1. Transcriptomic analyses

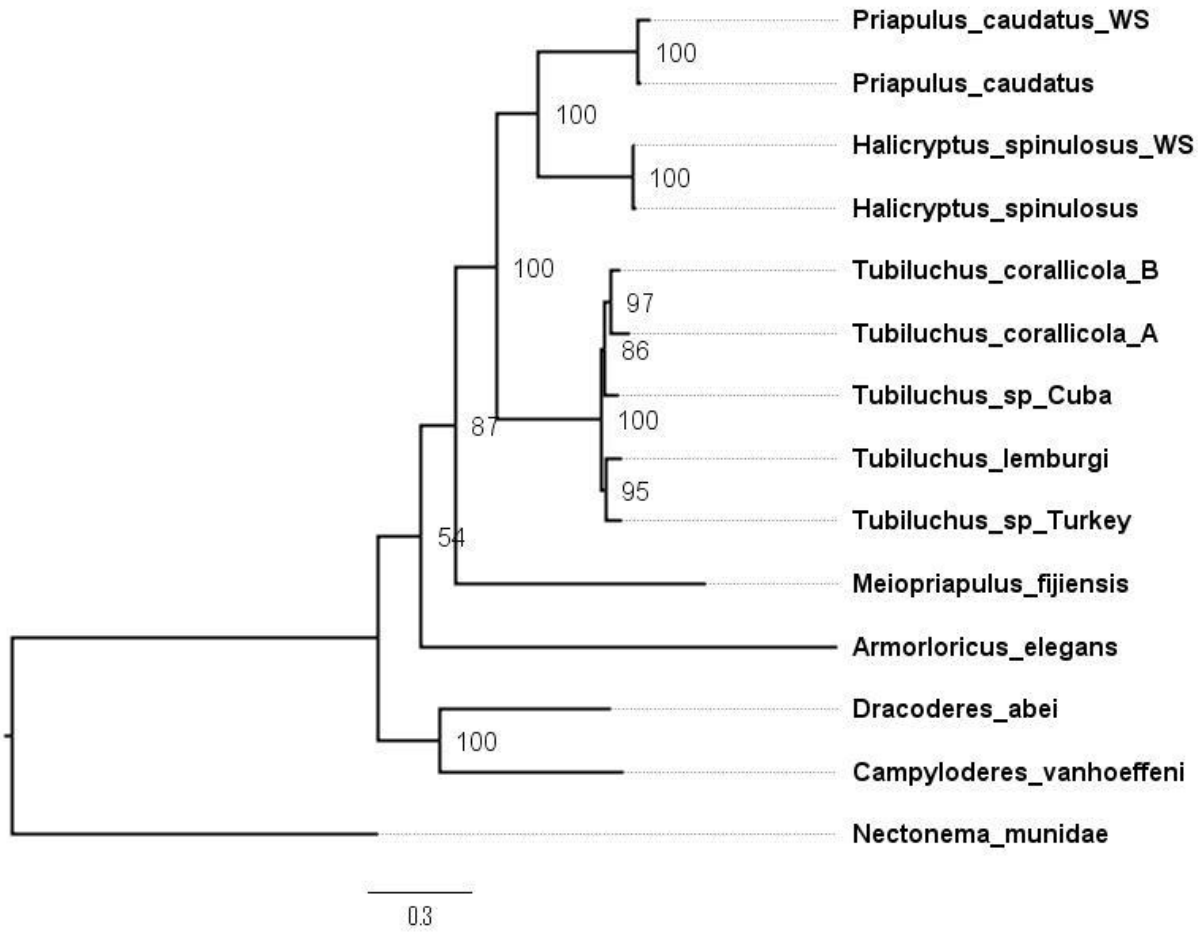
Supplementary table S1. Species with corresponding sequencing information of assembled transcriptomes. BUSCO values after assembly of transcriptomes. Bold species show newly generated transcriptomes.

Phylum	Species	Sequencing method	Read length	Amount of reads
Priapulida	*Halicryptus spinulosus*	HiSeq 2000	PE-100	243,230,884
	***Halicryptus spinulosus* (White Sea)**	NovaSeq 6000	PE-150	39,535,631
	Priapulus caudatus	HiSeq 2500	PE-100	13,127,836
	***Priapulus caudatus* (White Sea)**	NovaSeq 6000	PE-150	89,135,981
	***Tubiluchus corallicola* (A)**	NovaSeq 6000	PE-150	31,705,844
	Tubiluchus corallicola (B)	HiSeq 2500	PE-100	29,281,791
	Tubiluchus lemburgi	NovaSeq 6000	PE-150	23,149,413
	***Tubiluchus* sp. A (Cuba)**	NovaSeq 6000	PE-150	48,498,029
	***Tubiluchus* sp. B (Turkey)**	NovaSeq 6000	PE-150	38,035,180
	Meiopriapulus fijiensis	HiSeq 2500	PE-100	30,405,185
Kinorhyncha	*Dracoderes abei*	NovaSeq 6000	PE-150	46,182,895
	Campyloderes vanhoeffeni	NovaSeq 6000	PE-150	63,585,594
Loricifera	*Armorloricus elegans*	HiSeq 2500	PE-100	28,245,230
Nematomorpha	*Nectonema munidae*	HiSeq 2500	PE-100	79,884,095

Supplementary table S2. Information of occupancy matrixes of transcriptomic analyses. Percent values of matrices are rounded to the nearest 5.

Occupancy matrix	Minimum number of species	Gene regions	Amino acid sites
100%	14	11	3,912
95%	13	44	16,147
85%	12	116	48,416
80%	11	257	122,432
70%	10	526	265,933
65%	9	889	477,231
55%	8	1376	805,039
50%	7	2060	1,276,118

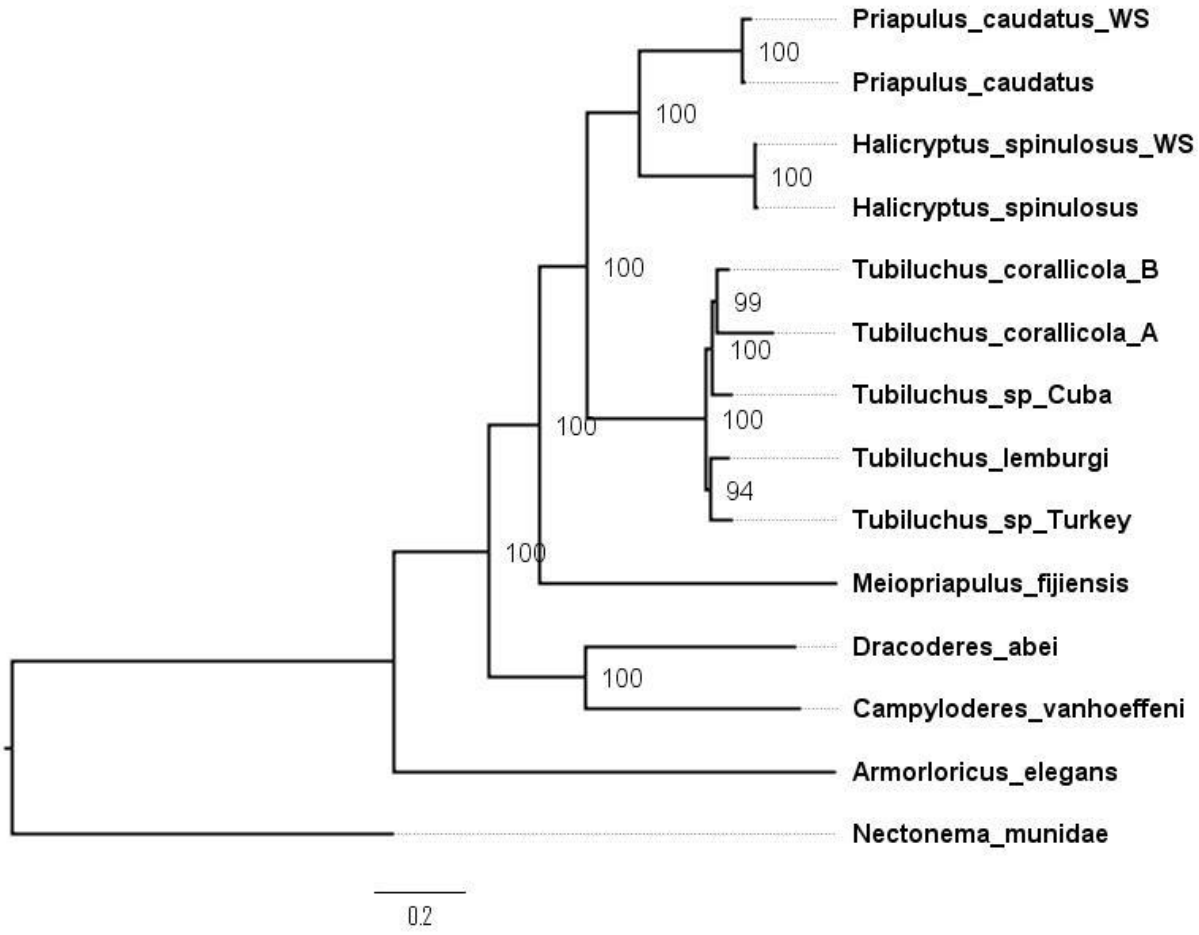
Maximum-likelihood species trees



Supplementary figure S1 Maximum-likelihood species tree from the 100 % occupancy matrix (14 species, 11 gene regions, 3912 amino acids) generated with IQ-Tree2. Support values are bootstrap.

Single-copy 100 % occupancy matrix (14 species, 11 gene regions, 3912 amino acids) (input file '100_supermatrix.fas')

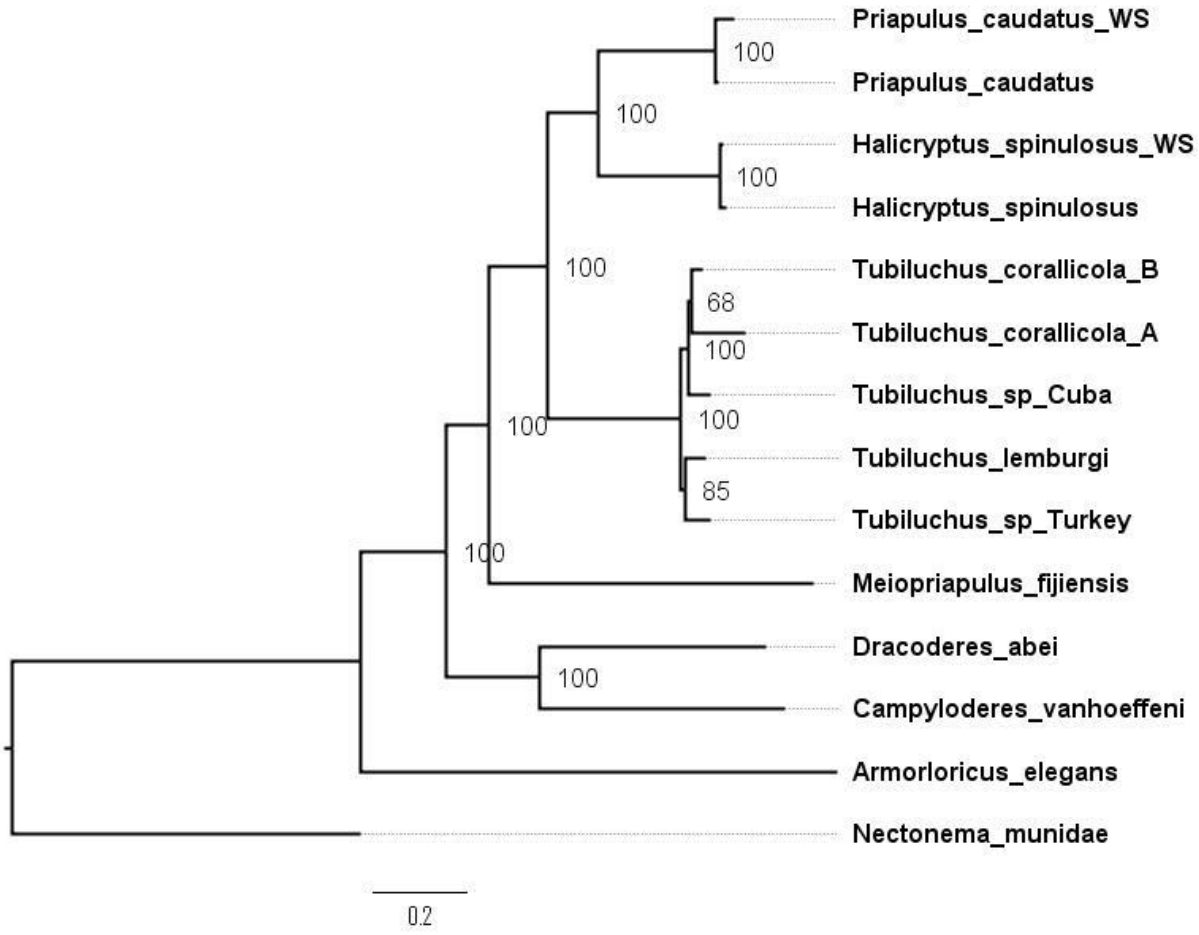
```
iqtree -s 100_supermatrix.fas -m MFP -B 1000 -bnni -T AUTO
```



Supplementary figure S2 Maximum-likelihood species tree from the 95 % occupancy matrix (≥13 species, 44 gene regions, 16,147 amino acids) generated with IQ-Tree2. Support values are bootstrap.

Single-copy 95 % occupancy matrix (≥13 species, 44 gene regions, 16,147 amino acids) (input file '95_supermatrix.fas')

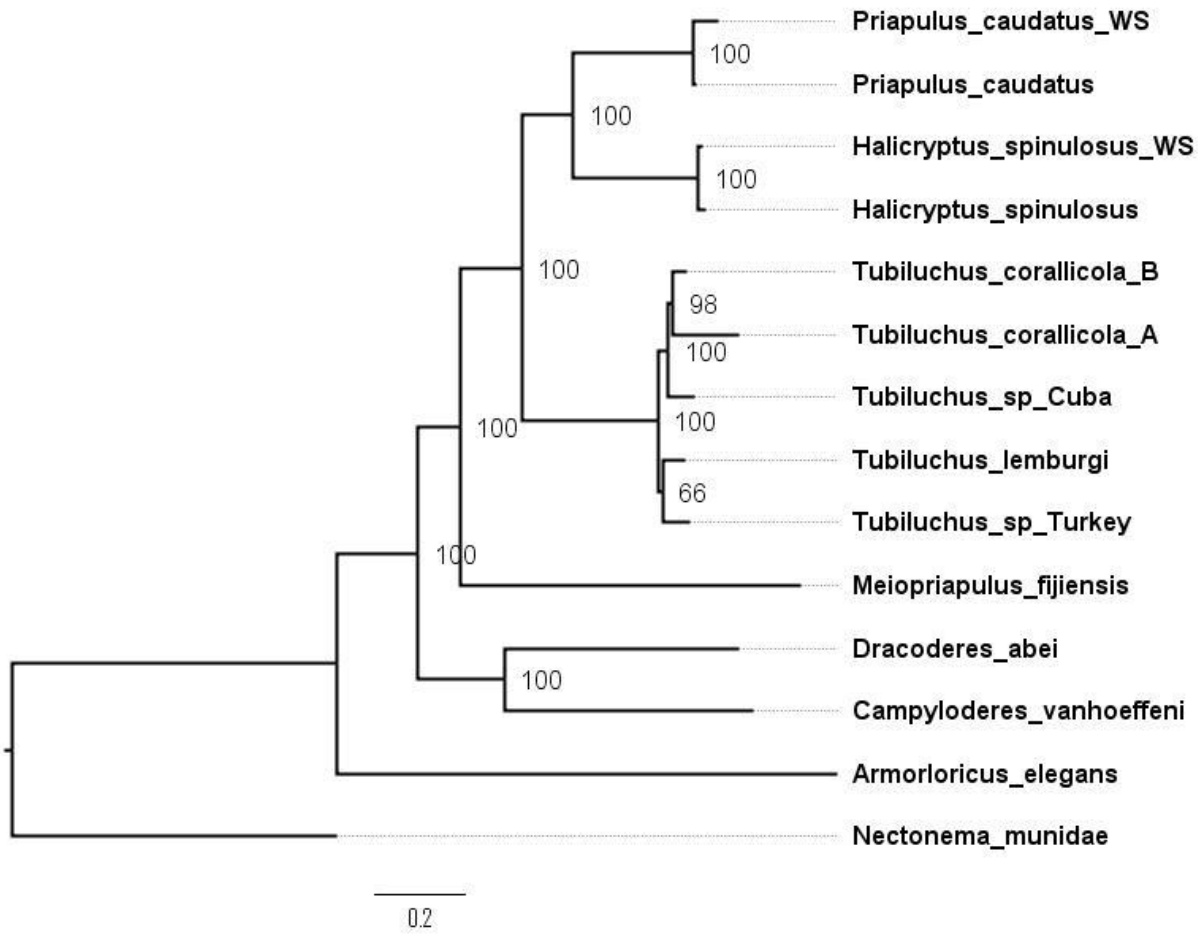
```
iqtree -s 95_supermatrix.fas -m MFP -B 1000 -bnni -T AUTO
```



Supplementary figure S3 Maximum-likelihood species tree from the 85 % occupancy matrix (≥12 species, 116 gene regions, 48,416 amino acids) generated with IQ-Tree2. Support values are bootstrap.

Single-copy 85 % occupancy matrix (≥12 species, 116 gene regions, 48,416 amino acids) (input file '85_supermatrix.fas')

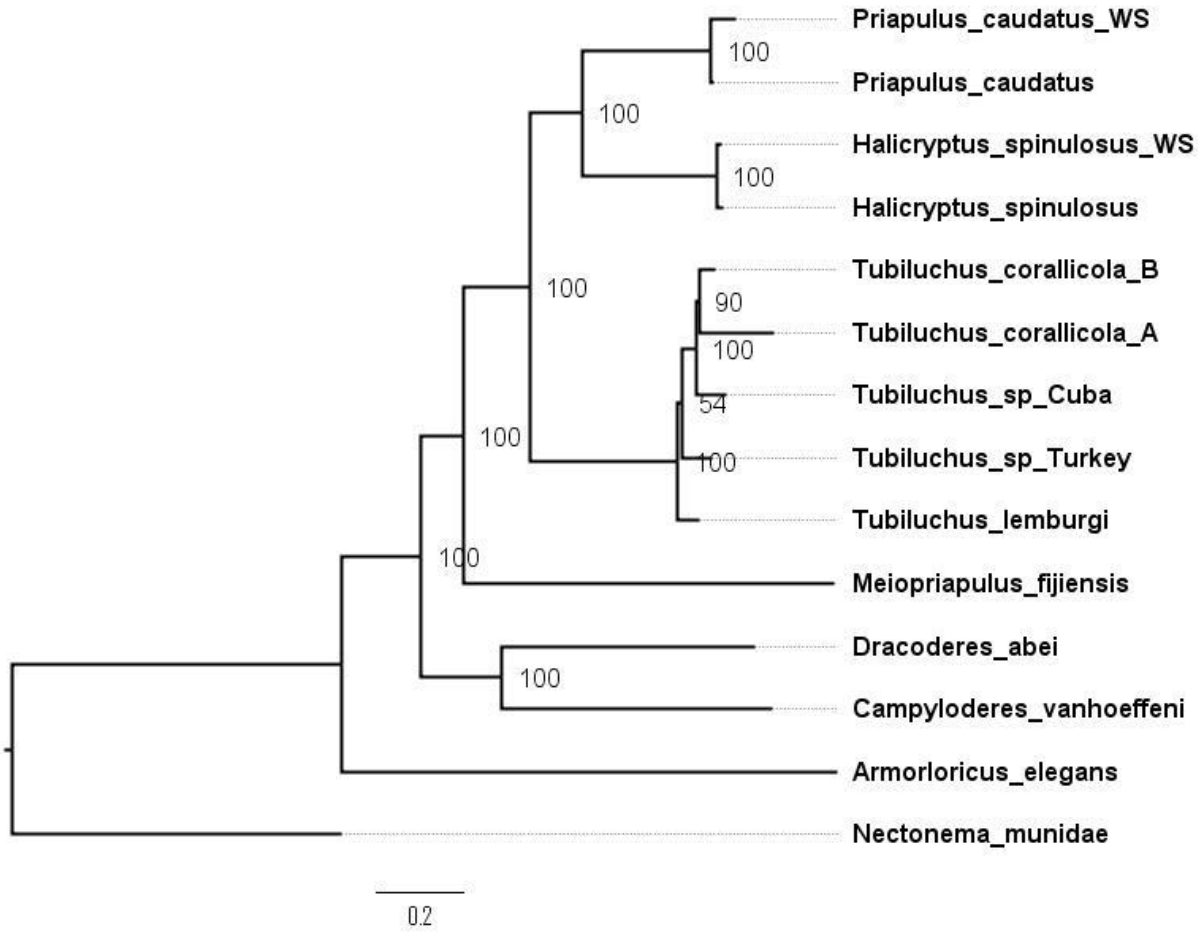
```
iqtree -s 85_supermatrix.fas -m MFP -B 1000 -bnni -T AUTO
```



Supplementary figure S4 Maximum-likelihood species tree from the 80 % occupancy matrix (≥11 species, 257 gene regions, 122,432 amino acids) generated with IQ-Tree2. Support values are bootstrap.

Single-copy 80 % occupancy matrix (≥11 species, 257 gene regions, 122,432 amino acids) (input file '80_supermatrix.fas')

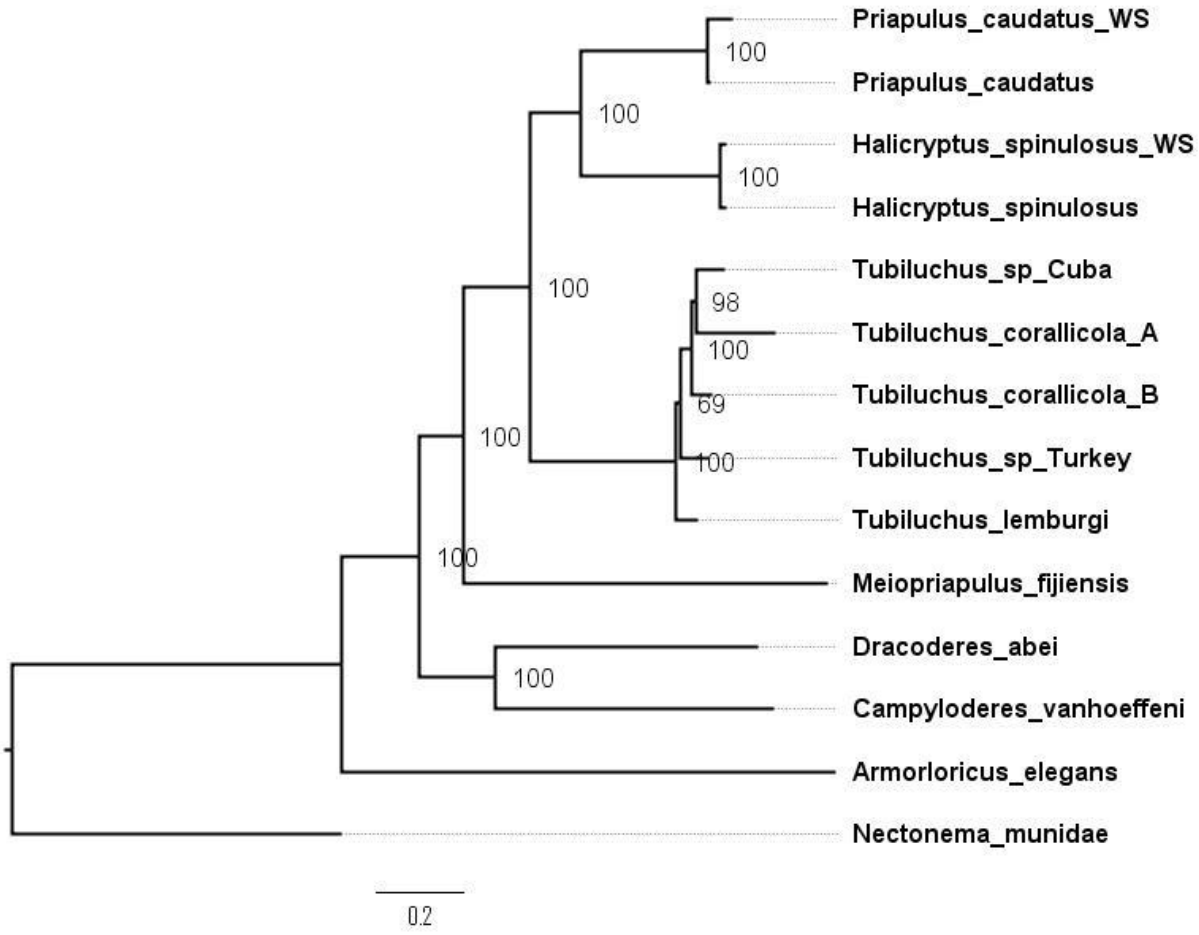
```
iqtree -s 80_supermatrix.fas -m MFP -B 1000 -bnni -T AUTO
```



Supplementary figure S5 Maximum-likelihood species tree from the 70 % occupancy matrix (≥10 species, 526 gene regions, 265,933 amino acids) generated with IQ-Tree2. Support values are bootstrap.

Single-copy 70 % occupancy matrix (≥10 species, 526 gene regions, 265,933 amino acids) (input file '70_supermatrix.fas')

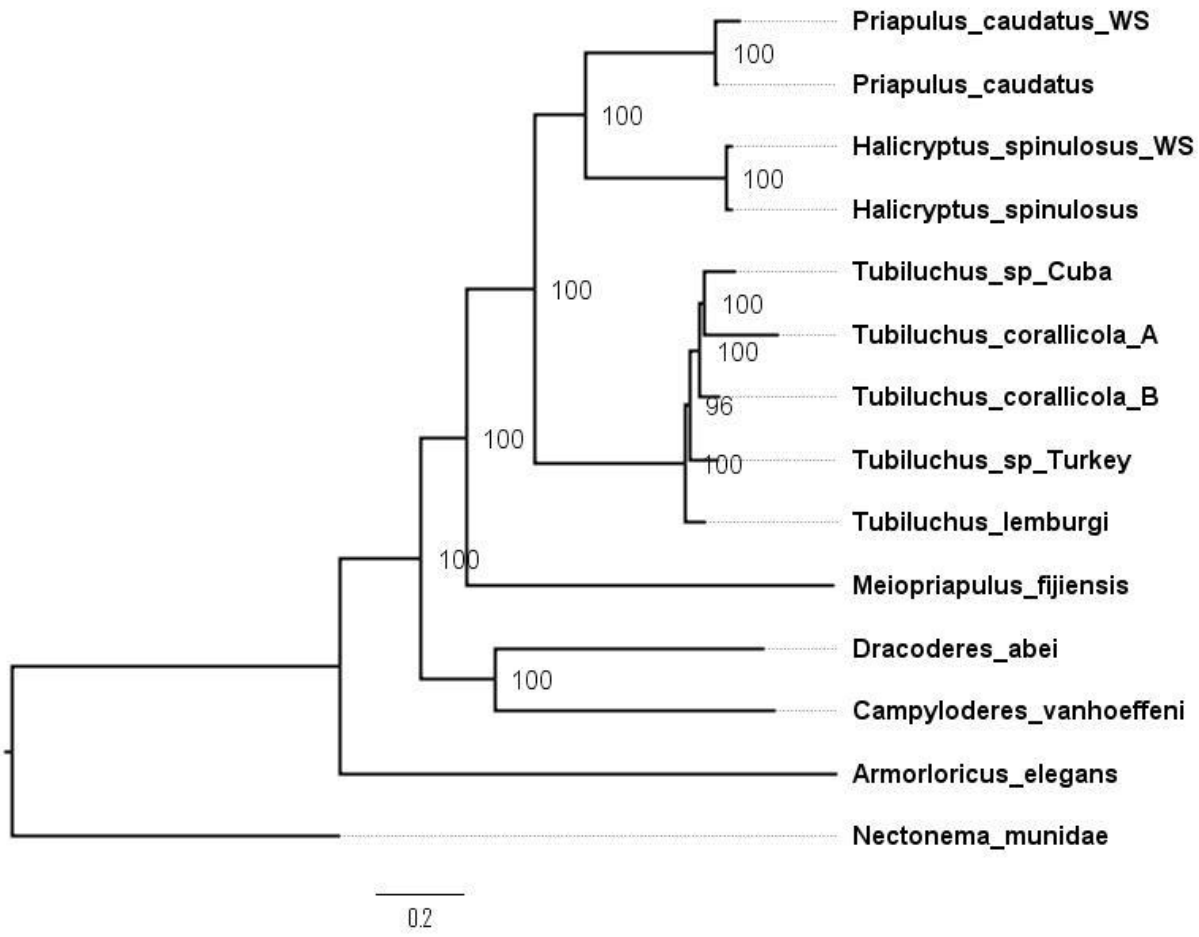
```
iqtree -s 70_supermatrix.fas -m MFP -B 1000 -bnni -T AUTO
```



Supplementary figure S6 Maximum-likelihood species tree from the 65 % occupancy matrix (≥9 species, 889 gene regions, 477,231 amino acids) generated with IQ-Tree2. Support values are bootstrap.

Single-copy 65 % occupancy matrix (≥9 species, 889 gene regions, 477,231 amino acids) (input file '65_supermatrix.fas')

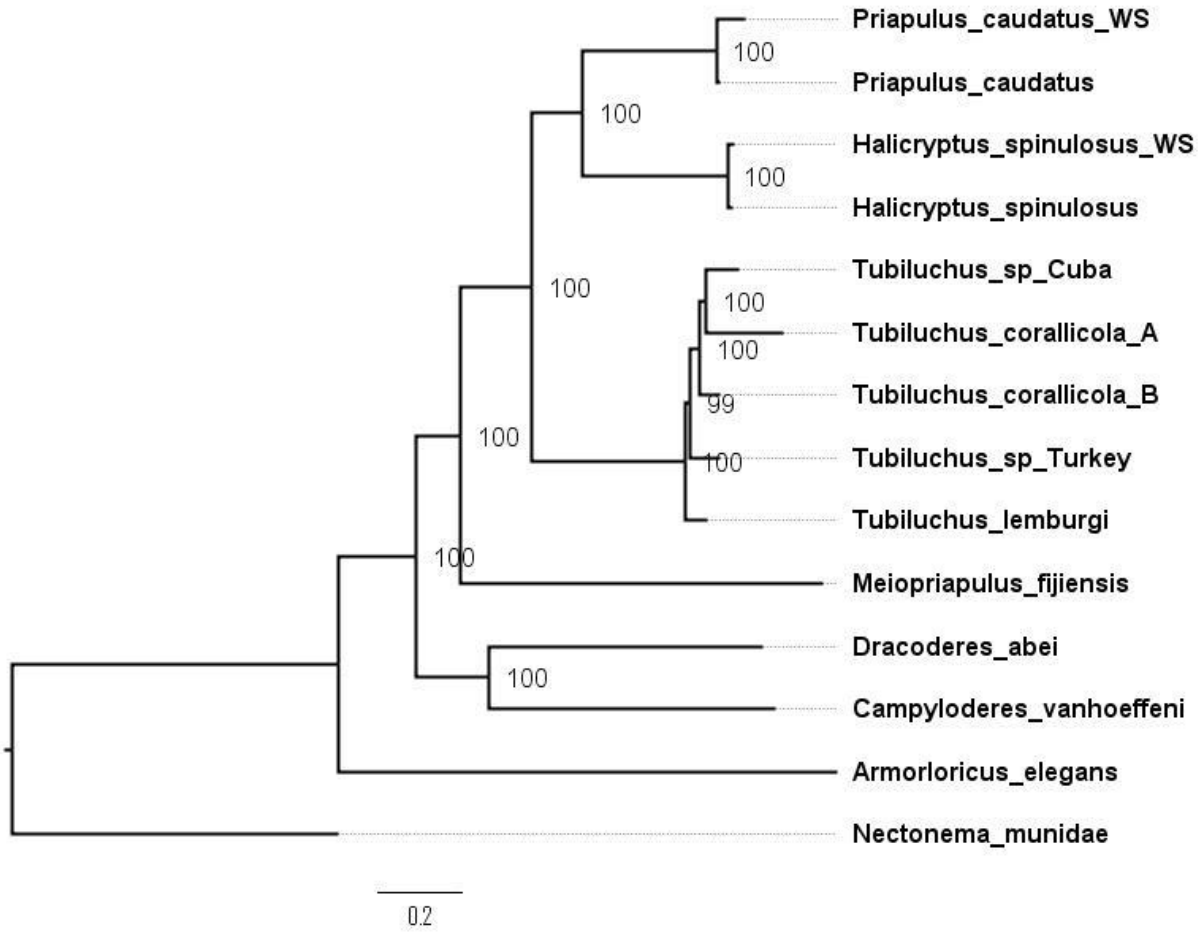
```
iqtree -s 65_supermatrix.fas -m MFP -B 1000 -bnni -T AUTO
```



Supplementary figure S7 Maximum-likelihood species tree from the 55 % occupancy matrix (≥8 species, 1376 gene regions, 805,039 amino acids) generated with IQ-Tree2. Support values are bootstrap.

Single-copy 55 % occupancy matrix (≥8 species, 1376 gene regions, 805,039 amino acids) (input file '55_supermatrix.fas')

```
iqtree -s 55_supermatrix.fas -m MFP -B 1000 -bnni -T AUTO
```

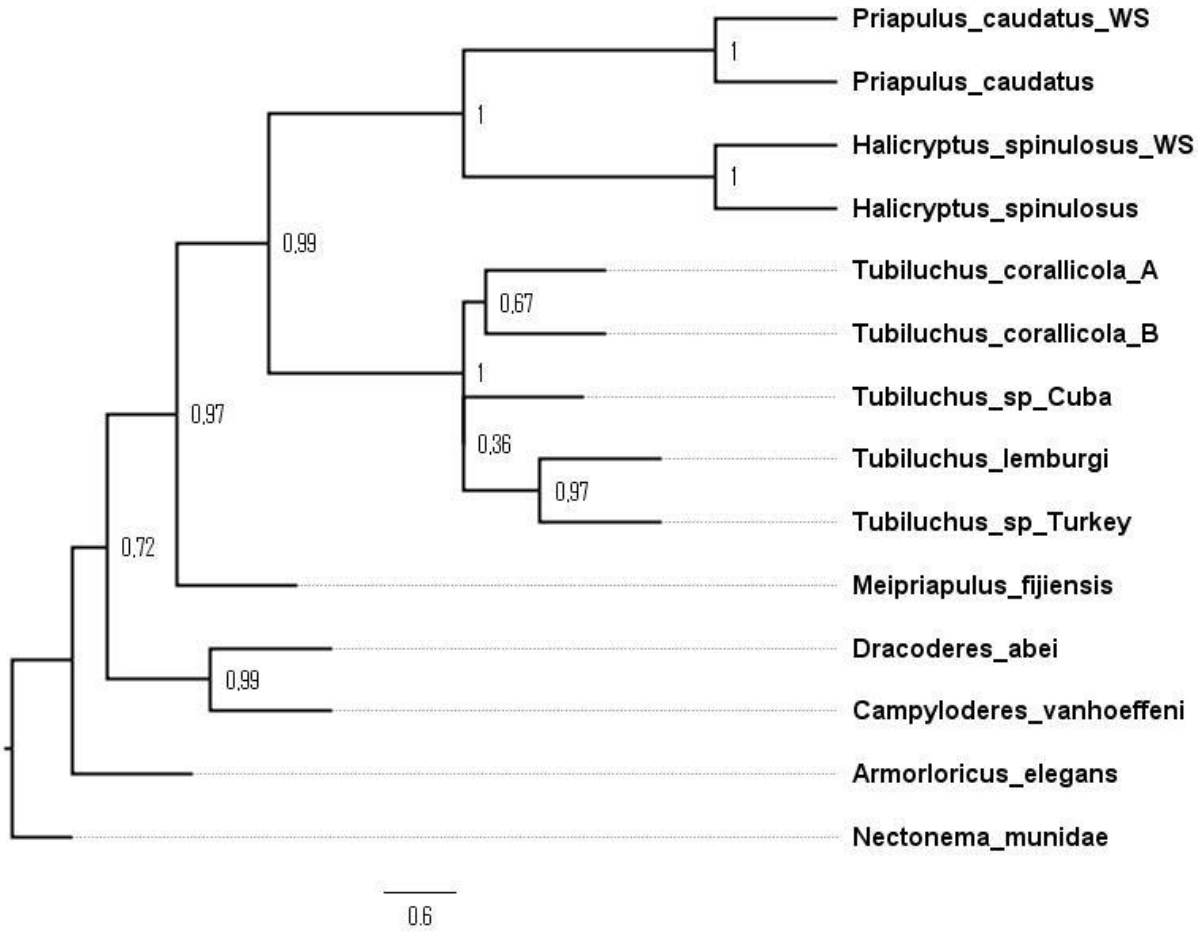


Supplementary figure S8 Maximum-likelihood species tree from the 50 % occupancy matrix (≥7 species, 2060 gene regions, 1,276,118 amino acids) generated with IQ-Tree2. Support values are bootstrap.

Single-copy 50 % occupancy matrix (≥7 species, 2060 gene regions, 1,276,118 amino acids) (input file '07_supermatrix.fas')

```
iqtree -s 50_supermatrix.fas -m MFP -B 1000 -bnni -T AUTO
```

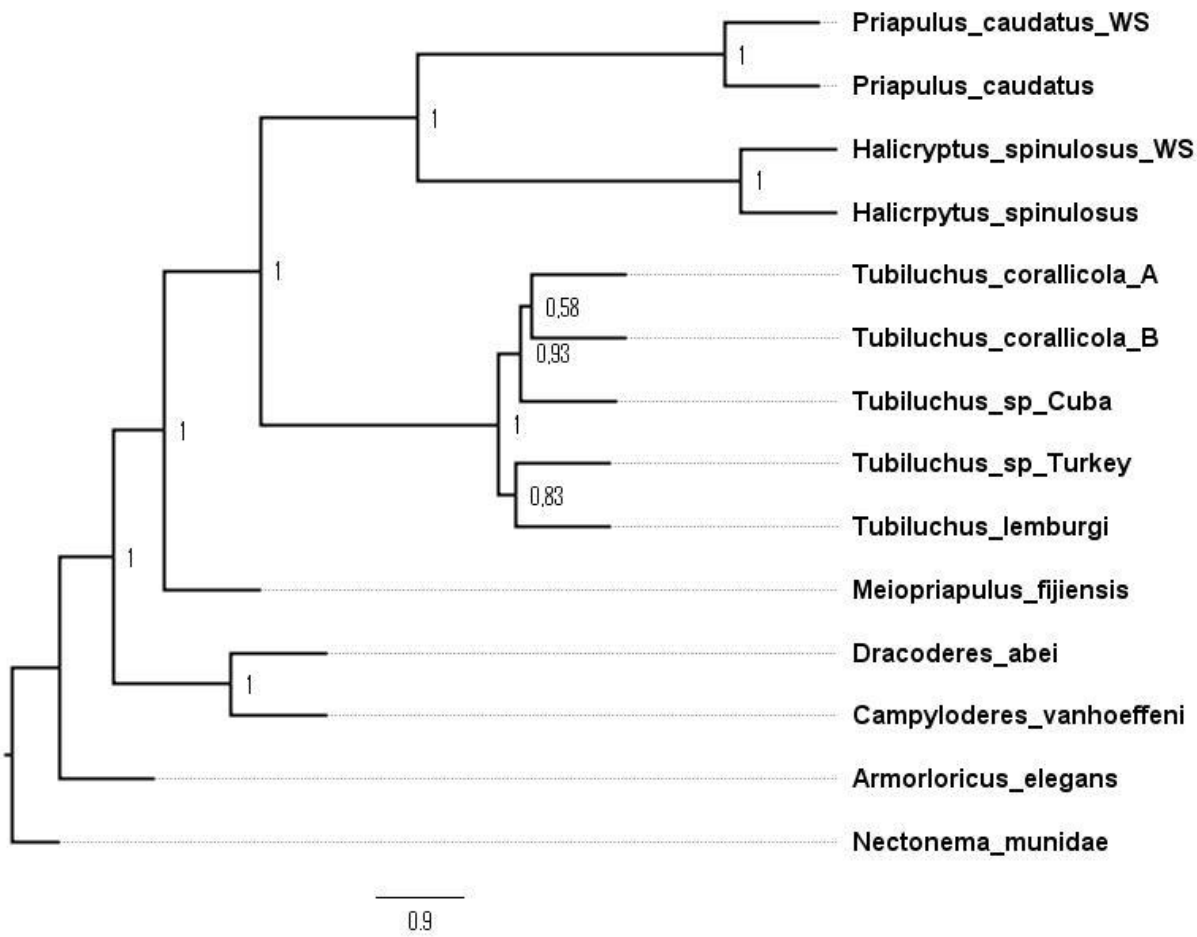
Coalescent-based species trees



Supplementary figure S9 Coalescent-based species tree from the 100 % occupancy matrix based on 11 single-copy gene trees generated with ASTRAL-III. Support values are local posterior probabilities.

Single-copy 100 % occupancy matrix (11 gene trees) (input file '14_concatenated_gene_trees.tre')

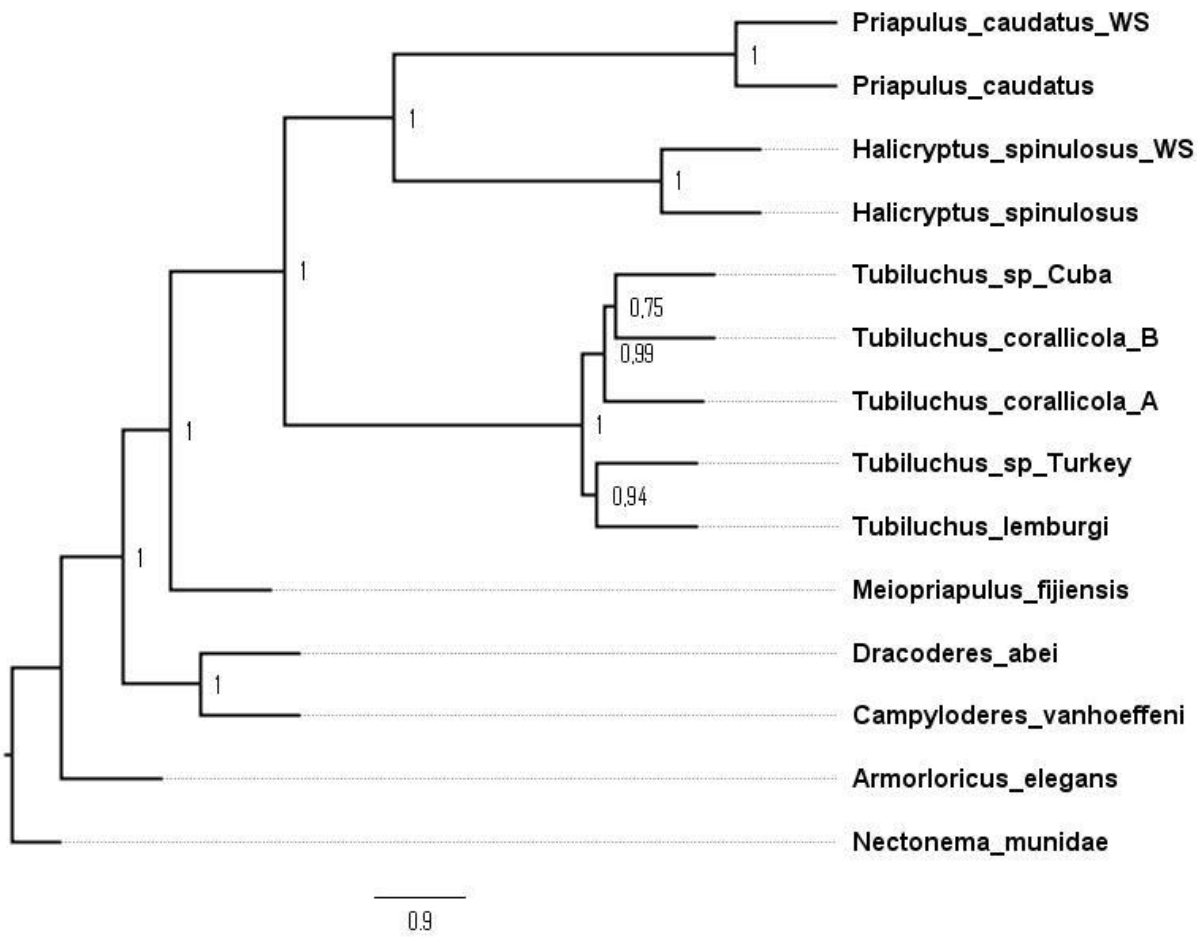
```
java -jar astral.5.7.8.jar -i 14_concatenated_gene_trees -o
14_astral.tre
```



Supplementary figure S10 Coalescent-based species tree from the 95 % occupancy matrix based on 44 single-copy gene trees generated with ASTRAL-III. Support values are local posterior probabilities.

Single-copy 95 % occupancy matrix (44 gene trees) (input file '13_concatenated_gene_trees.tre')

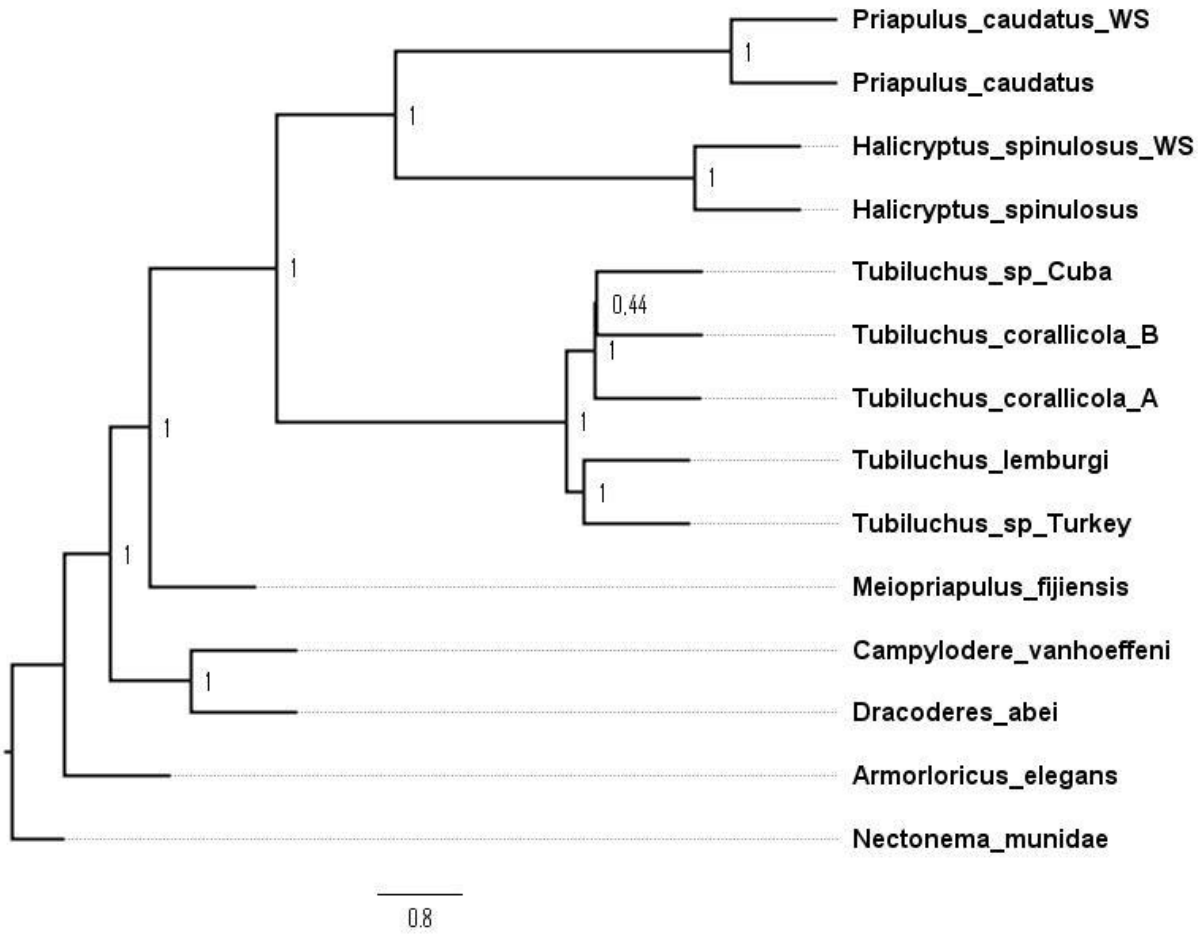
```
java -jar astral.5.7.8.jar -i 13_concatenated_gene_trees -o
13_astral.tre
```



Supplementary figure S11 Coalescent-based species tree from the 85 % occupancy matrix based on 116 single-copy gene trees generated with ASTRAL-III. Support values are local posterior probabilities.

Single-copy 85 % occupancy matrix (116 gene trees) (input file '12_concatenated_gene_trees.tre')

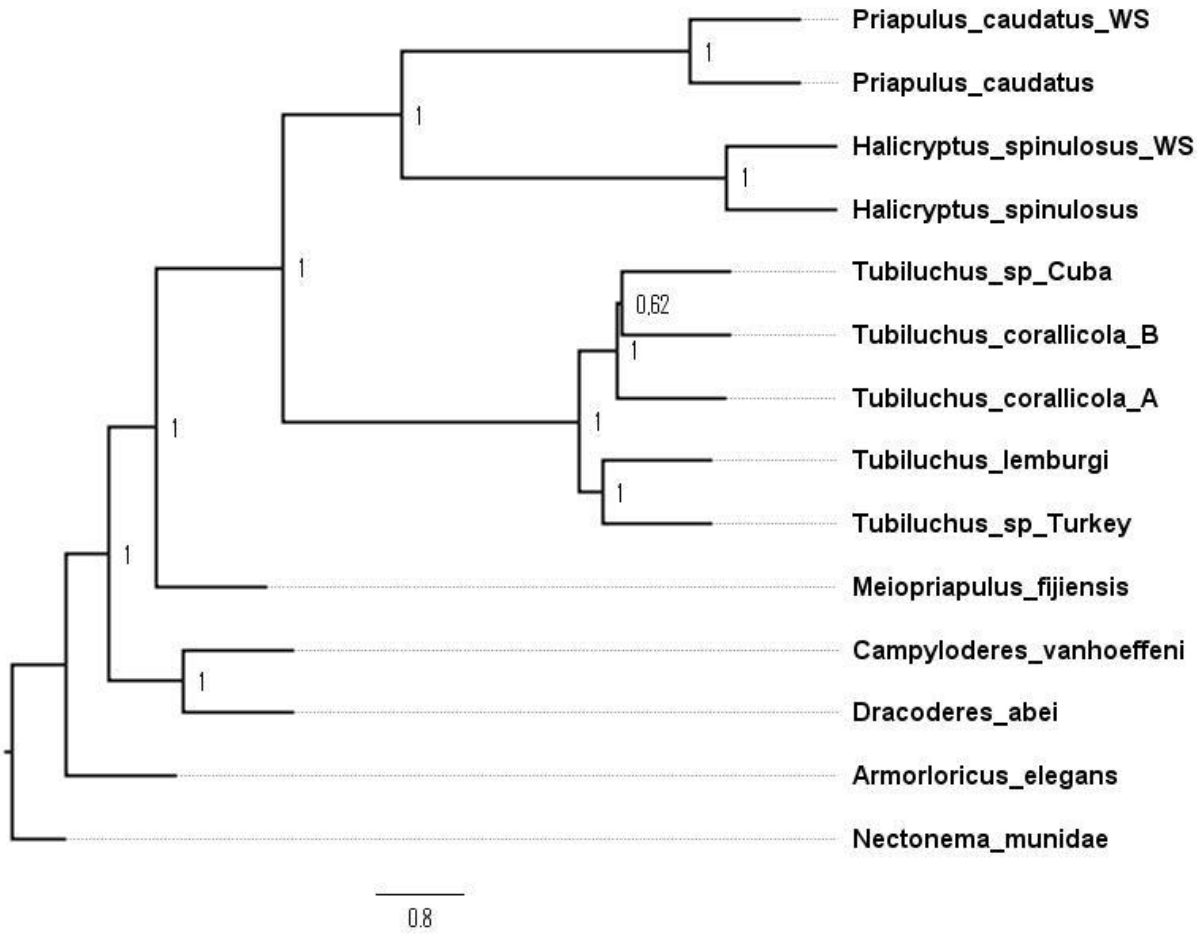
```
java -jar astral.5.7.8.jar -i 12_concatenated_gene_trees -o
12_astral.tre
```



Supplementary figure S12 Coalescent-based species tree from the 80 % occupancy matrix based on 257 single-copy gene trees generated with ASTRAL-III. Support values are local posterior probabilities.

Single-copy 80 % occupancy matrix (257 gene trees) (input file '11_concatenated_gene_trees.tre')

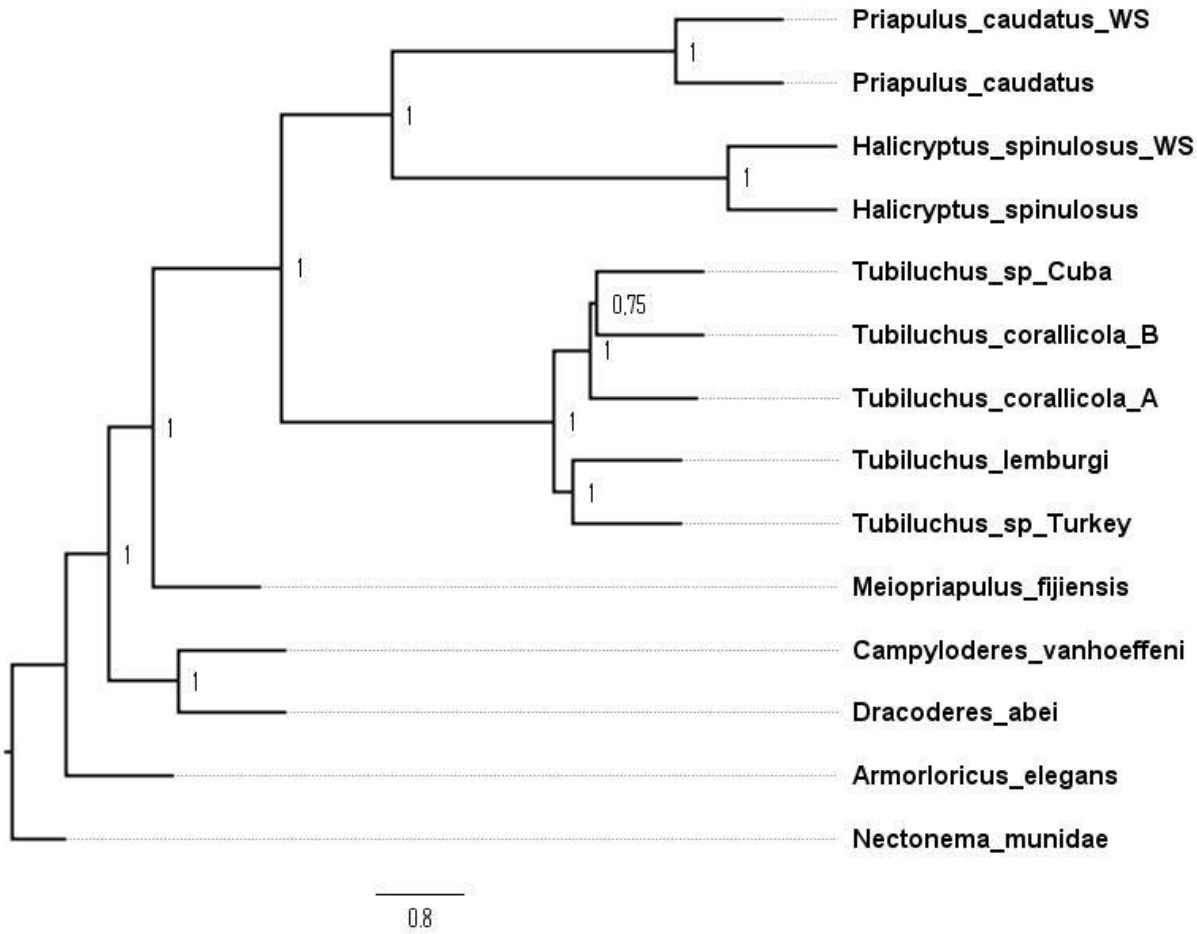
```
java -jar astral.5.7.8.jar -i 11_concatenated_gene_trees -o
11_astral.tre
```



Supplementary figure S13 Coalescent-based species tree from the 70 % occupancy matrix based on 526 single-copy gene trees generated with ASTRAL-III. Support values are local posterior probabilities.

Single-copy 70 % occupancy matrix (526 gene trees) (input file '10_concatenated_gene_trees.tre')

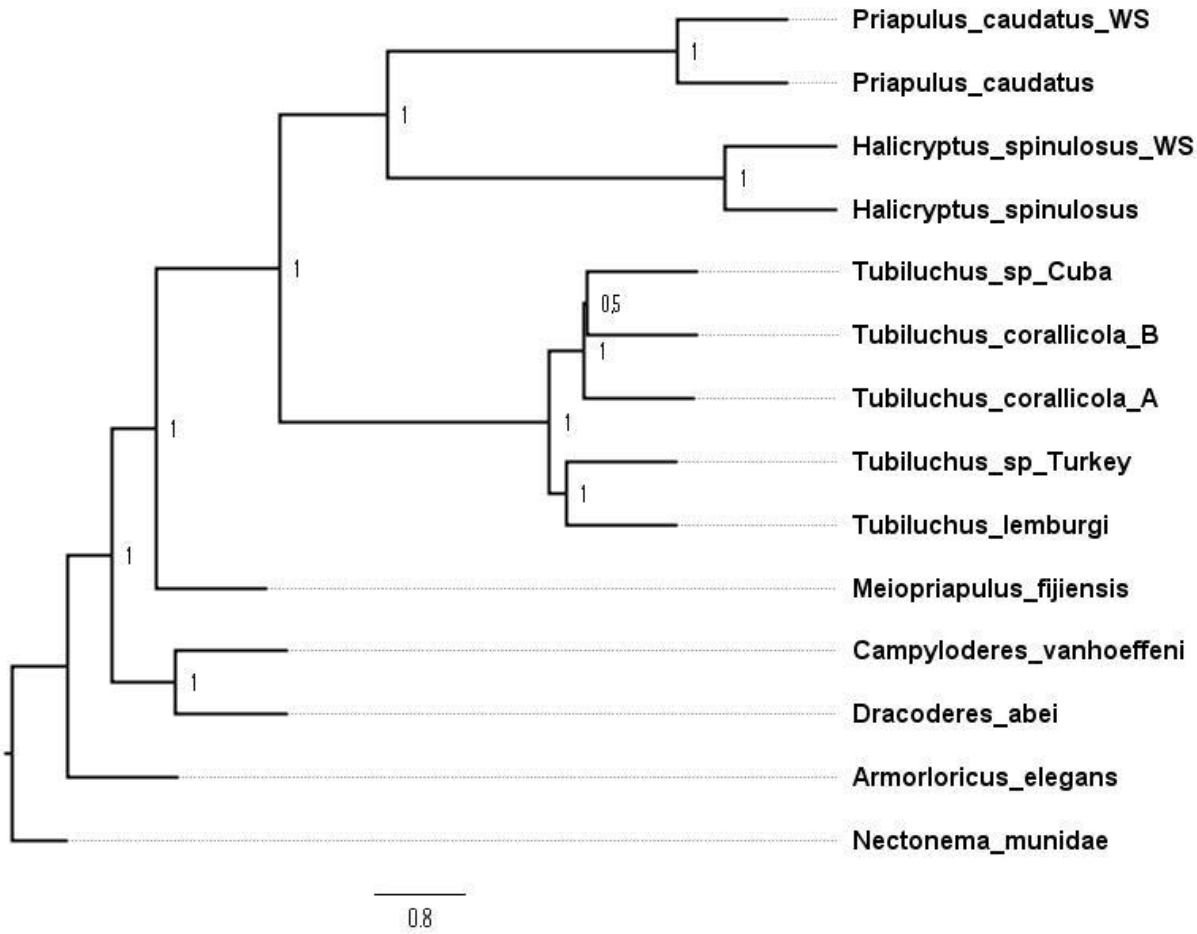
```
java -jar astral.5.7.8.jar -i 10_concatenated_gene_trees -o
10_astral.tre
```



Supplementary figure S14 Coalescent-based species tree from the 65 % occupancy matrix based on 889 single-copy gene trees generated with ASTRAL-III. Support values are local posterior probabilities.

Single-copy 65 % occupancy matrix (889 gene trees) (input file '09_concatenated_gene_trees.tre')

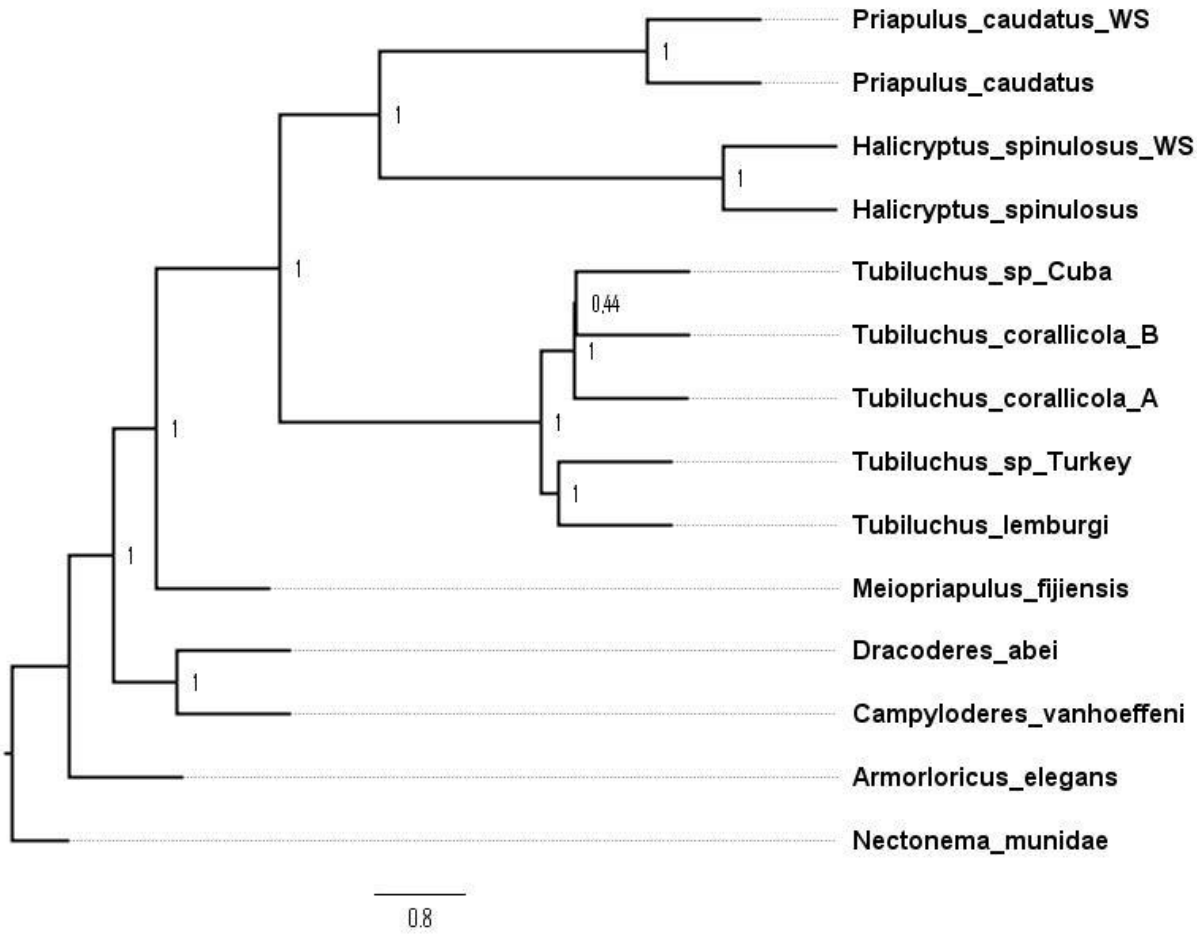
```
java -jar astral.5.7.8.jar -i 09_concatenated_gene_trees -o
09_astral.tre
```



Supplementary figure S15 Coalescent-based species tree from the 55 % occupancy matrix based on 1376 single-copy gene trees generated with ASTRAL-III. Support values are local posterior probabilities.

Single-copy 55 % occupancy matrix (1376 gene trees) (input file '08_concatenated_gene_trees.tre')

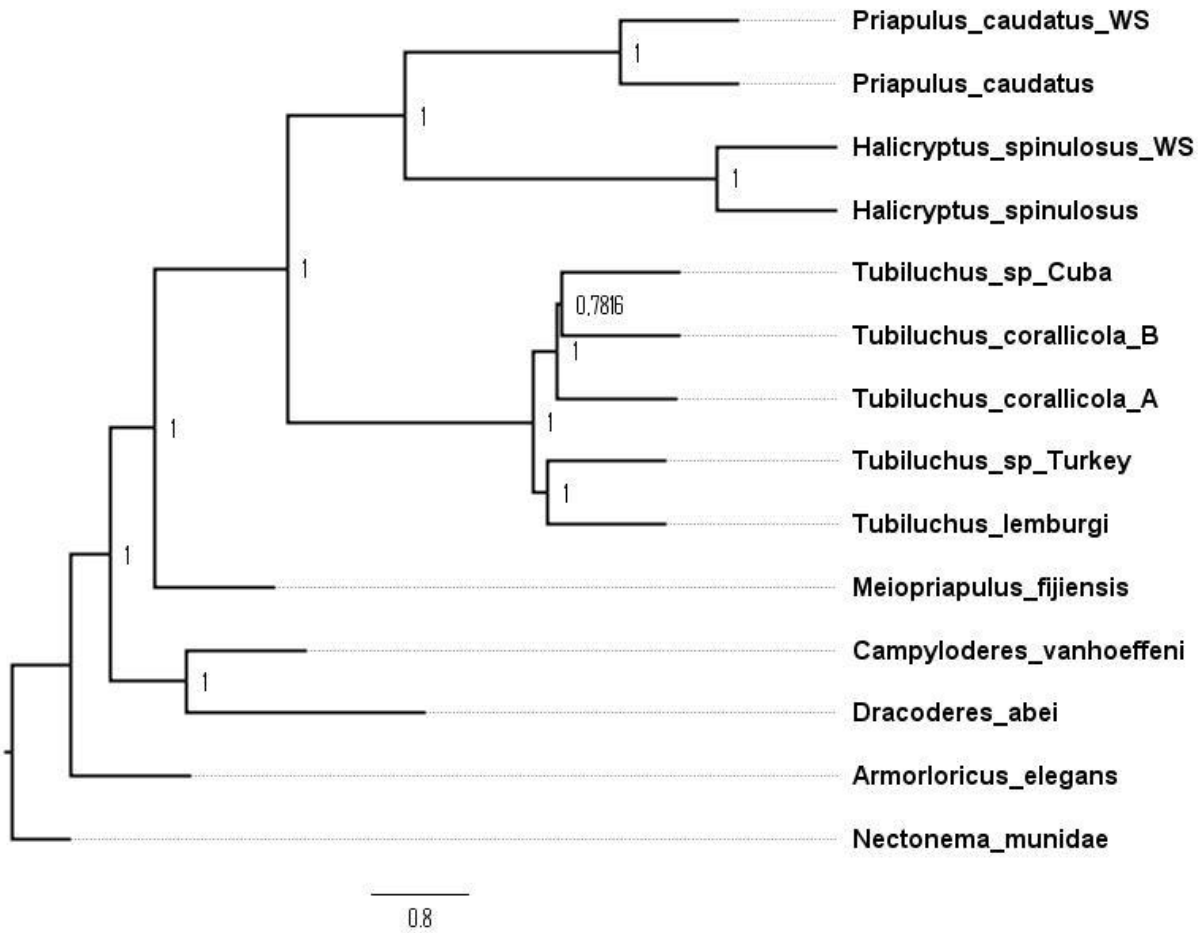
```
java -jar astral.5.7.8.jar -i 08_concatenated_gene_trees -o
08_astral.tre
```



Supplementary figure S16 Coalescent-based species tree from the 50 % occupancy matrix based on 2060 single-copy gene trees generated with ASTRAL-III. Support values are local posterior probabilities.

Single-copy 50 % occupancy matrix (2060 gene trees) (input file '07_concatenated_gene_trees.tre')

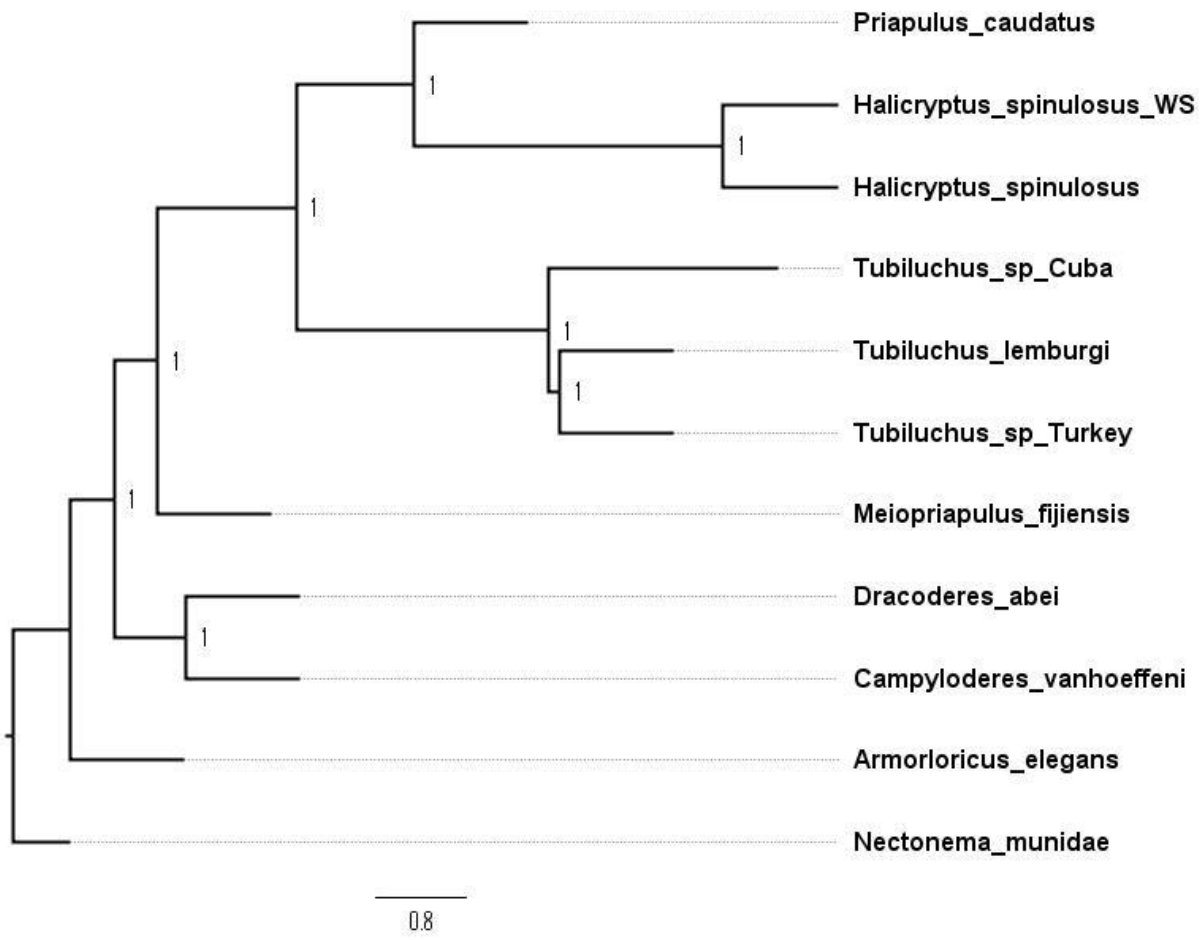
```
java -jar astral.5.7.8.jar -i 07_concatenated_gene_trees -o
07_astral.tre
```



Supplementary figure S17 Coalescent-based species tree of all 50,723 multi-copy gene regions generated by ASTRAL-Pro2. Support values are local posterior probabilities.

Multi-copy gene regions dataset with all transcriptomes (input file: ‘multicopy_all_genetrees.tre‘)

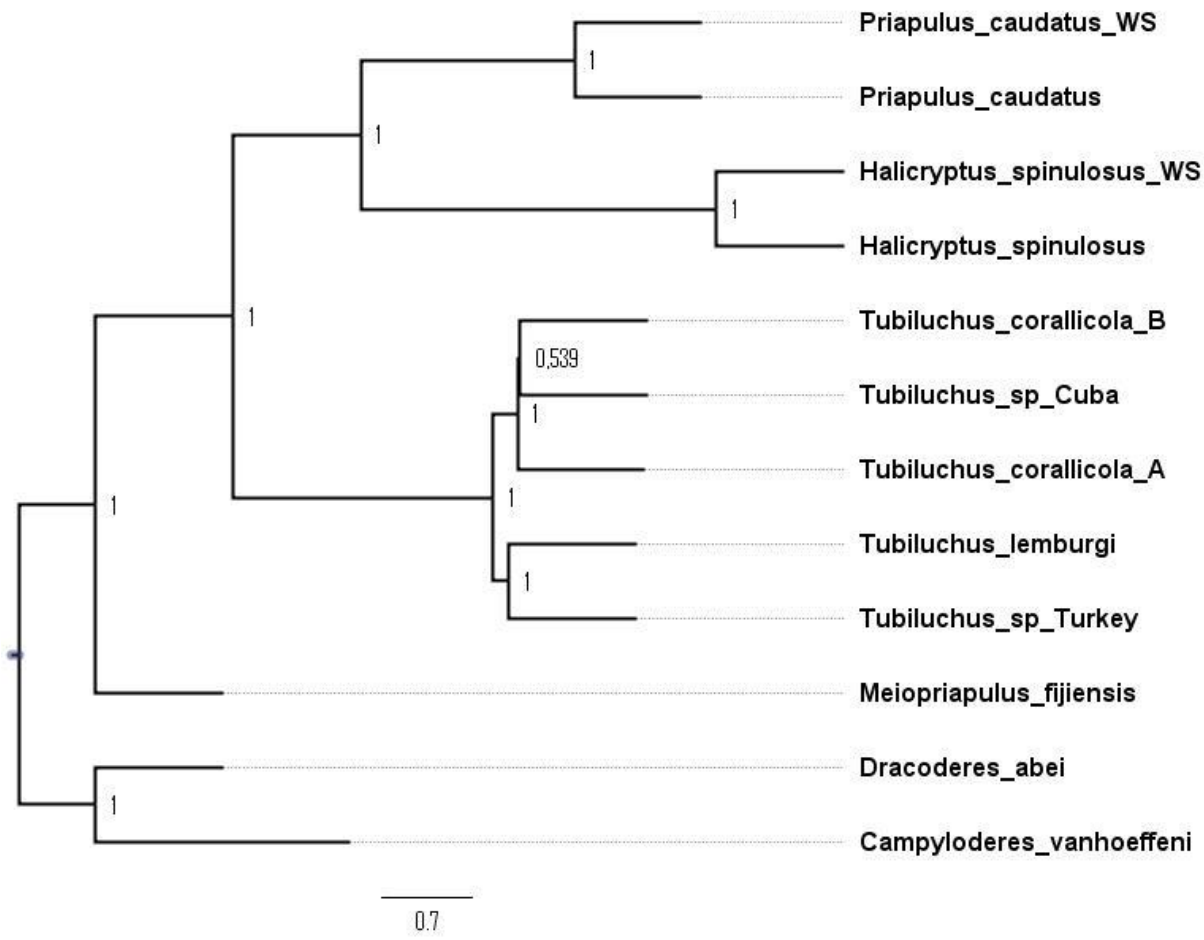
```
astral-pro –o multicopy_all_astralpro.tre –i
multicopy_all_genetrees.tre
```



Supplementary figure S18 Coalescent-based species tree from 45,078 multi-copy gene regions generated by ASTRAL-Pro2 after excluding transcriptomes with low BUSCO scores. Support values are local posterior probabilities.

Multi-copy dataset after removing transcriptomes with low BUSCO scores (input file 'multicopy_without_low_quality.tre')

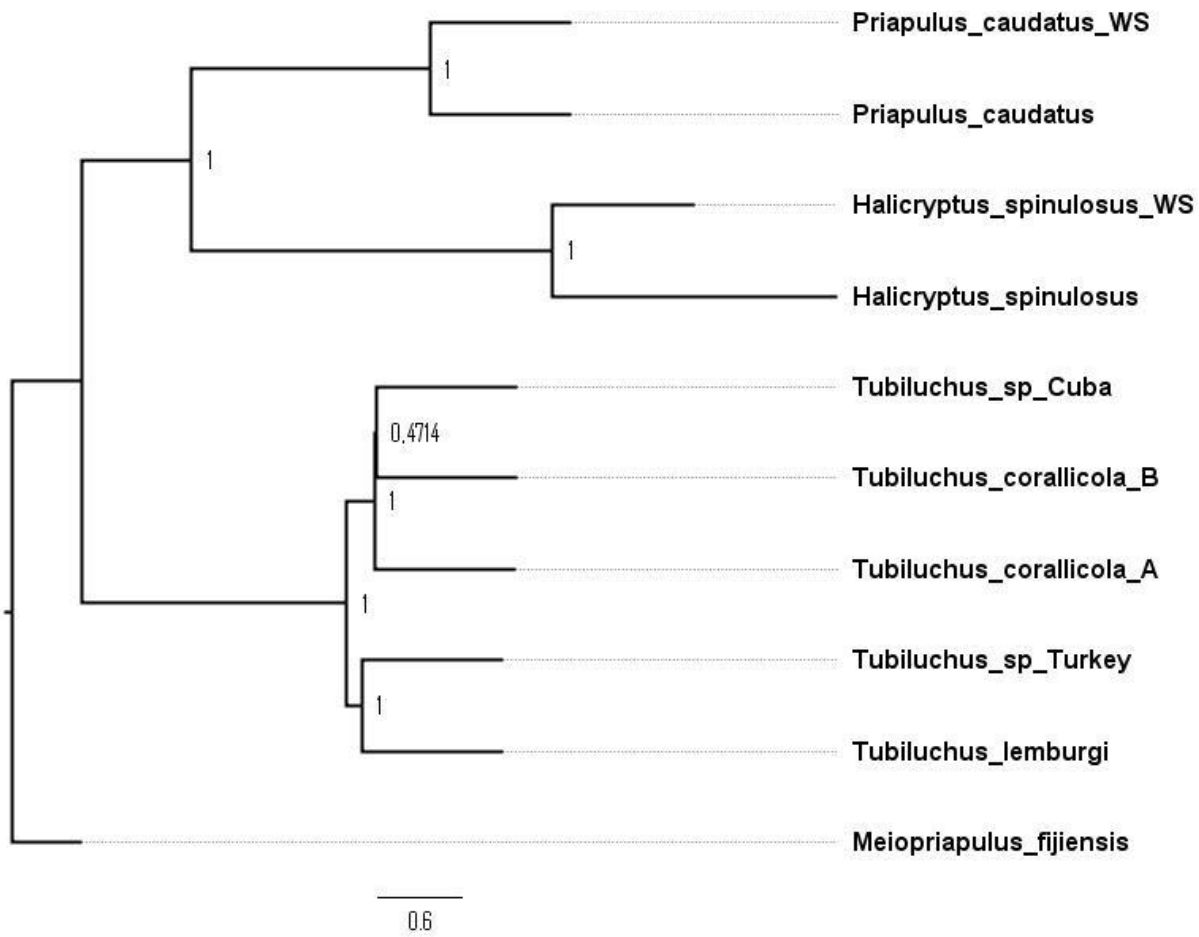
```
astral-pro –o multicopy_lowq_astralpro.tre –i
multicopy_without_low_quality.tre
```



Supplementary figure S19 Coalescent-based species tree from 43,952 multi-copy gene regions generated by ASTRAL-Pro2 after excluding outgroups that have long branches in IQ-Tree2 generated species trees. Support values are local posterior probabilities.

Multi-copy dataset after removing outgroup transcriptomes that have long branches in IQ-Tree2 species trees (input file 'multicopy_without_long_branches.tre')

```
astral-pro –o multicopy_lb_astralpro.tre –i
multicopy_without_long_branches.tre
```

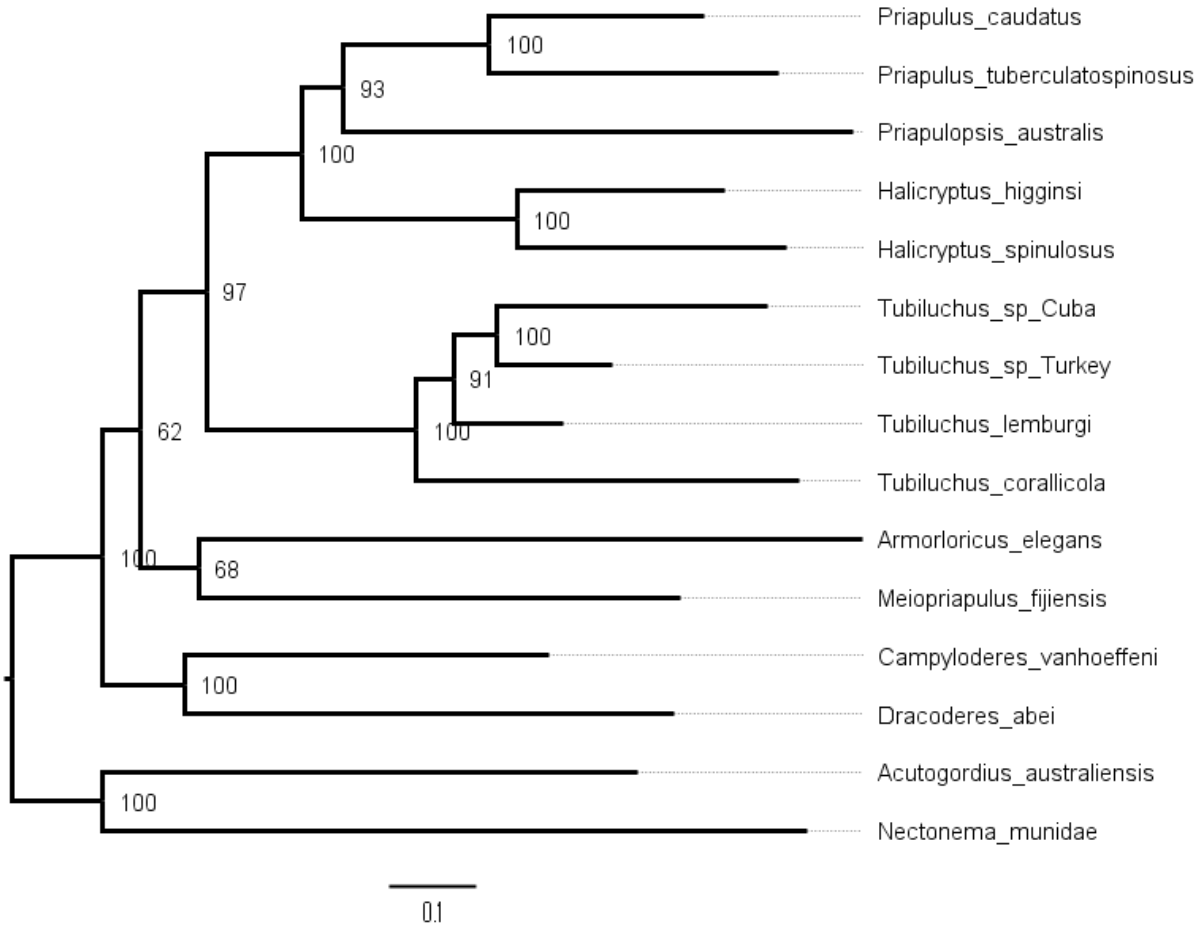


Supplementary figure S20 Coalescent-based species tree from 37,904 multi-copy gene regions generated by ASTRAL-Pro2 after excluding all outgroups. Support values are local posterior probabilities.

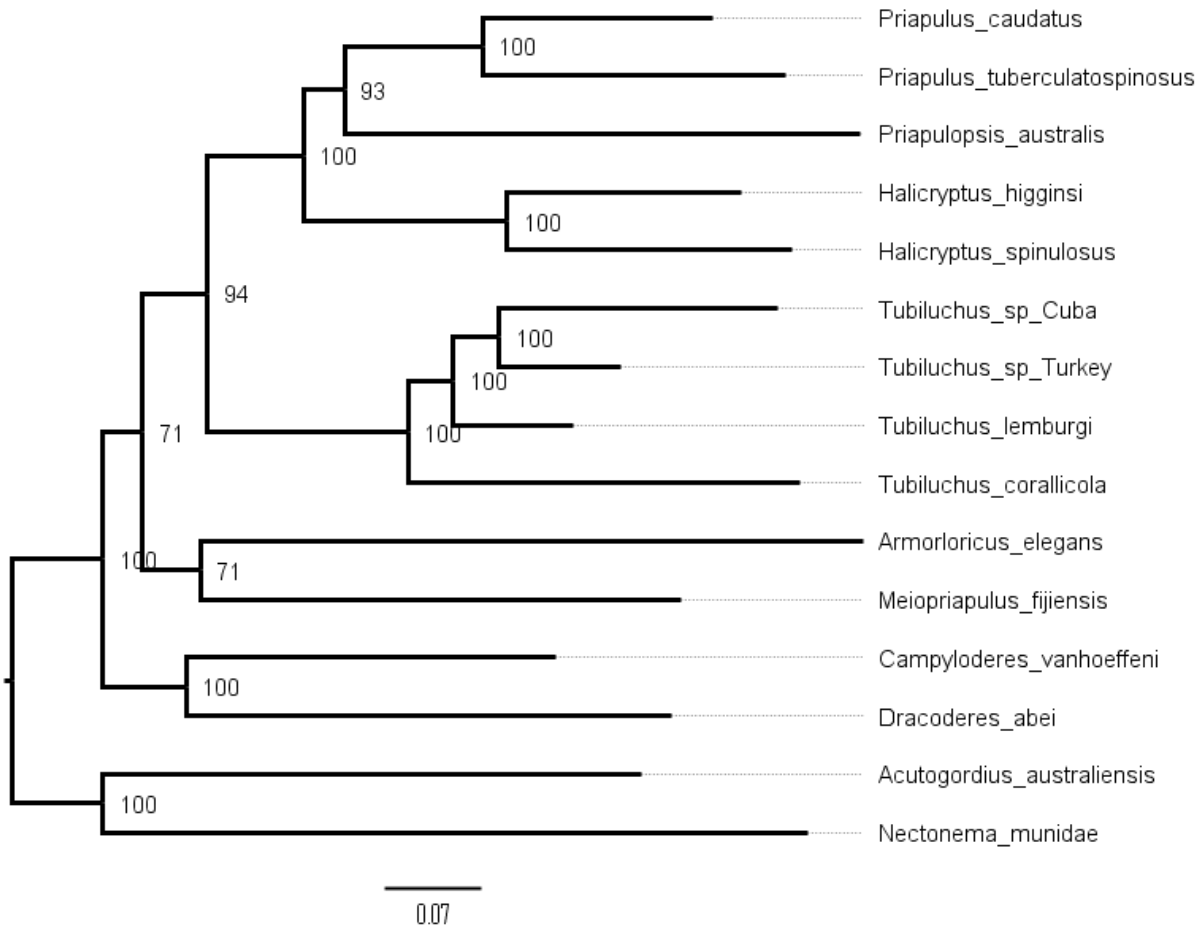
Multi-copy dataset after removing all outgroups (input file 'multicopy_without_outgroups.tre')

```
astral-pro –o multicopy_og_astralpro.tre –i
multicopy_without_outgroups.tre
```

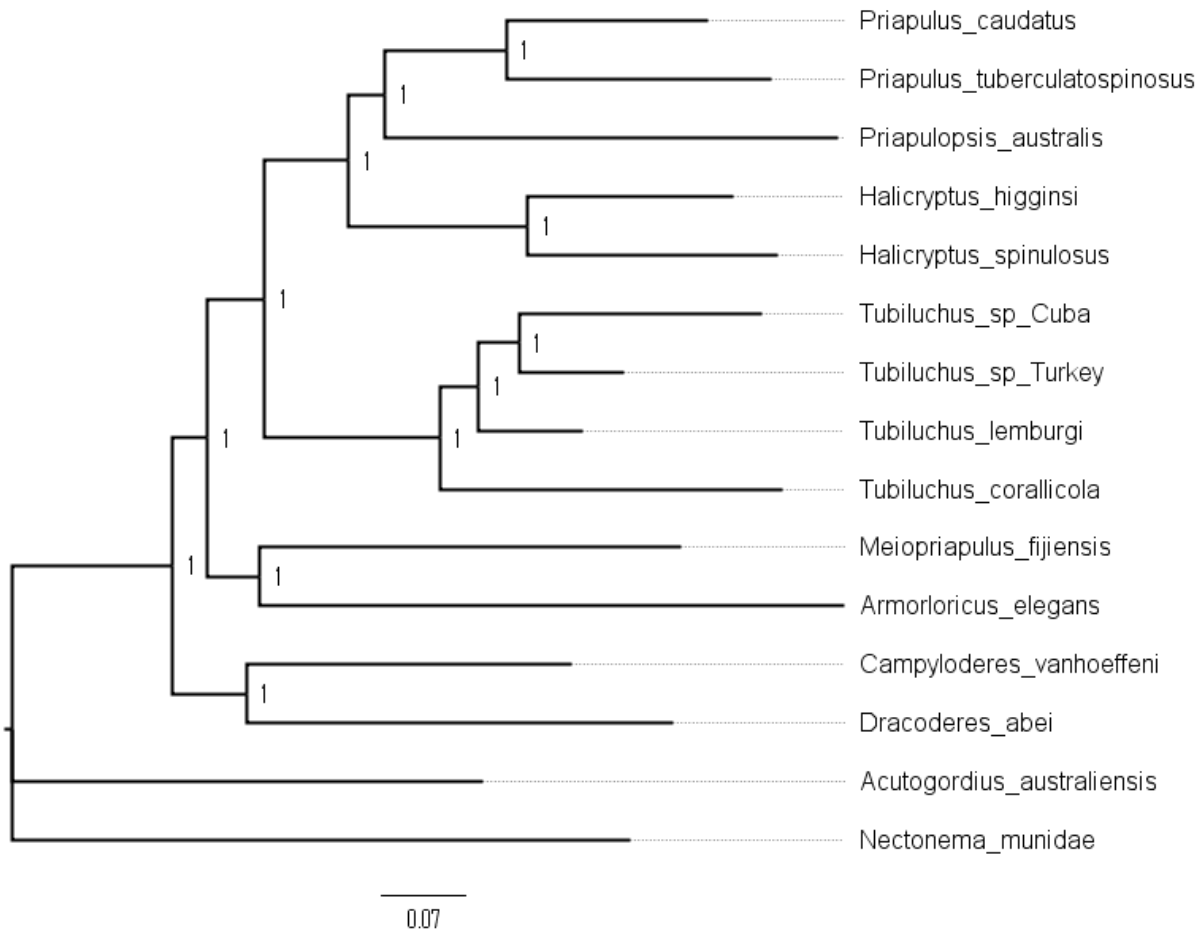
2. Combined (transcriptomic and genomic) analysis



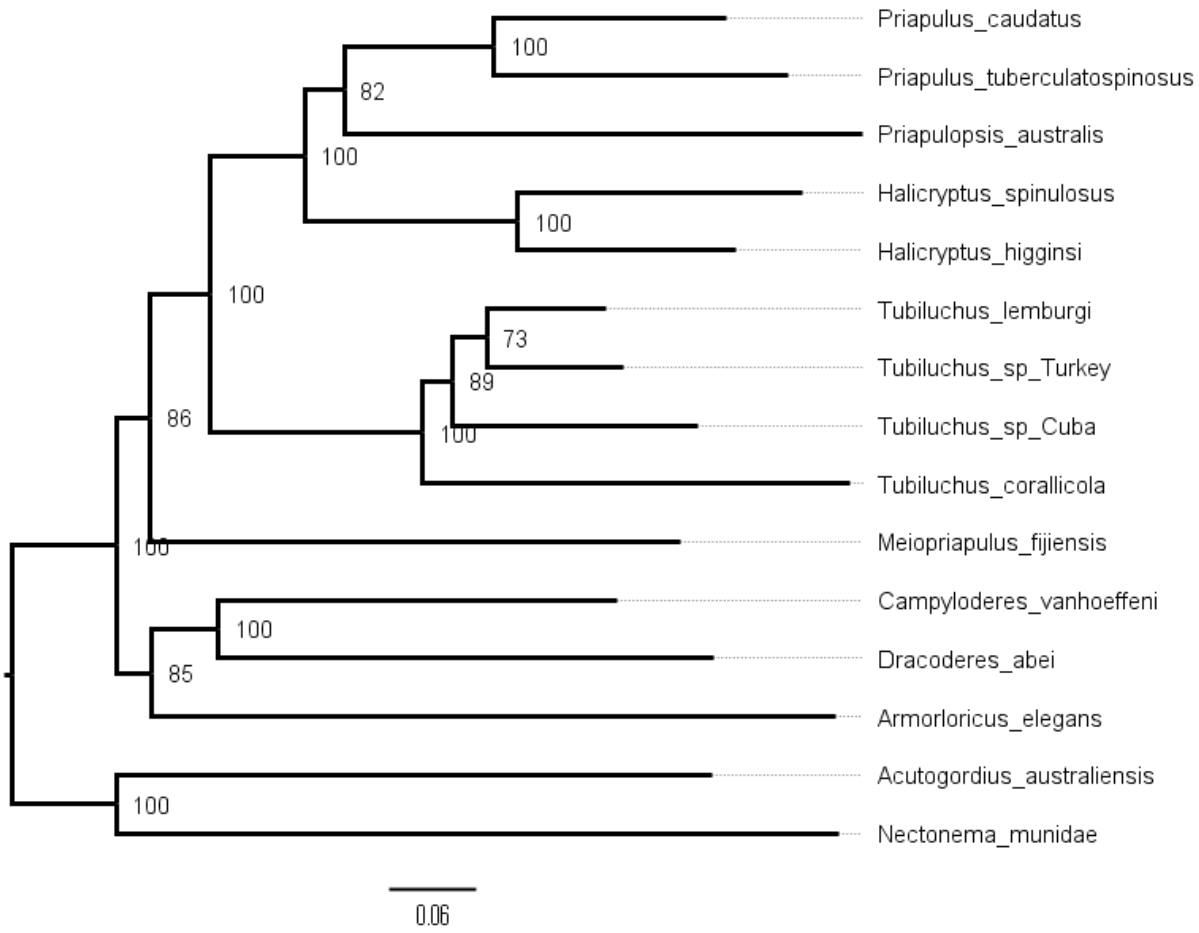
Supplementary figure S21 Maximum likelihood tree of the partitioned 90% occupancy matrix (208 loci) of the combined molecular data generated with IQ-Tree2. Support values are bootstrap values.



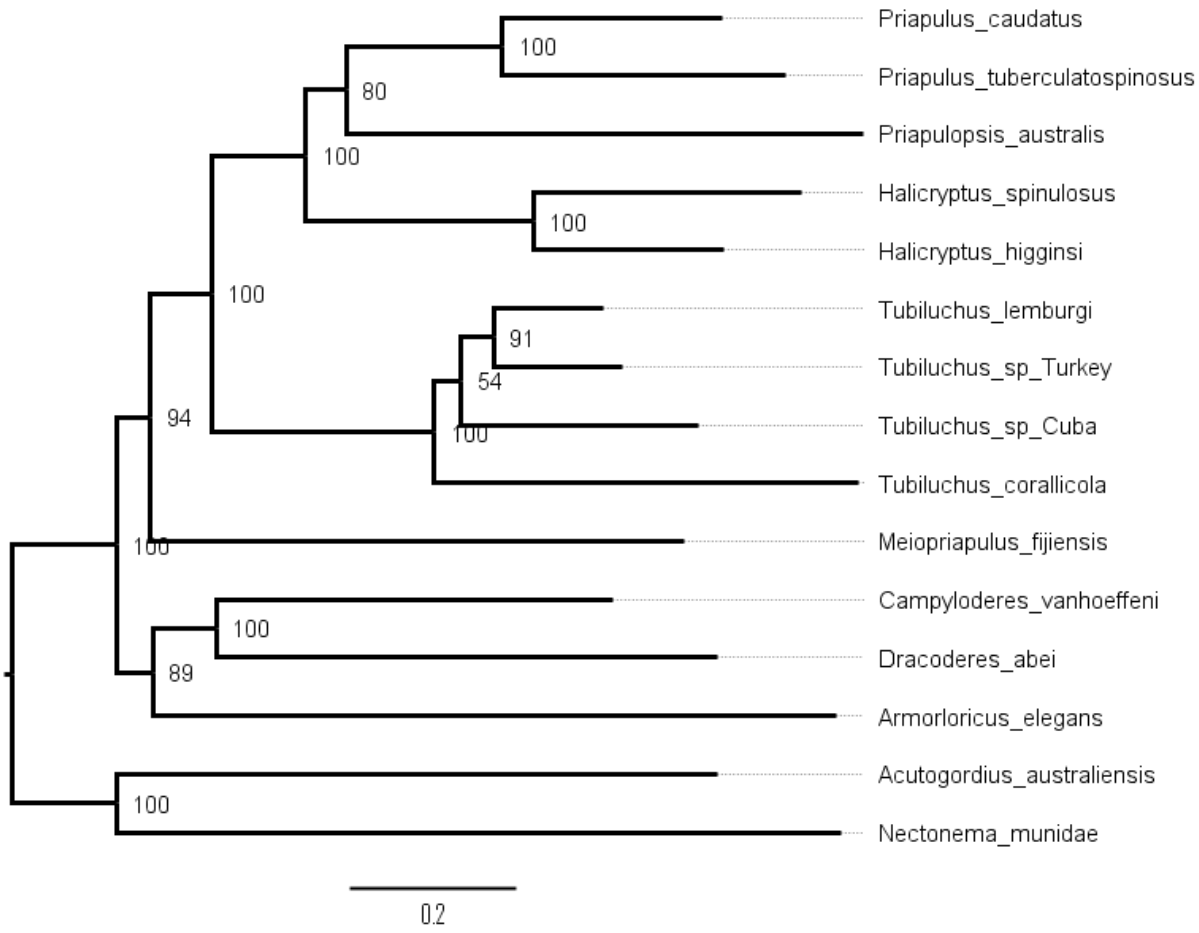
Supplementary figure S22 Maximum likelihood tree of the unpartitioned 90% occupancy matrix (208 loci) of the combined molecular data generated with IQ-Tree2. Support values are bootstrap values.



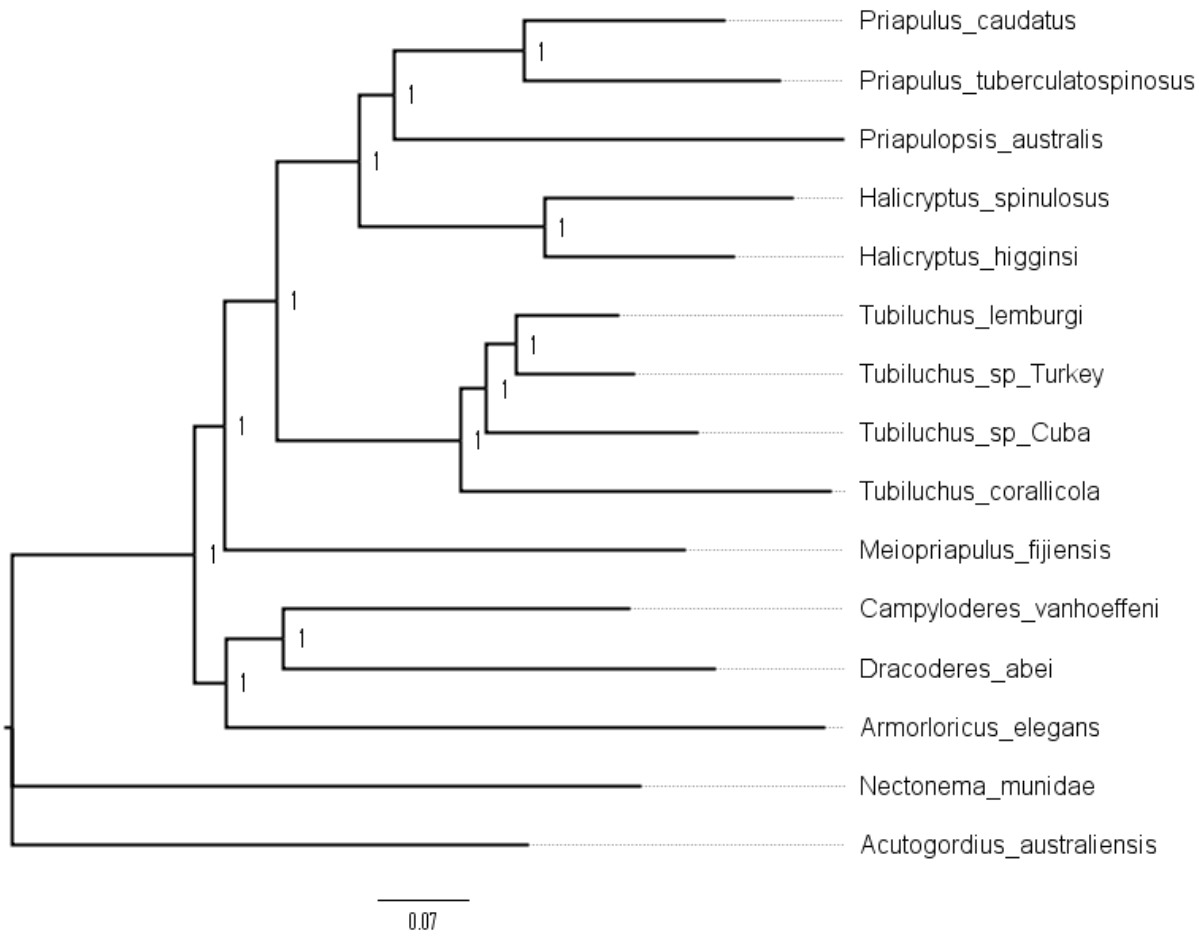
Supplementary figure S23 Bayesian tree of the unpartitioned 90% occupancy matrix (208 loci) of the combined molecular data generated with ExaBayes. Support values are posterior probabilities.



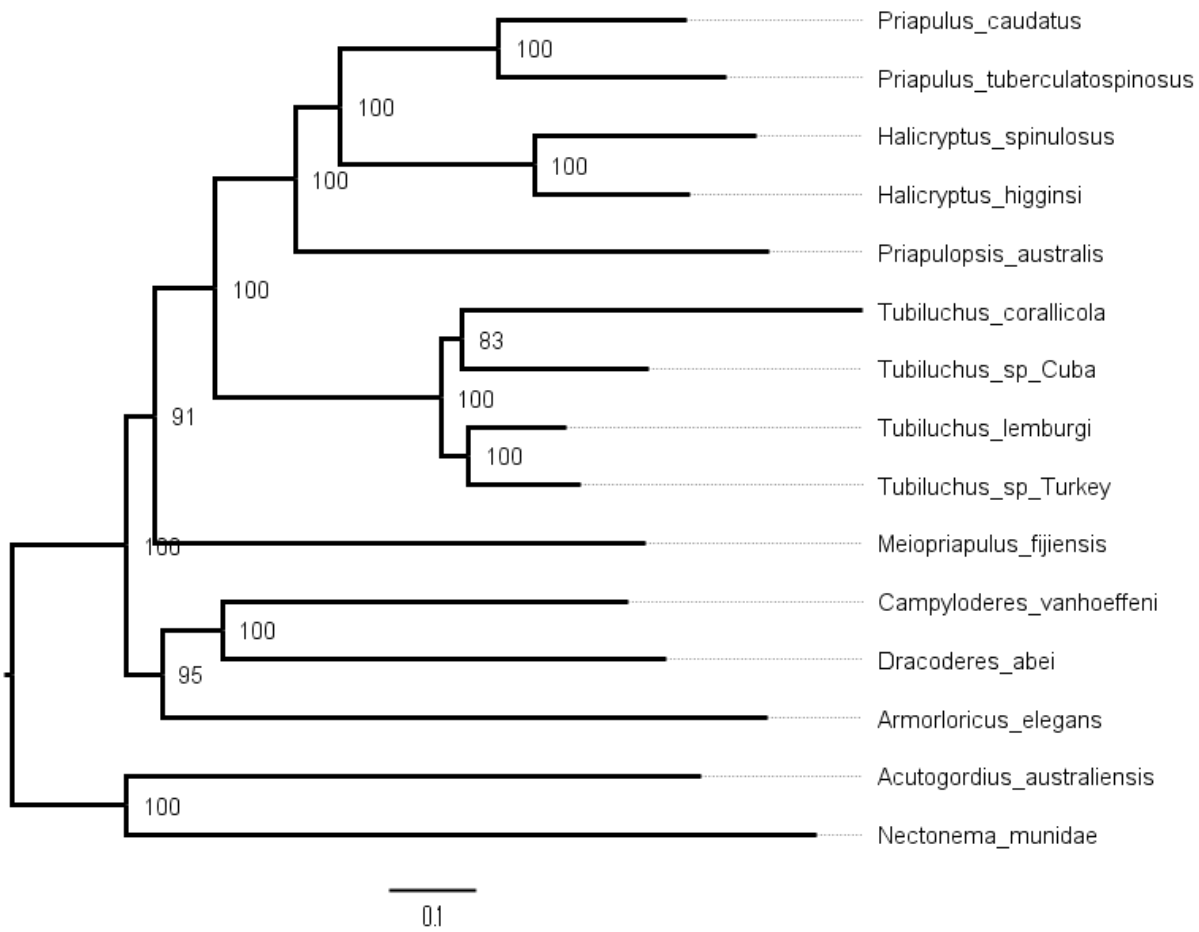
Supplementary figure S24 Maximum likelihood tree of the partitioned 80% occupancy matrix (378 loci) of the combined molecular data generated with IQ-Tree2. Support values are bootstrap values.



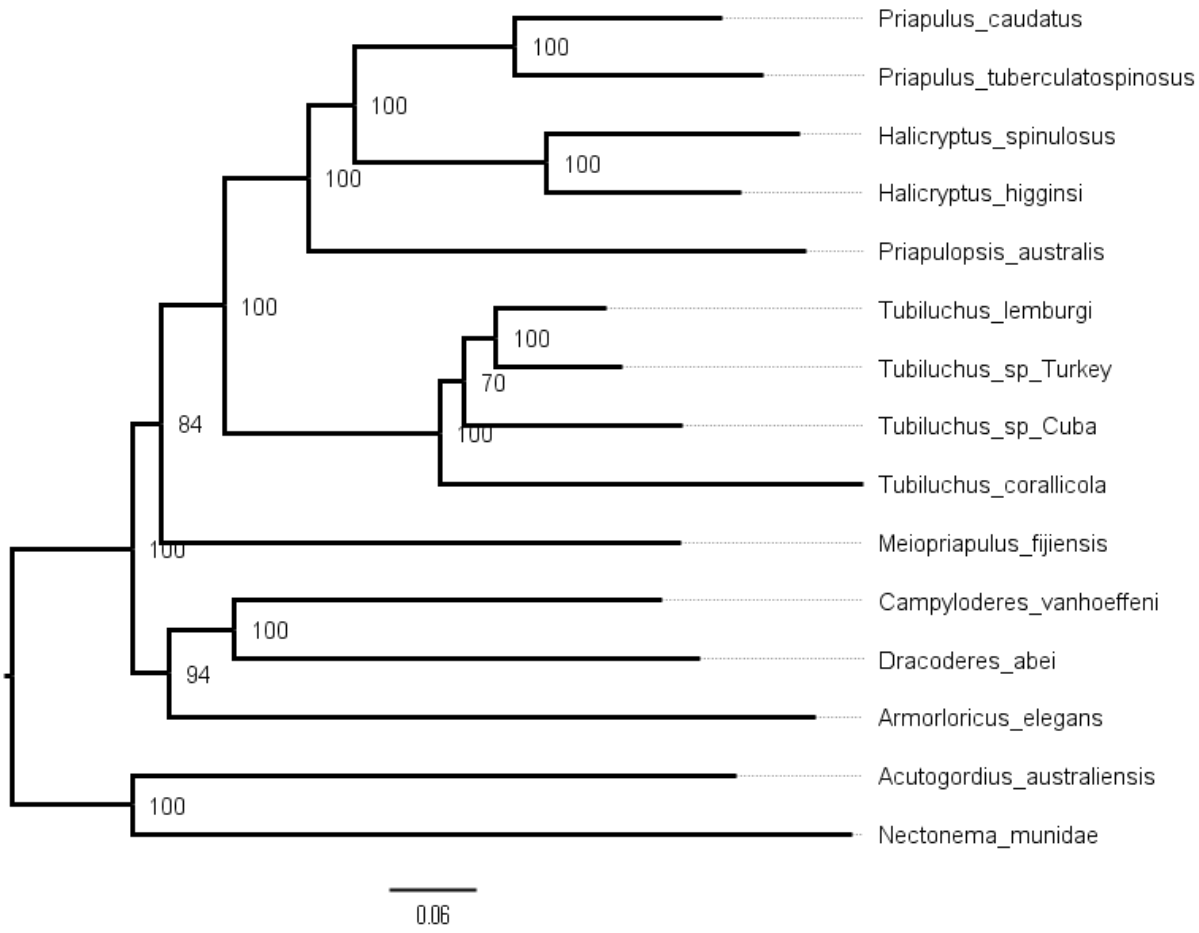
Supplementary figure S25 Maximum likelihood tree of the unpartitioned 80% occupancy matrix (378 loci) of the combined molecular data generated with IQ-Tree2. Support values are bootstrap values.



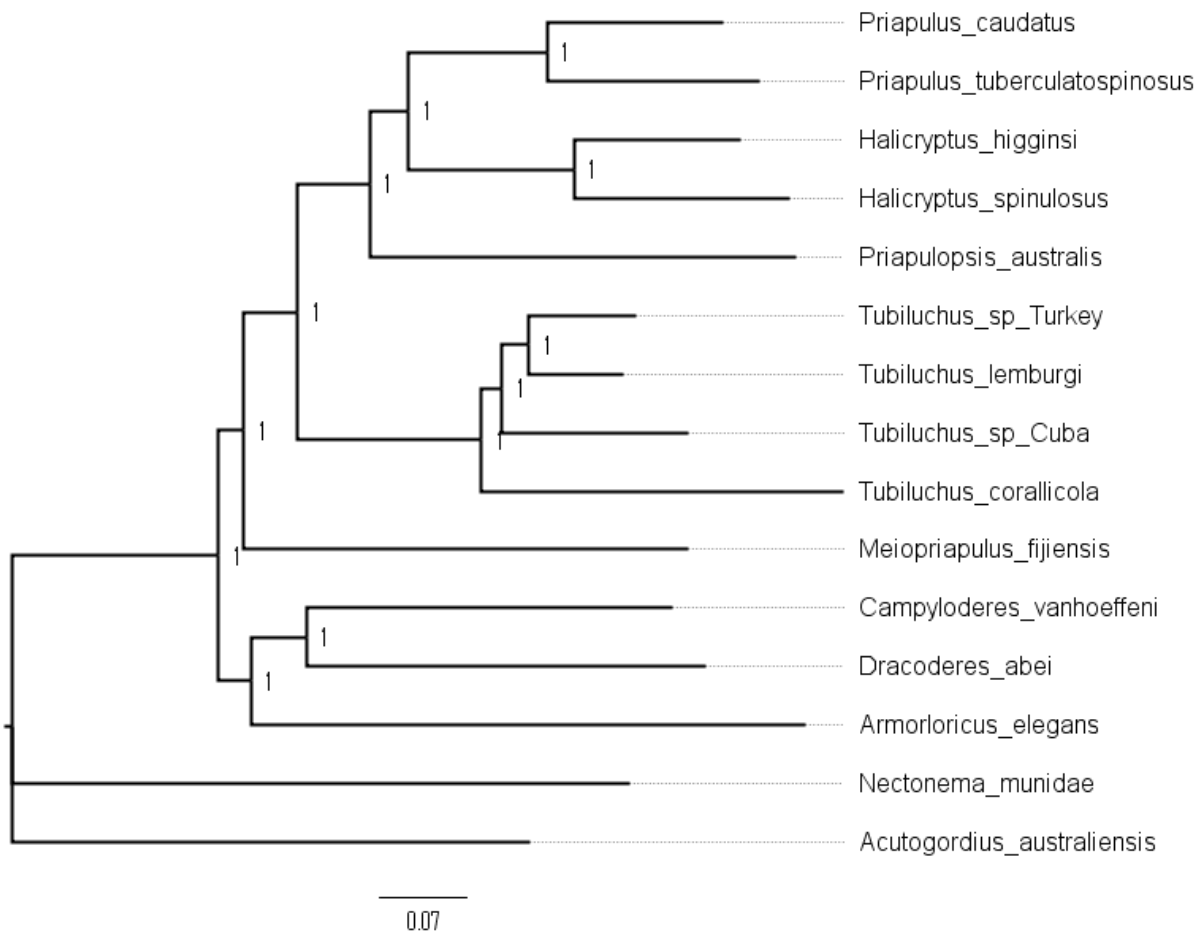
Supplementary figure S26 Bayesian tree of the unpartitioned 80% occupancy matrix (378 loci) of the combined molecular data generated with ExaBayes. Support values are posterior probabilities.



Supplementary figure S27 Maximum likelihood tree of the partitioned 70% occupancy matrix (724 loci) of the combined molecular data generated with IQ-Tree2. Support values are bootstrap values.



Supplementary figure S28 Maximum likelihood tree of the unpartitioned 70% occupancy matrix (724 loci) of the combined molecular data generated with IQ-Tree2. Support values are bootstrap values.



Supplementary figure S29 Bayesian tree of the unpartitioned 70% occupancy matrix (724 loci) of the combined molecular data generated with ExaBayes. Support values are posterior probabilities.