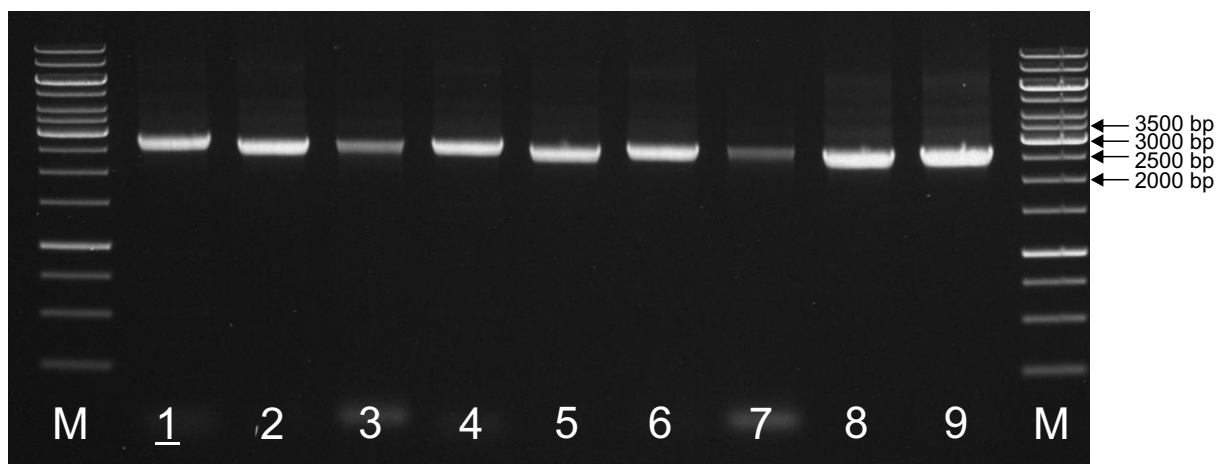
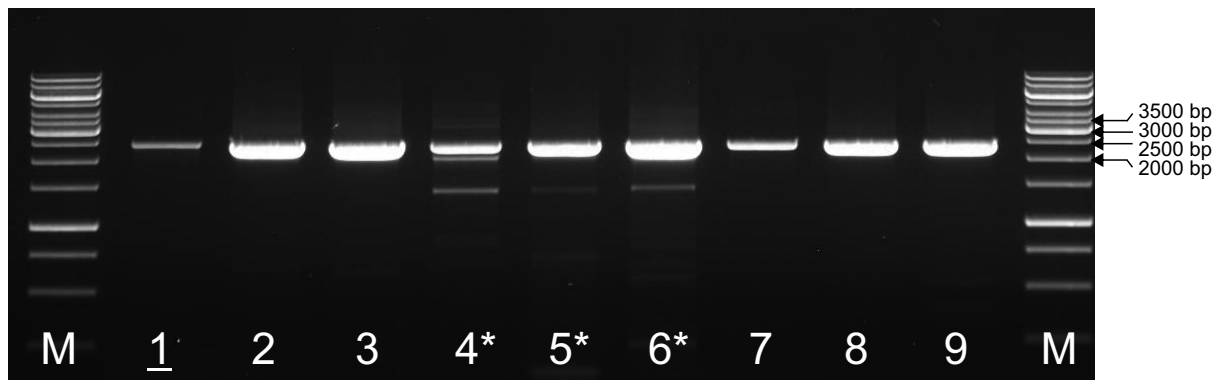


**Figure S1.** Representative agarose gels of CR1/CR2 amplicons obtained for *Sagittarius serpentarius* (A) and *Cathartes aura* (B). The numbering of amplicons separated in the agarose gel corresponds to the reaction numbered in Table S7. Lane M - GeneRuler 1 kb DNA Ladder (Thermo Scientific). All 18 reactions were run with the same procedure described in Materials and methods. Conditions occurred to be improper for three reactions marked with asterisk \* and run for *Cathartes aura* (B) with the use of primer no. 1068 (Table S7). Besides the most abundant amplicon with the length of about 2500 bp, some shorter unspecific fragments were also obtained. Finally, only the longest (underlined) and specific fragments were sequenced.

A

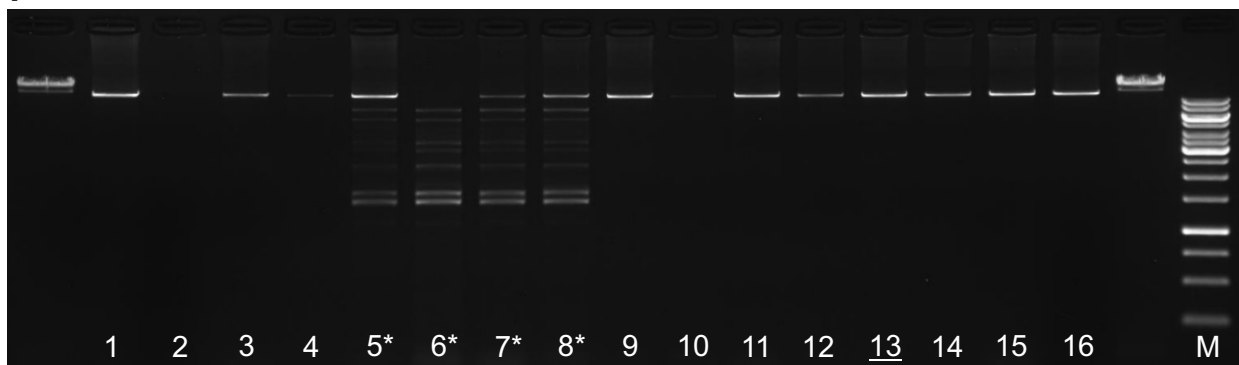


B

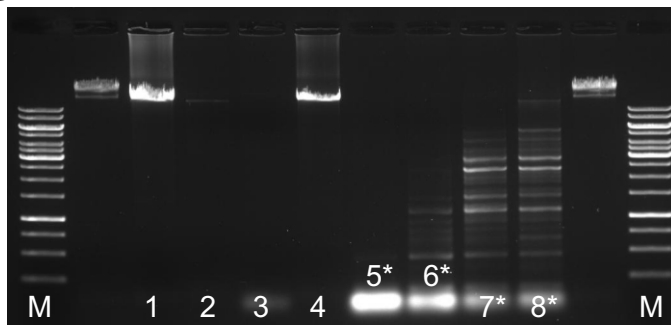


**Figure S2.** Representative agarose gels of L amplicons obtained for *Sarcoramphus papa* (A) and *Coragyps atratus* (B and C). The numbering of amplicons separated in the agarose gel corresponds to the reaction numbered in Table S2. Lane M - GeneRuler 1 kb DNA Ladder (Thermo Scientific). The predicted length of L amplicons was about 13,300 bp as calculated based on the position of appropriate forward and reverse primers (see Table S2). It is much greater than the longest fragment of the mass marker with the length of 10,000 bp. Such specific and efficient products were obtained for 9 or 3 out of 16 reactions run for *Sarcoramphus papa* and *Coragyps atratus*, respectively. In the case of reactions marked with asterisk \*, in which primer no. 1115 (Table S1) was used, many shorter unspecific amplicons were obtained. Only the underlined specific amplicons were finally sequenced.

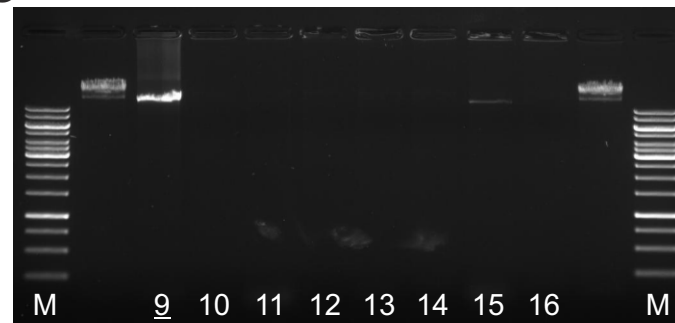
A



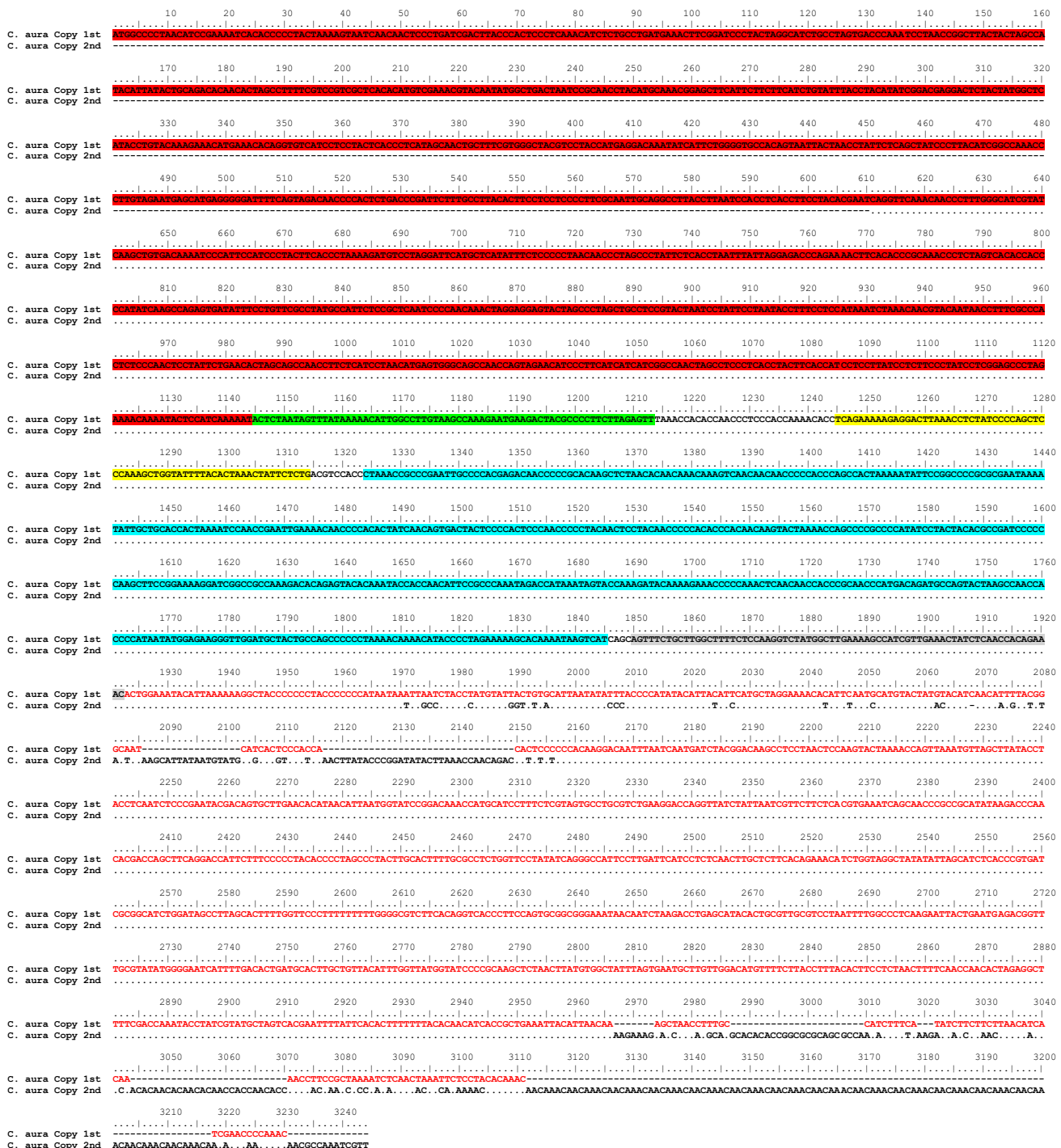
B



C



**Figure S3.** Alignment of tandemly duplicated segment from *cytb* to CR with its first copy in *Cathartes aura*, *Cathartes burrovianus*, *Coragyps atratus*, *Sagittarius serpentarius*, *Sarcoramphus papa* and *Vultur gryphus* mitogenomes. Dots in the second copy sequence indicate residues identical with those in the first copy. **Cytochrome b genes (in the 1st copies)** and ***cytb* pseudogene (in the 2nd copies)** are marked in **red background**, **tRNA-Thr** copies in **green background**, **tRNA-Pro** copies in **yellow background**, **ND6** copies in **blue background**, **tRNA-Glu** copies in **grey background**. **Two copies of control region** are in **red font**.



[illegible]

1130 1140 1150 1160 1170 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280

C. atratus Copy 1st  
C. atratus Copy 2nd

1290 1300 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430 1440

C. atratus Copy 1st  
C. atratus Copy 2nd

1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560 1570 1580 1590 1600

C. atratus Copy 1st  
C. atratus Copy 2nd

1610 1620 1630 1640 1650 1660 1670 1680 1690 1700 1710 1720 1730 1740 1750 1760

C. atratus Copy 1st  
C. atratus Copy 2nd

1770 1780 1790 1800 1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920

C. atratus Copy 1st  
C. atratus Copy 2nd

1930 1940 1950 1960 1970 1980 1990 2000 2010 2020 2030 2040 2050 2060 2070 2080

C. atratus Copy 1st  
C. atratus Copy 2nd

2090 2100 2110 2120 2130 2140 2150 2160 2170 2180 2190 2200 2210 2220 2230 2240

C. atratus Copy 1st  
C. atratus Copy 2nd

2250 2260 2270 2280 2290 2300 2310 2320 2330 2340 2350 2360 2370 2380 2390 2400

C. atratus Copy 1st  
C. atratus Copy 2nd

2410 2420 2430 2440 2450 2460 2470 2480 2490 2500 2510 2520 2530 2540 2550 2560

C. atratus Copy 1st  
C. atratus Copy 2nd

2570 2580 2590 2600 2610 2620 2630 2640 2650 2660 2670 2680 2690 2700 2710 2720

C. atratus Copy 1st  
C. atratus Copy 2nd

2730 2740 2750 2760 2770 2780 2790 2800 2810 2820 2830 2840 2850 2860 2870 2880

C. atratus Copy 1st  
C. atratus Copy 2nd

2890 2900 2910 2920 2930 2940 2950 2960 2970 2980 2990 3000 3010 3020 3030 3040

C. atratus Copy 1st  
C. atratus Copy 2nd

3050 3060 3070 3080 3090 3100 3110 3120 3130 3140 3150 3160 3170 3180 3190 3200

C. atratus Copy 1st  
C. atratus Copy 2nd

3210 3220 3230 3240

C. atratus Copy 1st  
C. atratus Copy 2nd

10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160

S. serpentarius Copy 1st  
S. serpentarius Copy 2nd

170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320

S. serpentarius Copy 1st  
S. serpentarius Copy 2nd

330 340 350 360 370 380 390 400 410 420 430 440 450 460 470 480

S. serpentarius Copy 1st  
S. serpentarius Copy 2nd

490 500 510 520 530 540 550 560 570 580 590 600 610 620 630 640

S. serpentarius Copy 1st  
S. serpentarius Copy 2nd

650 660 670 680 690 700 710 720 730 740 750 760 770 780 790 800

S. serpentarius Copy 1st  
S. serpentarius Copy 2nd

810 820 830 840 850 860 870 880 890 900 910 920 930 940 950 960

S. serpentarius Copy 1st  
S. serpentarius Copy 2nd

970 980 990 1000 1010 1020 1030 1040 1050 1060 1070 1080 1090 1100 1110 1120

S. serpentarius Copy 1st  
S. serpentarius Copy 2nd

1130 1140 1150 1160 1170 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280

S. serpentarius Copy 1st  
S. serpentarius Copy 2nd

1290 1300 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430 1440

S. serpentarius Copy 1st  
S. serpentarius Copy 2nd

1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560 1570 1580 1590 1600

S. serpentarius Copy 1st  
S. serpentarius Copy 2nd

1610 1620 1630 1640 1650 1660 1670 1680 1690 1700 1710 1720 1730 1740 1750 1760

S. serpentarius Copy 1st  
S. serpentarius Copy 2nd

1770 1780 1790 1800 1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920

S. serpentarius Copy 1st  
S. serpentarius Copy 2nd

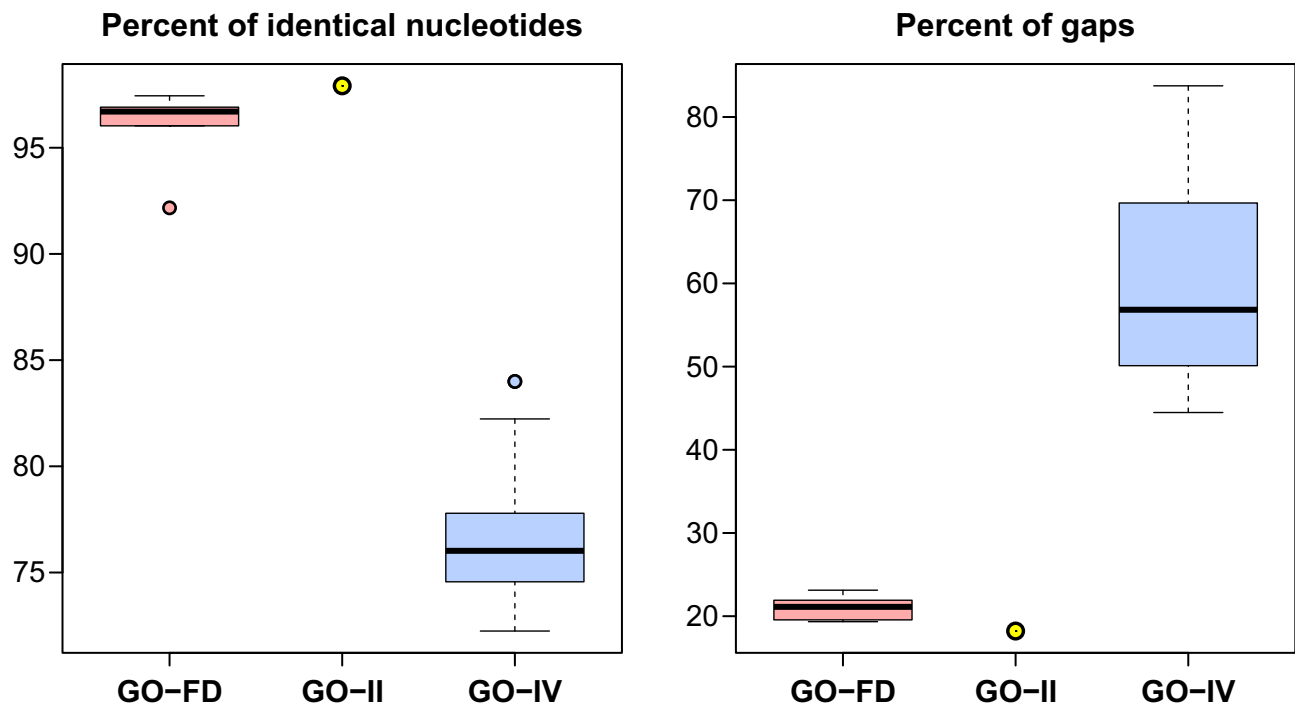
1930 1940 1950 1960 1970 1980 1990 2000 2010 2020 2030 2040 2050 2060 2070 2080

S. serpentarius Copy 1st  
S. serpentarius Copy 2nd

[illegible]

[illegible]

**Figure S4.** Percent of identity and gaps between alignments of duplicated control regions in three rearrangement types of Cathartiformes and Accipitriformes (GO-FD, GO-II, GO-IV, shown in Figure 1). The thick line indicates the median, the boxes show the quartile range and the whiskers denote the range without outliers.







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TGC GCGGT CAGTAAGATCCATCAGCCCGAGCTTCAGGCCCATTTCTTTCCCGCTACACCCCTAGCCCTACTTGCACCTTTTGCGCCTCTGGTTCCCTCGGT CAGGCGCCATC  
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GAACGCTTTGGTGTATGGCGTAAAGATATGGGGAATCACCTTAGCACTGATGCACCTTTGTCTTCCATT CAGTATTGATATCTCTTACATAATCCCTATTATGTTACT  
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***Bubo scandiacus* mitogenome from ND5 to 12S rRNA (MG681084.1)**

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***Ketupa blackstoni* mitogenome from ND5 to 12S rRNA (LC099104)**

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*Ketupa flavipes* mitogenome from ND5 to 12S rRNA (LC099100.1)

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***Strix occidentalis* mitogenome from ND5 to 12S rRNA (MF431746.1)**

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***Strix uralensis* mitogenome from ND5 to 12S rRNA (MG681081.1)**

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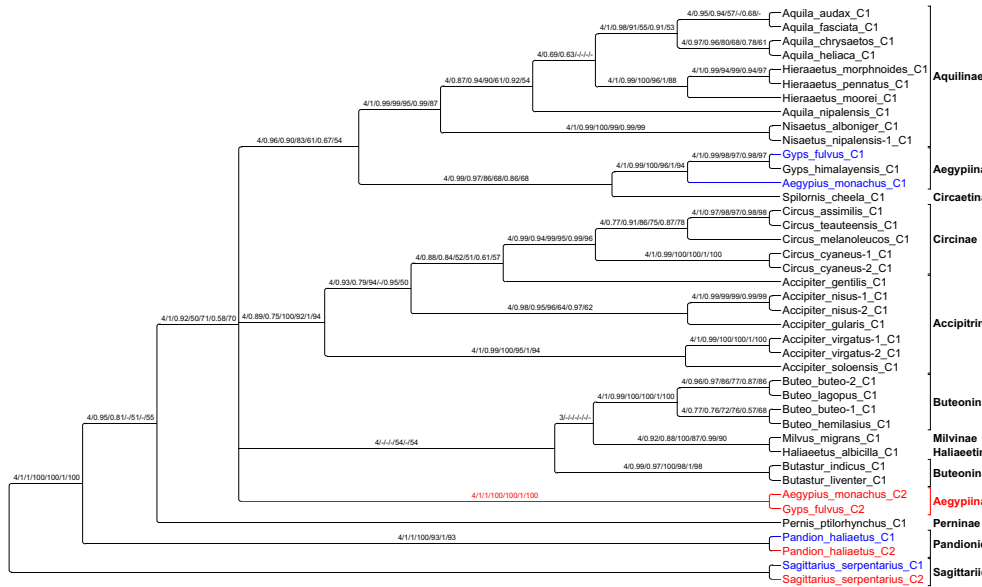
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**Figure S6.** Consensus cladograms of phylogenetic trees inferred in four programs, MrBayes, PhyloBayes, IQ-TREE and (more)PhyML, based on three types of data sets of control regions (all sites, repeats masked or removed) for Accipitriformes and related groups. Pairs of CRs from the same species are colored, whereas CRs without the second copy in the tree are in black. The blue and red colors indicate the corresponding first and second copies of CR, respectively. The taxa names are in the format Genus\_species-X\_CY, where X is the individual number (if present) and Y is the number of control region, i.e. 1 or 2. The values at nodes, in the following order N/MB/PB/SH-I/BP-I/SH-P/BP-P, indicate the number of trees containing a given node (N), posterior probabilities found in MrBayes (MB) and PhyloBayes (PB), as well as SH-aLRT and nonparametric bootstrap support values calculated in IQ-TREE (SH-I and BP-I) and (more)PhyML (SH-P and BP-P). The posterior probabilities < 0.5 and the percentages < 50% were indicated by a dash “-.” SH-aLRT means approximate likelihood ratio test based on Shimodara-Hasegawa procedure. See Table S6 for details about the data sets.

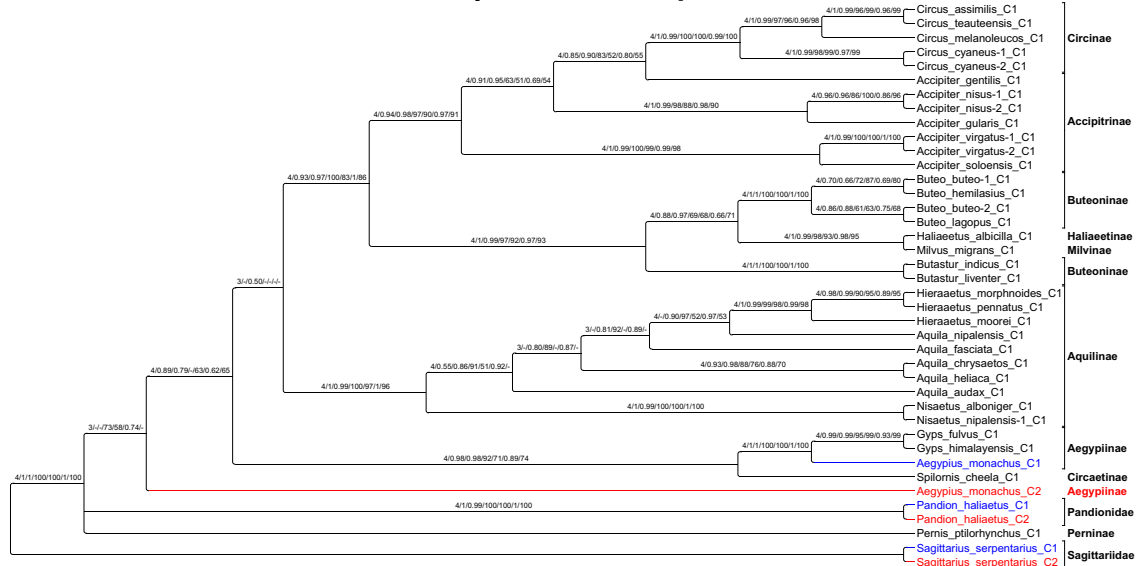
## Inclusion of Accipitriformes and all sites



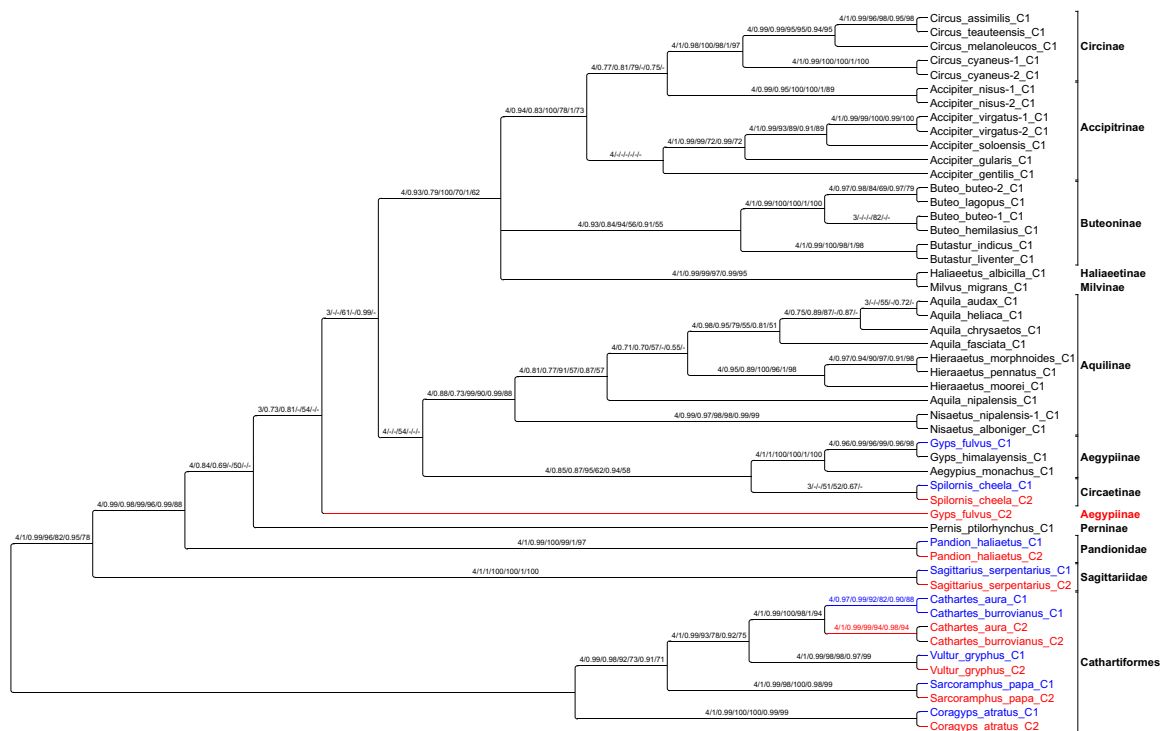
## Inclusion of Accipitriformes, Cathartiformes, Strigiformes and all sites



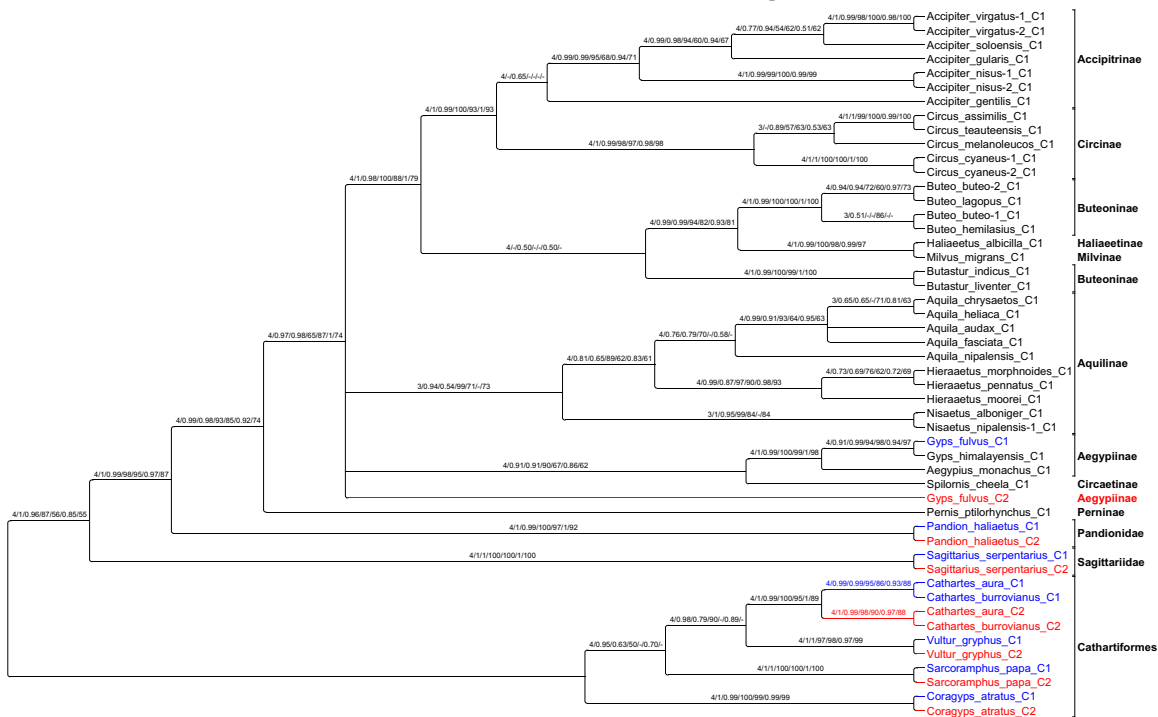
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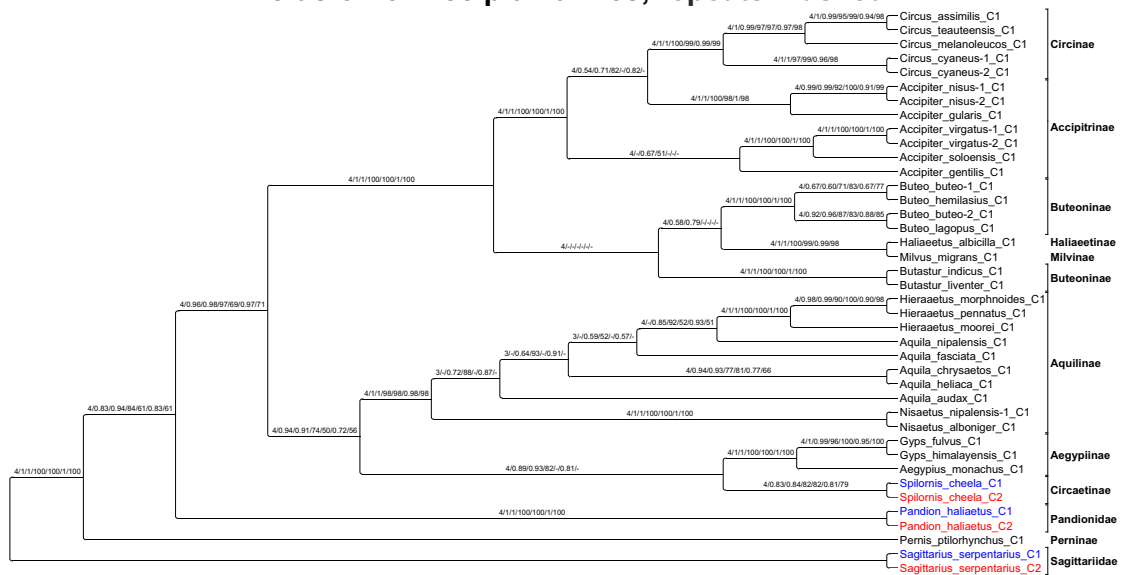
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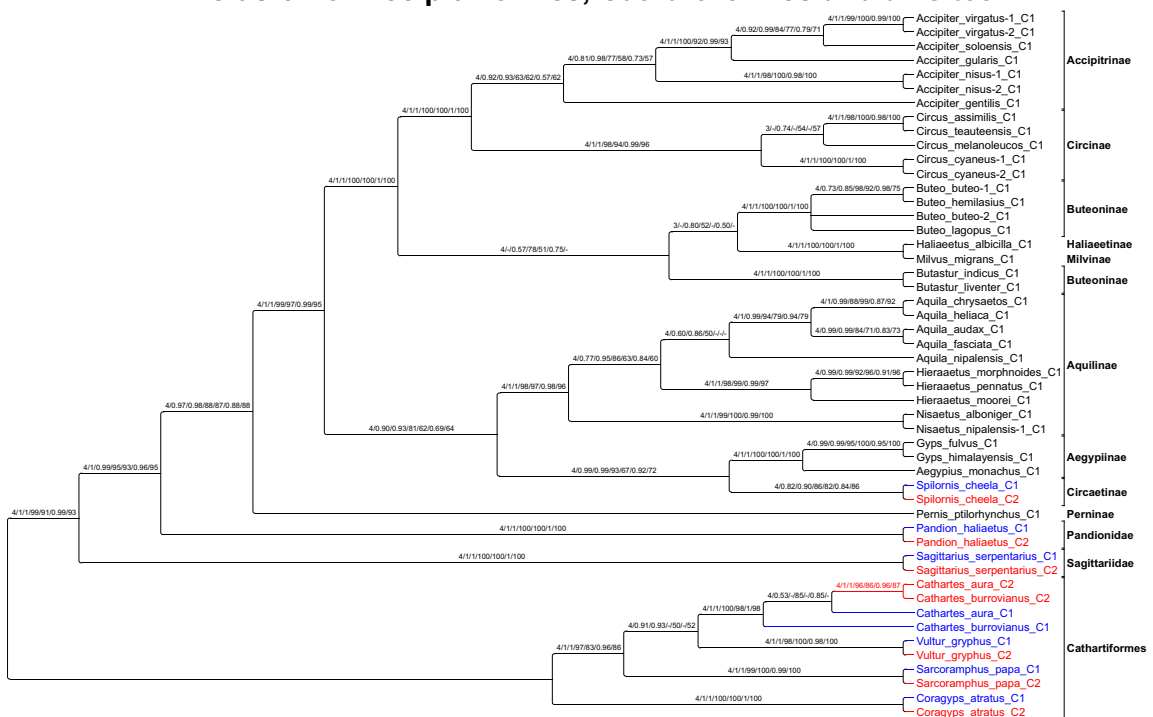
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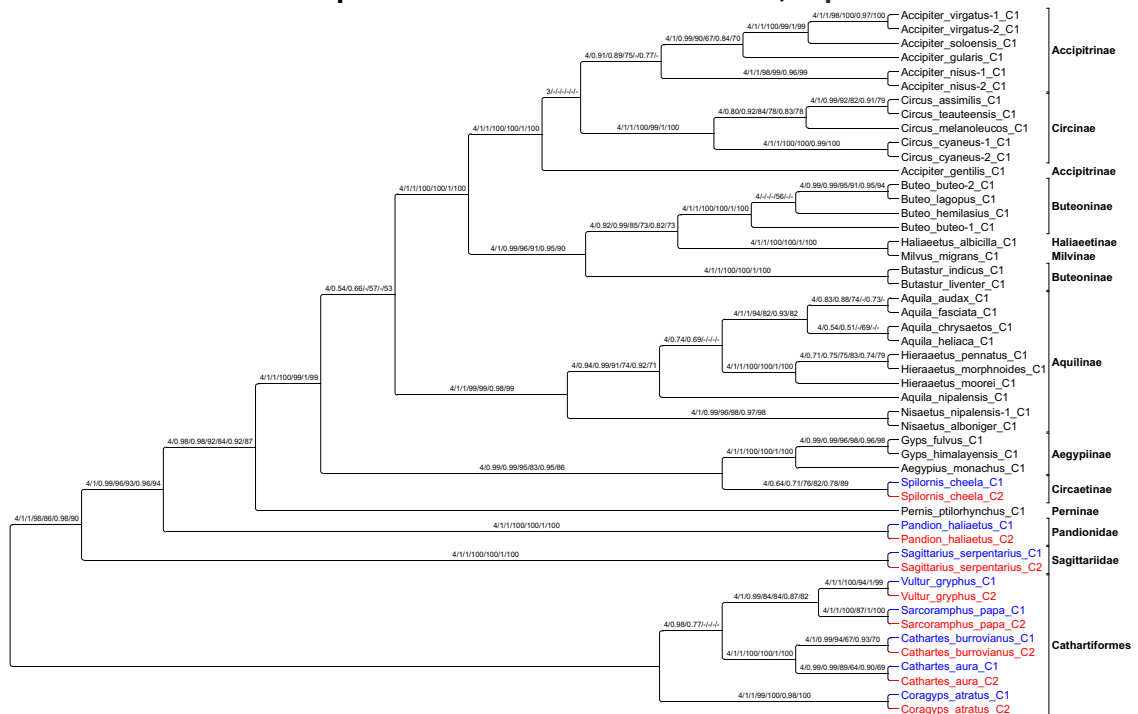
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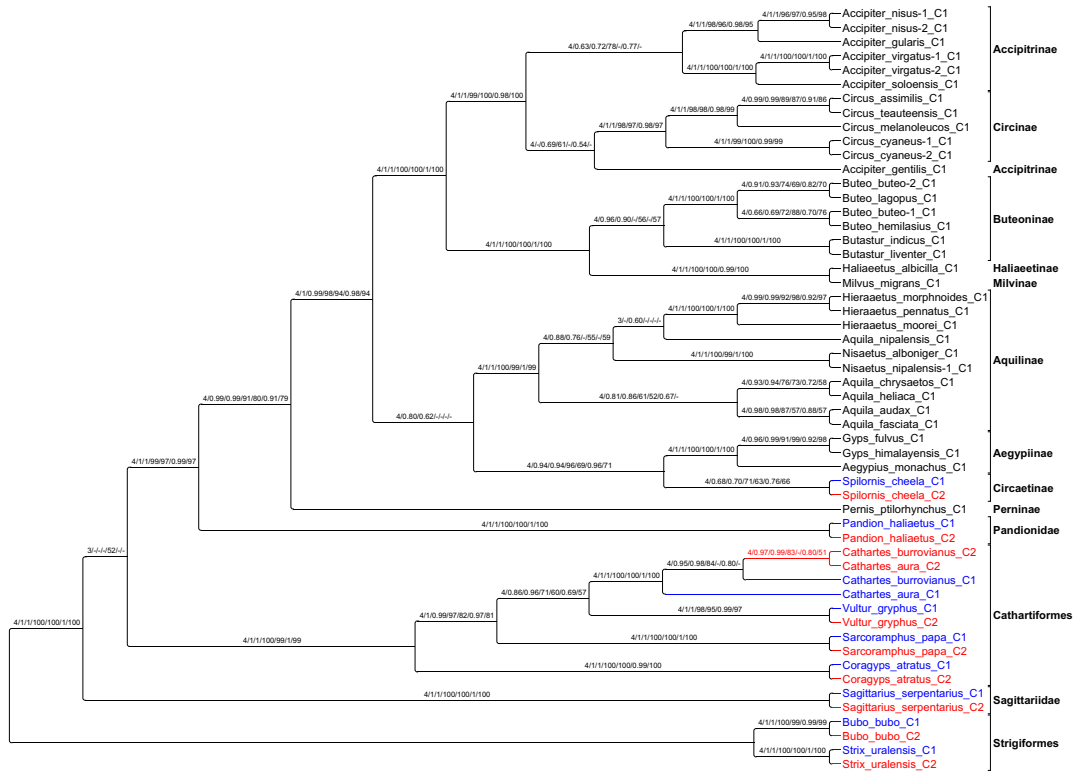
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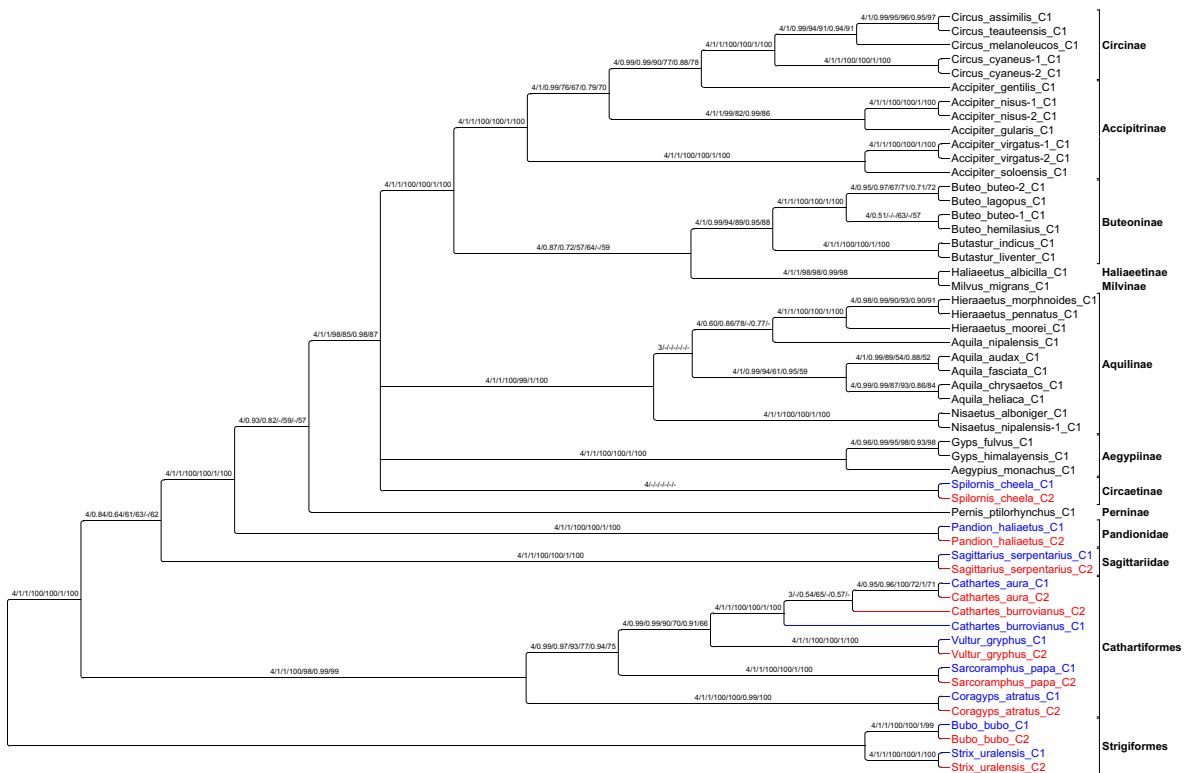
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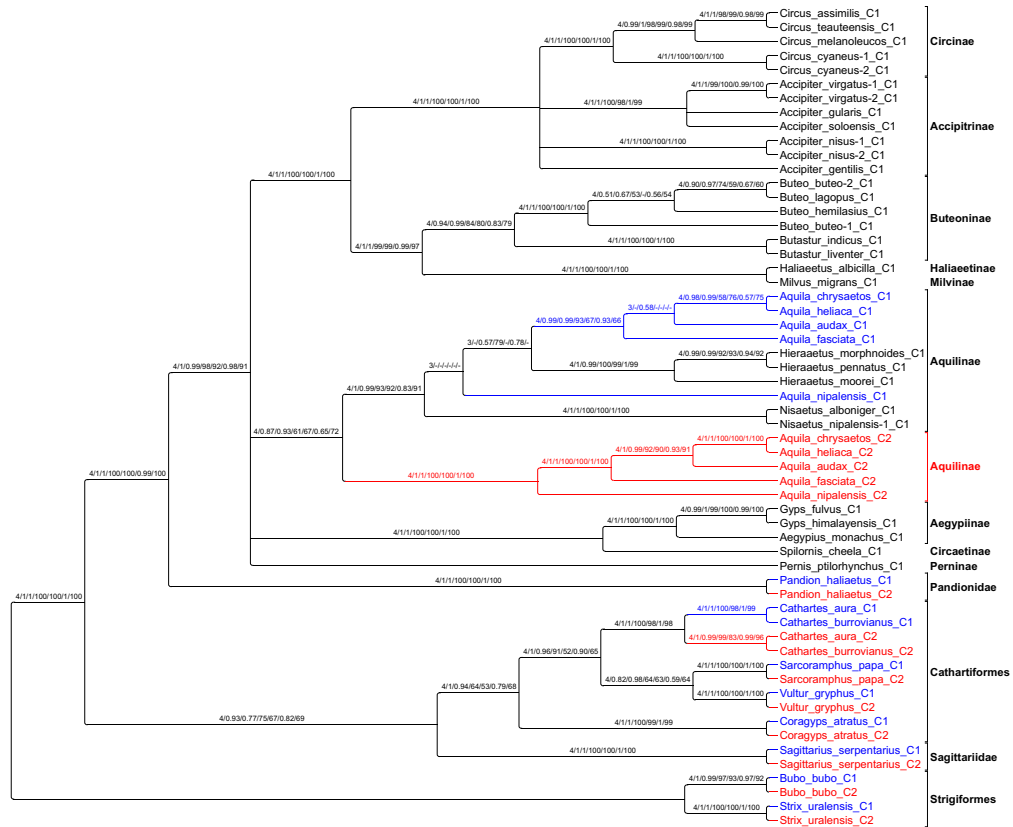
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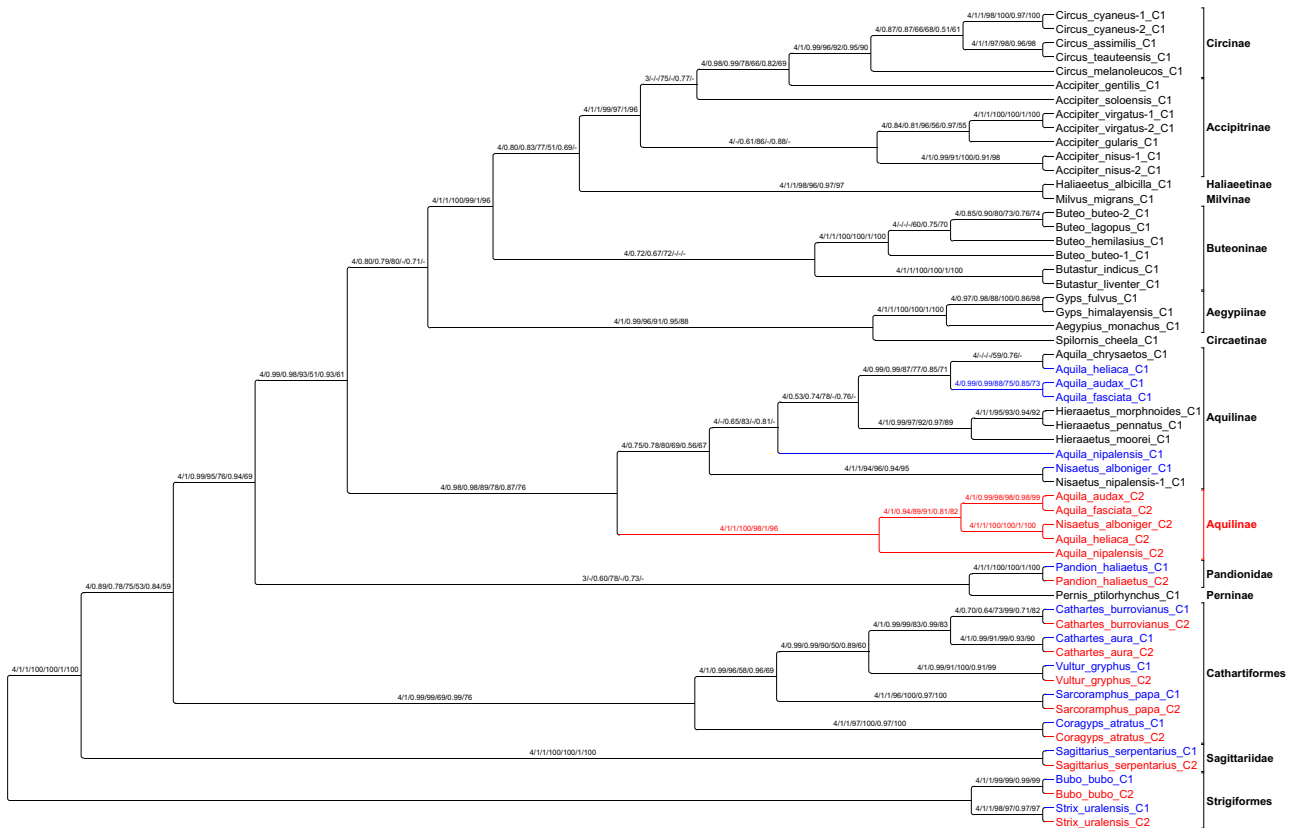
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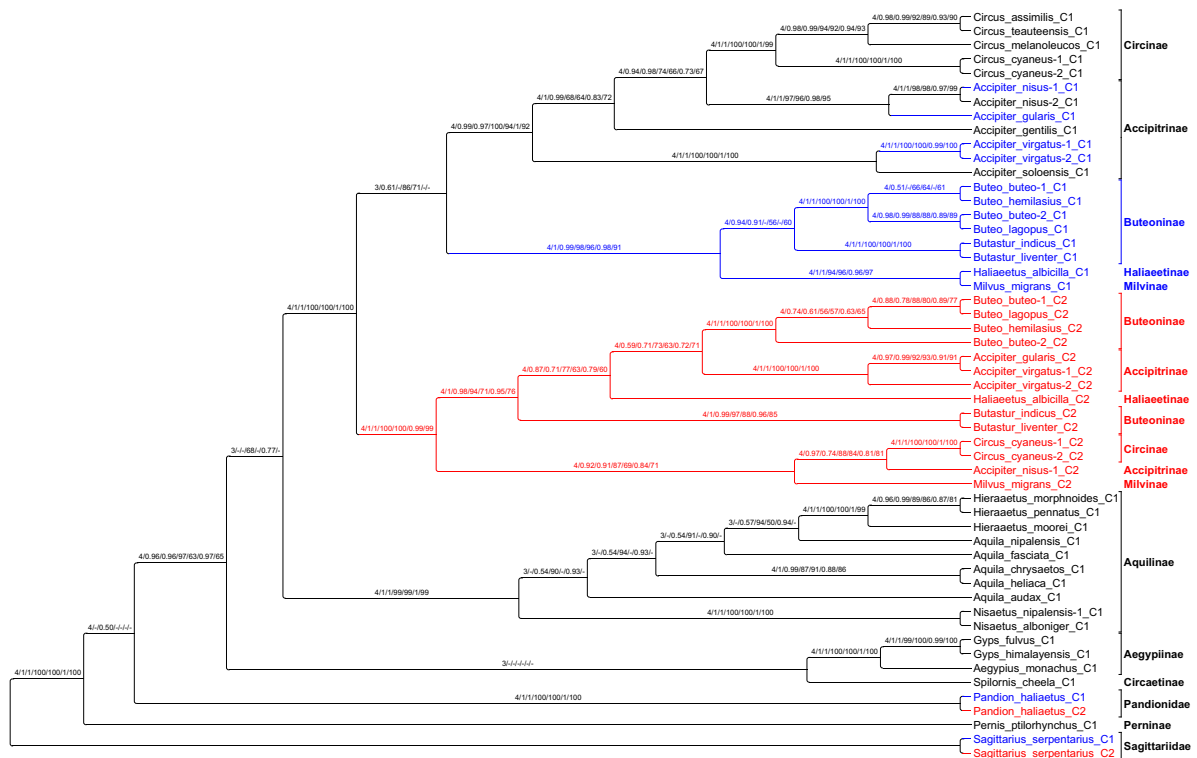
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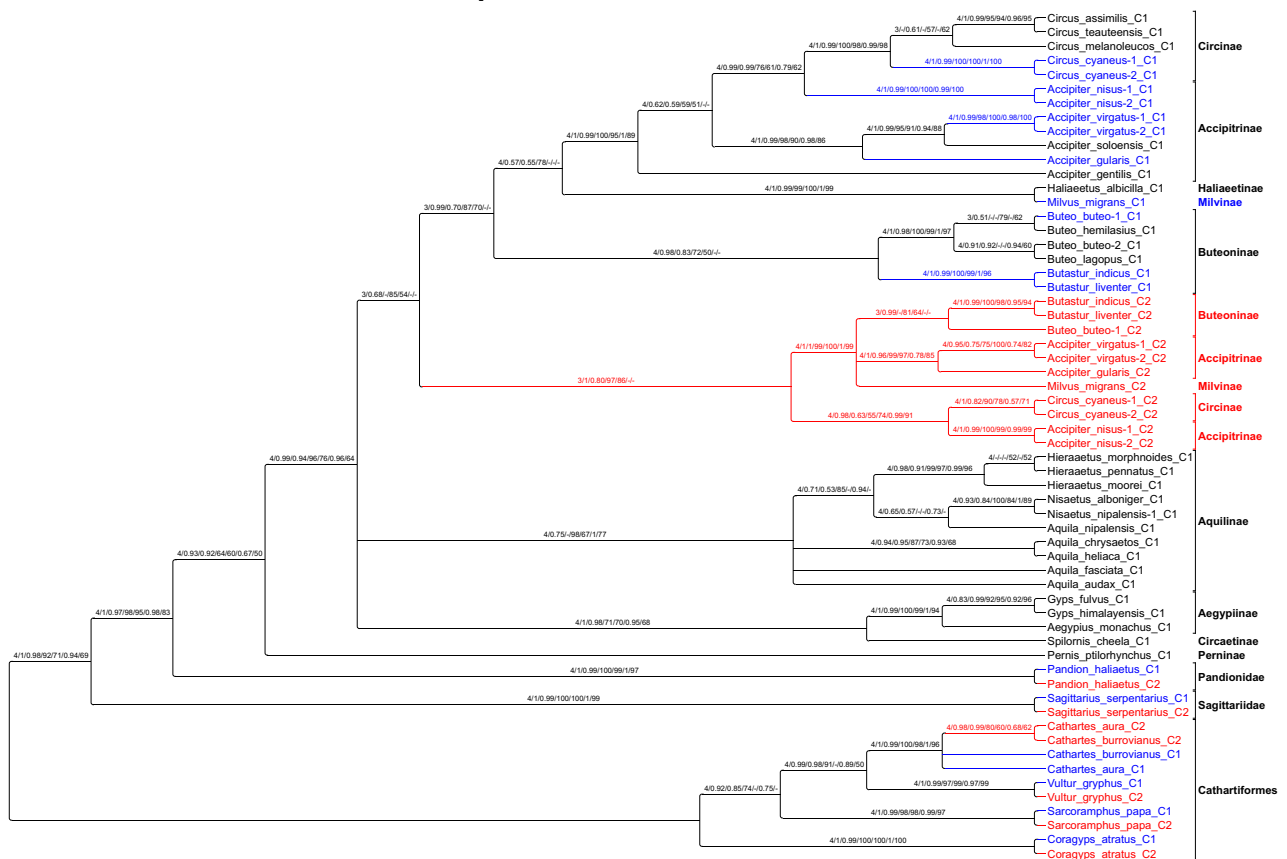
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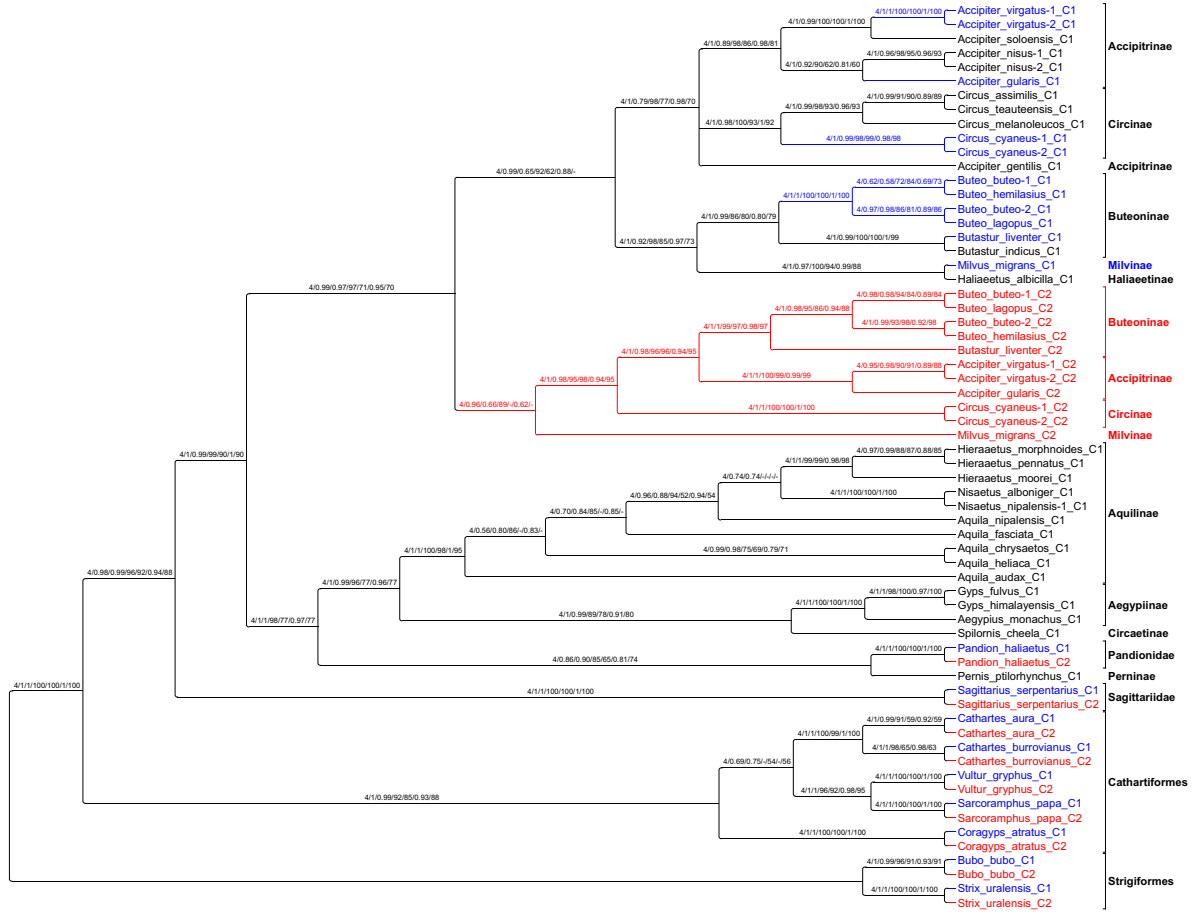
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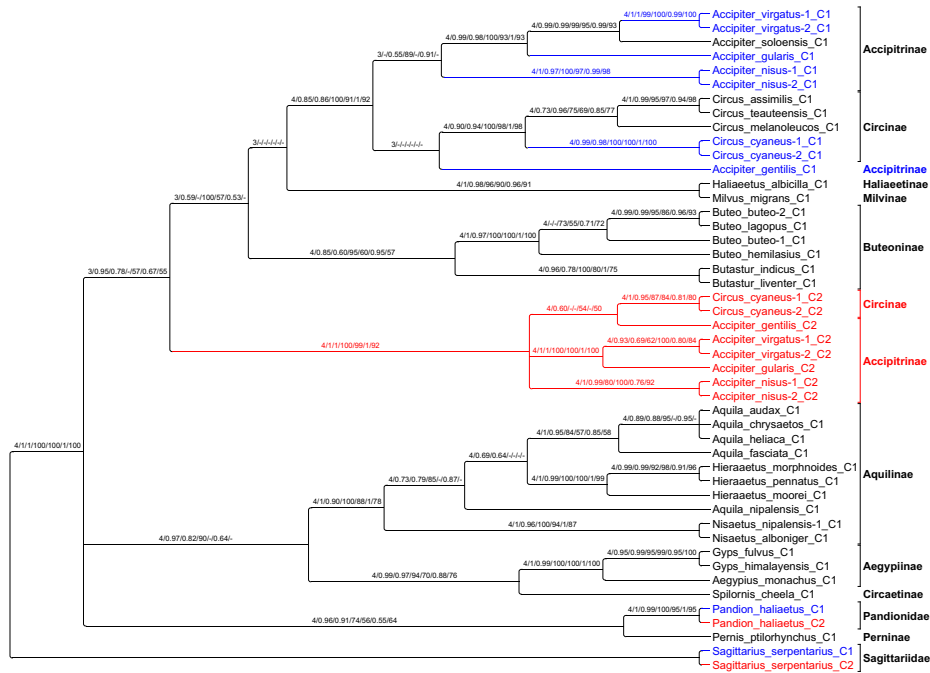
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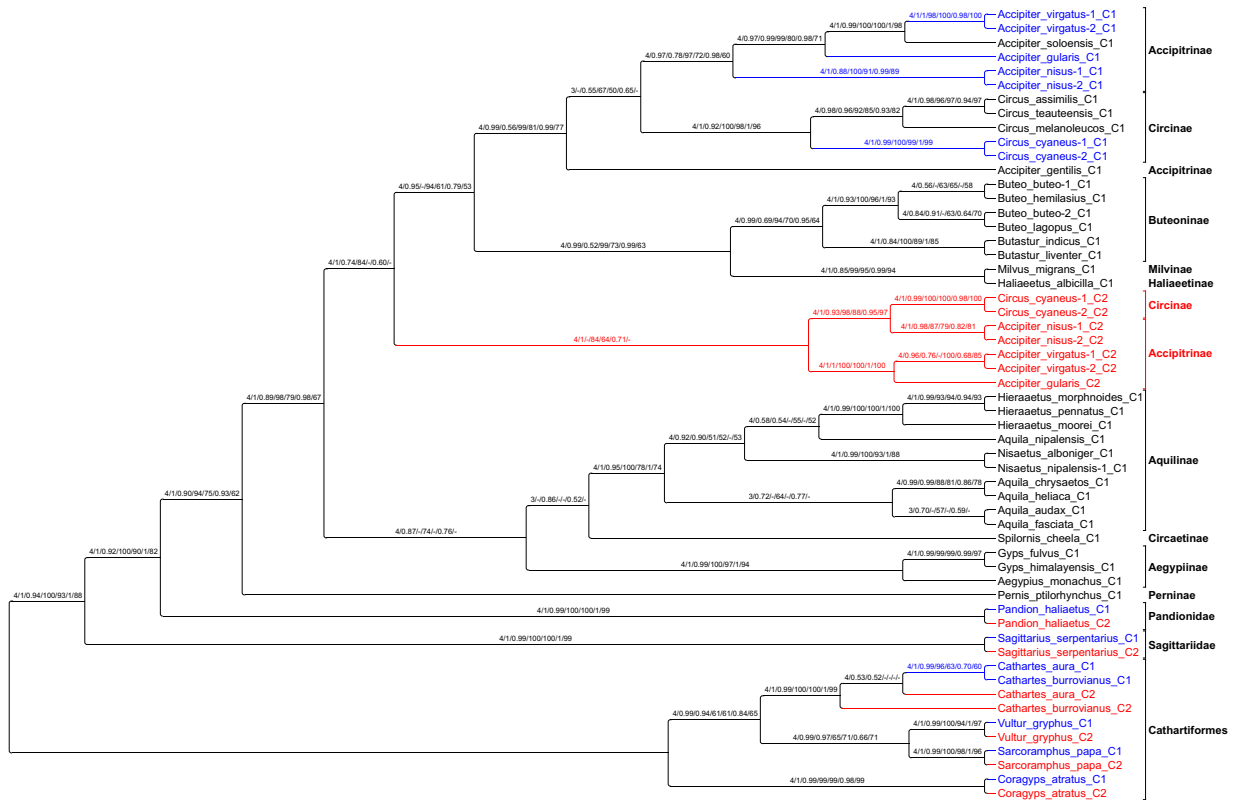


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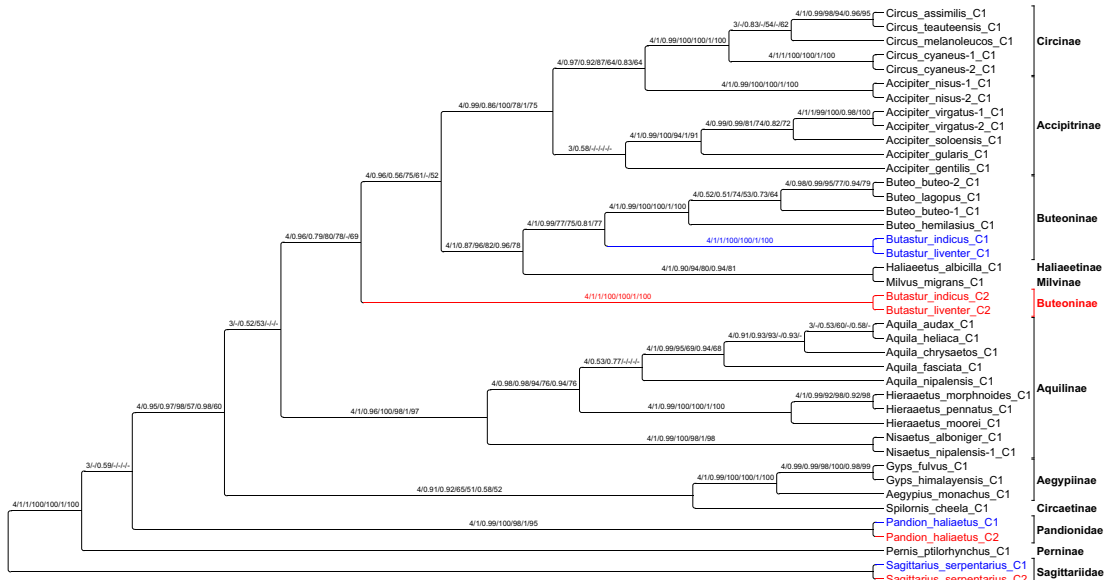




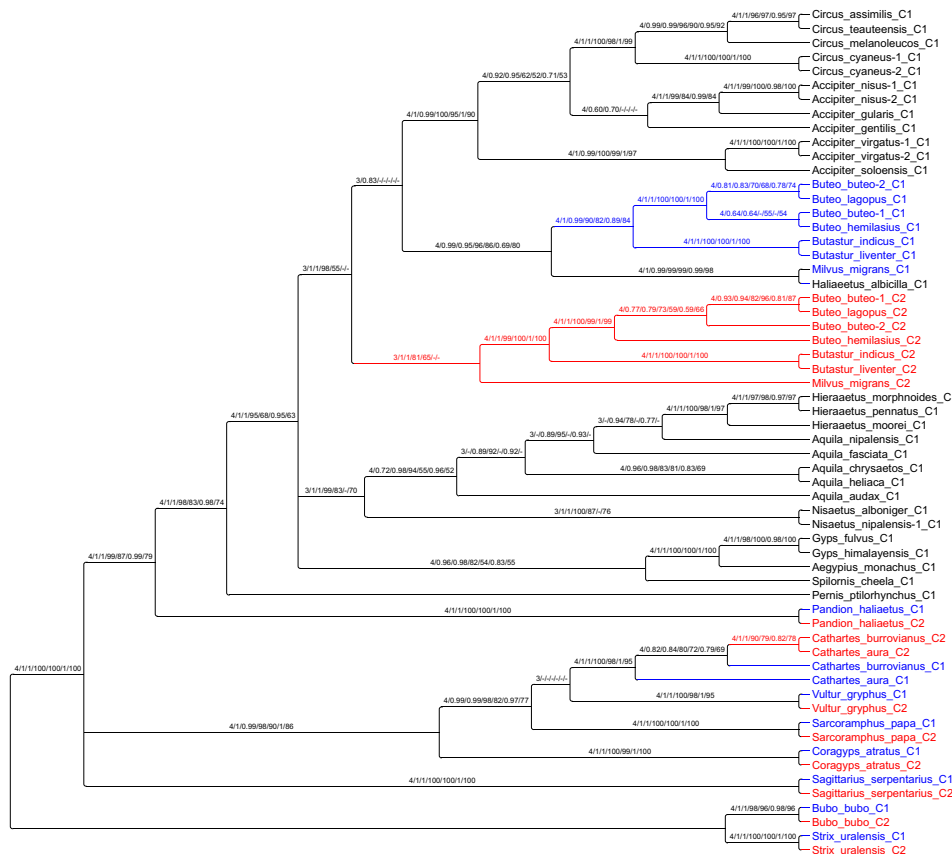
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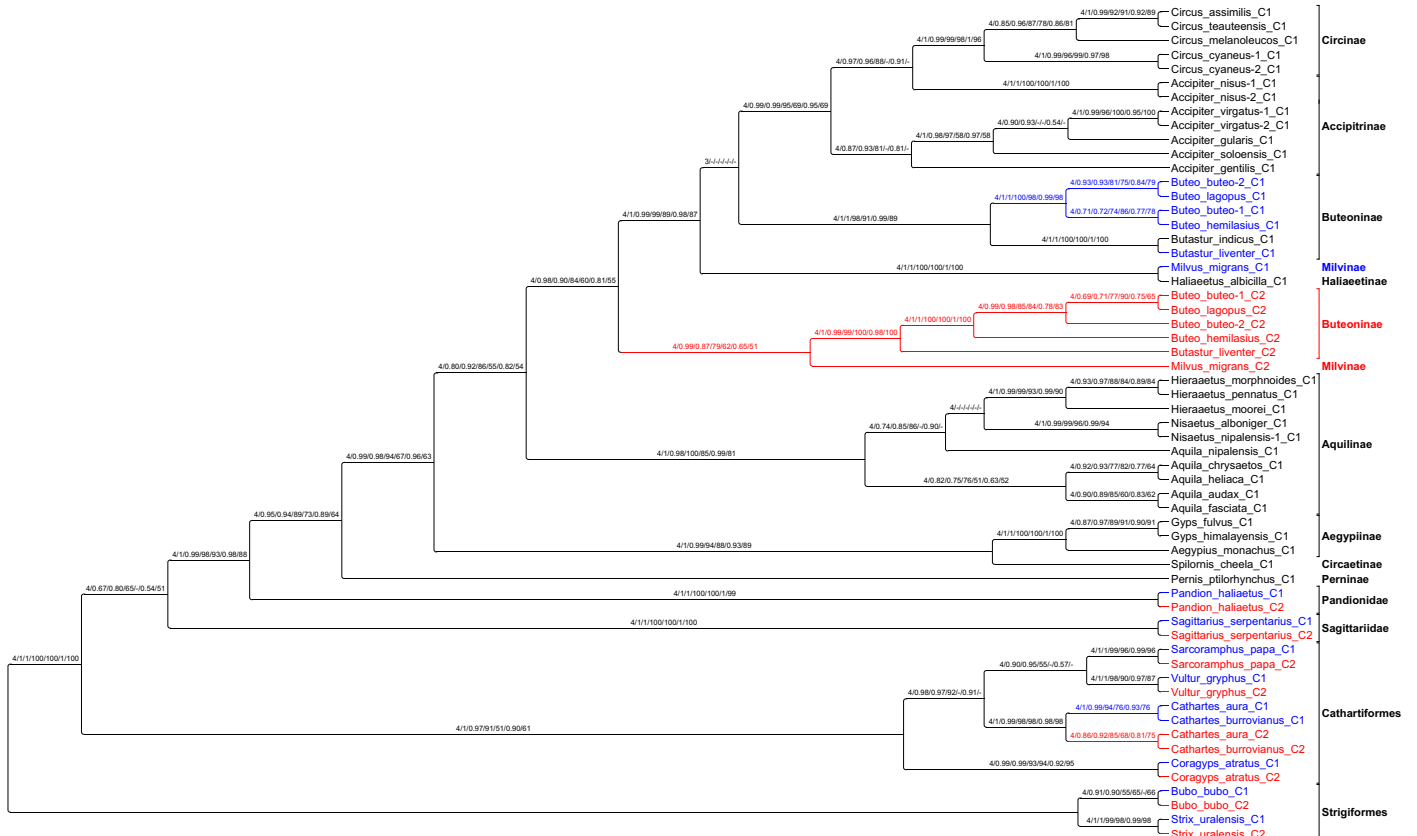
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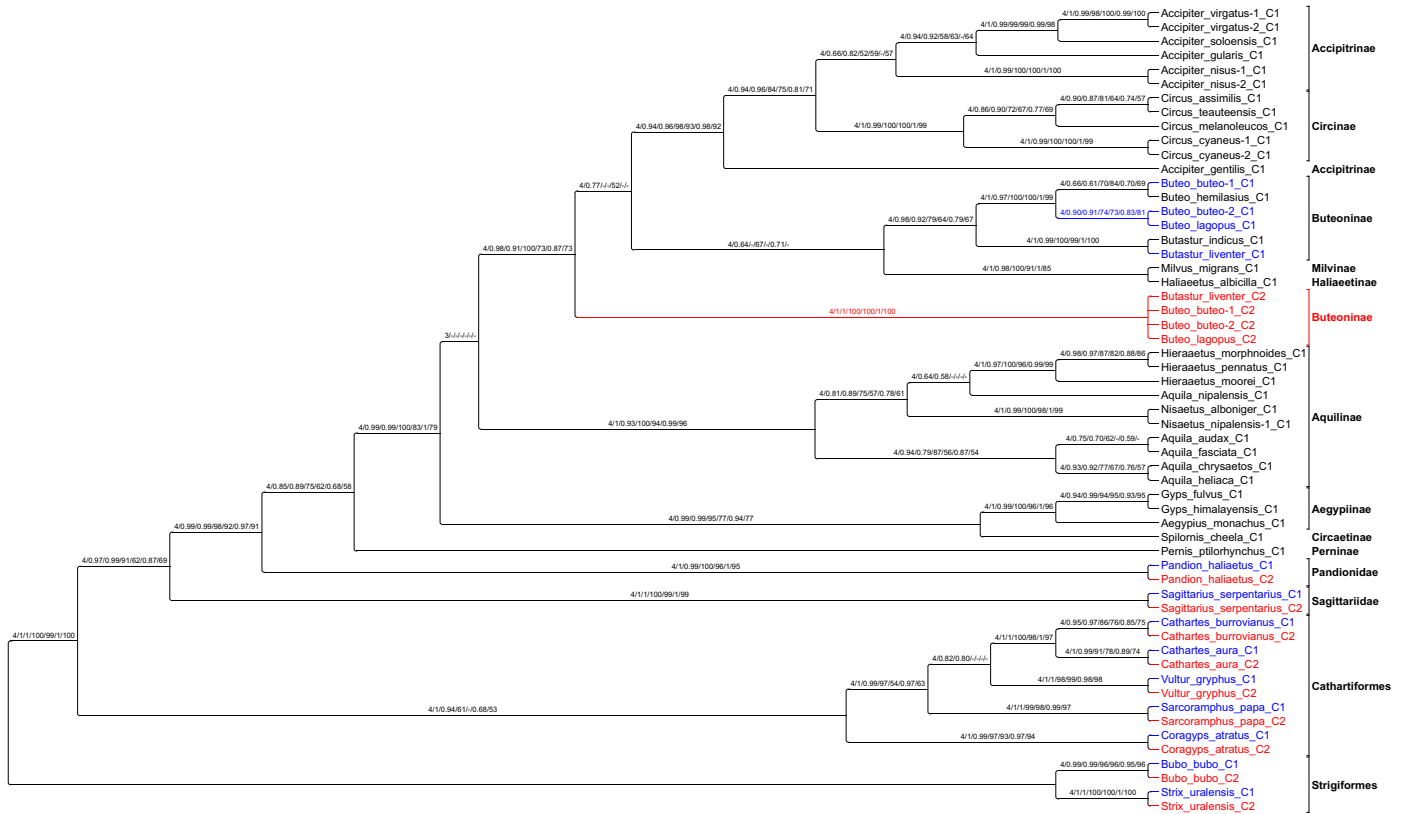
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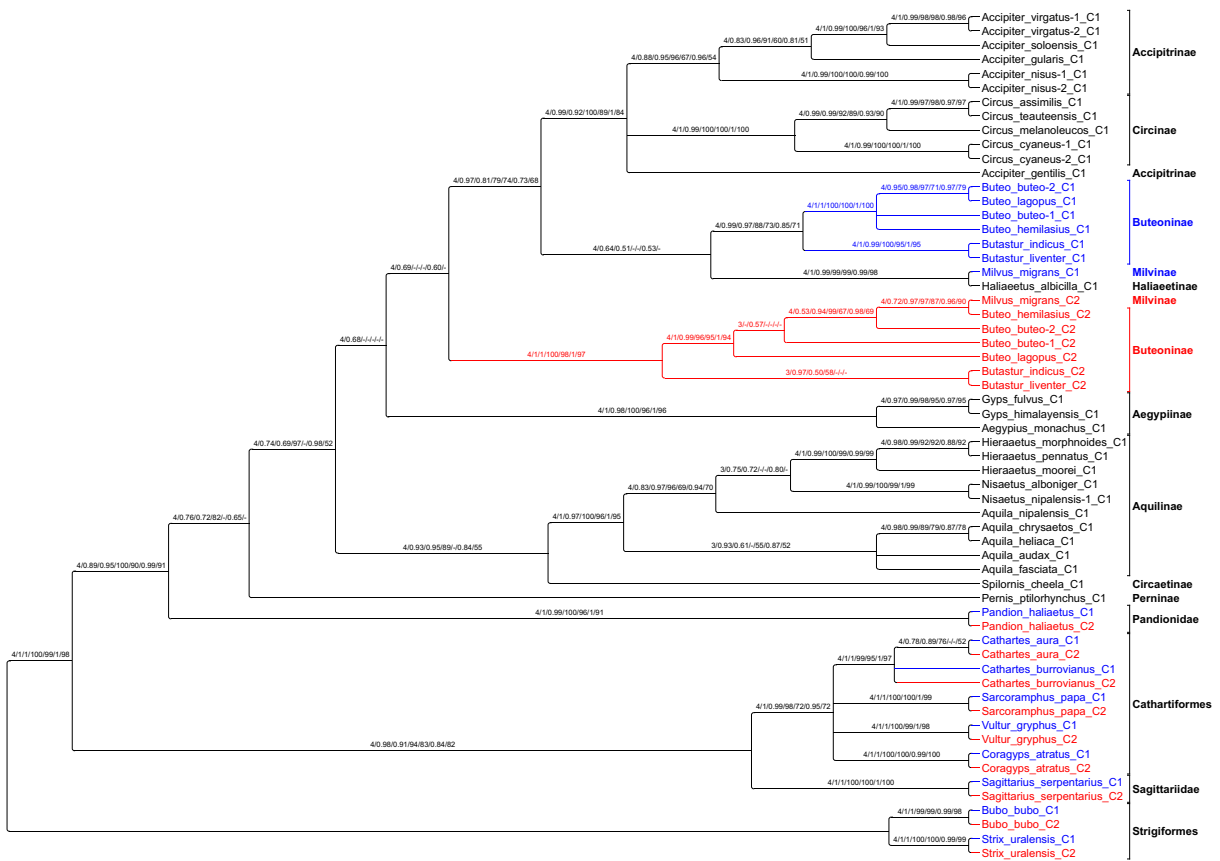
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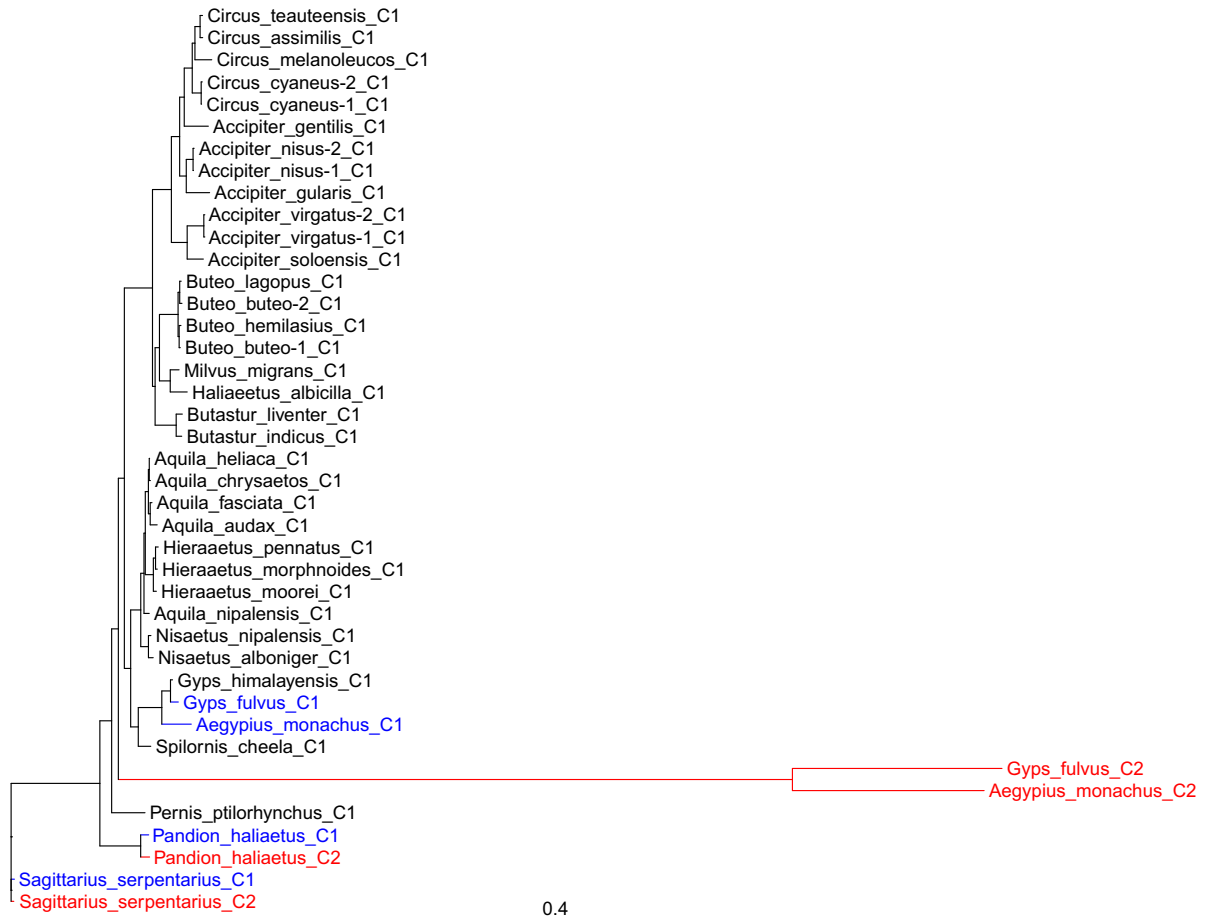


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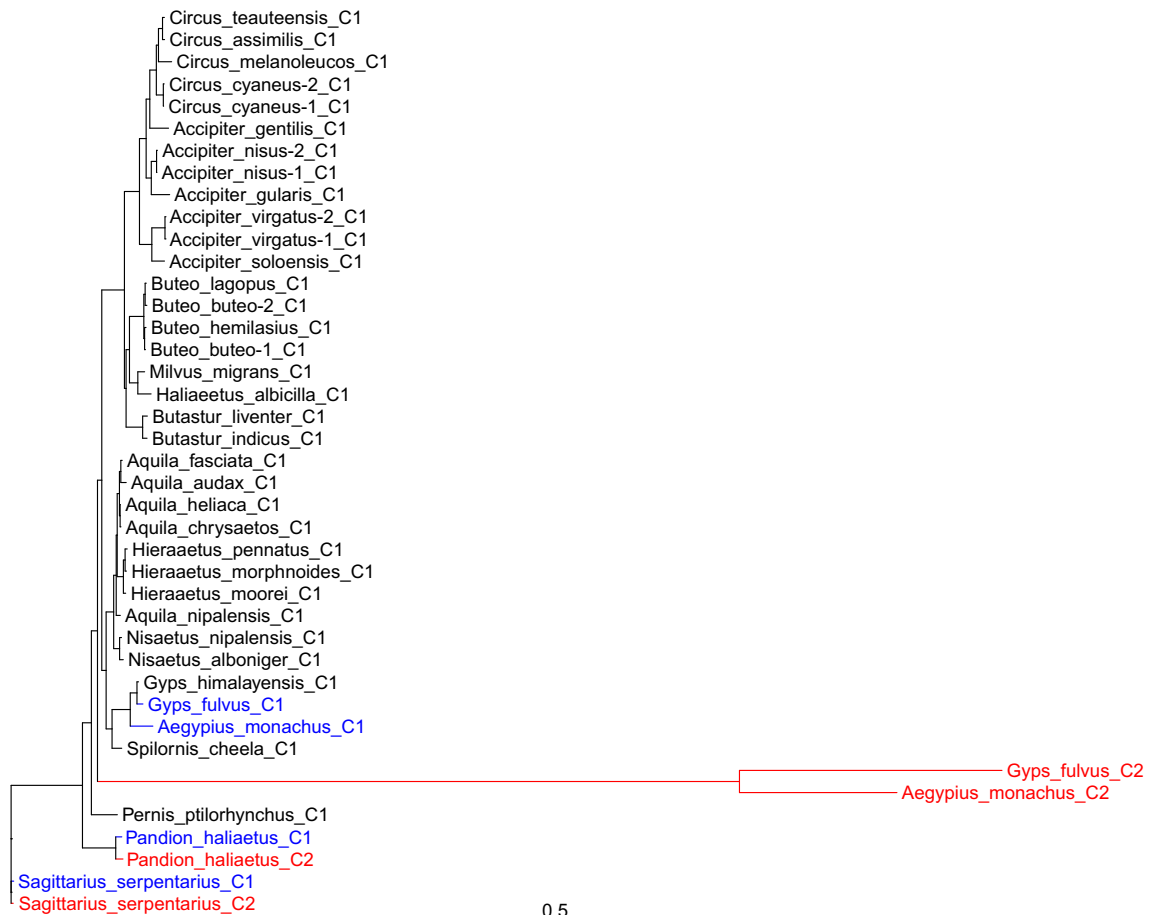


**Figure S7.** Phylograms obtained in four programs, MrBayes, PhyloBayes, IQ-TREE and (more)PhyML, based on three types of data sets of control regions (all sites, repeats masked or removed) for Accipitriformes and related groups. Pairs of CRs from the same species are colored, whereas CRs without the second copy in the tree are in black. The blue and red colors indicate the corresponding first and second copies of CR, respectively. The taxa names are in the format Genus\_species-X\_CY, where X is the individual number (if present) and Y is the number of control region, i.e. 1 or 2. See Table S6 for details about the data sets.

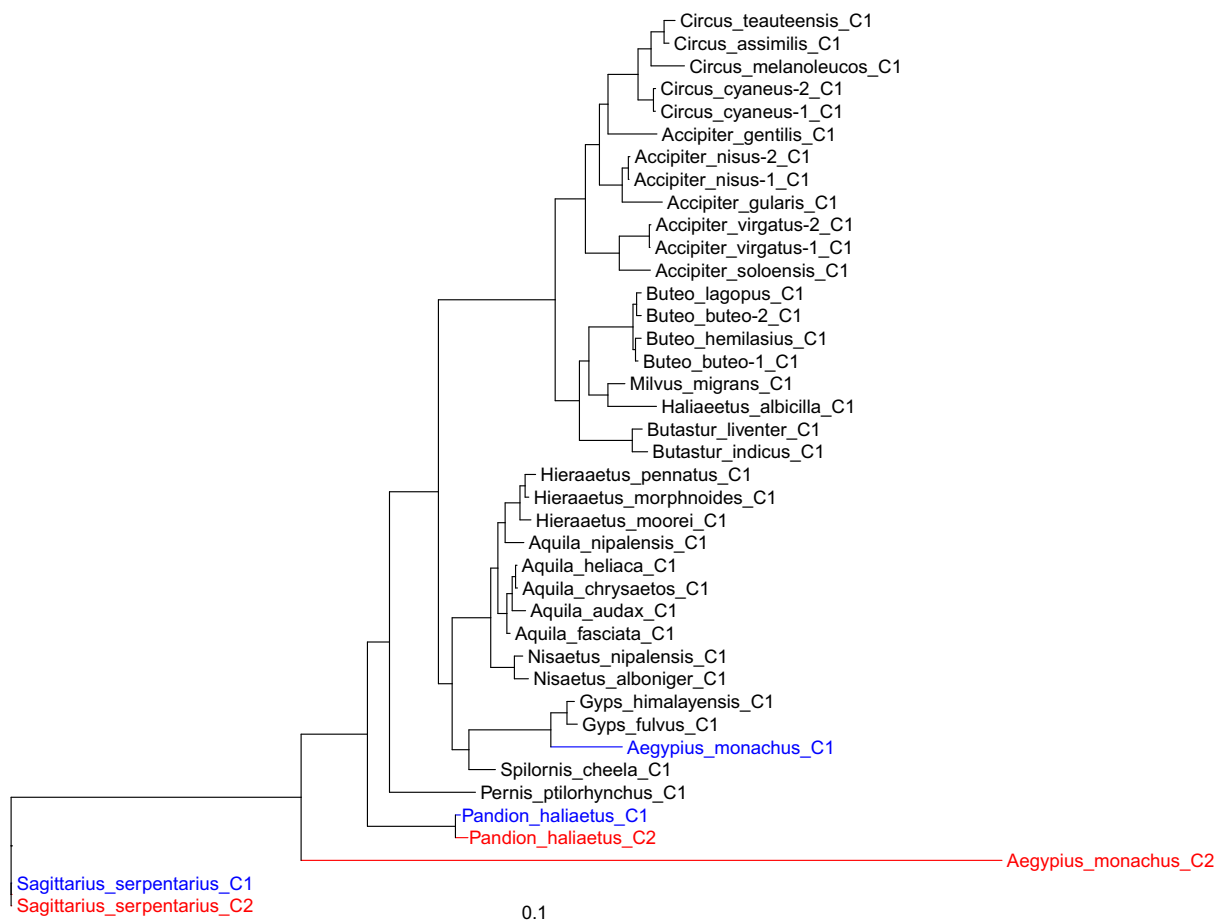
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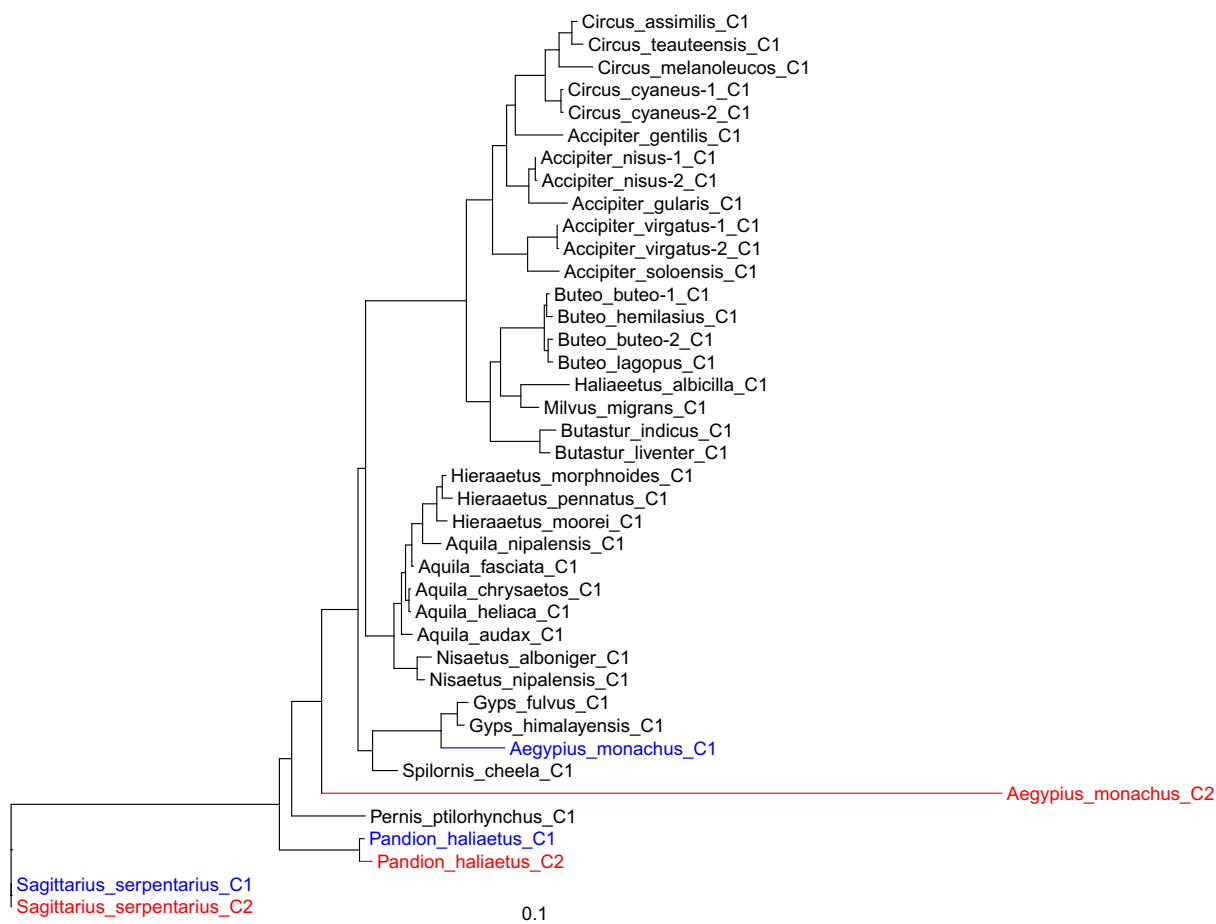
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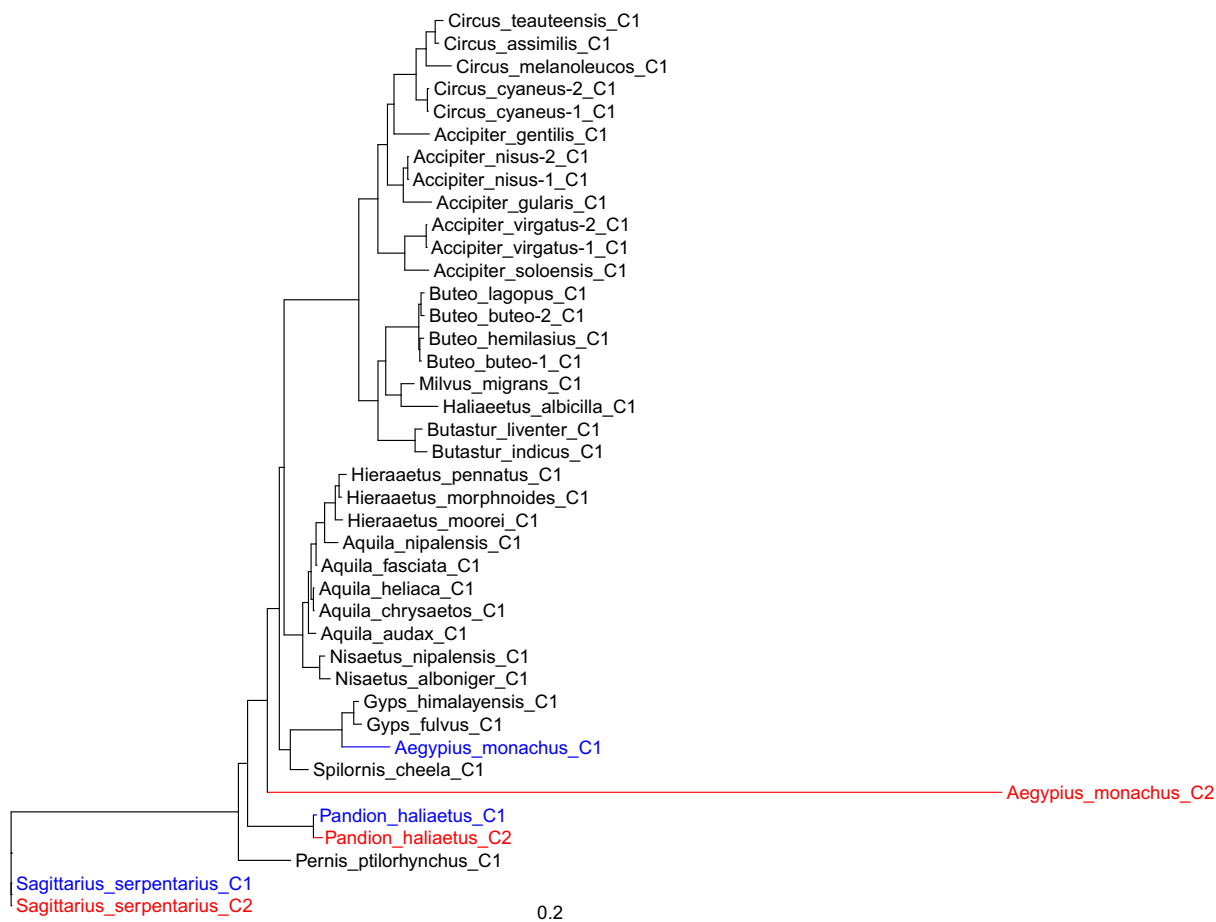
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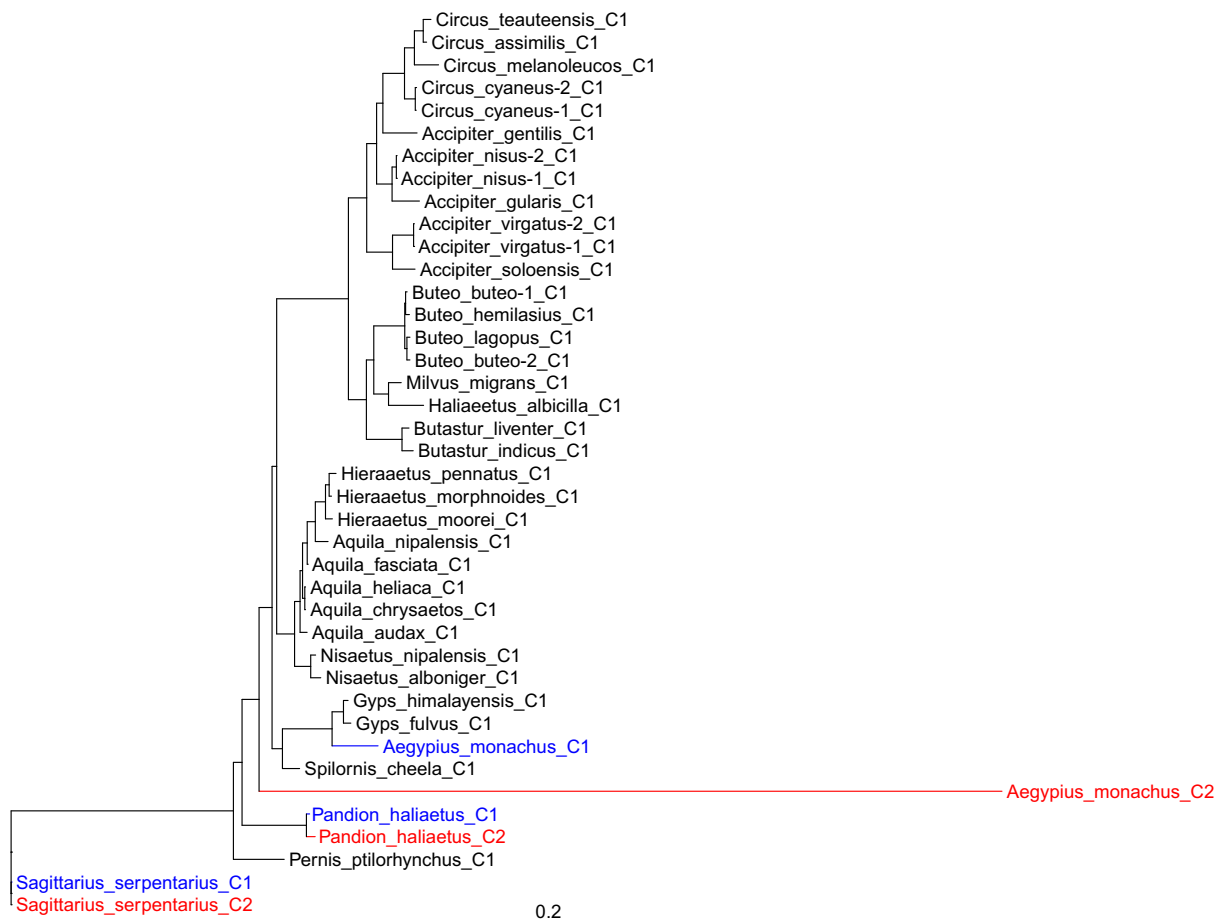
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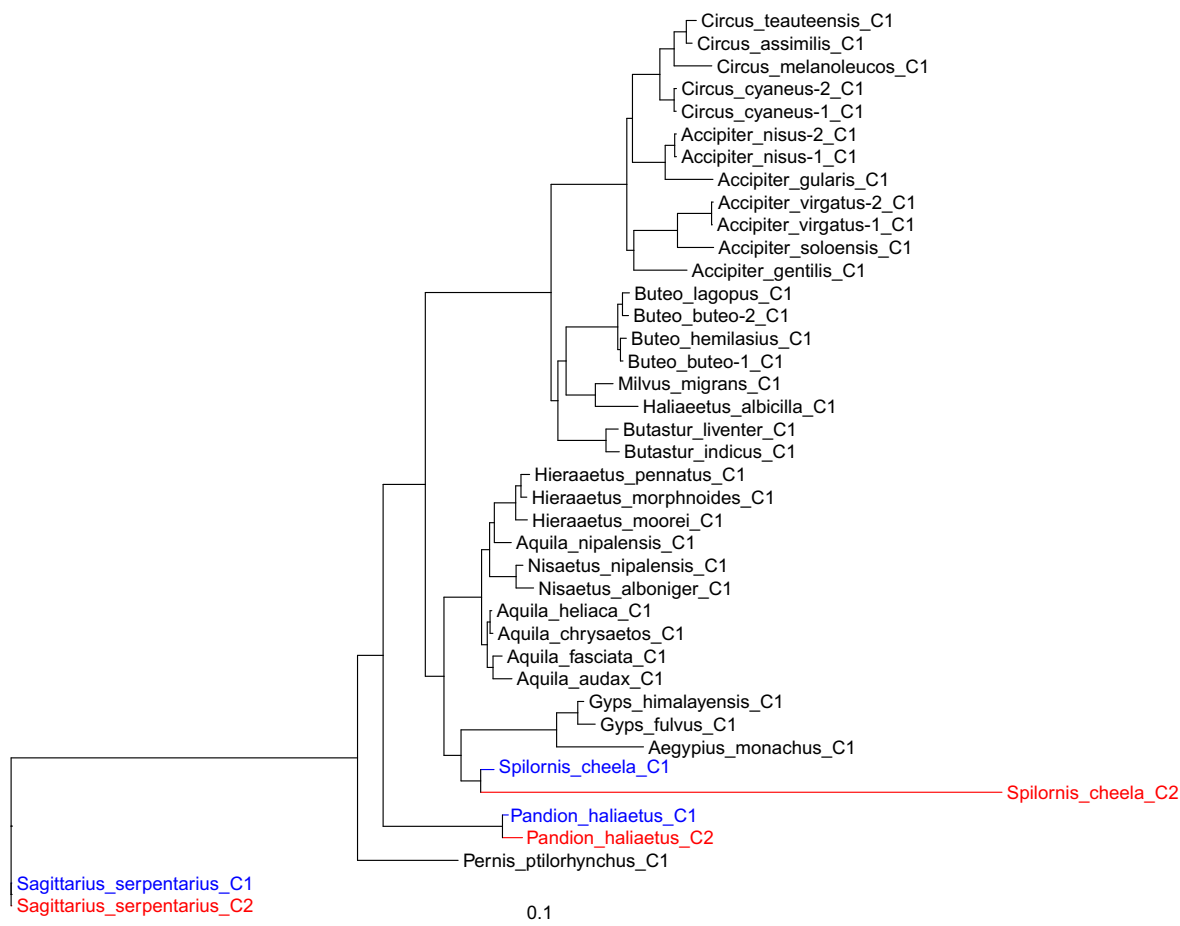
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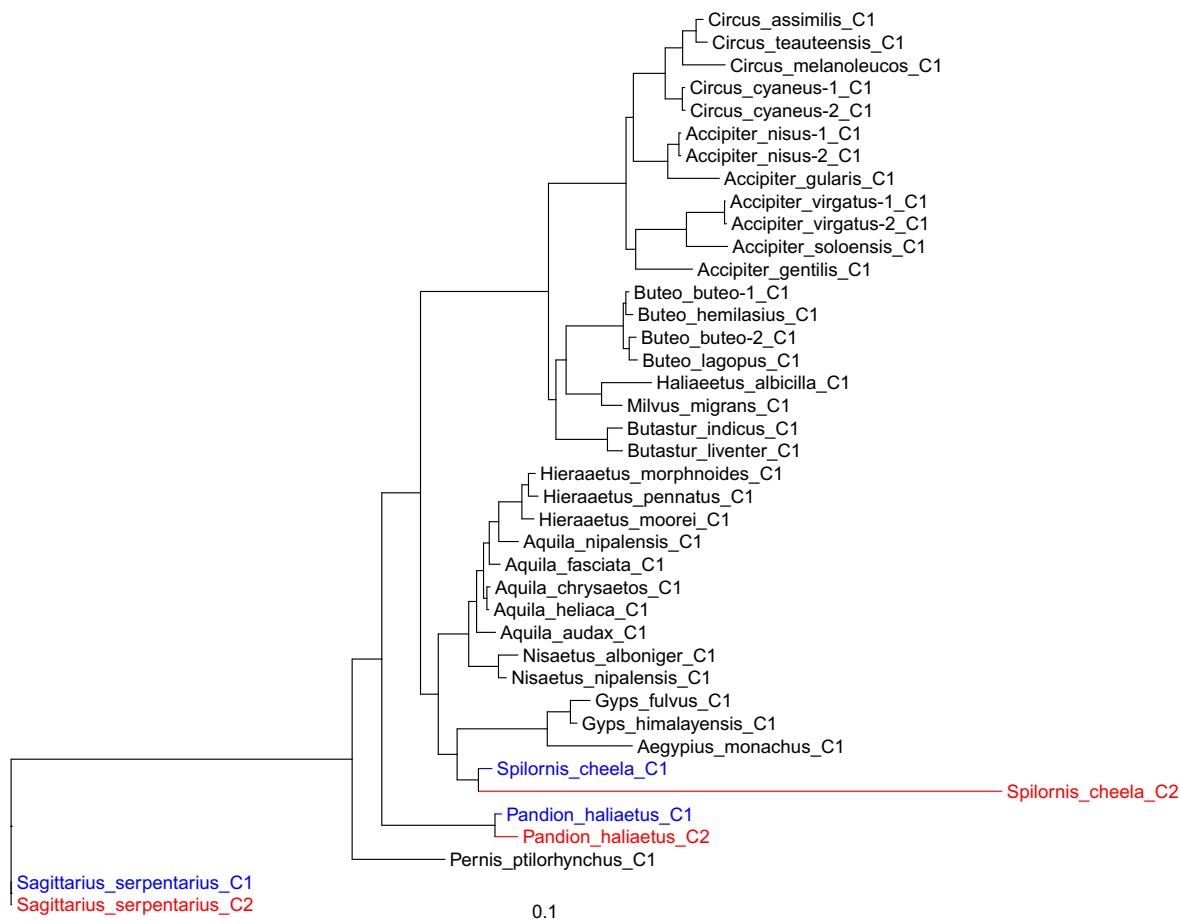
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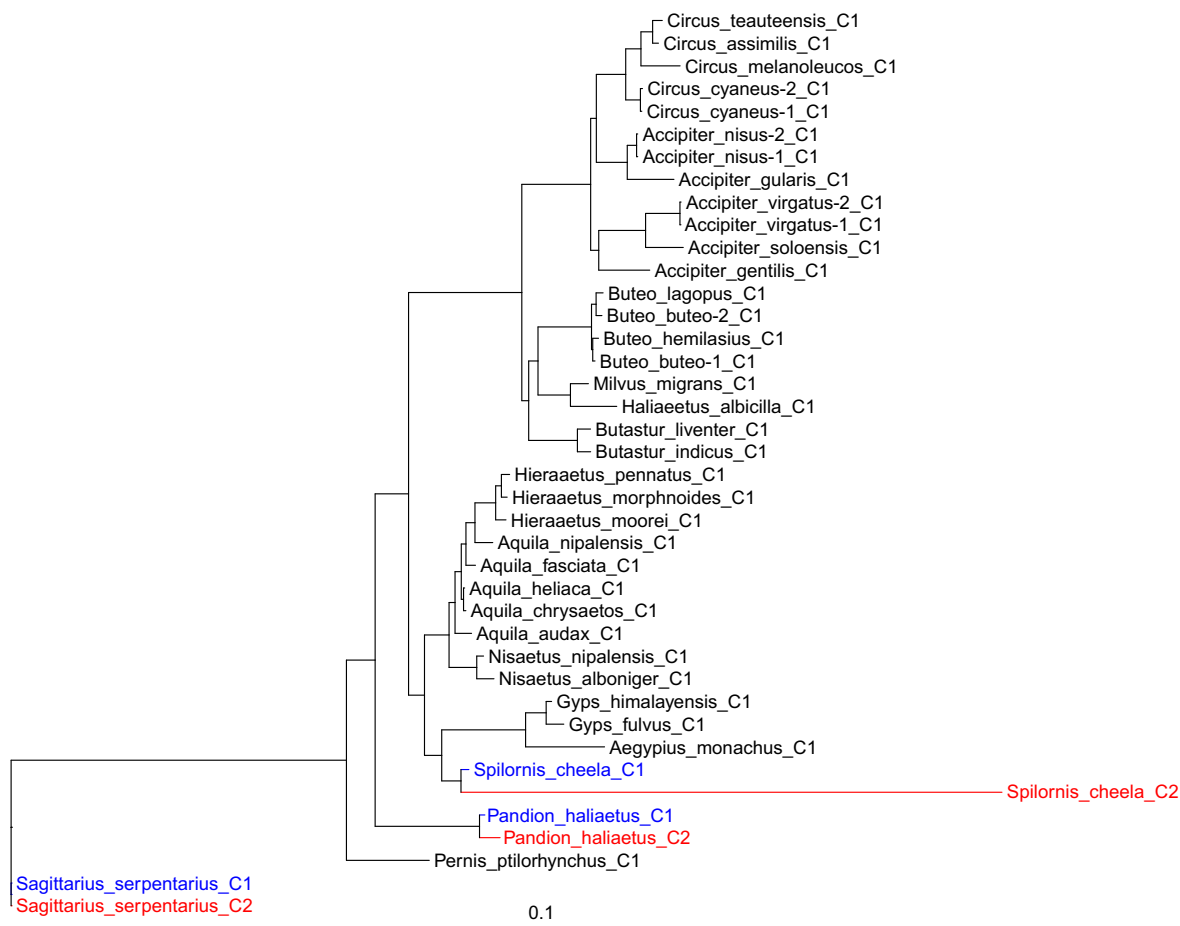


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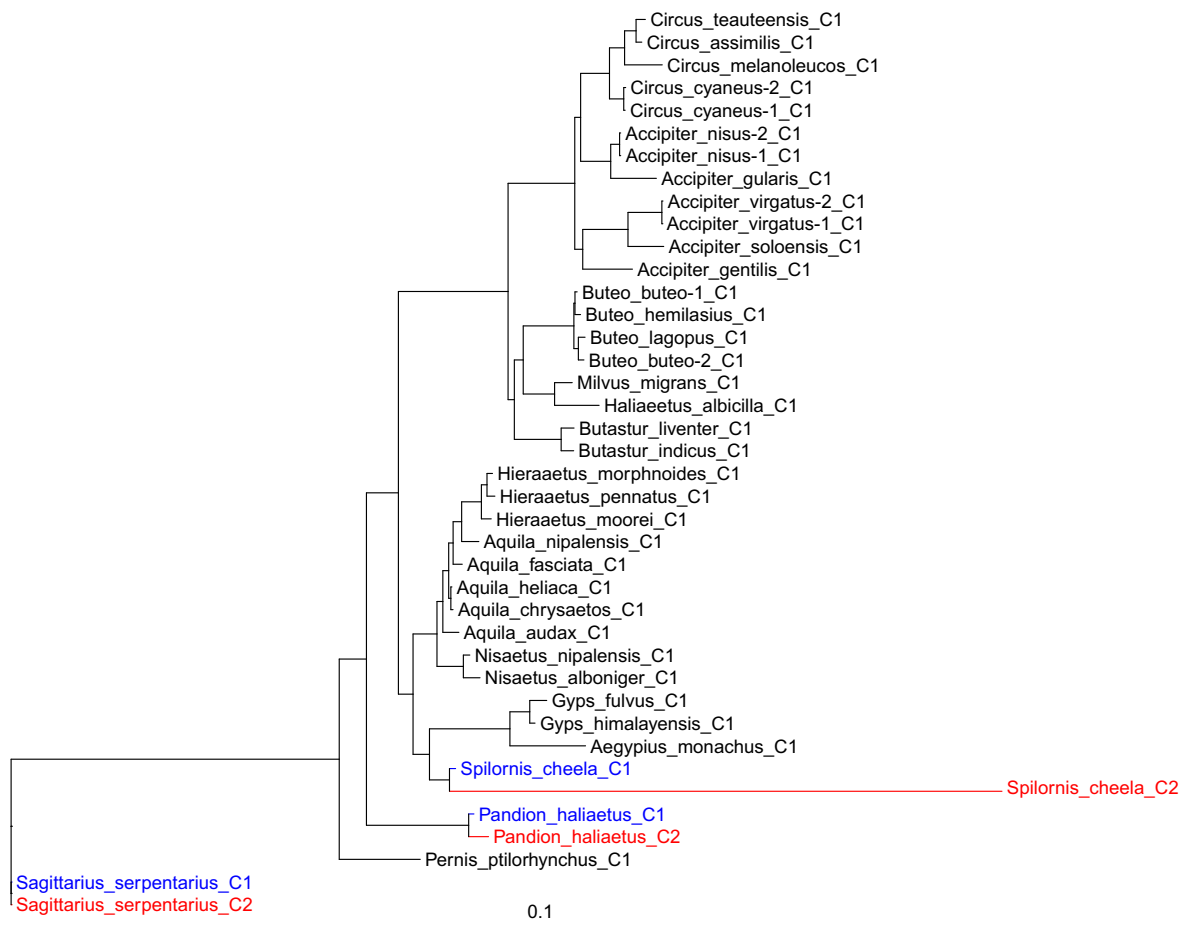




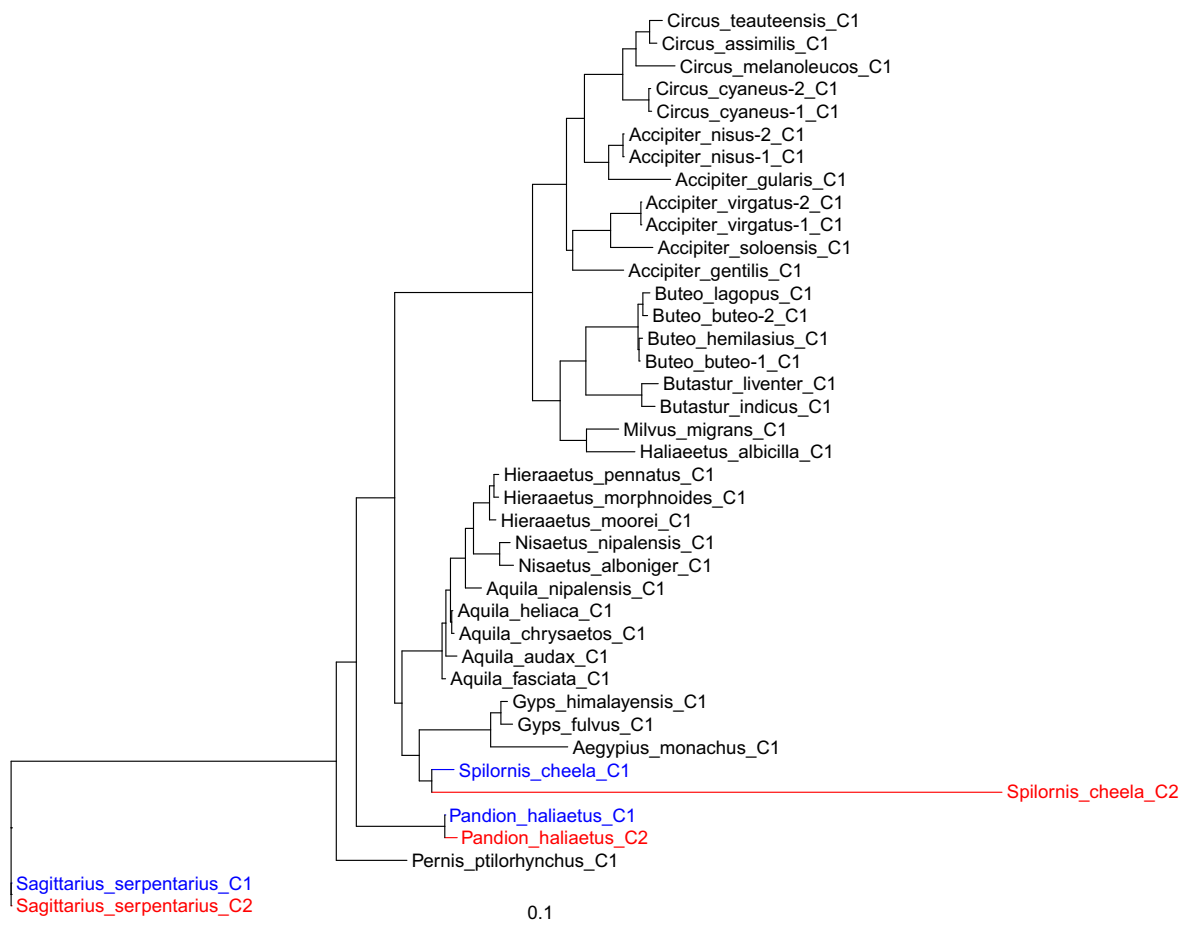
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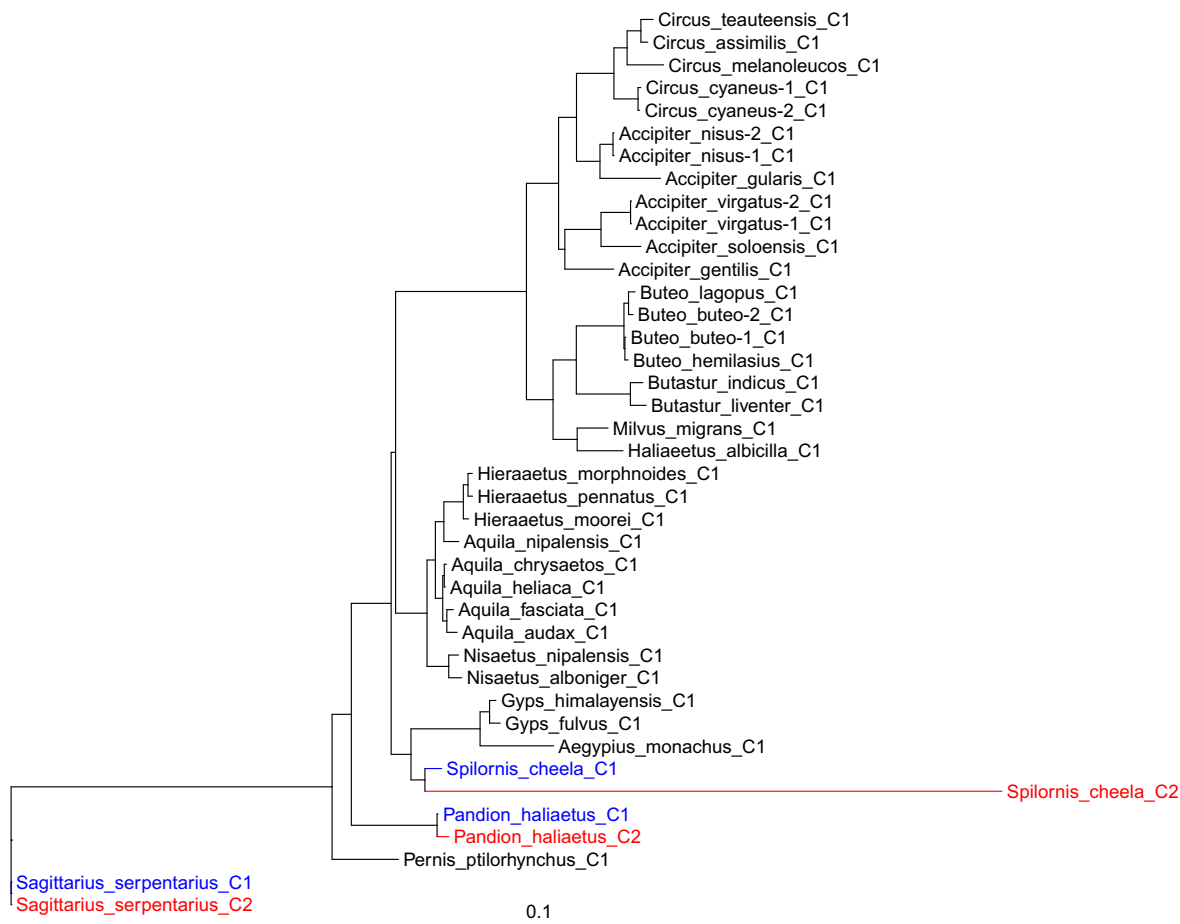
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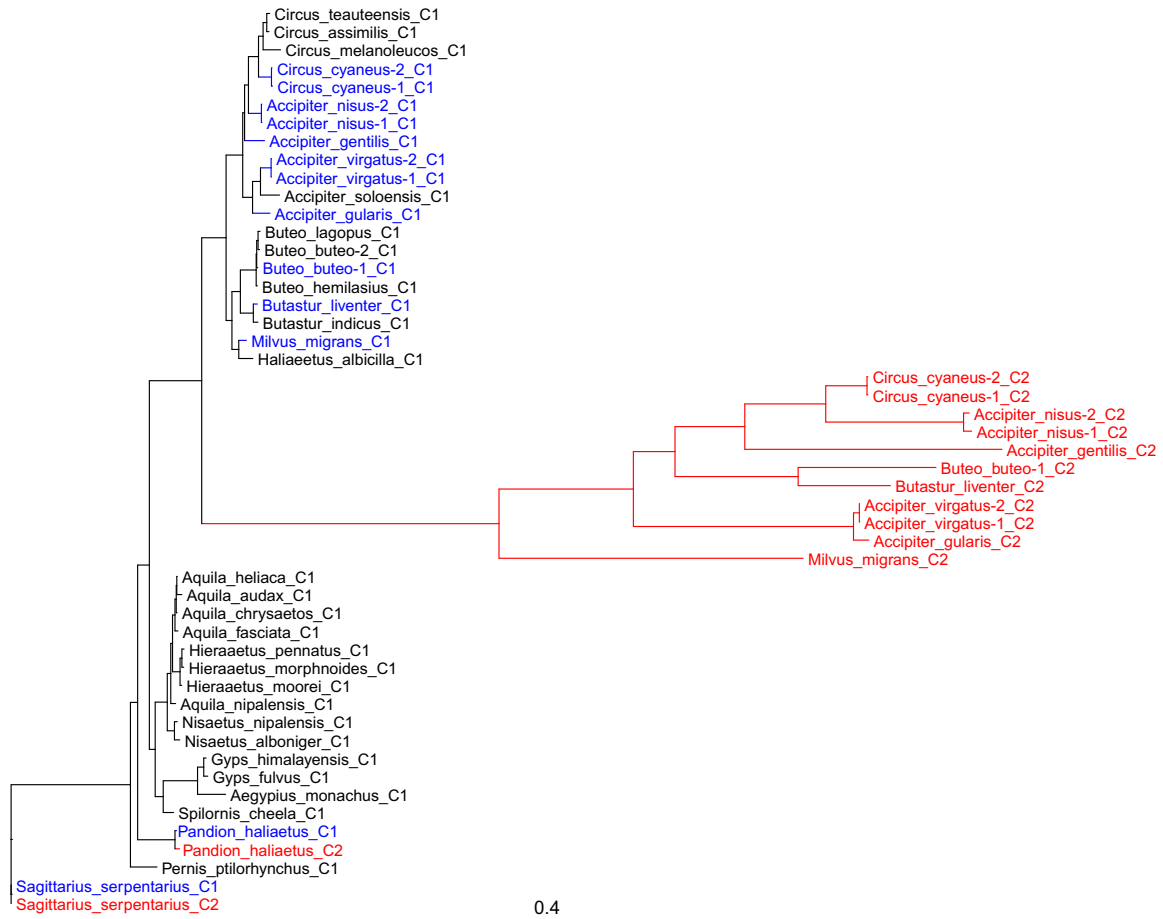
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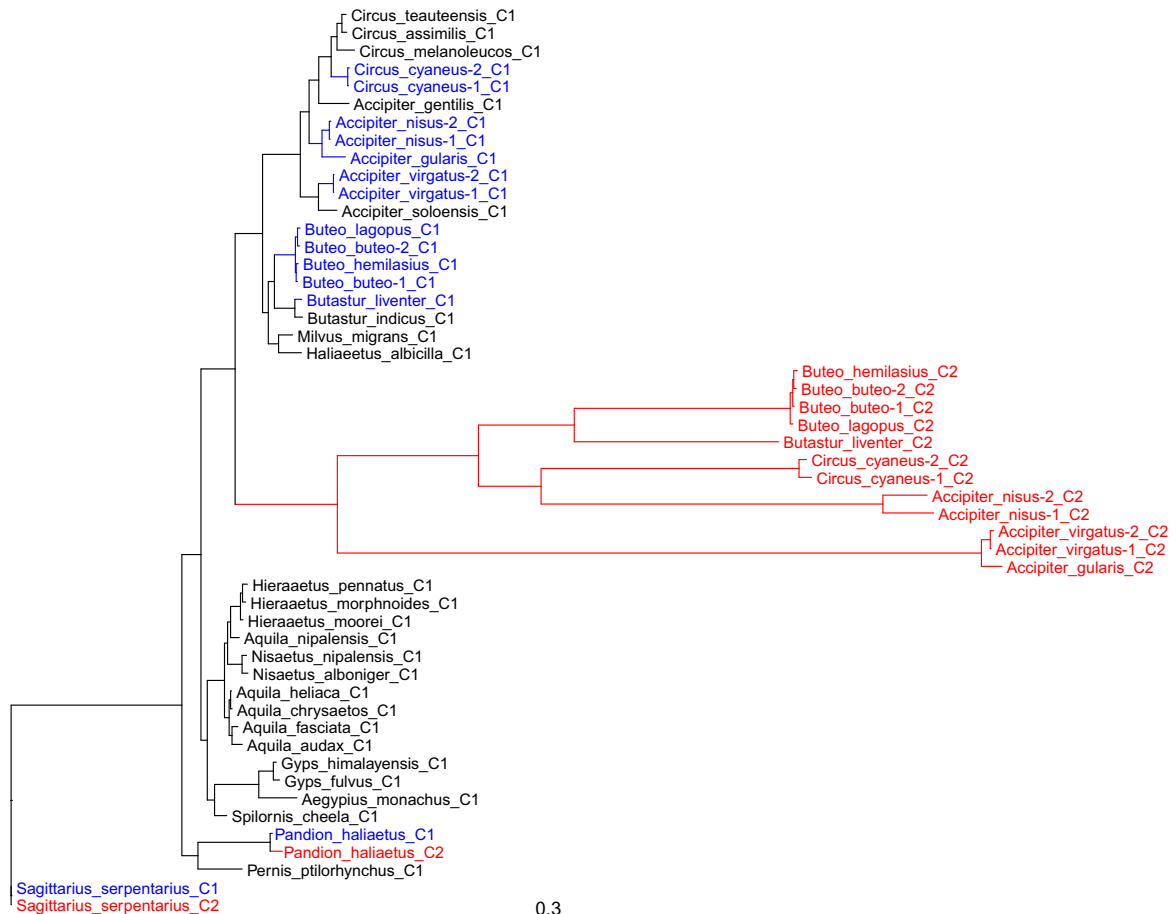
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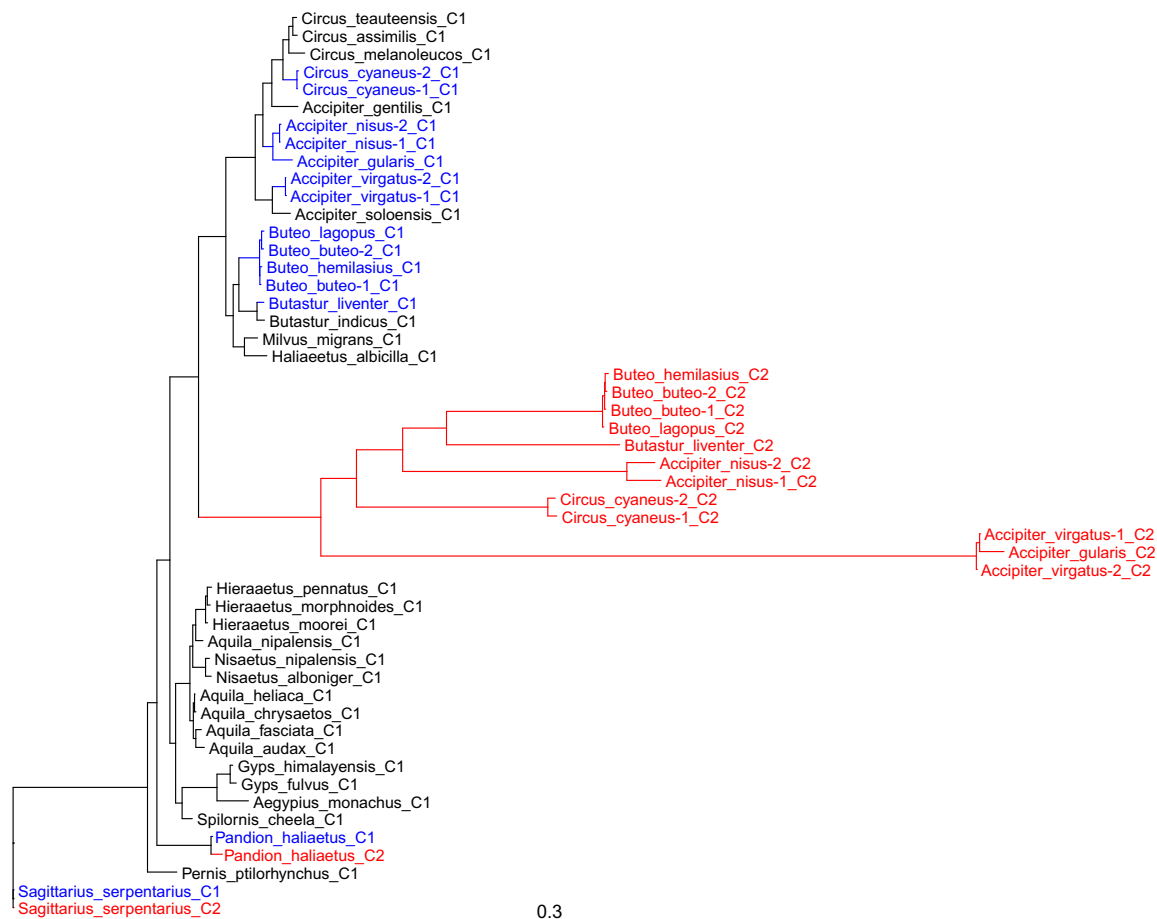
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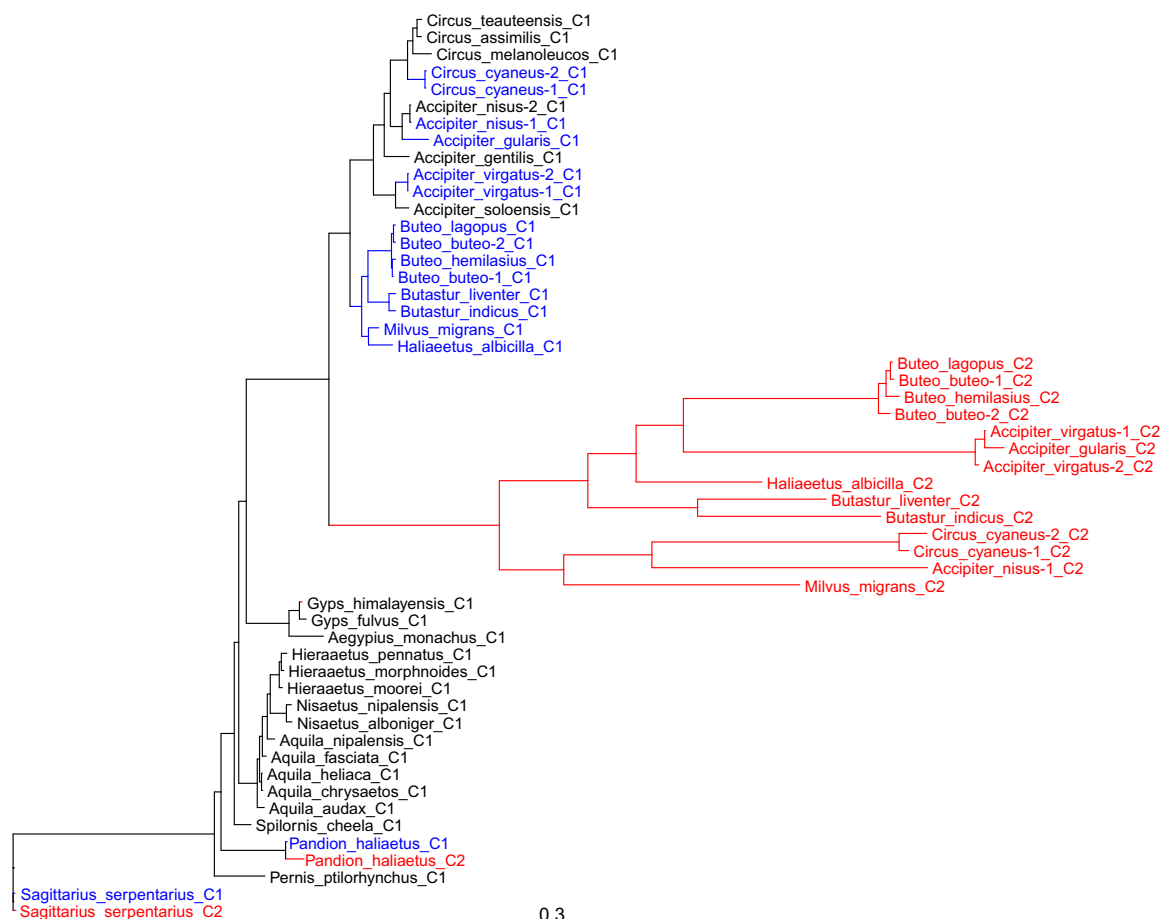
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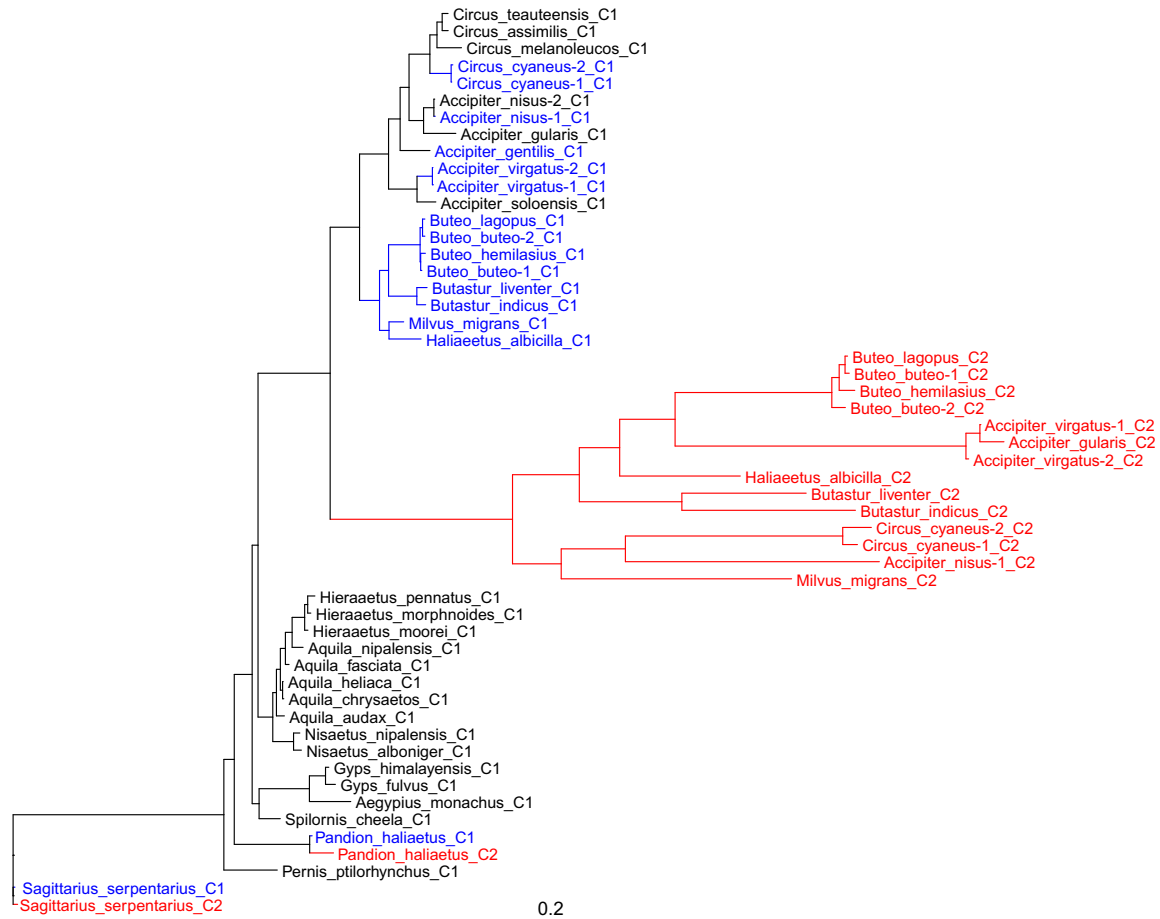
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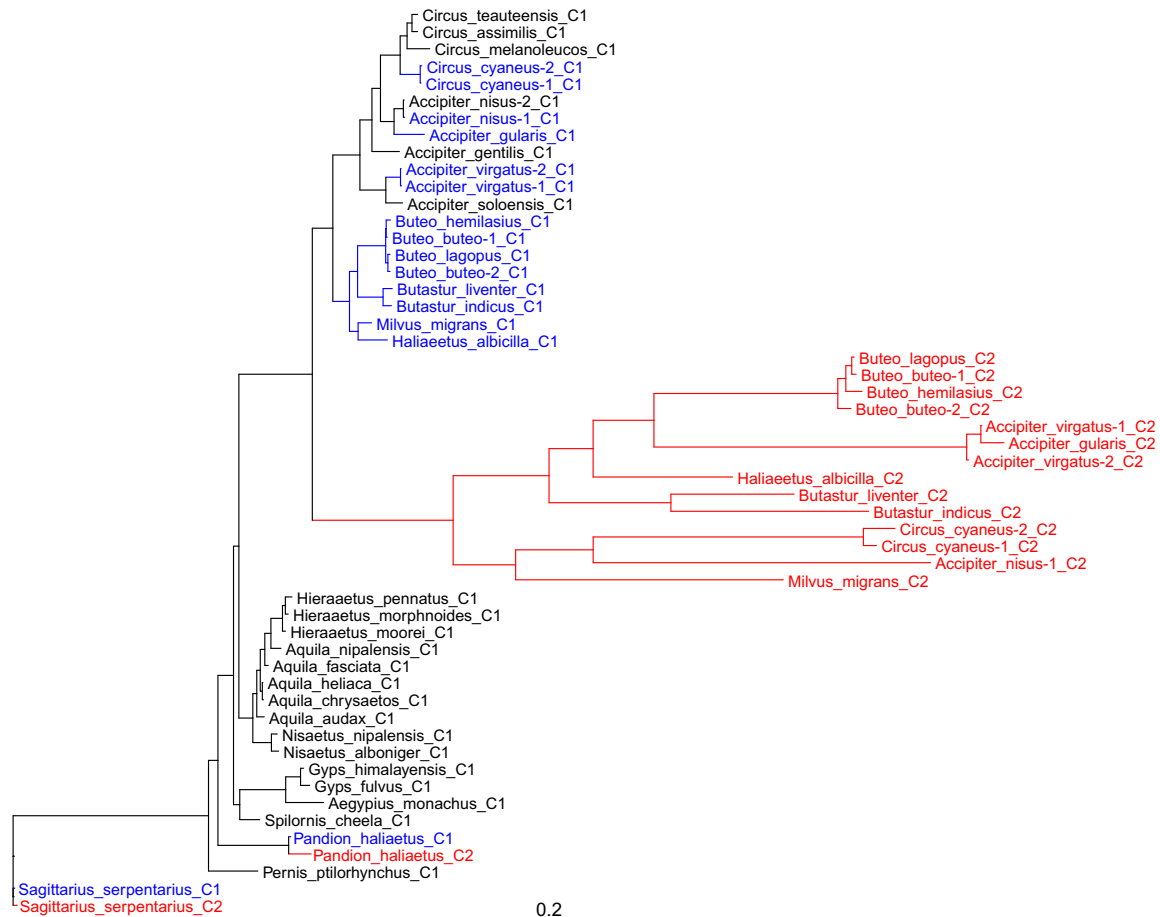
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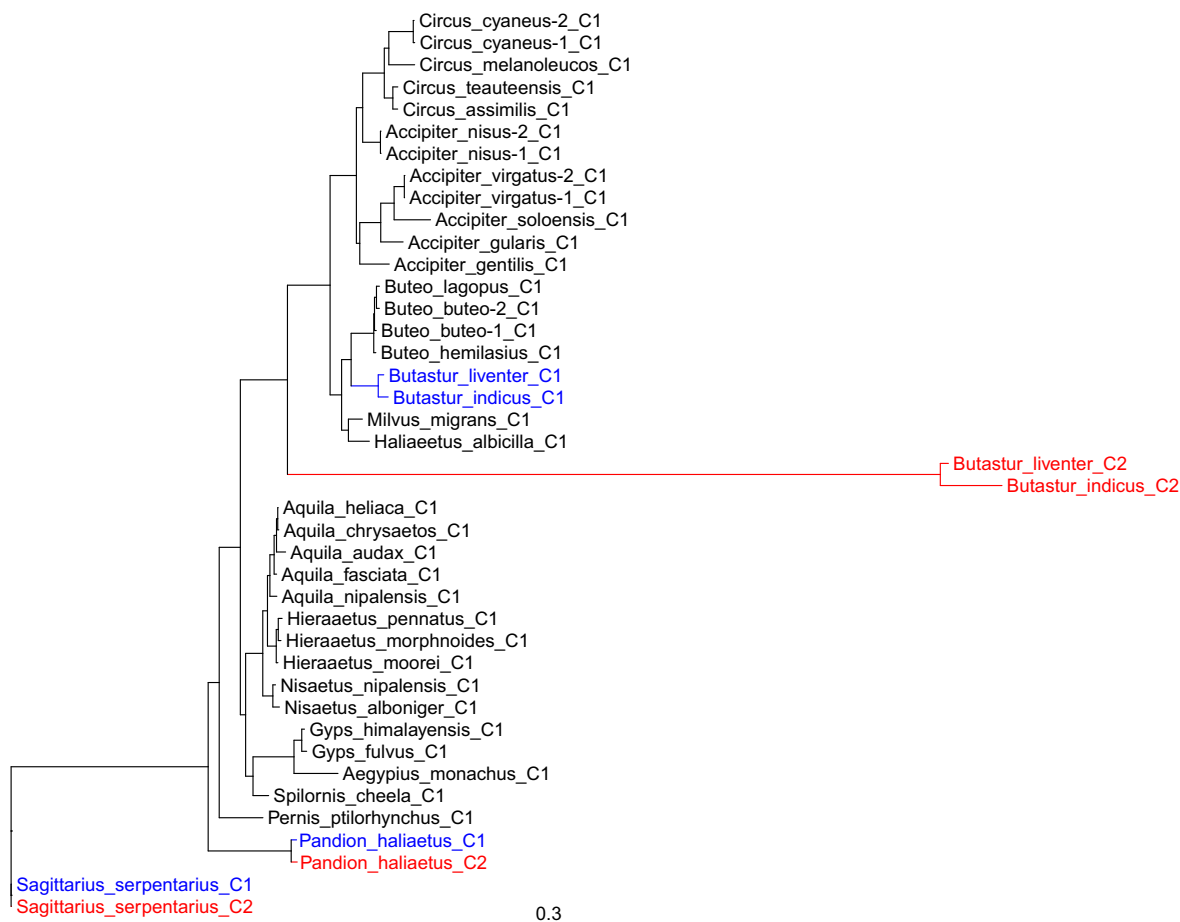
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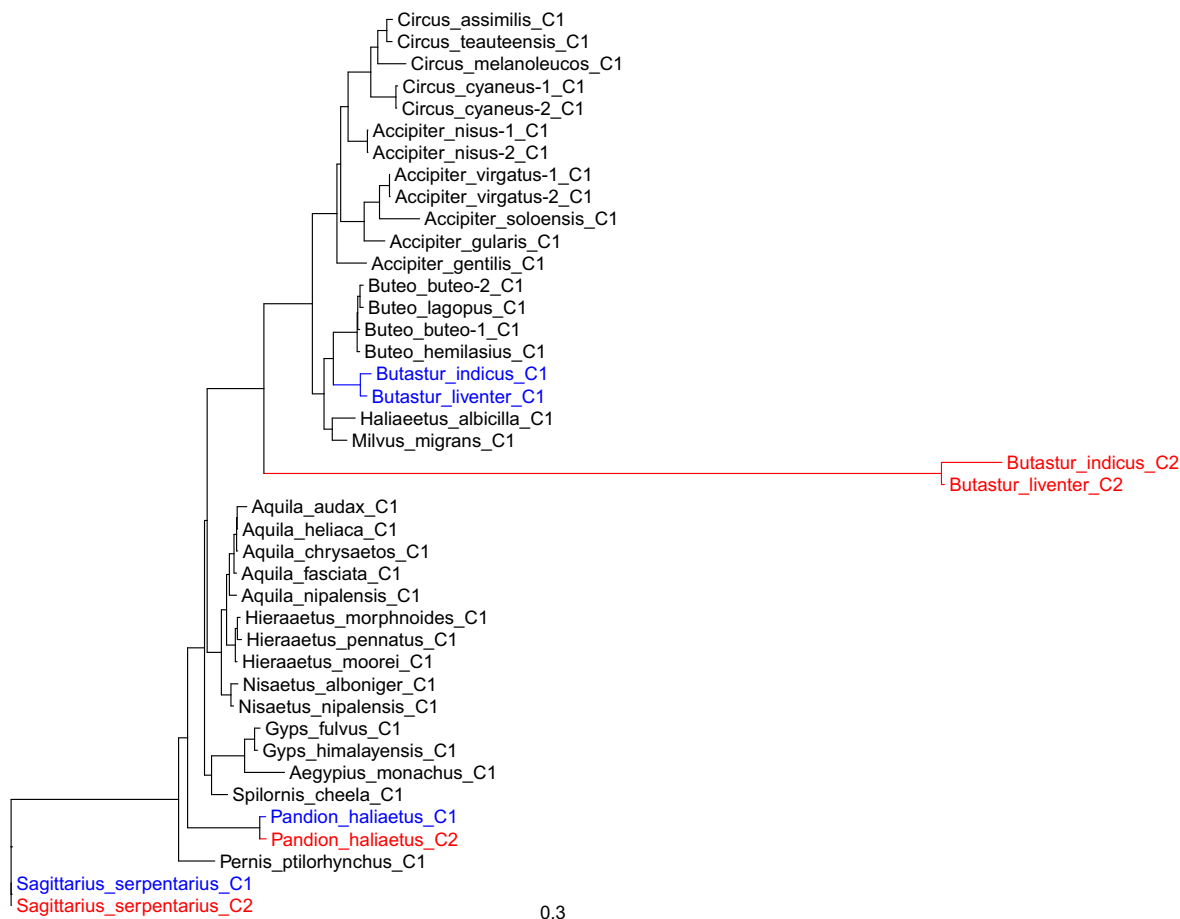
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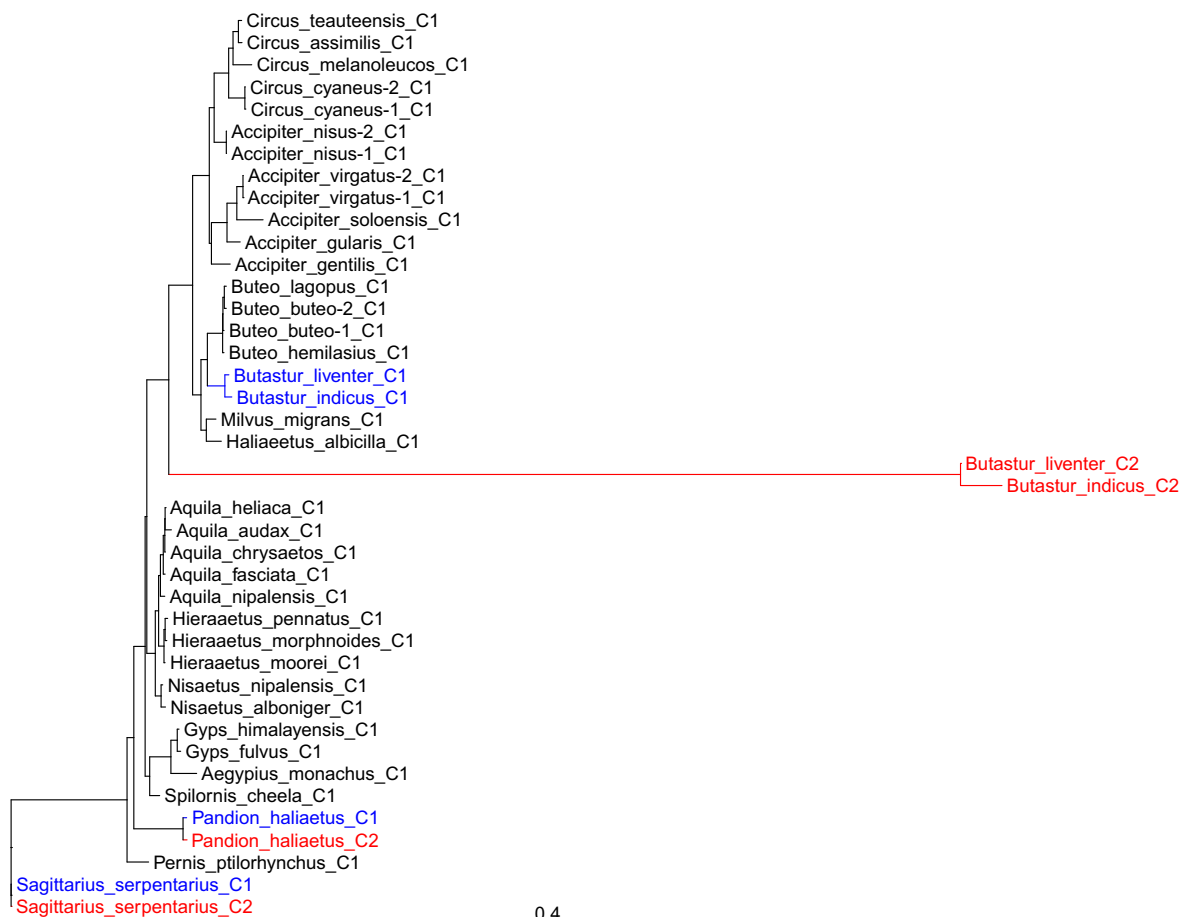
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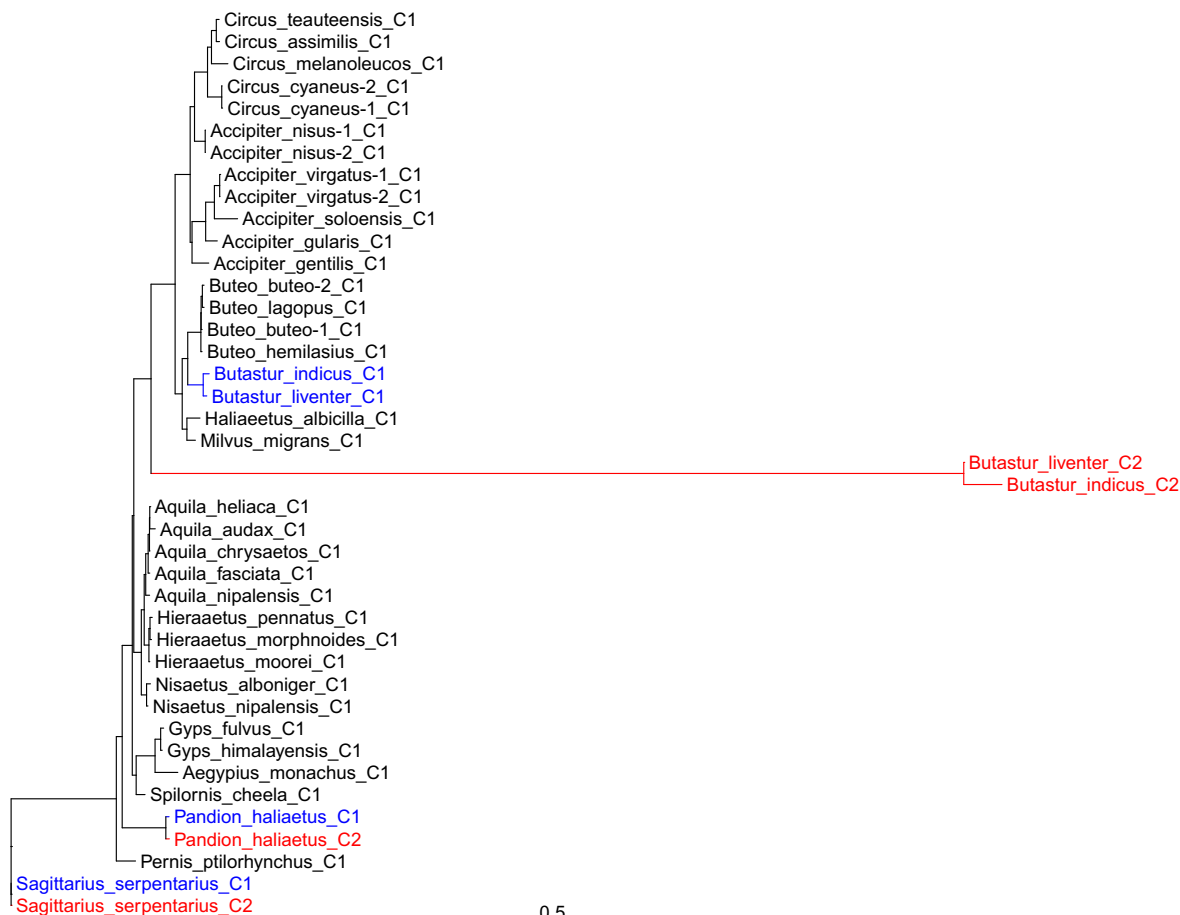
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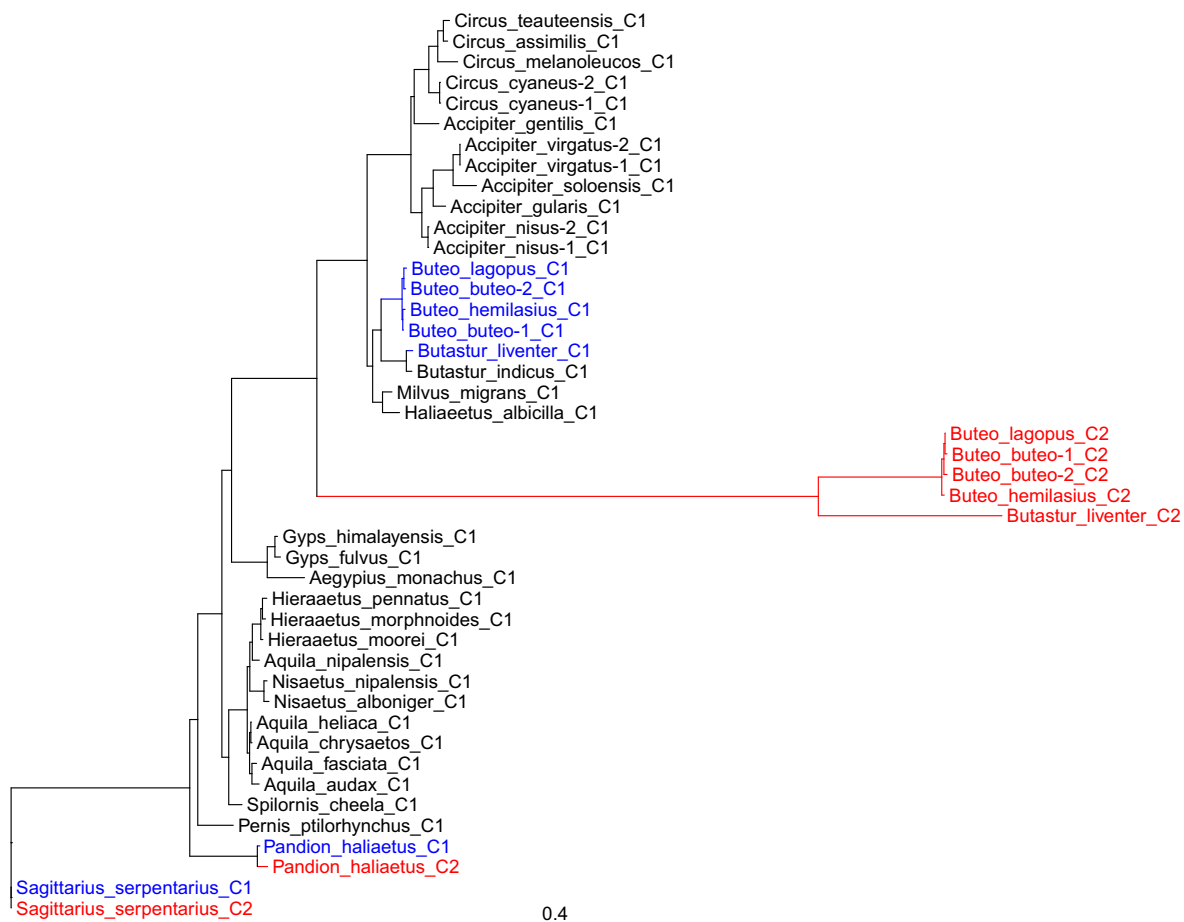
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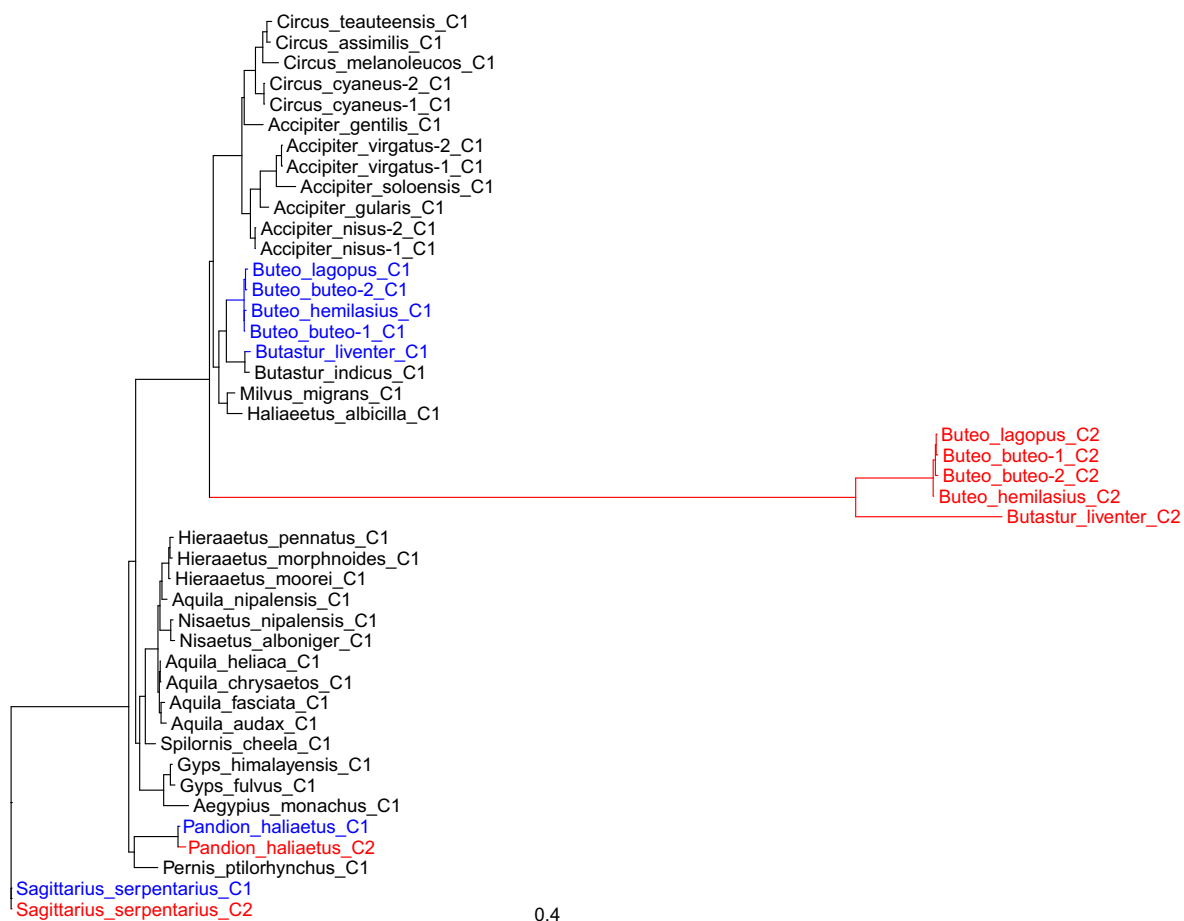
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## Repeats masked, MrBayes

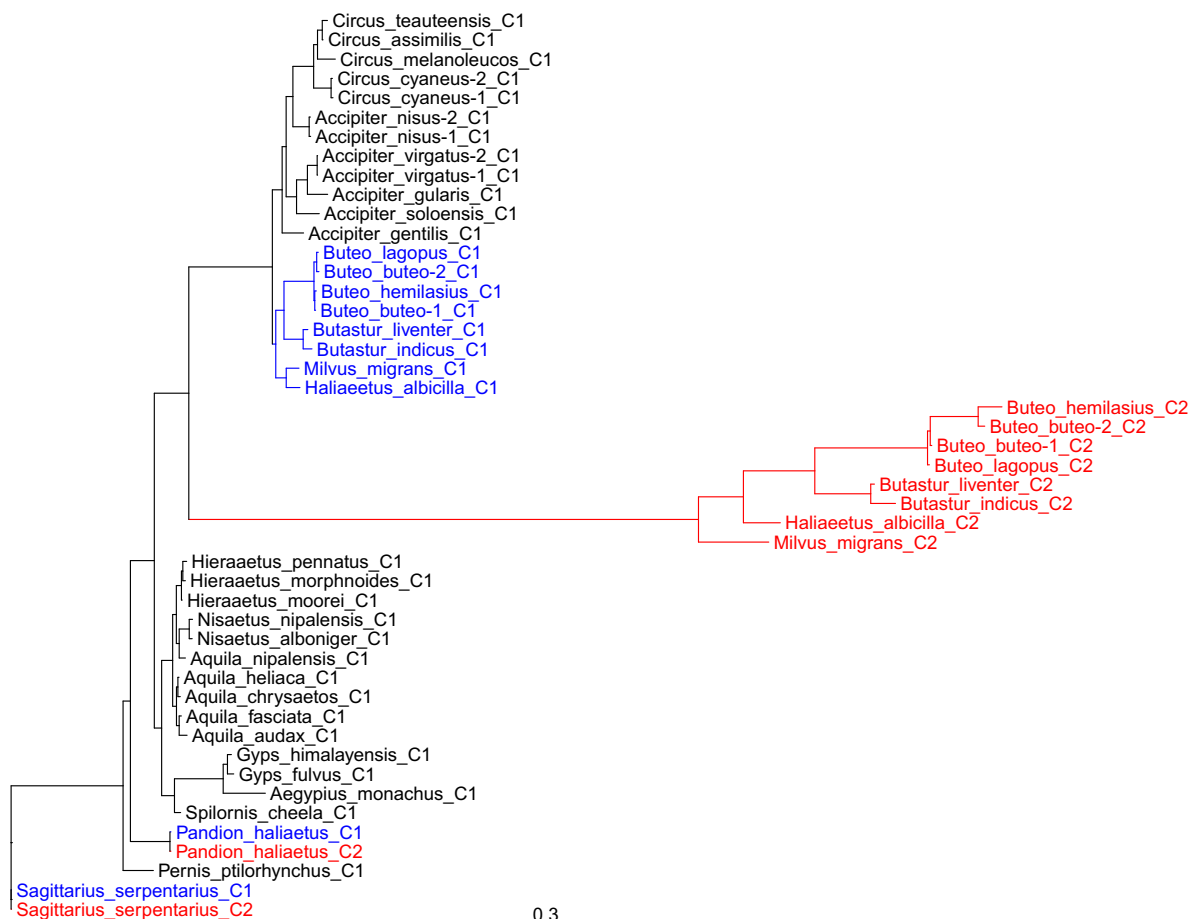


## Repeats masked, IQ-Tree

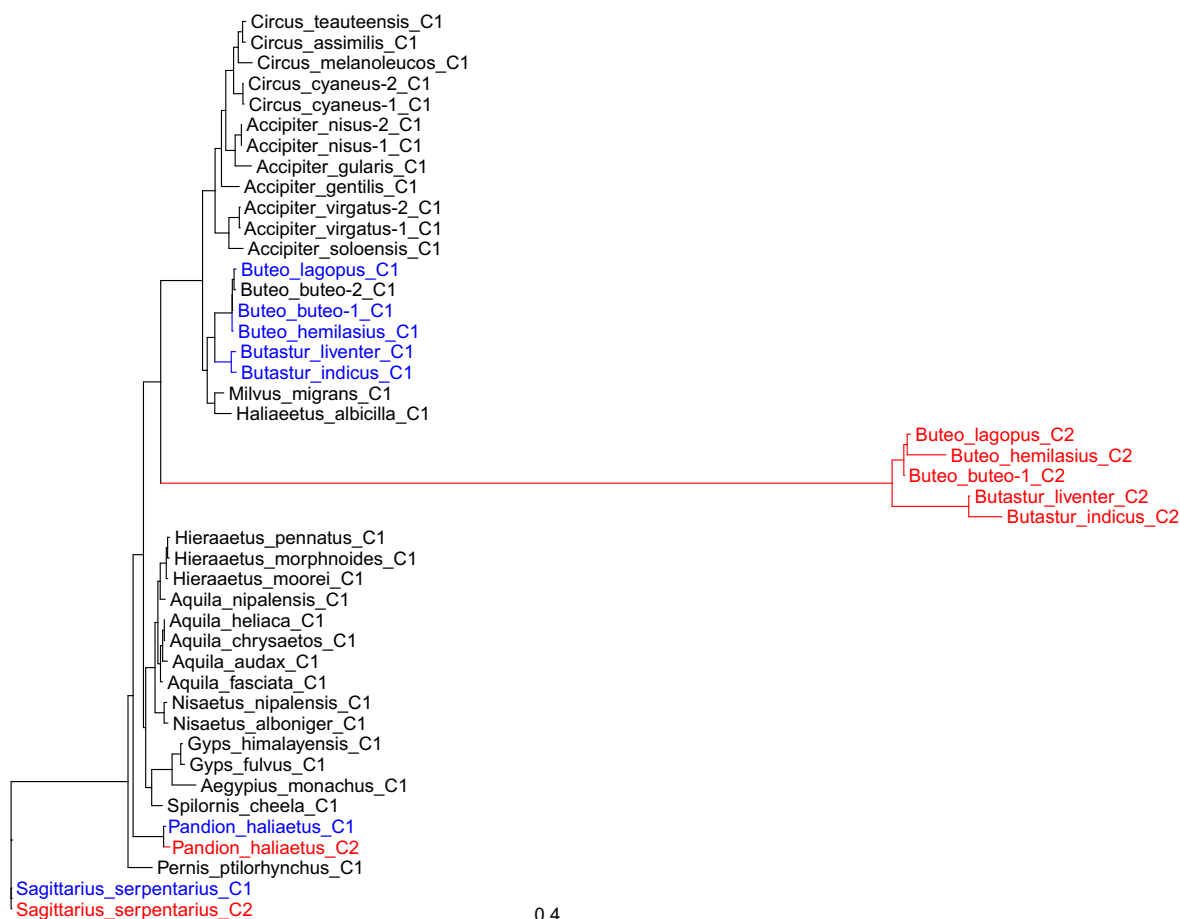




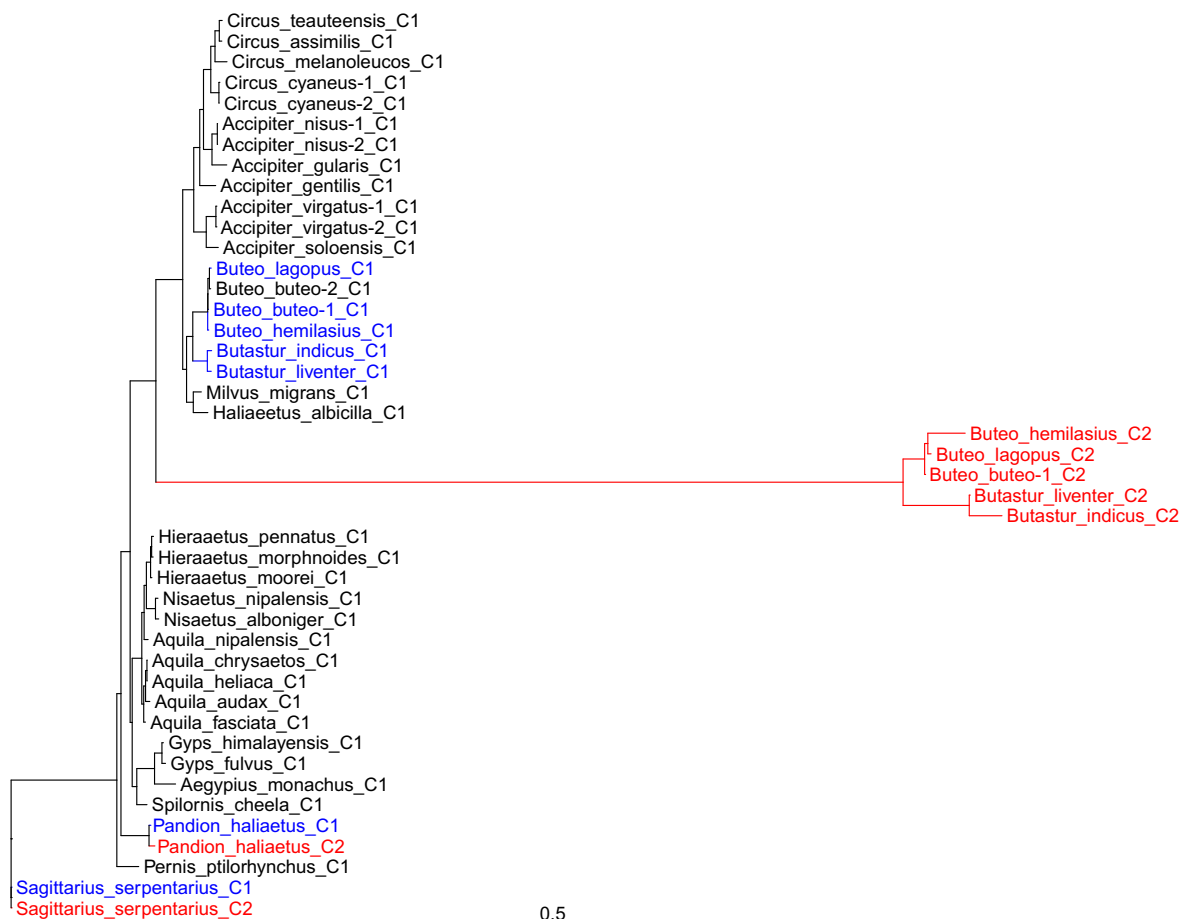
## Repeats removed, MrBayes



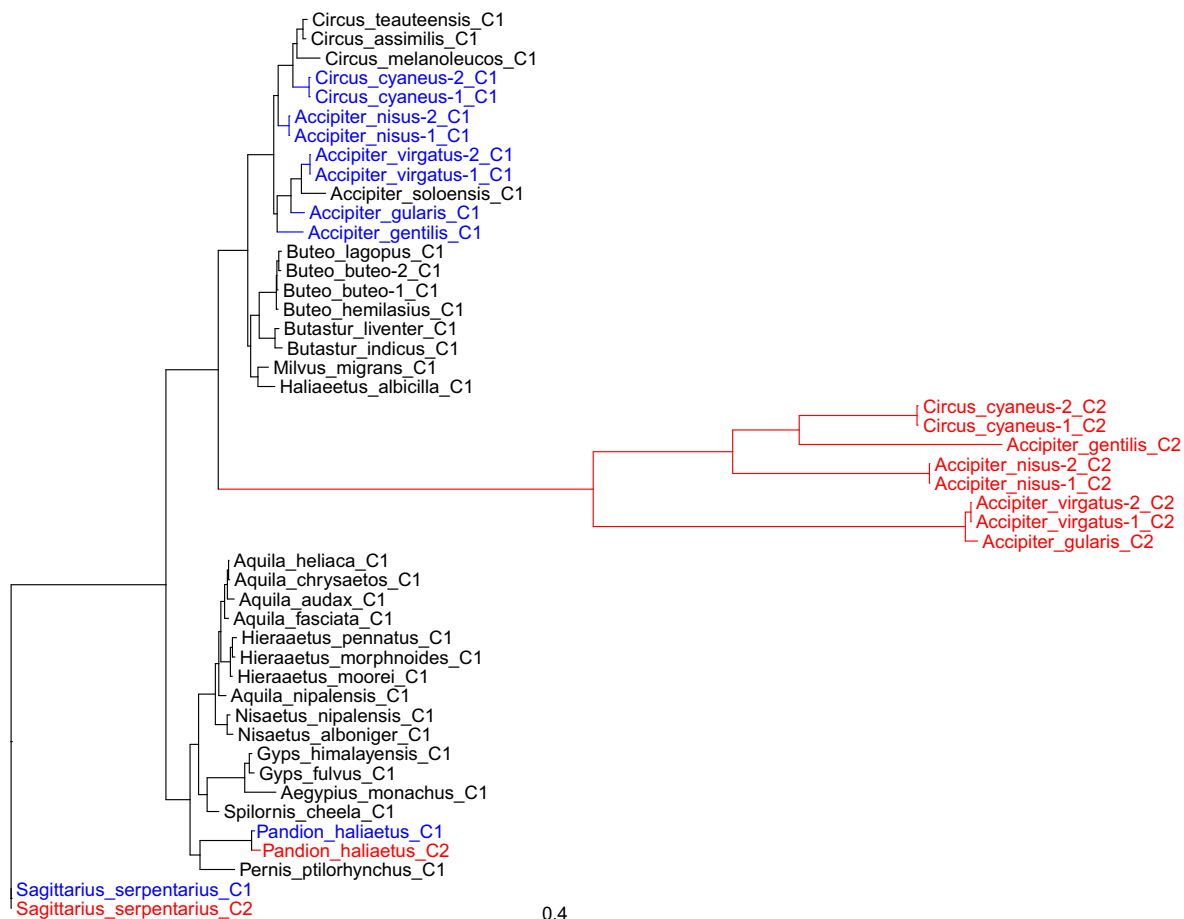
## Repeats removed, IQ-Tree



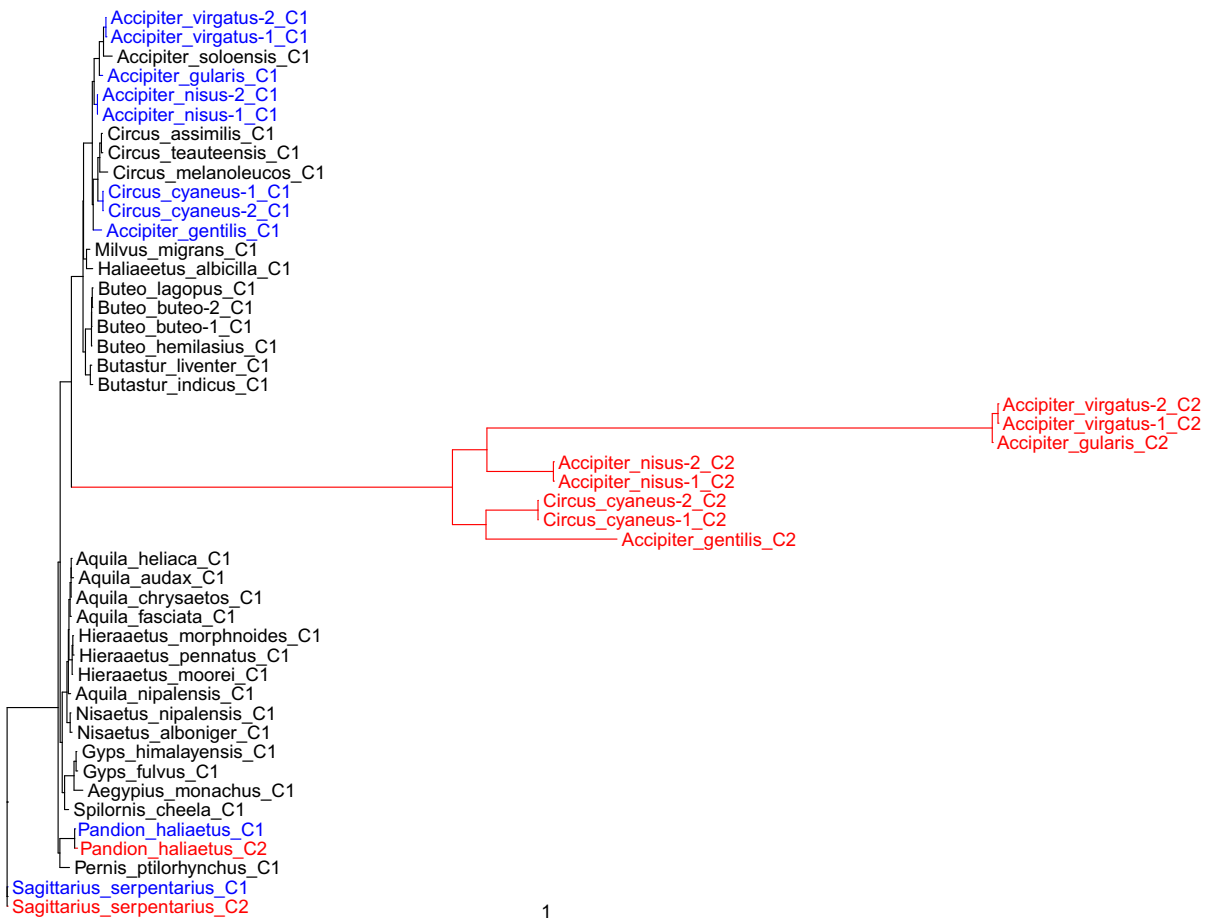
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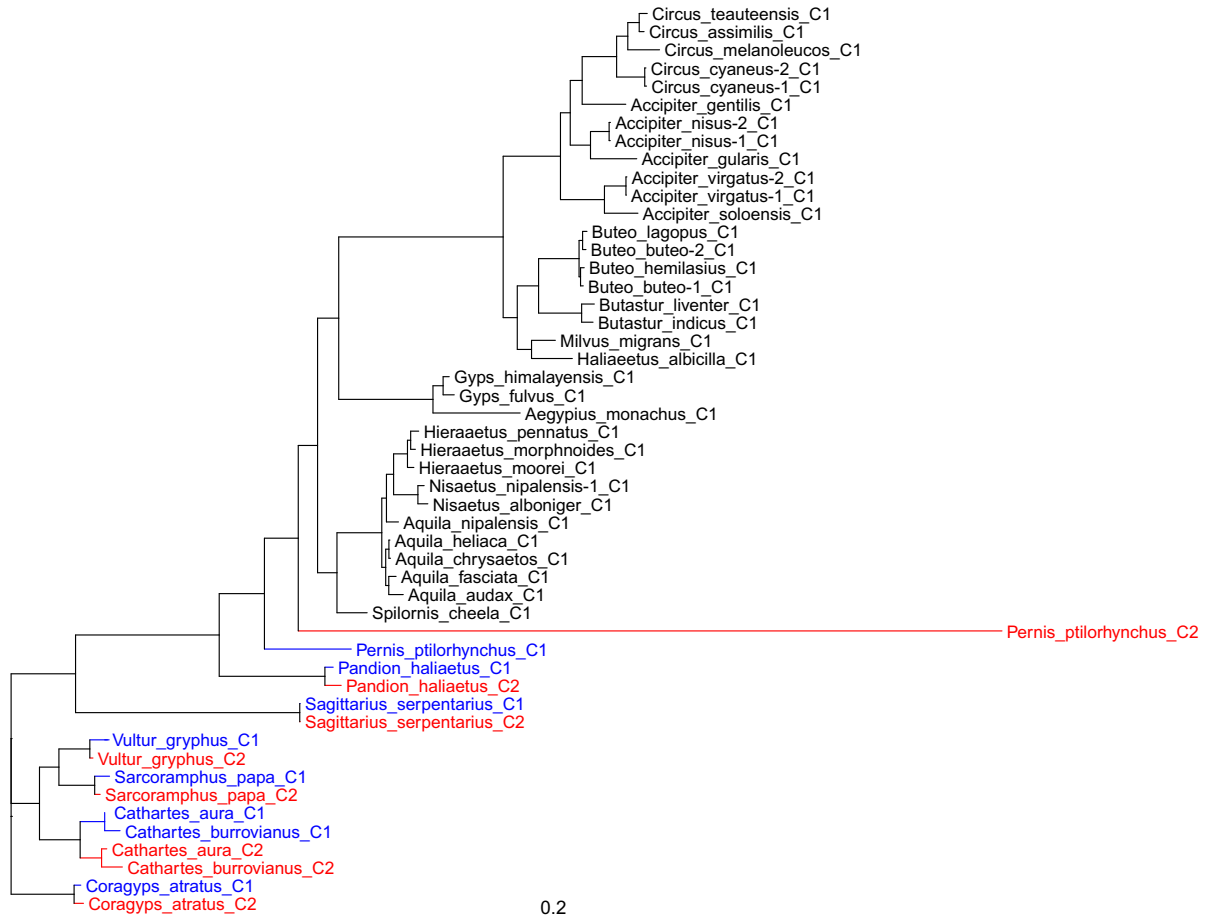
## All sites, MrBayes



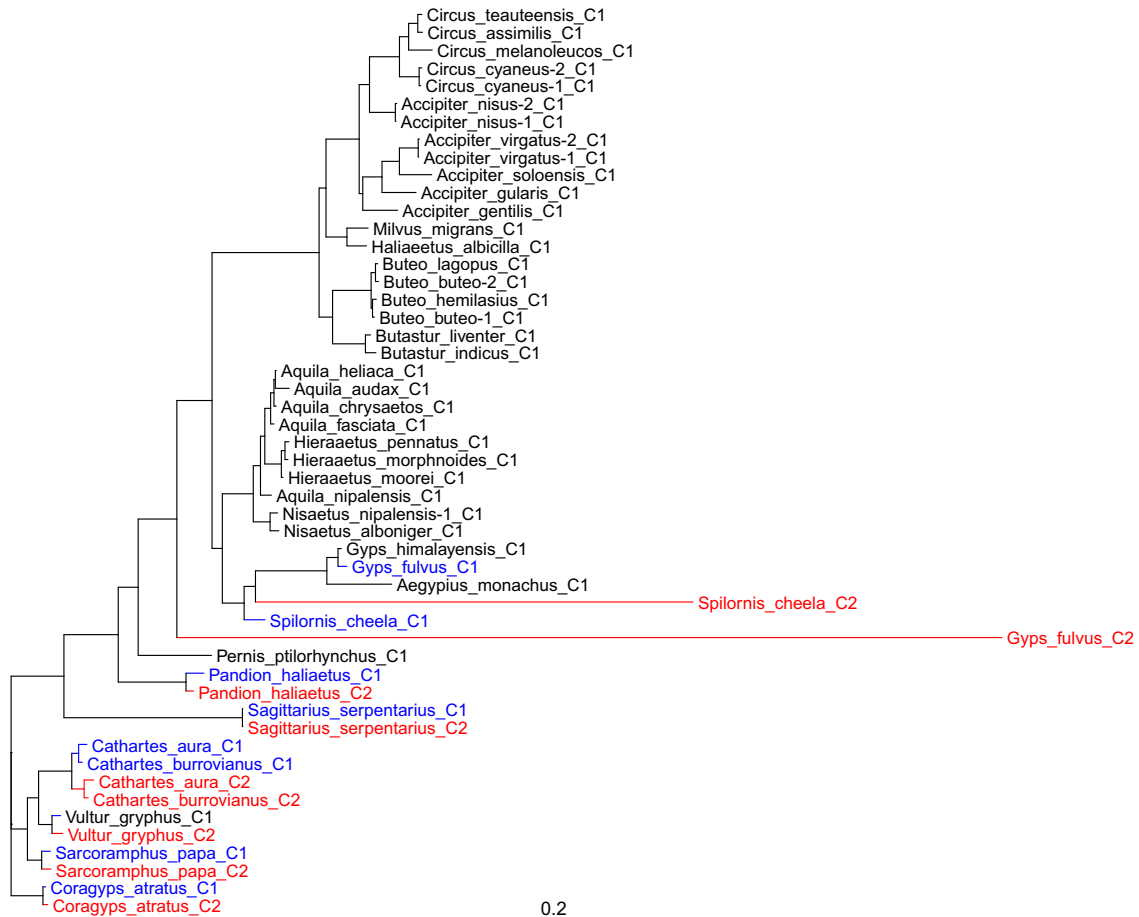
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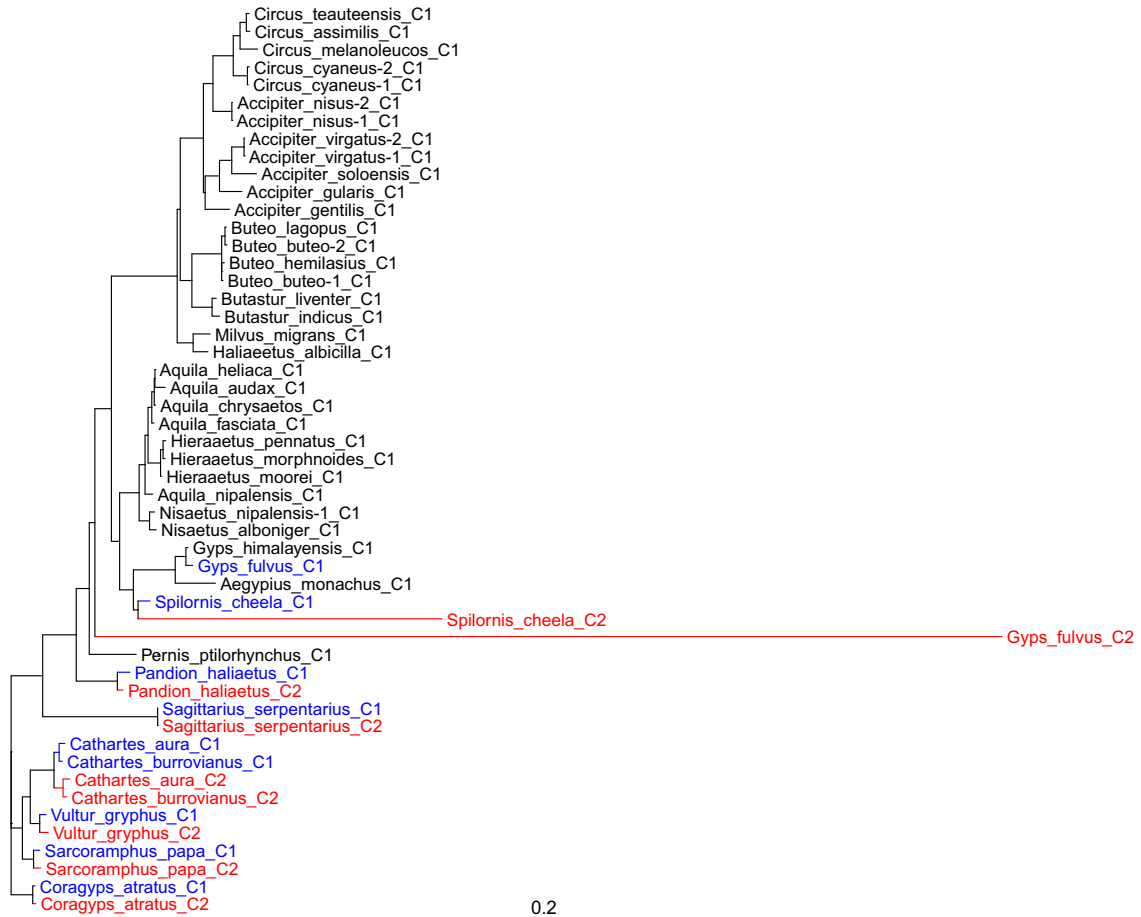
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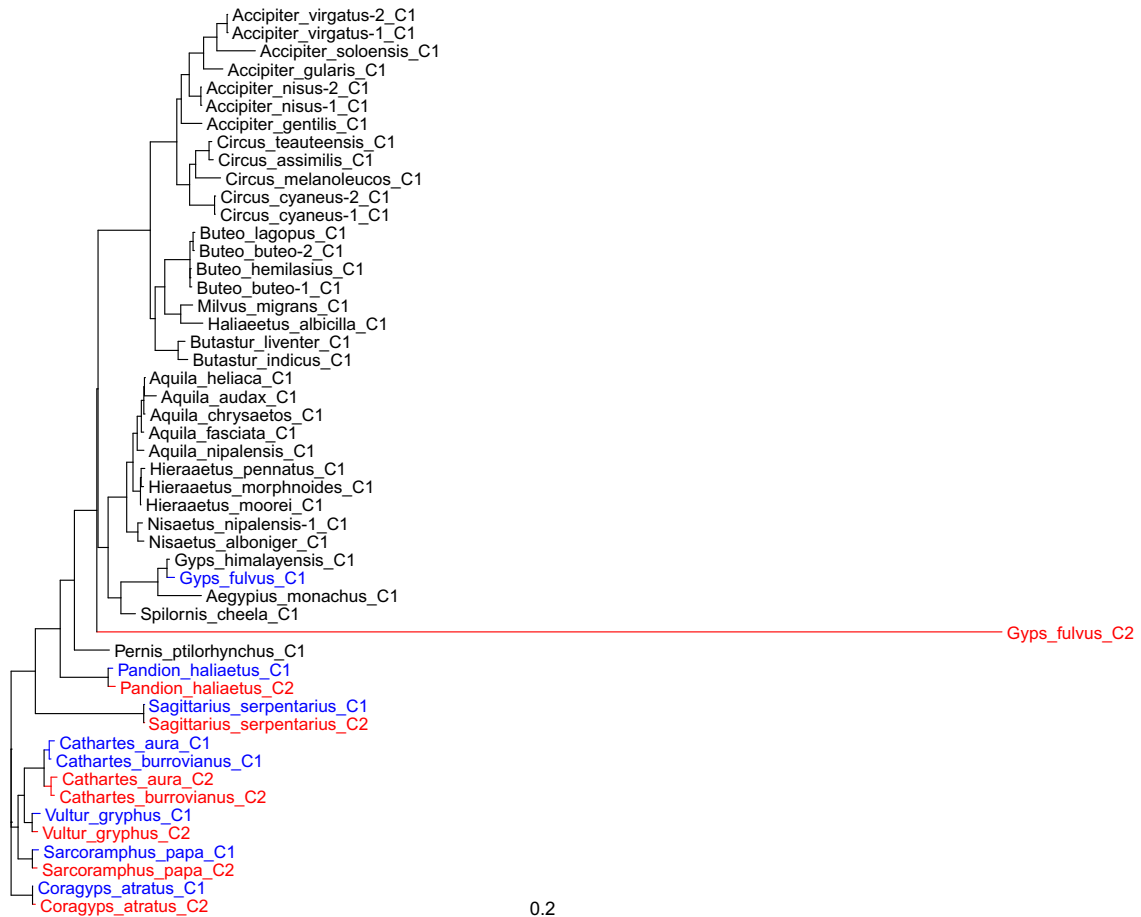
## All sites, MrBayes



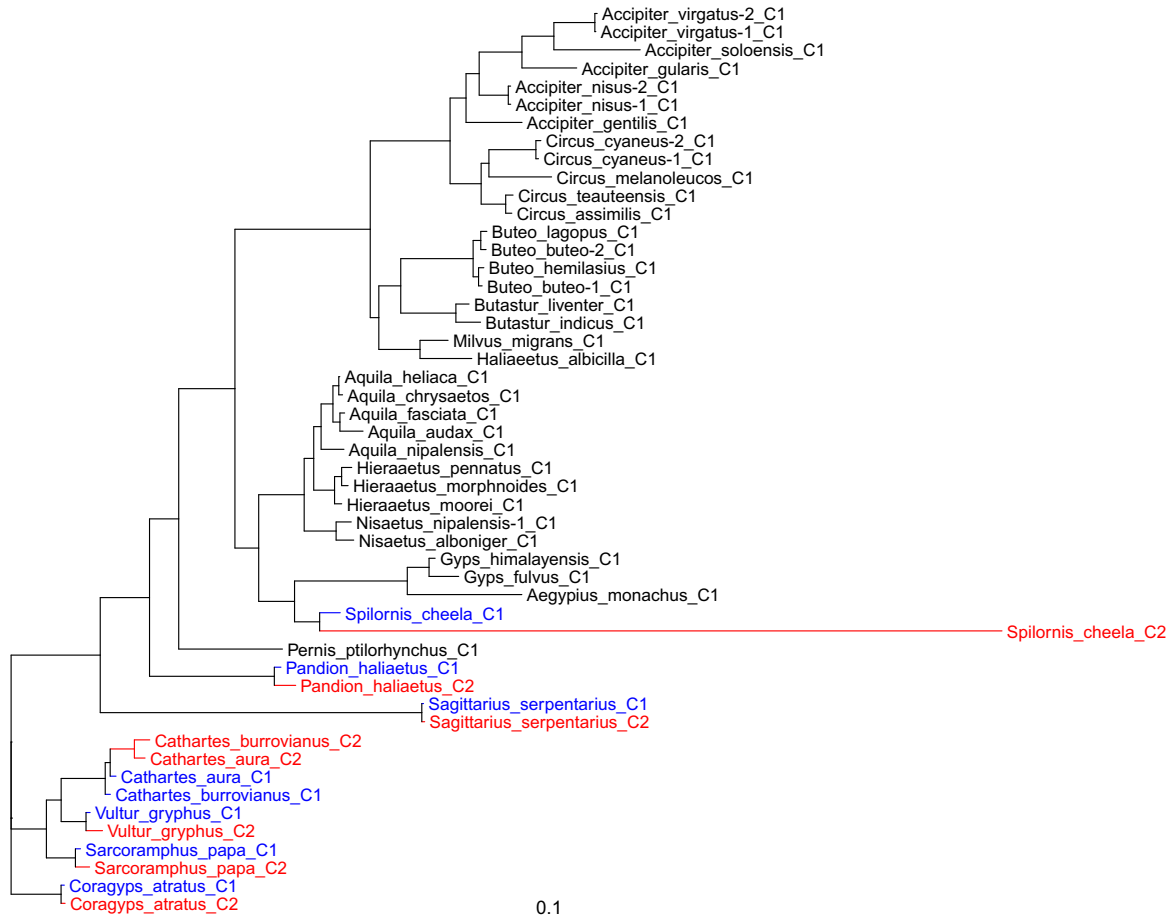
## All sites, IQ-Tree



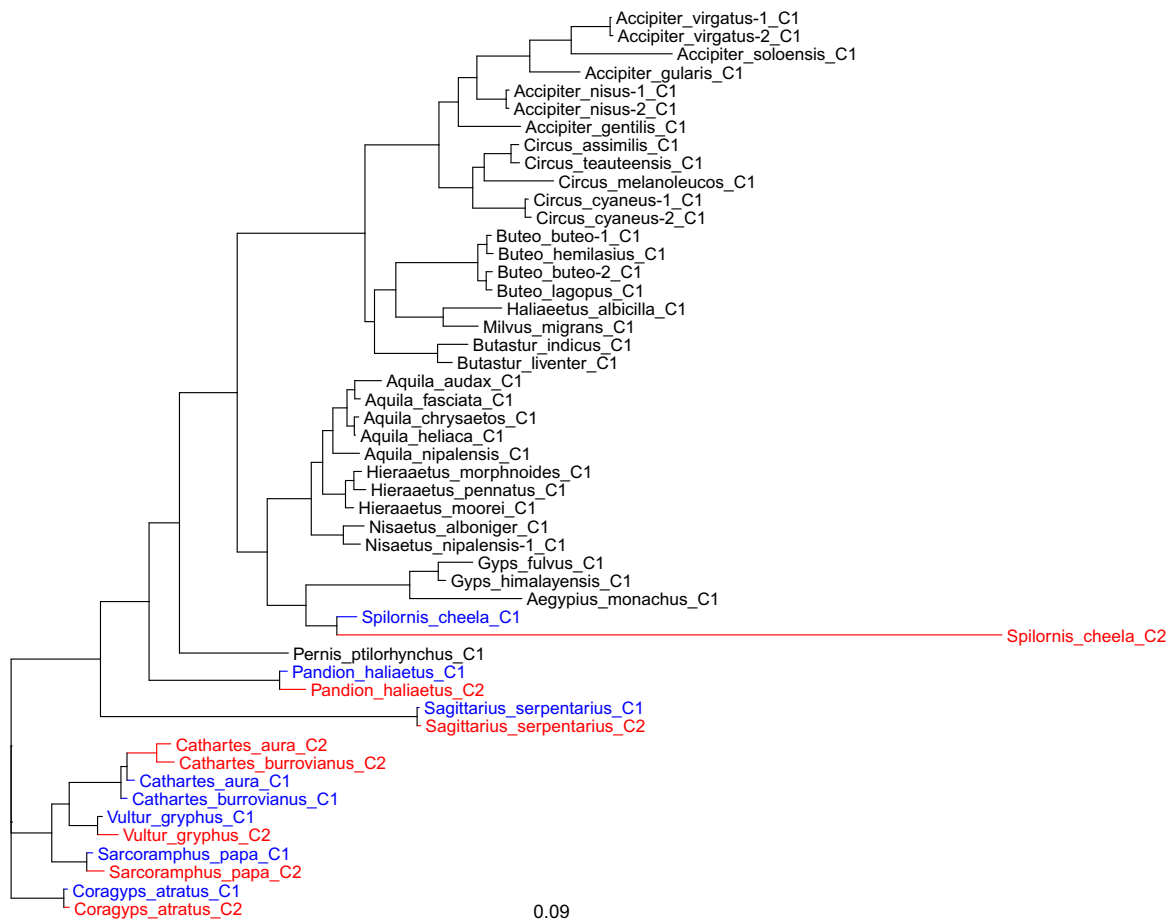
## All sites, IQ-Tree



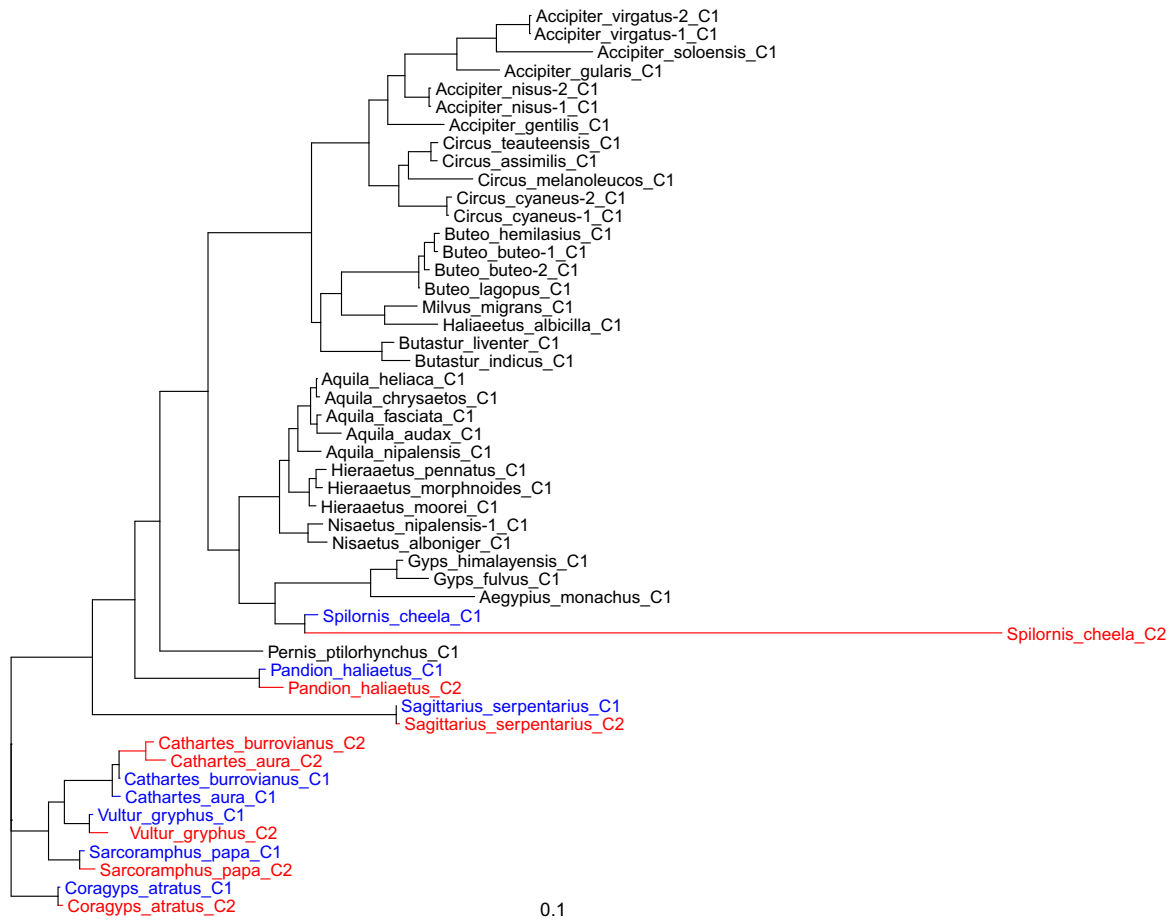
## All sites, MrBayes



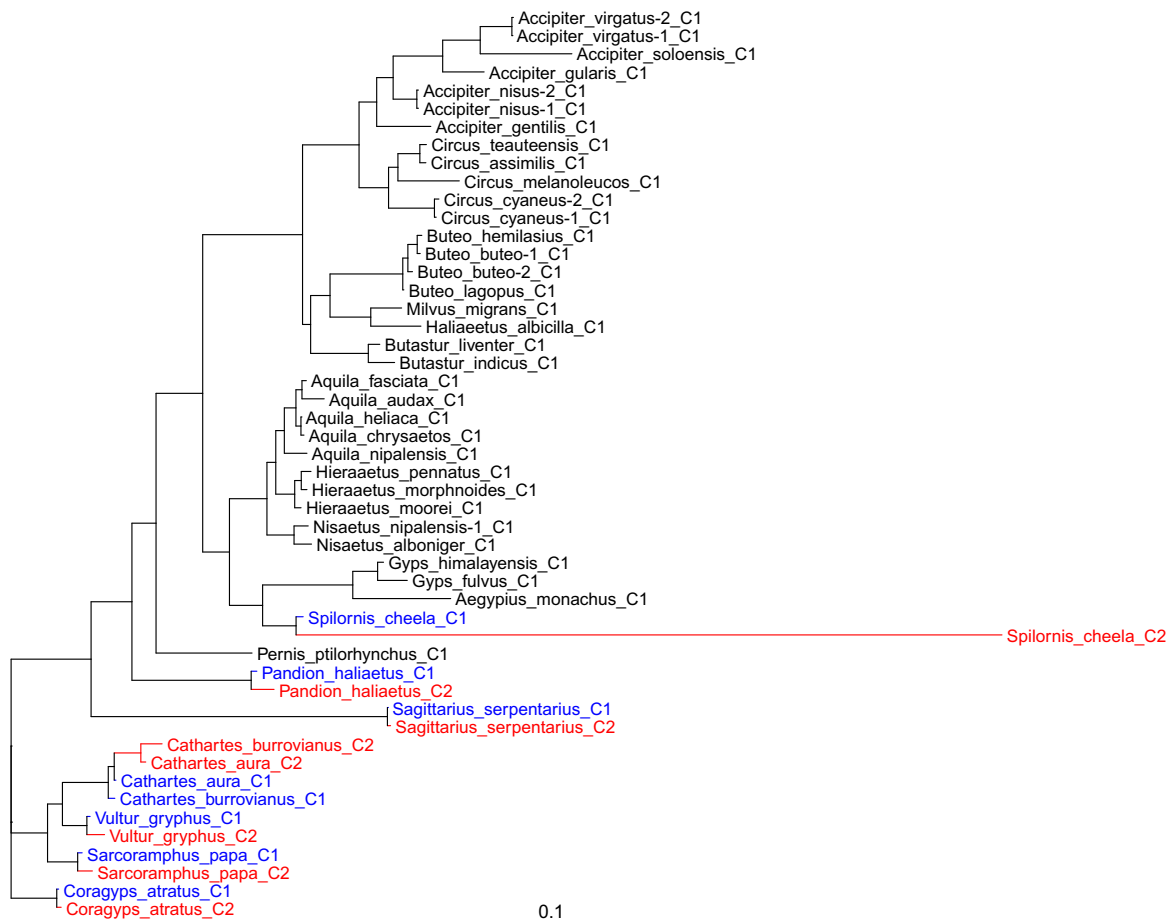
## All sites, PhyloBayes



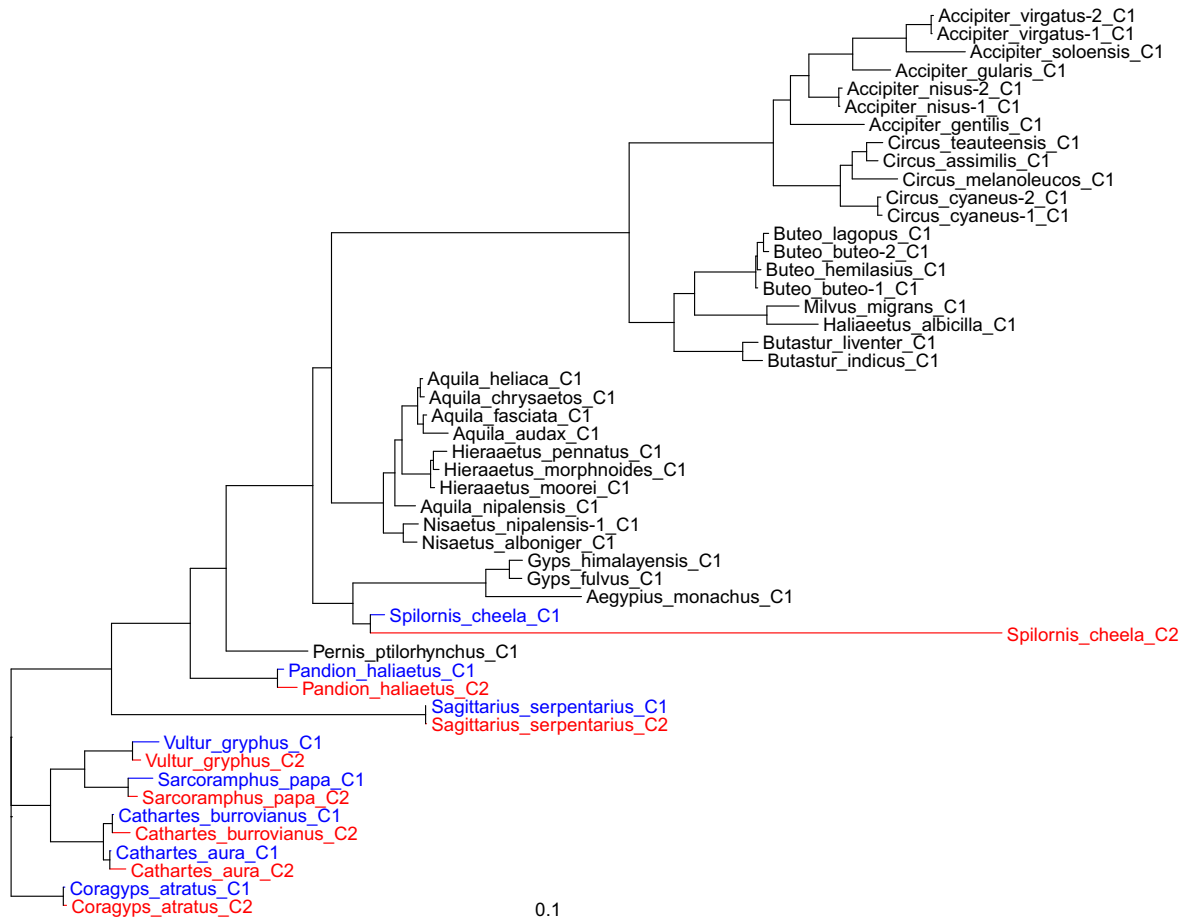
## All sites, IQ-Tree



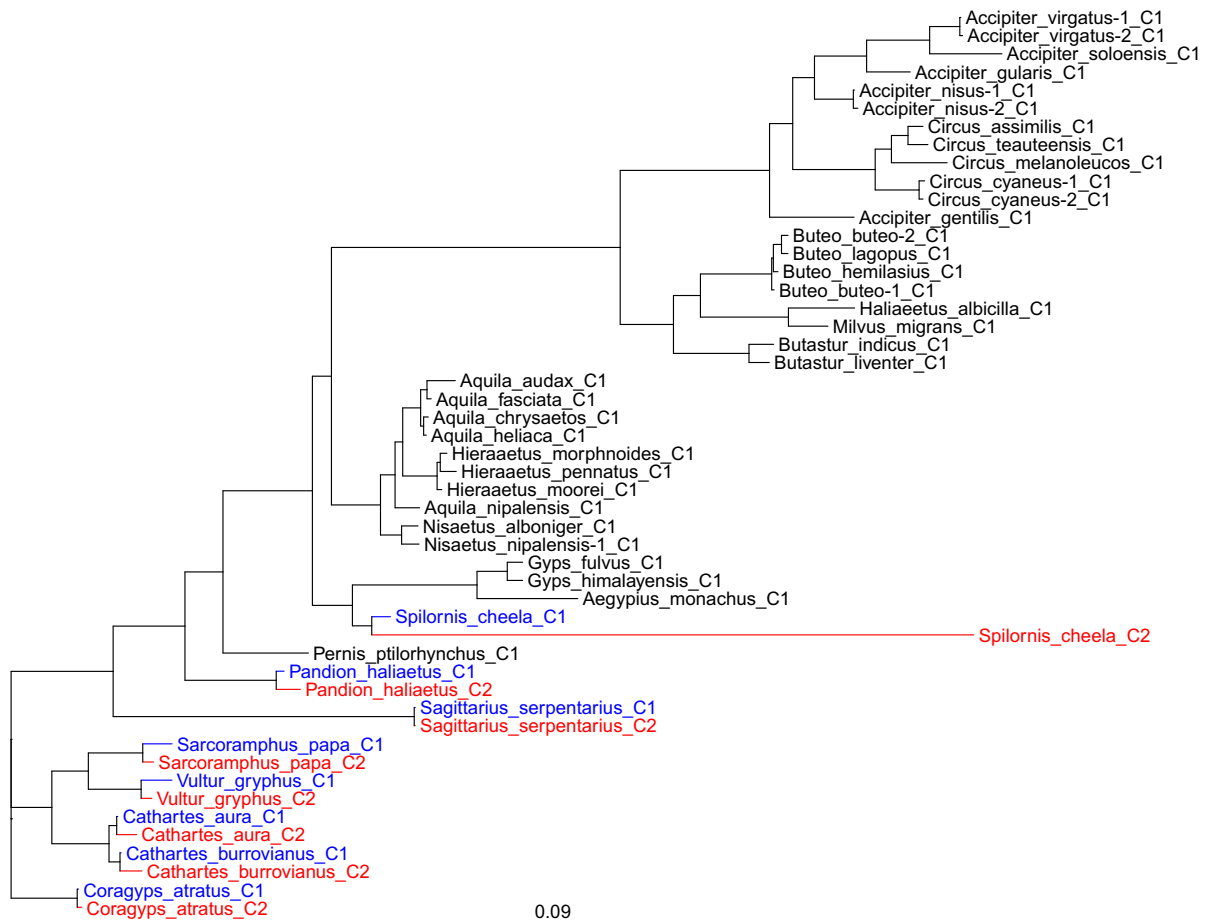
## All sites, PhyML



## Repeats masked, MrBayes

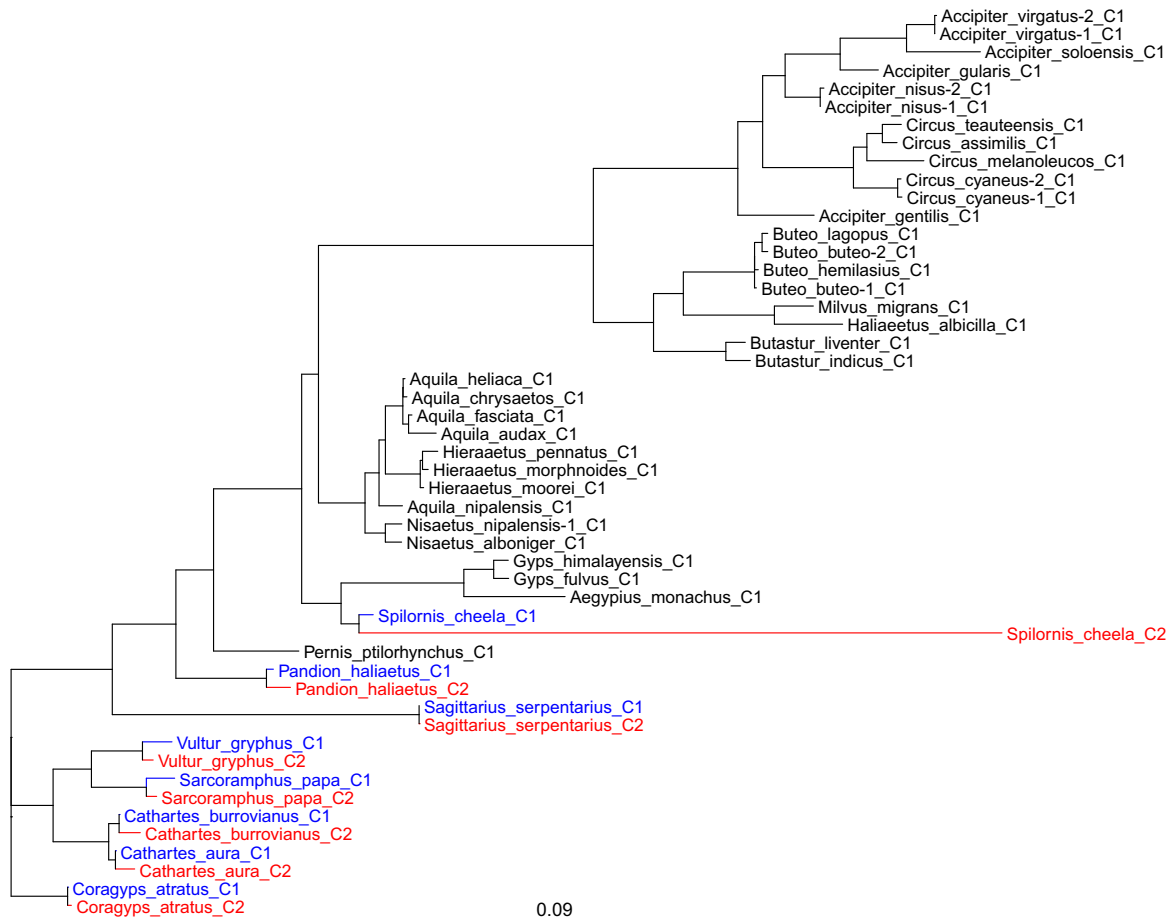


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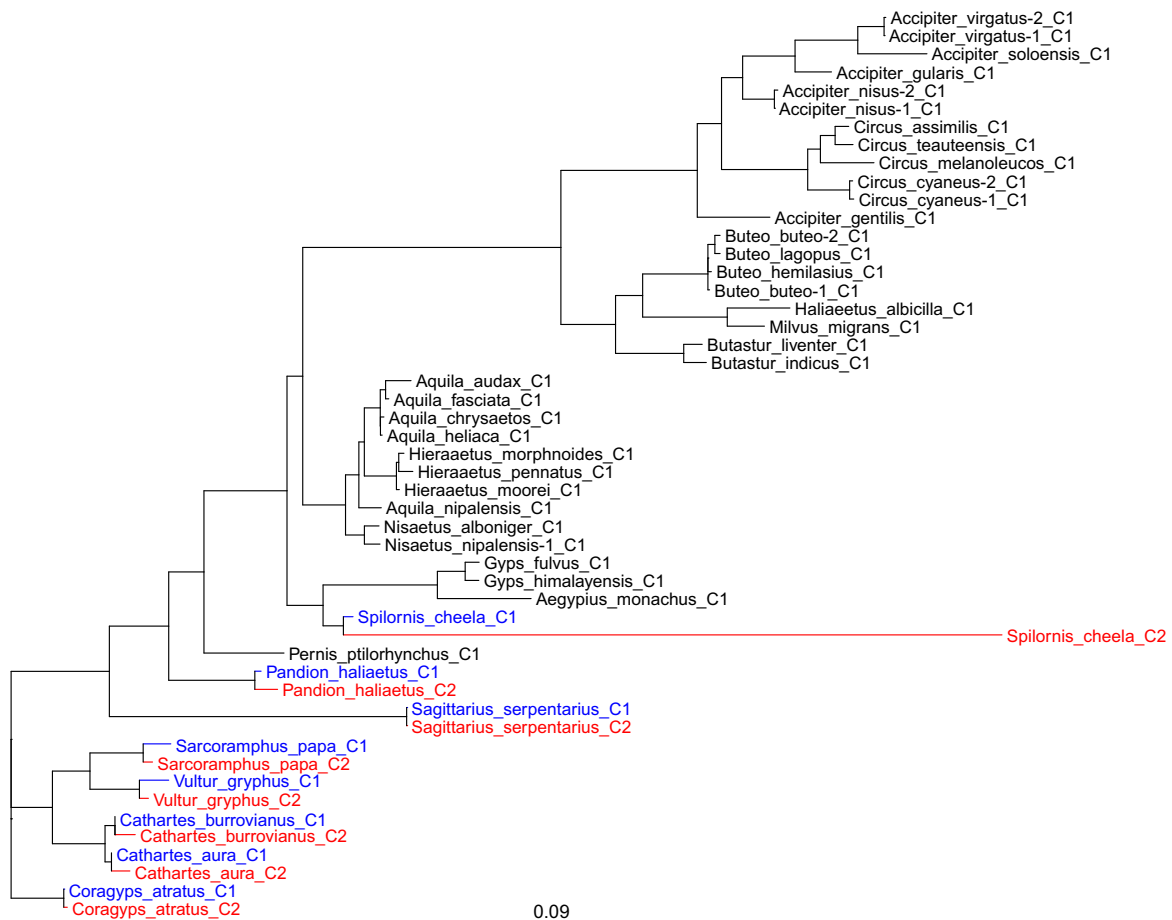




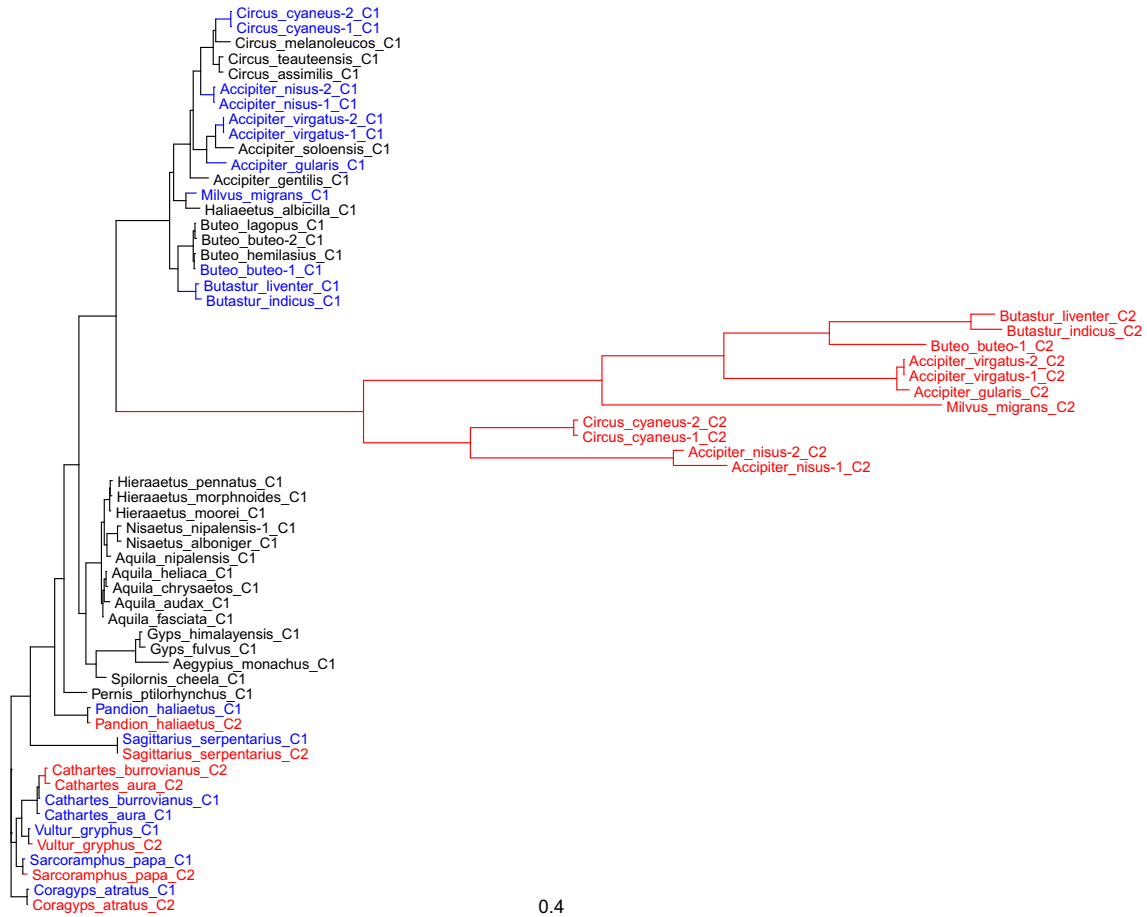
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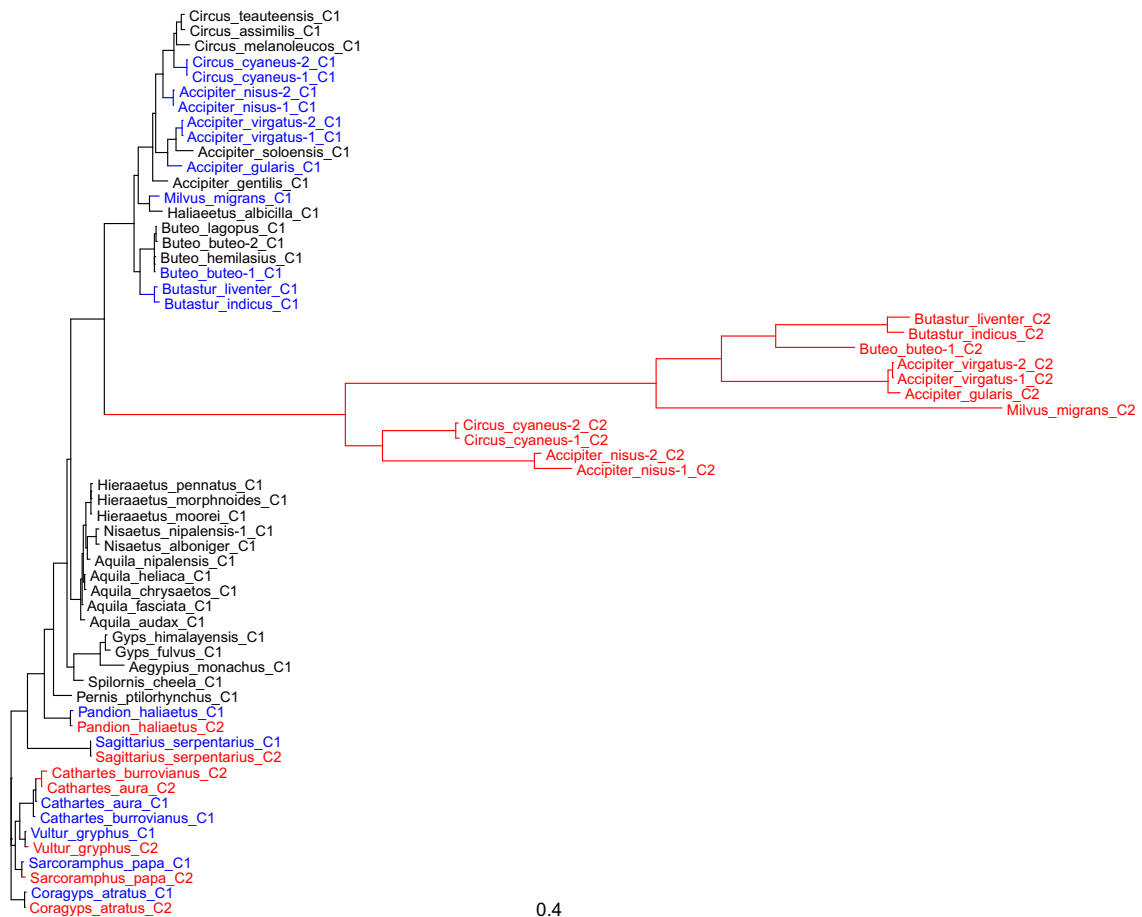
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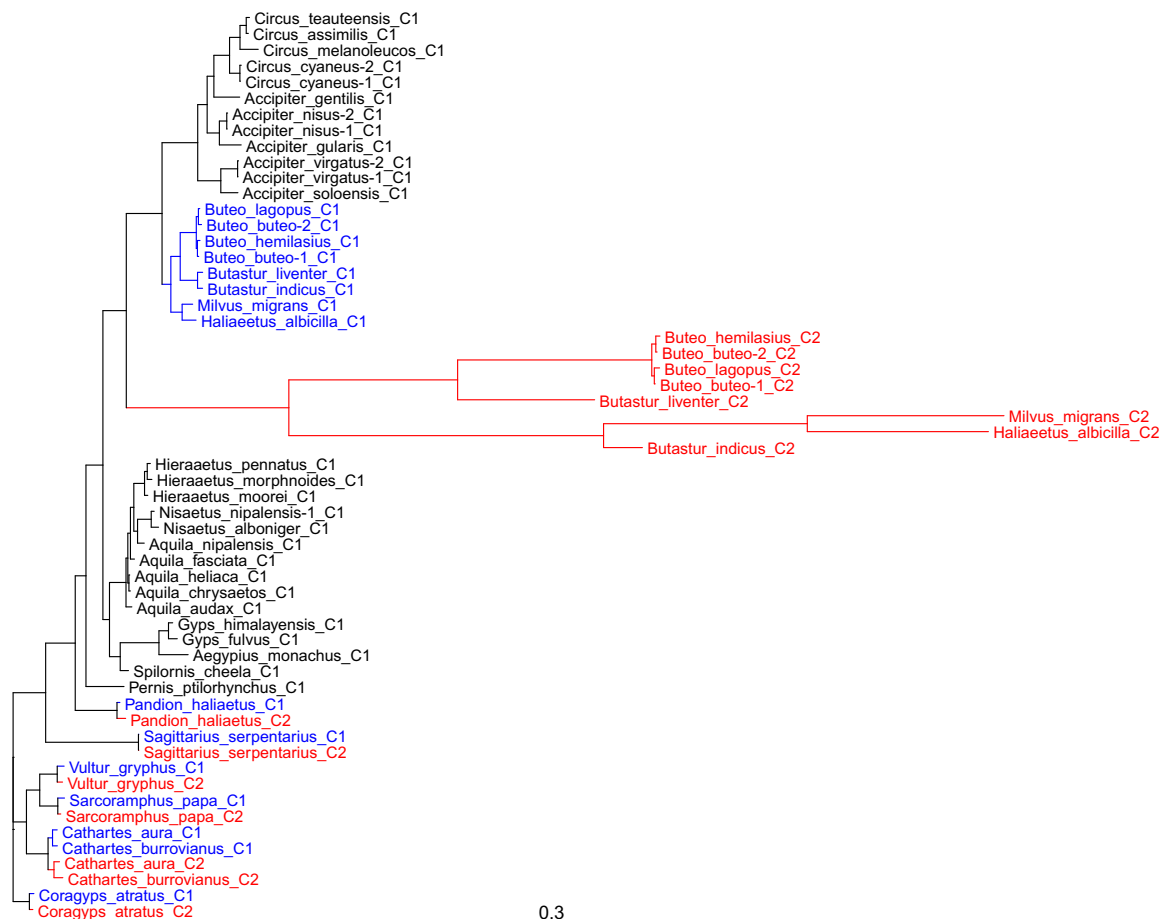
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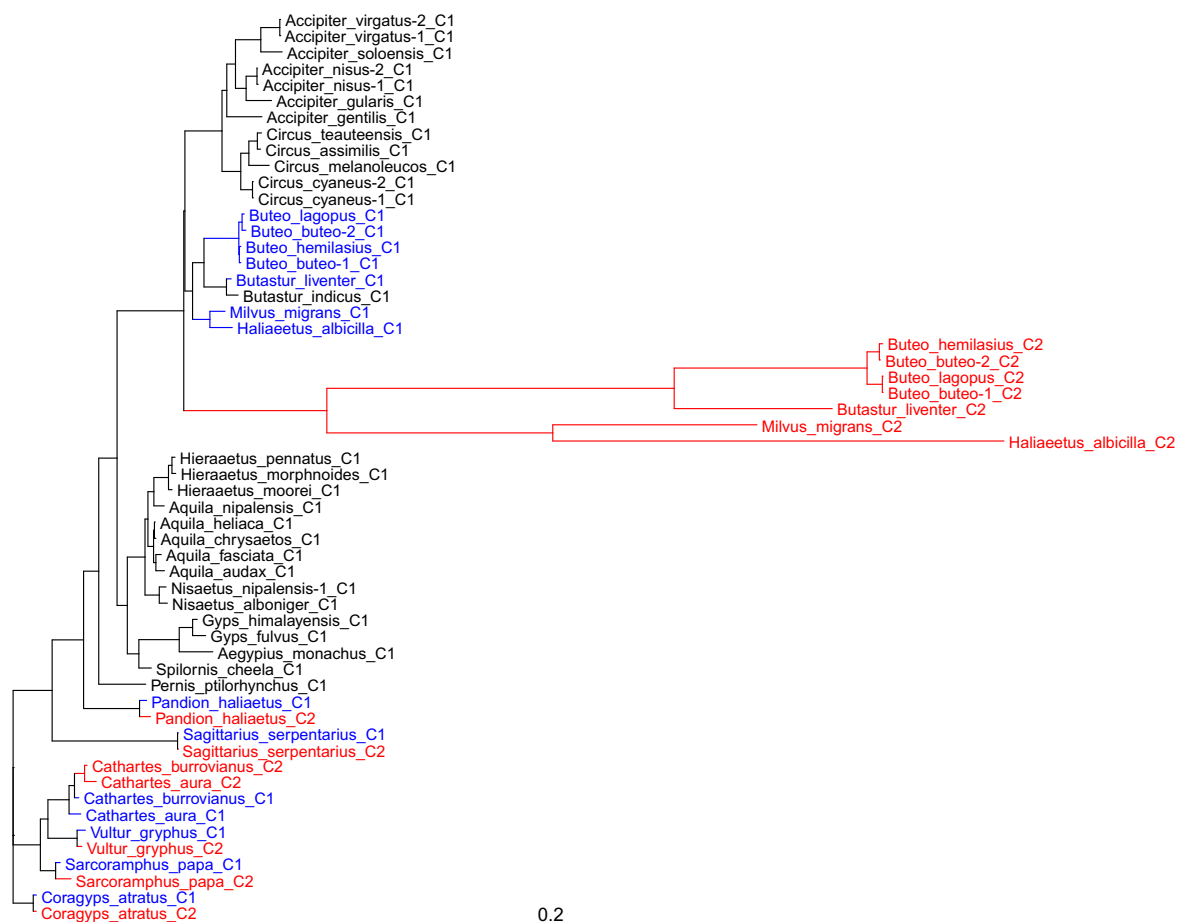
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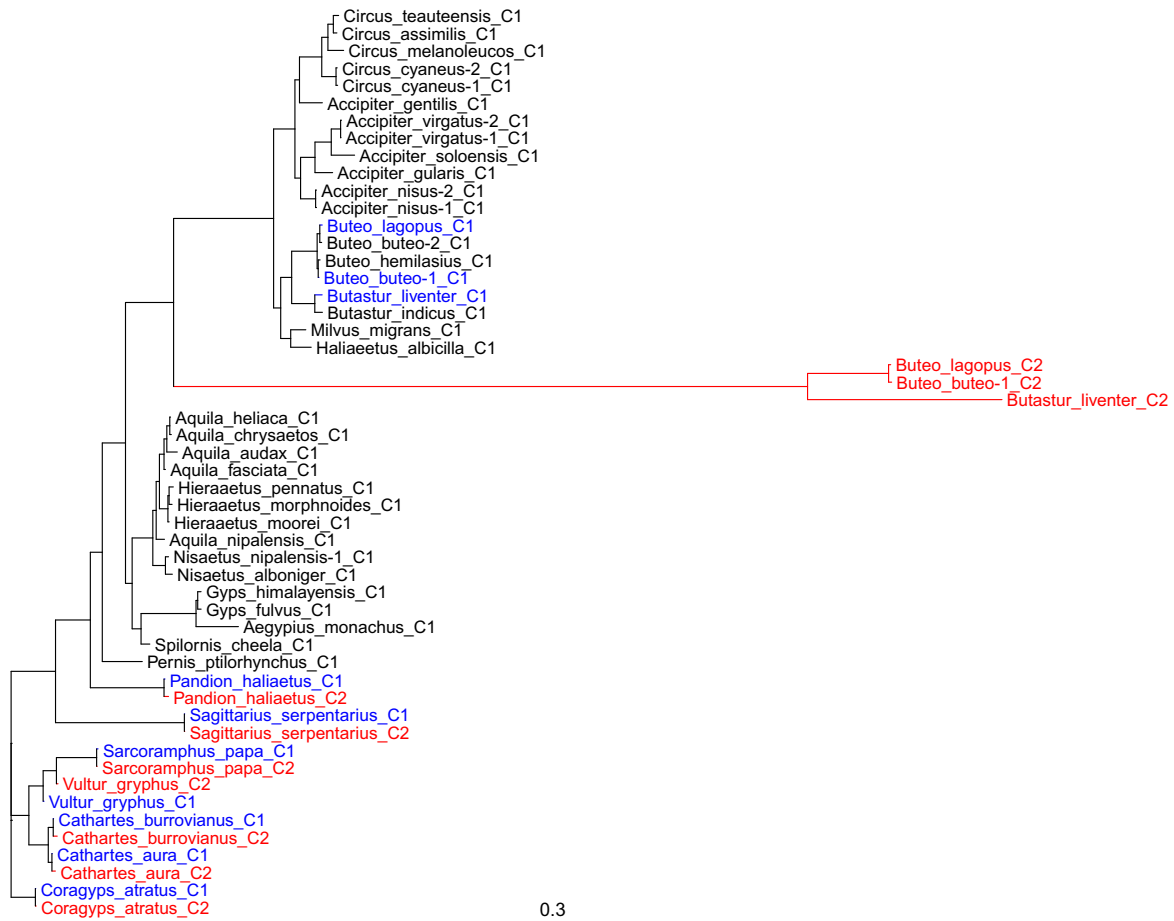
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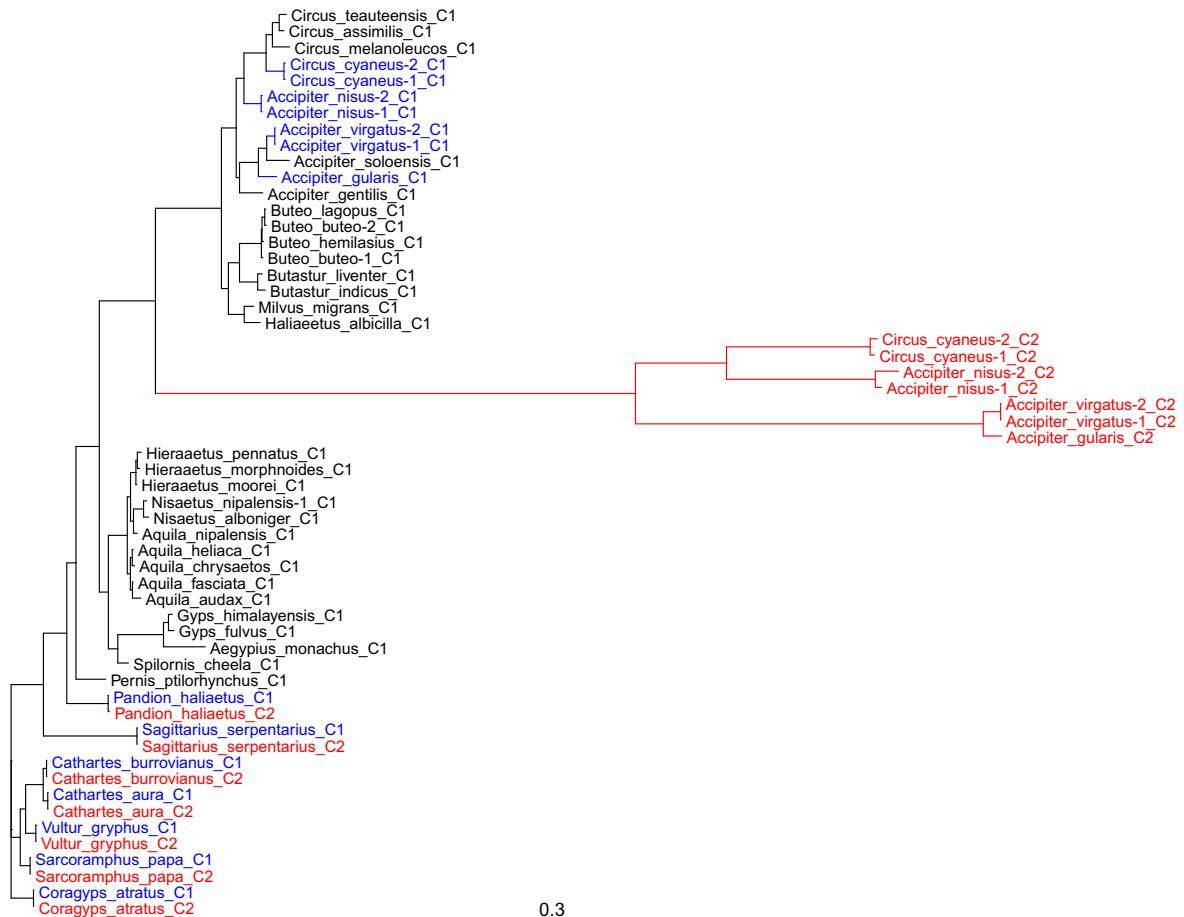
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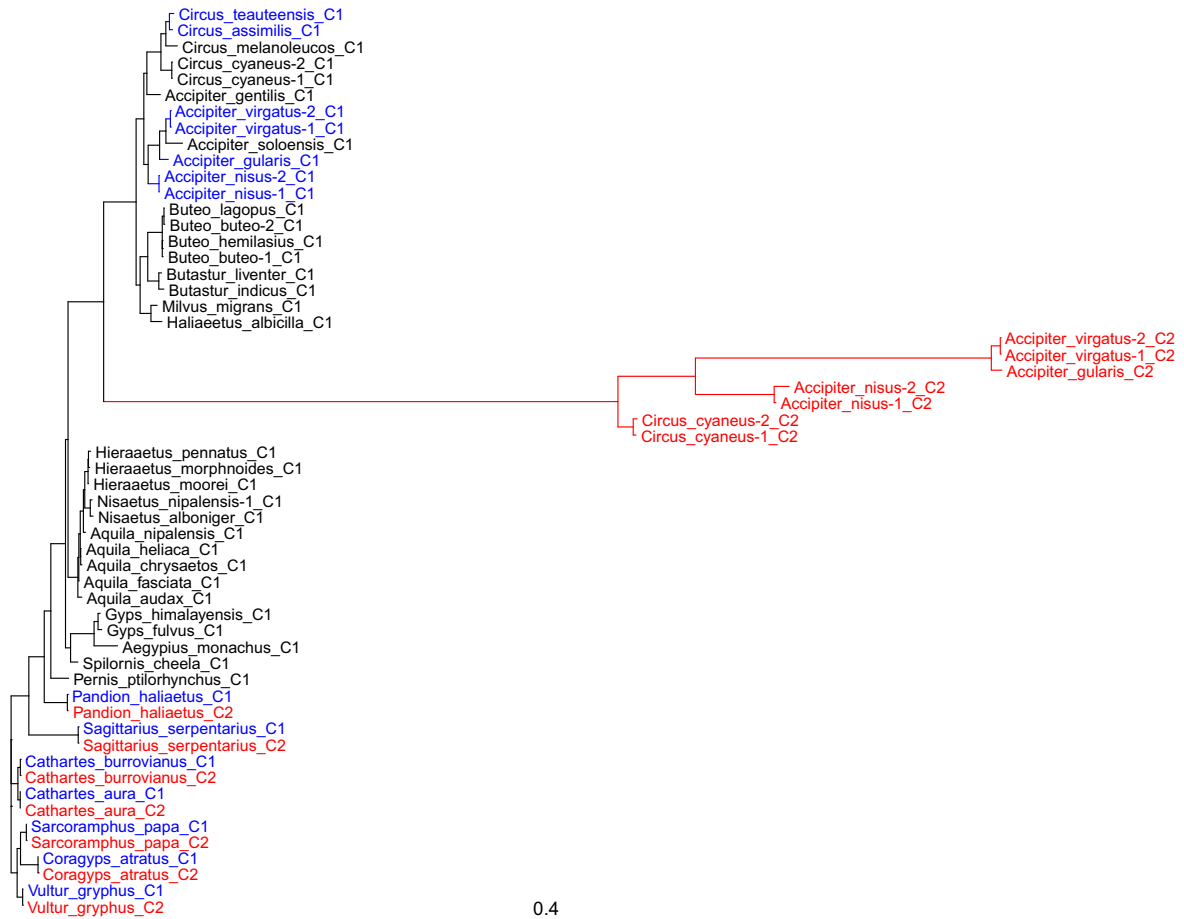
## Repeats removed, MrBayes



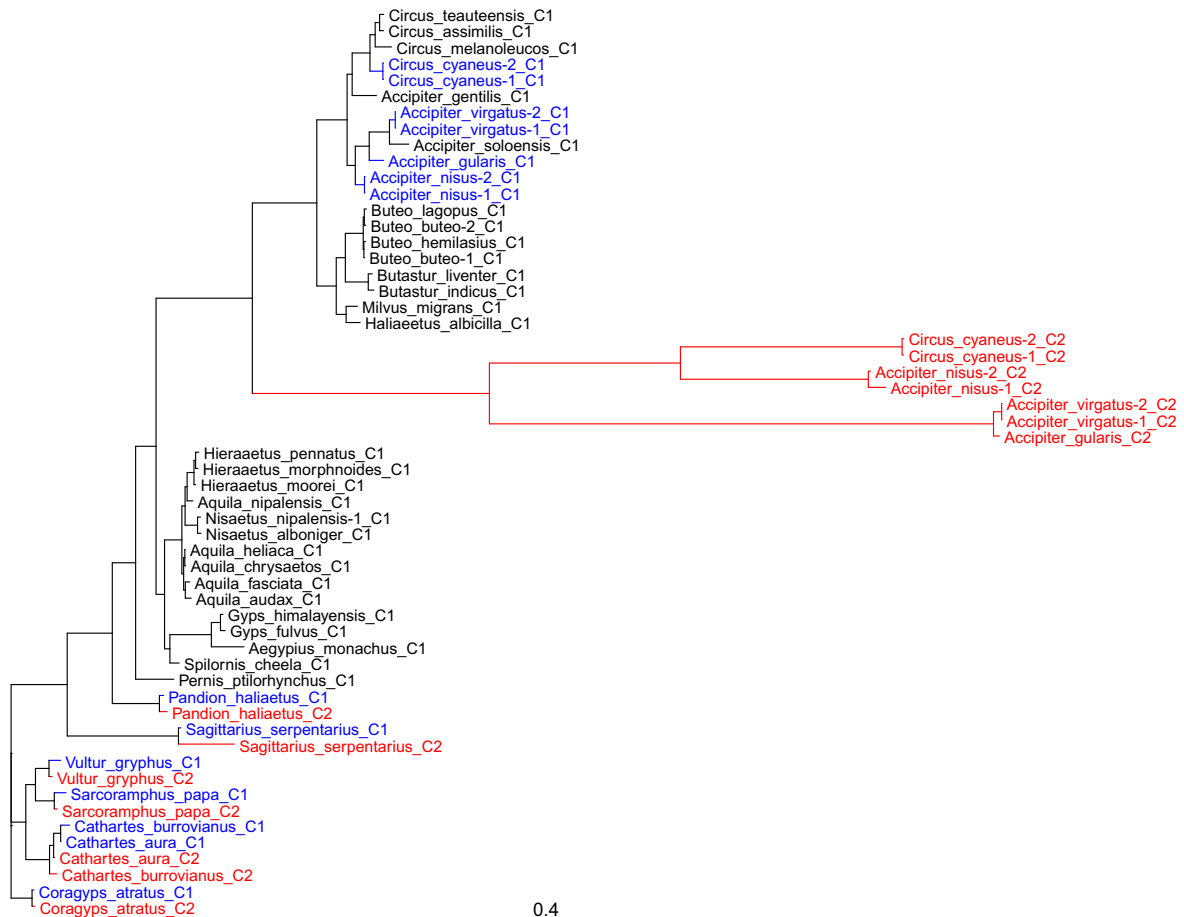
## All sites, MrBayes



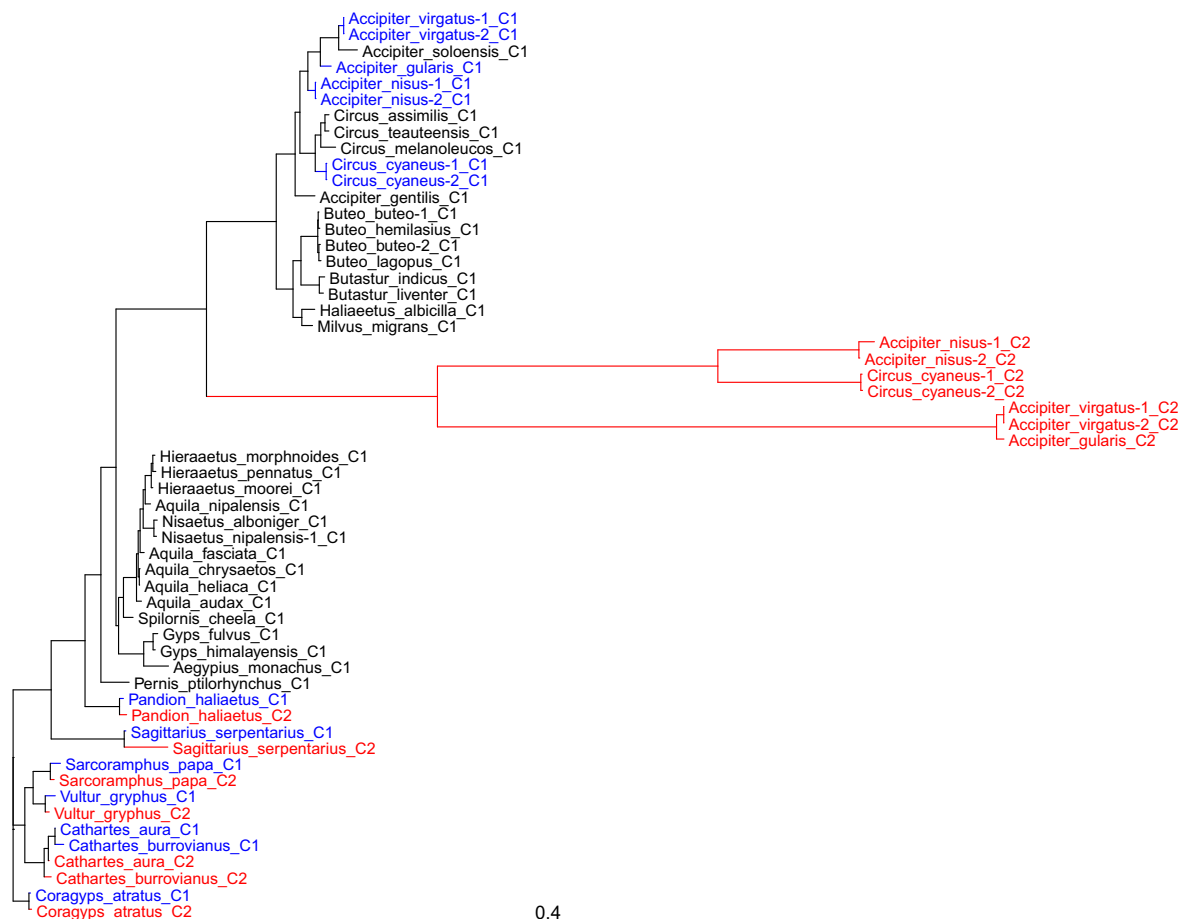
## All sites, IQ-Tree



## Repeats masked, MrBayes



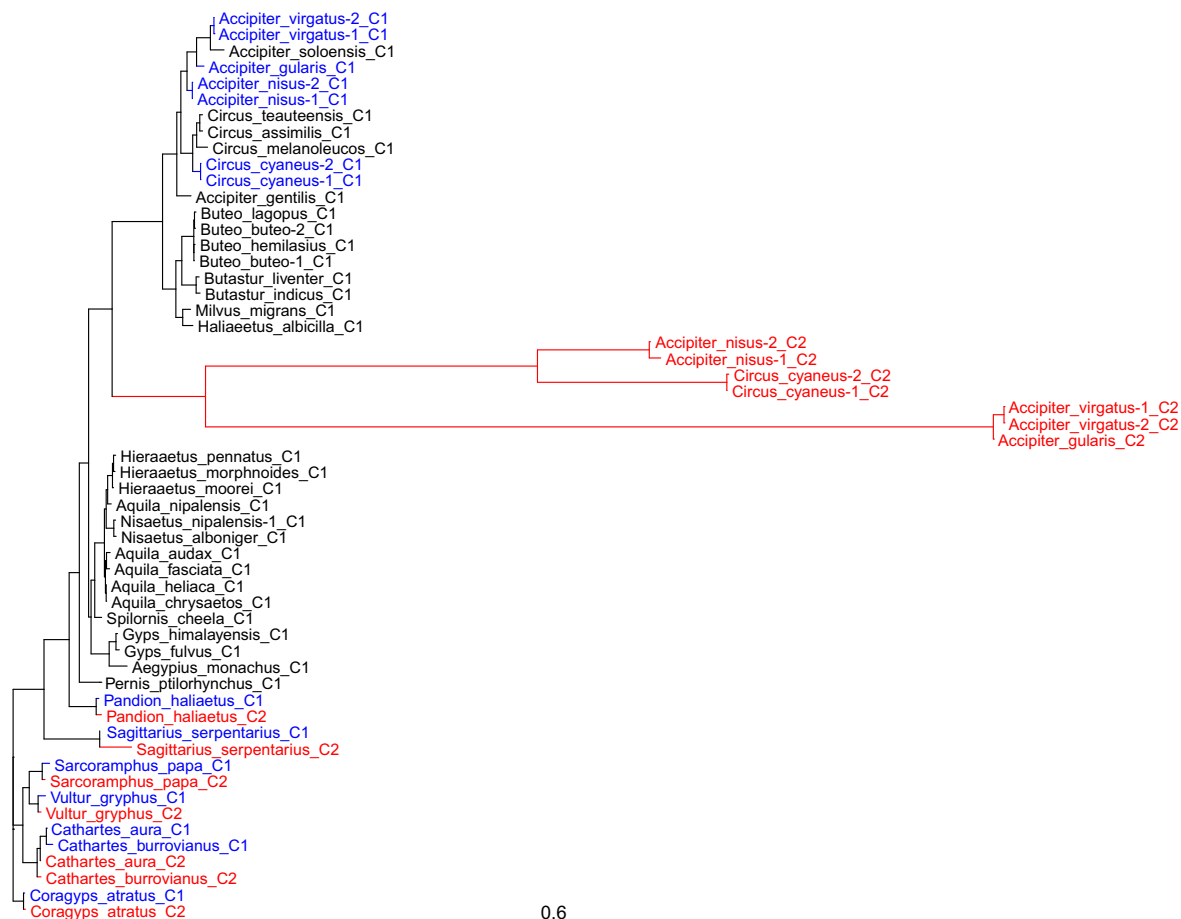
## Repeats masked, PhyloBayes



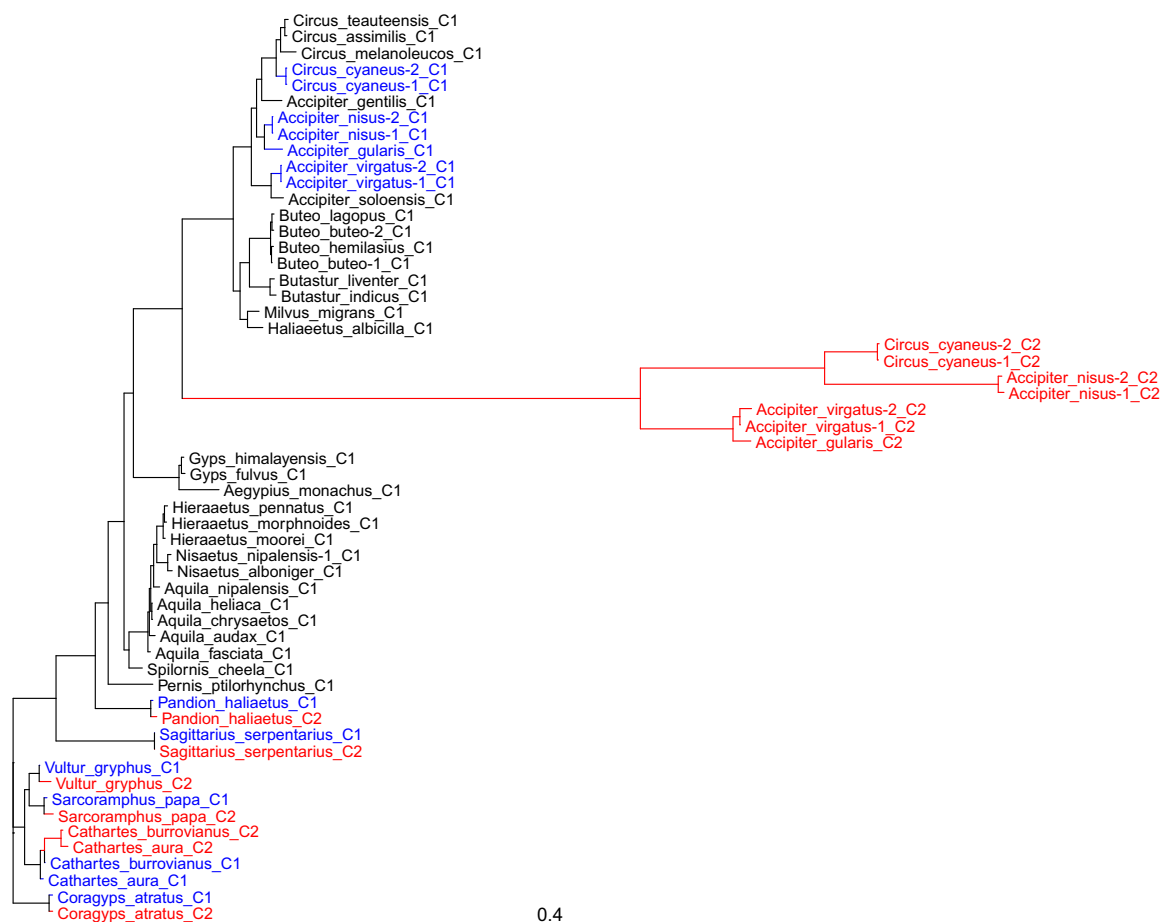
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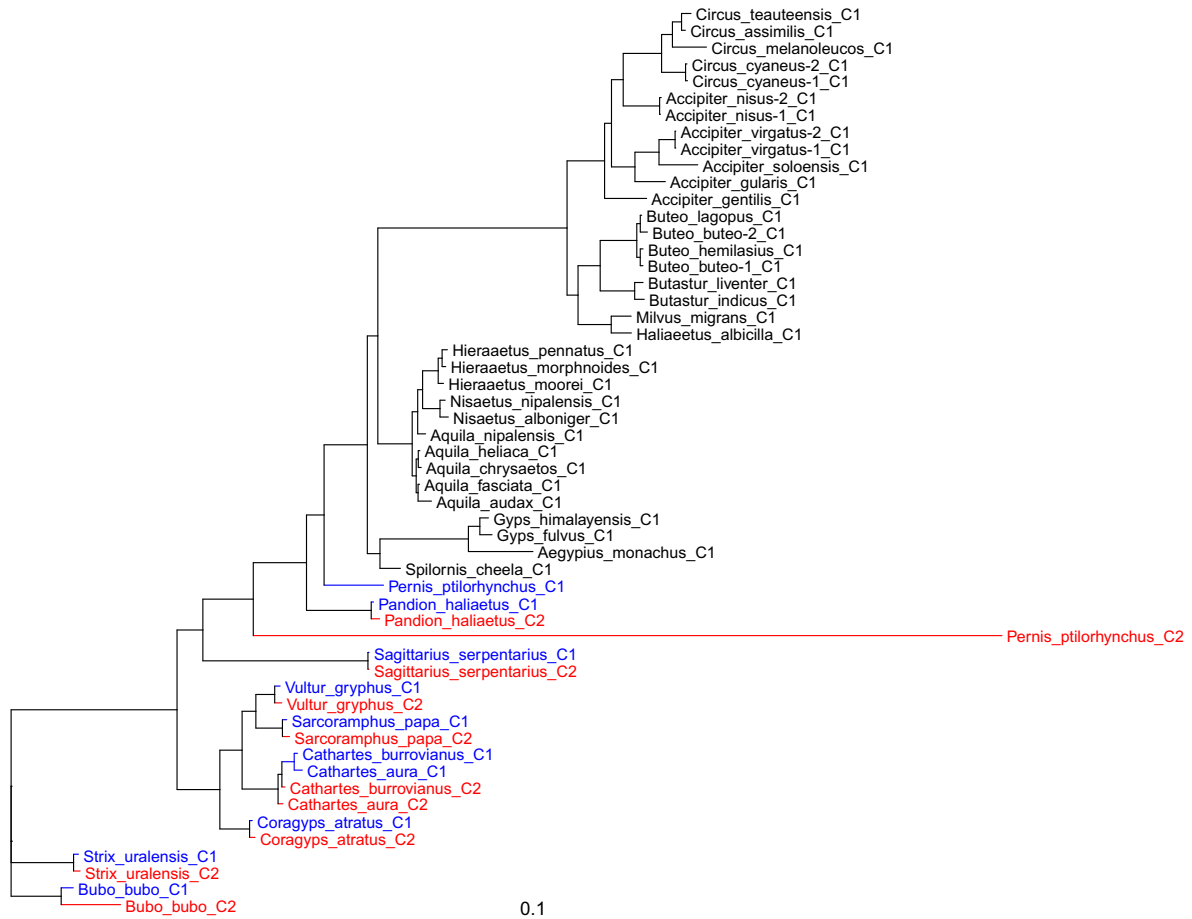
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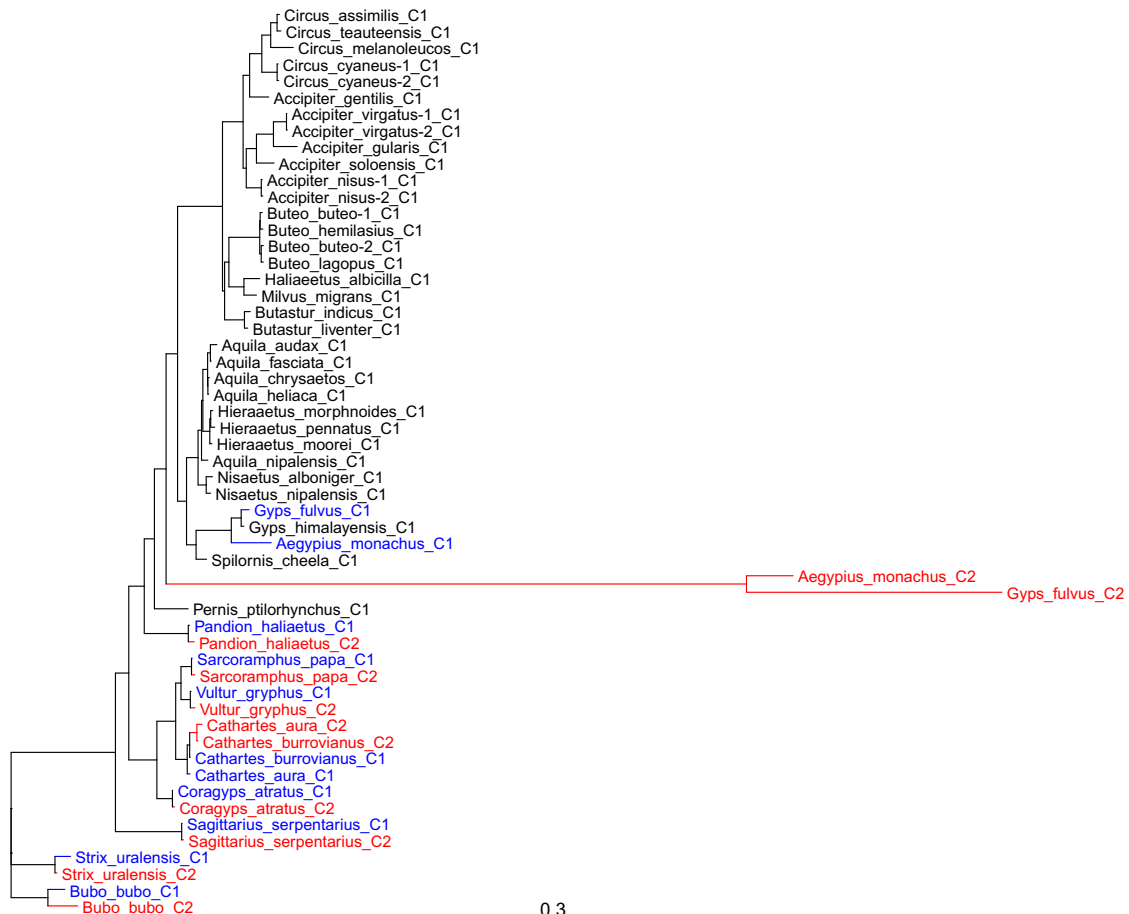
## Repeats removed, MrBayes



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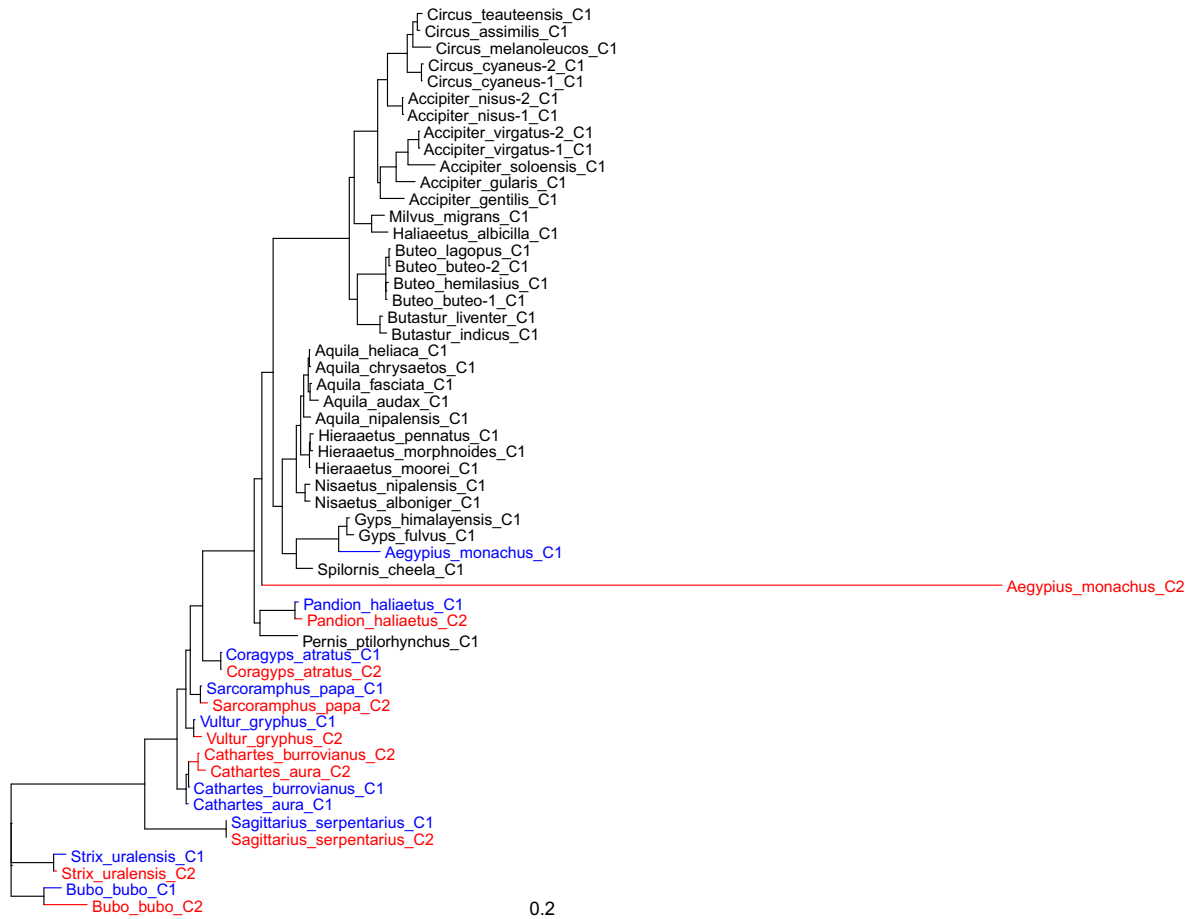


## All sites, PhyloBayes

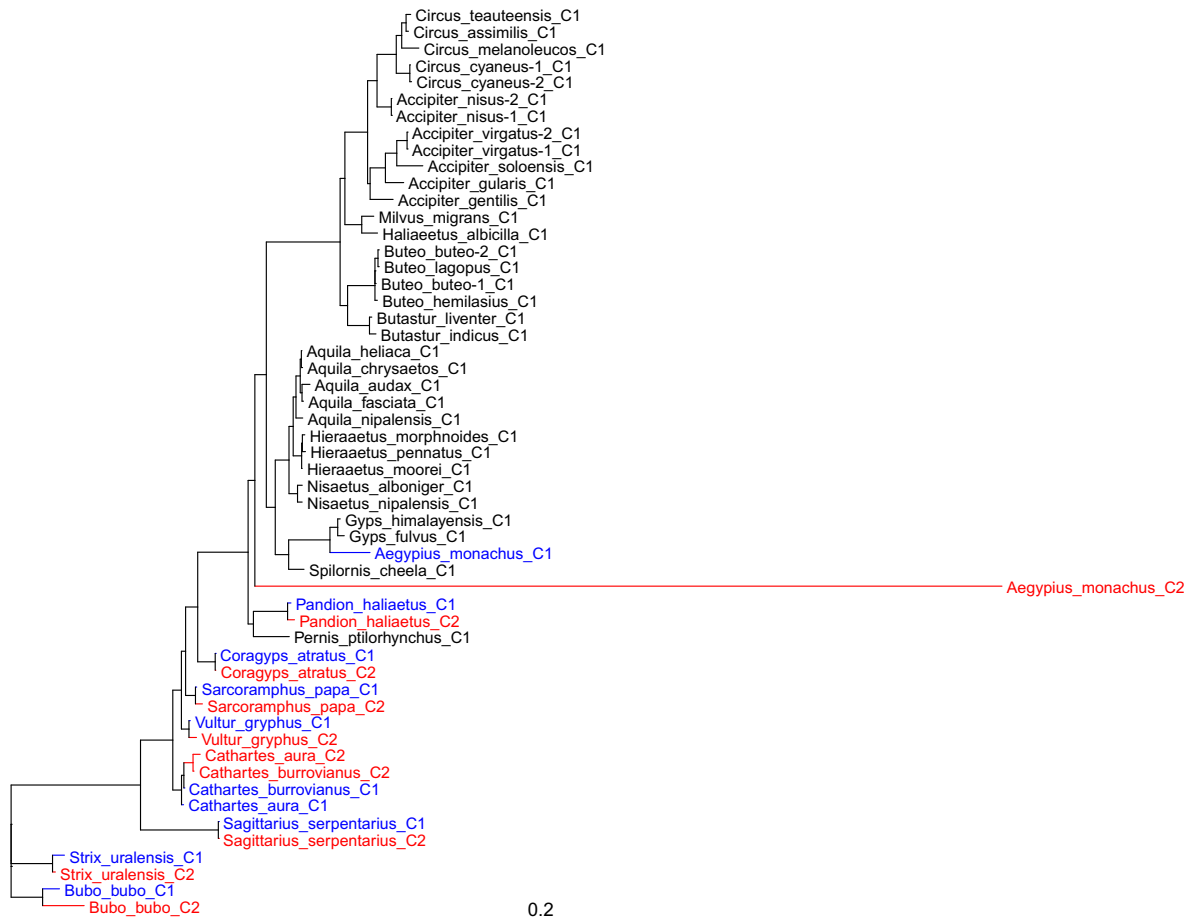




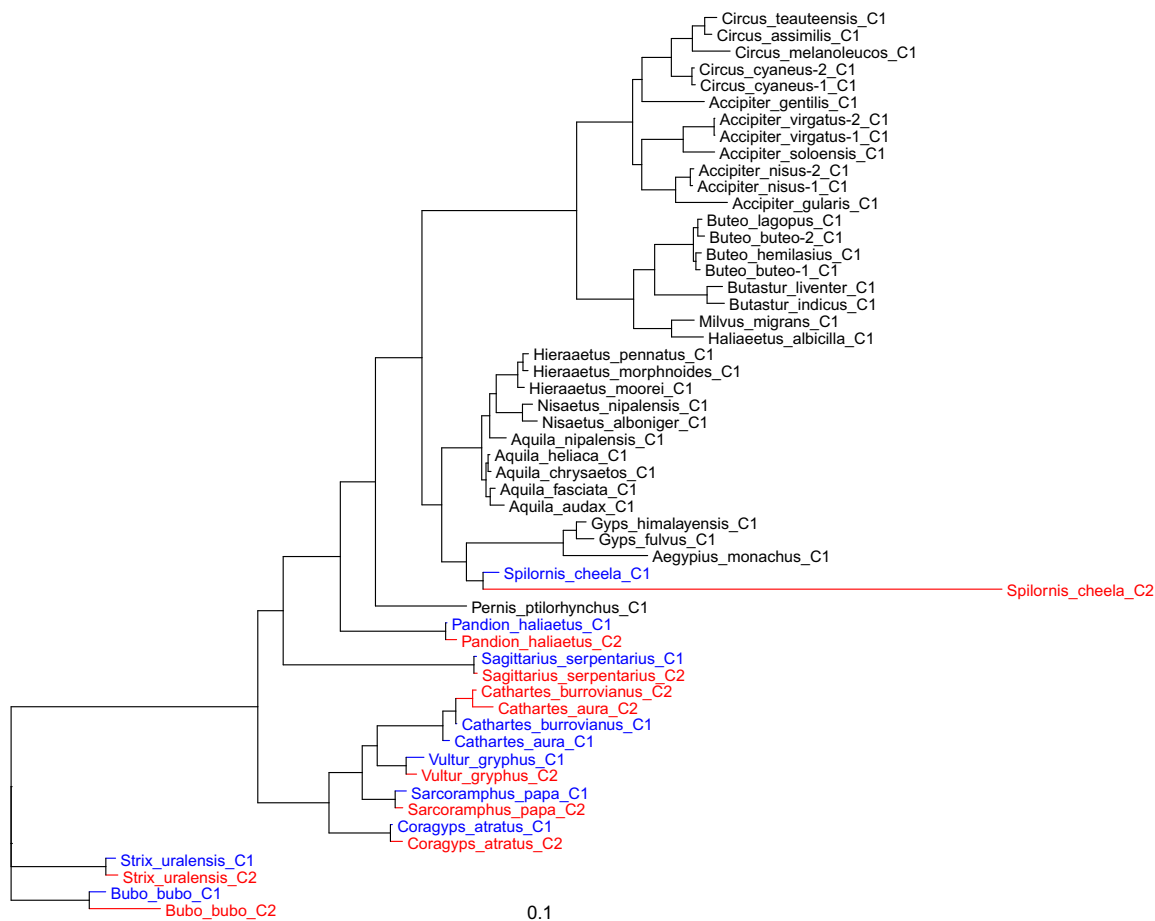
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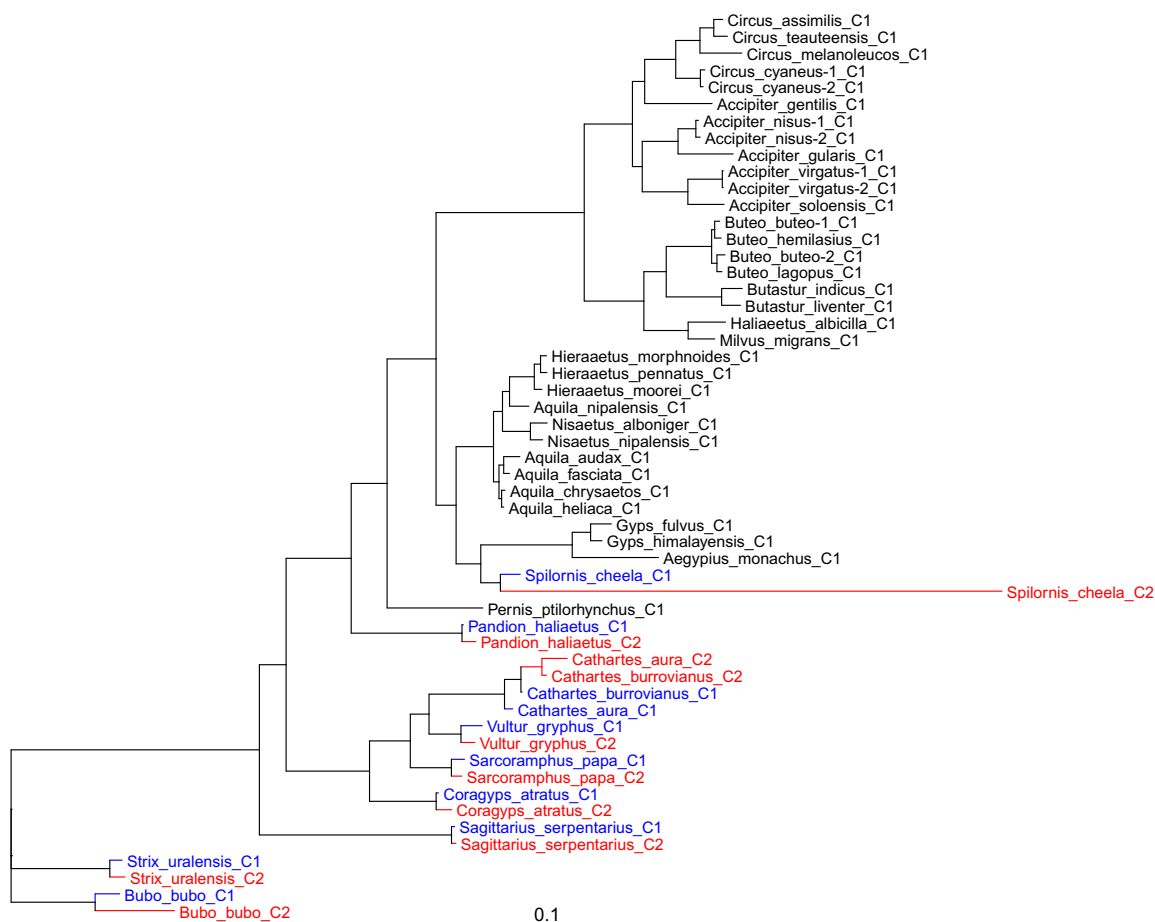
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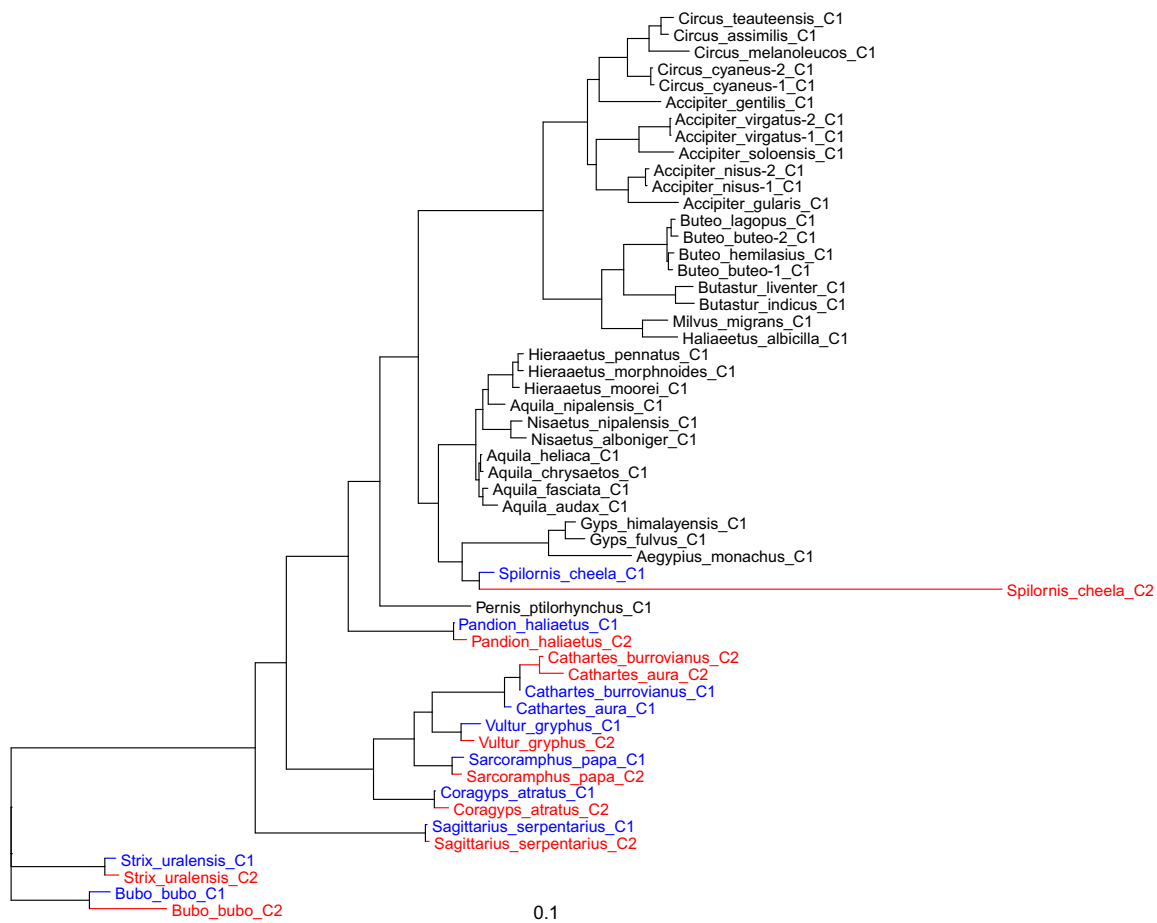
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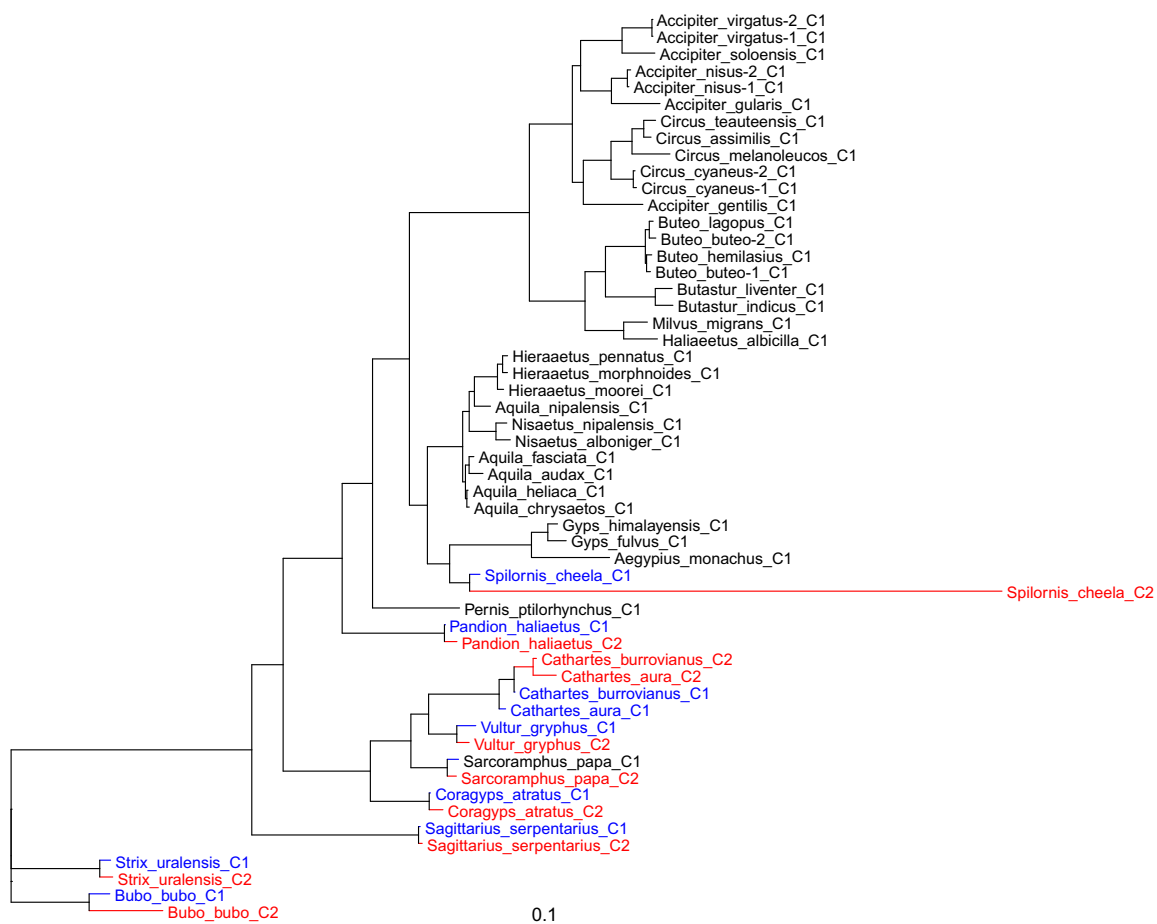
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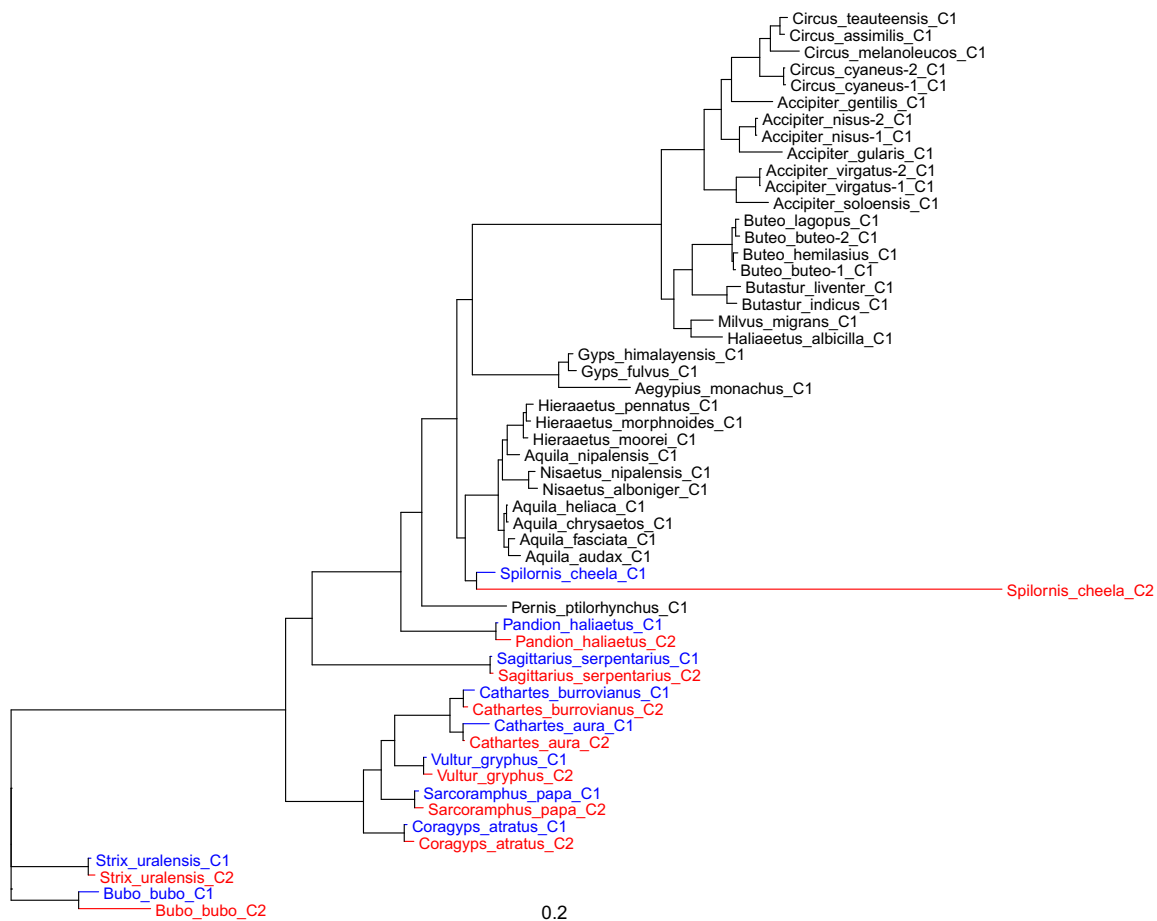
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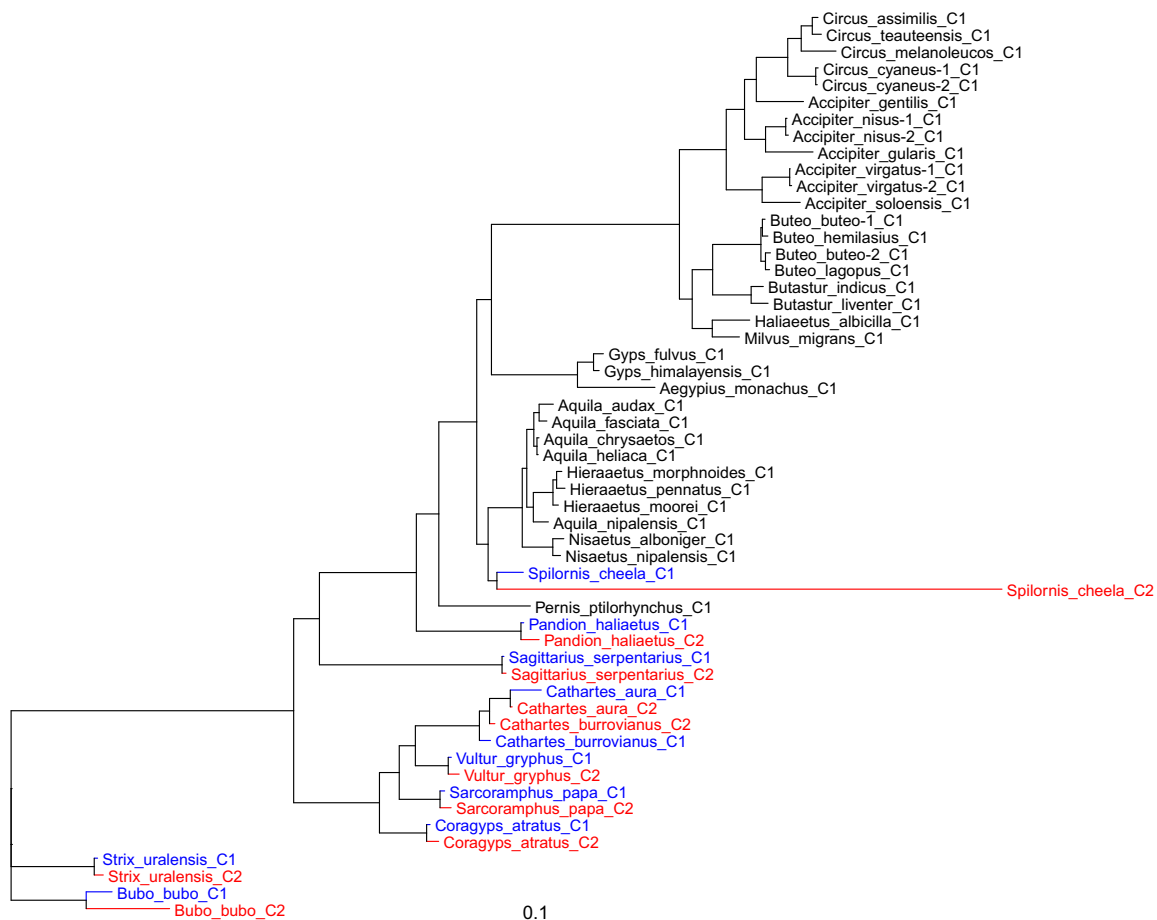
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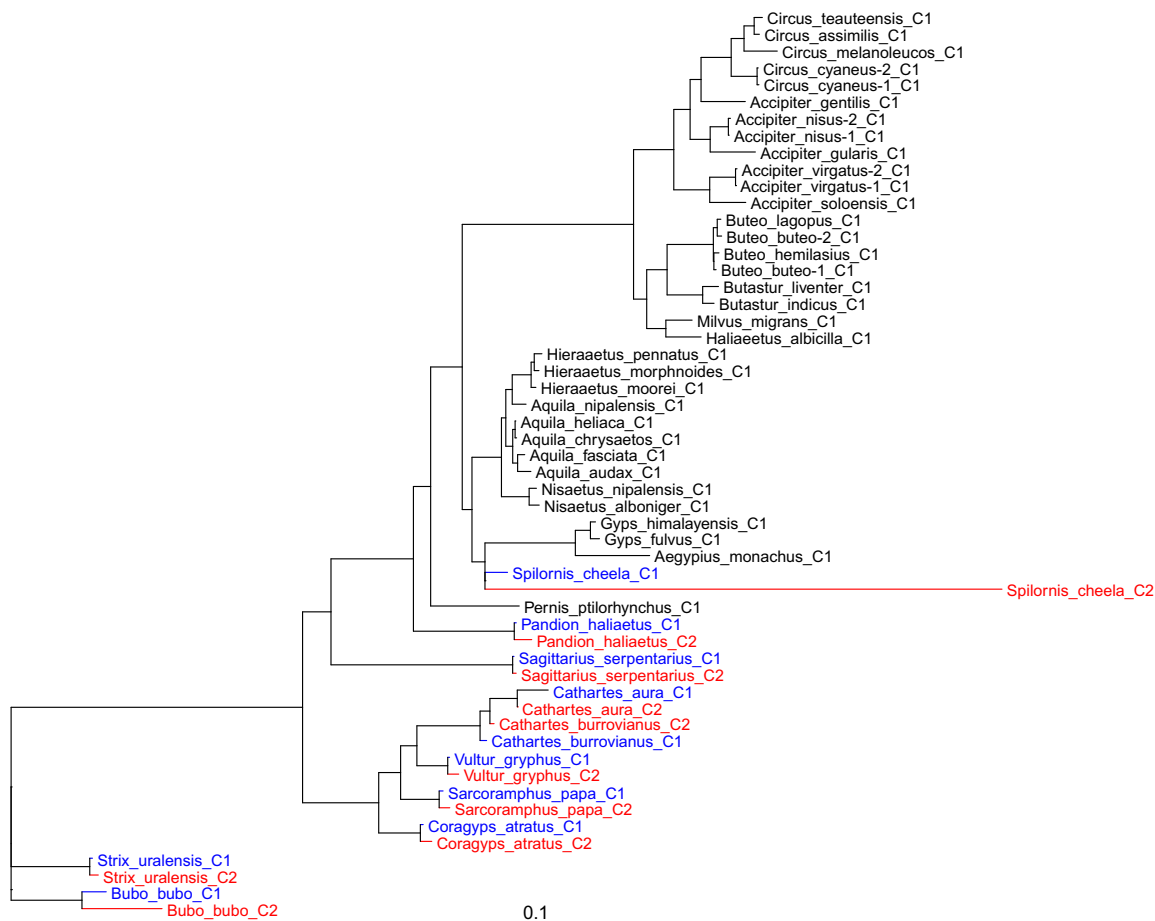
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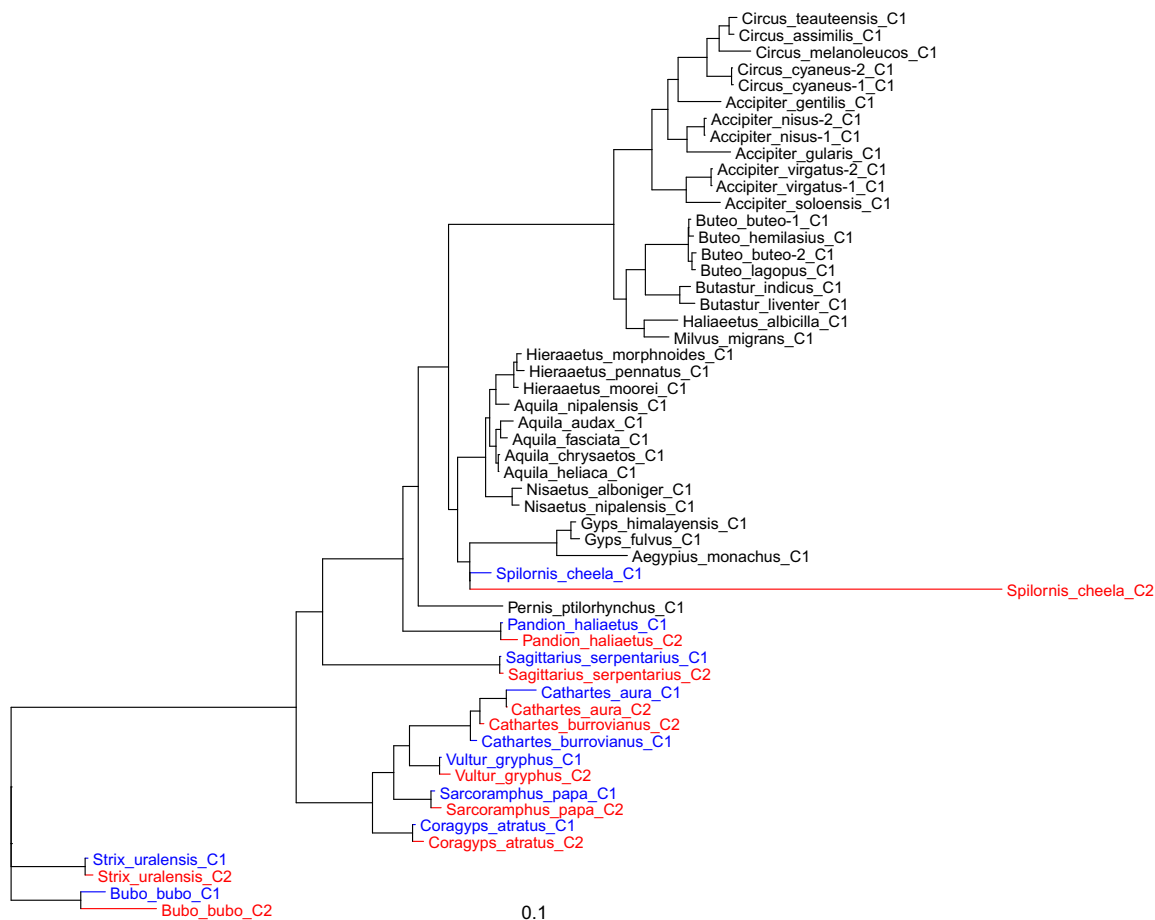
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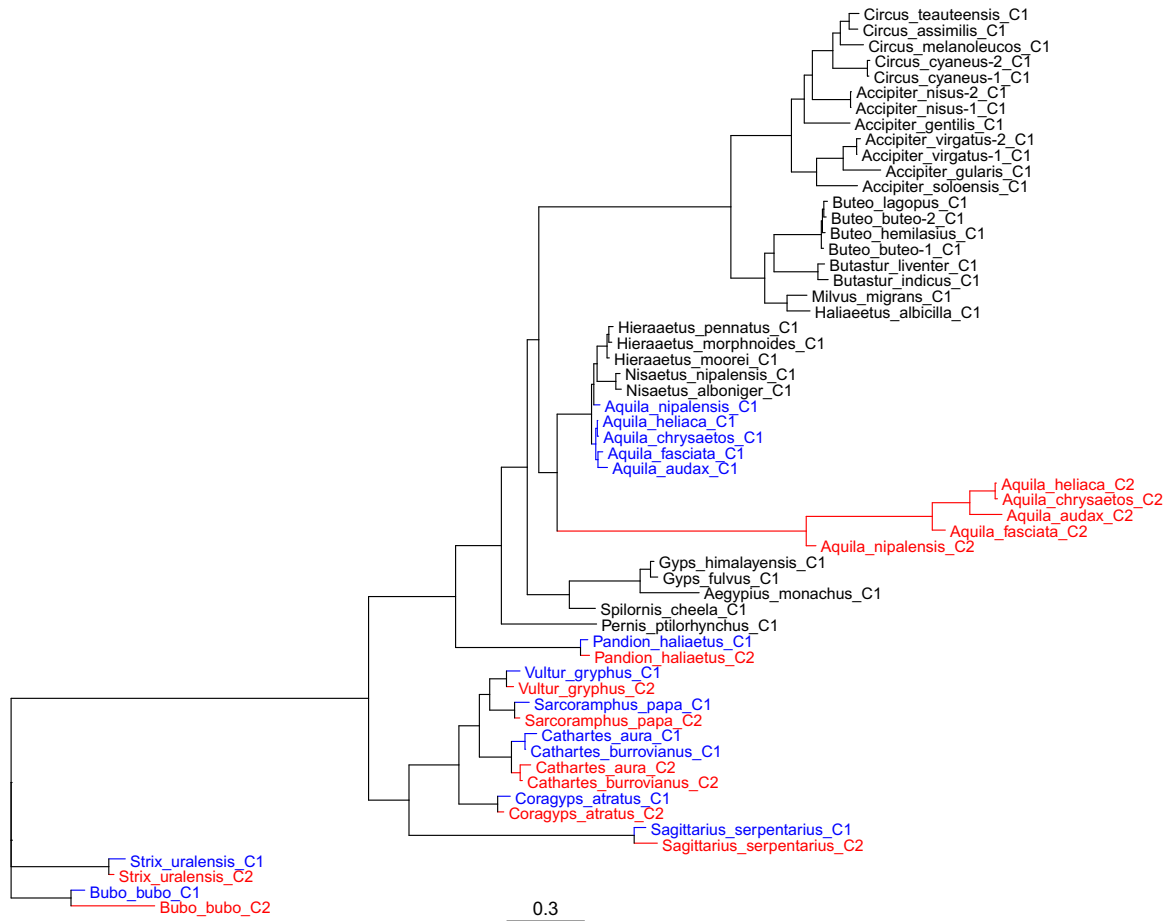
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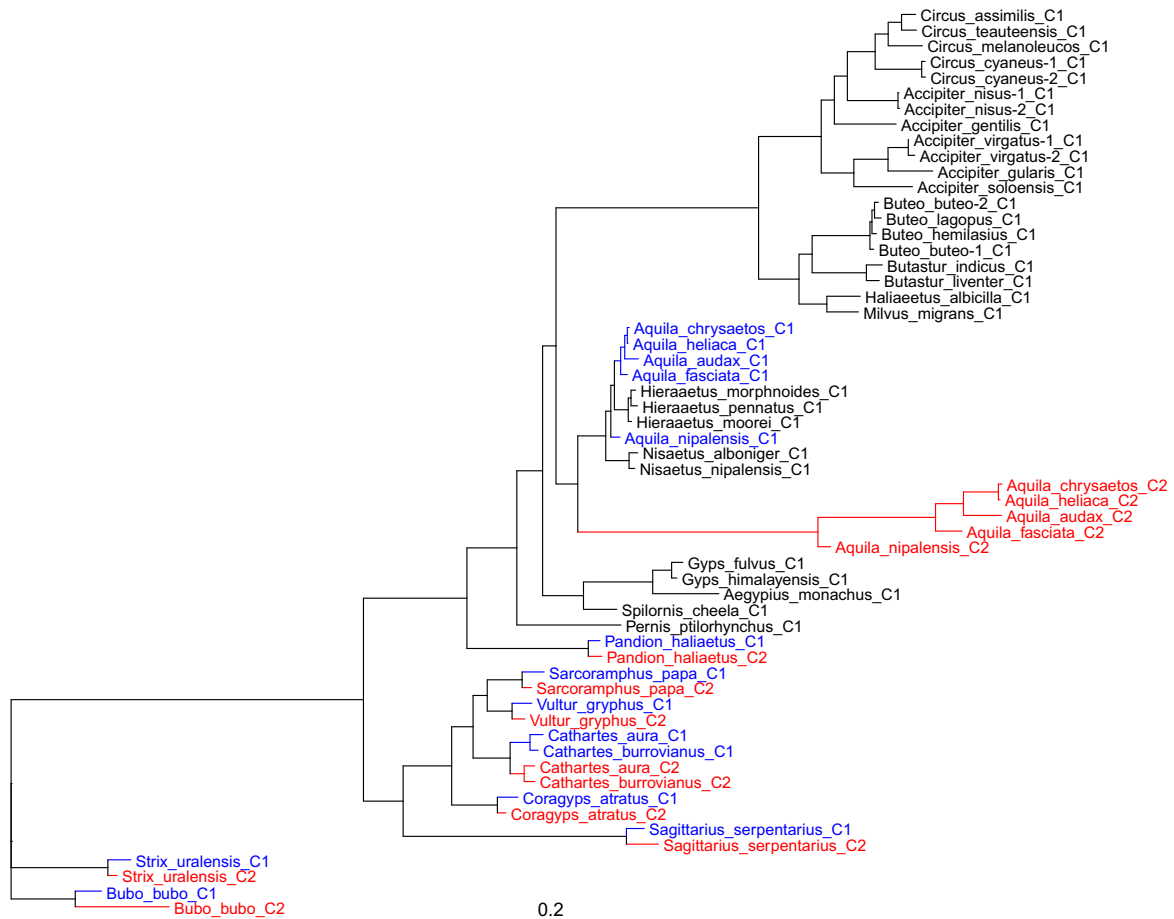
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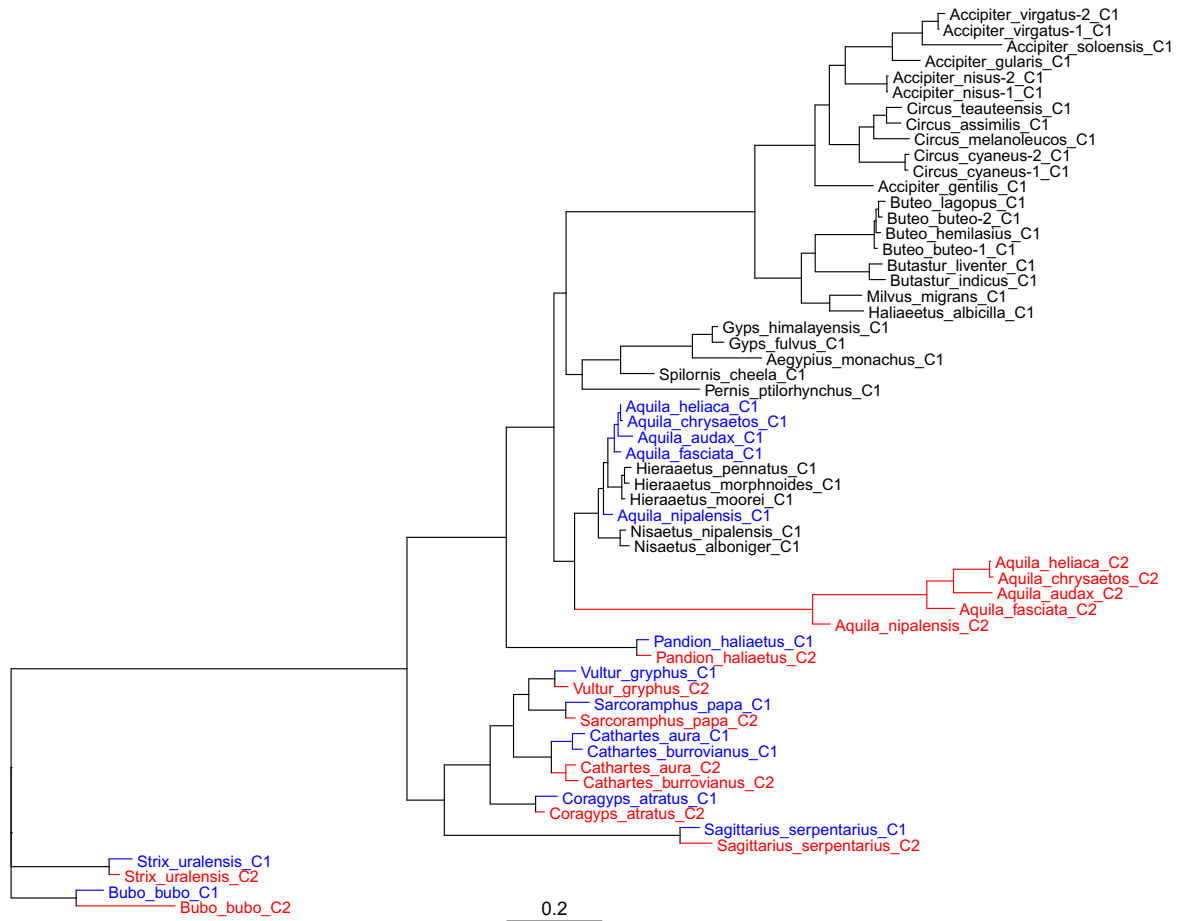
## All sites, MrBayes



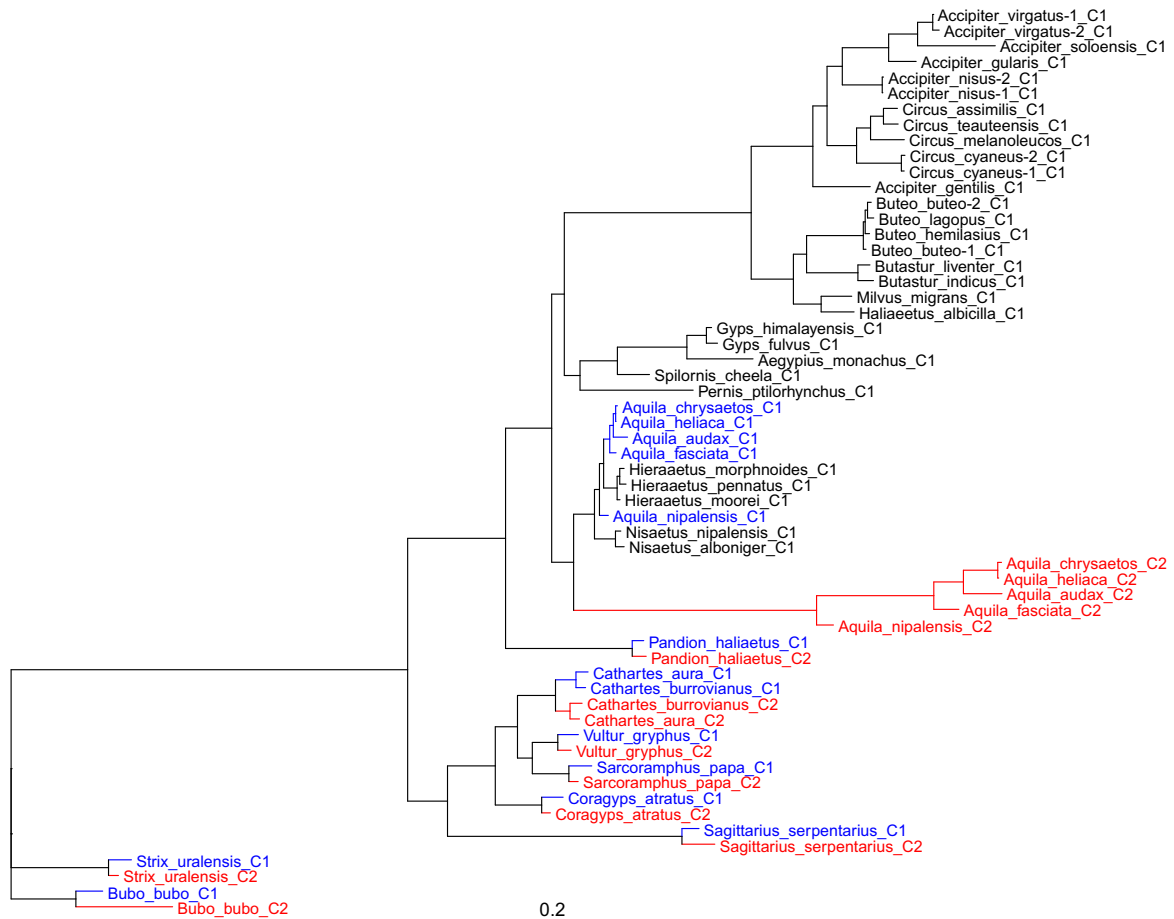
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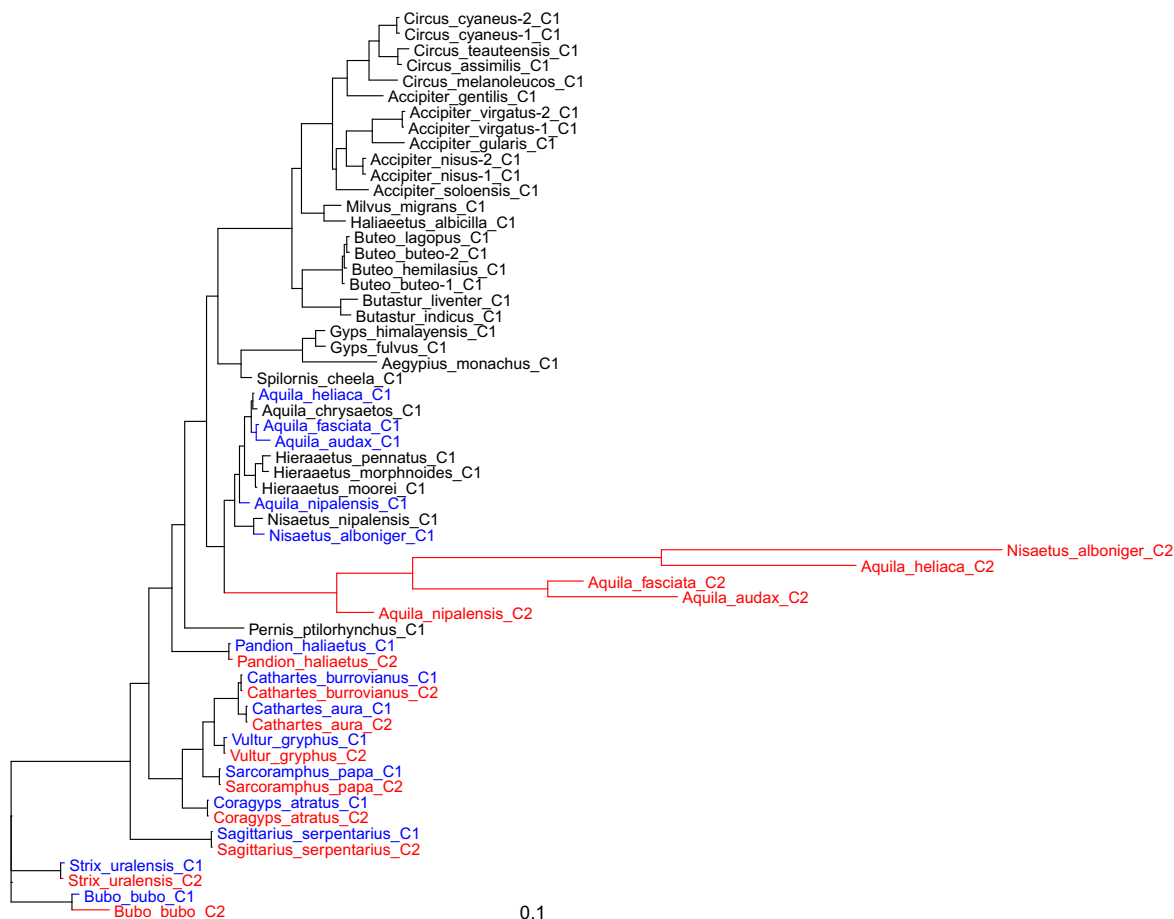
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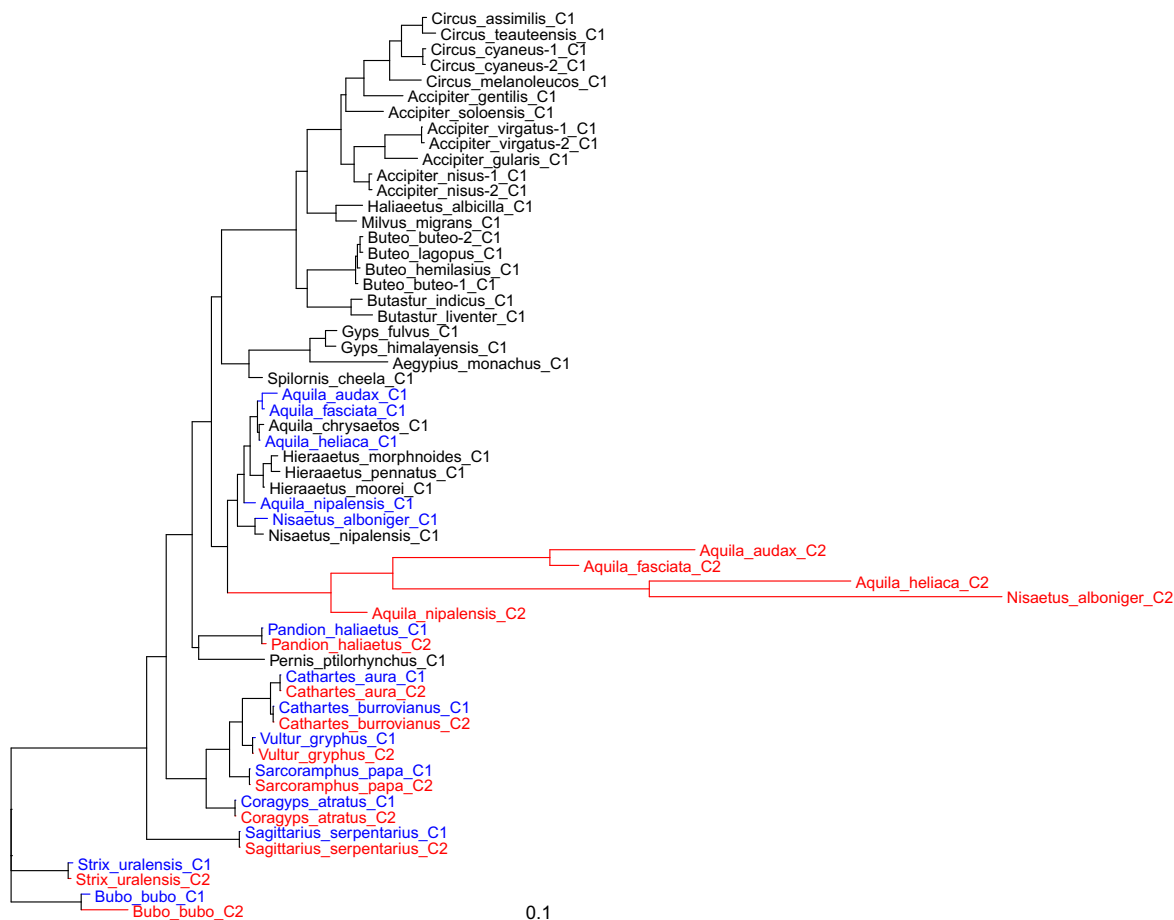
## All sites, PhyML



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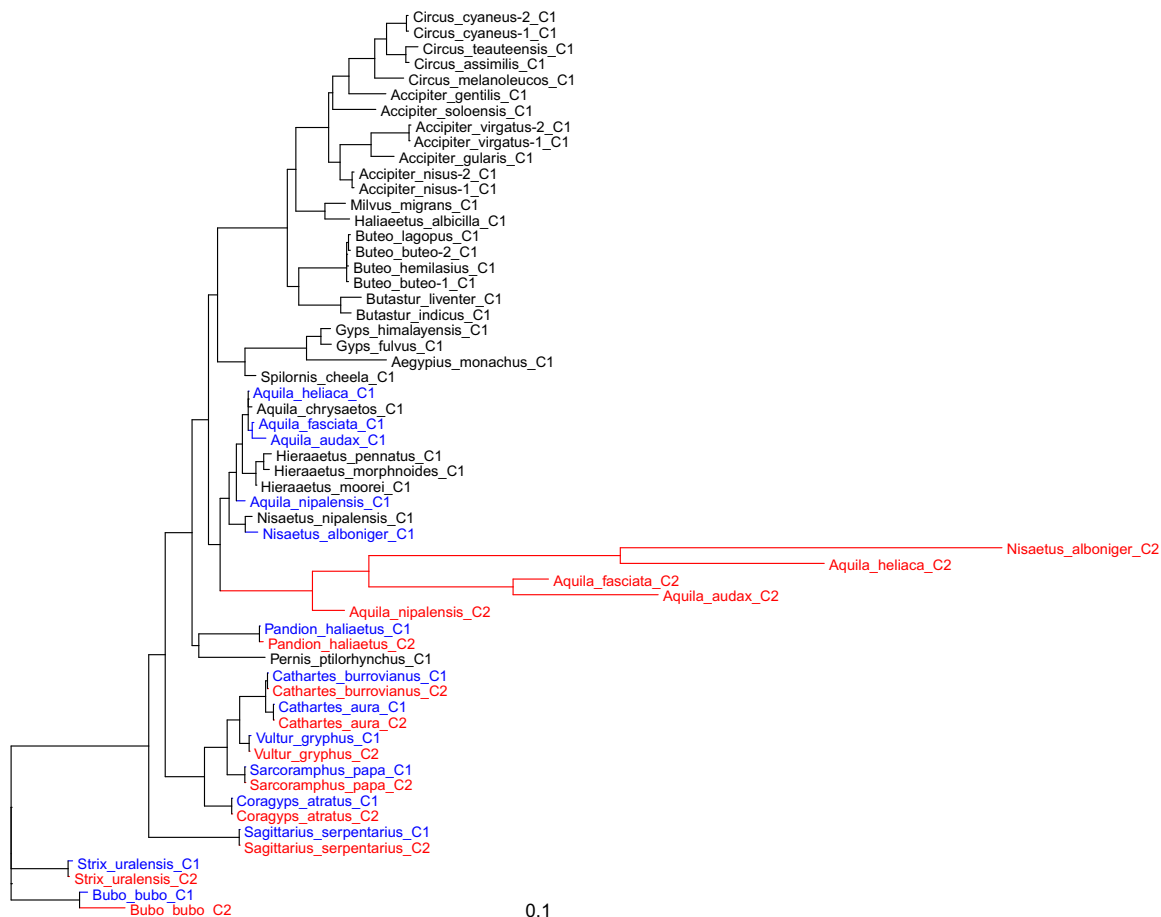


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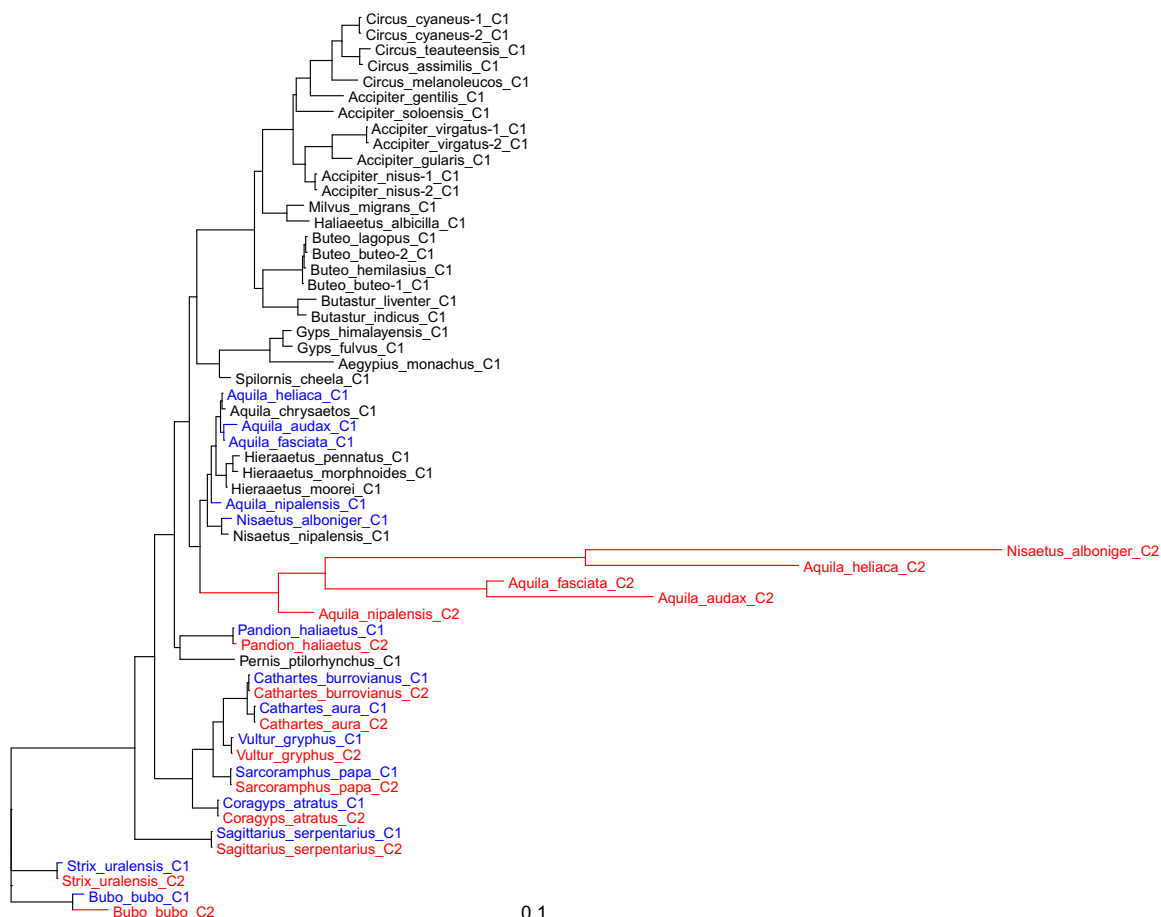




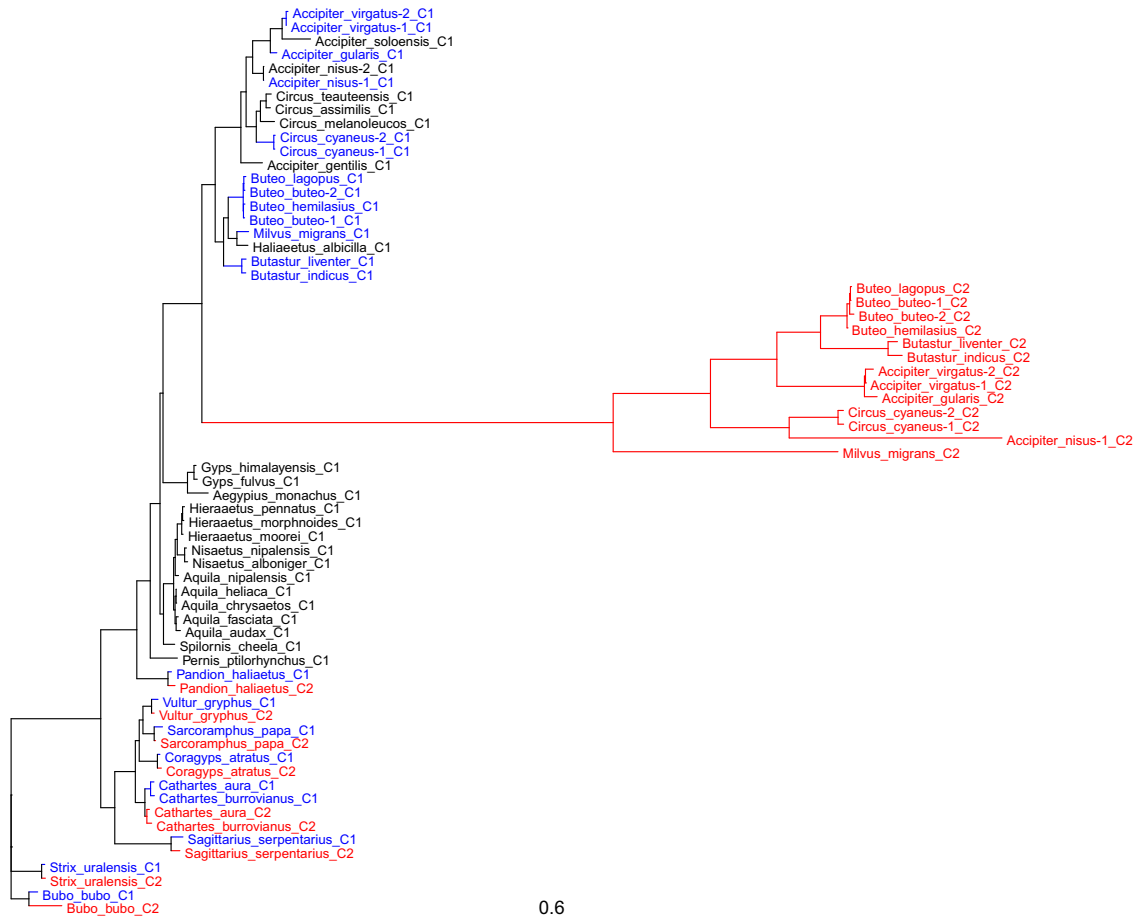
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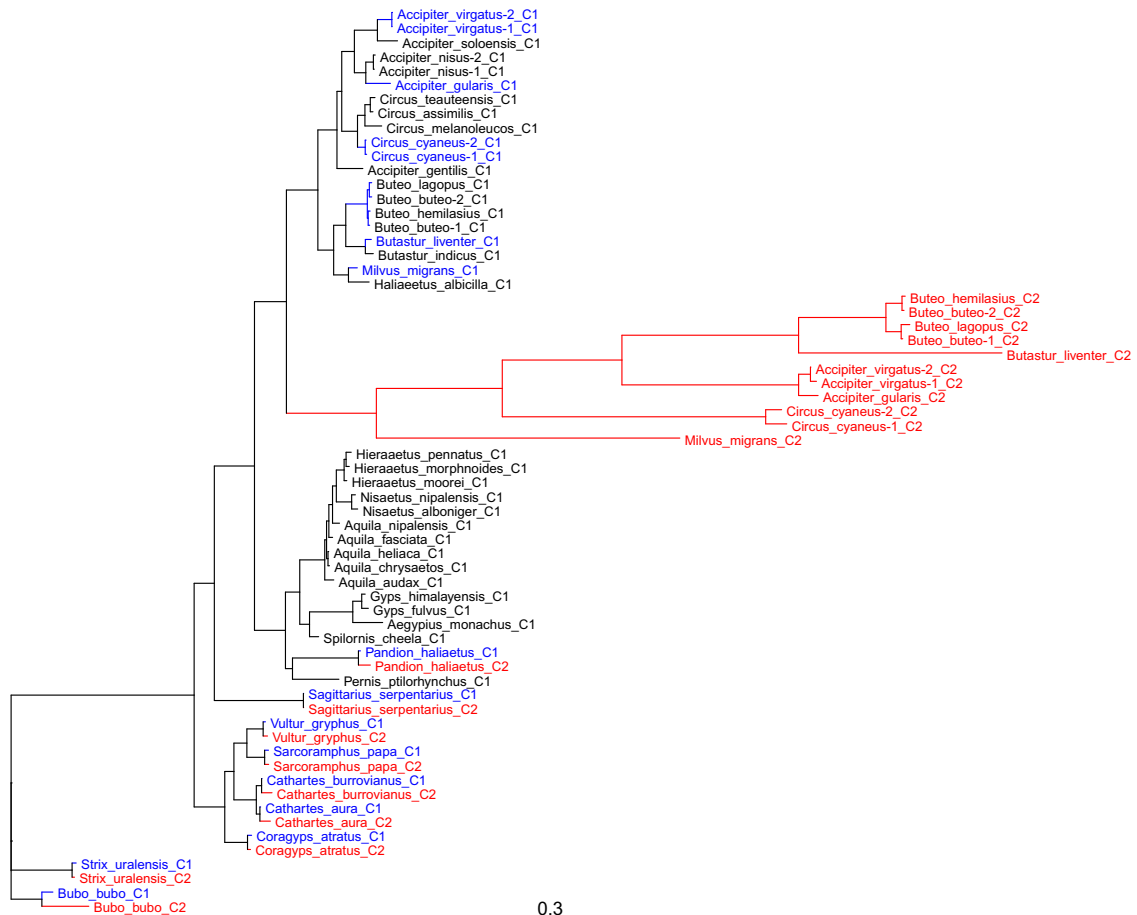
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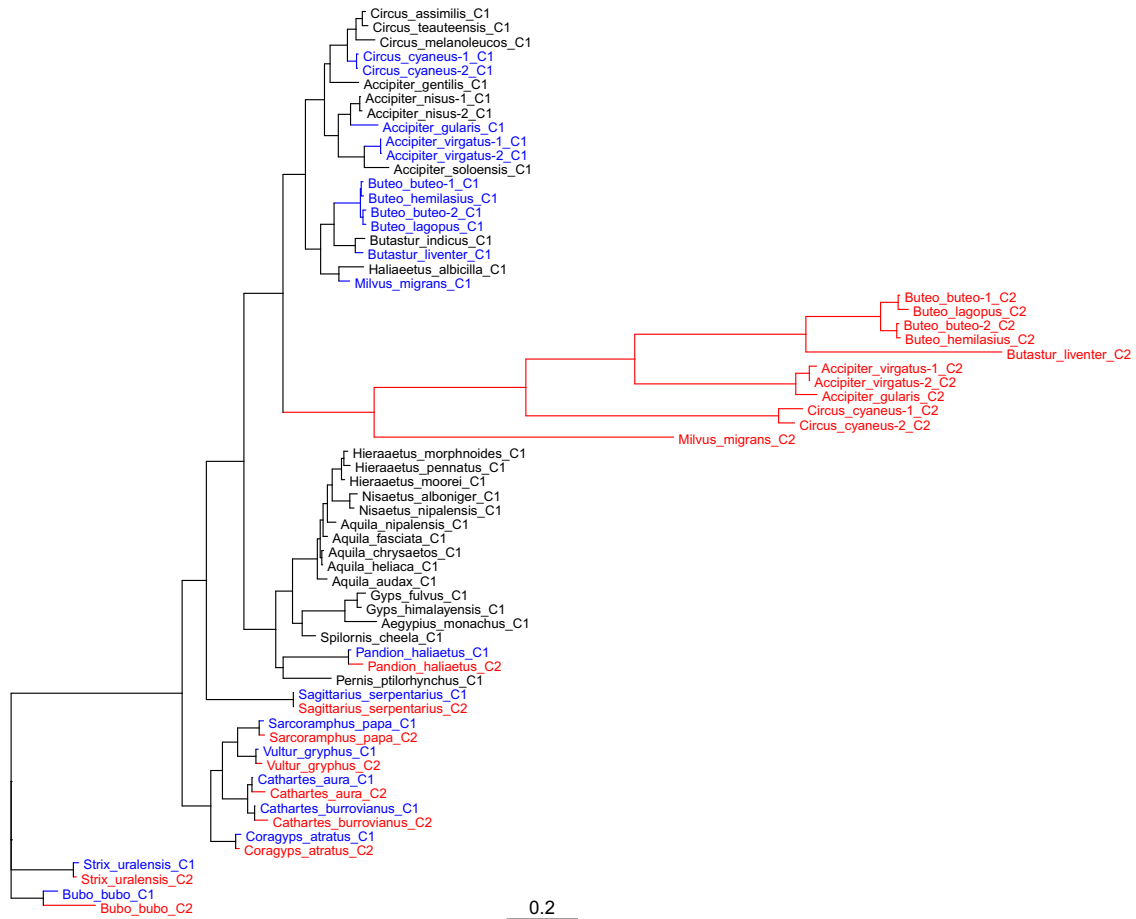
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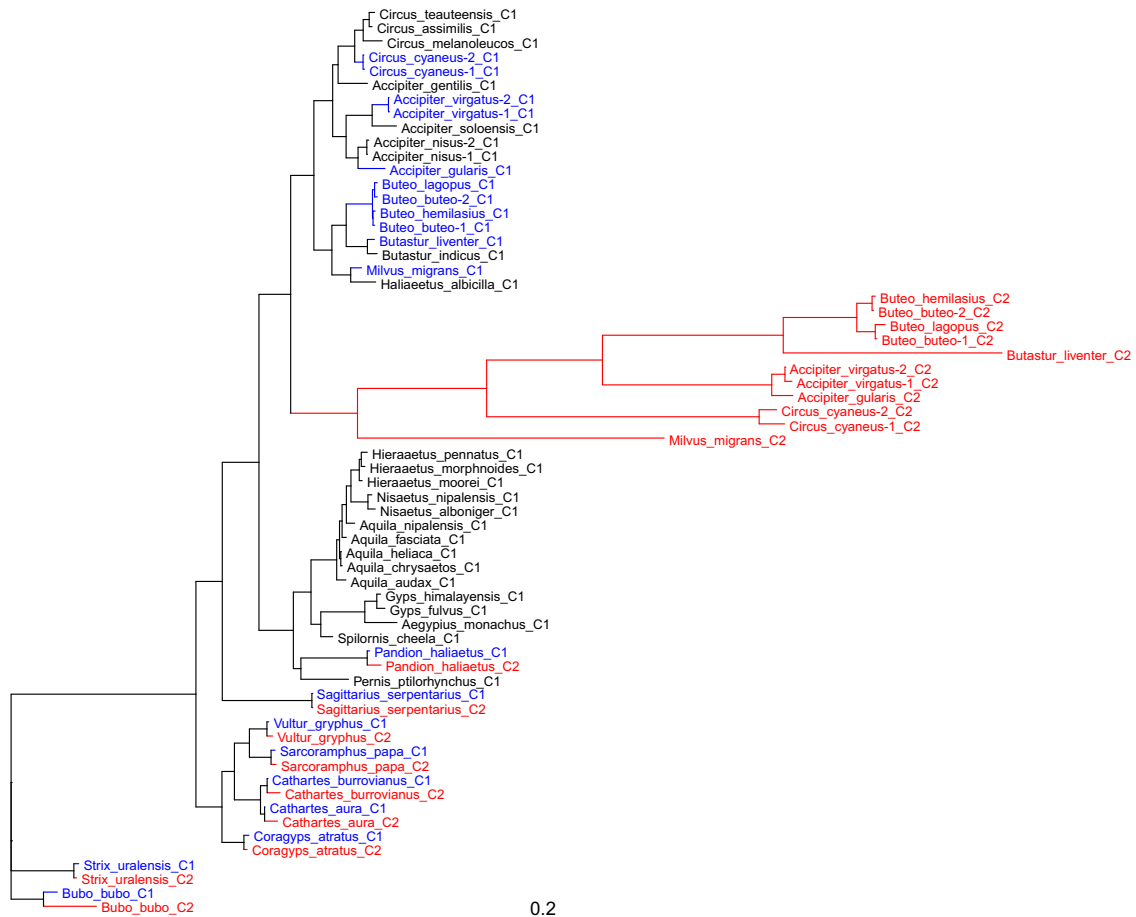
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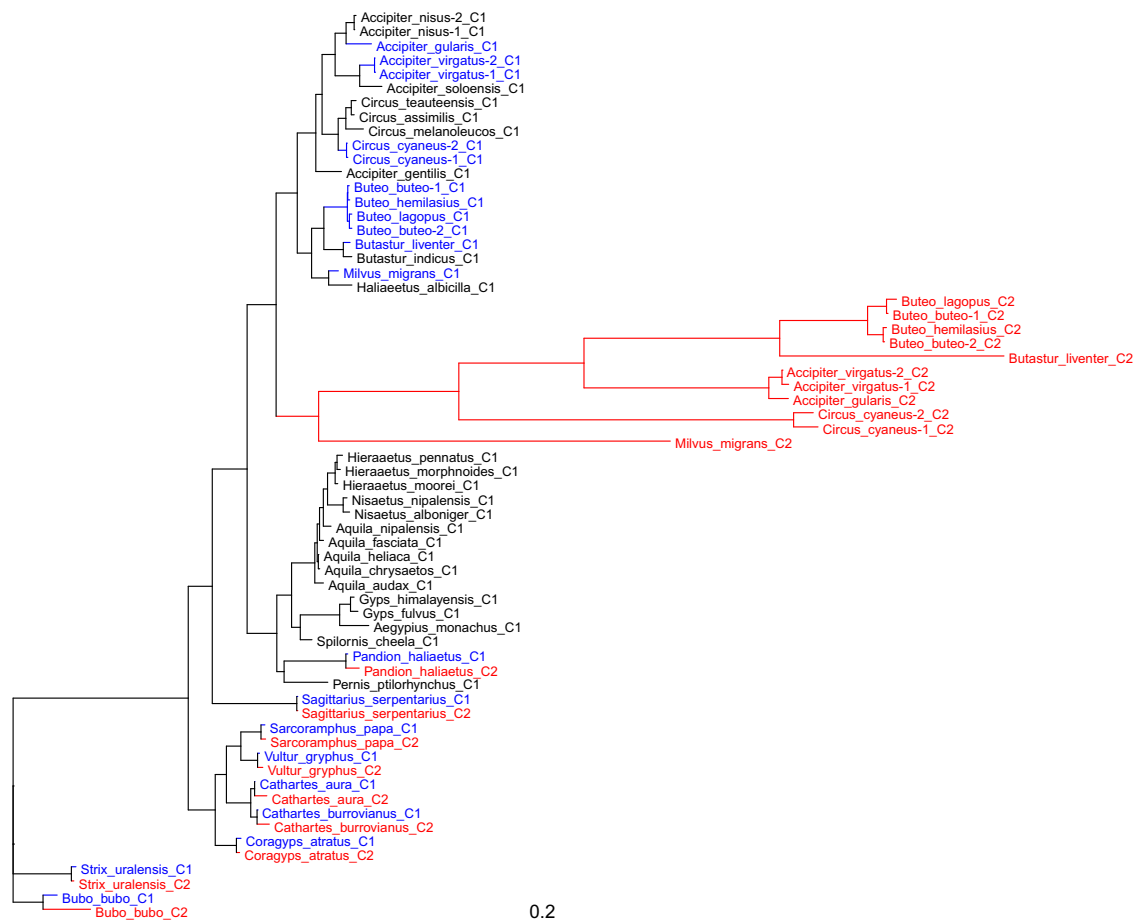
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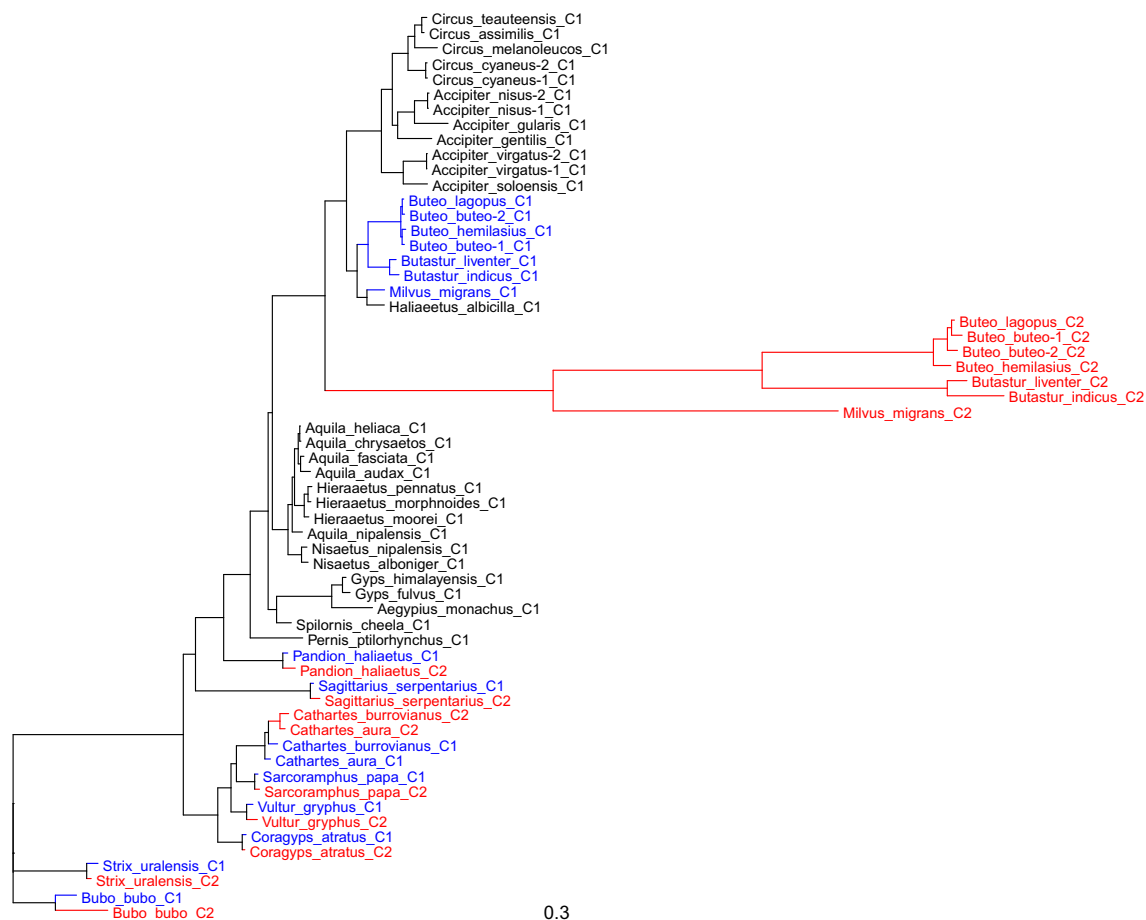
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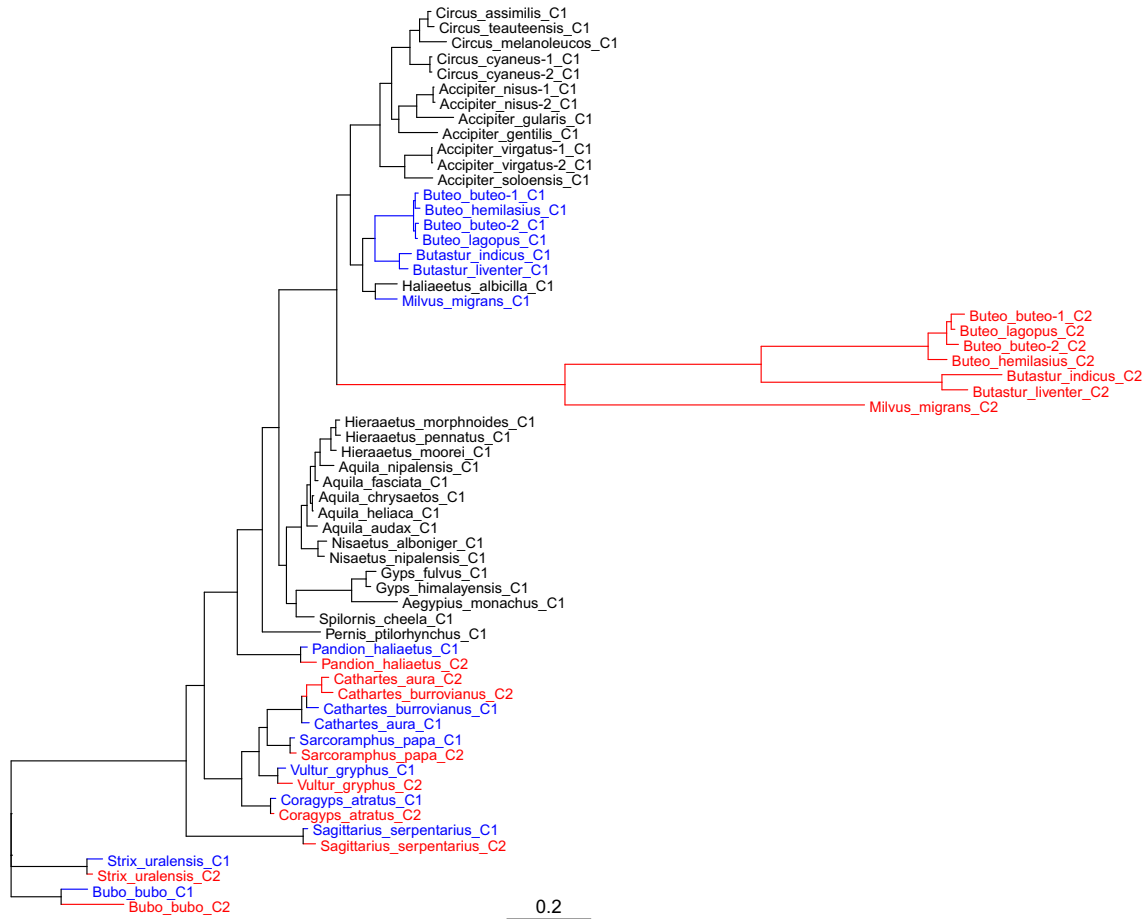
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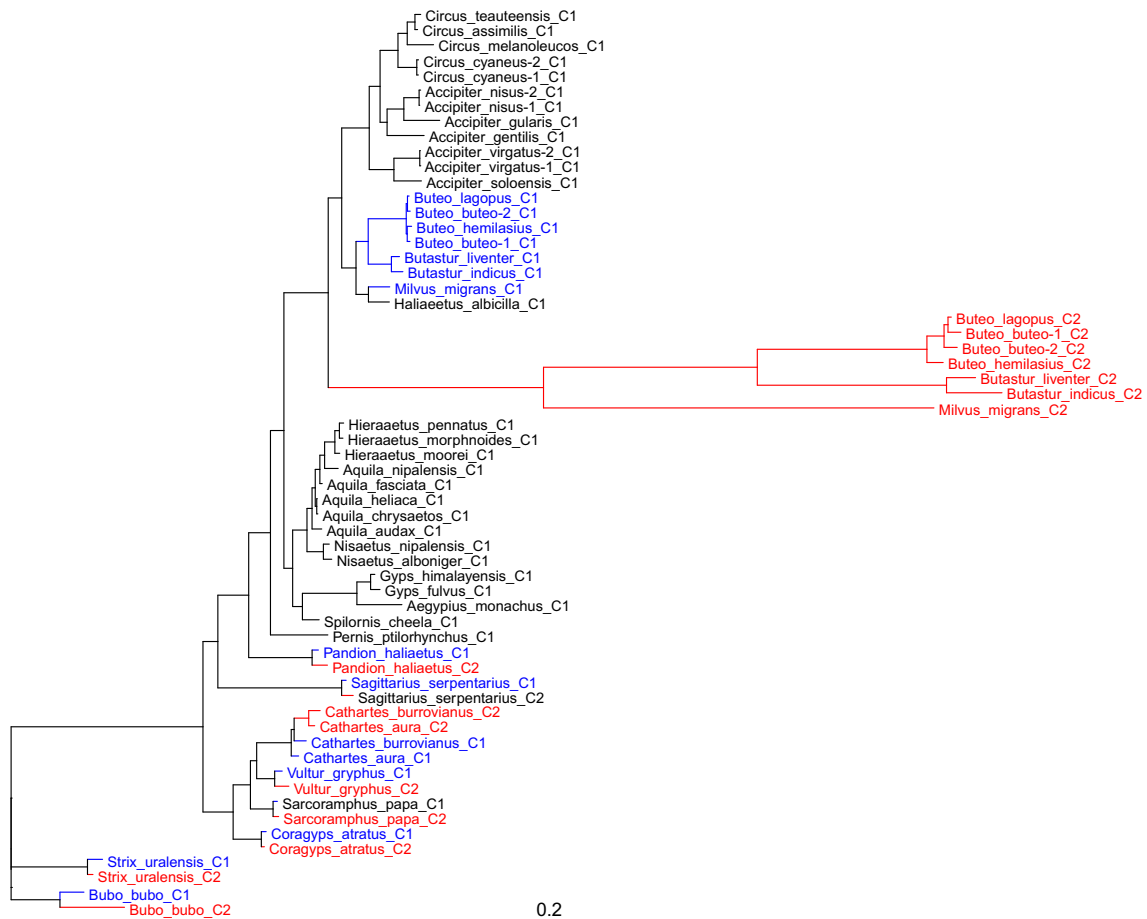
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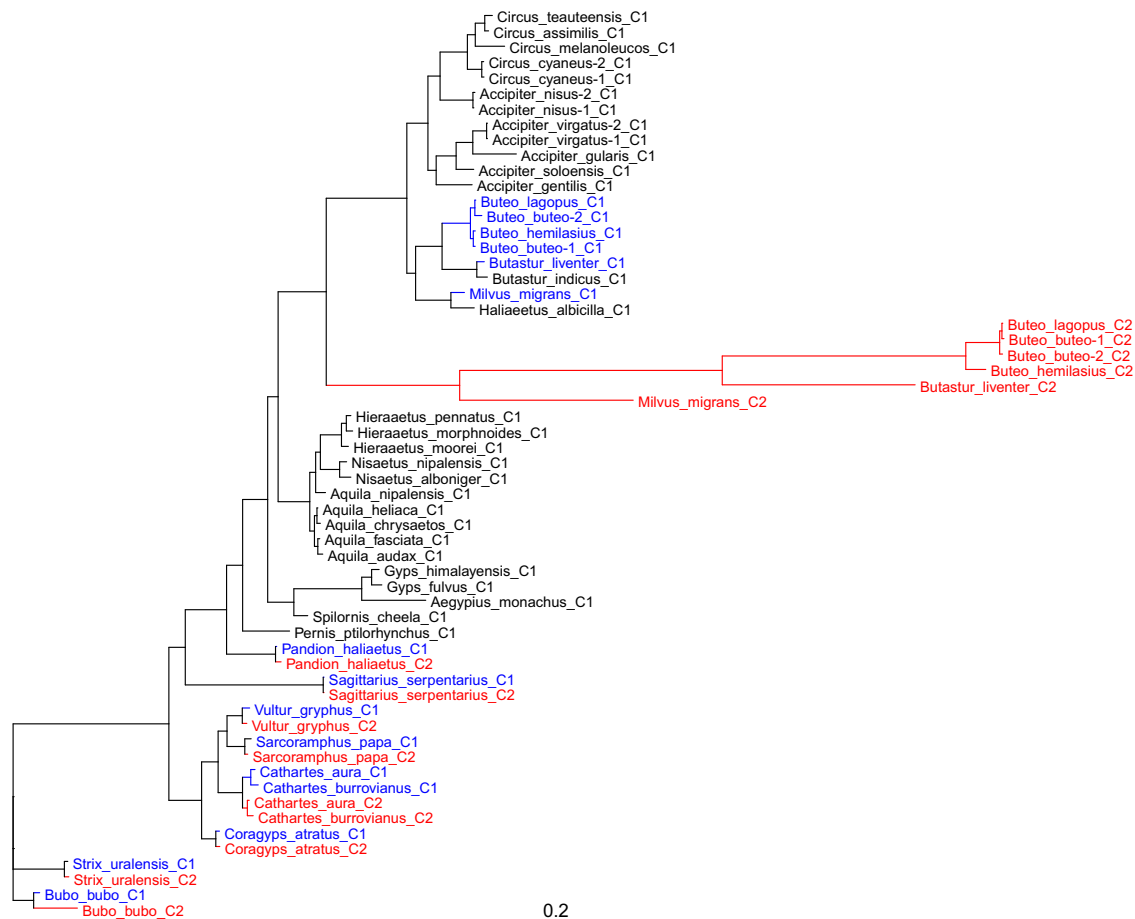
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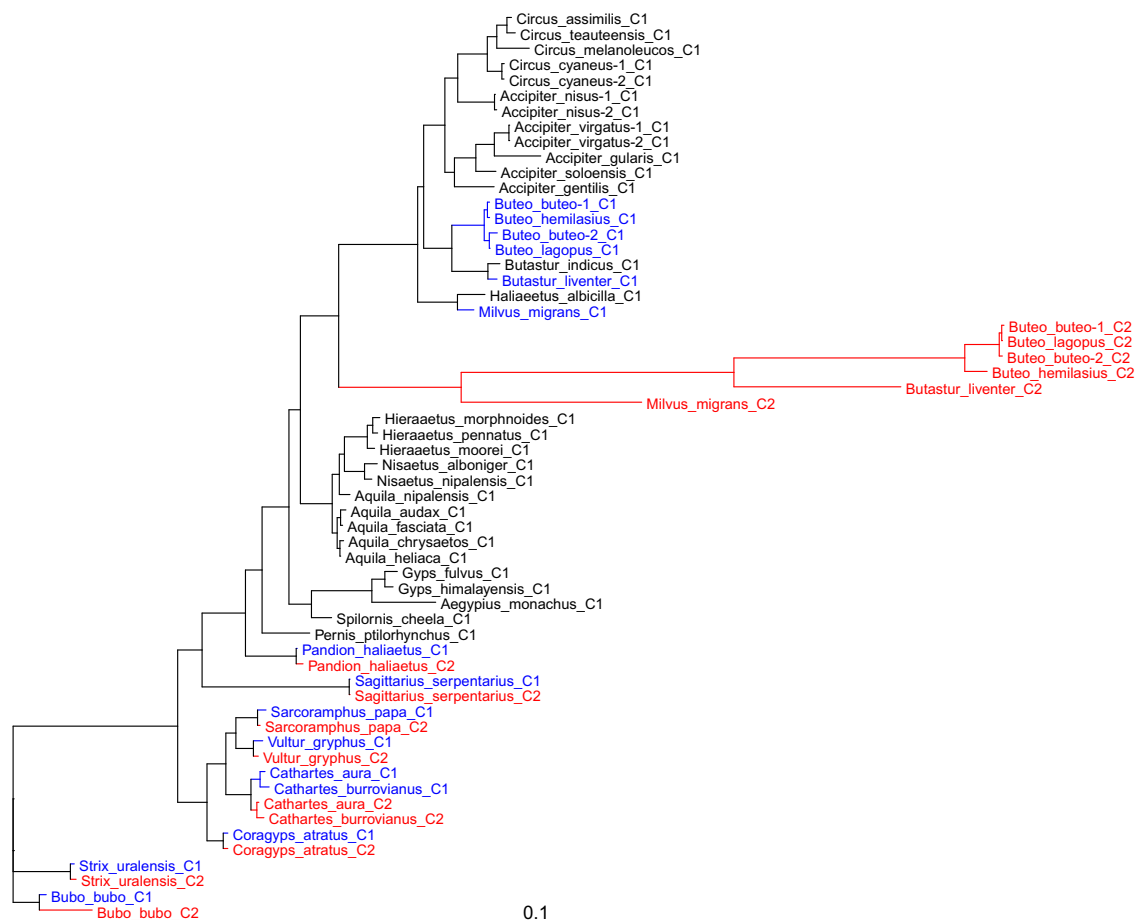
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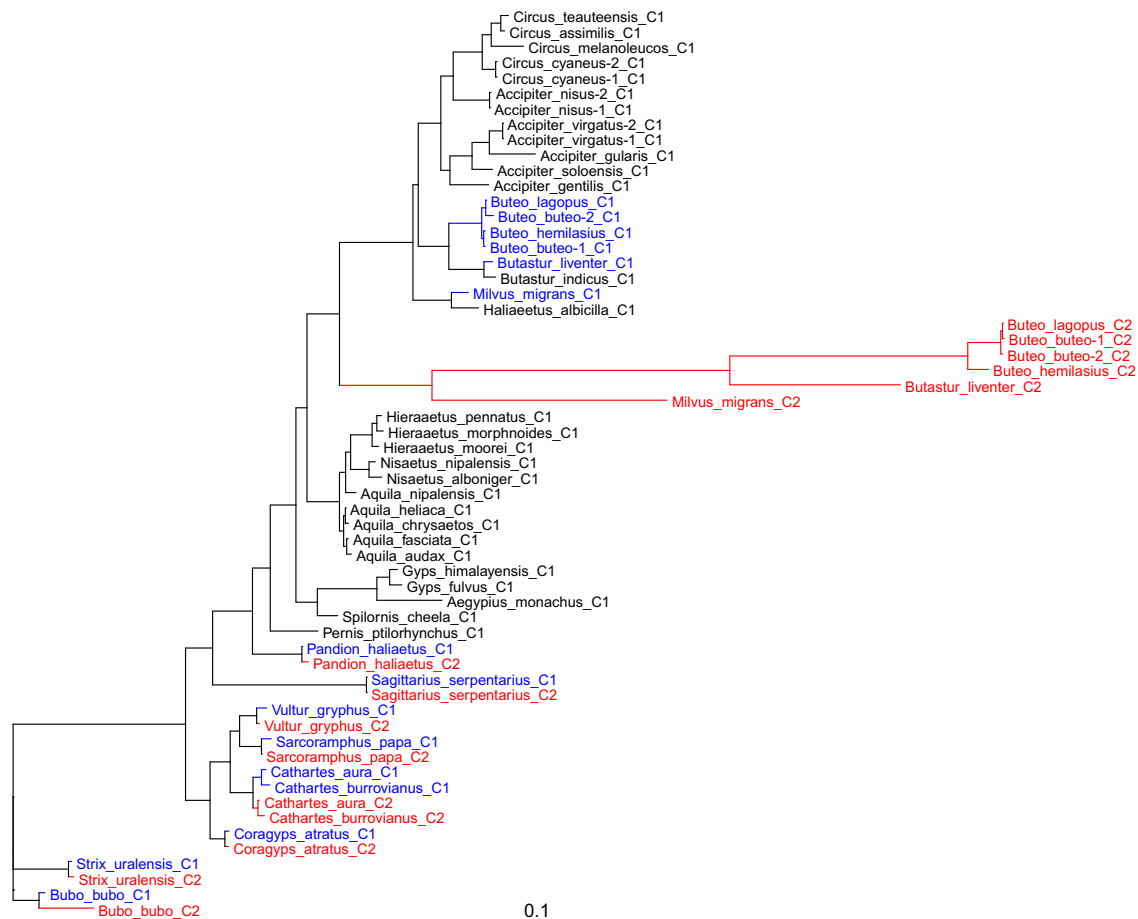
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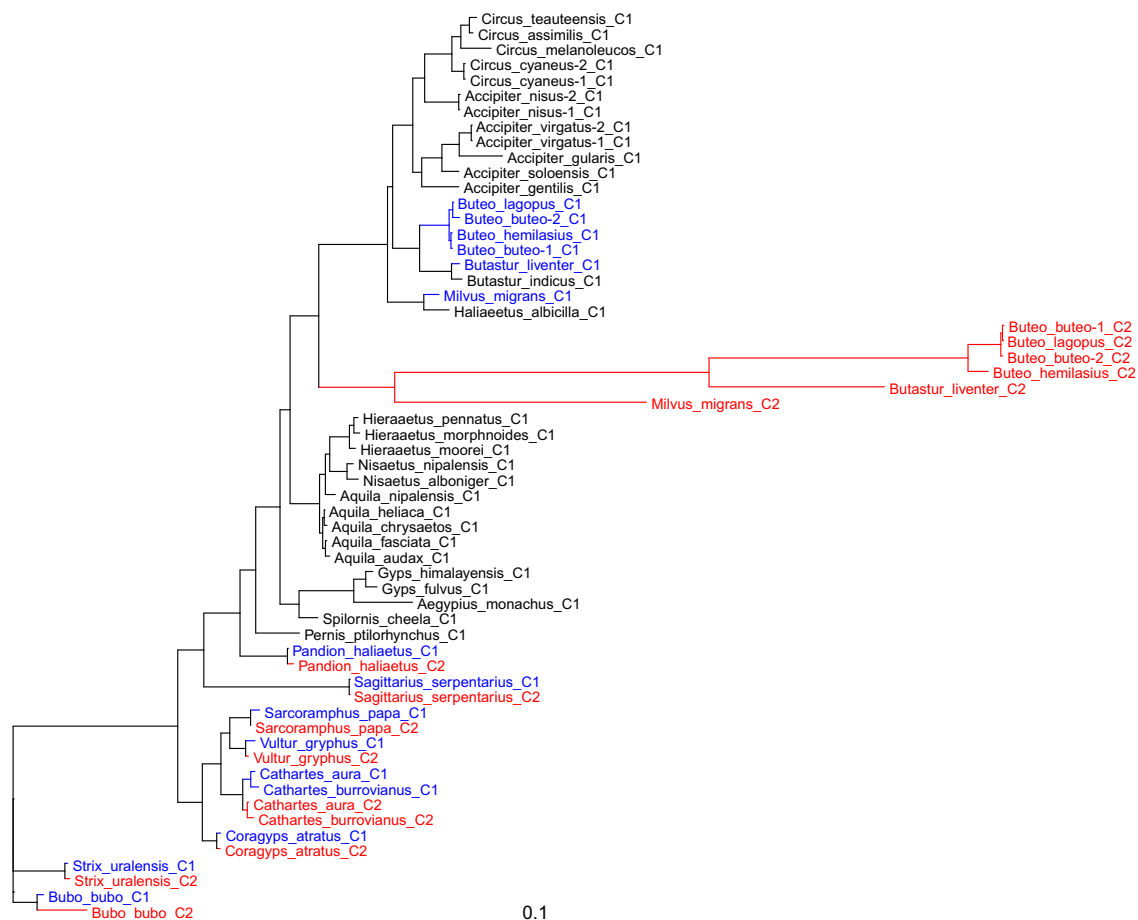
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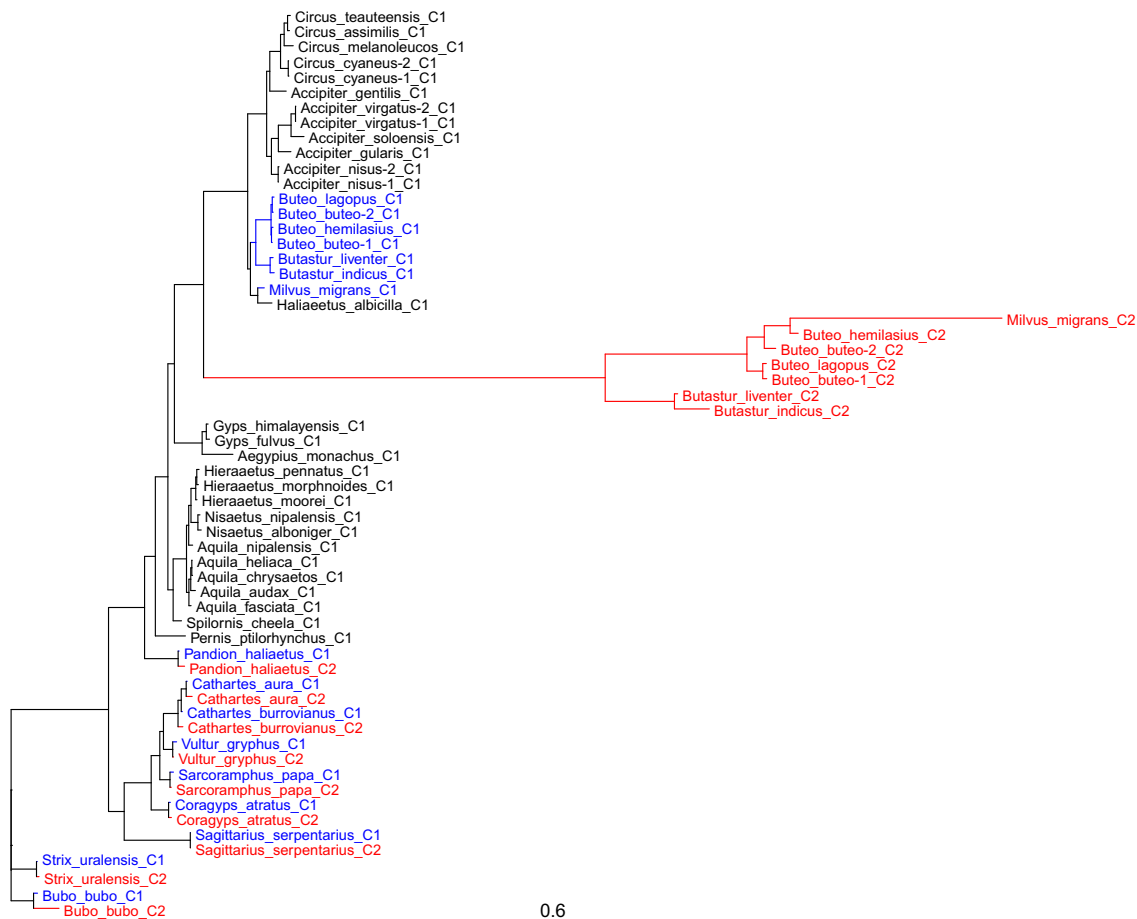
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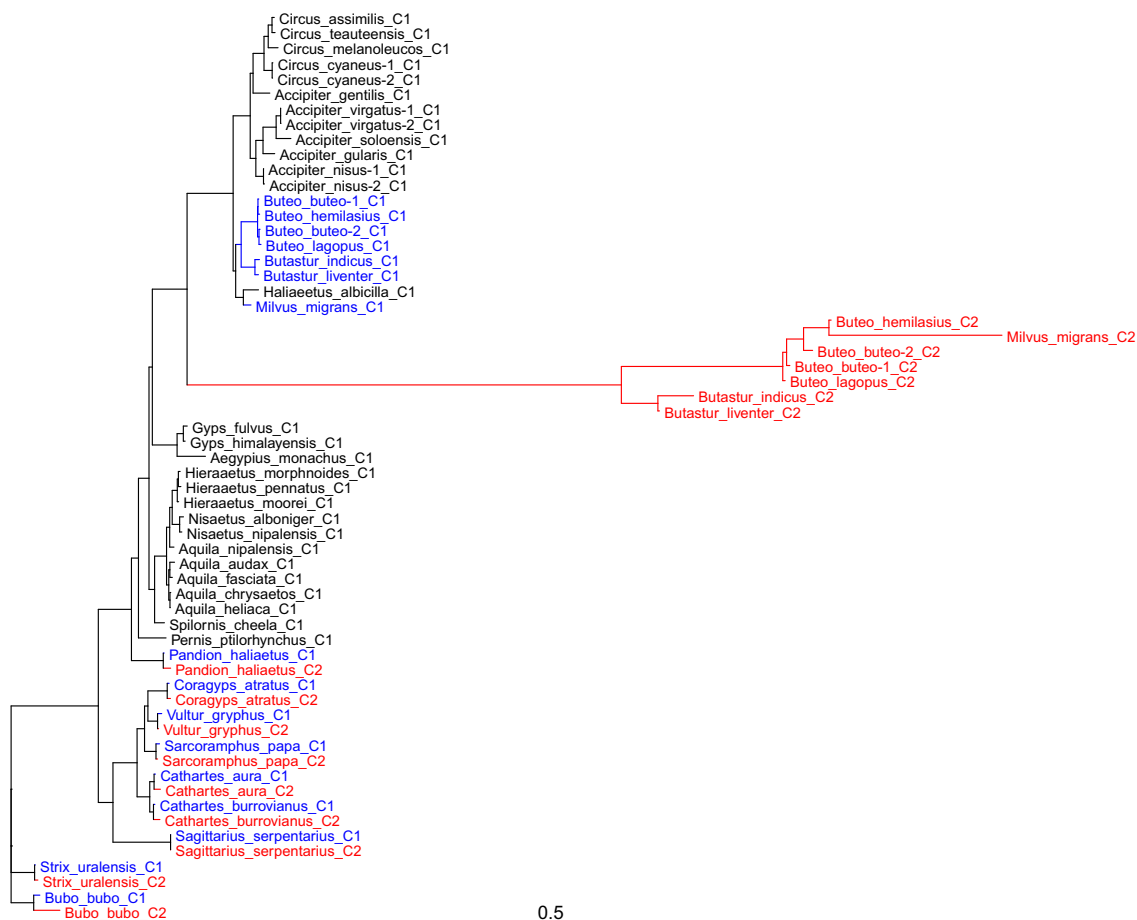
## Repeats masked, PhyML



## Repeats removed, MrBayes

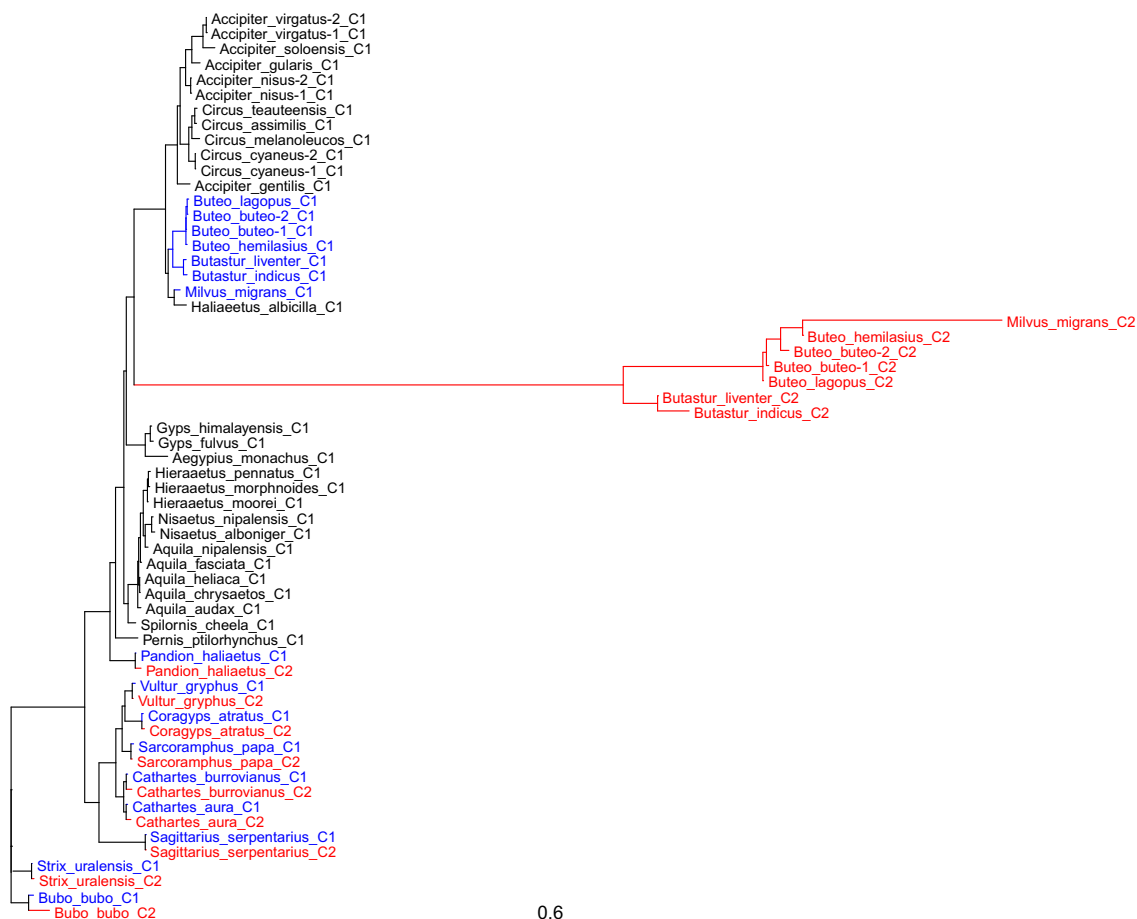


## Repeats removed, PhyloBayes





## Repeats removed, IQ-Tree

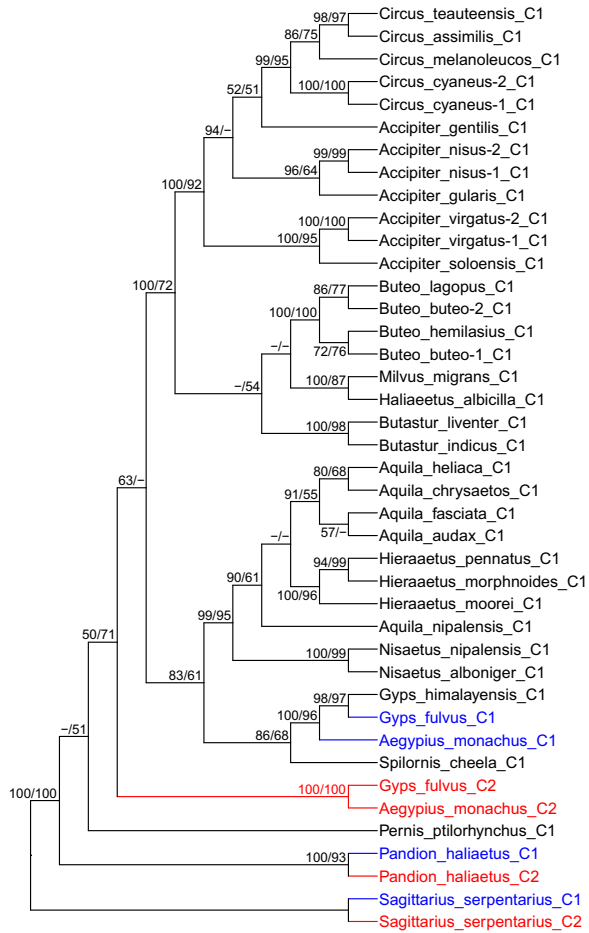


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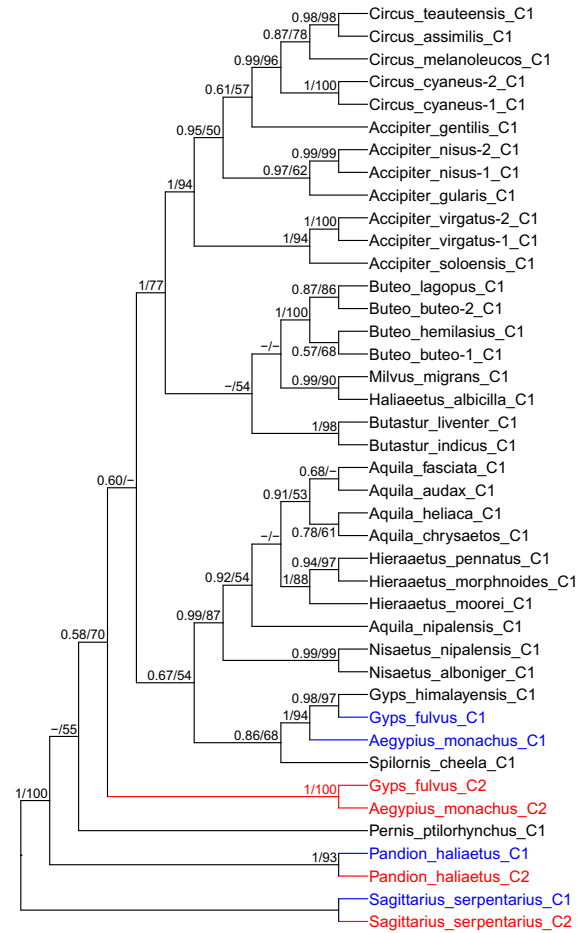


**Figure S8.** Cladograms obtained in four programs, MrBayes, PhyloBayes, IQ-TREE and (more)PhyML, based on three types of data sets of control regions (all sites, repeats masked or removed) for Accipitriformes and related groups. Pairs of CRs from the same species are colored, whereas CRs without the second copy in the tree are in black. The blue and red colors indicate the corresponding first and second copies of CR, respectively. The taxa names are in the format Genus\_species-X\_CY, where X is the individual number (if present) and Y is the number of control region, i.e. 1 or 2. The values at nodes indicate: posterior probabilities (for MrBayes and PhyloBayes), as well as support values obtained in approximate likelihood ratio test based on Shimodara-Hasegawa procedure and nonparametric bootstrap (for IQ-TREE and (more)PhyML). The posterior probabilities < 0.5 and the percentages < 50% were indicated by a dash “-.” See Table S6 for details about the data sets.

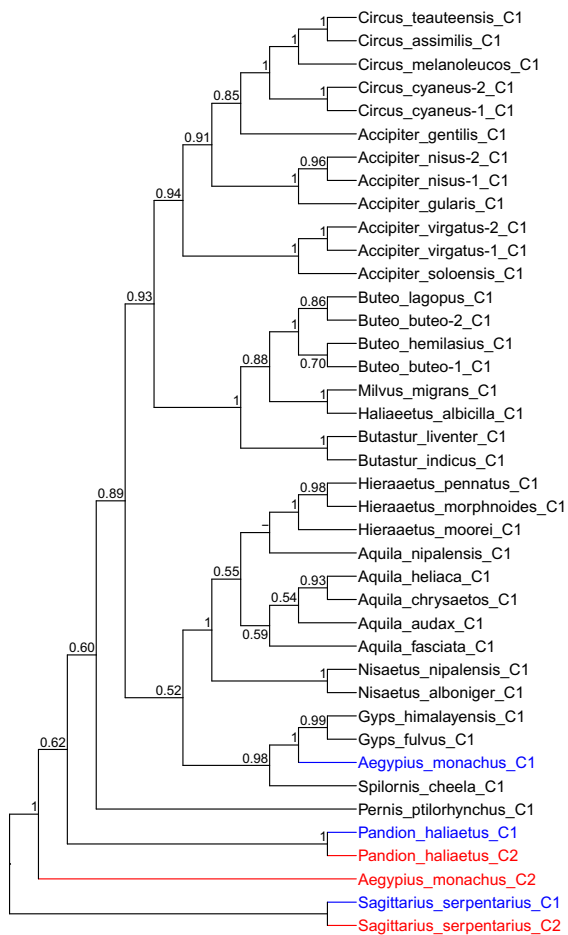
All sites, IQ-Tree



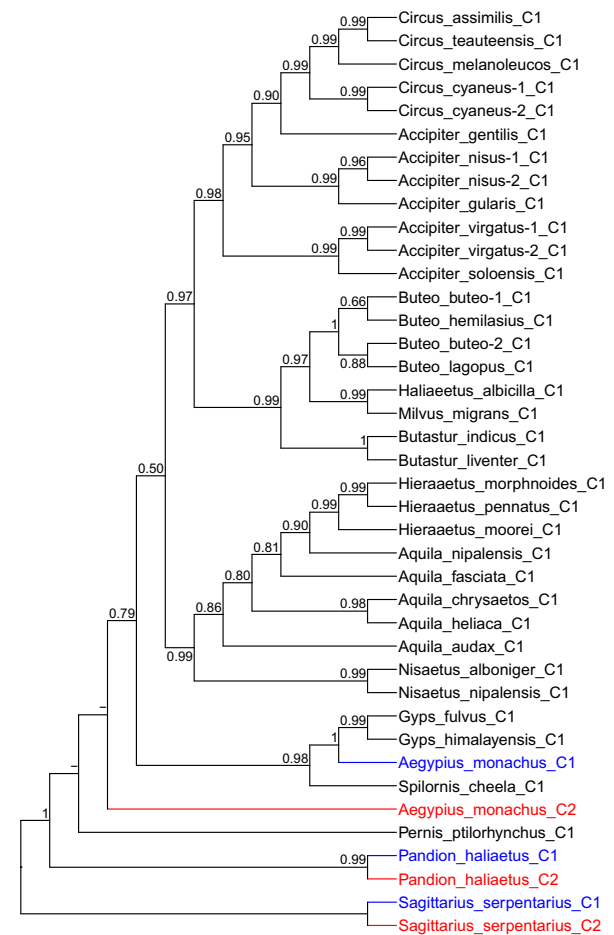
All sites, PhyML



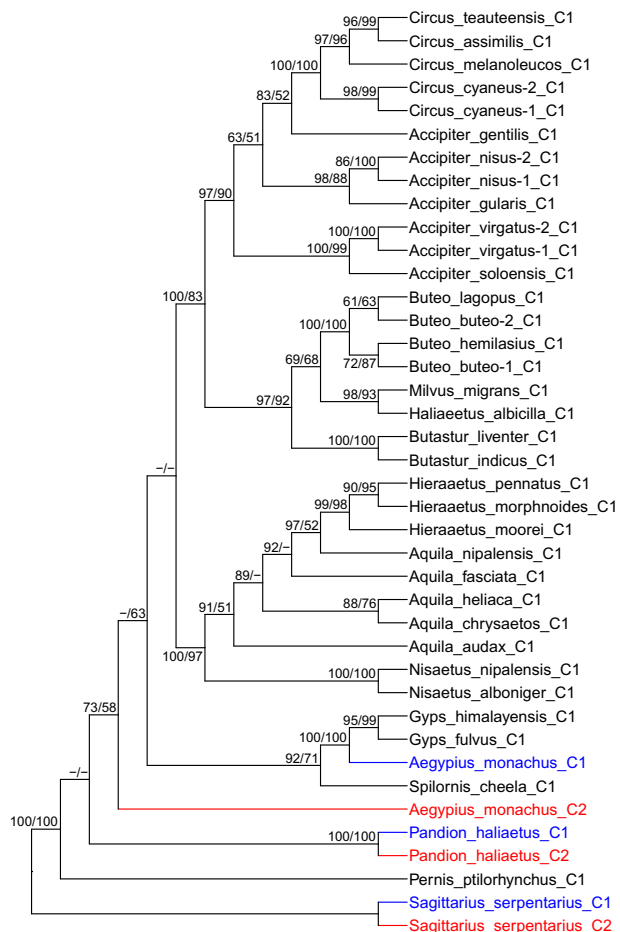
Repeats removed, MrBayes



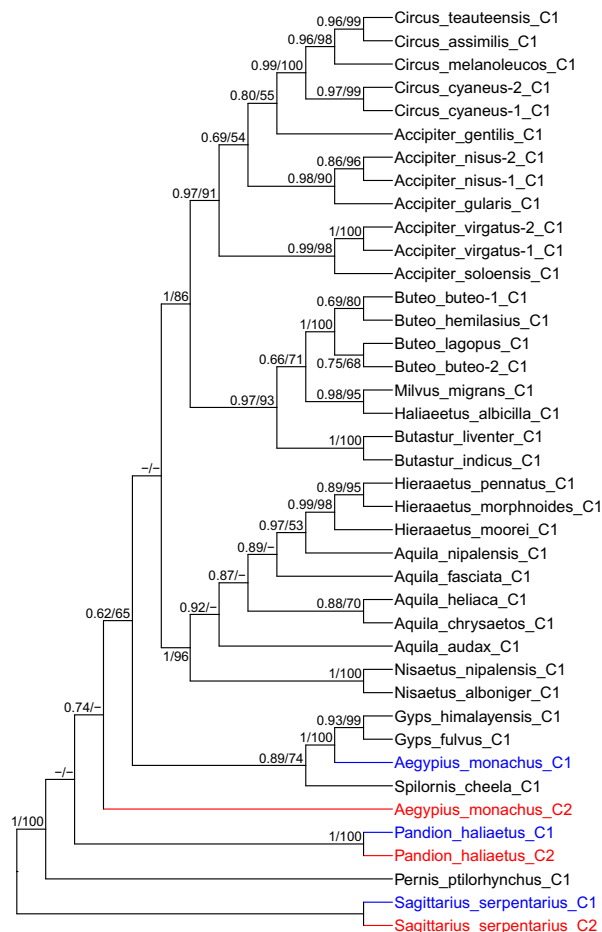
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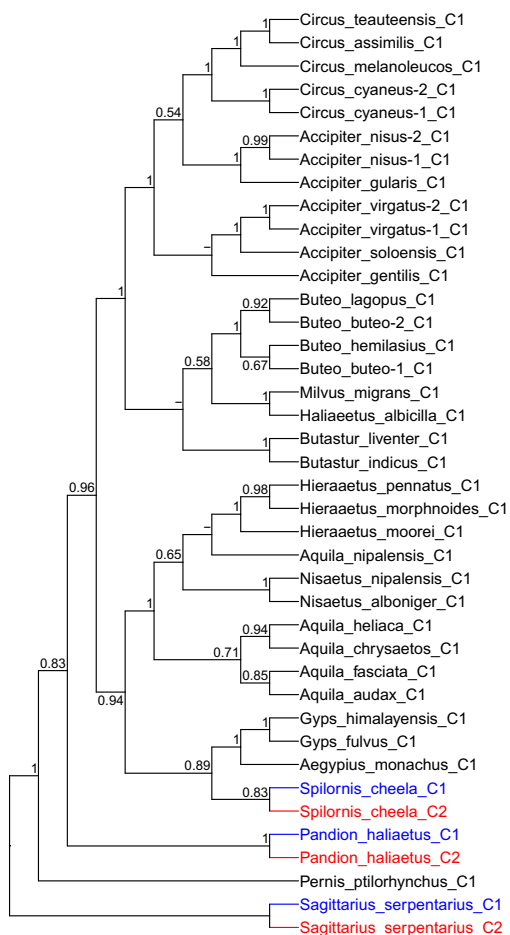
Repeats removed, IQ-Tree



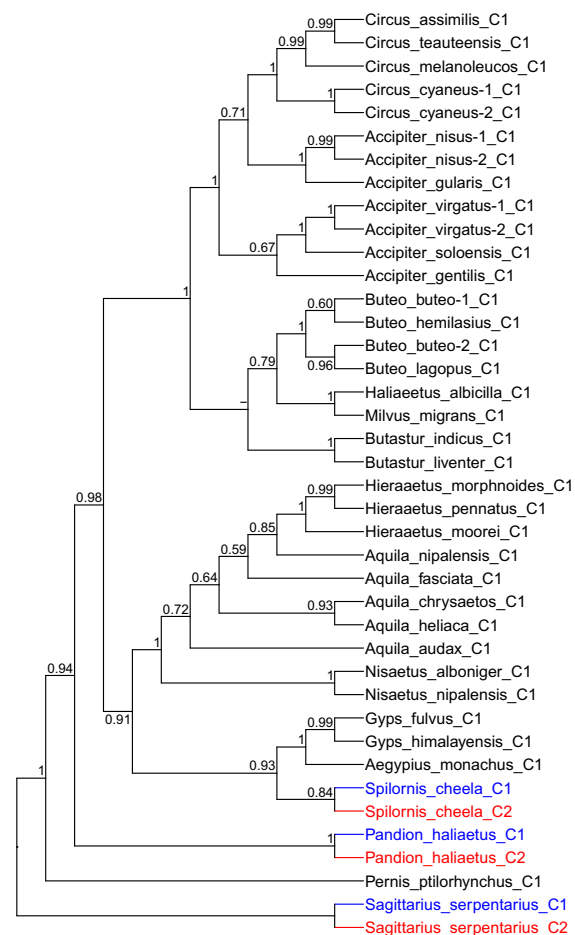
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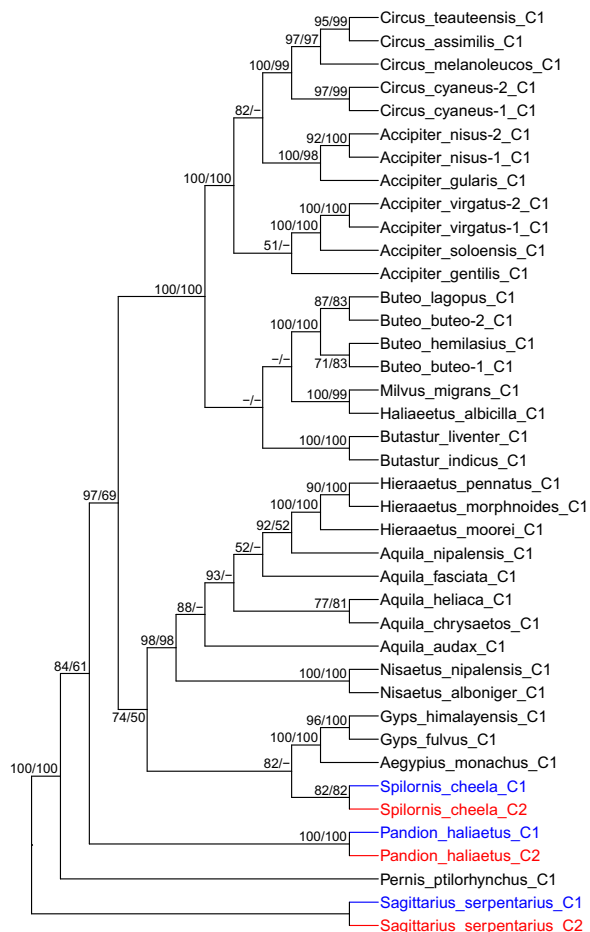
Repeats masked, MrBayes



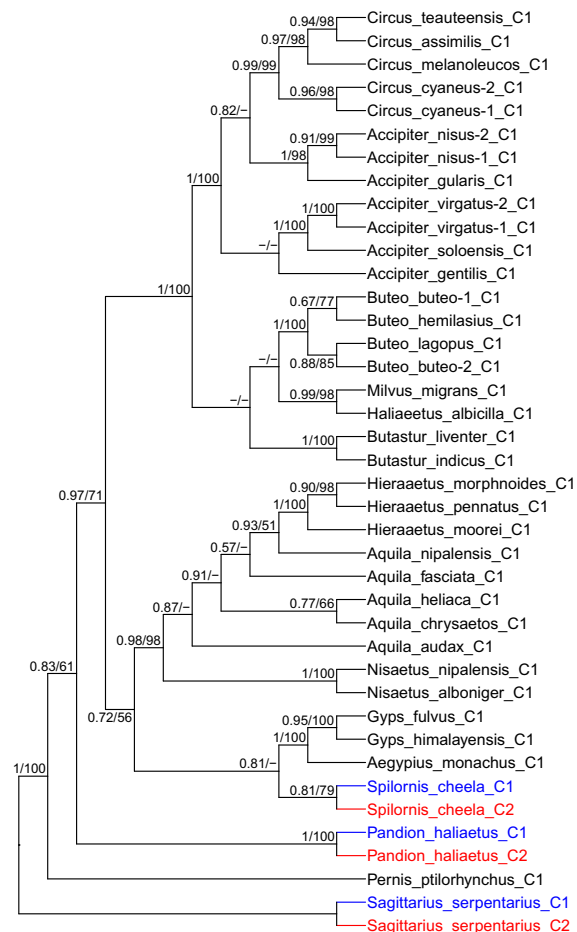
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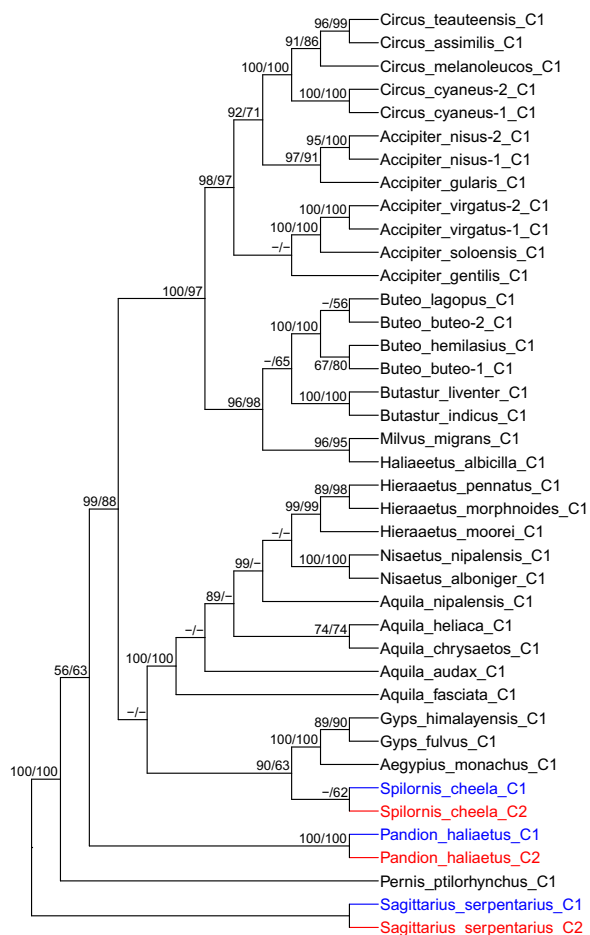
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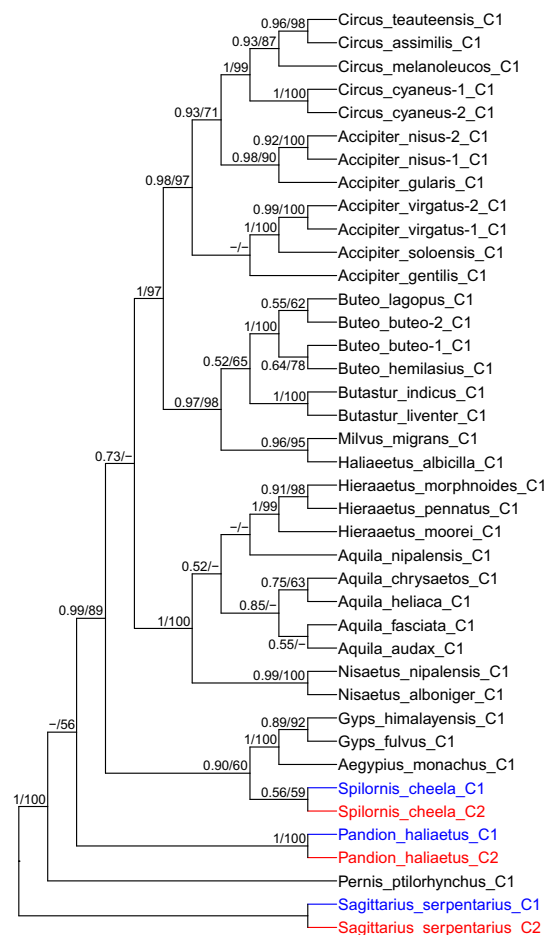
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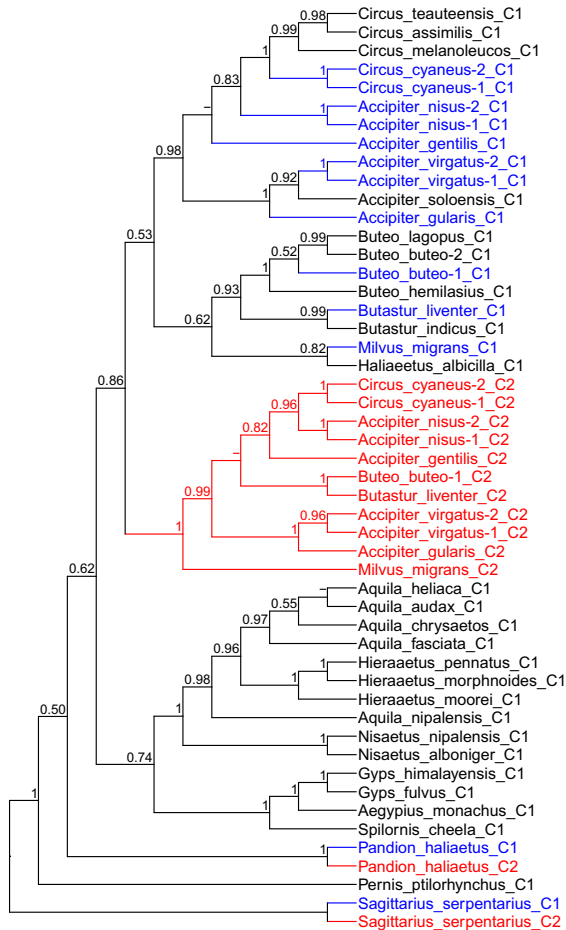
Repeats removed, IQ-Tree



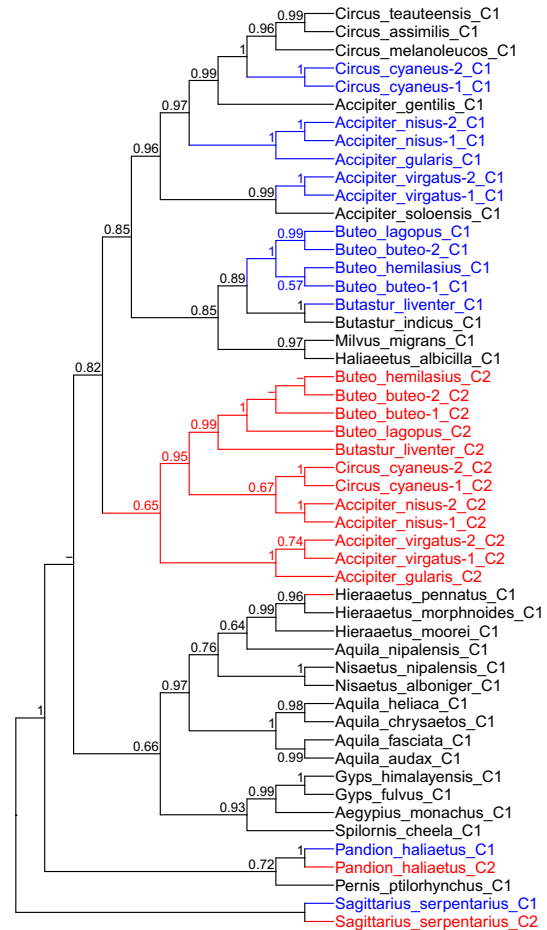
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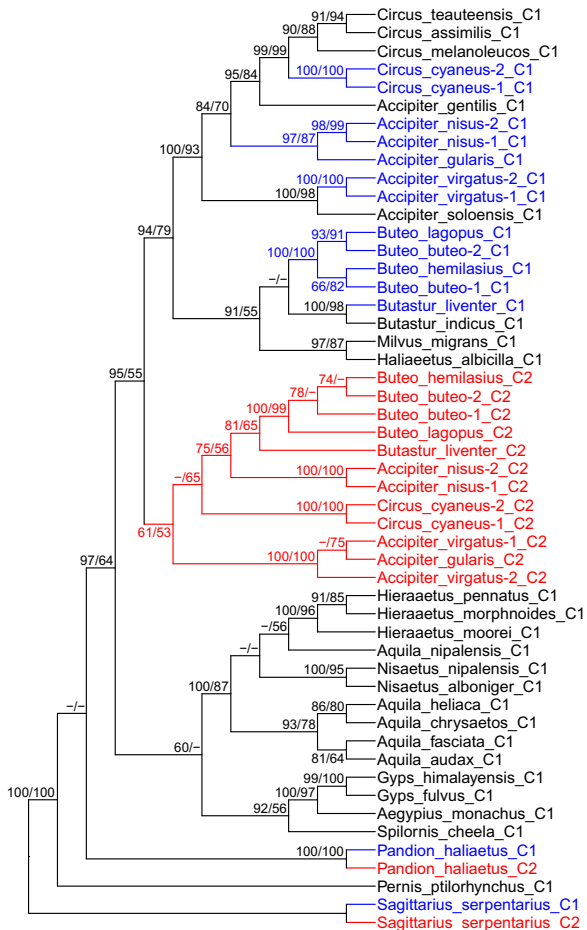
All sites, MrBayes



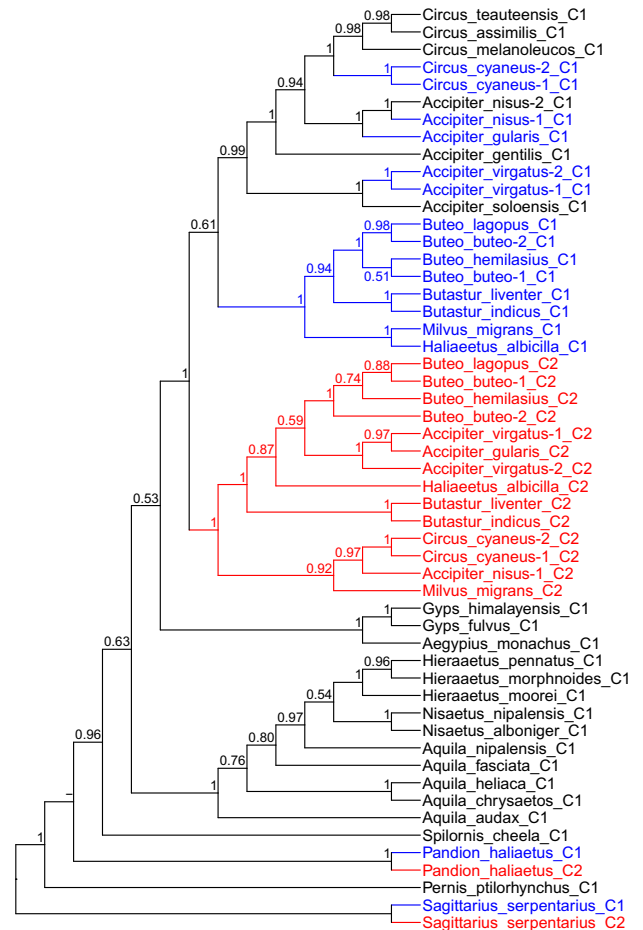
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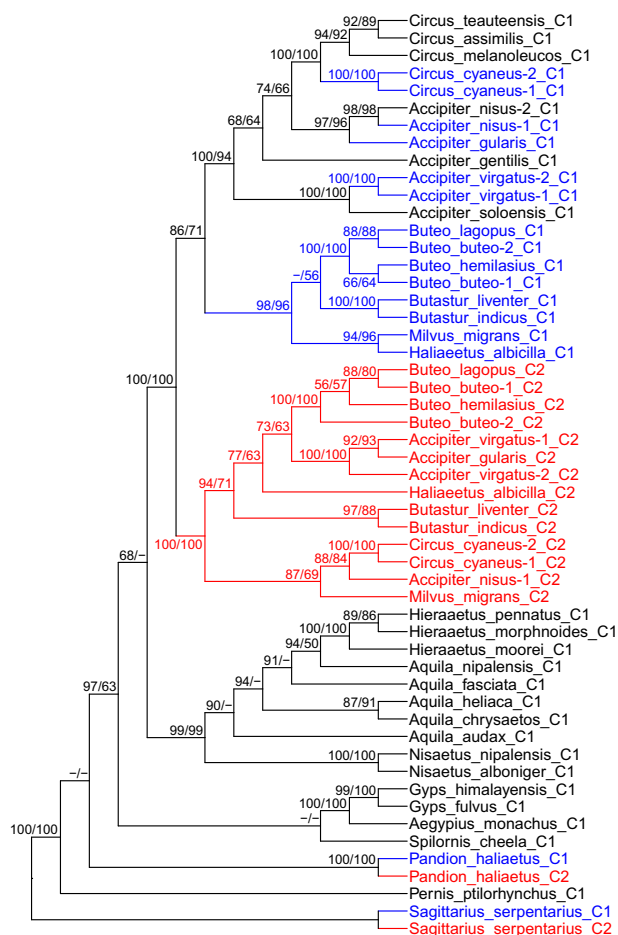
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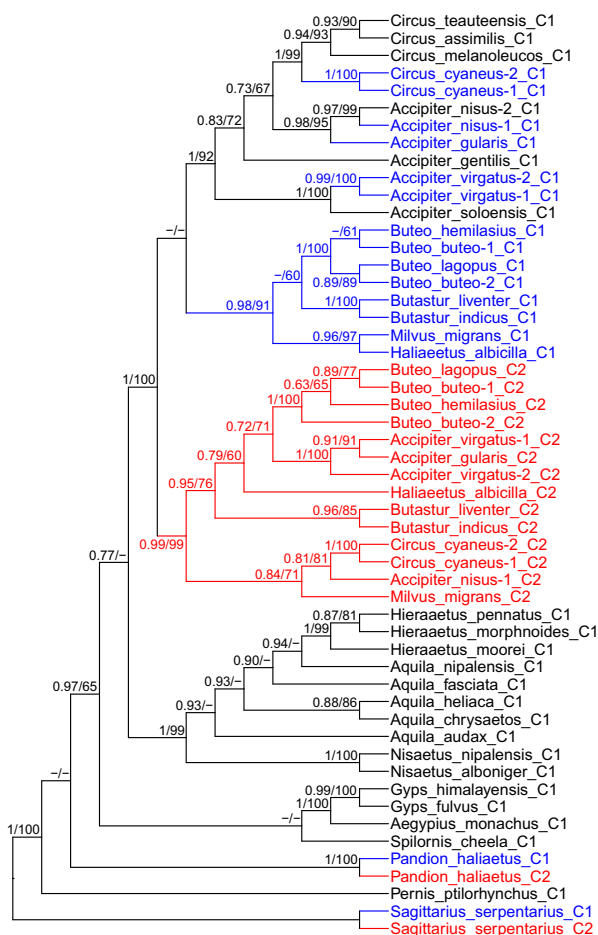
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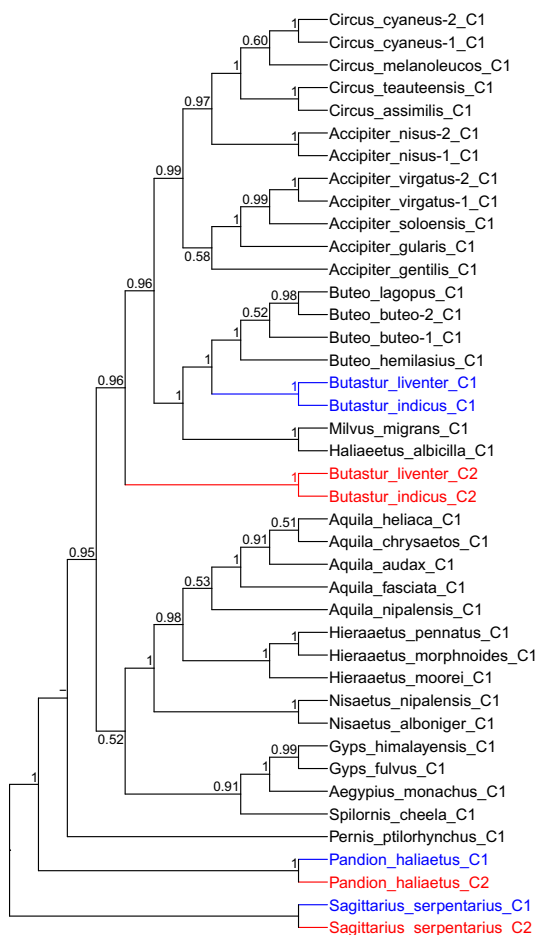
Repeats removed, IQ-Tree



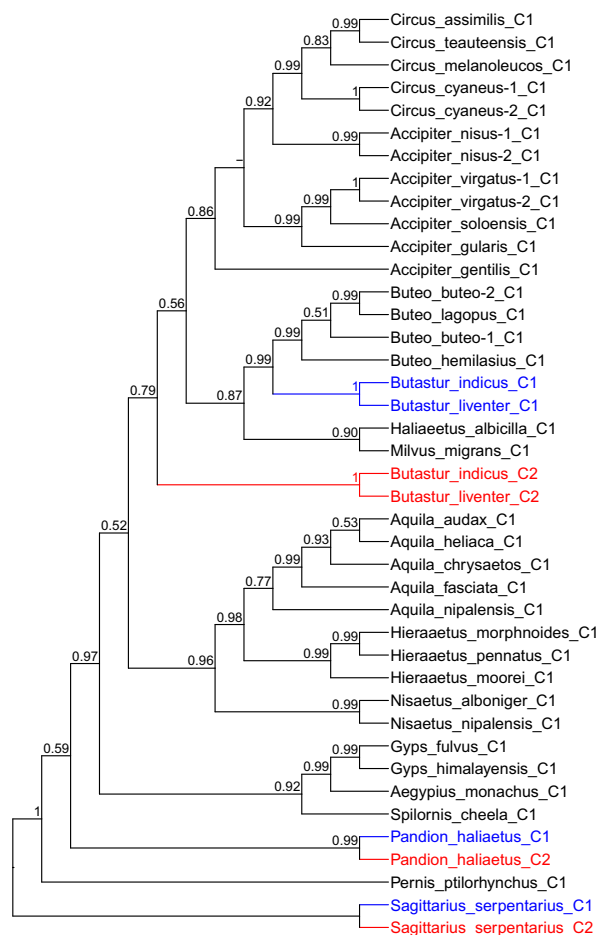
Repeats removed, PhyML



All sites, MrBayes

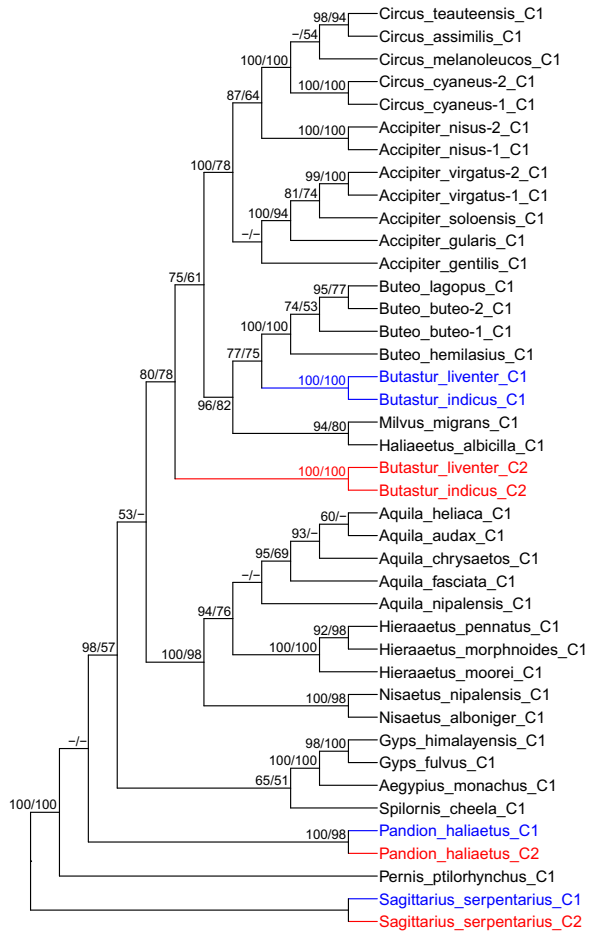


All sites, PhyloBayes

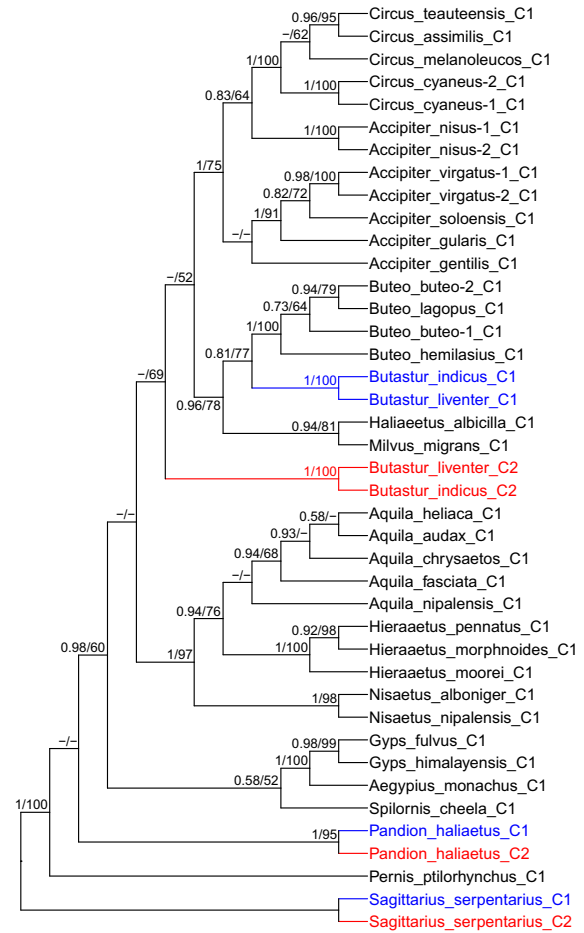




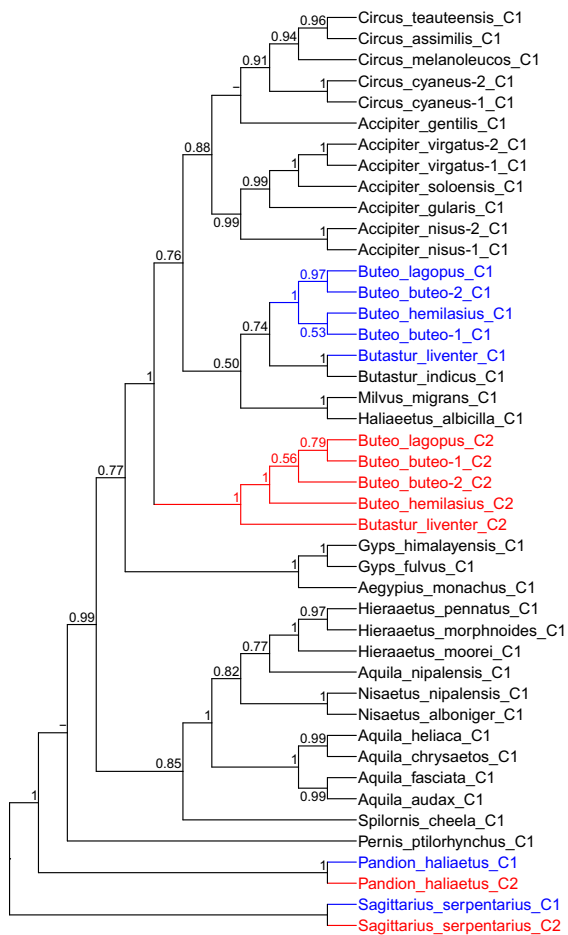
All sites, IQ-Tree



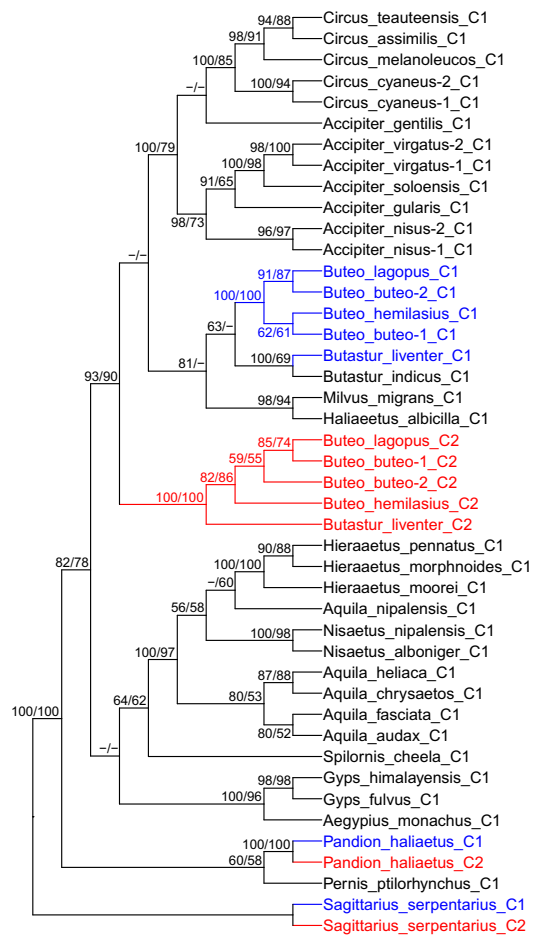
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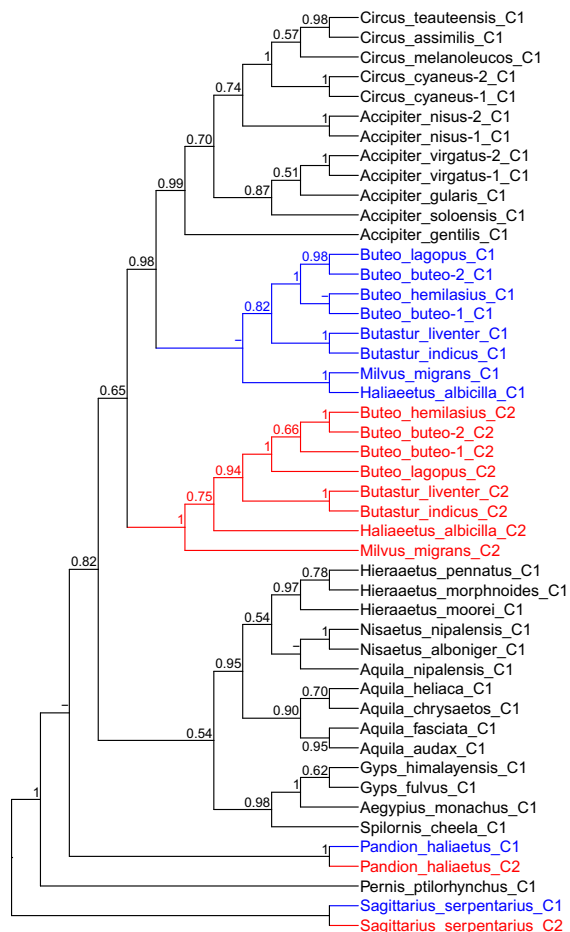


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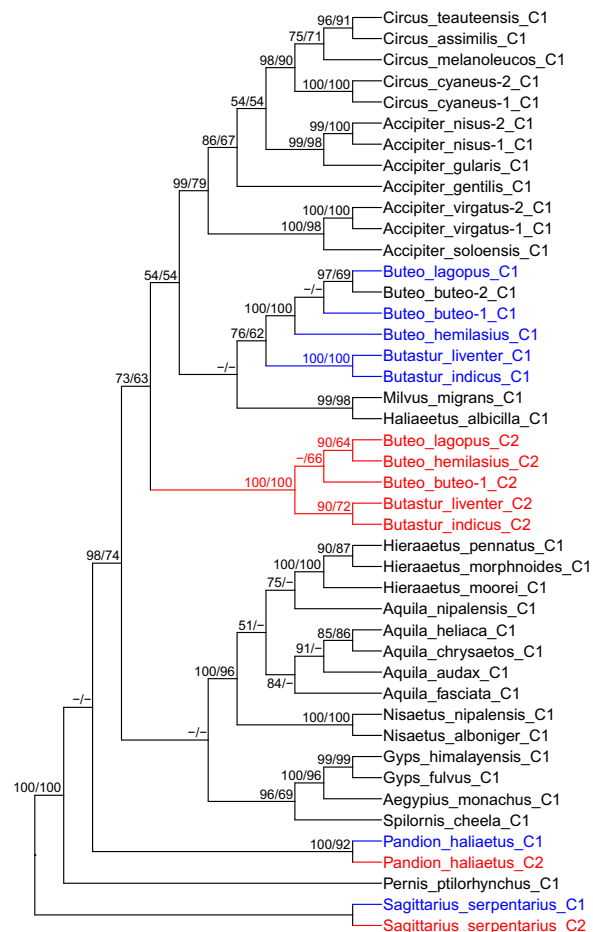




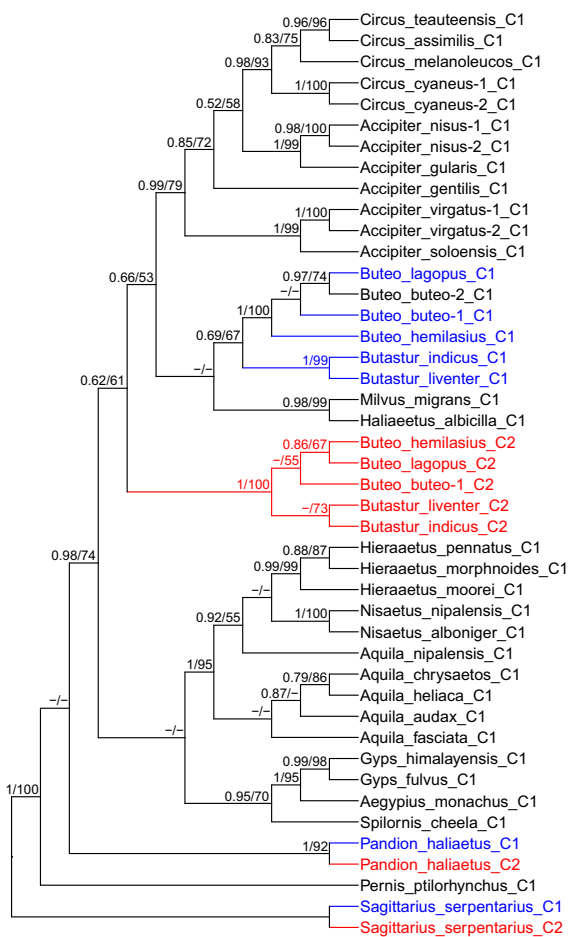
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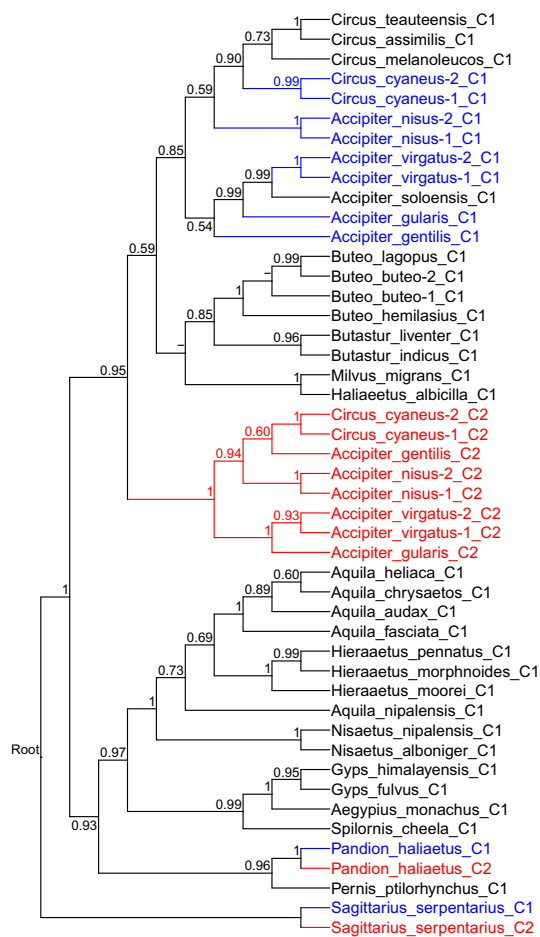
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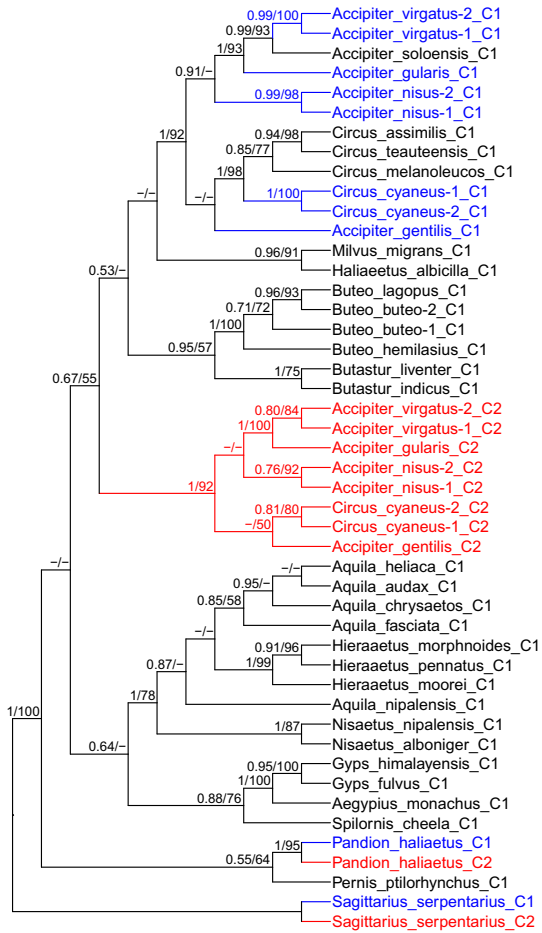
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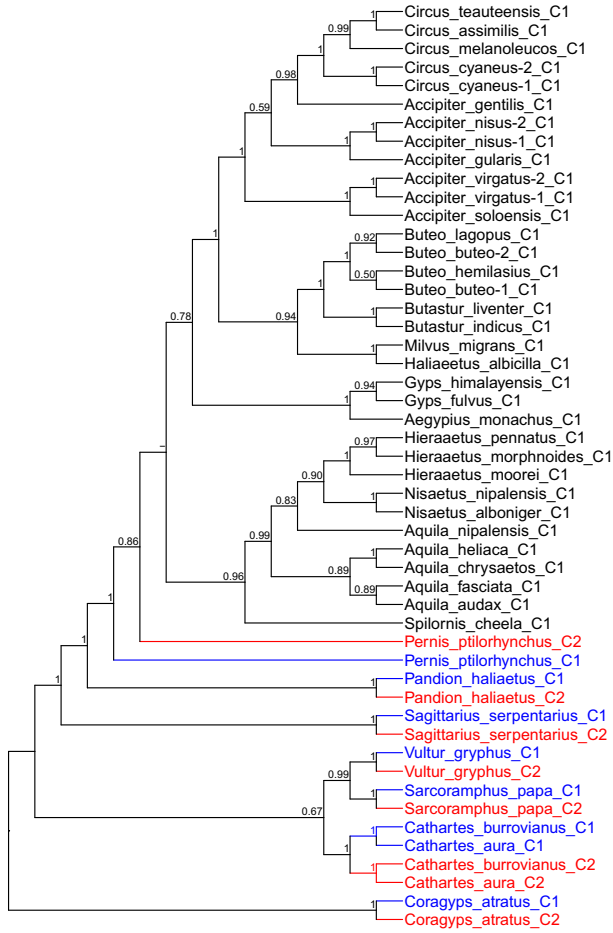
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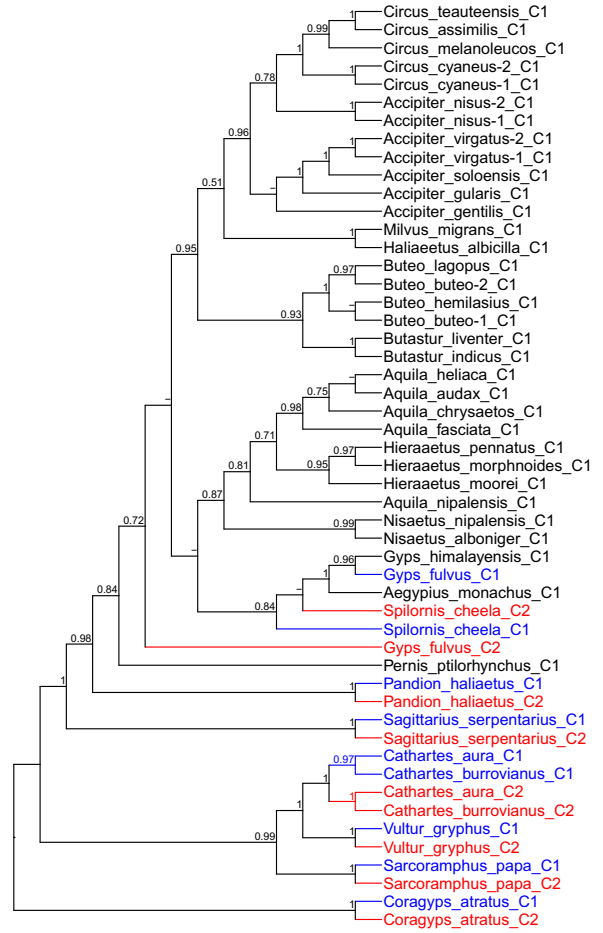
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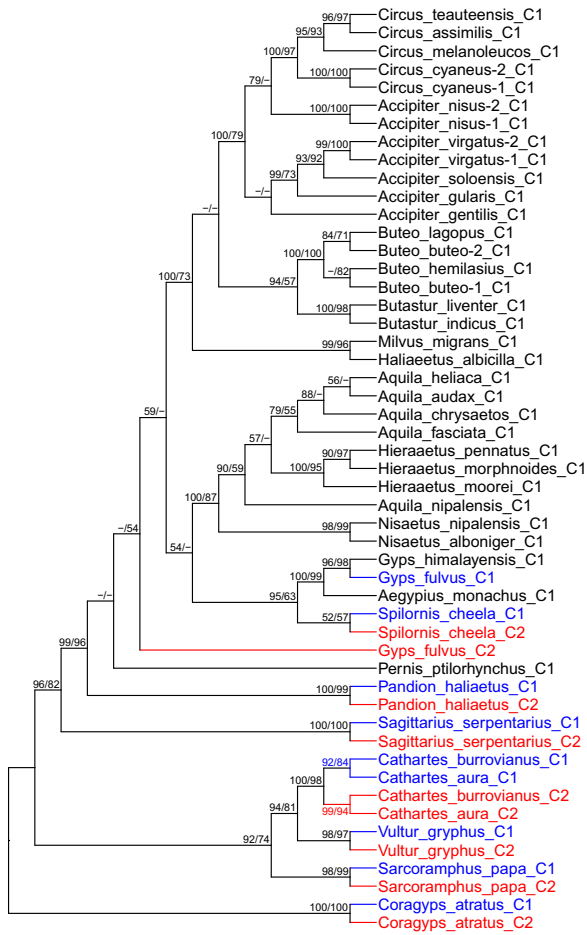
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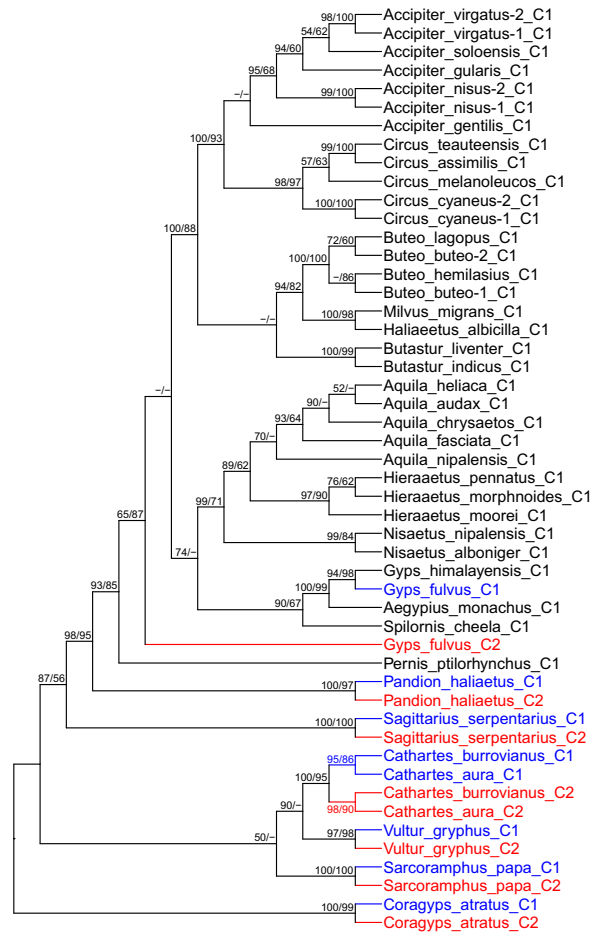
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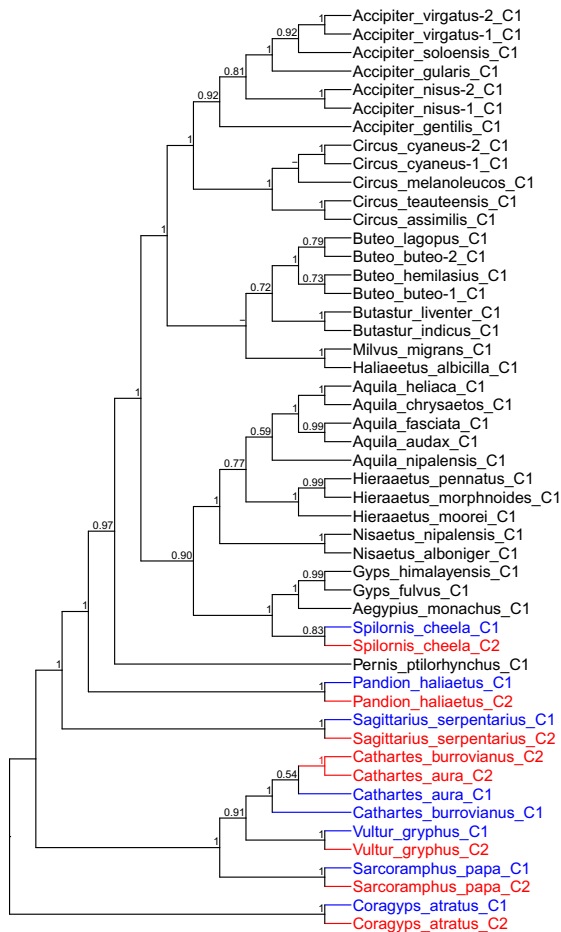
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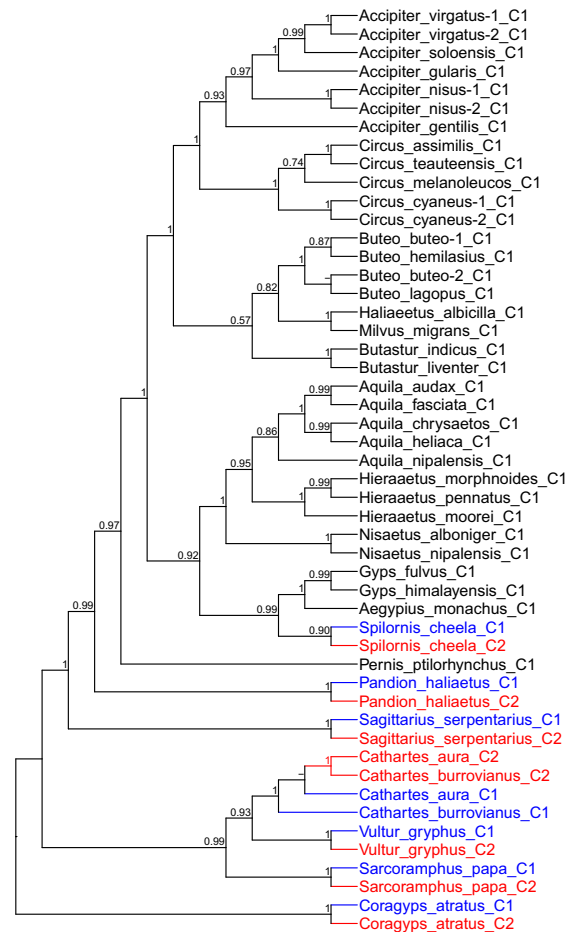
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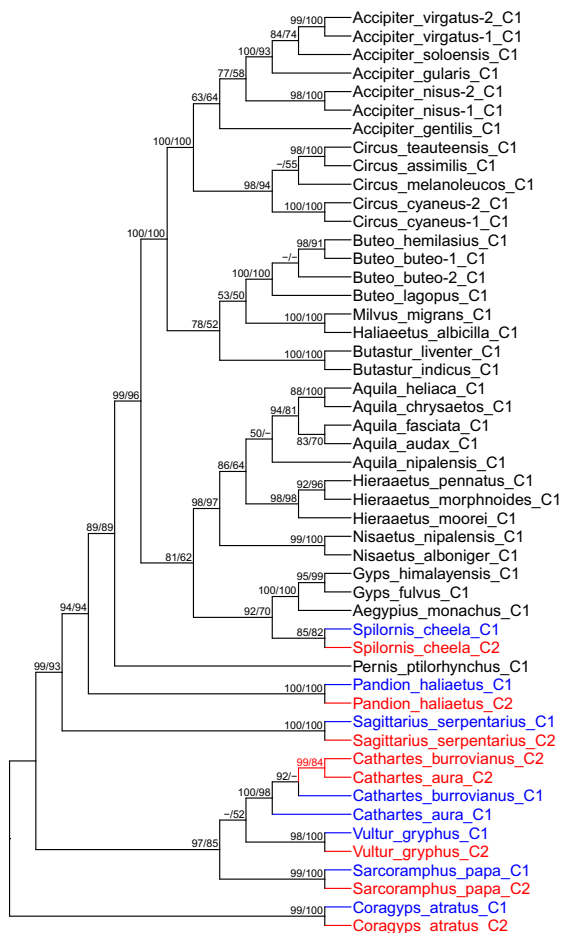
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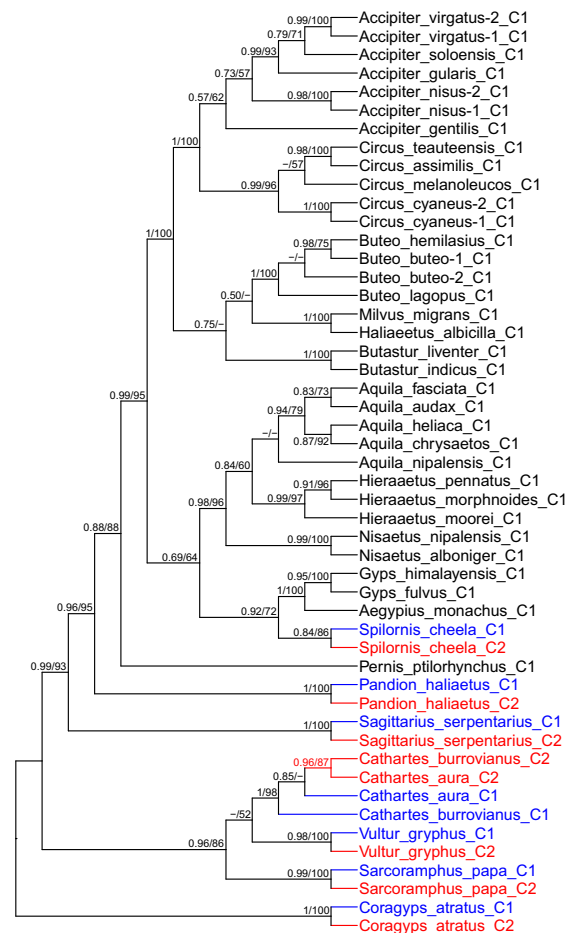
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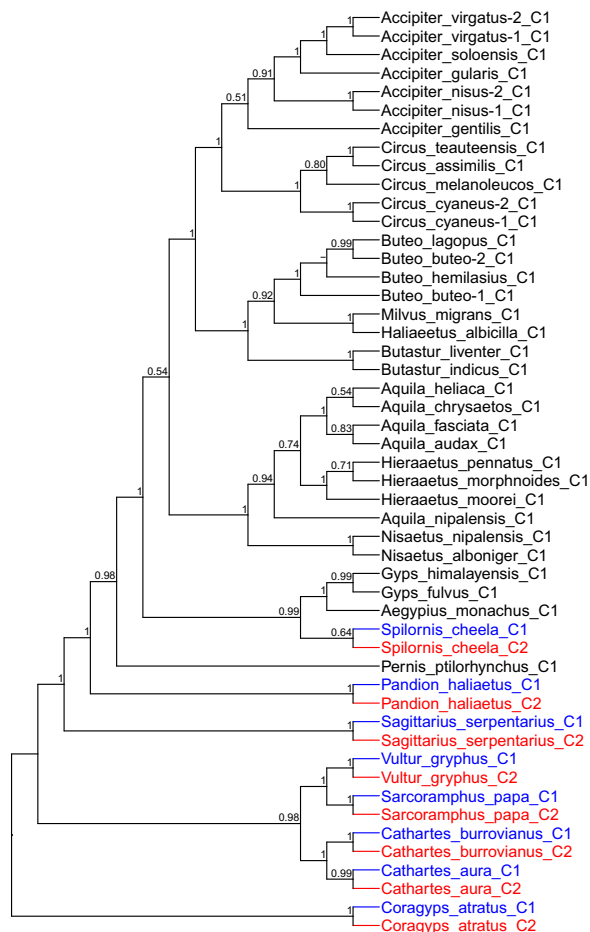
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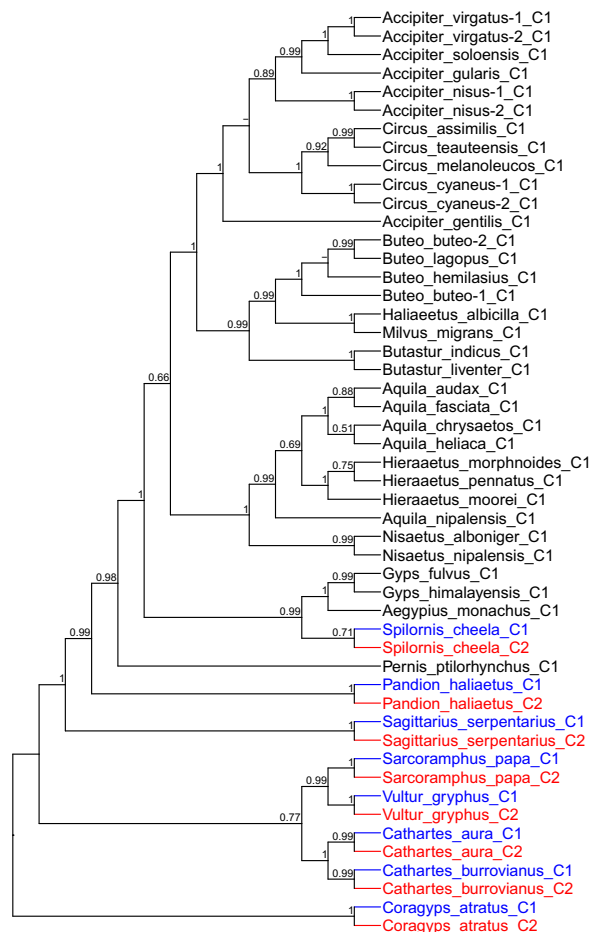
All sites, PhyML



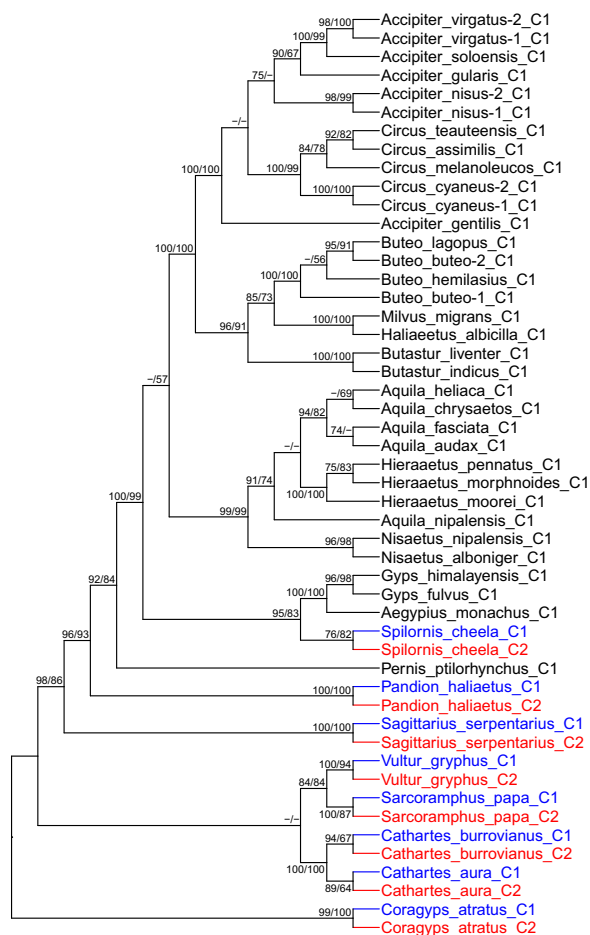
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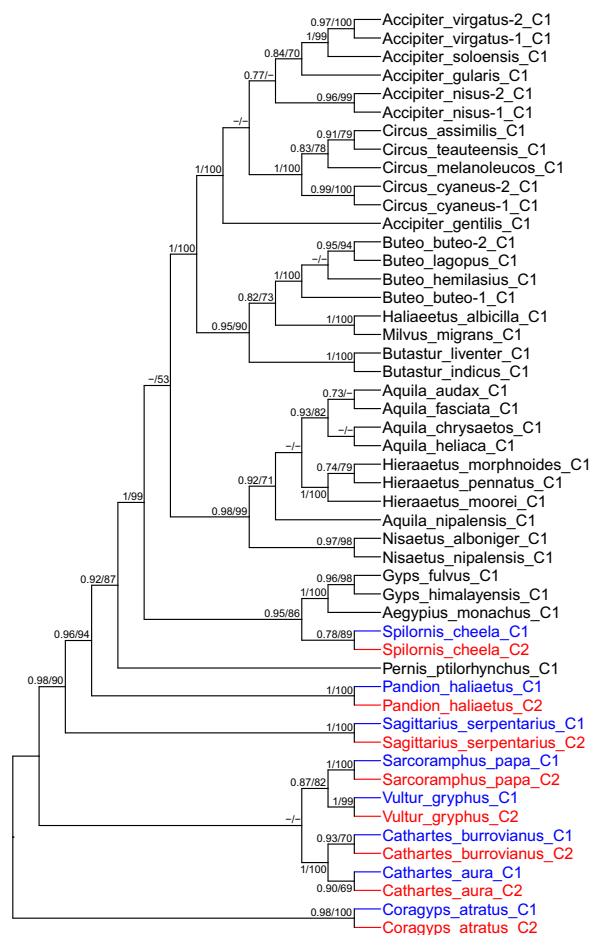
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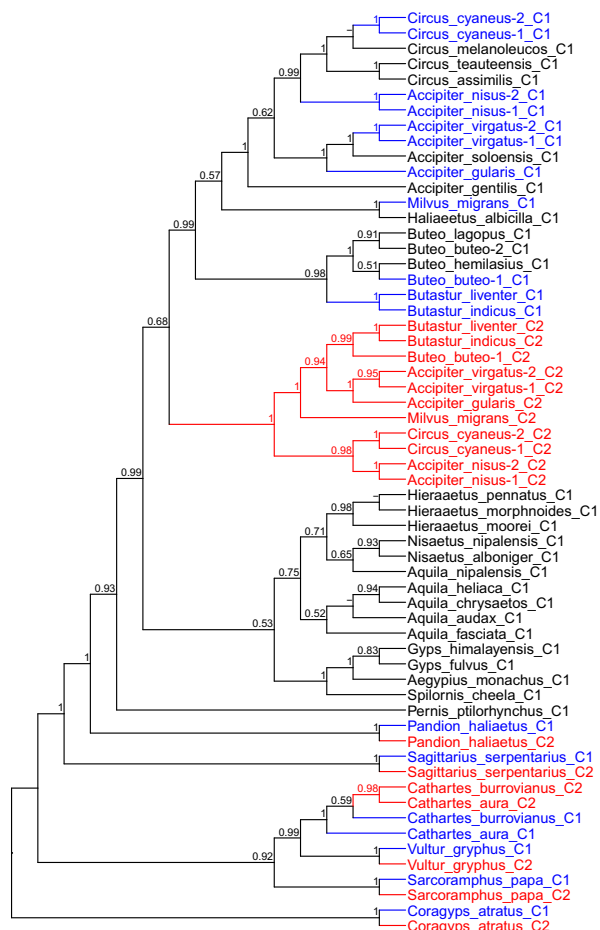
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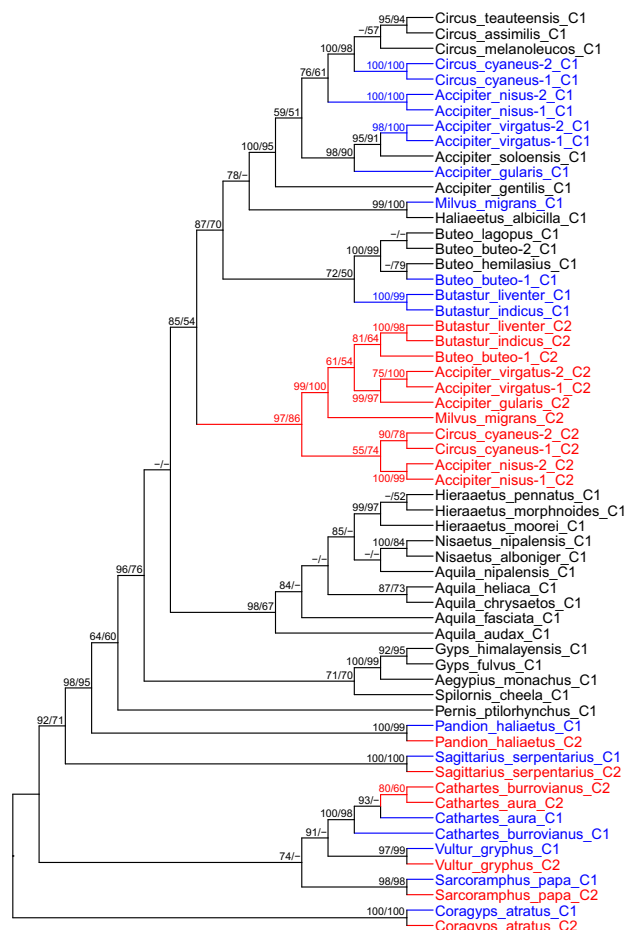
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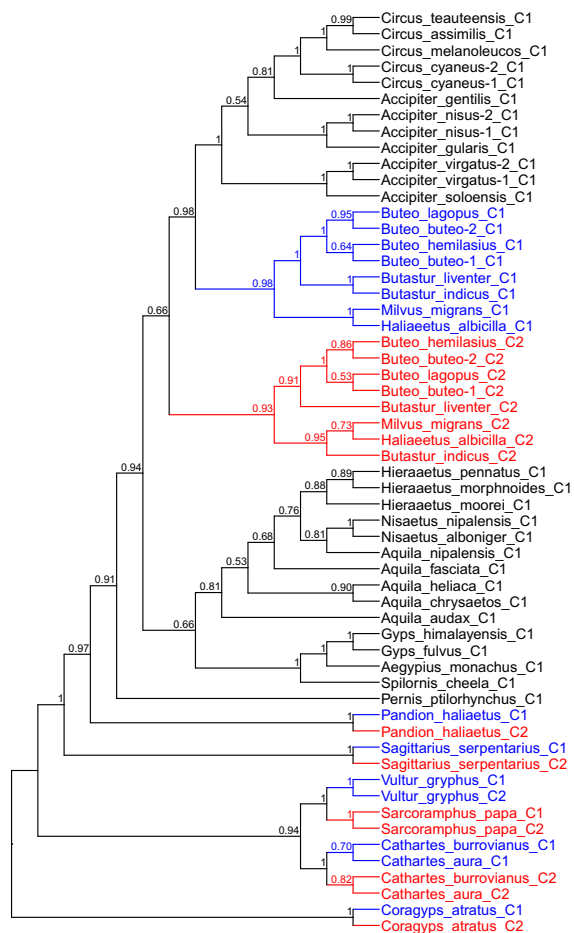
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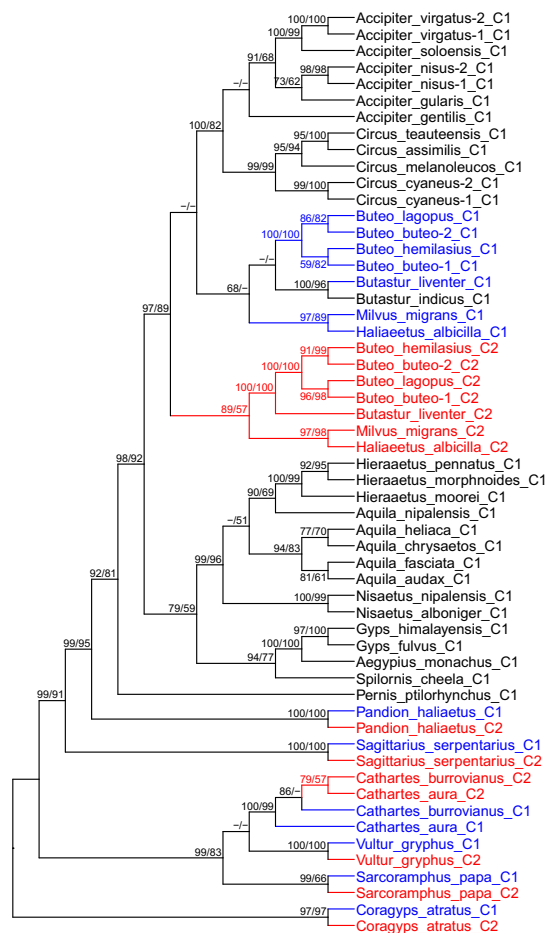
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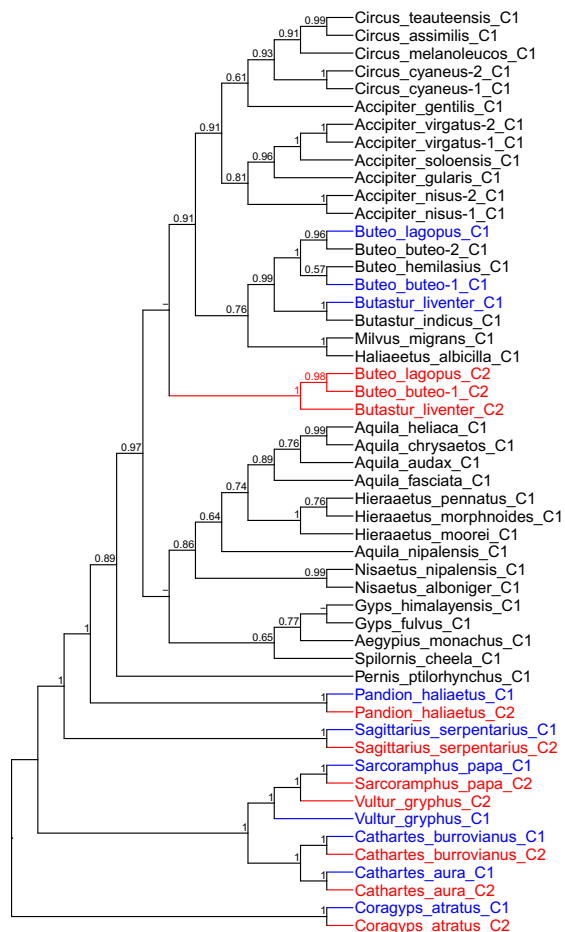


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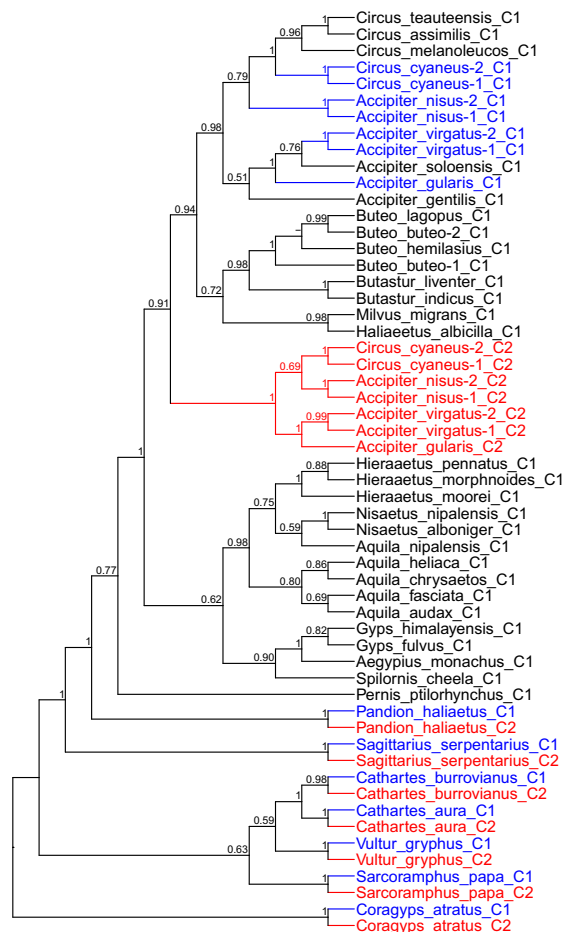




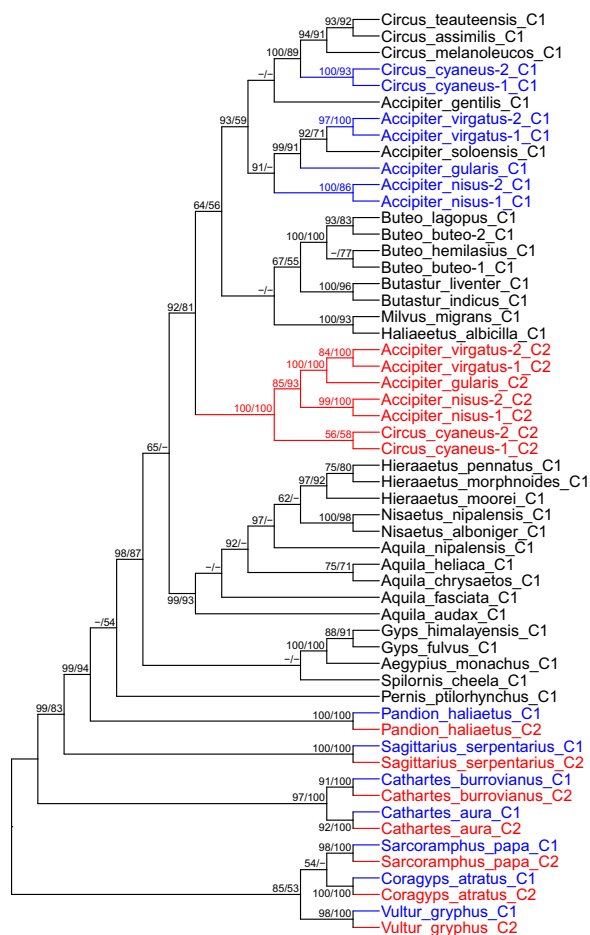
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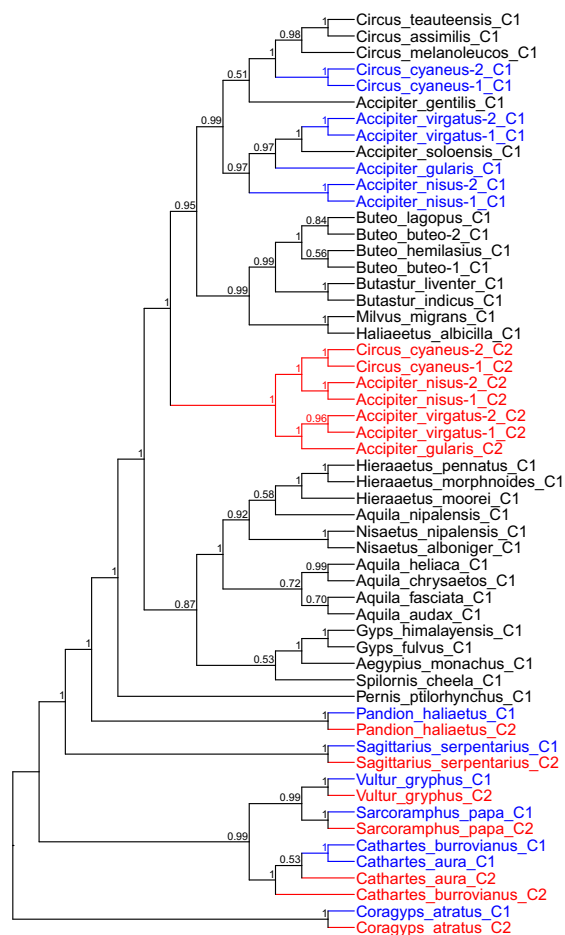
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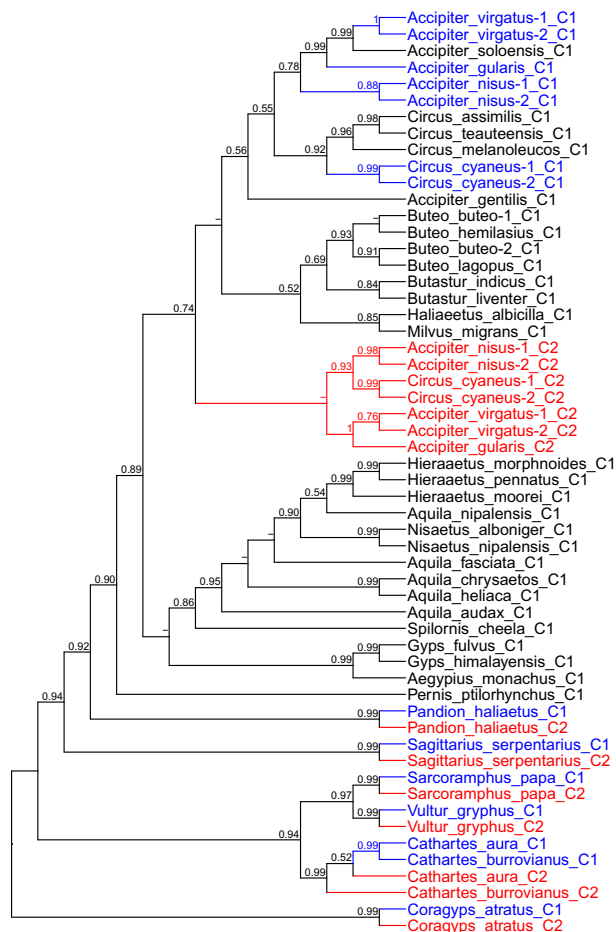
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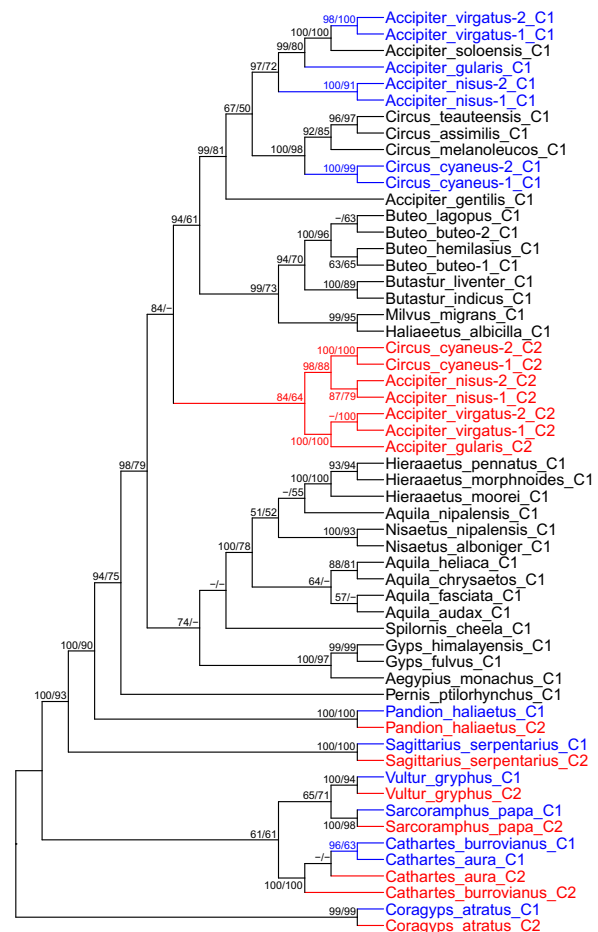
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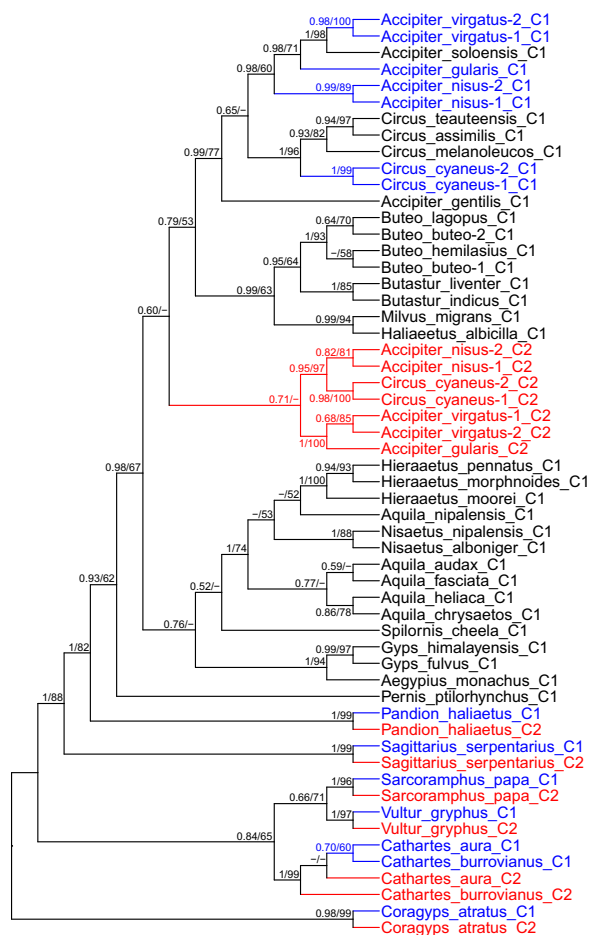
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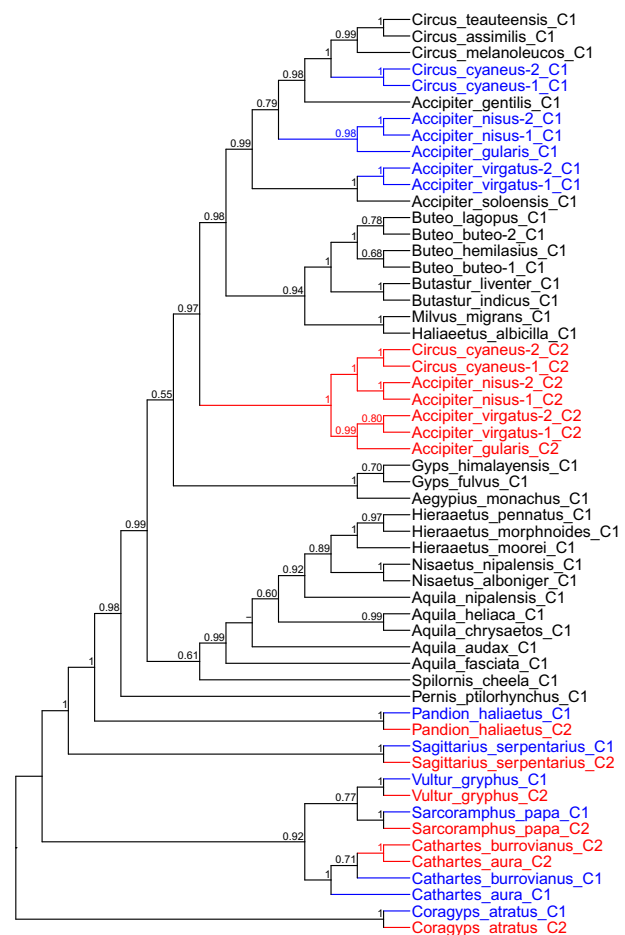
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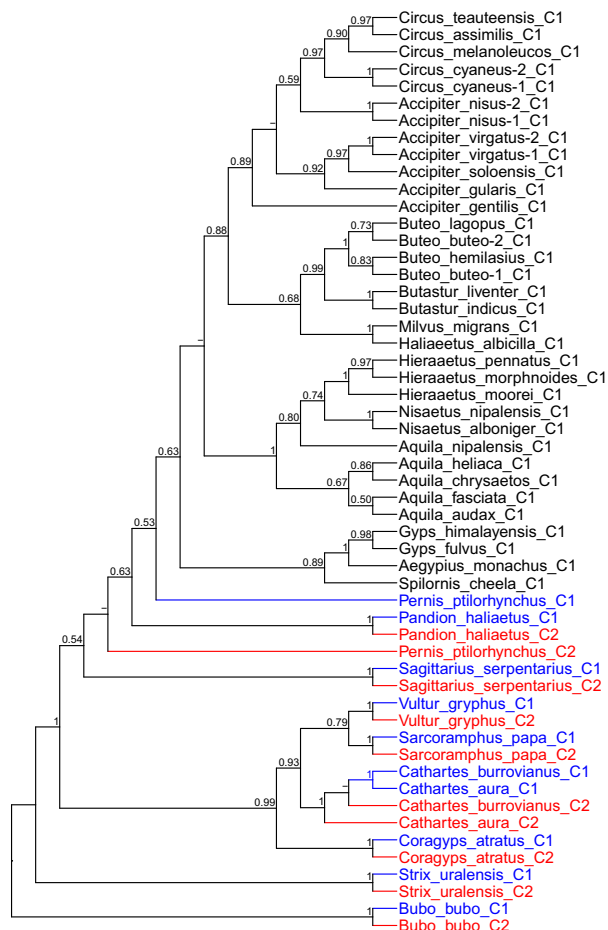


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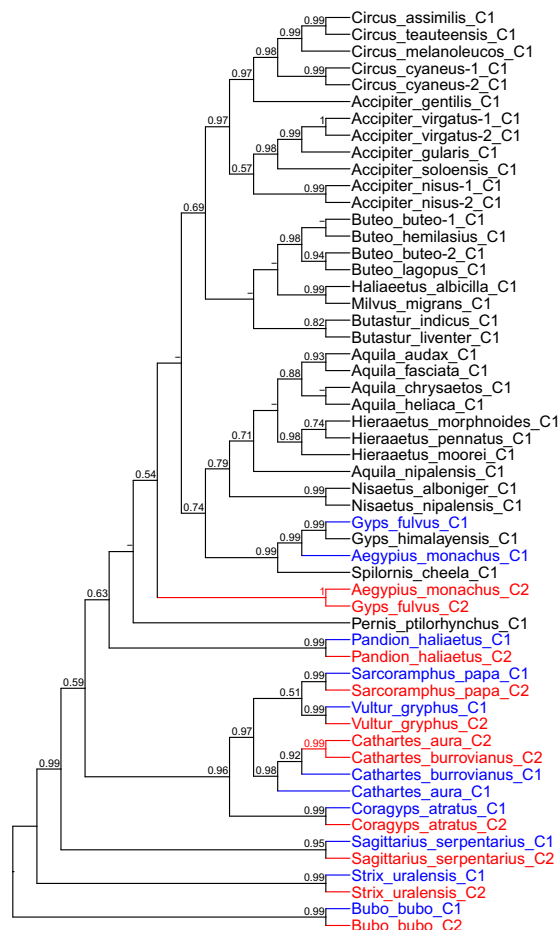




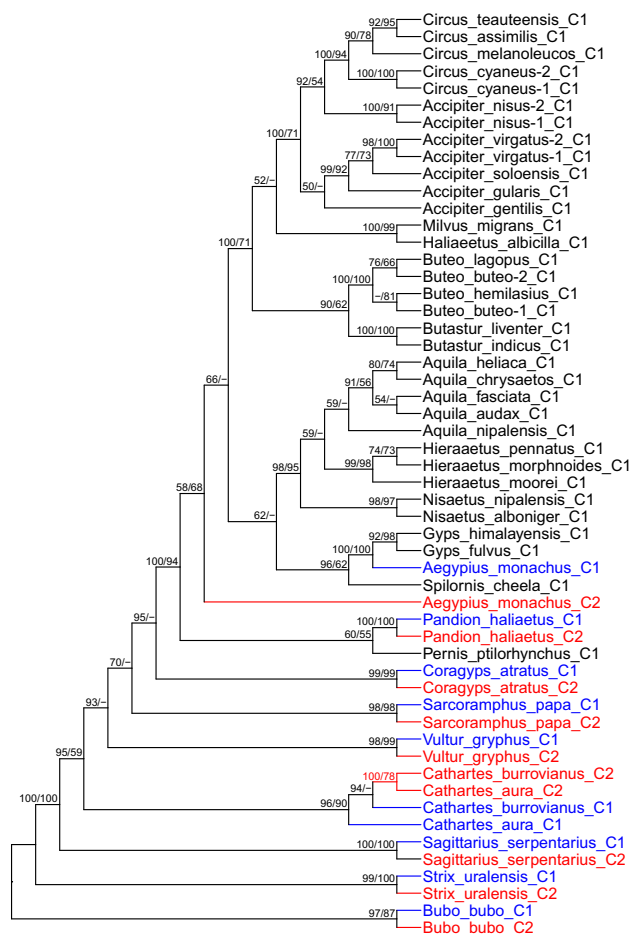
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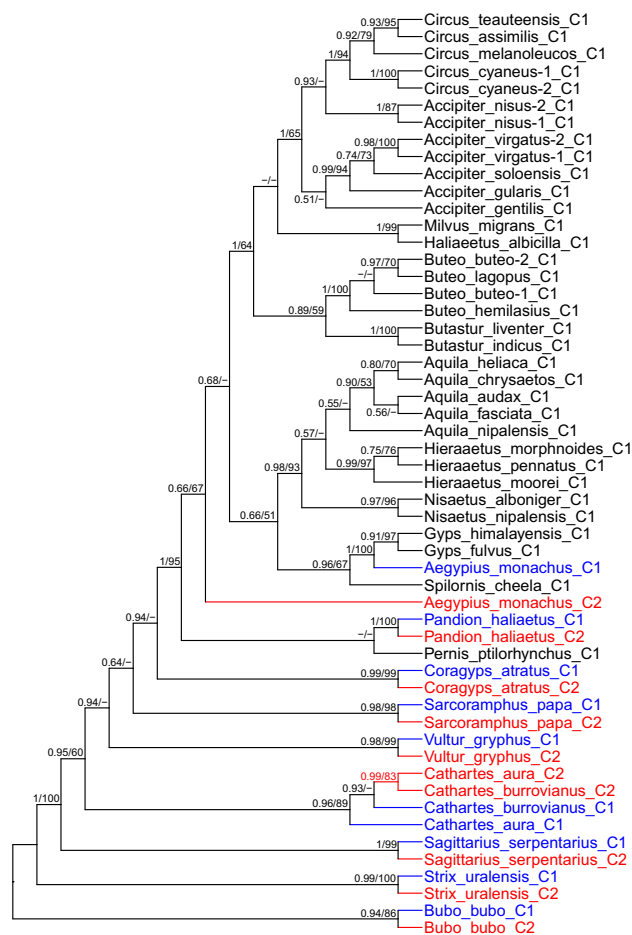
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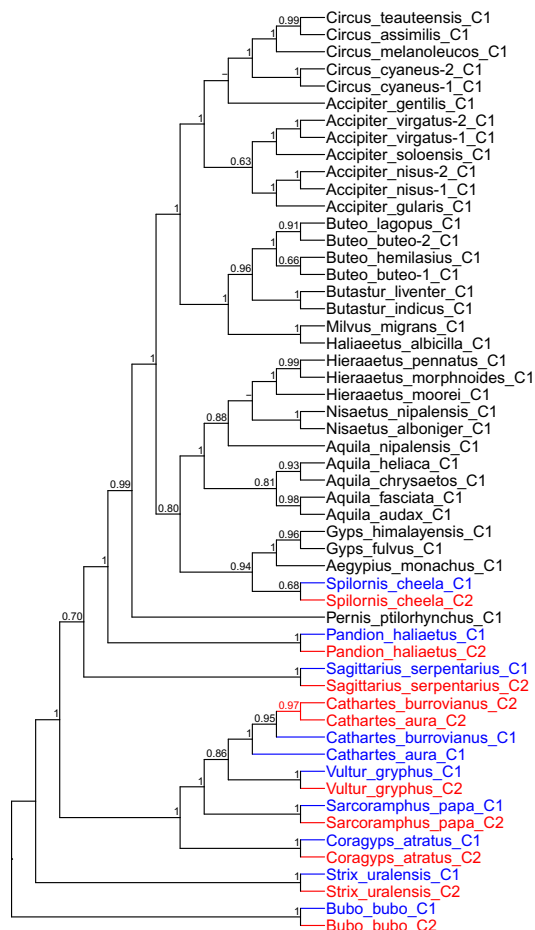
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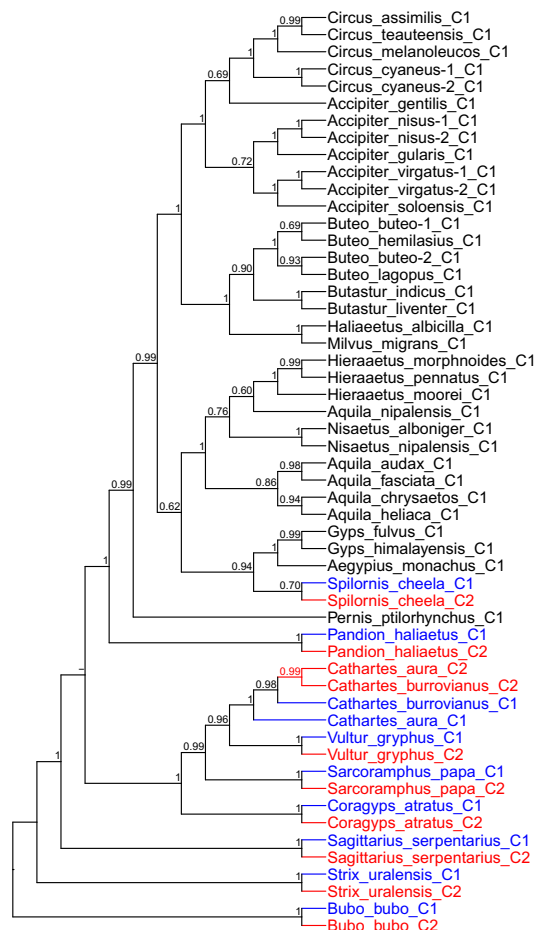
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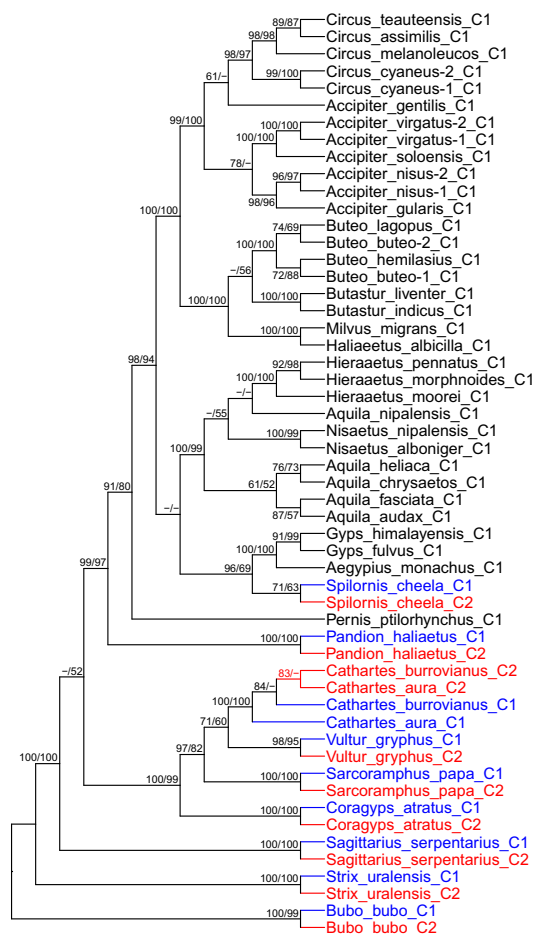
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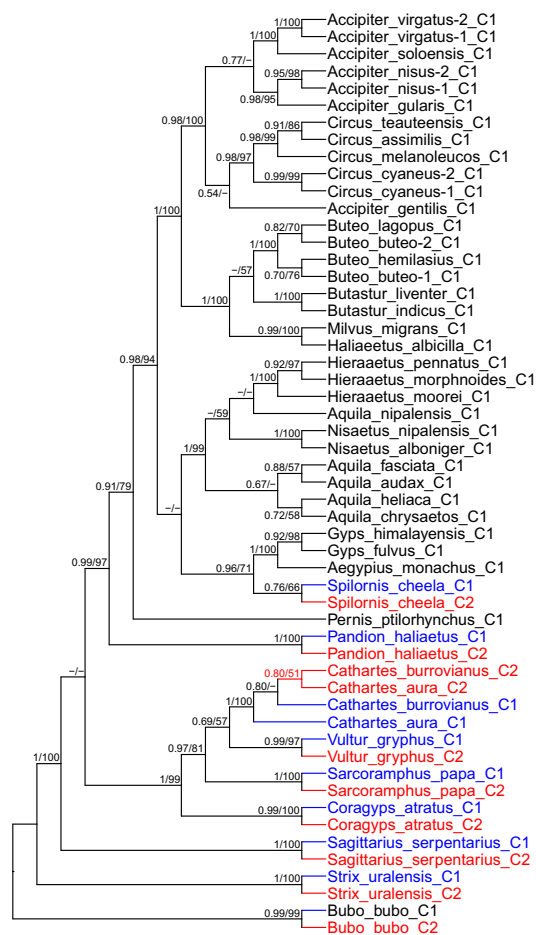
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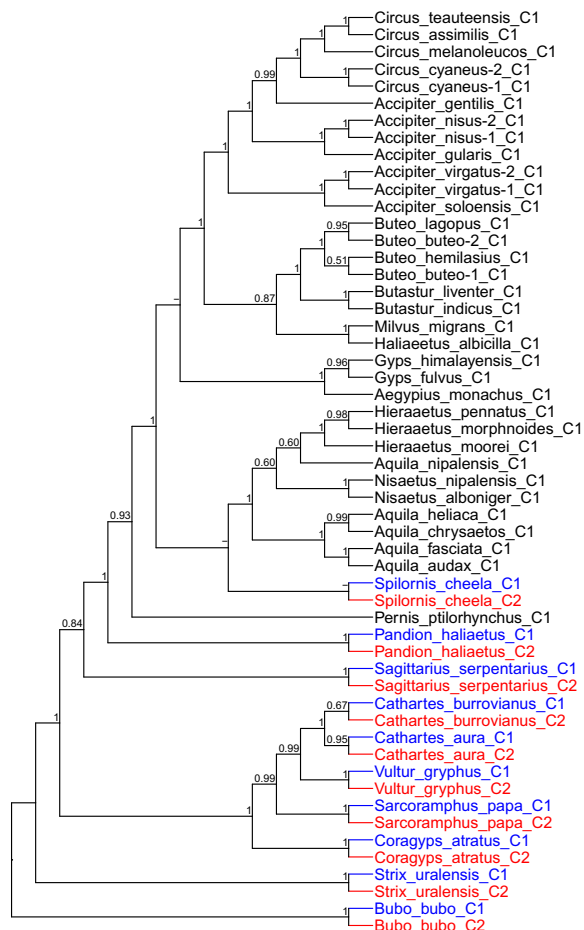
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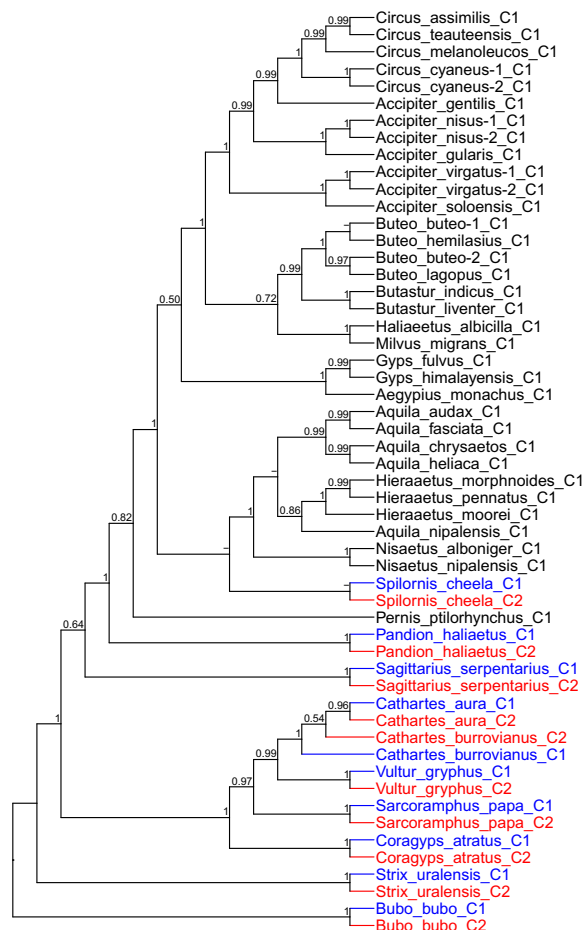
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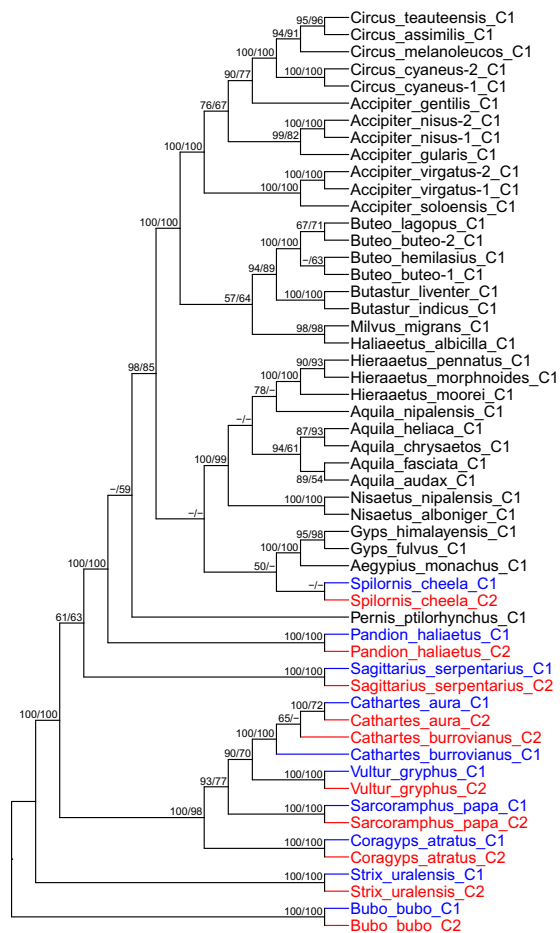
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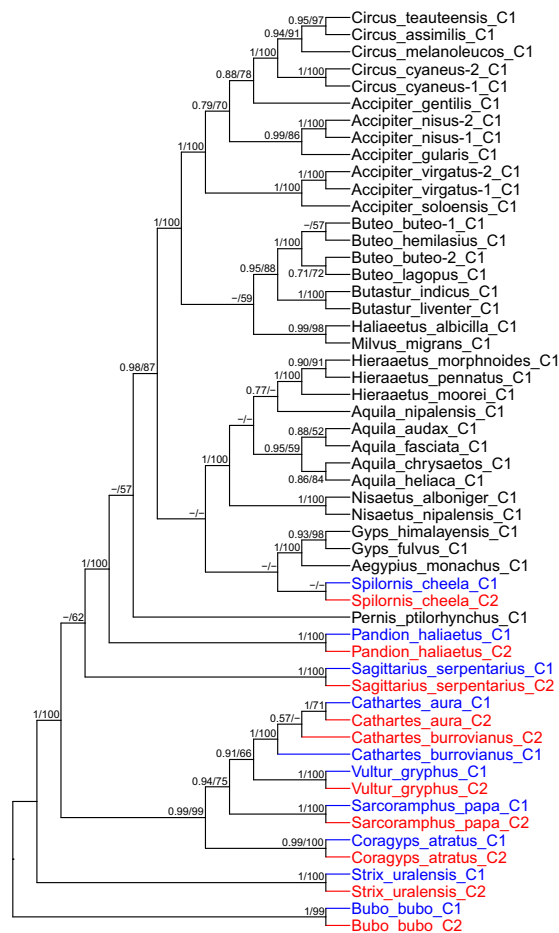
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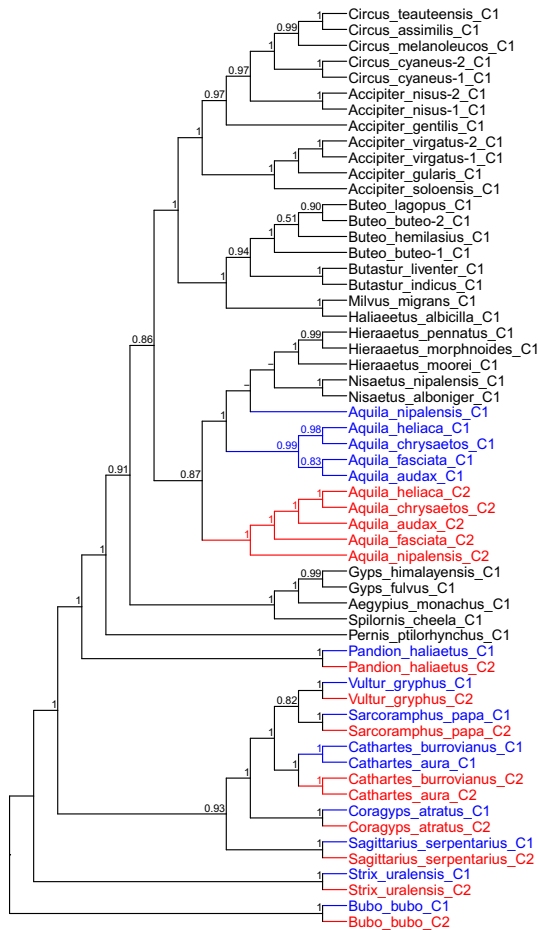
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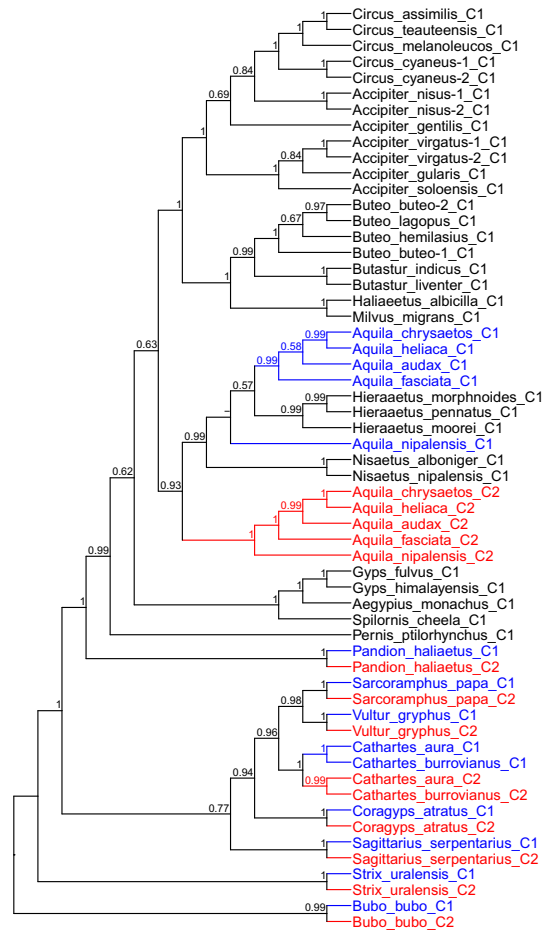
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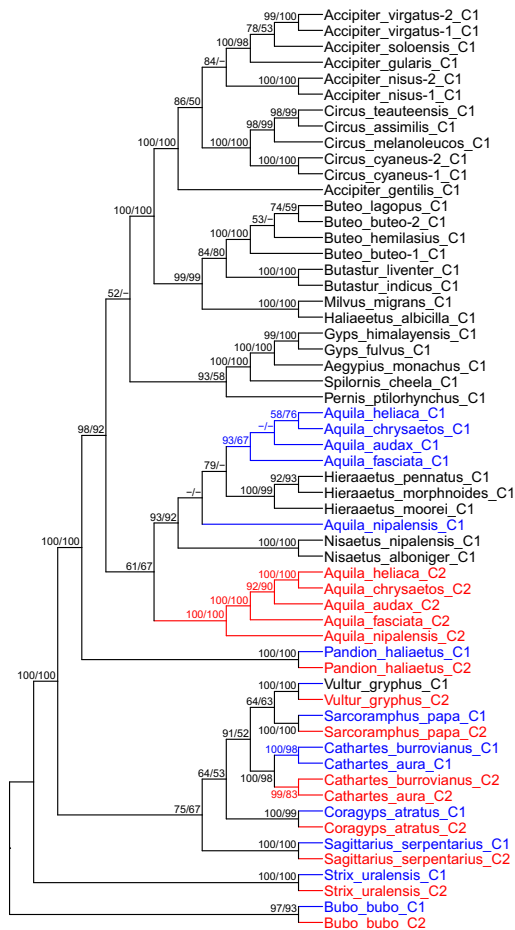
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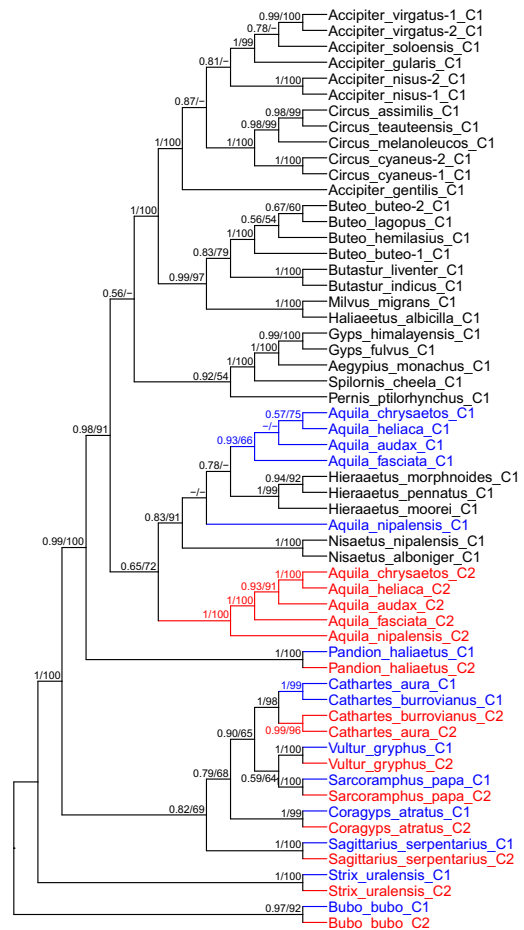
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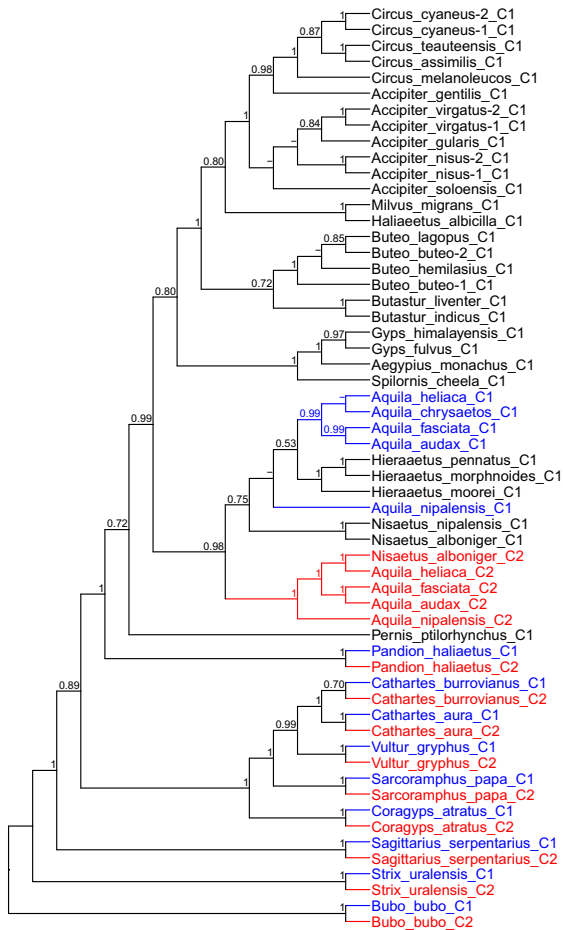
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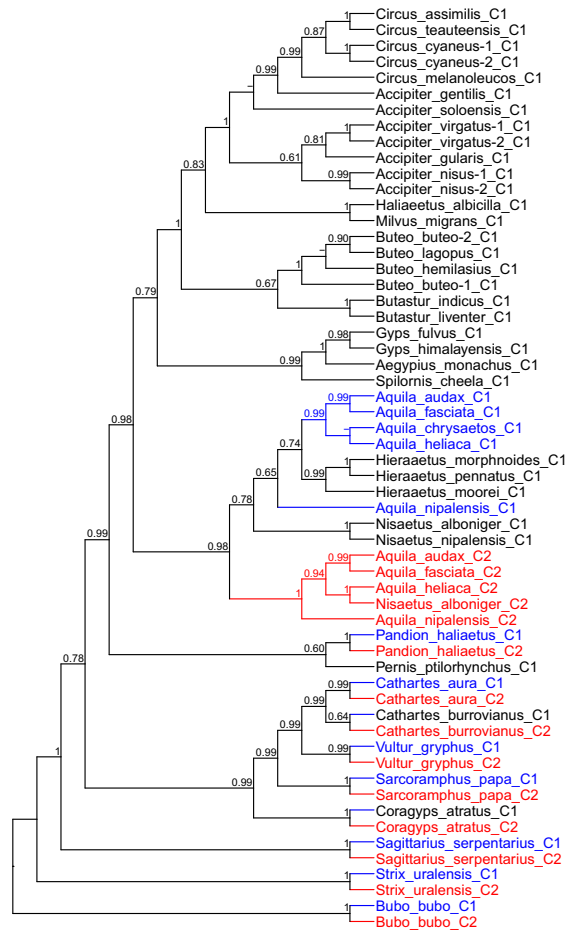
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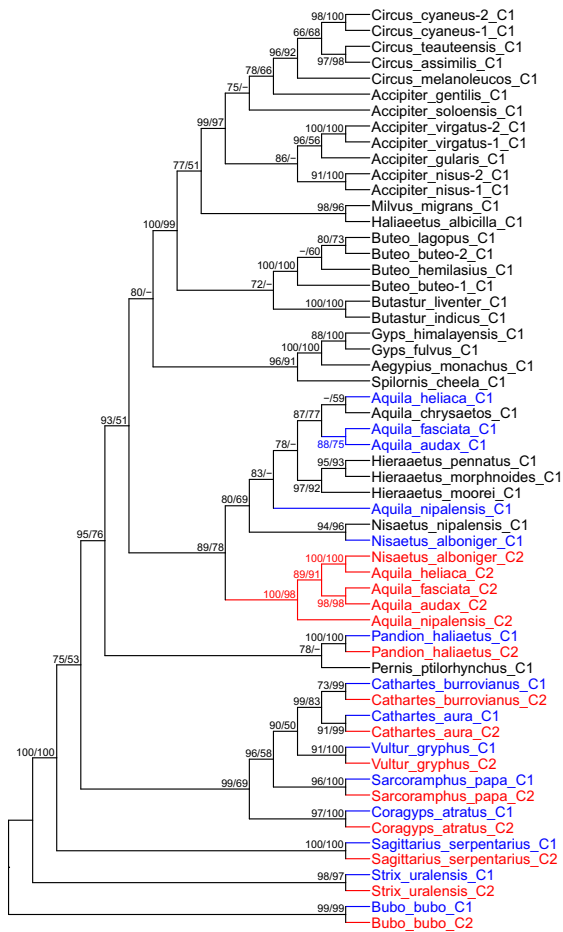
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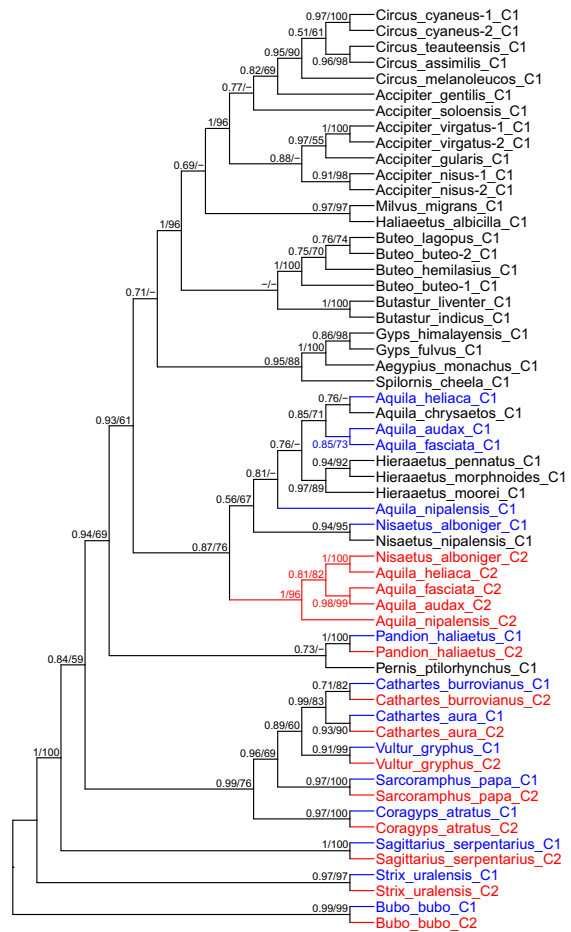
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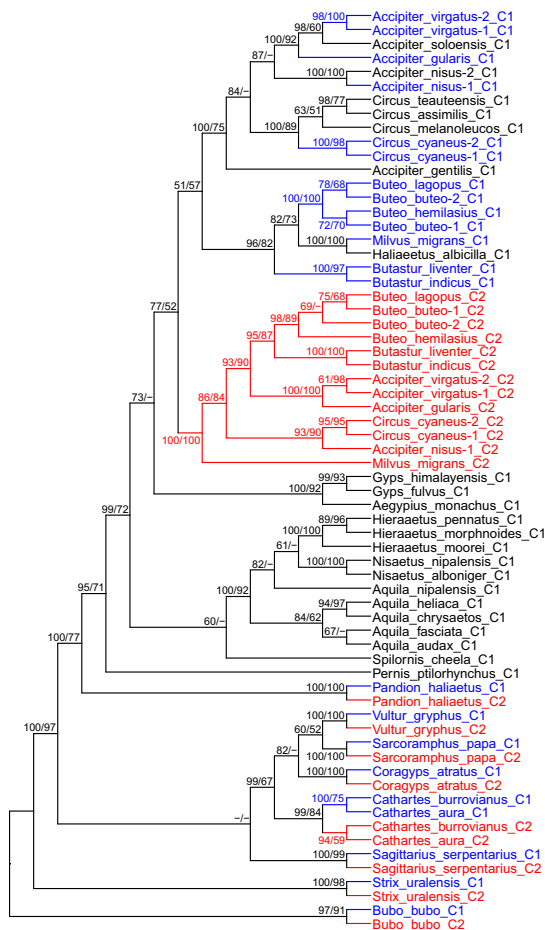
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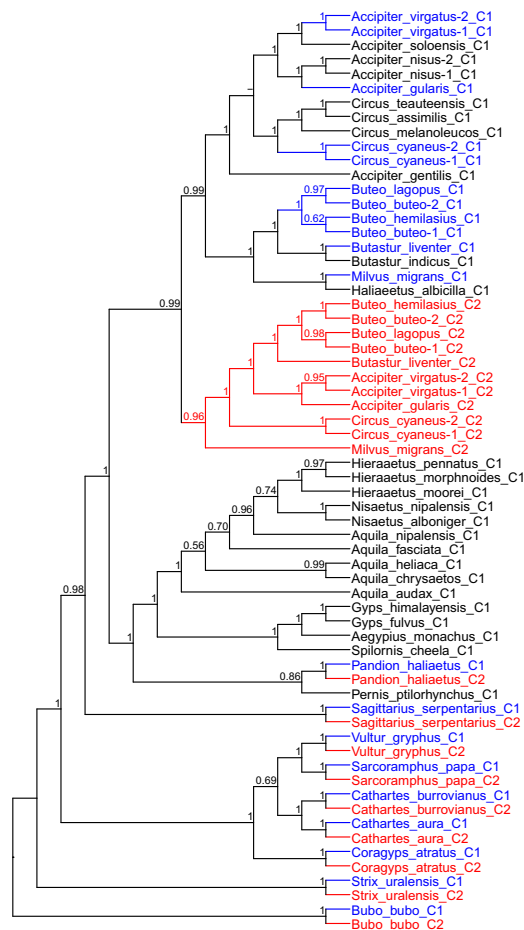
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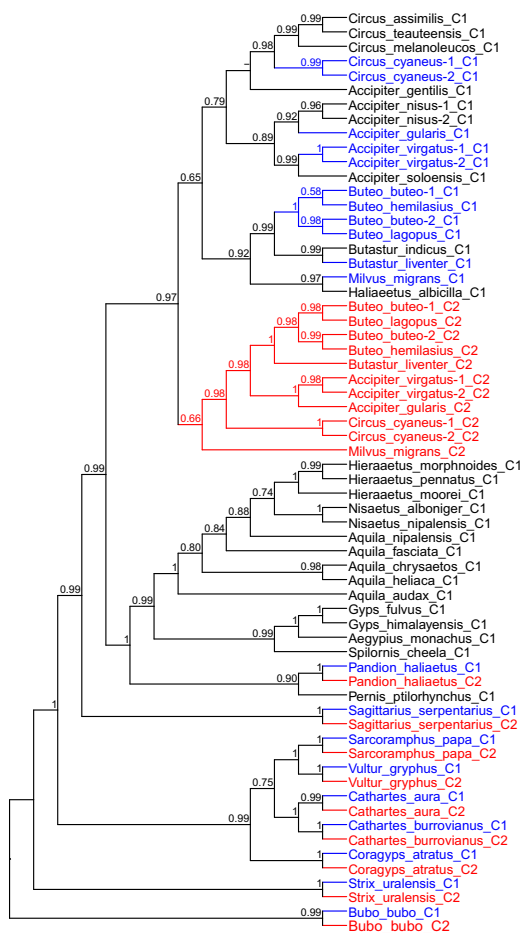
All sites, IQ-Tree



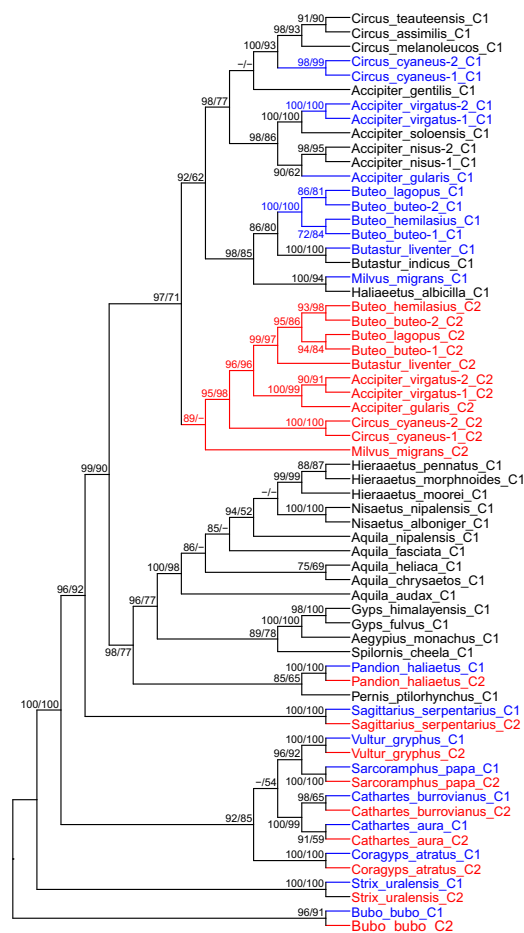
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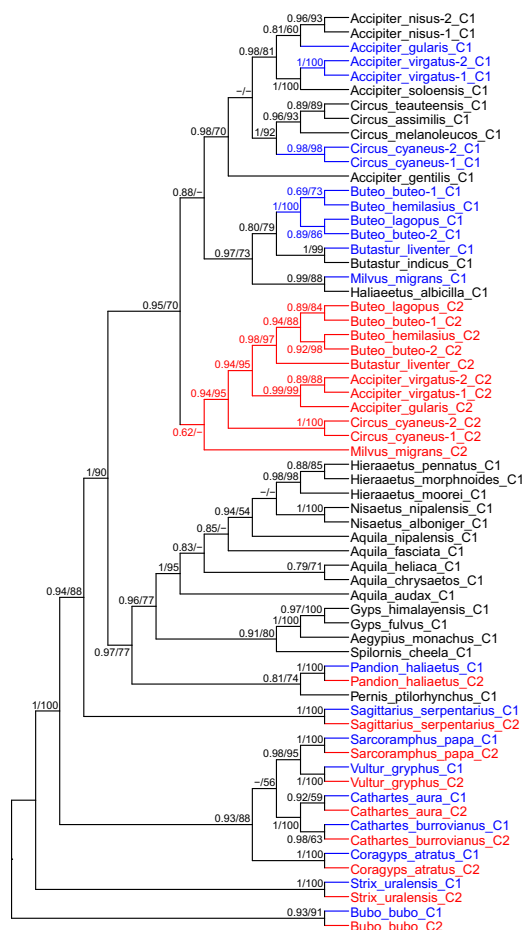


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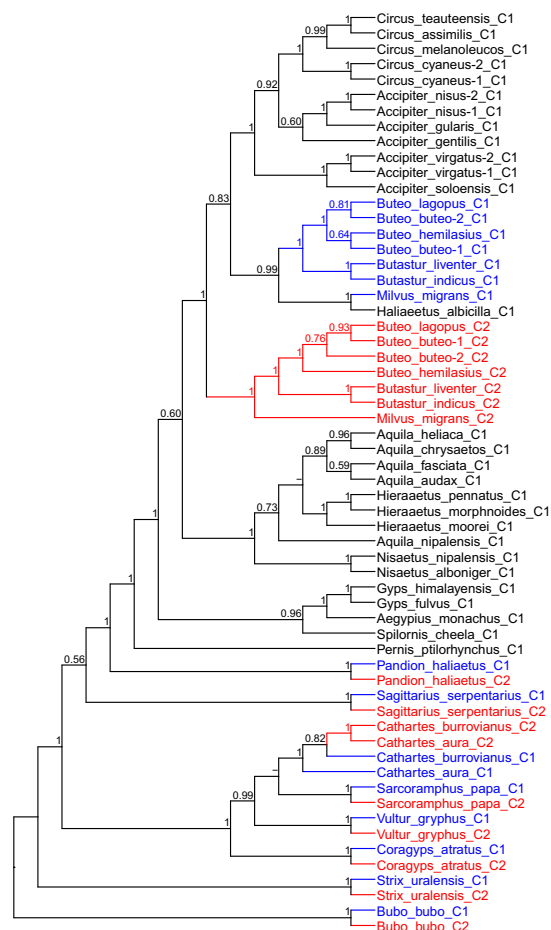




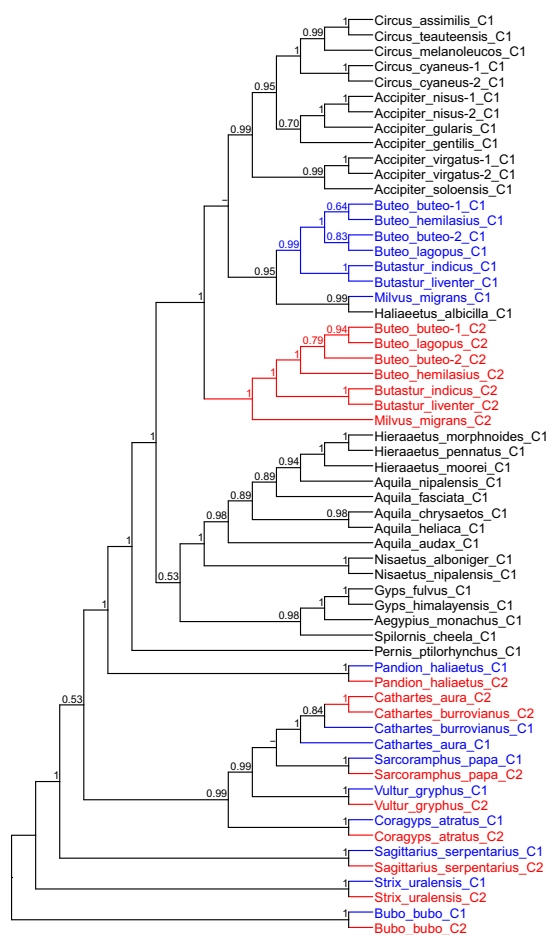
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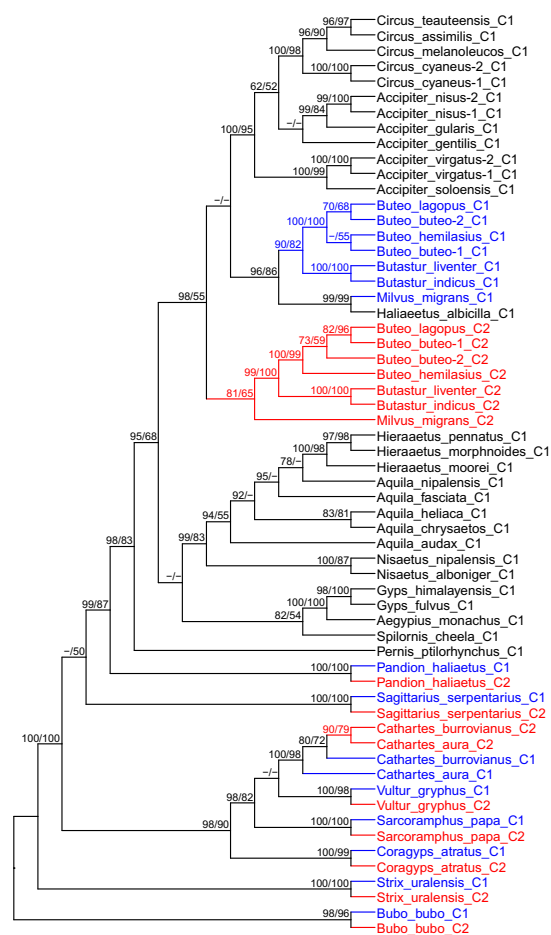
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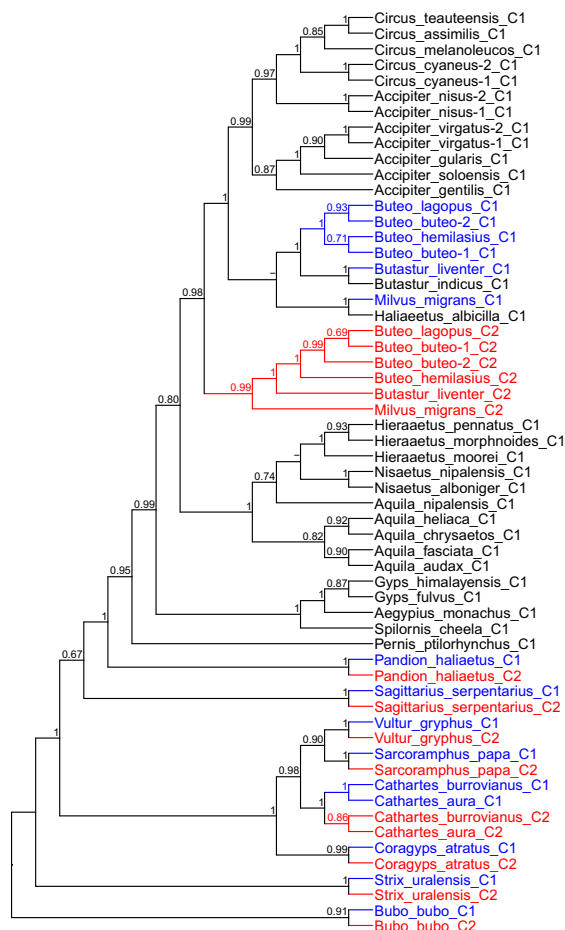
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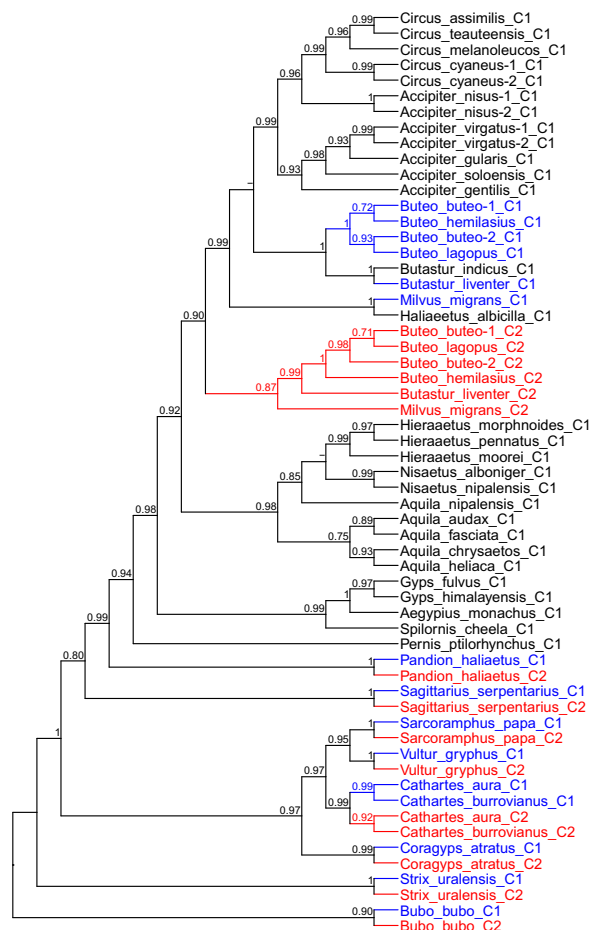
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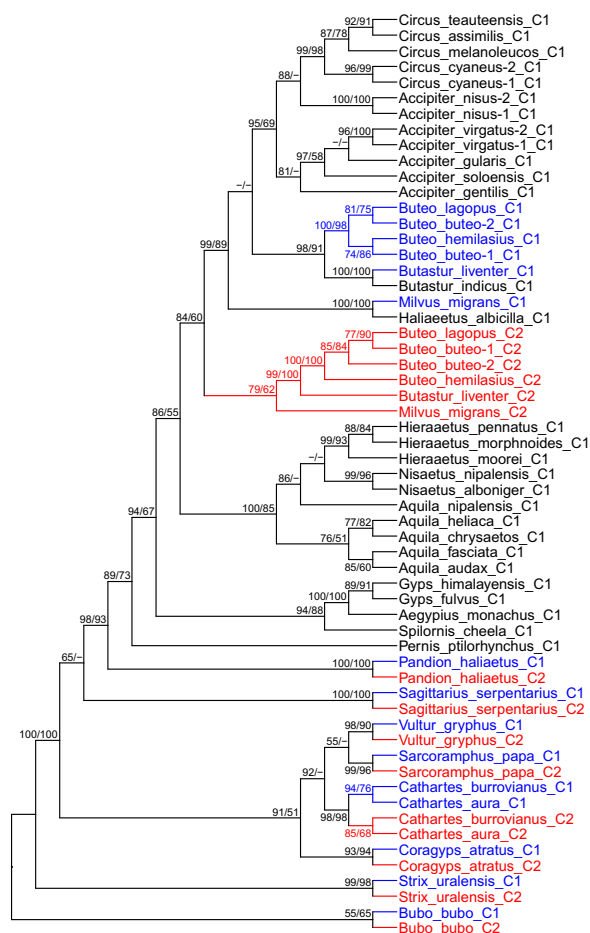
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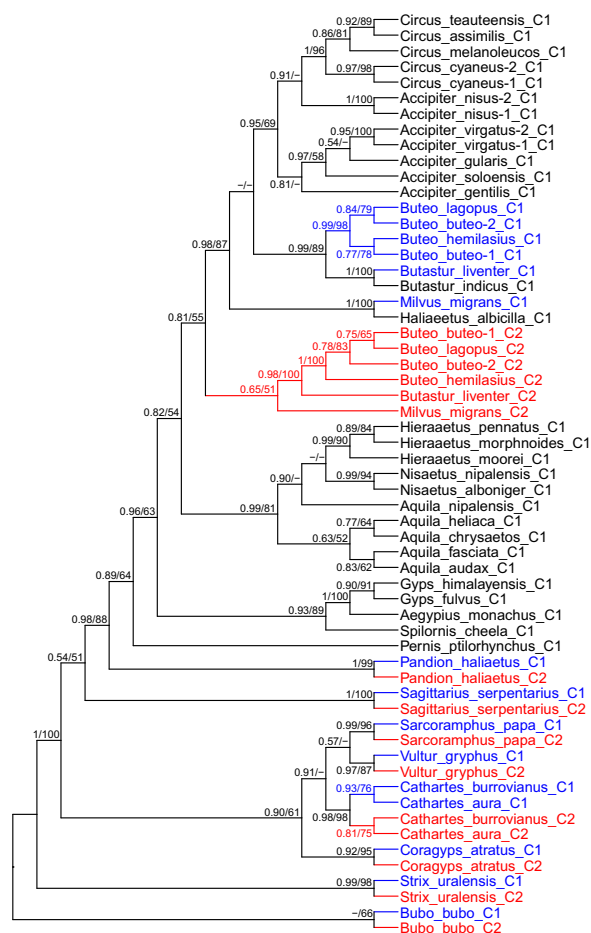
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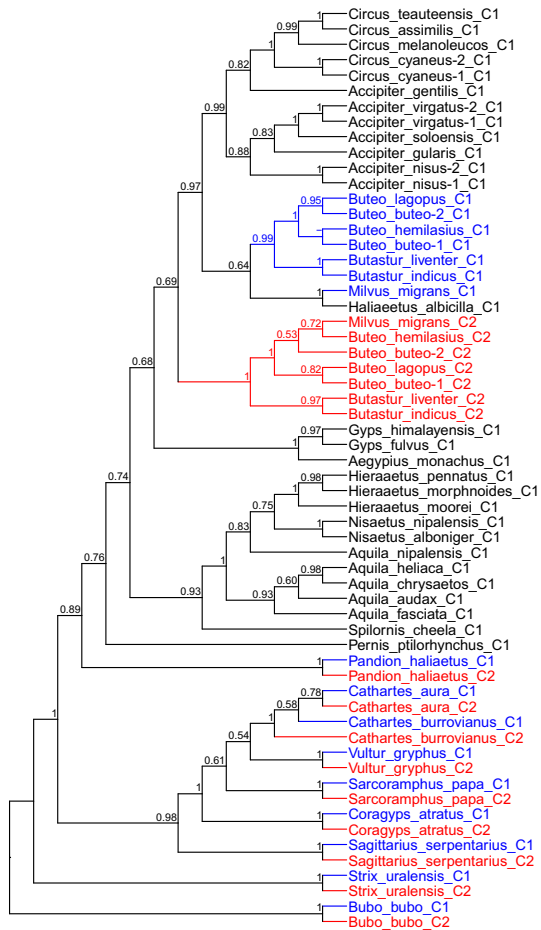


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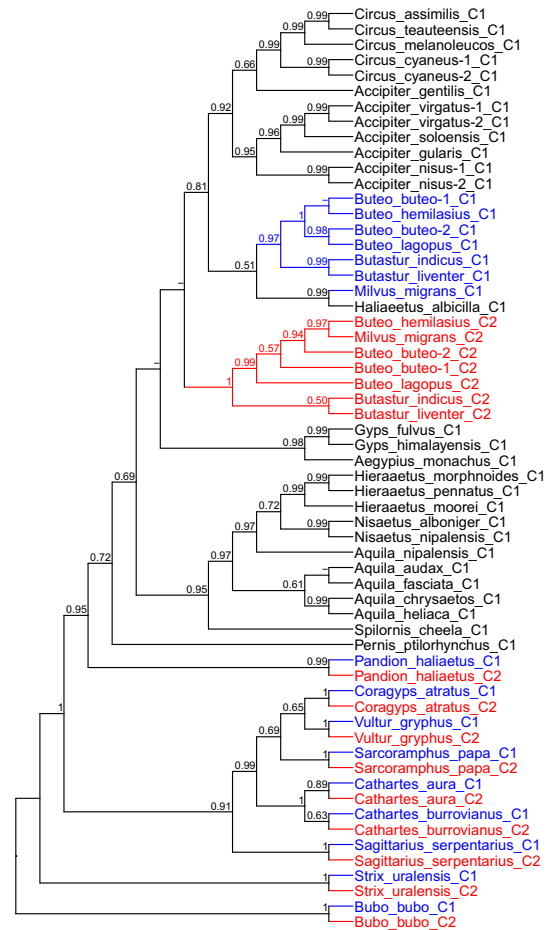




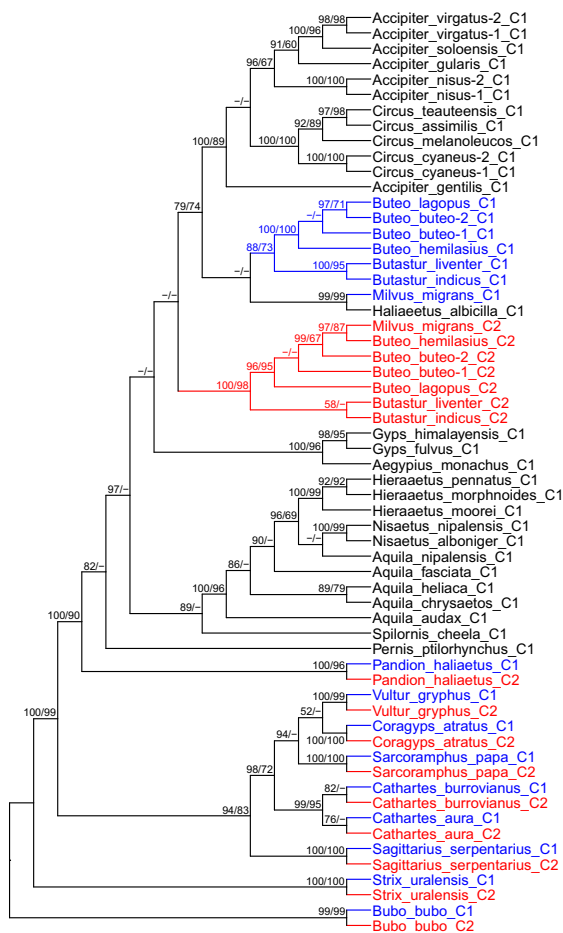
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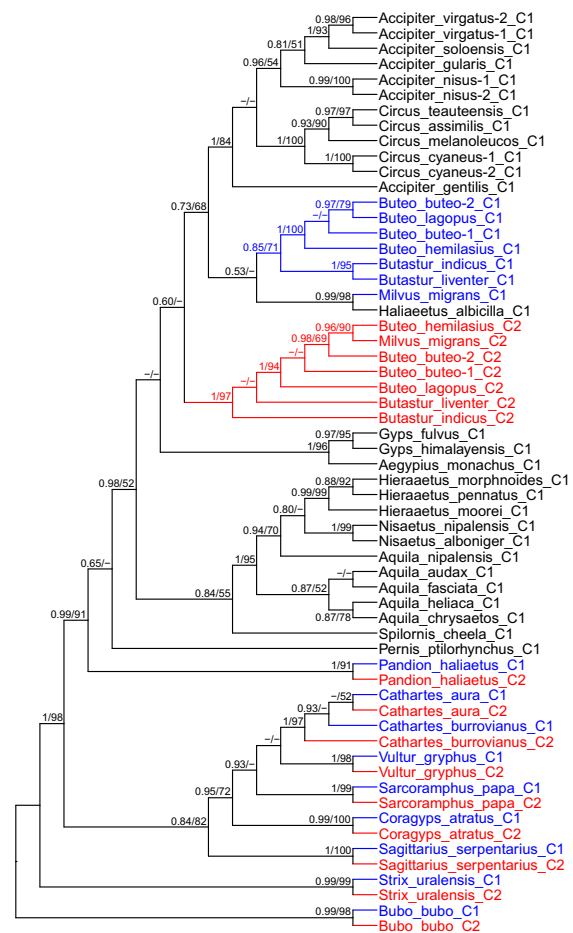
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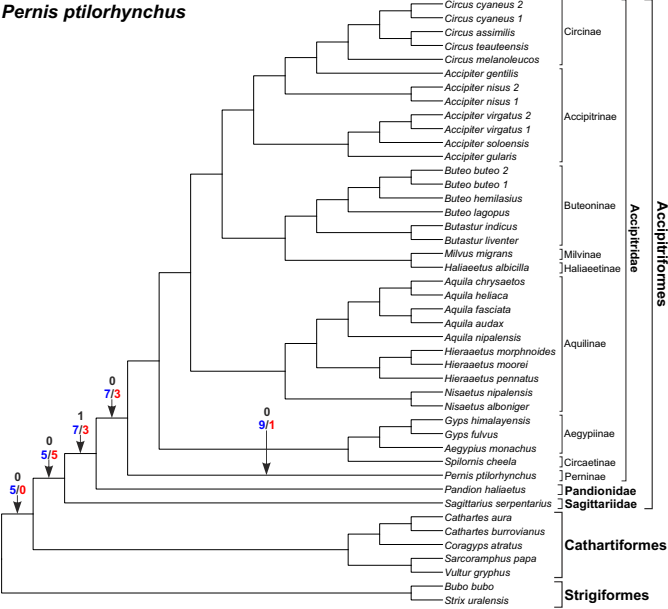


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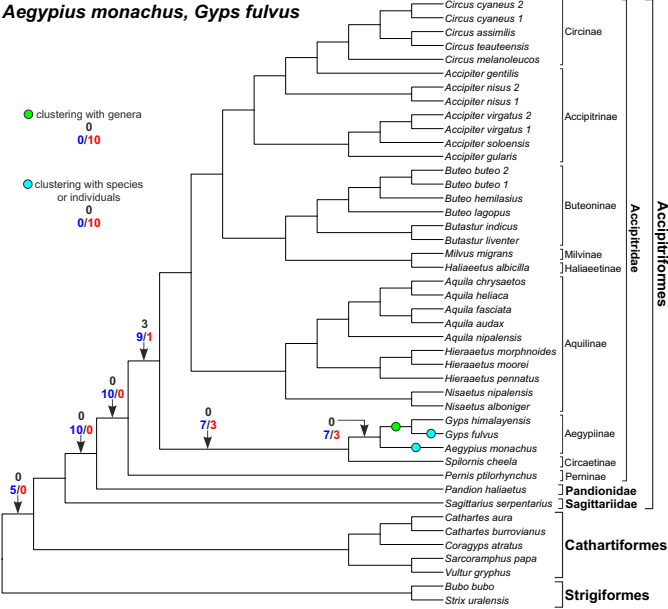


**Figure S9.** Observed and potential phylogenetic positions of second control region copies from given species and groups of Accipitriformes. The positions in major branches are indicated by arrows. The positions within the given groups are marked by circles. In the case of Aquilinae, Accipitrinae, Circinae, Buteoninae, Haliaeetinae and Milvinae, also other not shown positions were considered as obtained in the phylogenetic trees and those assuming the monophyly of some genera, e.g. *Accipiter*. The number of phylogenetic trees supporting the given position are shown in black fonts; the number of tree topology tests that did not reject the given position are shown in blue fonts; the number of tree topology tests that rejected the given position are shown in red fonts. The number after species name indicates the number of individual.

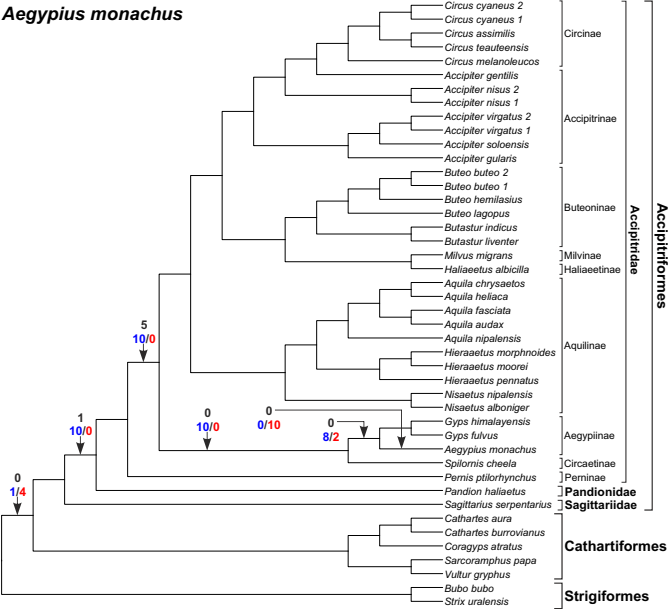
*Pernis ptilorhynchus*



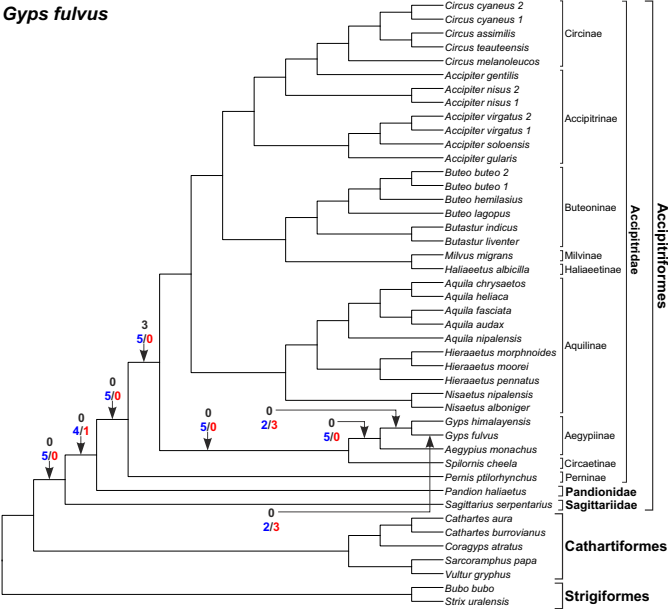
*Aegypius monachus, Gyps fulvus*



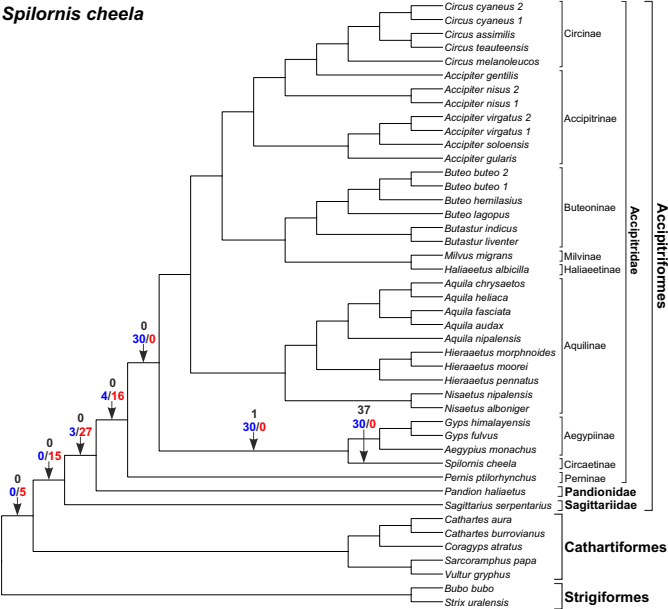
*Aegypius monachus*



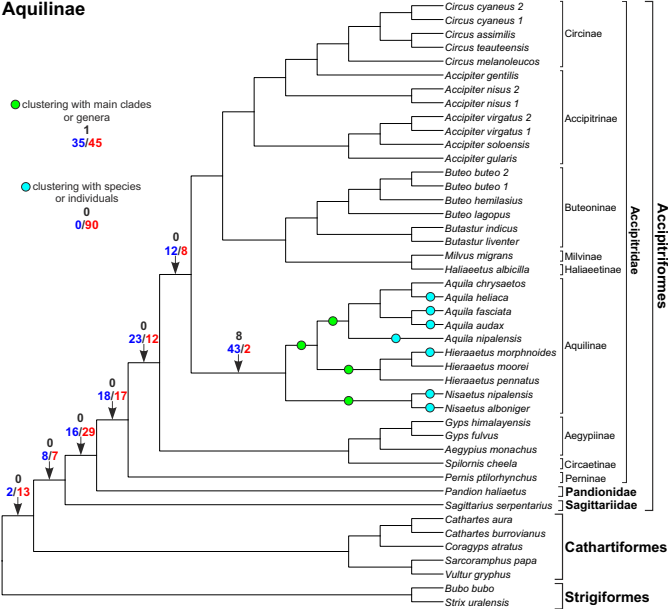
*Gyps fulvus*



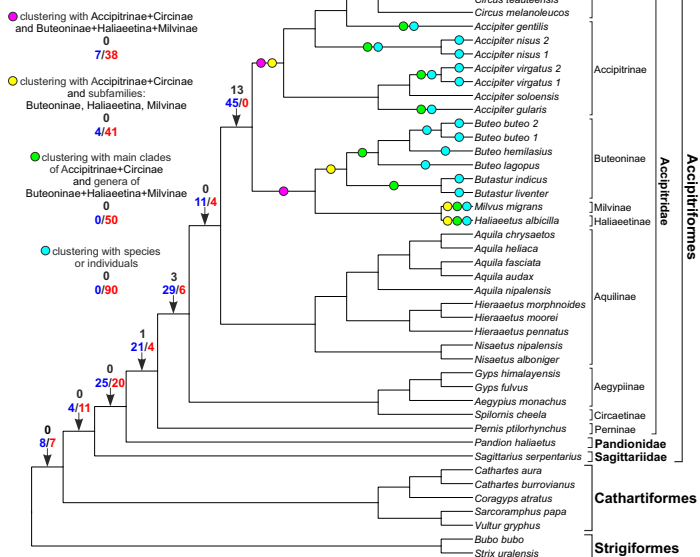
*Spilornis cheela*



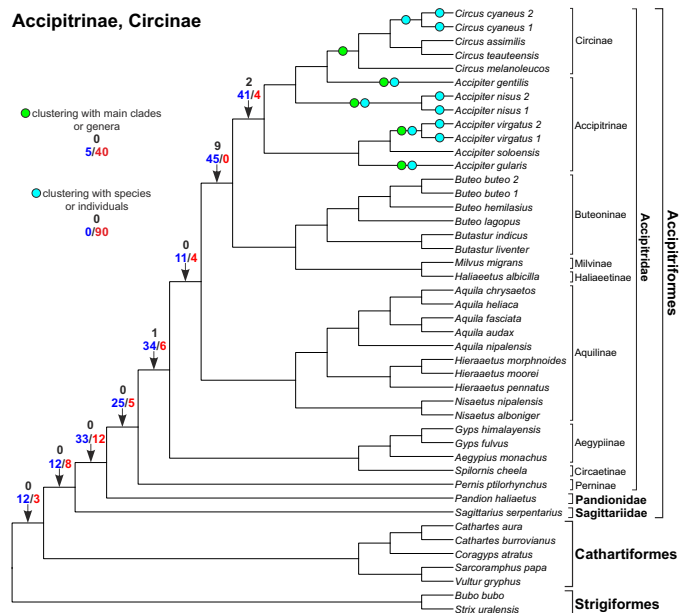
*Aquilinae*



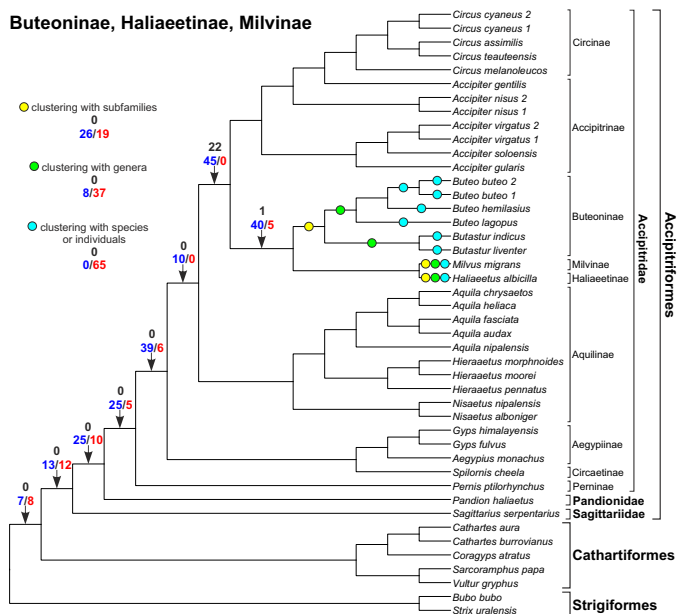
## Accipitrinae, Circinae Buteoninae, Haliaeetinae, Milvinae



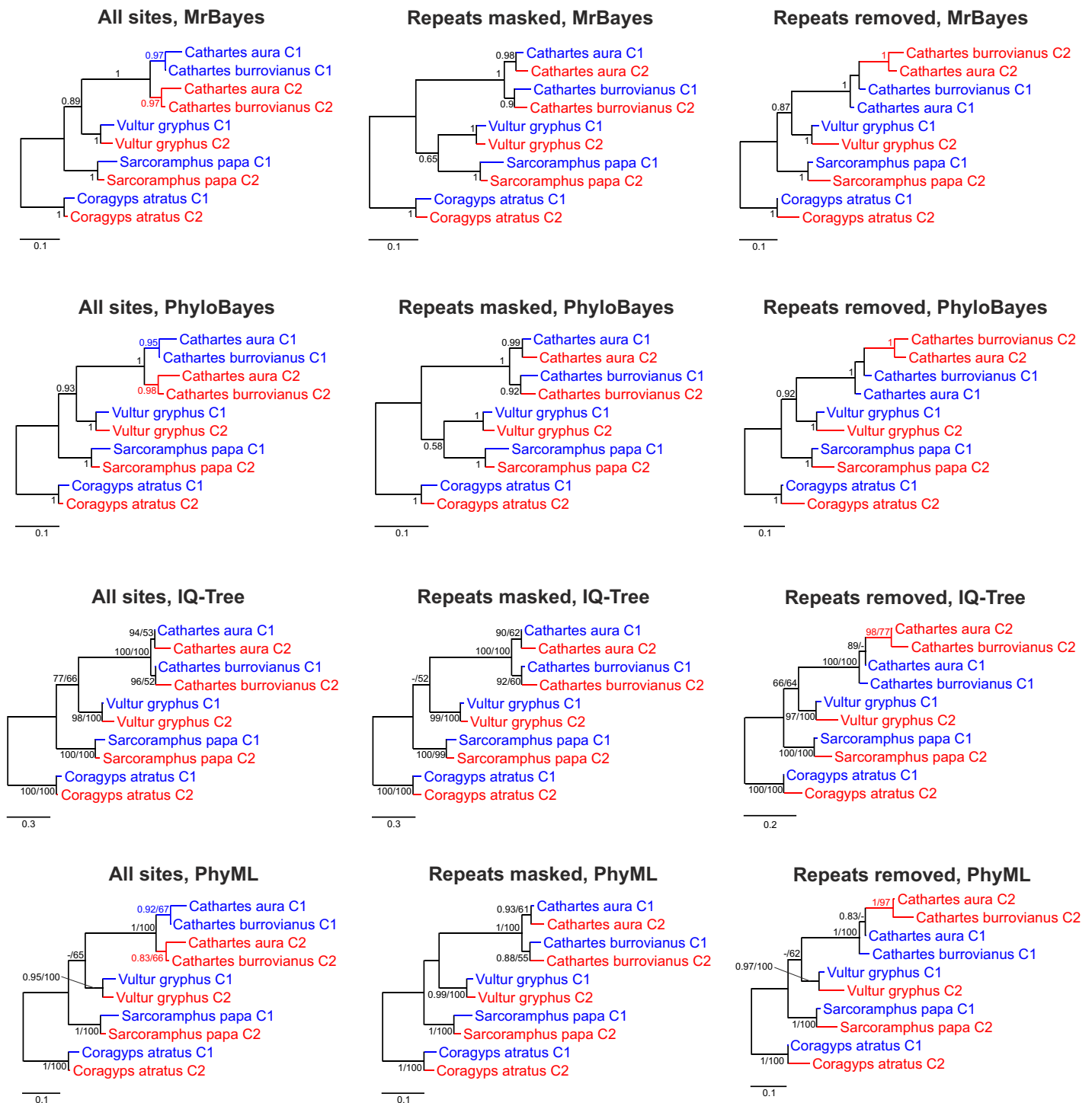
## Accipitrinae, Circinae



## Buteoninae, Haliaeetinae, Milvinae



**Figure S10.** Phylograms obtained in four programs, MrBayes, PhyloBayes, IQ-TREE and (more)PhyML, based on three types of data sets of control regions (all sites, repeats masked or removed) for Cathartiformes. The blue and red colors indicate the corresponding first and second copies of CR, respectively. The values at nodes indicate: posterior probabilities (for MrBayes and PhyloBayes), as well as support values obtained in the approximate likelihood ratio test based on Shimodara-Hasegawa procedure and nonparametric bootstrap (for IQ-TREE and (more)PhyML). The posterior probabilities < 0.5 and the percentages < 50% were indicated by a dash “-.” The number after C indicates the number of control region.





**Figure S11.** Differences between log-likelihood values for individual alignment sites of Cathartiformes control regions for two tree topologies t1 and t2 assuming different relationships between CRs of *Cathartes* (the plot inside). Above and under the plot, there are these two trees and alignment fragments supporting the given topology. The position of these fragments are marked in the plot. The label C1 and C2 as well as blue and red colors indicate the corresponding first and second copies of CR, respectively. Ca - *Cathartes aura*, Cb - *Cathartes burrovianus*.

