTGTTC
species 4   GTACTATGGTT
species 5   GAAGACTGTGA
species 6   GCAGCAGGCTC
species 7   GTACTAGGTTT
```

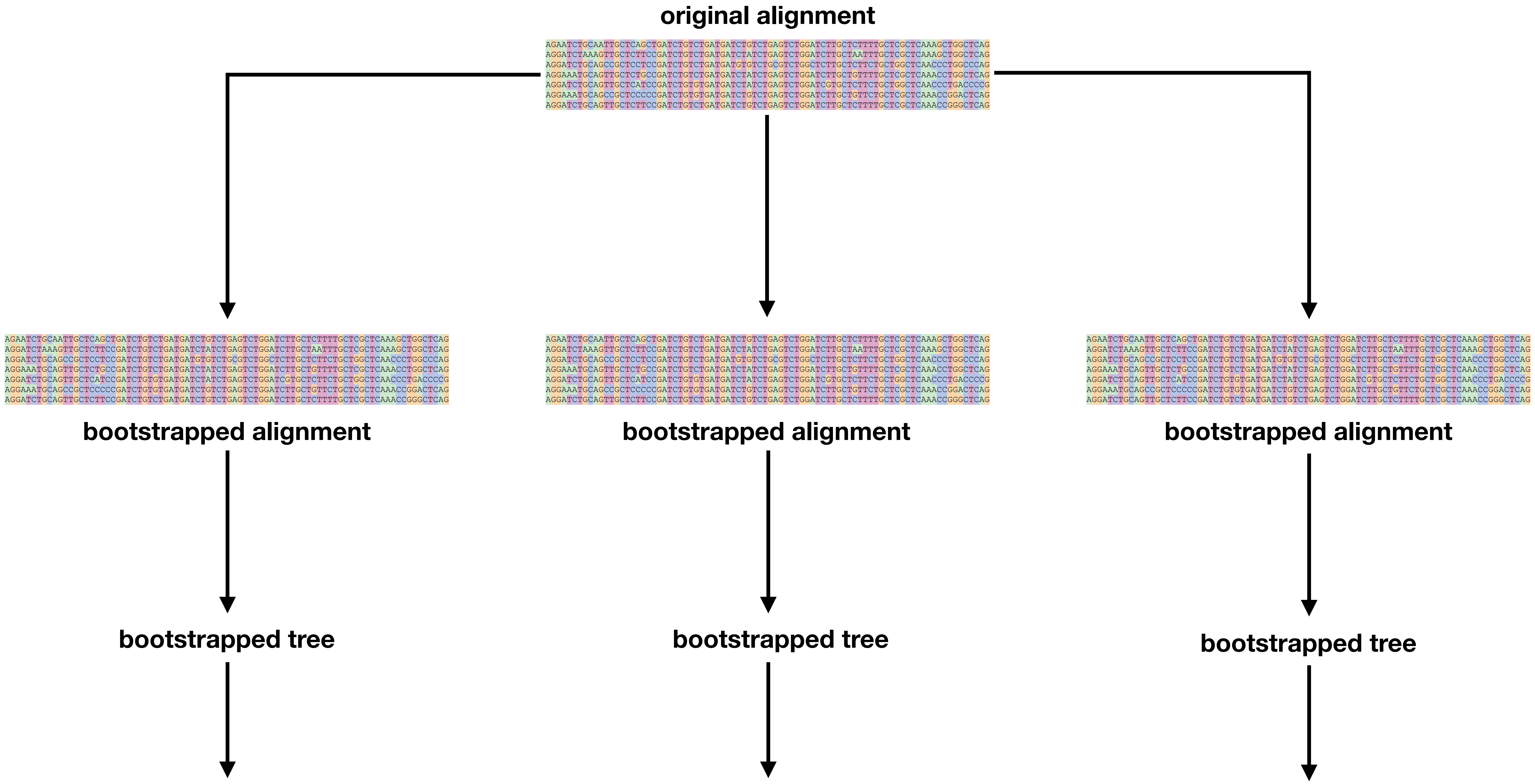


```
species 1    AGAATCTGCAATTGCTCAGCTGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAAGCTGGCTCAG
species 2    AGGATCTAAAGTTGCTCTTCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTAATTTGCTCGCTCAAAGCTGGCTCAG
species 3    AGGATCTGCAGCCGCTCCTCCGATCTGTCTGATGATGTGTCTGCGTCTGGCTCTTGCTCTTCTGCTGGCTCAACCCTGGCCCAG
species 4    cGGAAATGCAGTTGCTCTGCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTGTTTTGCTCGCTCAAACCTGGCTCAG
species 5    AGGATCTGCAGTTGCTCATCCGATCTGTGTGATGATCTATCTGAGTCTGGATCGTGCTCTTCTGCTGGCTCAACCCTGACCCCG
species 6    AGGAAATGCAGCCGCTCCCCCGATCTGTGTGATGATCTGTCTGAGTCTGGATCTTGCTGTTCTGCTCGCTCAACCGGACTCAG
species 7    AGGATCTGCAGTTGCTCTTCCGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAACCGGGCTCAG
```

```
species 1    GAACAATGGTA…                                          …AGTAAA
species 2    GTACTATGTTT…                                          …TGTTAT
species 3    GCACCCTGTTC…                                          …CGTCAC
species 4    GTACTATGGTT…                                          …TGTTAT
species 5    GAAGACTGTGA…                                          …AGTAAA
species 6    GCAGCAGGCTC…                                          …CGGCAC
species 7    GTACTAGGTTT…                                          …TGGTAT
```

Sampling with replacement!

Is the same information present in this bootstrapped alignment?



original alignment

bootstrapped alignment

bootstrapped alignment

bootstrapped alignment

bootstrapped tree

bootstrapped tree

bootstrapped tree

node	split is present?	split is present?	split is present?	BPs
1	✅	✅	✅	100%
2	✅	❌	❌	33%
3	✅	✅	✅	100%
4	❌	❌	❌	0%
5	❌	✅	✅	66%
..	…	…	…	…
last	✅	✅	✅	100%

parametric bootstrapping

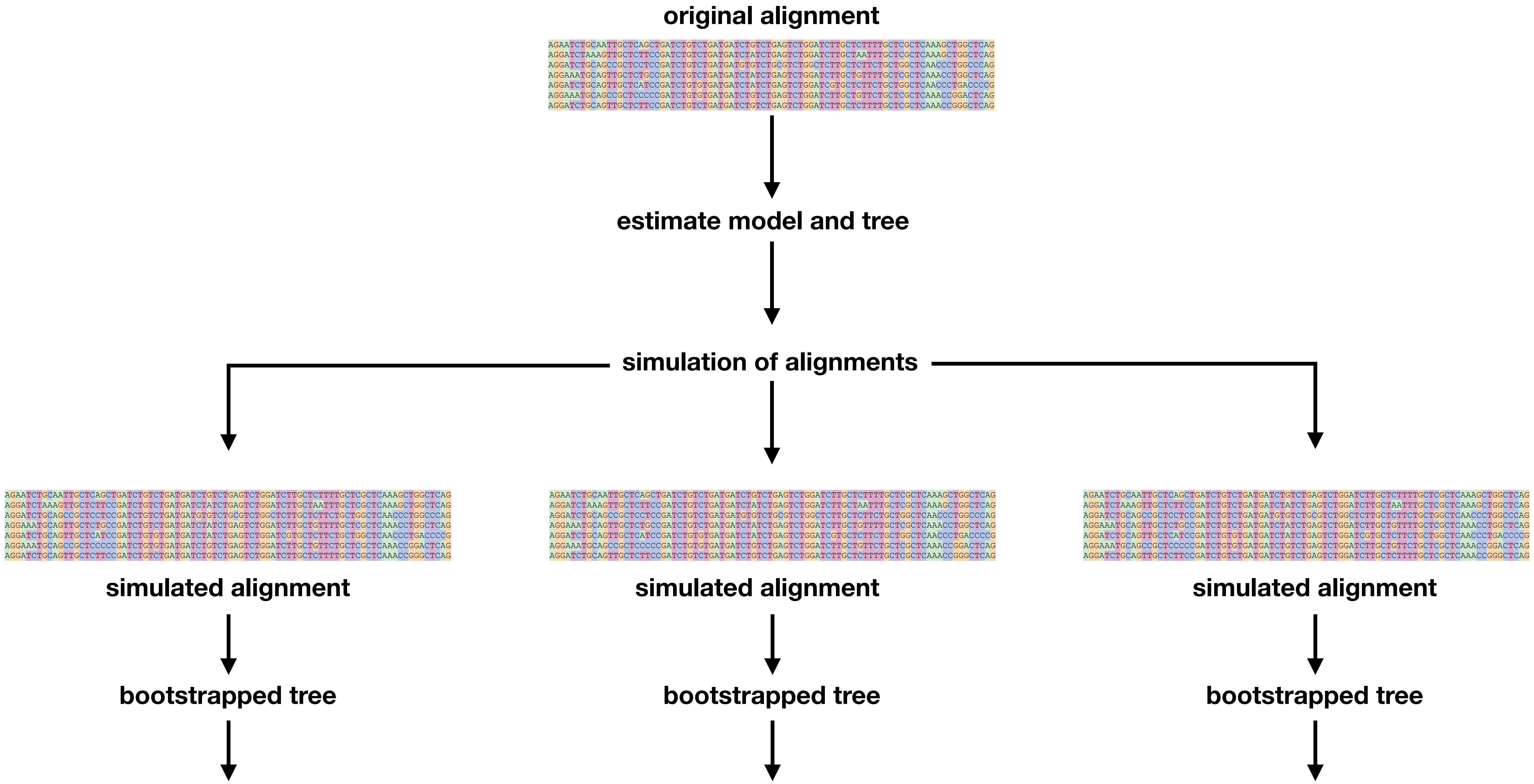
A **model-based approach** to estimate support using simulated data.

This is the process step-by-step:

- estimate from the original alignment **X**:
  - model of substitution
  - a phylogenetic tree **T**
- use the model and tree T to simulate replicate alignments $\mathbf{X_i}$
- phylogenetic trees $\mathbf{T_i}$ are inferred for each of the alignments $\mathbf{X_i}$
- branch support values are derived - as in non-parametric bootstrapping - by computing the percentage with which a certain branch occurs in the set of generated trees $\mathbf{T_i}$

⚠️ Important:

- parametric bootstrap values **depend heavily on the model** estimated from the original data
- if the model is misspecified, support values may be biased or misleading



original alignment

estimate model and tree

simulation of alignments

simulated alignment **simulated alignment** **simulated alignment**

bootstrapped tree **bootstrapped tree** **bootstrapped tree**

node	**split is present?**	**split is present?**	**split is present?**	**BPs**
1	✅	✅	✅	100%
2	✅	❌	❌	33%
3	✅	✅	✅	100%
4	❌	❌	❌	0%
5	❌	✅	✅	66%
..	…	…	…	…
last	✅	✅	✅	100%

transfer bootstrap expectation (TBE)

The bootstrap is used extensively 🤩 but scaling to thousands of tips introduces new challenges 🤯. Felsenstein BP yield **low support on deep branches**

Think about a deep branch in a tree with a thousand tips. Even if 999 out of 1000 taxa are placed correctly but one rogue taxon jumps to the wrong side, the entire branch is scored as absent.

This is due to its **binary scoring** system: a branch is either **present** or **absent** in a replicate tree.

Lemoine et al. 2018 introduced TBE which uses a **transfer index based on gradual distances** of a branch in the replicate tree to the reference branch, capturing partial support in bootstrap trees. In detail:

- for a reference clade **R**, split the tips into two complementary groups.
- for each bipartition **B** in the bootstrap tree, compute the transfer distance: that is the minimum number of tips that need to be moved from one side to the other so that **B** matches **R**
- select the branch with the smallest transfer distance - i.e. the best-matching clade
- compute the transfer index from this match: $\text{Transfer Index} = 1 - \dfrac{\text{Transfer Distance}}{\min(|R|, |\neg R|)}$
- averaging the transfer indices across all replicates gives the TBE value for the clade **R**

jackknife

A resampling method related to BPs but uses **deletion rather than resampling** with replacement.

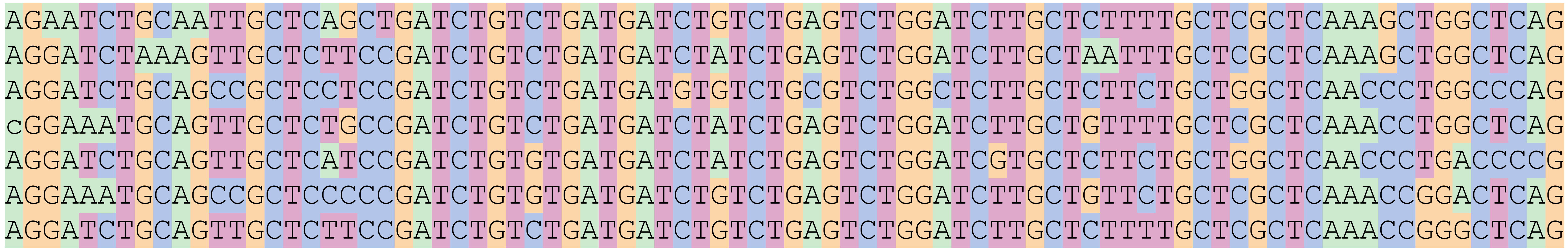
This is the process step-by-step:

- generate a jackknifed alignment from the starting alignment
    - randomly delete a fixed proportion of alignment columns
    - typically, 50% of sites are removed per replicate as in Felsenstein 1985
    - equivalent to drawing n/2 sites without replacement from an alignment of length n
- build a tree from the jackknifed alignment with the same method as for the original alignment
- repeat the process many times and store all resulting jackknife trees
- the **jackknife proportion** is the percentage of trees containing a given bipartition.

It reflects the consistence with the sequence data support that branch.

Jacknife is typically shown on the "*best tree*" or the majority-rule consensus of jackknife trees.

… a parametric version of the jackknife is also possible, but not shown!



```
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species 2   AGGATCTAAAGTTGCTCTTCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTAATTTGCTCGCTCAAAGCTGGCTCAG
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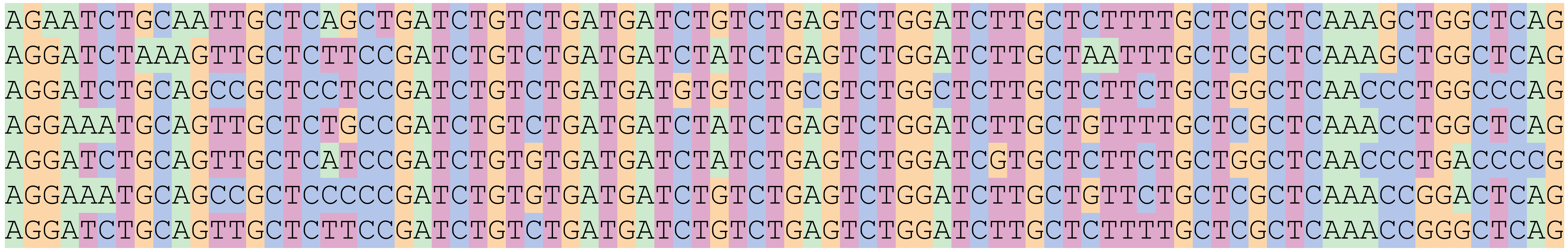

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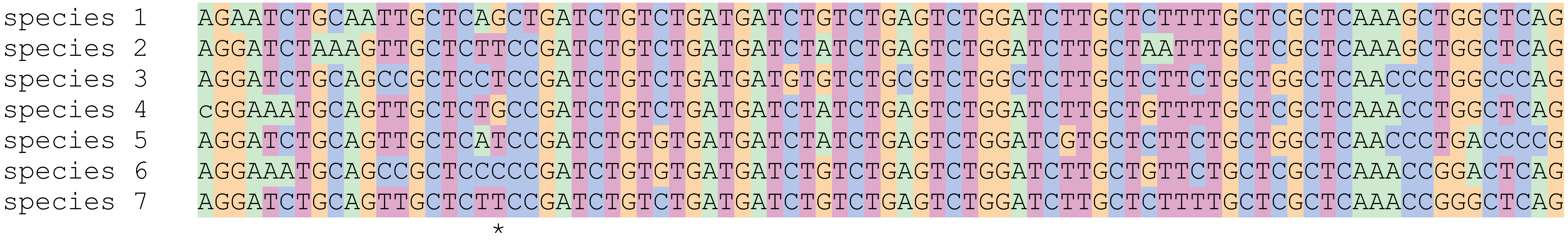

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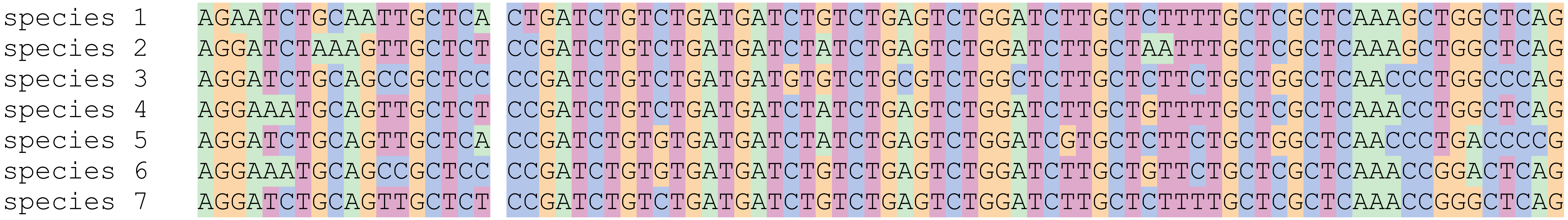

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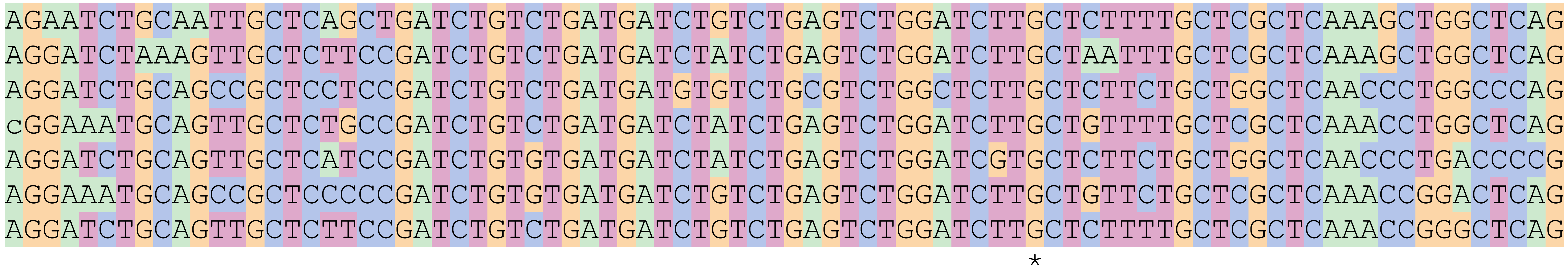

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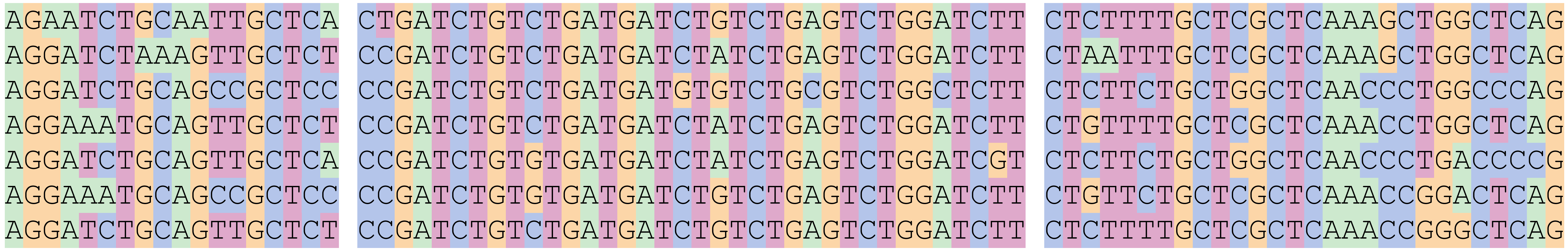

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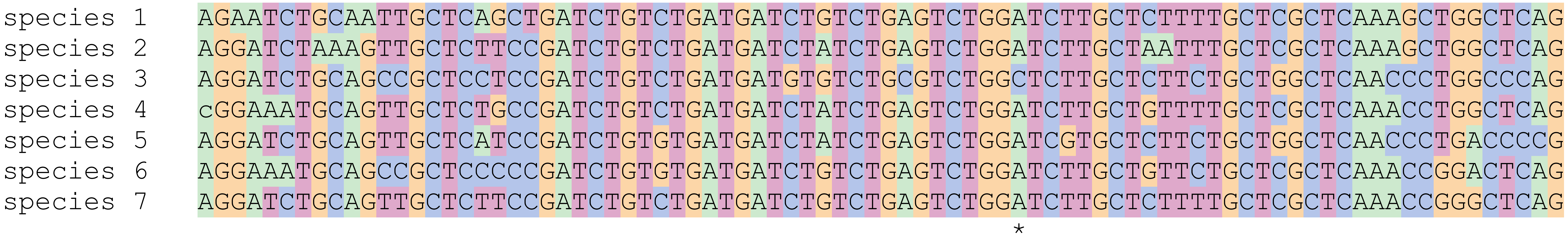

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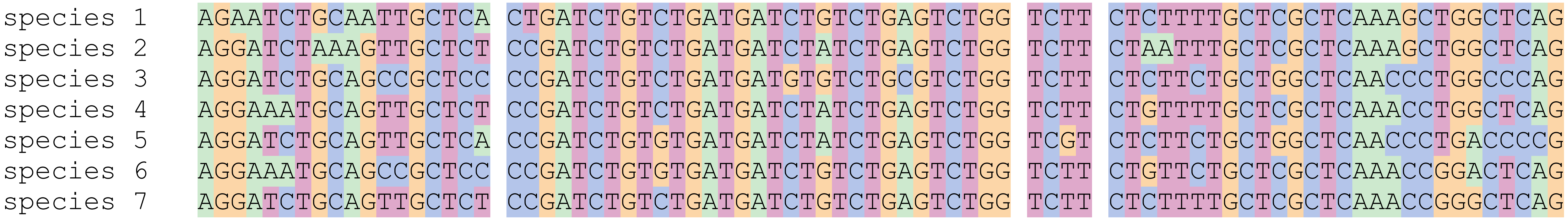

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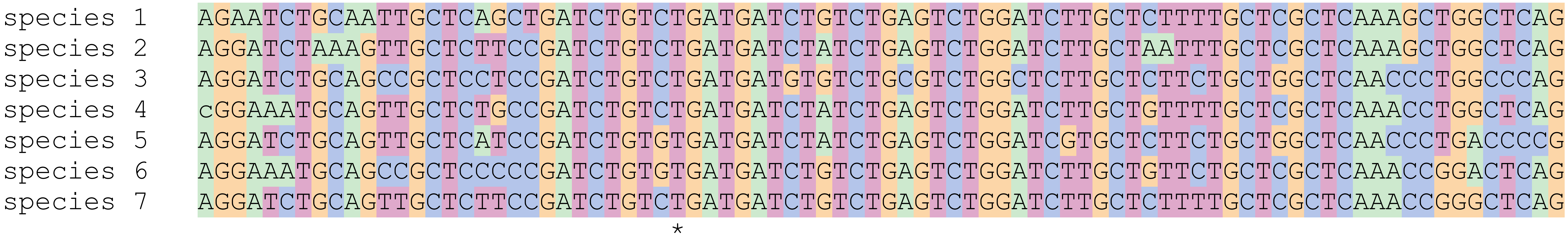

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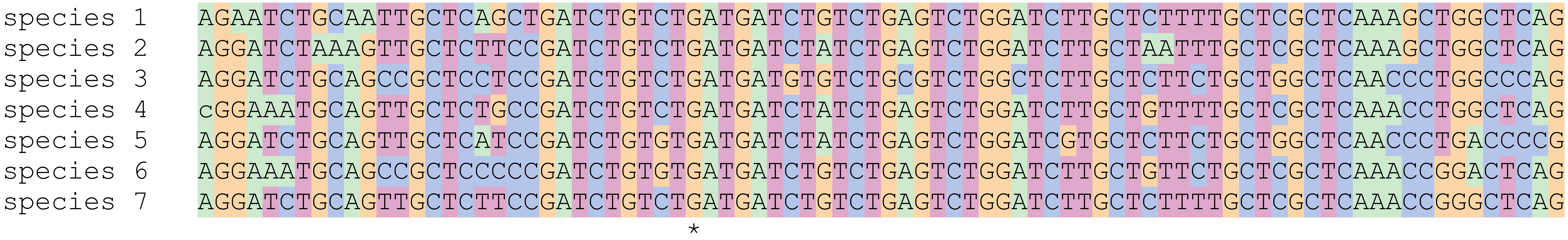

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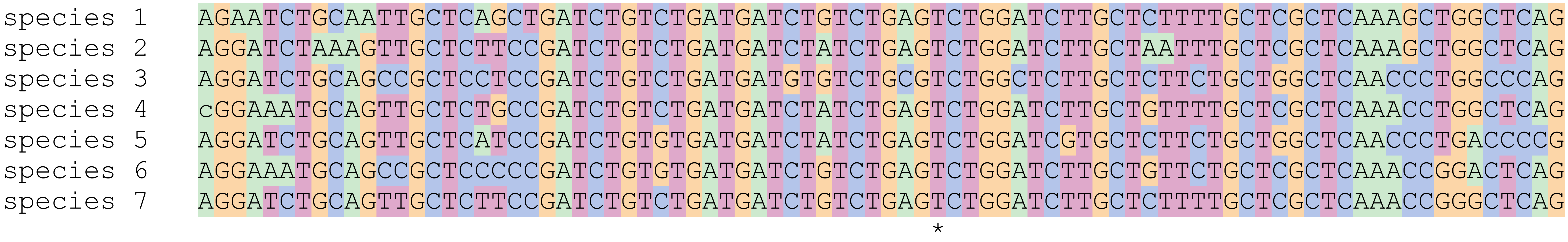

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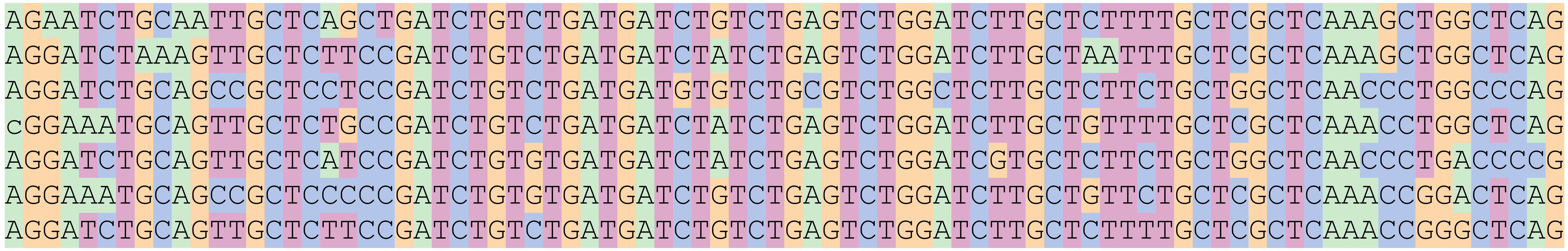

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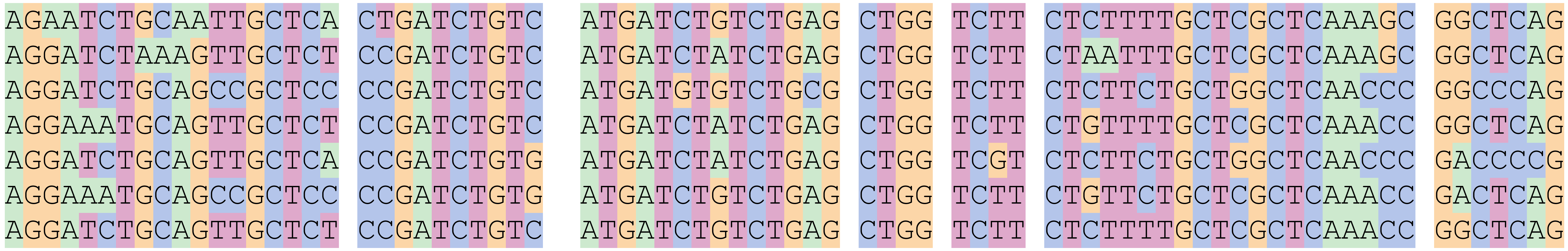

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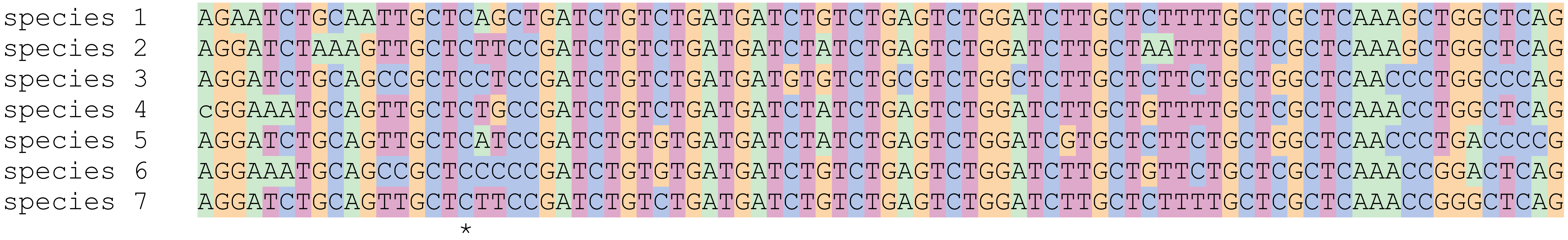

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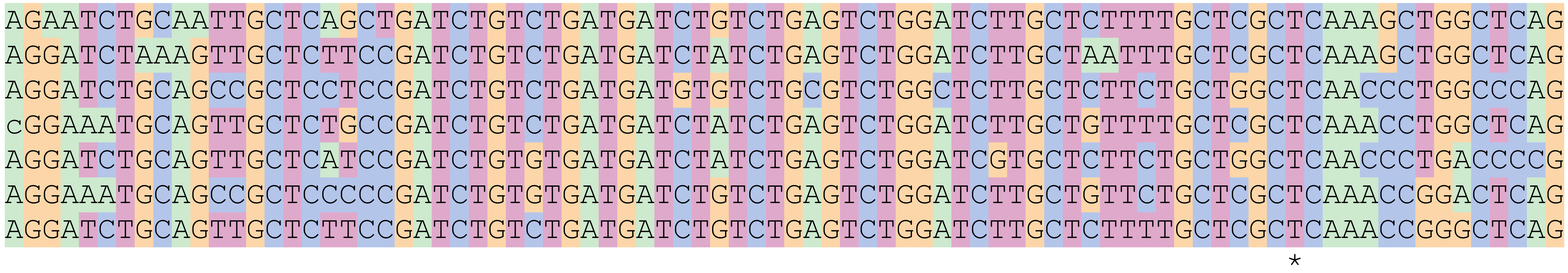

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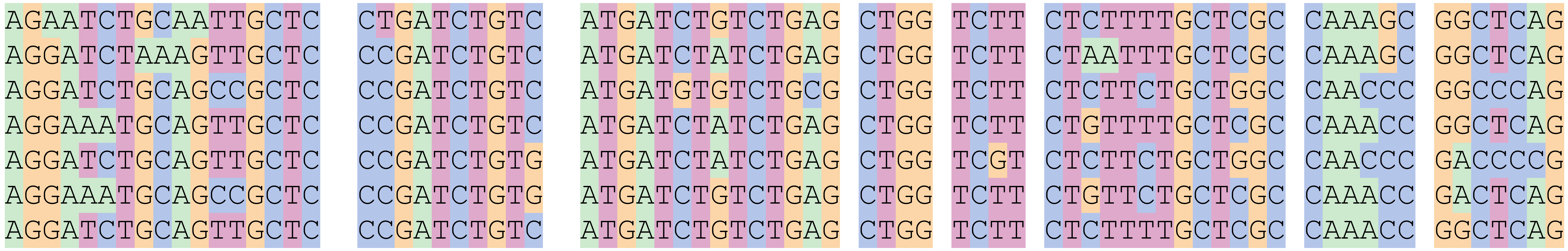

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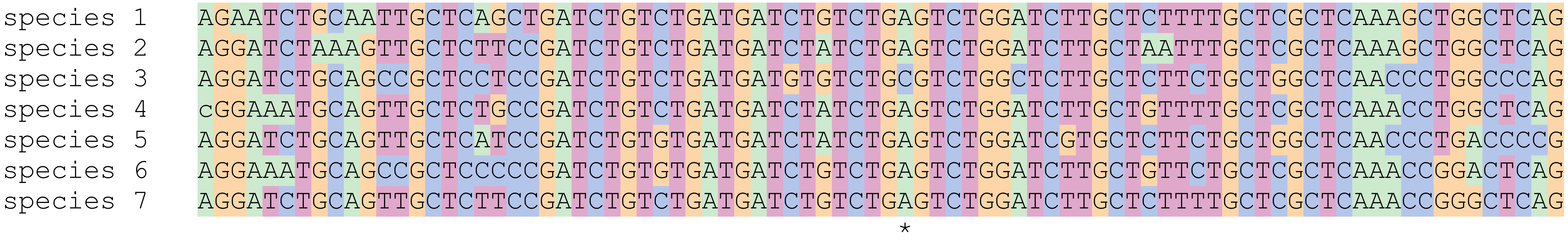

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species 2   AGGATCTAAAGTTGCTC  CCGATCTGTC  ATGATCTATCTG G CTGG TCTT CTAATTTGCTCGC CAAAGC GGCTCAG
species 3   AGGATCTGCAGCCGCTC  CCGATCTGTC  ATGATGTGTCTG G CTGG TCTT CTCTTCTGCTGGC CAACCC GGCCCAG
species 4   AGGAAATGCAGTTGCTC  CCGATCTGTC  ATGATCTATCTG G CTGG TCTT CTGTTTTGCTCGC CAAACC GGCTCAG
species 5   AGGATCTGCAGTTGCTC  CCGATCTGTG  ATGATCTATCTG G CTGG TCGT CTCTTCTGCTGGC CAACCC GACCCCG
species 6   AGGAAATGCAGCCGCTC  CCGATCTGTG  ATGATCTGTCTG G CTGG TCTT CTGTTCTGCTCGC CAAACC GACTCAG
species 7   AGGATCTGCAGTTGCTC  CCGATCTGTC  ATGATCTGTCTG G CTGG TCTT CTCTTTTGCTCGC CAAACC GGCTCAG
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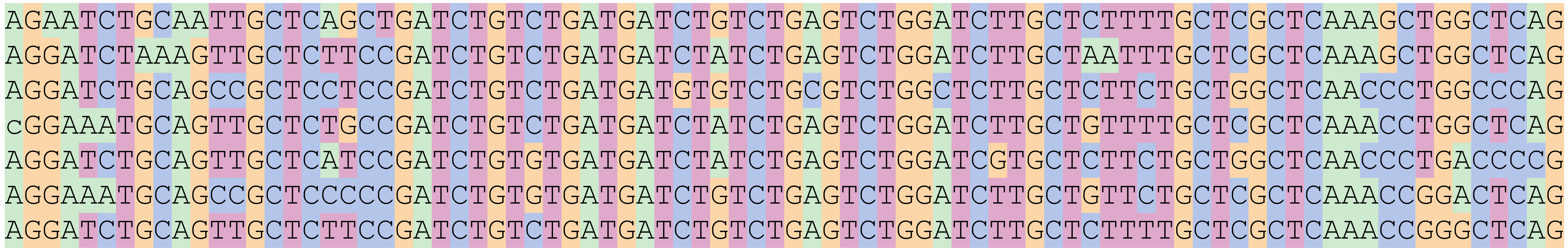

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species 1    AGAATCTGCAATTGCTCAGCTGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAAGCTGGCTCAG
species 2    AGGATCTAAAGTTGCTCTTCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTAATTTGCTCGCTCAAAGCTGGCTCAG
species 3    AGGATCTGCAGCCGCTCCTCCGATCTGTCTGATGATGTGTCTGCGTCTGGCTCTTGCTCTTCTGCTGGCTCAACCCTGGCCCAG
species 4    cGGAAATGCAGTTGCTCTGCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTGTTTTGCTCGCTCAAACCTGGCTCAG
species 5    AGGATCTGCAGTTGCTCATCCGATCTGTGTGATGATCTATCTGAGTCTGGATCGTGCTCTTCTGCTGGCTCAACCCTGACCCCG
species 6    AGGAAATGCAGCCGCTCCCCCGATCTGTGTGATGATCTGTCTGAGTCTGGATCTTGCTGTTCTGCTCGCTCAAACCGGACTCAG
species 7    AGGATCTGCAGTTGCTCTTCCGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAACCGGGCTCAG
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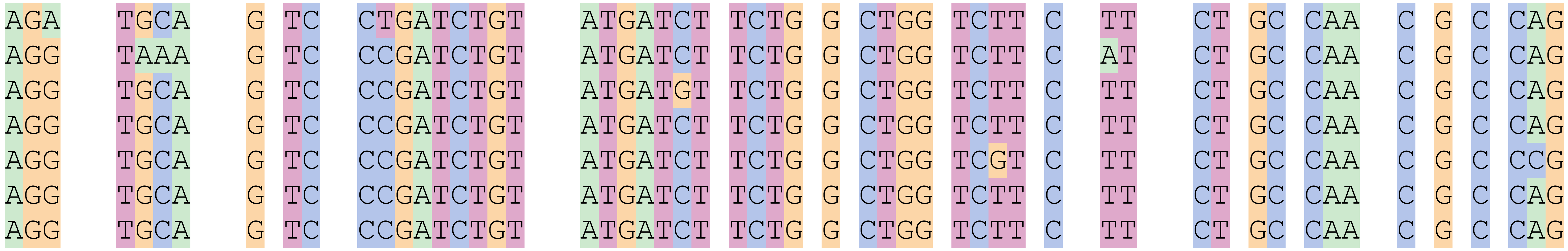

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species 1    AGA   TGCA   G TC  CTGATCTGT   ATGATCT TCTG G CTGG TCTT C  TT   CT GC CAA  C G C CAG
species 2    AGG   TAAA   G TC  CCGATCTGT   ATGATCT TCTG G CTGG TCTT C  AT   CT GC CAA  C G C CAG
species 3    AGG   TGCA   G TC  CCGATCTGT   ATGATGT TCTG G CTGG TCTT C  TT   CT GC CAA  C G C CAG
species 4    AGG   TGCA   G TC  CCGATCTGT   ATGATCT TCTG G CTGG TCTT C  TT   CT GC CAA  C G C CAG
species 5    AGG   TGCA   G TC  CCGATCTGT   ATGATCT TCTG G CTGG TCGT C  TT   CT GC CAA  C G C CCG
species 6    AGG   TGCA   G TC  CCGATCTGT   ATGATCT TCTG G CTGG TCTT C  TT   CT GC CAA  C G C CAG
species 7    AGG   TGCA   G TC  CCGATCTGT   ATGATCT TCTG G CTGG TCTT C  TT   CT GC CAA  C G C CAG
```

```
species 1    AGAATCTGCAATTGCTCAGCTGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAAGCTGGCTCAG
species 2    AGGATCTAAAGTTGCTCTTCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTAATTTGCTCGCTCAAAGCTGGCTCAG
species 3    AGGATCTGCAGCCGCTCCTCCGATCTGTCTGATGATGTGTCTGCGTCTGGCTCTTGCTCTTCTGCTGGCTCAACCCTGGCCCAG
species 4    cGGAAATGCAGTTGCTCTGCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTGTTTTGCTCGCTCAAACCTGGCTCAG
species 5    AGGATCTGCAGTTGCTCATCCGATCTGTGTGATGATCTATCTGAGTCTGGATCGTGCTCTTCTGCTGGCTCAACCCTGACCCCG
species 6    AGGAAATGCAGCCGCTCCCCCGATCTGTGTGATGATCTGTCTGAGTCTGGATCTTGCTGTTCTGCTCGCTCAAACCGGACTCAG
species 7    AGGATCTGCAGTTGCTCTTCCGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAACCGGGCTCAG
```

```
species 1    AGATGCAGTCCTGATCTGTATGATCTTCTGGCTGGTCTTCTTCTGCCAACGCCAG
species 2    AGGTAAAGTCCCGATCTGTATGATCTTCTGGCTGGTCTTCATCTGCCAACGCCAG
species 3    AGGTGCAGTCCCGATCTGTATGATGTTCTGGCTGGTCTTCTTCTGCCAACGCCAG
species 4    AGGTGCAGTCCCGATCTGTATGATCTTCTGGCTGGTCTTCTTCTGCCAACGCCAG
species 5    AGGTGCAGTCCCGATCTGTATGATCTTCTGGCTGGTCGTCTTCTGCCAACGCCCG
species 6    AGGTGCAGTCCCGATCTGTATGATCTTCTGGCTGGTCTTCTTCTGCCAACGCCAG
species 7    AGGTGCAGTCCCGATCTGTATGATCTTCTGGCTGGTCTTCTTCTGCCAACGCCAG
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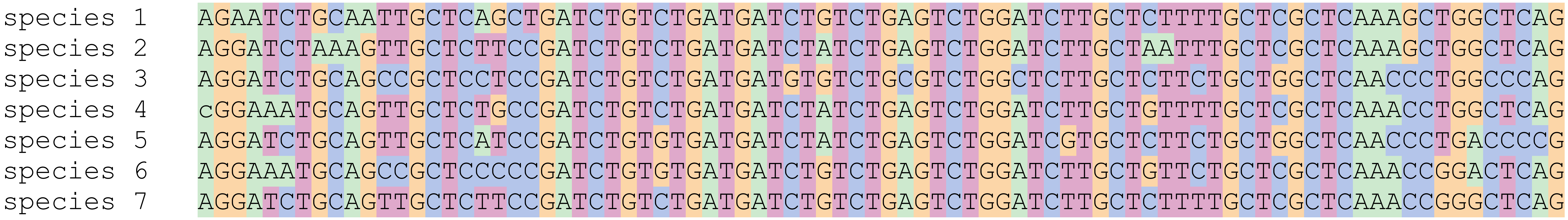

```
species 1   AGAATCTGCAATTGCTCAGCTGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAAGCTGGCTCAG
species 2   AGGATCTAAAGTTGCTCTTCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTAATTTGCTCGCTCAAAGCTGGCTCAG
species 3   AGGATCTGCAGCCGCTCCTCCGATCTGTCTGATGATGTGTCTGCGTCTGGCTCTTGCTCTTCTGCTGGCTCAACCCTGGCCCAG
species 4   cGGAAATGCAGTTGCTCTGCCGATCTGTCTGATGATCTATCTGAGTCTGGATCTTGCTGTTTTGCTCGCTCAAACCTGGCTCAG
species 5   AGGATCTGCAGTTGCTCATCCGATCTGTGTGATGATCTATCTGAGTCTGGATCGTGCTCTTCTGCTGGCTCAACCCTGACCCCG
species 6   AGGAAATGCAGCCGCTCCCCCGATCTGTGTGATGATCTGTCTGAGTCTGGATCTTGCTGTTCTGCTCGCTCAAACCGGACTCAG
species 7   AGGATCTGCAGTTGCTCTTCCGATCTGTCTGATGATCTGTCTGAGTCTGGATCTTGCTCTTTTGCTCGCTCAAACCGGGCTCAG
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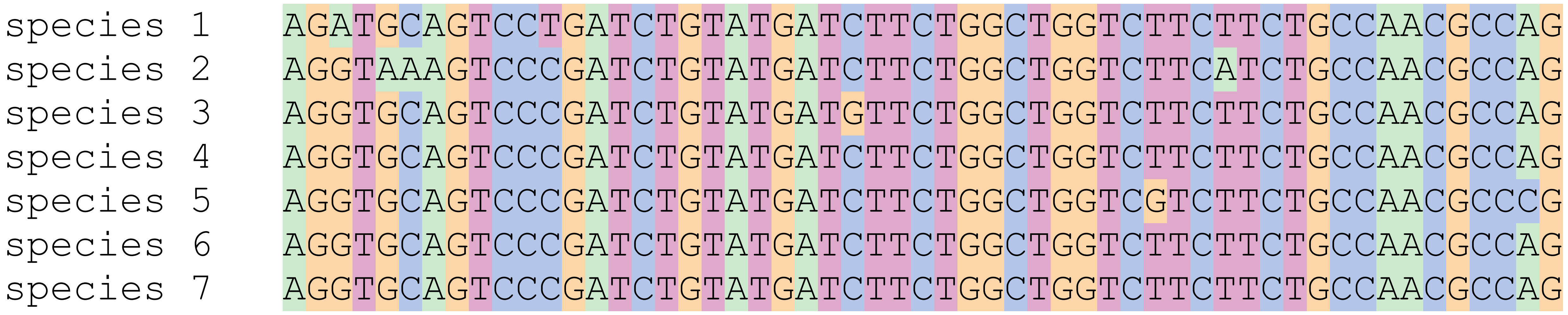
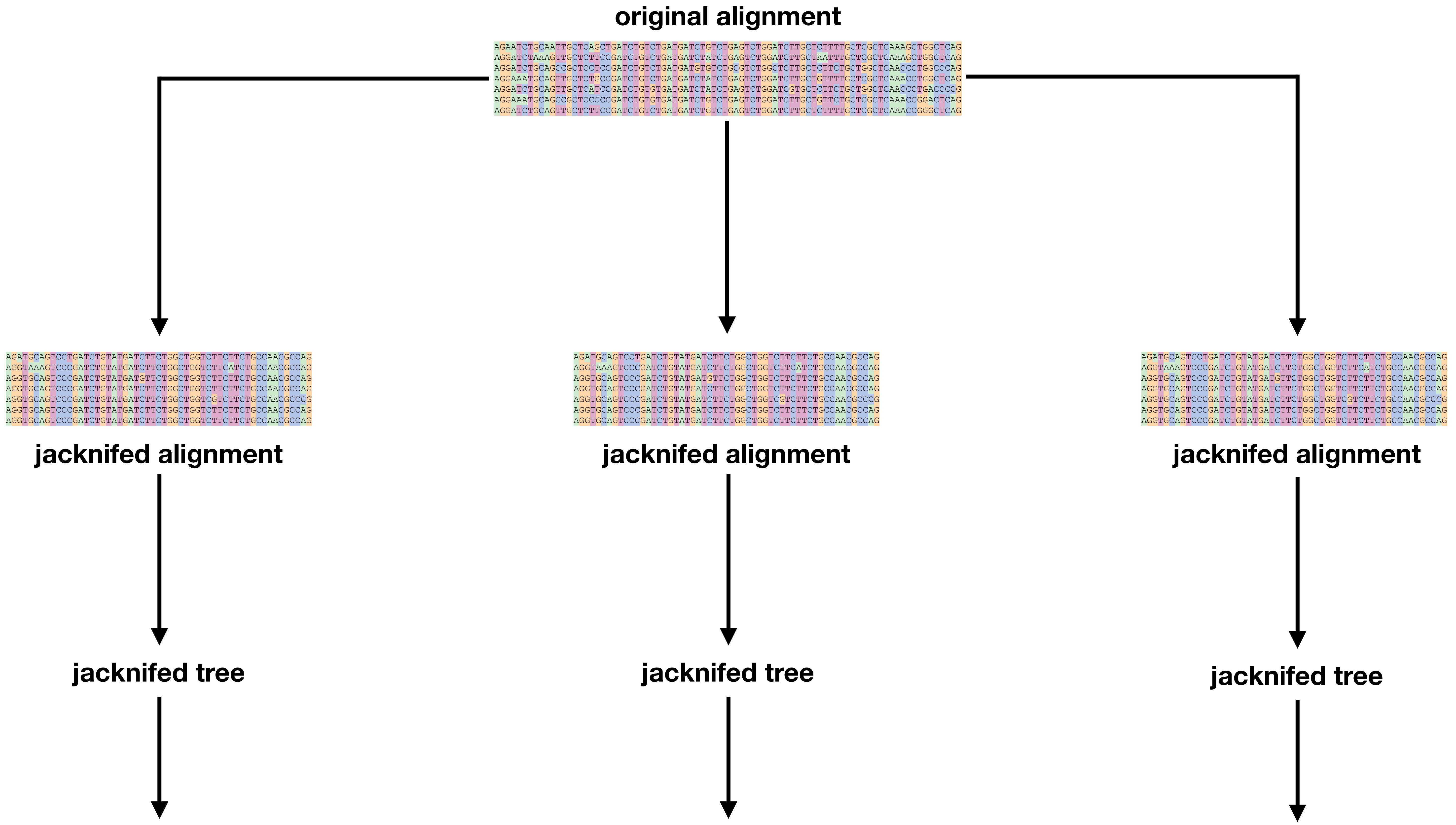


```
species 1   AGATGCAGTCCTGATCTGTATGATCTTCTGGCTGGTCTTCTTCTGCCAACGCCAG
species 2   AGGTAAAGTCCCGATCTGTATGATCTTCTGGCTGGTCTTCATCTGCCAACGCCAG
species 3   AGGTGCAGTCCCGATCTGTATGATGTTCTGGCTGGTCTTCTTCTGCCAACGCCAG
species 4   AGGTGCAGTCCCGATCTGTATGATCTTCTGGCTGGTCTTCTTCTGCCAACGCCAG
species 5   AGGTGCAGTCCCGATCTGTATGATCTTCTGGCTGGTCGTCTTCTGCCAACGCCCG
species 6   AGGTGCAGTCCCGATCTGTATGATCTTCTGGCTGGTCTTCTTCTGCCAACGCCAG
species 7   AGGTGCAGTCCCGATCTGTATGATCTTCTGGCTGGTCTTCTTCTGCCAACGCCAG
```

Sampling without replacement!

Is the same information present in the jacknifed alignment?



original alignment

jacknifed alignment

jacknifed alignment

jacknifed alignment

jacknifed tree

jacknifed tree

jacknifed tree

node	branch is present?	branch is present?	branch is present?	JF
1	✅	✅	✅	100%
2	✅	❌	❌	33%
3	✅	✅	✅	100%
4	❌	❌	❌	0%
5	❌	✅	✅	66%
..	…	…	…	…
last	✅	✅	✅	100%

approximate Likelihood Ratio Test (aLRT)

aLRT is a fast, branch-specific metric, related to the **conventional Likelihood Ratio Test (LRT)**.

The standard LRT uses the test statistics $2(L_1 - L_0)$:

L_1 - **alternative hypothesis**: is the log-likelihood of the current tree
L_0 - **null hypothesis**: is the log-likelihood of the same tree, but with the branch of interest collapsed

aLRT approximation: $2(L_1 - L_2)$ where L_2 is the second best NNI configuration around the branch.

Why use L_2 in aLRT?

- **Much faster** than evaluating a collapsed model or full likelihood surface …
  L_2 is computed by optimising only over the branch of interest and the four adjacent branches
  other parameters are fixed at their optimal values corresponding to the best ML tree

- Allows efficient estimation of **branch-specific support**, especially in large trees
  focuses on local rearrangements around a branch - just 4 adjacent branches!

⚠ Local rearrangements approach may miss topologies distant to current one but with higher lnL!

Posterior Probabilities (PPs)

Bayesian Inference is associated to **Posterior Probabilities** - also called clade credibility values.

- frequency that a bipartition appears in sampled trees at the stationary phase of MCMC chain
- ranges from 0 to 1 - a PP of 0.95 means that the clade appeared in 95% of sampled trees

⚠️ important:

- PPs tend to be higher than non-parametric BPs
- sensitive to model choice and priors

Concordance Factors (CFs)

Quantify how many genes or sites support each branch in a reference tree.

Two types of CFs:

- gene Concordance Factor **(gCF)**:
  proportion of **gene trees** that contain a specific branch (or split).

- site Concordance Factor **(sCF)**:
  proportion of **informative sites** in the alignment that support a branch

Interpreting CFs values:

- High (e.g., >70%) - strong signal and high agreement across loci/sites
- Moderate (40–70%) - moderate support; possible discordance
- Low (<40%) - weak support or substantial gene tree conflict

⚠️ Different support **metrics** have different **pitfalls**:

- parametric supports assume the evolutionary model is the correct one.
- standard metrics - PPs or BPs - can support conflicting topologies.
- …

🙃 Good practices:

- different support metrics must be compared
- observe consistent outcomes from different approaches

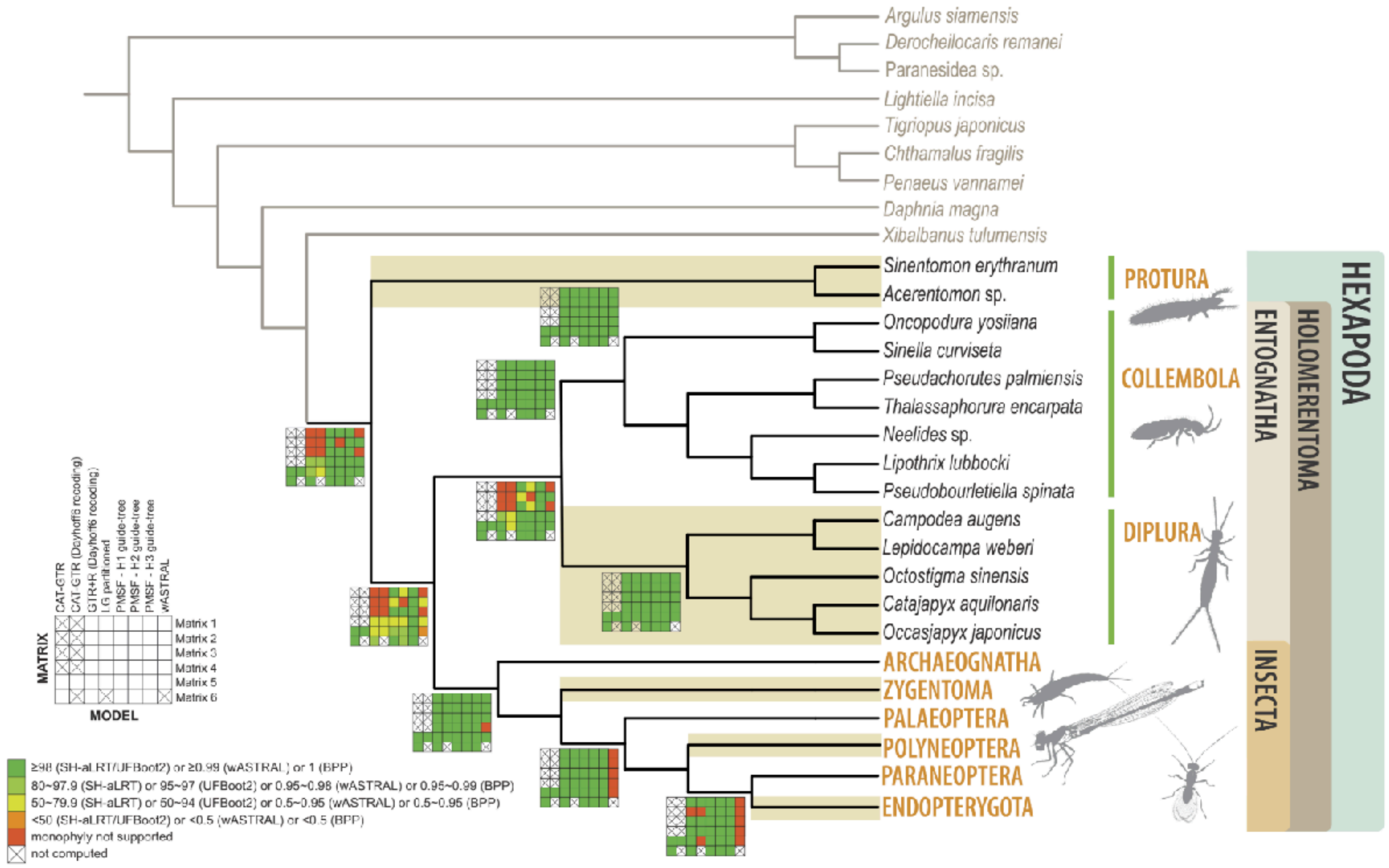


Fig. 2. Phylogeny of the noninsect hexapods. Main topology inferred from the Matrix 6 using the Bayesian across-site compositional heterogeneity CAT-GTR model implemented in PhyloBayes. Node supports from all analyses are indicated by the colored squares (The node supports of each phylogenetic tree is shown in *SI Appendix*, Supplementary Appendix B). Only the lowest support values are shown when different matrices or different models produced conflicting results. [H1.guide: Collembola + (Protura + (Diplura + Insecta)); H2.guide: (Collembola + Protura) + (Diplura + Insecta); H3.guide: Protura + ((Collembola + Diplura) + Insecta)]. Collembola + Diplura = original circumscription of Entognatha; Entognatha + Insecta = Holomerentoma (a.k.a. Holomera).

FINISH