

Supplementary information

Birds have peramorphic skulls, too: Anatomical Network Analyses reveal oppositional heterochronies in avian skull evolution

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1. Robustness of the results

Skull modularity of Tyrannosauridae. For the most part, the connectivity modularity pattern of the adult *Tarbosaurus* resembles that of *Tyrannosaurus* from the Werneburg et al.¹ study (Supplementary Fig. 1). Both species share the same number of modules. However, the assignment of some bones is not identical. While in *Tyrannosaurus*, for instance, the nasals are part of the skull roof module that includes also the lacrimal, prefrontal, frontal and postorbital, they belong to the preorbital module in *Tarbosaurus*. As we used a different cluster method (*Ward.2D* instead of *UPGMA*) and treated the fused elements like the nasals, frontals, parietals and vomers as one unit, these discrepancies are probably related to differences in the methodology.

Impact of phylogeny. The repetition of the *pFDA* and ancestral state reconstruction analyses using the crown-group topology of Ericson et al.² does not change the general outcome of the results. As for the Hackett et al.³ topology, non-avian theropods cluster closer with juvenile than adult birds (Supplementary data 2 file). Mapping *PCo1* and *PCo2* onto both trees, the substitution of adult crown-group species with the juvenile specimens is more parsimonious for both topologies (using the standard deviation as a proxy for parsimony). This effect becomes even clearer after *PCo* values are standardized for early juvenile size (i.e., residual values; Supplementary data 2 file). This signal retains after reshuffling the terminal taxa 1,000 times across the topology. This is also true for *PCo1* when the log-transformed product of ancestral values is used as a proxy for parsimony. In contrast, for *PCo2* the signal is only present between adults and juveniles standardized for size (Supplementary Fig. 4). Nevertheless, we consider the heterochronic signal to be robust with respect to the phylogenetic relationships.

Impact of network parameters on PCoA. In a recent study Esteve-Altava et al.⁴ applied multidimensional scaling in form of principal component analysis (*PCA*), using a different set of the network parameters, including nodes (*N*), links (*K*), density (*D*), clustering coefficient (*C*), path length (*L*), heterogeneity (*H*), assortativity (*A*), and parcellation (*P*). To test, if the selection of network parameters affects the outcome of the *PCoA*, we applied different combinations of parameters and compared the distribution of the resulting *PCo1* and *PCo2* values with the original ones (Supplementary Fig. 5). In all cases, ordinary least square linear regression analyses indicate a highly significant correlation between the outcome of the original and alternative *PCoA*'s. This is even the case for *PCo1*, if only the two parameters *N* and *K* are selected (Supplementary data 2 file). These comparisons indicate that the outcome of our original analysis seems to be robust in terms of the selection of network parameters.

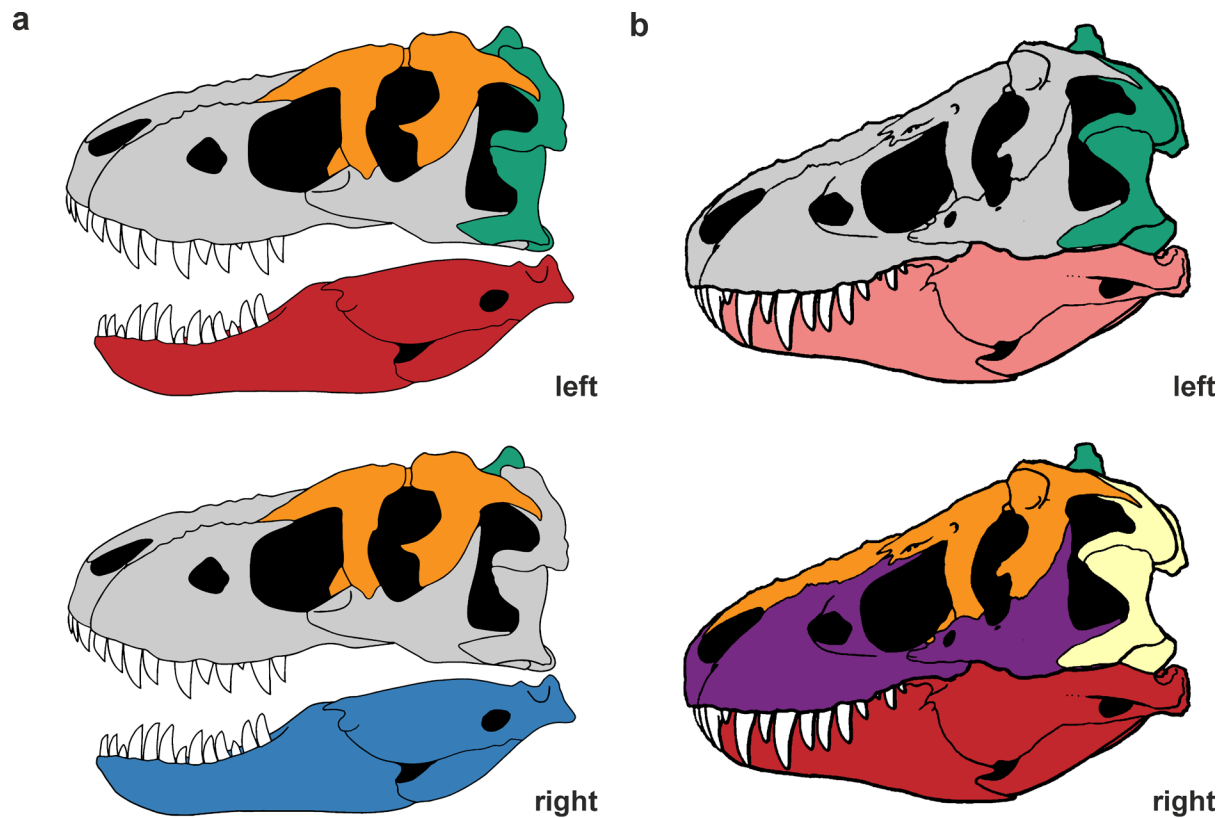
Impact of module asymmetry. As reported in the main text, many species in our dataset have a left-right asymmetry in terms of module distribution. This asymmetry might be partly explainable with the methodology, in which modularity is computed in the basis of cluster analysis with dichotomous branching¹. To test this, we applied three alternative network analyses for the basal avialan *Archaeopteryx lithographica*: 1) skull configuration including only paired bones, 2) skull configuration including only paired bones from the left side, and 3) skull configuration including only paired bones from the left side and unpaired bones (see Supplementary Table S1). The deletion of unpaired bones, results in a fully symmetric modularity between the left and right skull side, in which *A. lithographica* possesses left and right snout modules including the zygomatic arches and majority of palatal bones, left and right skull roof/temporal modules and left and right mandible modules. This pattern is more or less similar to the module distribution of the left skull side in the original analysis (Supplementary Fig. 6). Analyzing only one skull side reveals quite similar module distributions, although here the zygomatic arch and palatal bones form an own module (Supplementary Fig. 7). Based on these comparisons, we conclude that module asymmetry is at least partly driven by the presence of unpaired elements, but the phenomenon has to be tested in more detail in the future. However, as the deletion of the *S*-modules and *Q*-modules and the corresponding $Q_{\max}$ parameter from the *PCoA* did not change the general outcome of the analysis, module asymmetry seems not to affect our macroevolutionary findings.

2. Anatomical Network Analysis (*AnNA*) vs. *EMMLi* analysis

While *AnNA* estimates the number of modules based on the number of bones and their connections to each other (see connectivity modularity)⁵, *EMMLi* based on geometric morphometric shape data (see variational modularity)⁶. For adult bird skulls, both analyses produced a different number of modules, in which *EMMLi* produces a higher number of

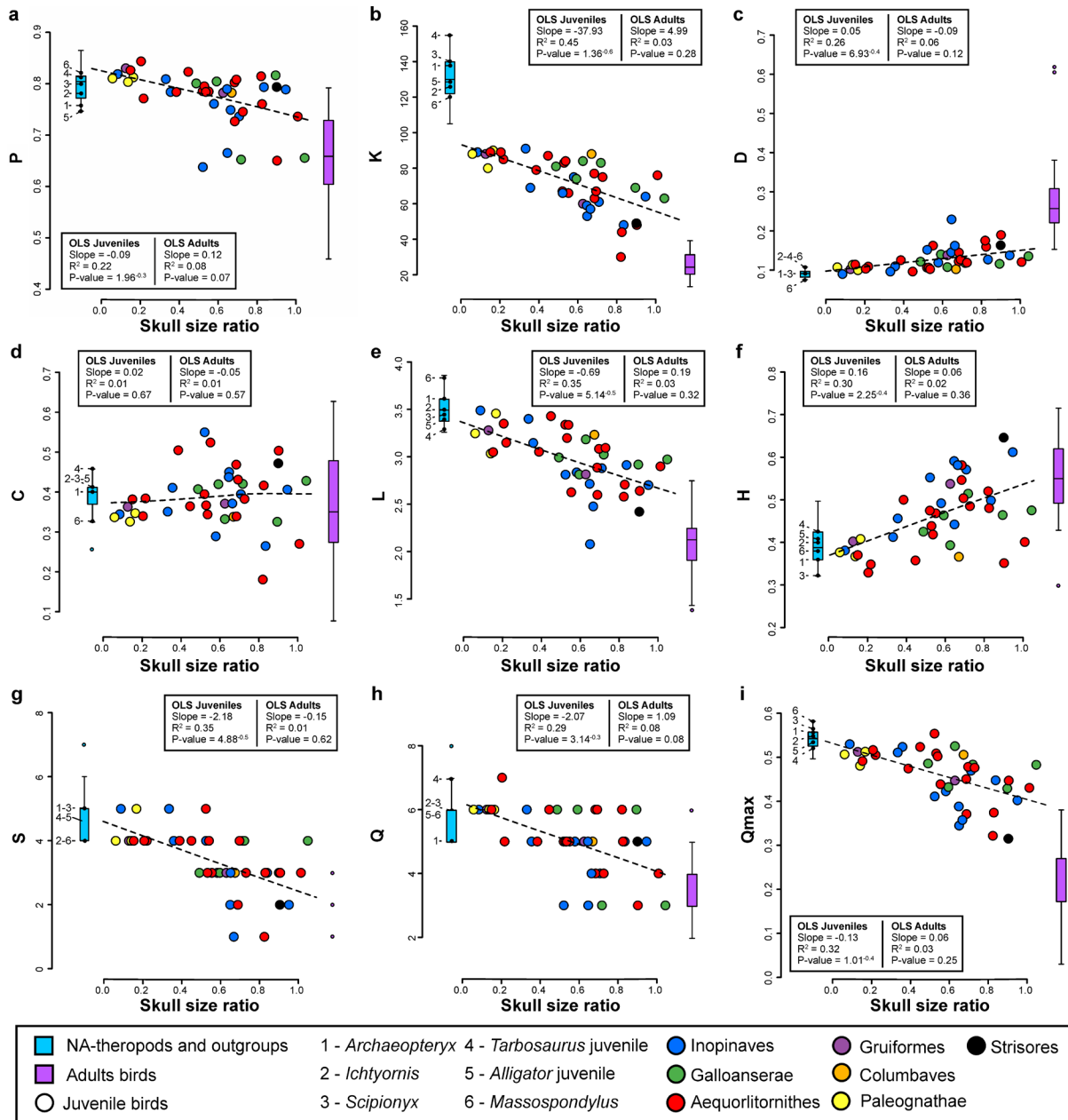
modules than *AnNA*^{1,7,8}. This discrepancy is obviously a result of the different methodological approaches, measuring different types of modularity. However, as indicated by our study, juvenile birds actually possess a higher number of modules than adults, which fall in the range of the *EMMLi* approach. Thus, it could be possible that the latter approach is just less sensitive to ontogenetic changes that include a reduction of modules due to bone fusion. In this case, *EMMLi* reproduces the juvenile condition with a high module number as the best solution for geometric morphometric shape data. How this outcome is dependent on the respective (semi-)landmark concept and the superimposition method has to be tested in the future⁹. Nevertheless, both methodologies provide congruent results, as non-avian theropods have a higher cranial modularity than crown group birds^{1,7,8}.

3. Supplementary figures



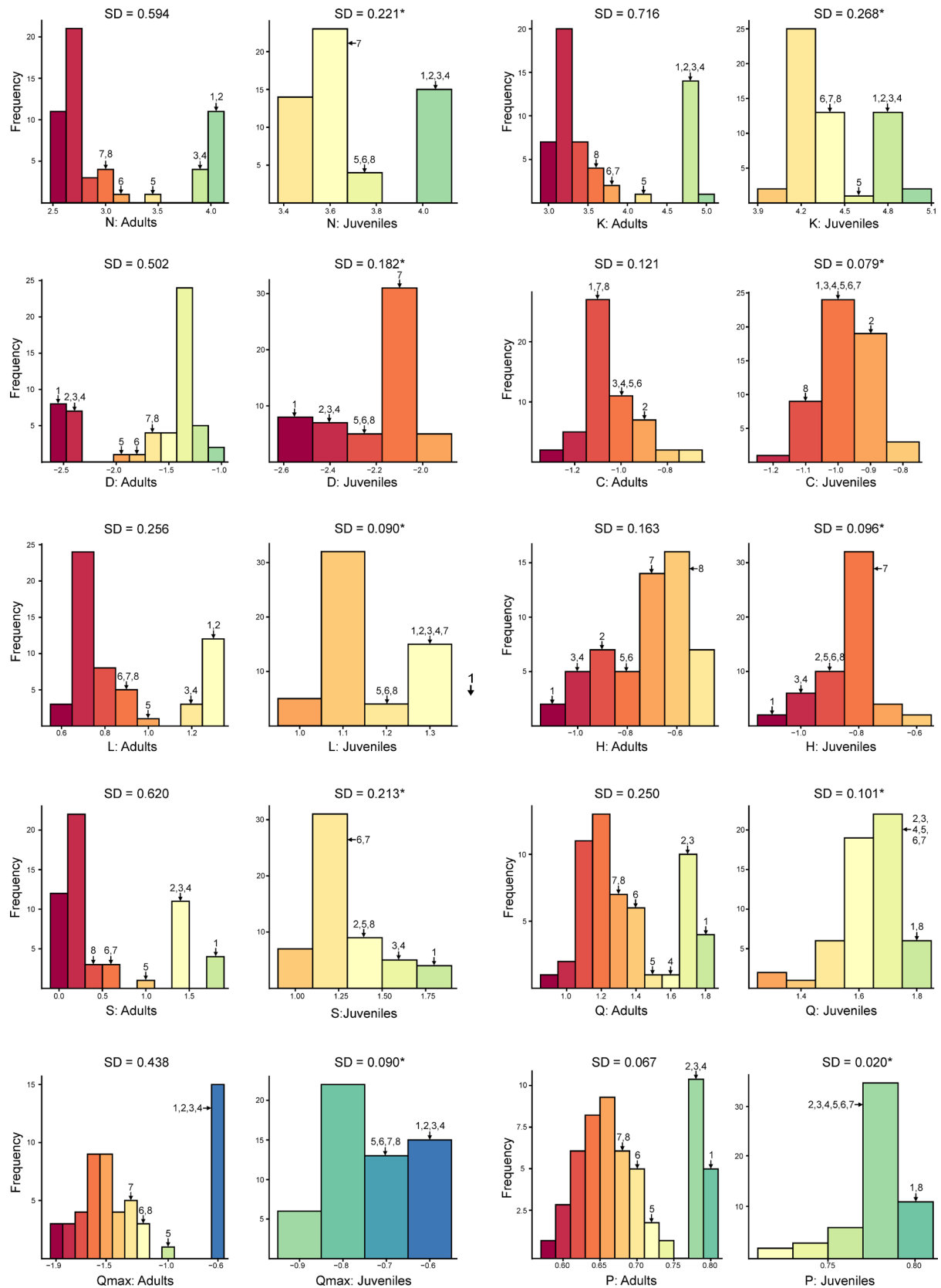
Supplementary Figure 1 | Comparison of skull modularity in large bodied

tyrannosaurids. **a**, *Tarbosaurus bataar* (modified after Hurum & Sabath¹⁰) using *Ward.2D* cluster (this study). **b**, *Tyrannosaurus rex* (modified after Carr¹¹) using *UPGMA* cluster¹.



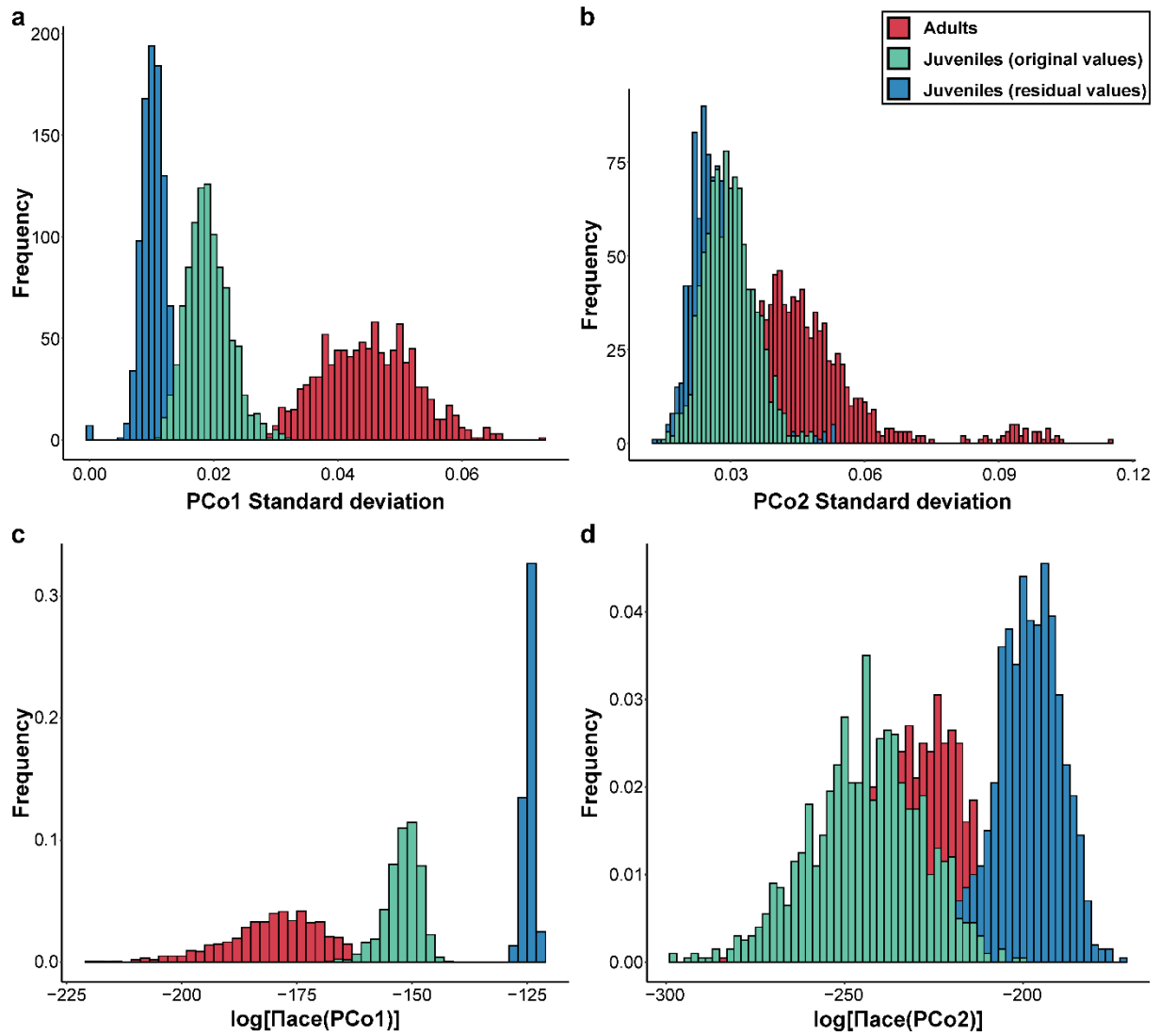
Supplementary Figure 2 | Range of number of network parameters in non-avian archosaurs, juvenile and adult birds. a, Parcellation (P). **b,** Number of connections (K). **c,** Density of connections (D). **d,** Mean clustering coefficient (C). **e,** Mean shortest path length (L). **f,** Variance of connectivity (H). **g,** Number of S -modules. **h,** Number of Q -modules. **i,** The strength of modular organization is expressed by the parameter Q_{max} . For juvenile birds, the range of network parameters is plotted against relative skull size (ratio of skull box volume). Results of ordinary least square regression analysis (OLS) describing the correlation

between network parameters and relative skull size of juvenile and adult birds are given in the box. The plot for the number of nodes (N) is given in Fig. 2 of the main text.

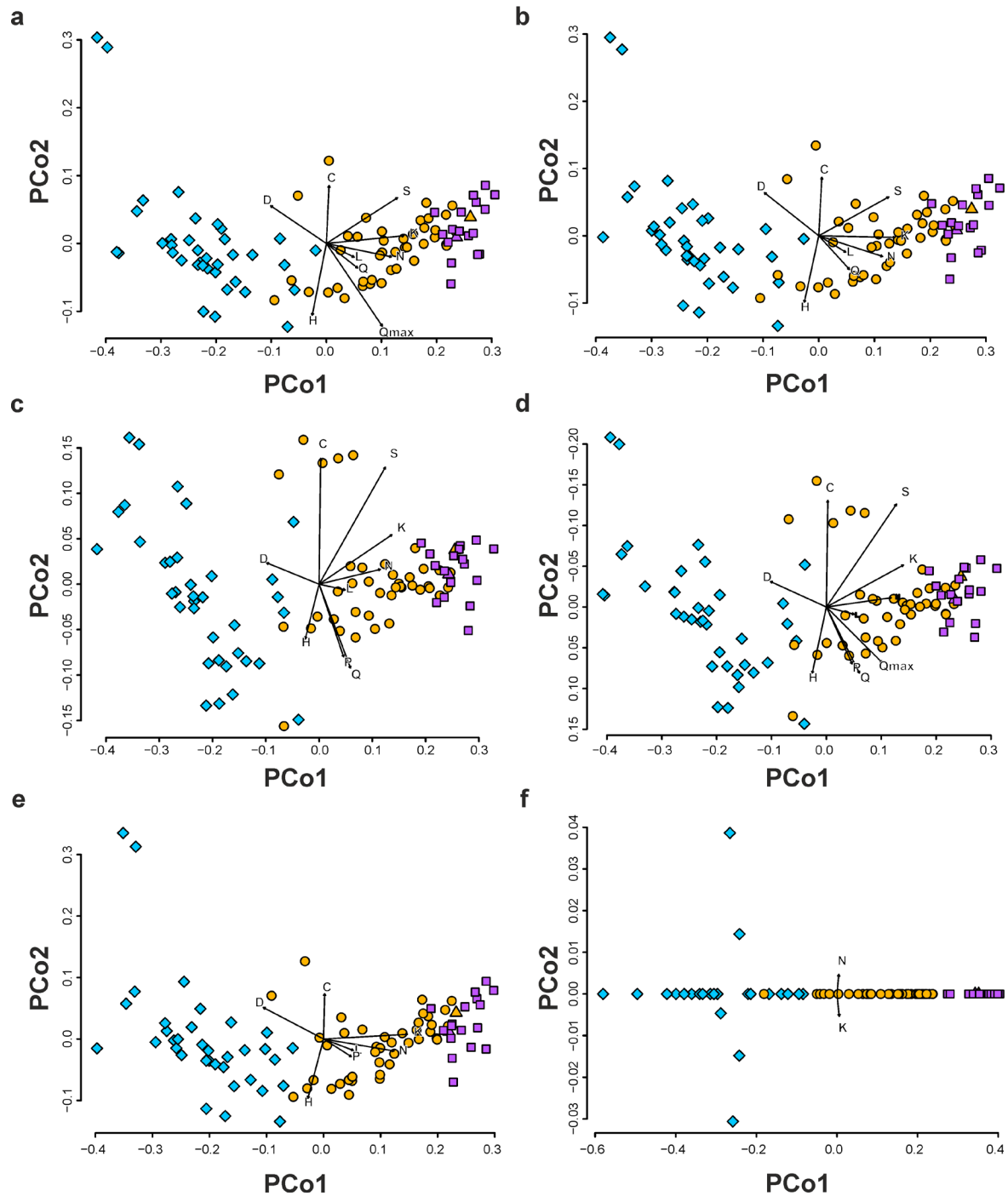


Supplementary Figure 3 | Histograms summarizing the distribution of ancestral values all network parameters and *PCo1* for adult and juvenile crown birds (original values).

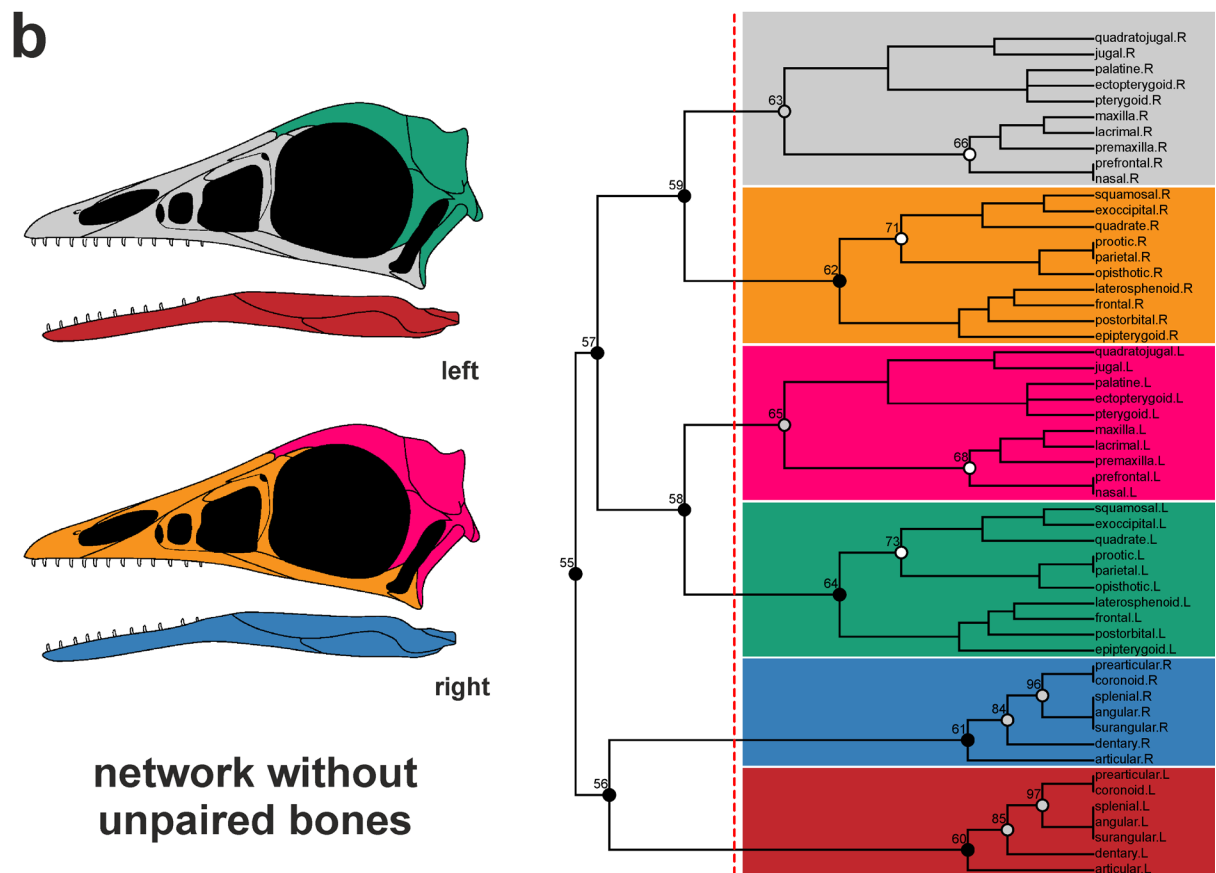
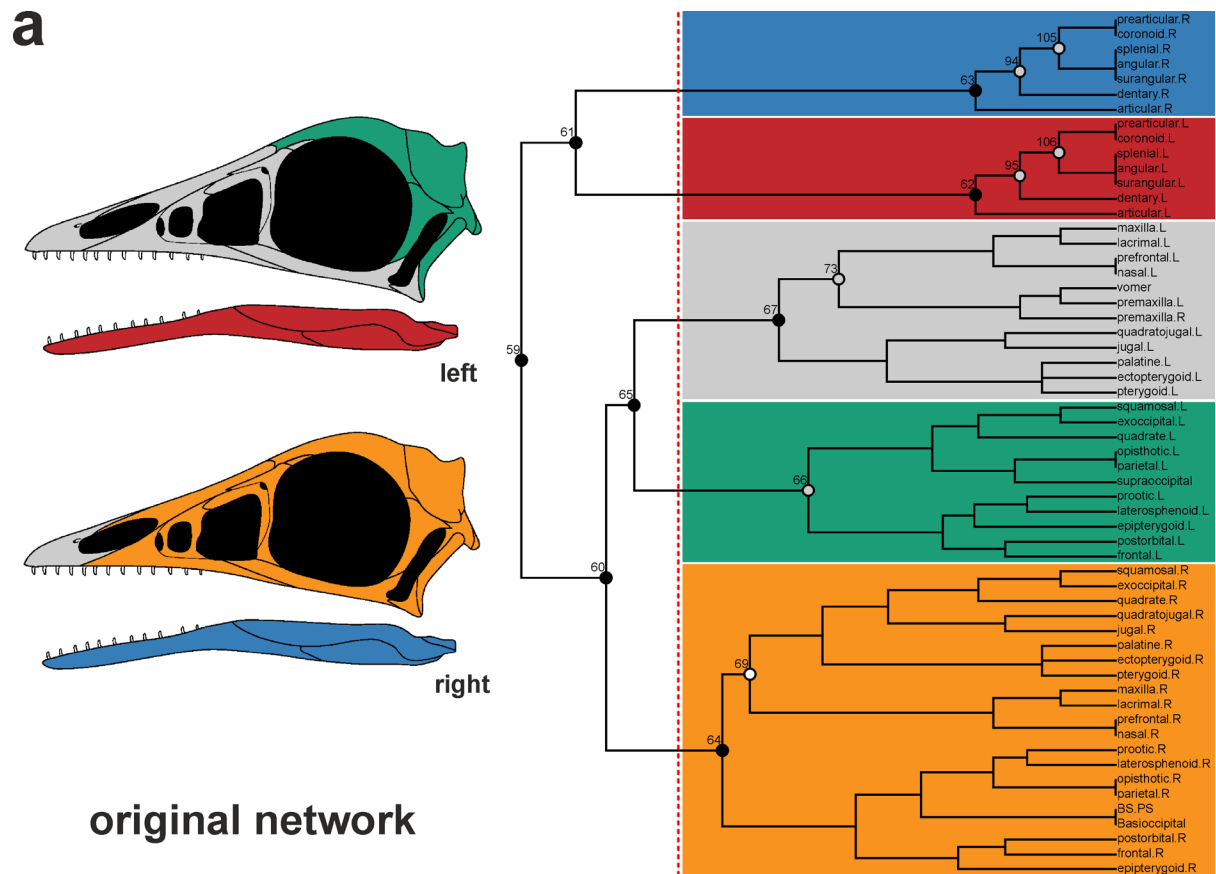
For all parameters, the hypothetical substitution of adult crown birds by their juvenile counterparts leads to a more parsimonious evolution as indicated by the standard deviation *SD* (*). (1) Theropoda; (2) Coelurosauria; (3) Eumaniraptora; (4) Avialae; (5) Ornithurae; (6) Aves; (7) Neognathae; (8) Palaeognathae.



Supplementary Figure 4 | Histograms of ancestral values of *PCo1* and *PCo2* after permutation over 1,000 trees. a, Standard deviation of ancestral values of *PCo1*. **b,** Standard deviation of ancestral values of *PCo2*, **c,** Log-transformed product of ancestral values of *PCo1*. **d,** Log-transformed product of ancestral values of *PCo2*.



Supplementary Figure 5 | Principal coordinate analyses based on different network parameters. **a**, original *PCoA*. **b**, *PCoA* without modular partition $Q_{\max}$. **c**, *PCoA* with parcellation P and without $Q_{\max}$. **d**, *PCoA* with P and $Q_{\max}$. **e**, *PCoA* with P and without S -modules, Q -modules and $Q_{\max}$. **f**, *PCoA* just based on number of bones N and bone contacts K .

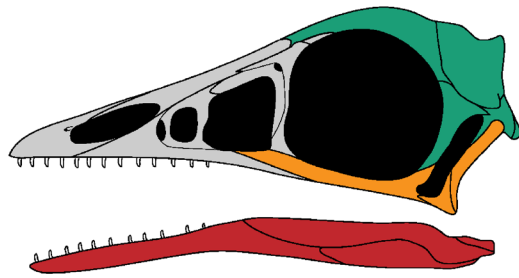


Supplementary Figure 6 | Alternative modularity for *Archaeopteryx lithographica*

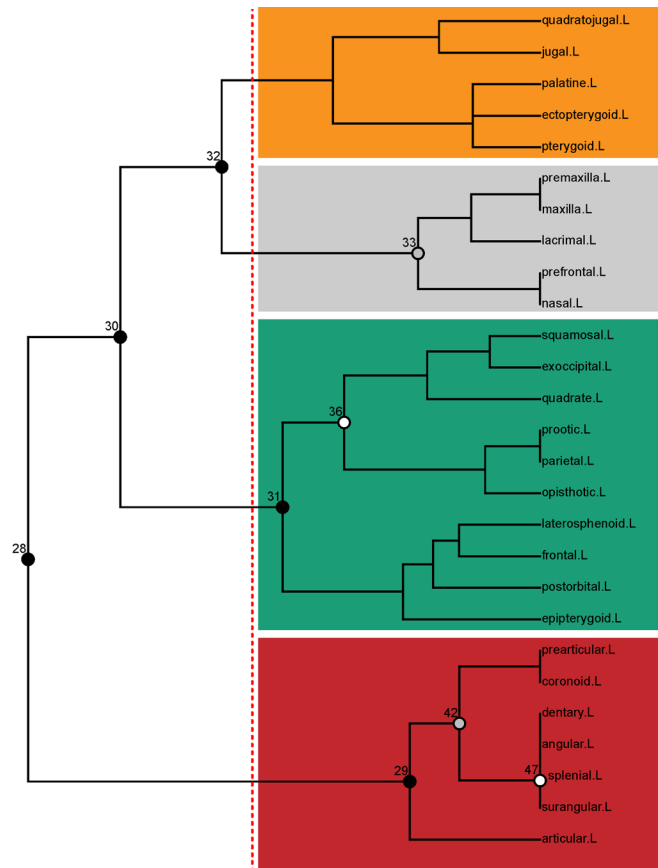
(modified after Rauhut¹²). a, Left-right modularity based on original *Ward.D2* cluster. **b,**

Left-right modularity based on *Ward.D2* cluster with unpaired bones excluded.

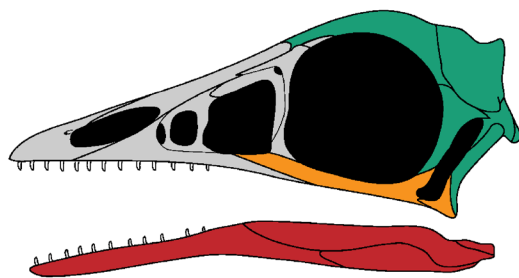
a



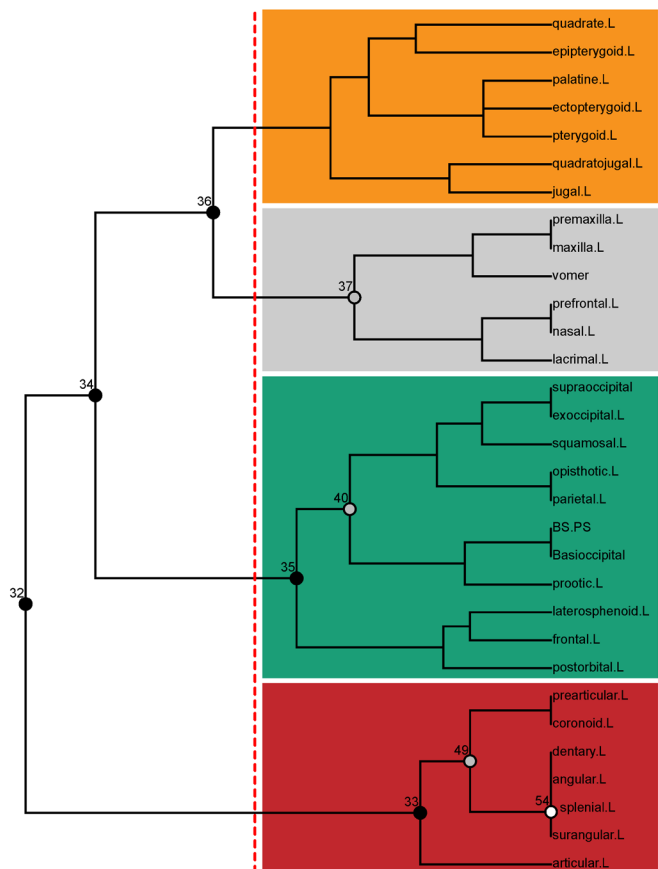
**network of left side
without unpaired bones**



b



**network of left side
with unpaired bones**



Supplementary Figure 7 | Alternative modularity for *Archaeopteryx lithographica*

(modified after Rauhut¹²). **a**, Modularity based on original *Ward.D2* cluster including paired bones from the left skull side. **b**, Modularity based on original *Ward.D2* cluster including unpaired bones and paired bones from the left skull side.

4. References

1. Werneburg, I., Esteve-Altava, B., Bruno, J., Ladeira, M. T. & Diogo, R. Unique skull network complexity of *Tyrannosaurus rex* among land vertebrates. *Sci. Rep.* **9**, 1520 (2019).
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3. Hackett, S. J. et al. A phylogenomic study of birds reveals their evolutionary history. *Science* **320**, 1763–1768 (2008).
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5. Skull network analysis results

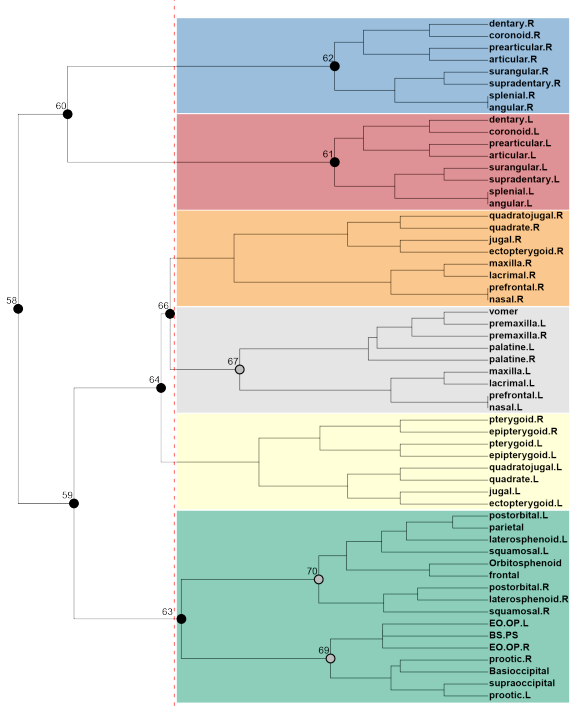
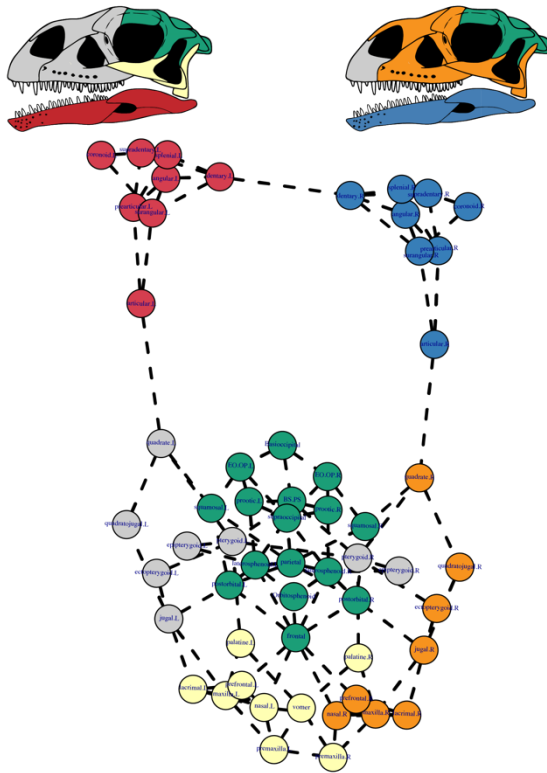
The skull modularity of non-avian dinosaurs and the ontogenetic pairs of crown birds is documented by the network model and the *Ward.2D* cluster. The latter shows the hierarchical organization of each anatomical network, in which the horizontal dashed line marks the partition into *Q*-modules, while filled circles at nodes mark the statistical significance of *S*-modules (white, p-value < 0.05; grey, p-value < 0.01; black, p-value < 0.001). Based on the *Q*-modules in the cluster, the network and a skull reconstruction were colour coded to illustrate skull modularity. All sources for skull reconstructions are listed in Supplementary data 2 file.

Massospondylus

L

Adult

R

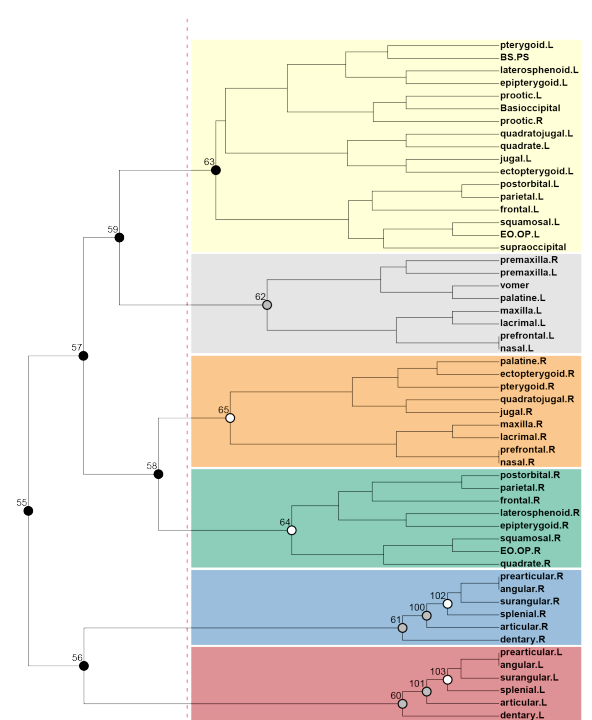
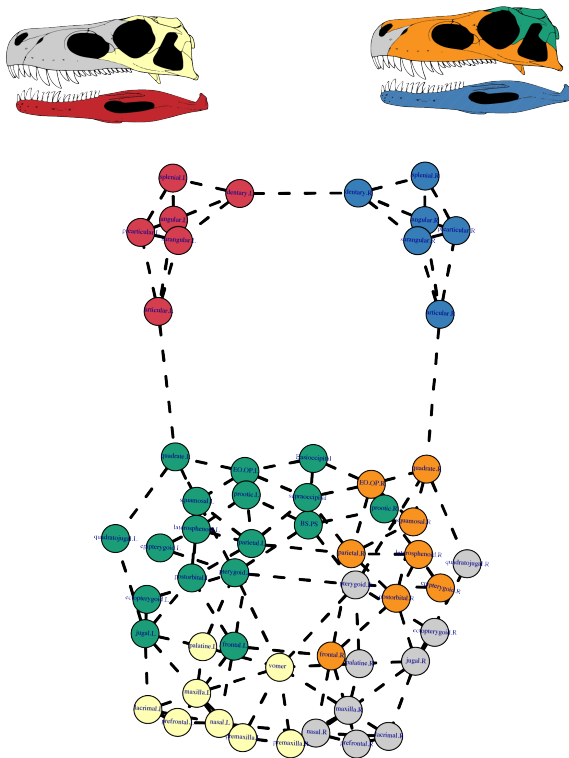


Herrerasaurus

L

Adult

R

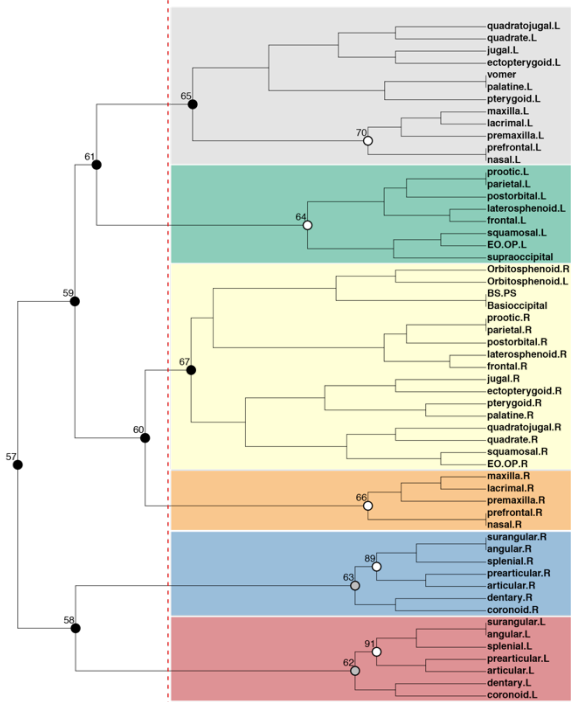
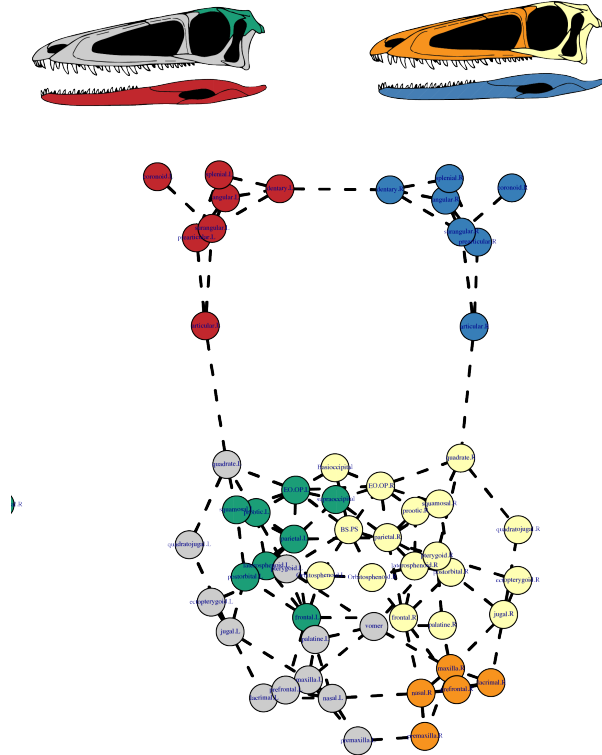


Coelophysis

L

Adult

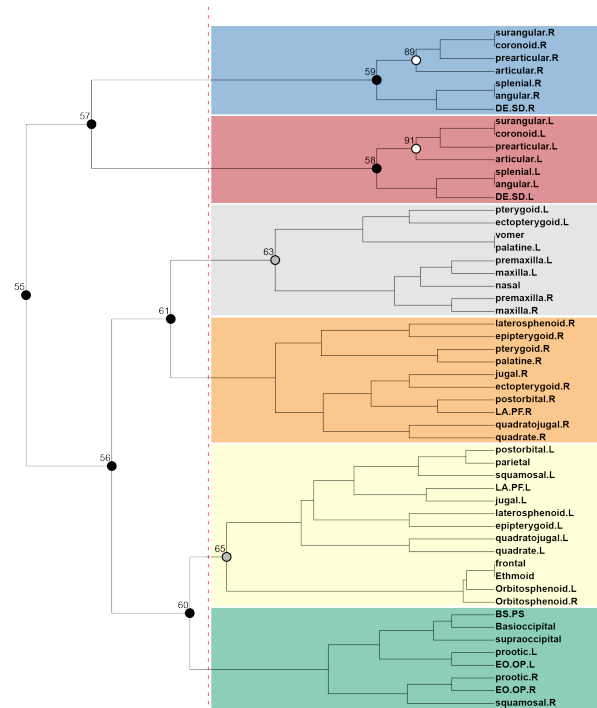
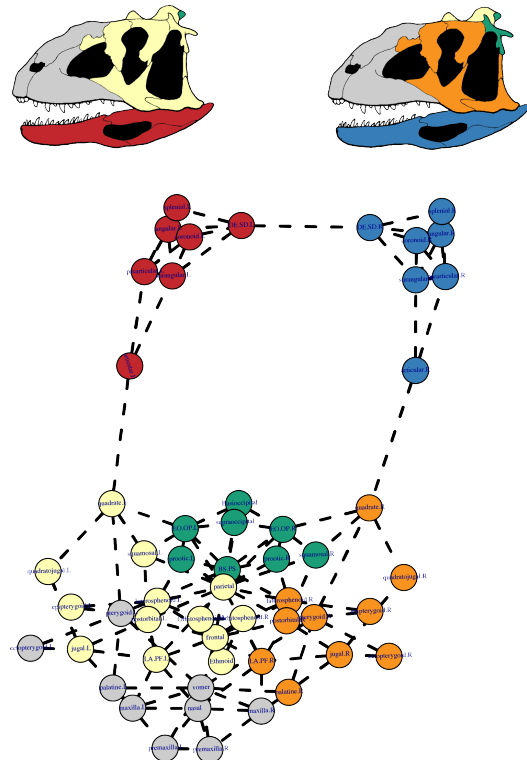
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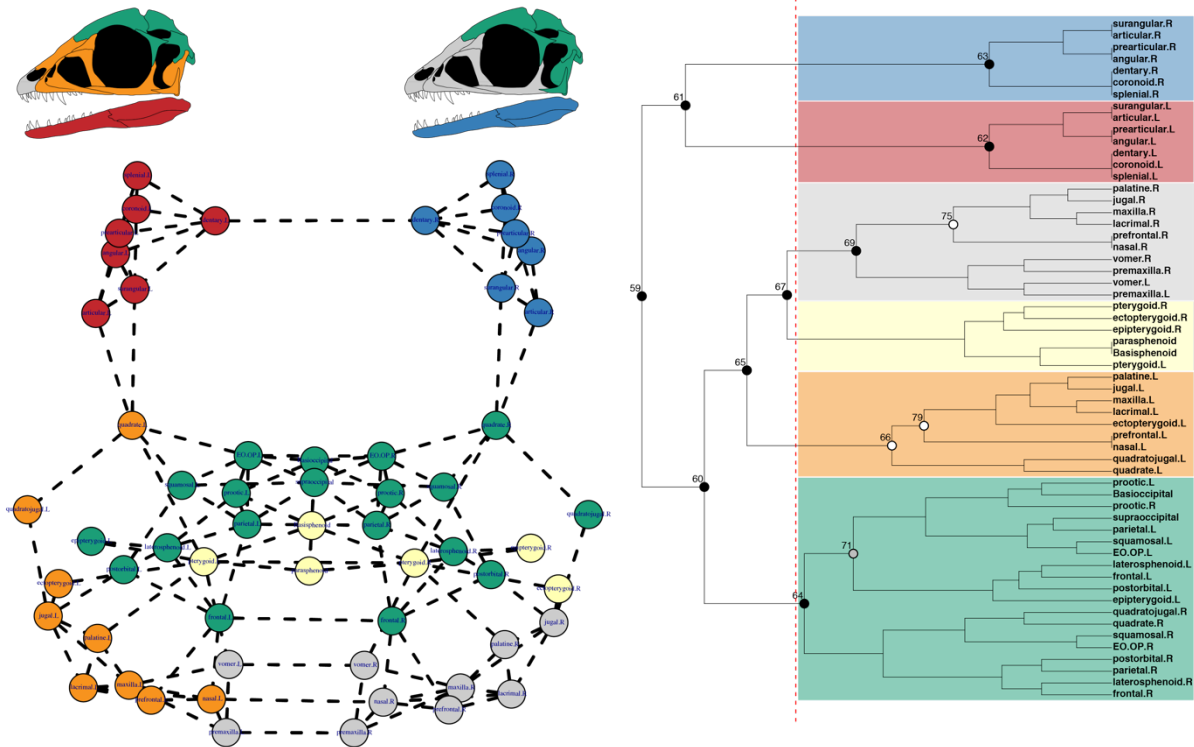
Majugasaurus

L

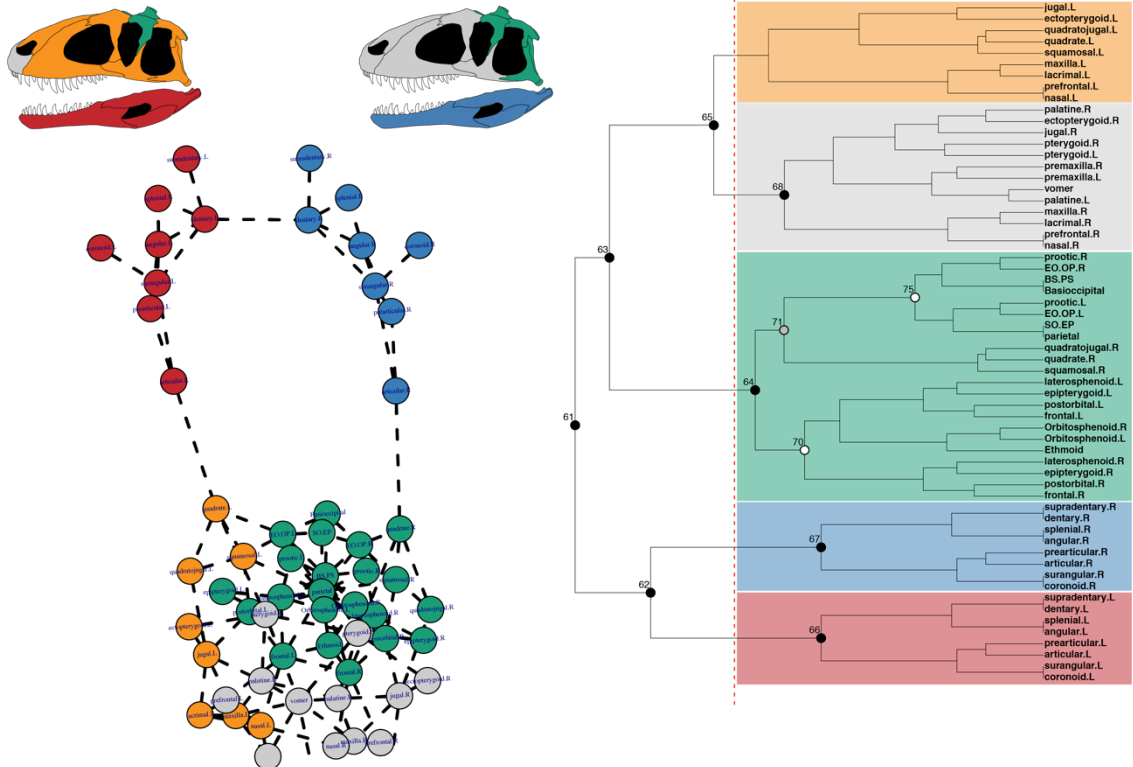
Adult



R I



R

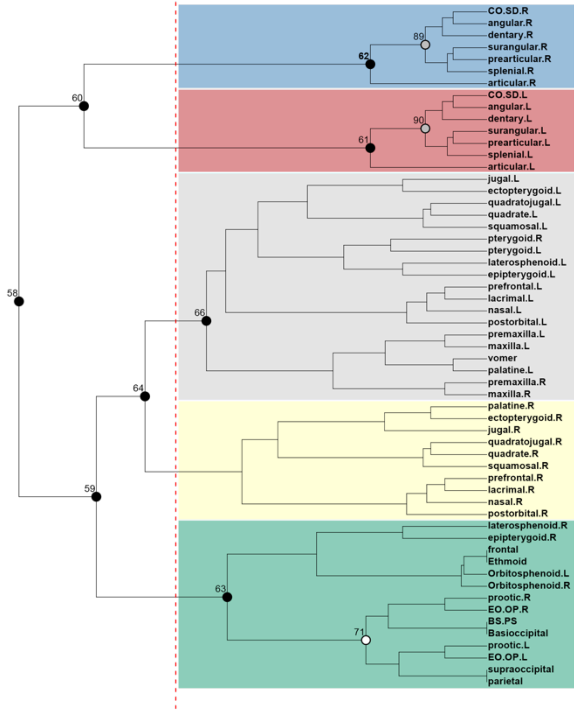
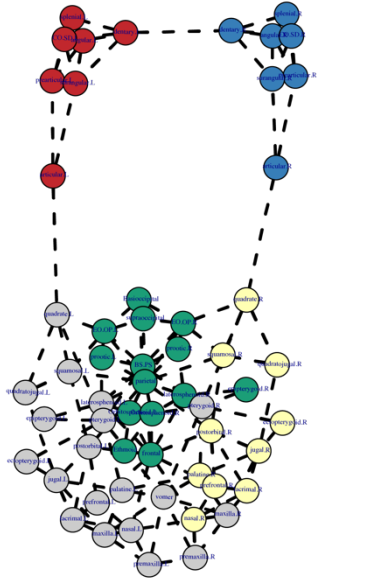
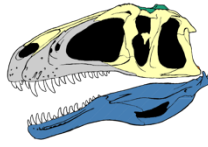


Acrocanthosaurus

L

Adult

R

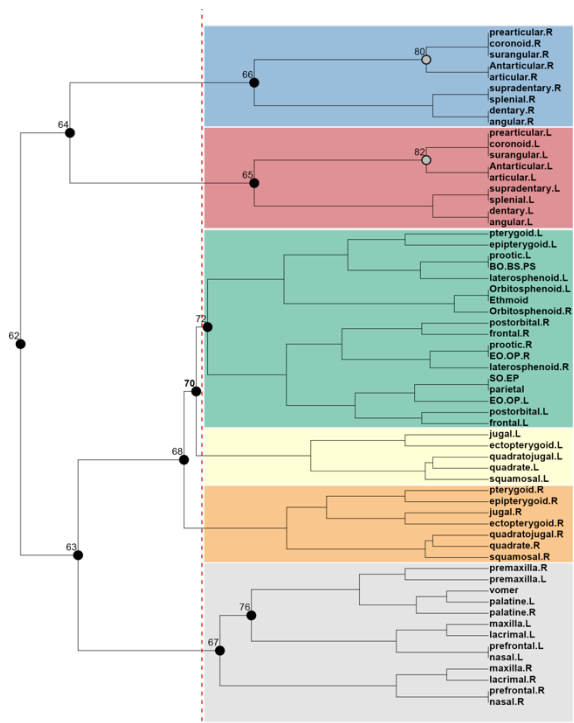
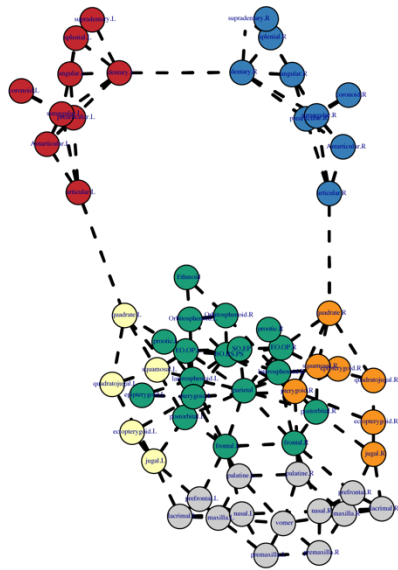


Allosaurus

L

Adult

R

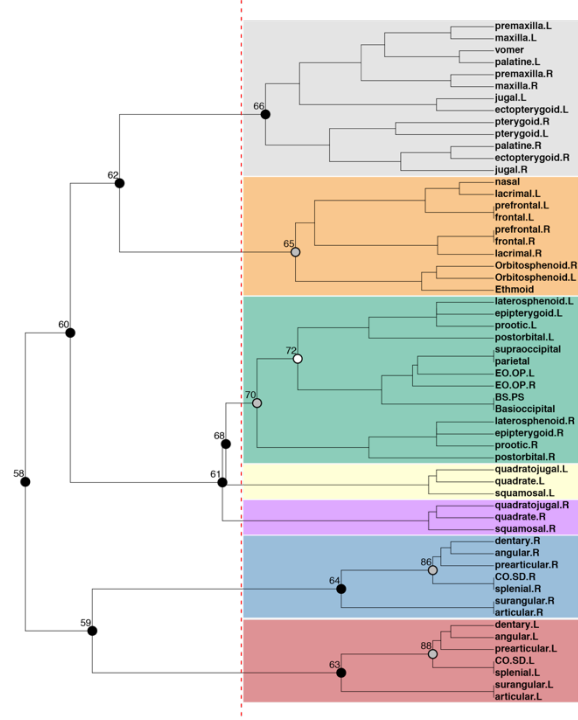
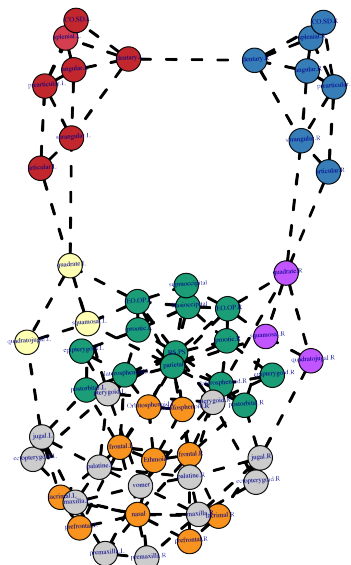
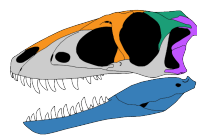
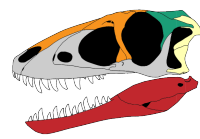


Tarbosaurus

L

Juvenile

R

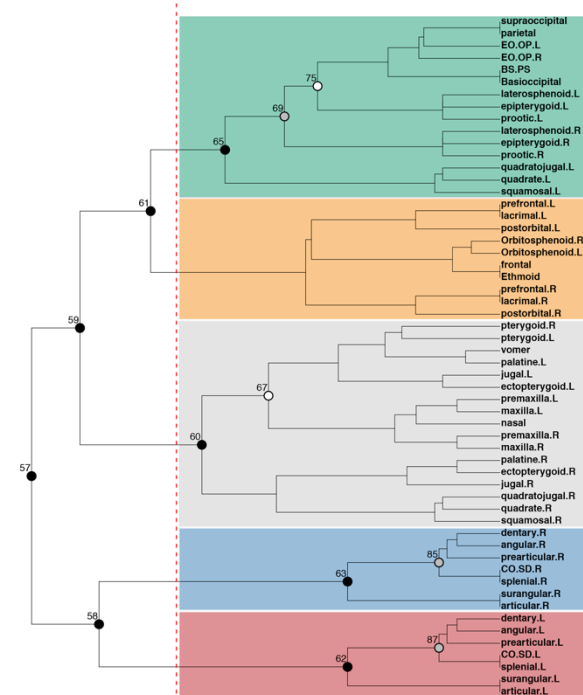
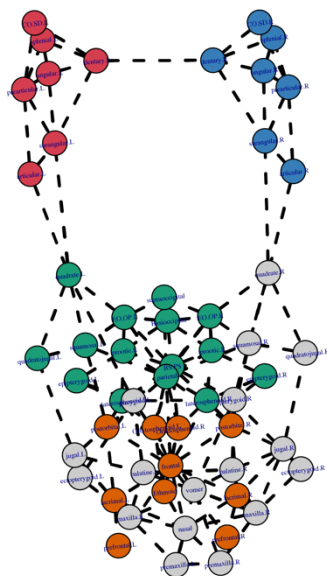
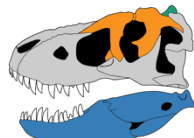


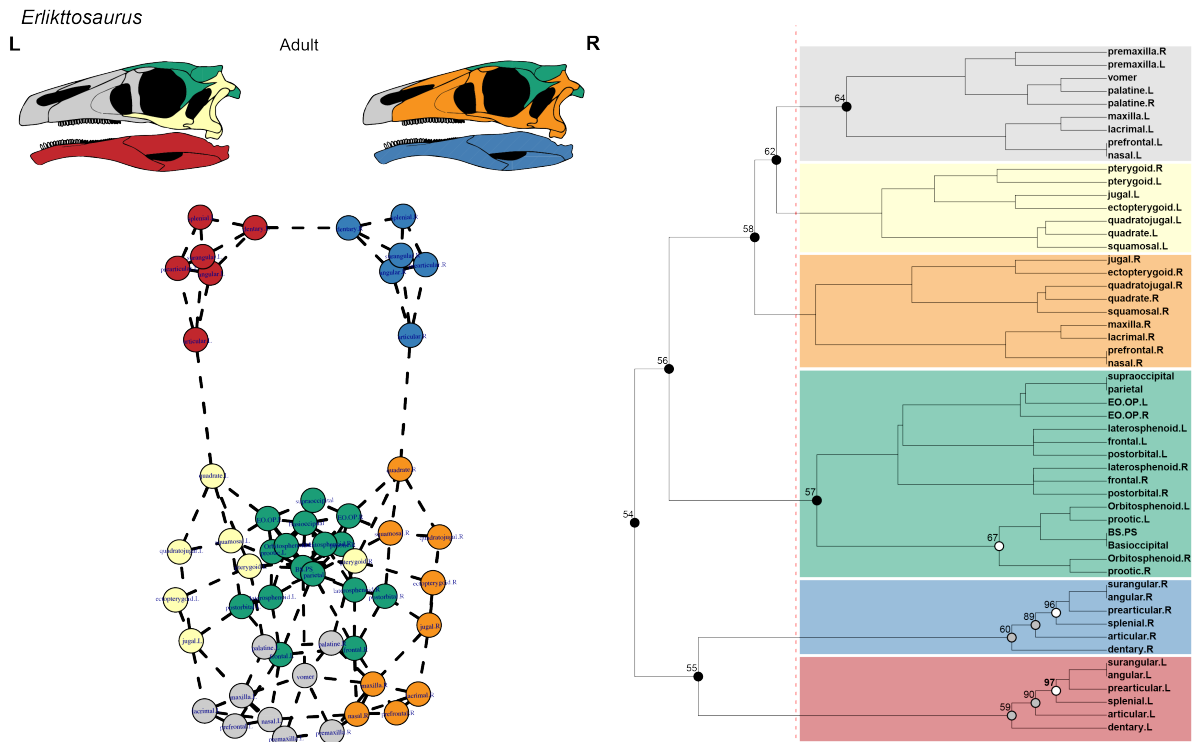
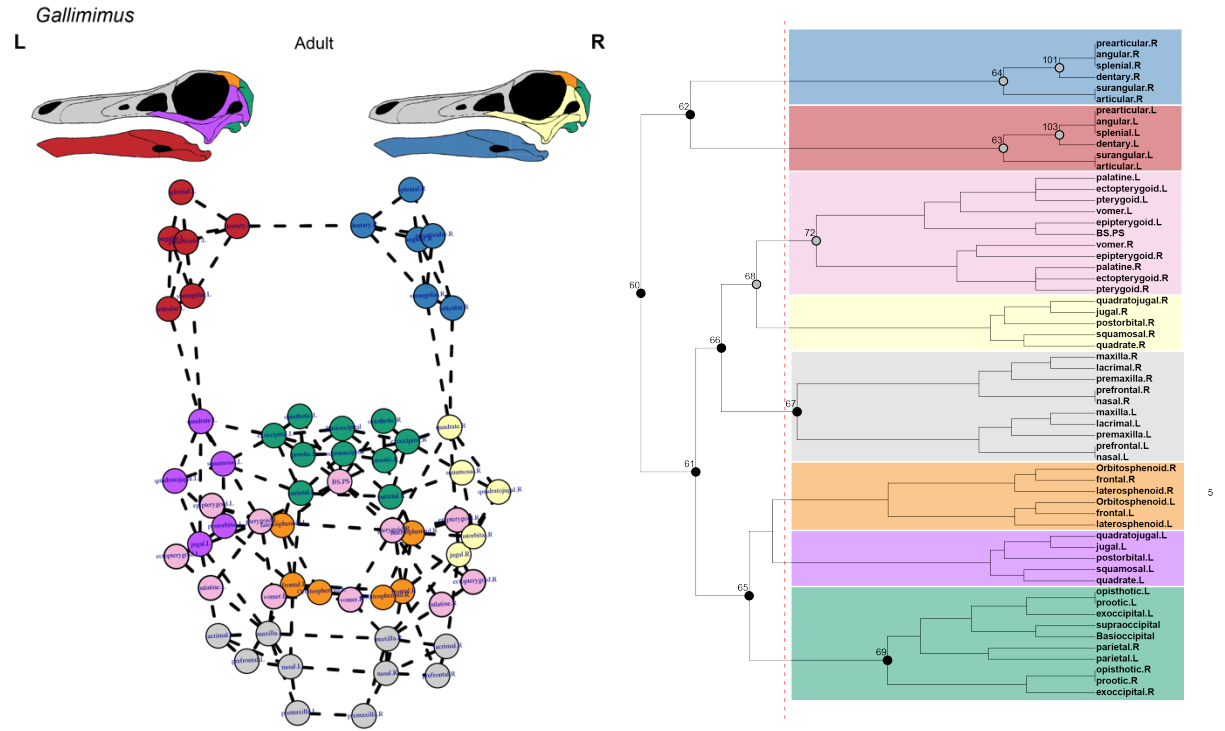
Tarbosaurus

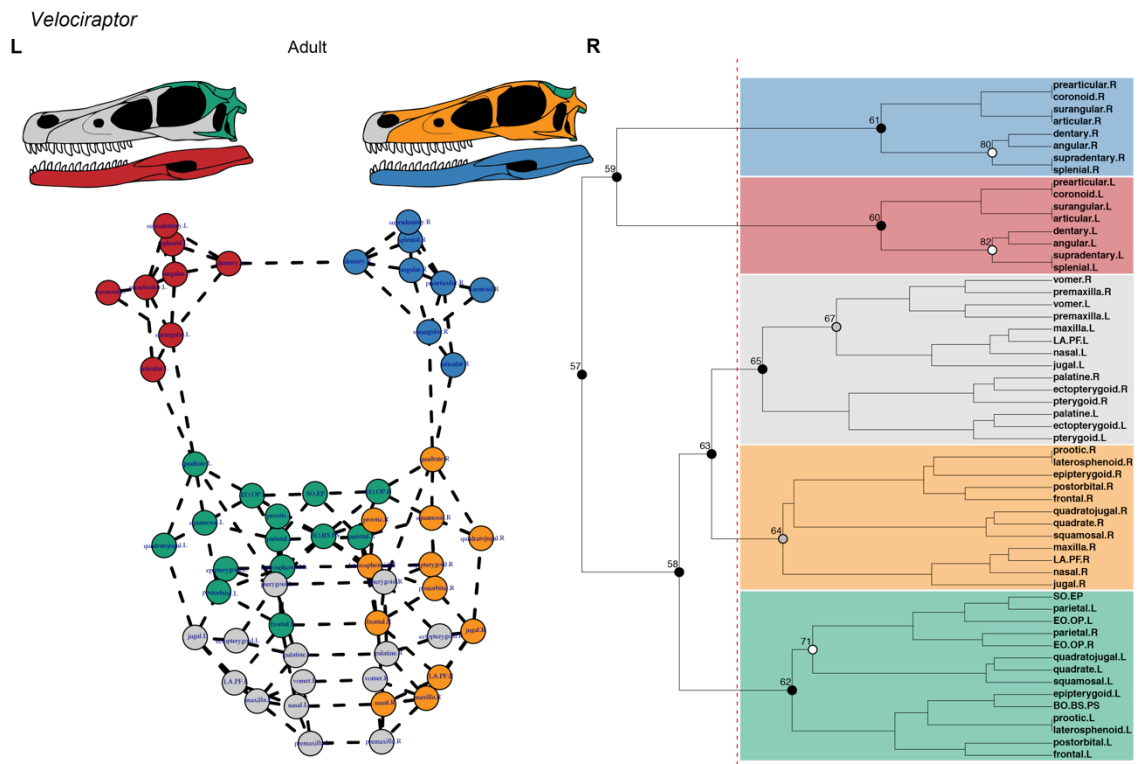
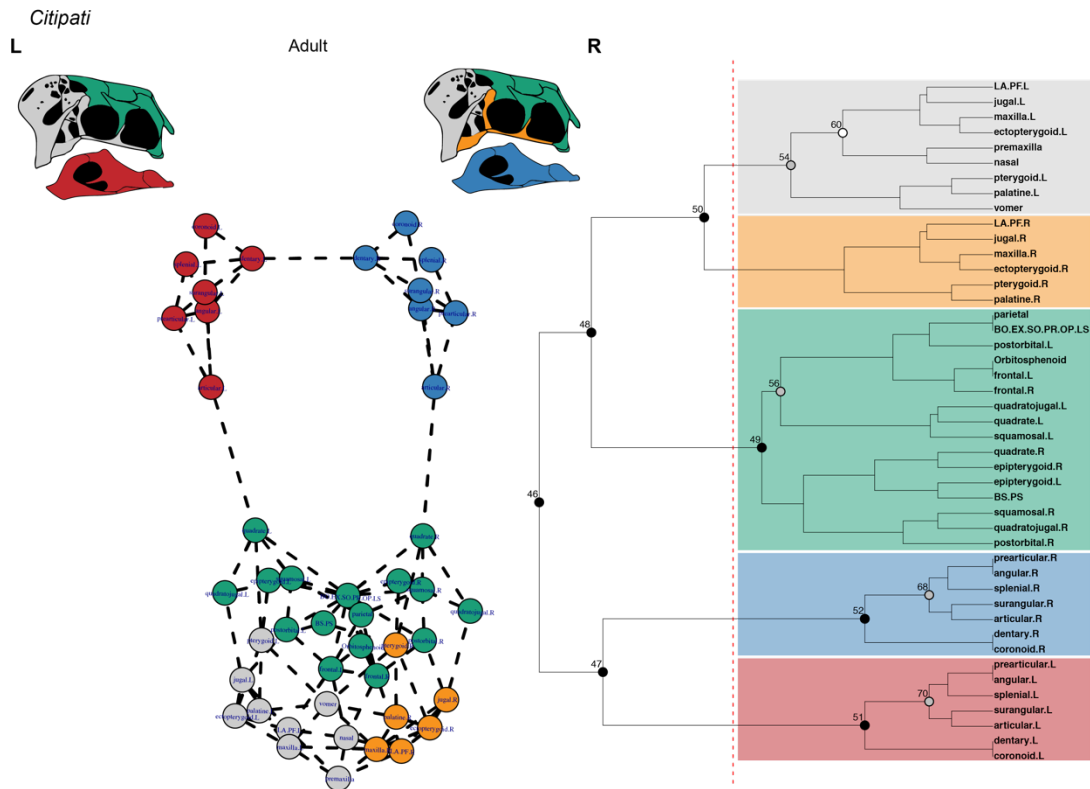
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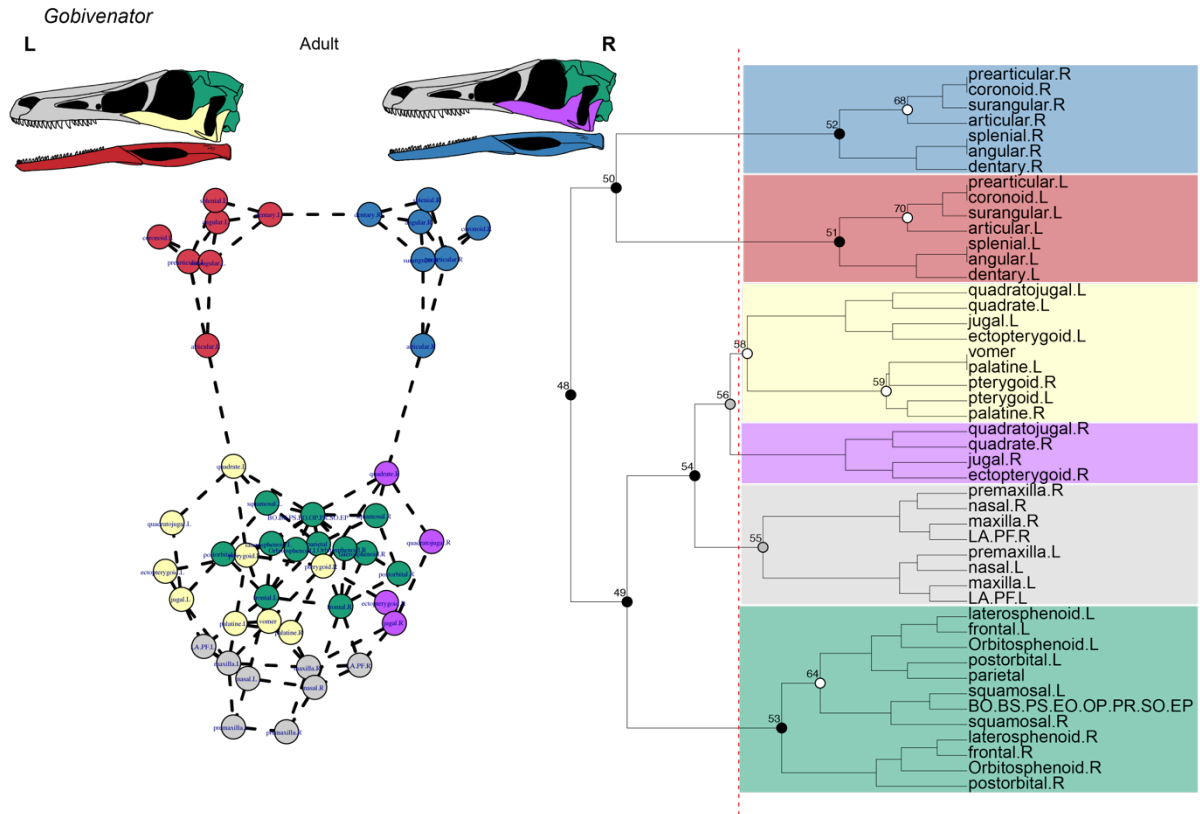
Adult

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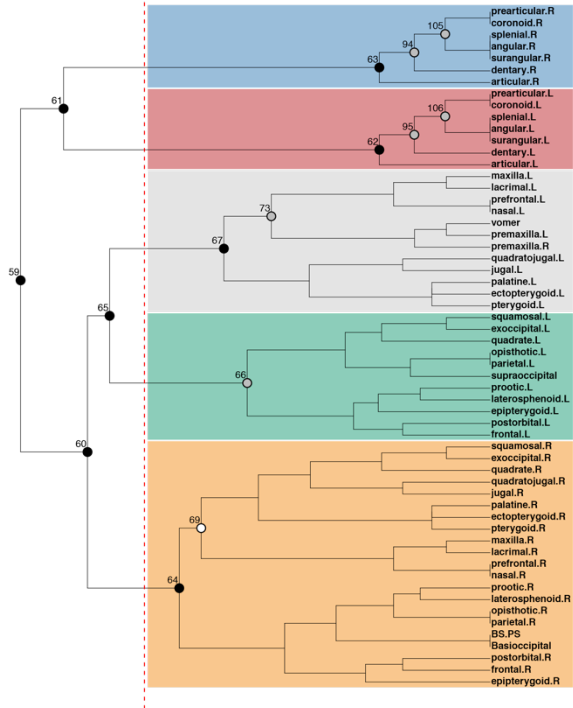
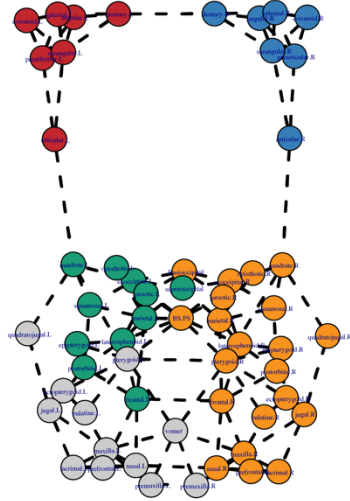
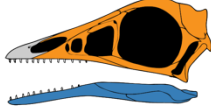
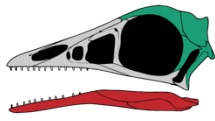


Archaeopteryx

L

Adult

R

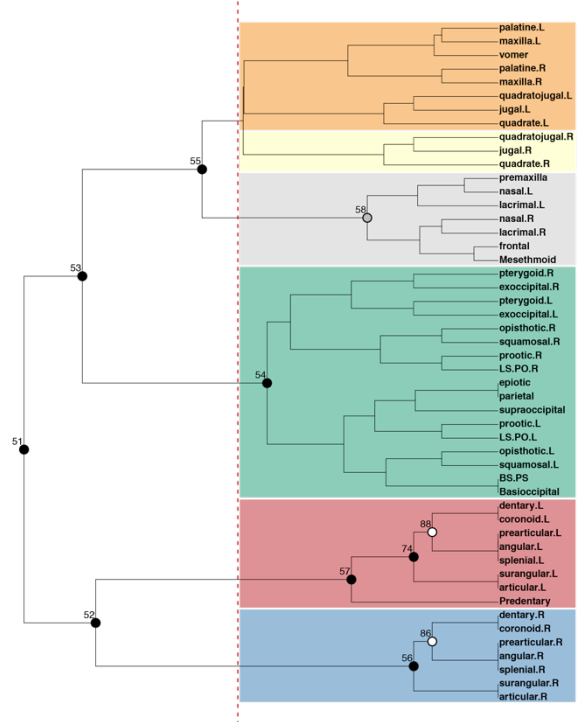
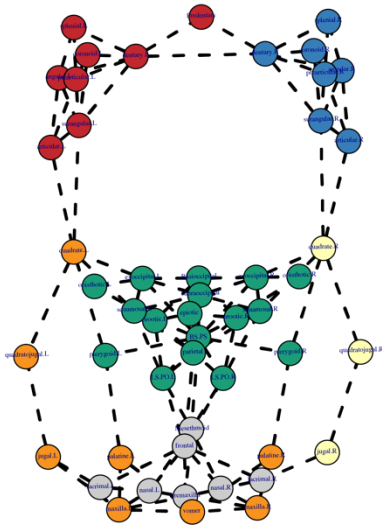
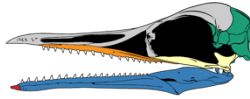


Ichthyornis

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Adult

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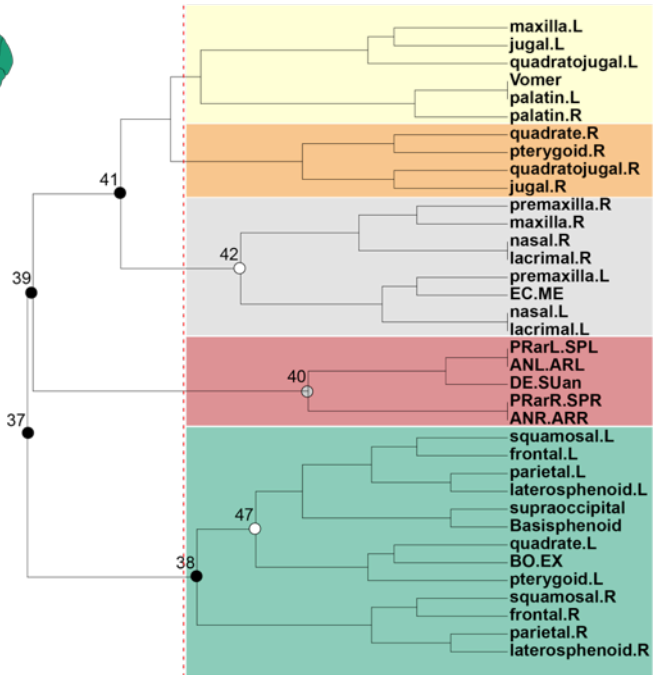
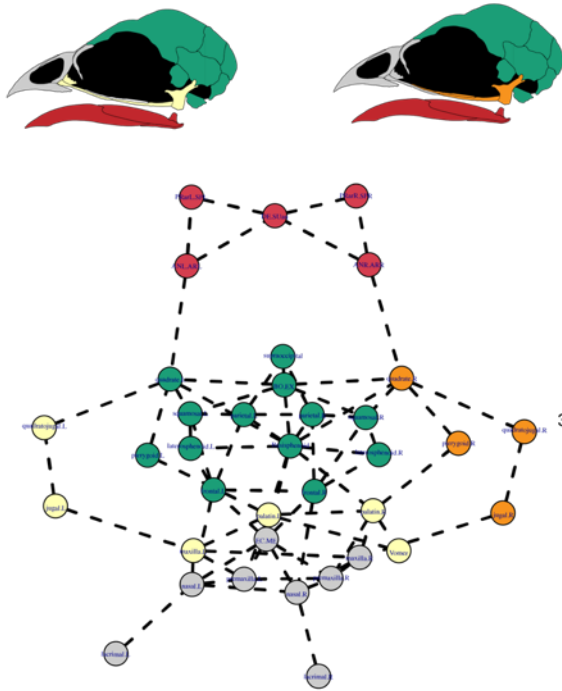


Gyps fulvus

L

Juvenile

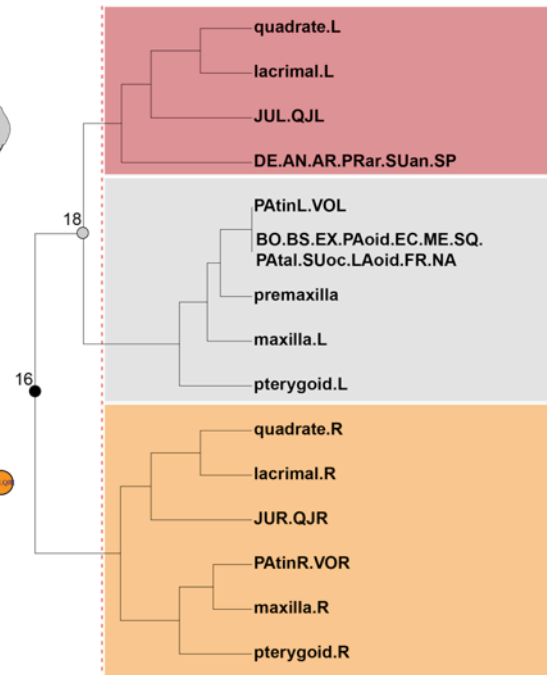
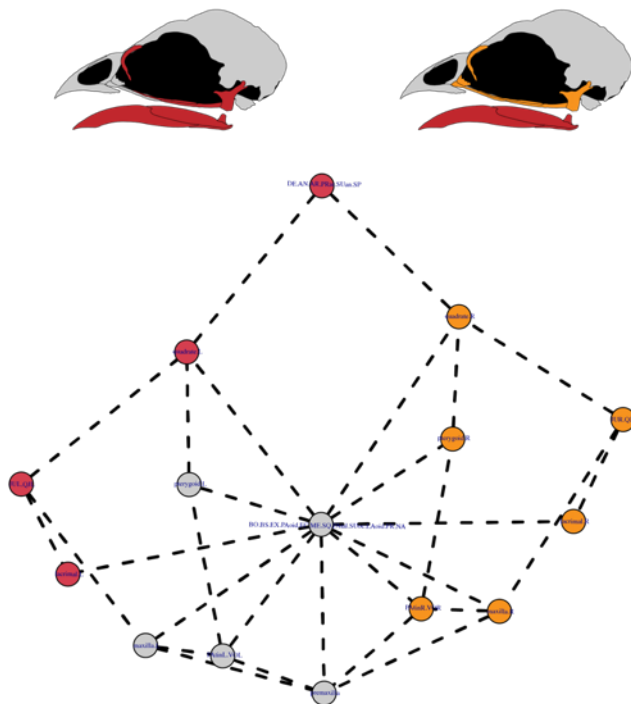
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Adult

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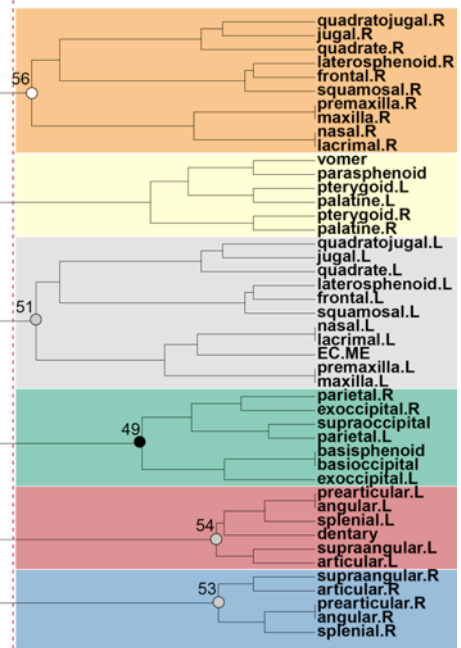
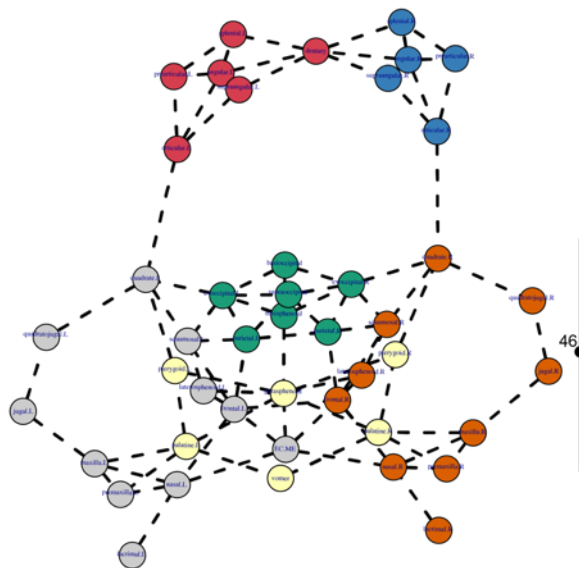


Gypaetus barbatus

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Juvenile

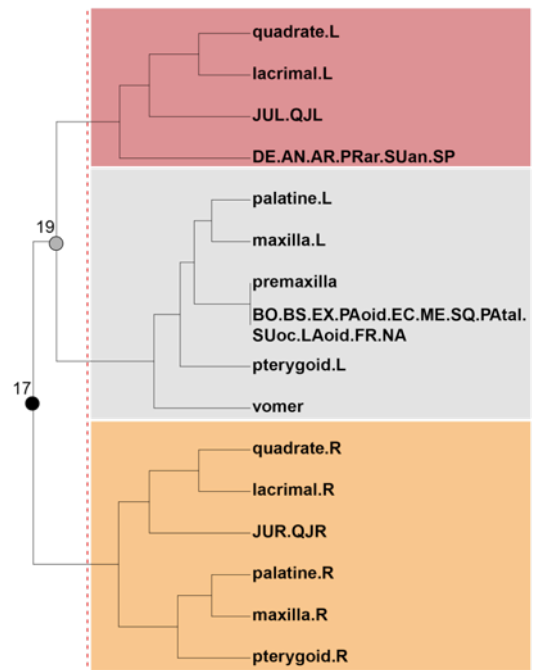
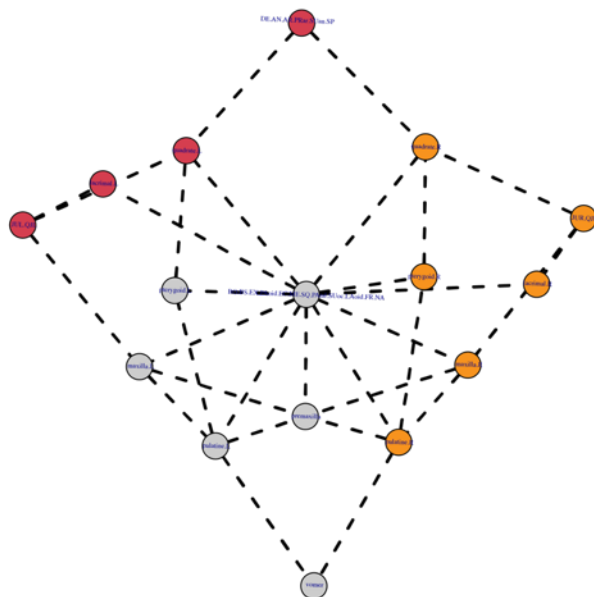
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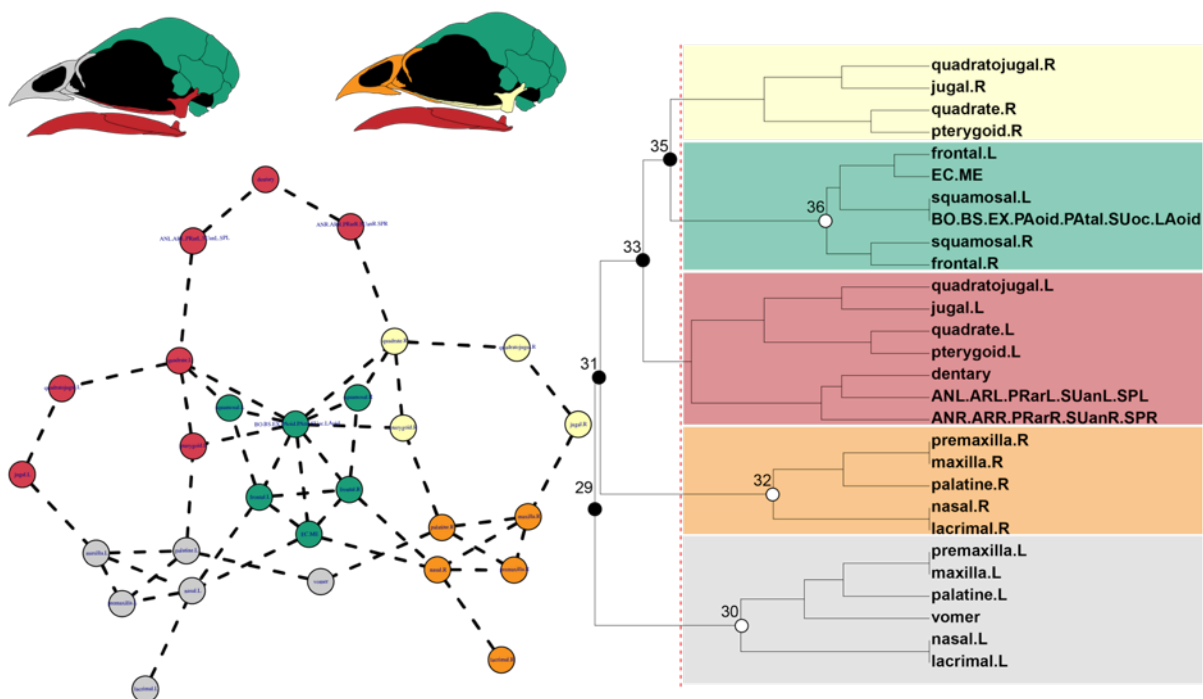
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Adult

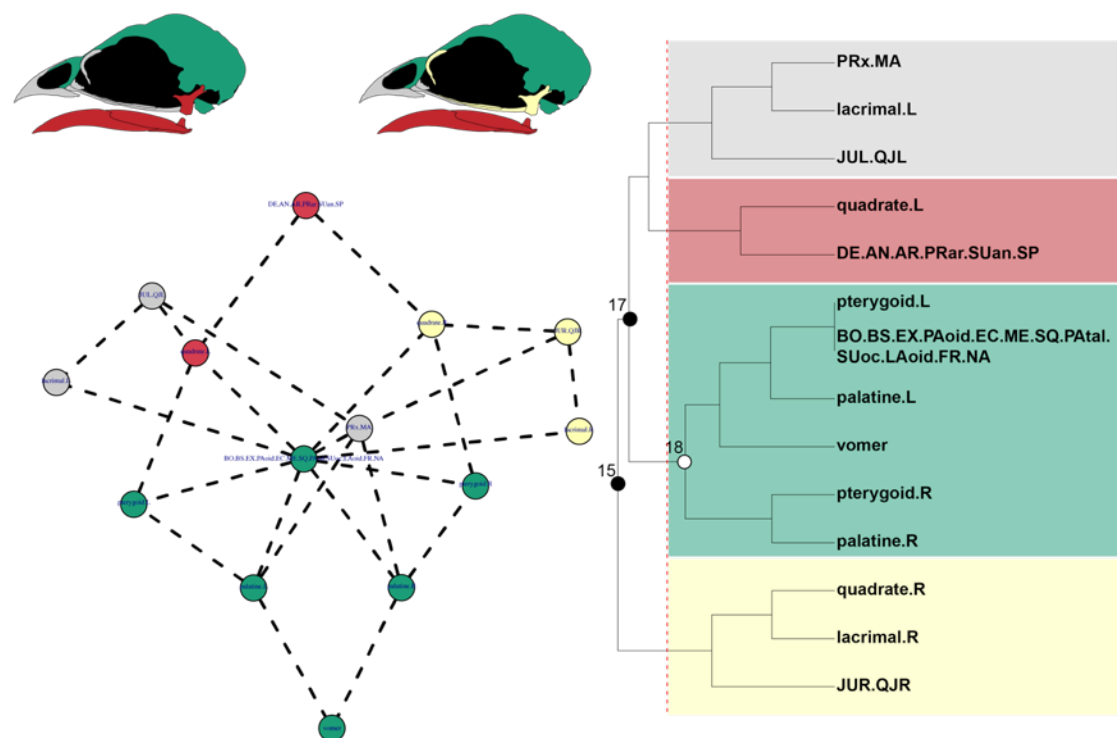
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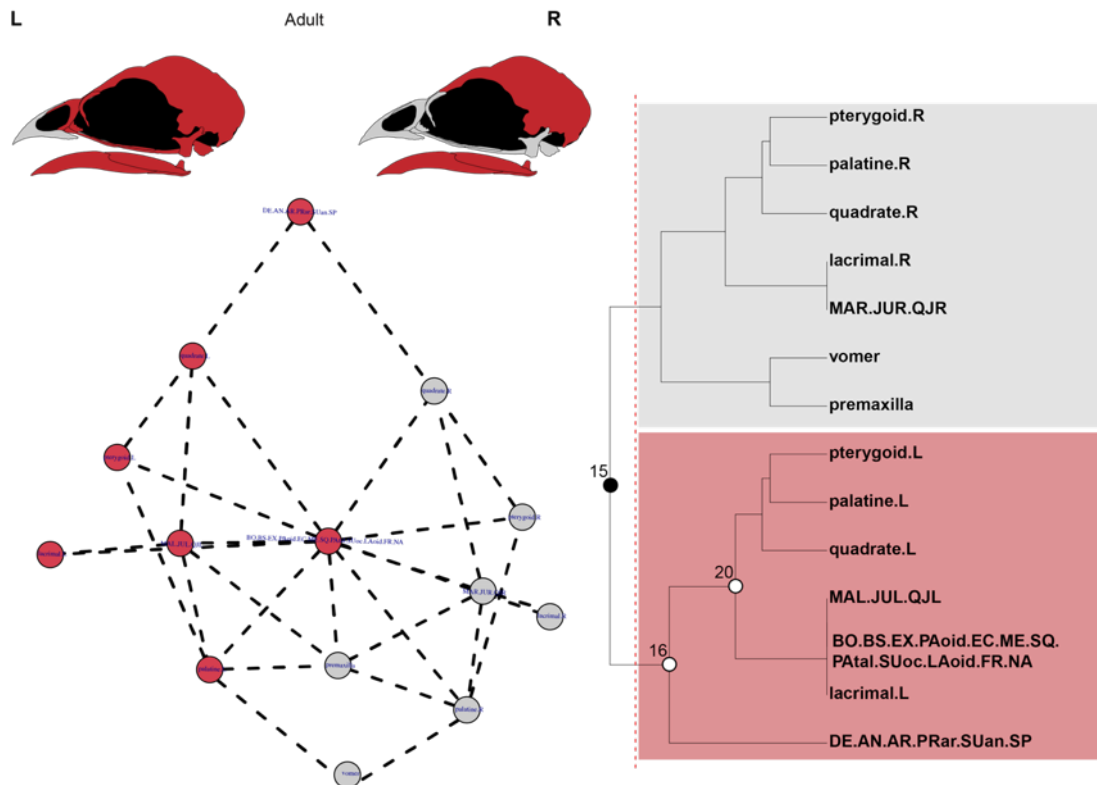
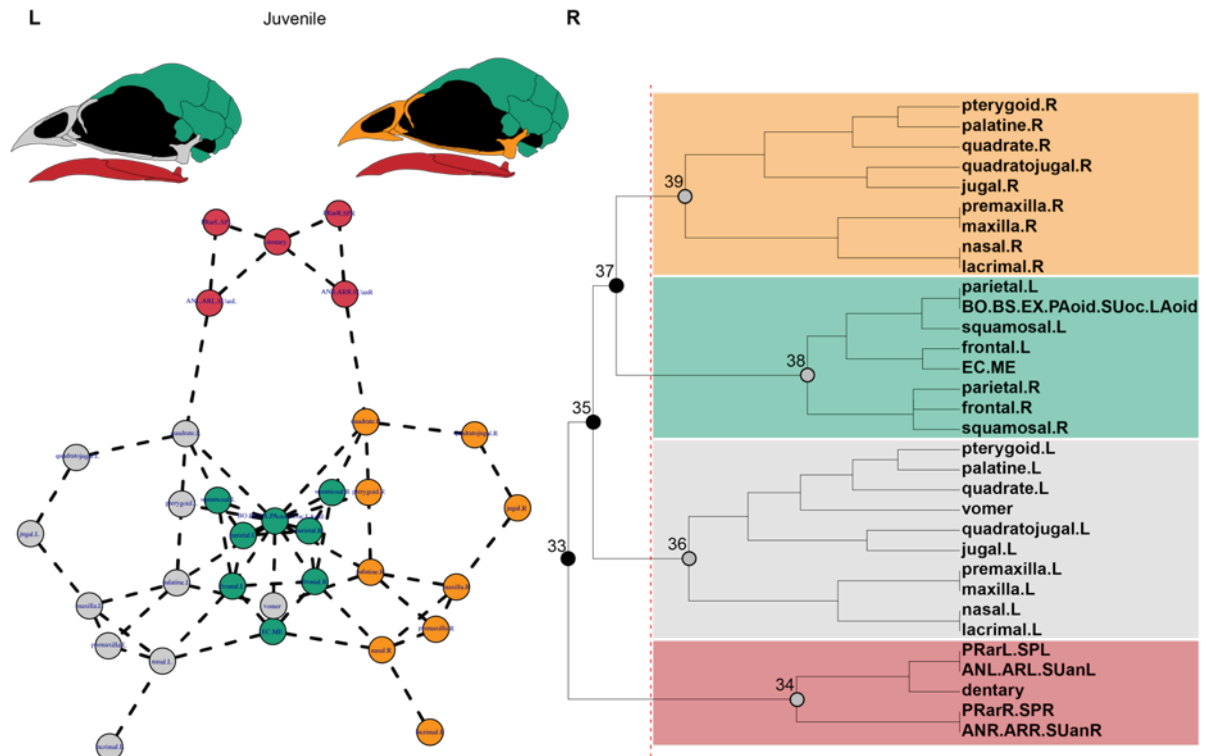
L Juvenile R



L Adult R



Falco tinnunculus

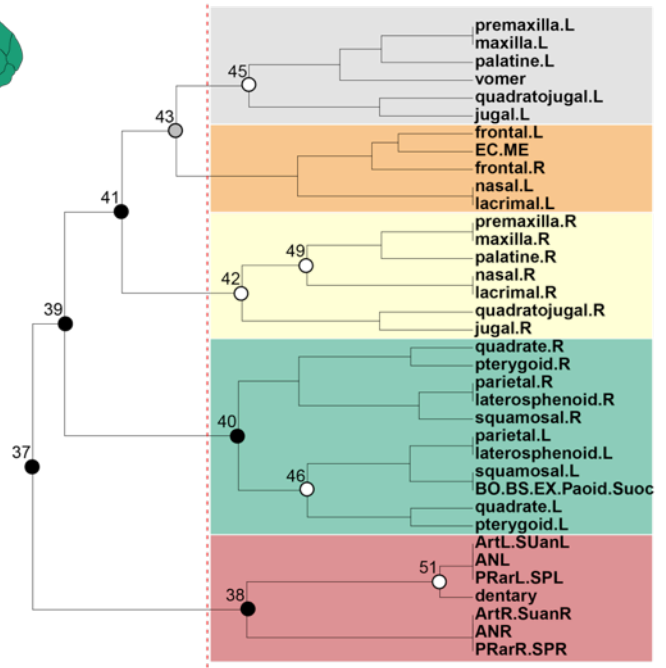
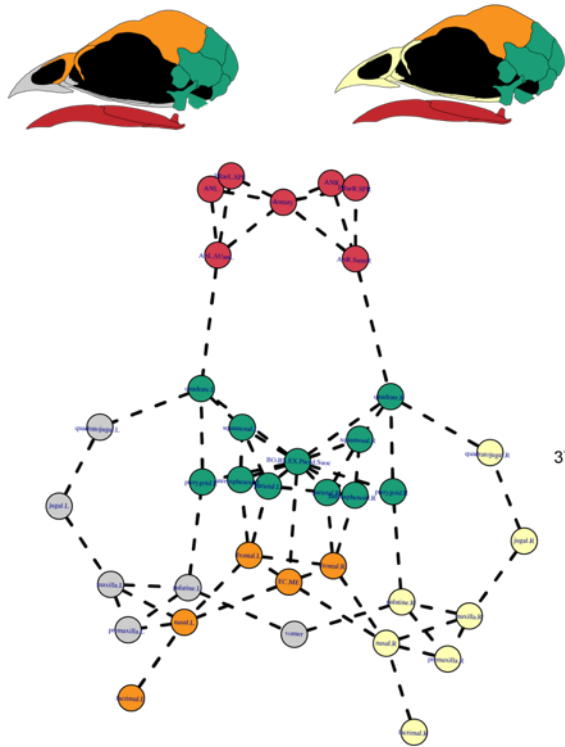


Tyto alba

L

Juvenile

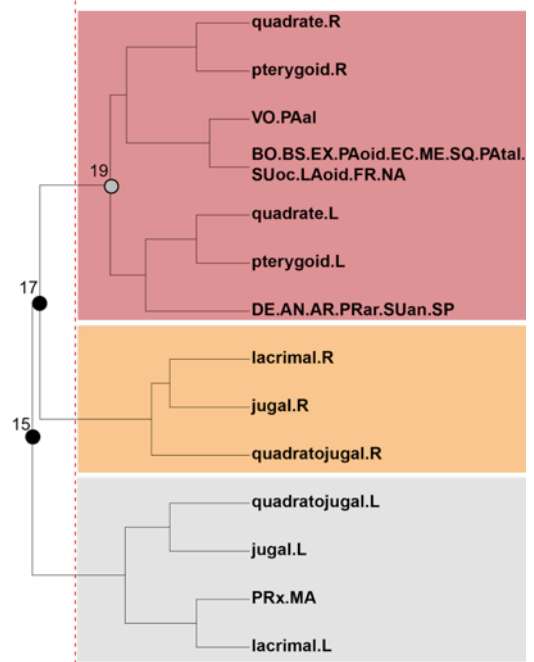
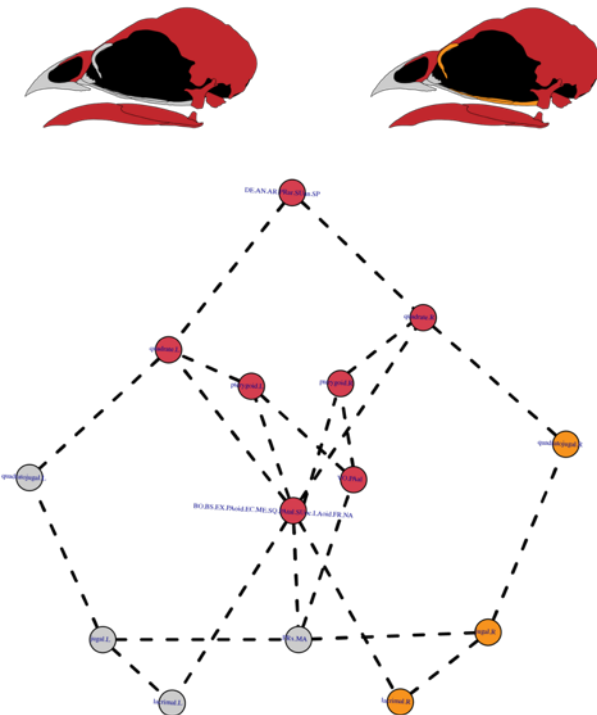
R



L

Adult

R

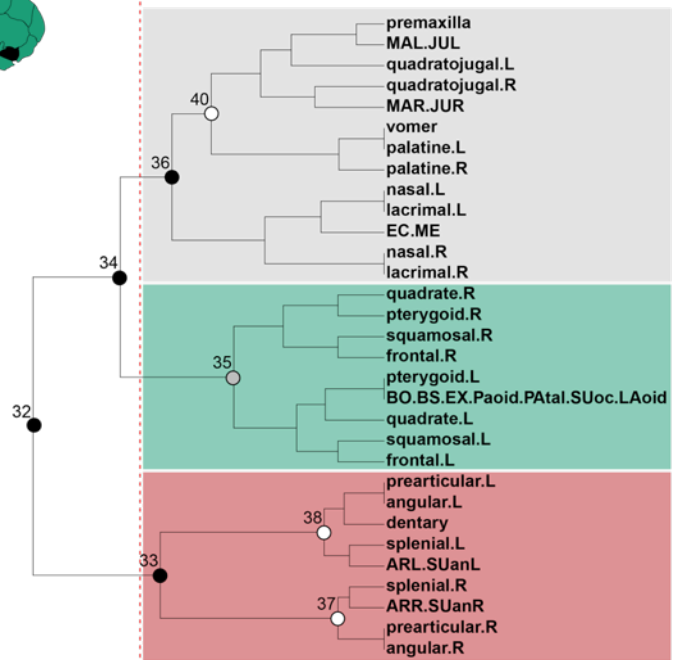
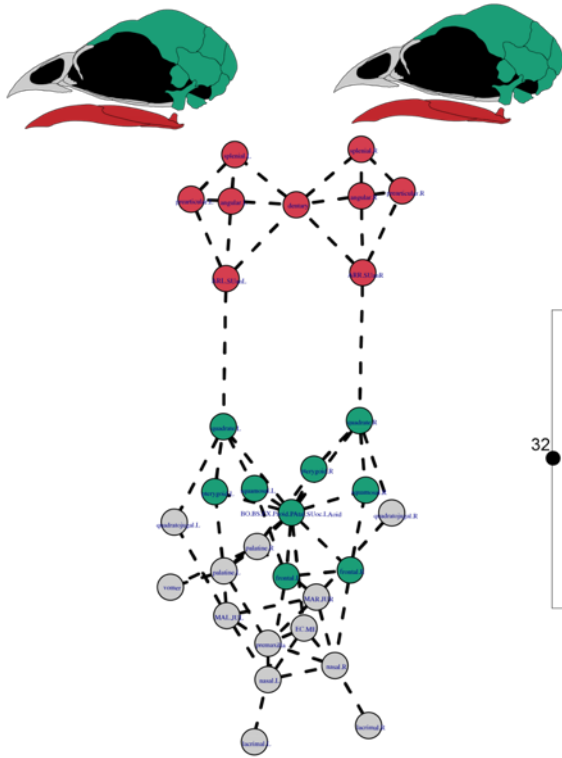


Anas crecca

L

Juvenile

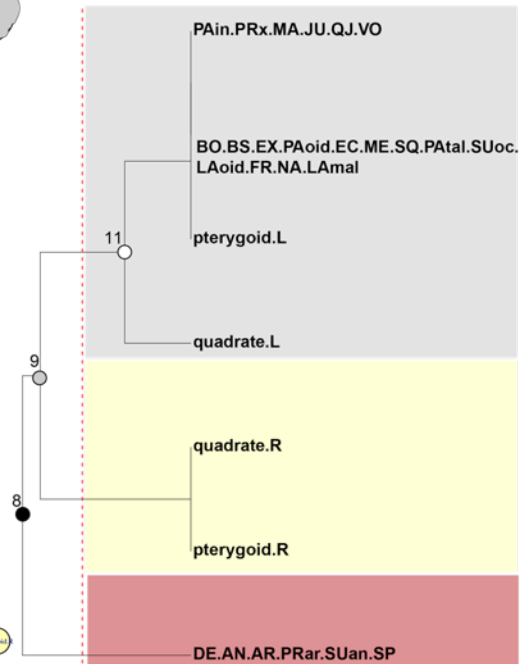
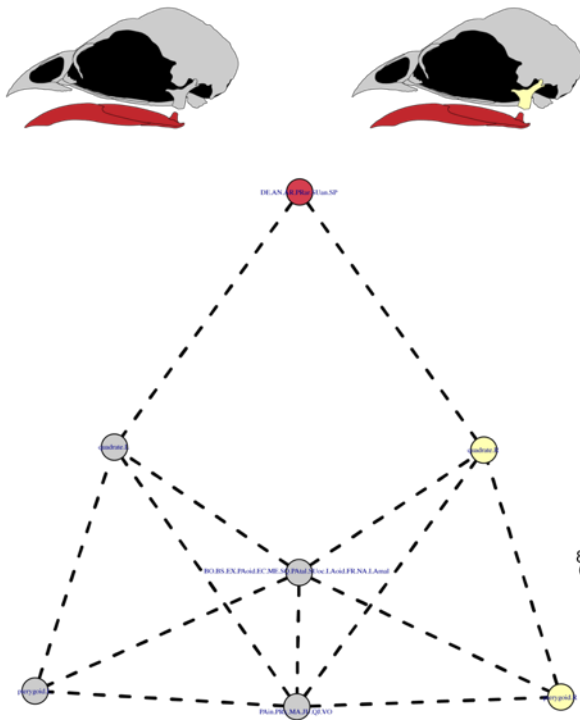
R



L

Adult

R

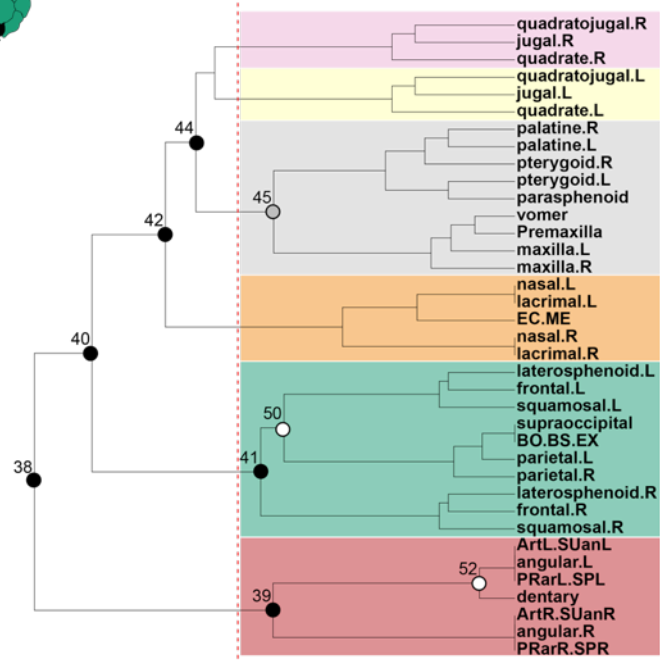
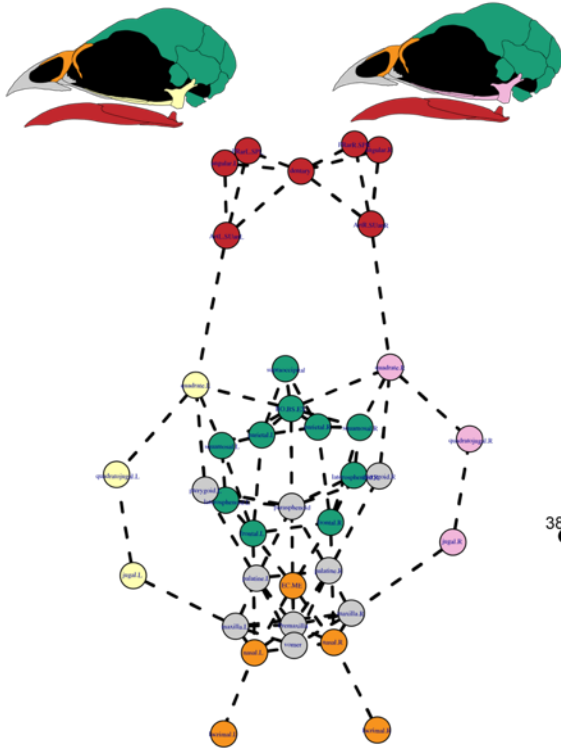


Anser anser

L

Juvenile

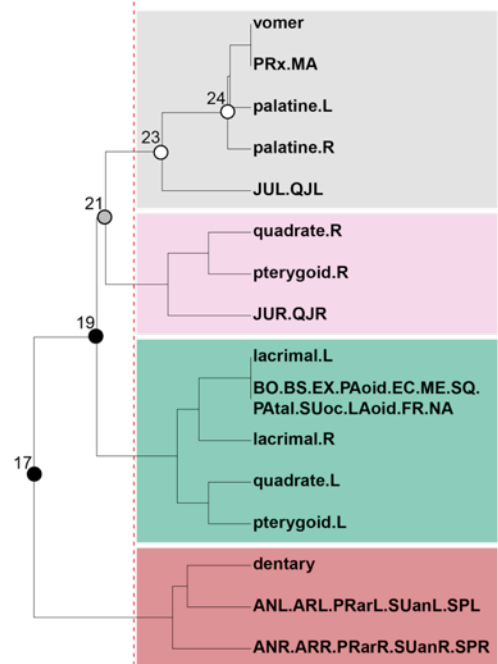
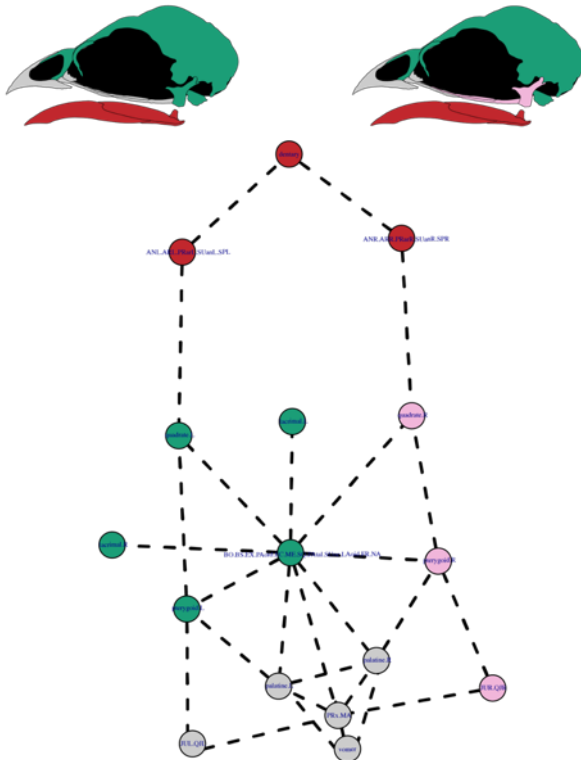
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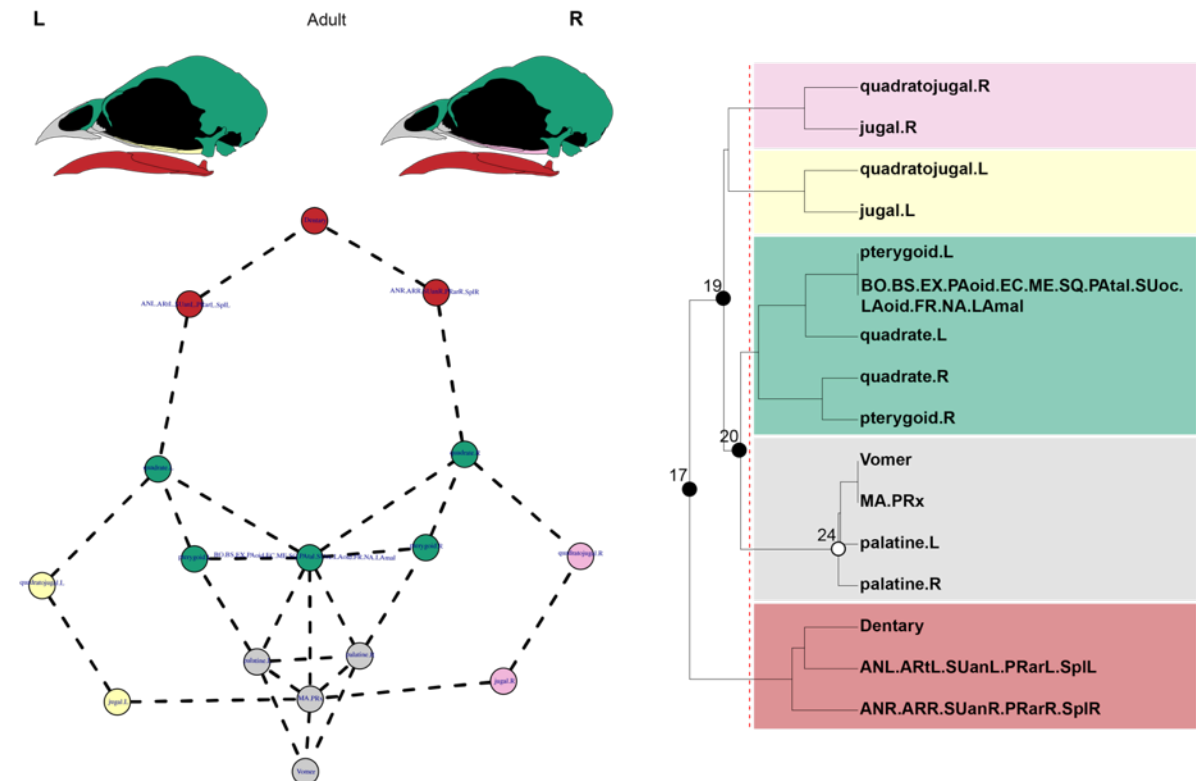
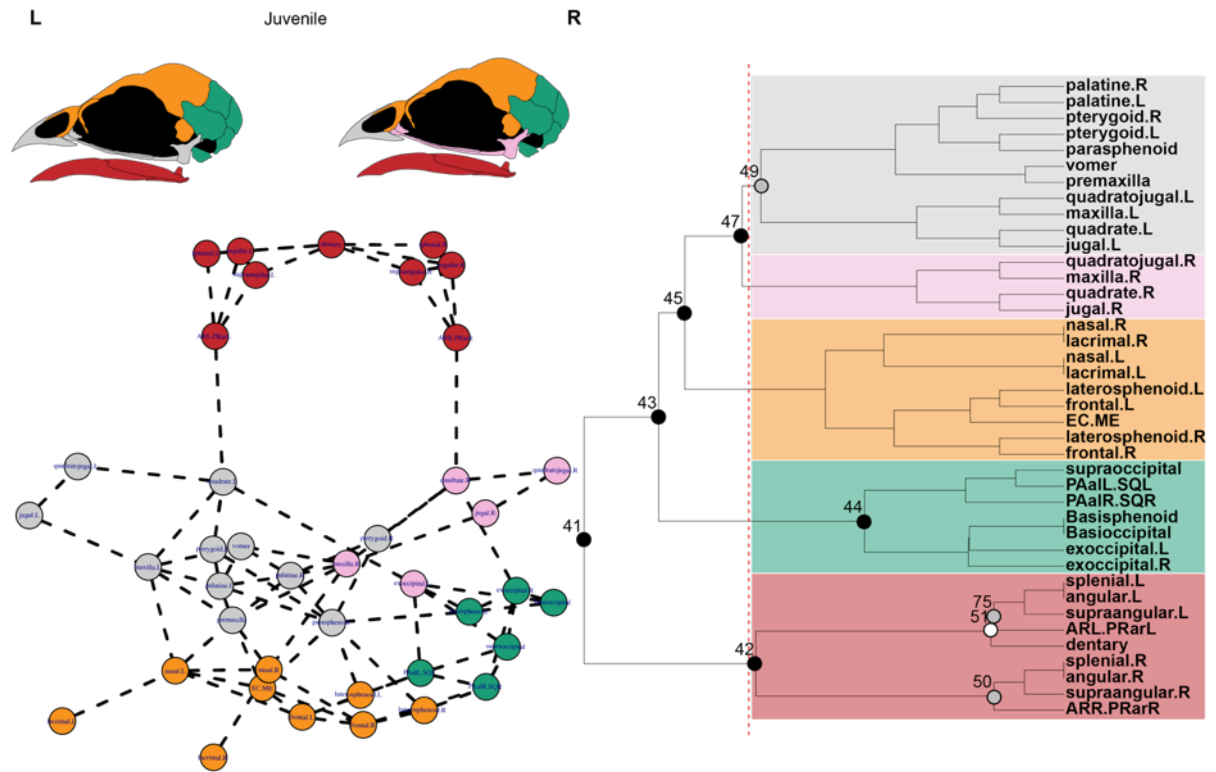
L

Adult

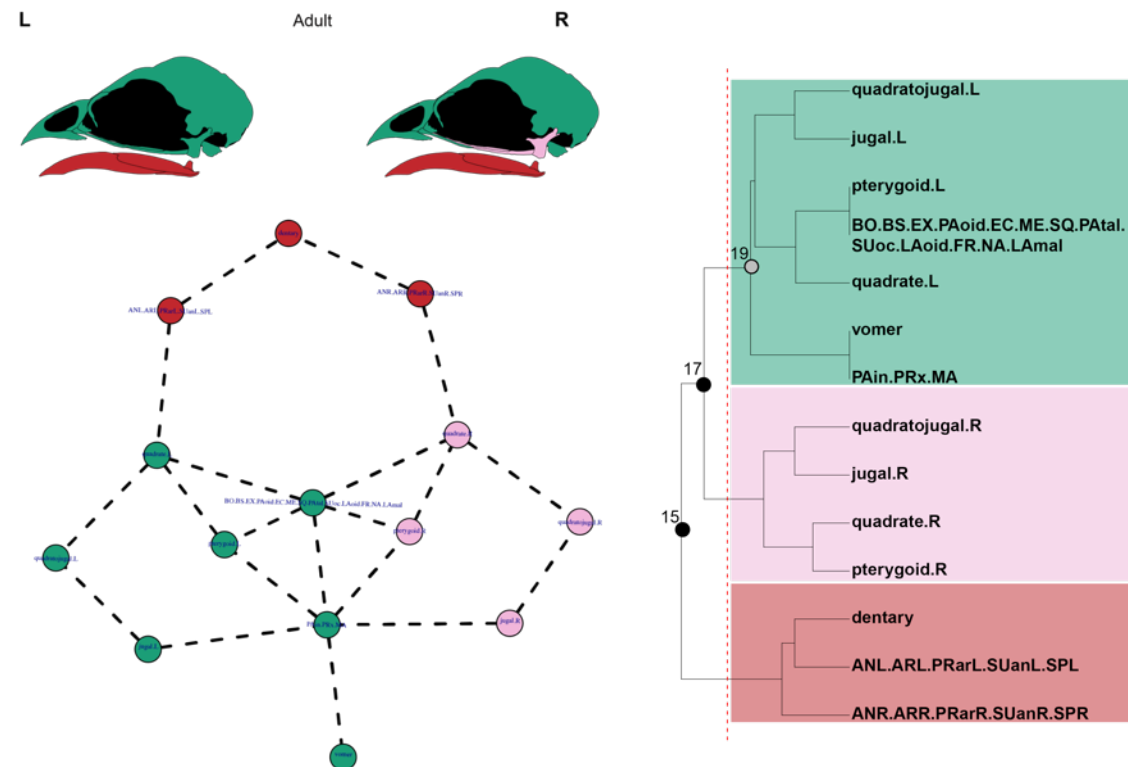
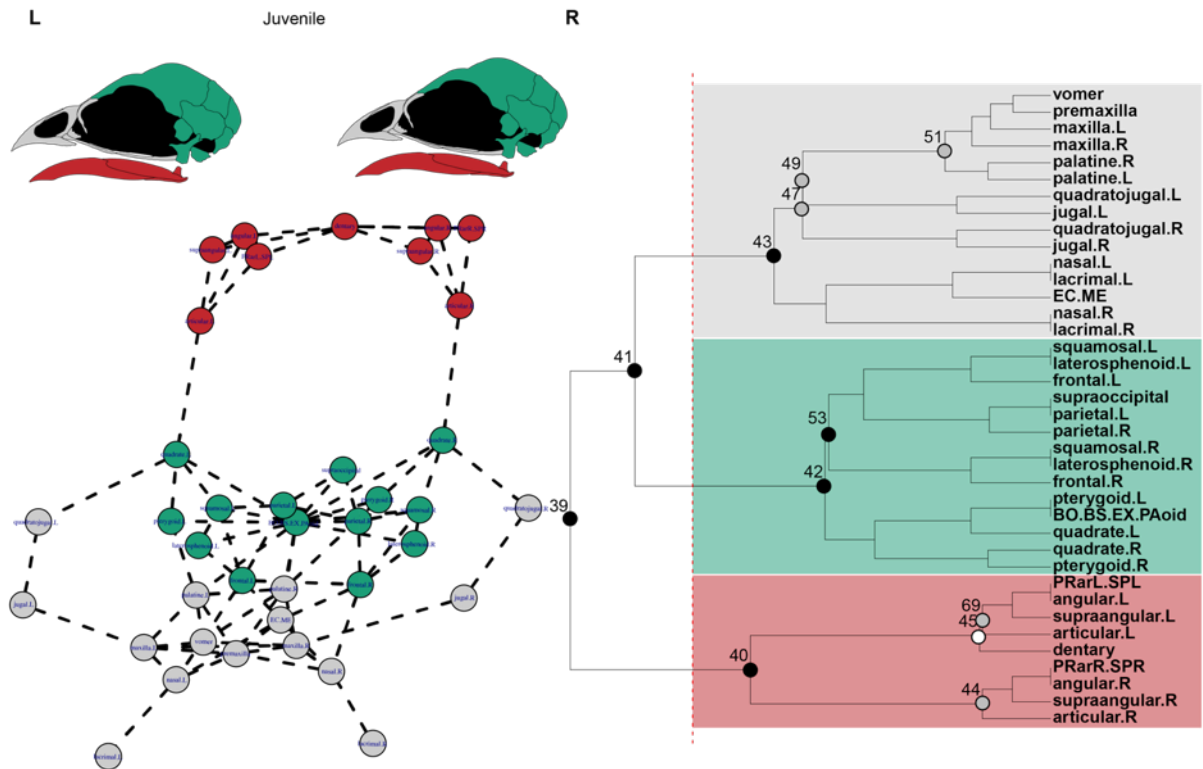
R



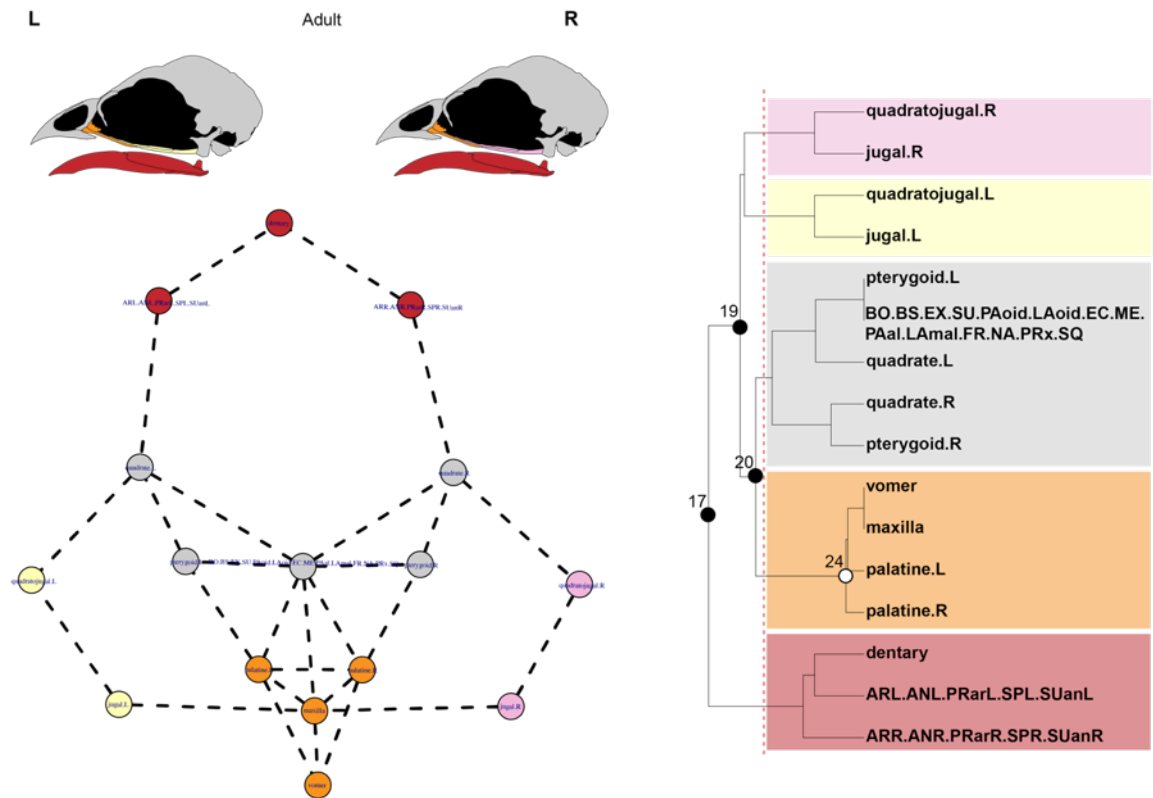
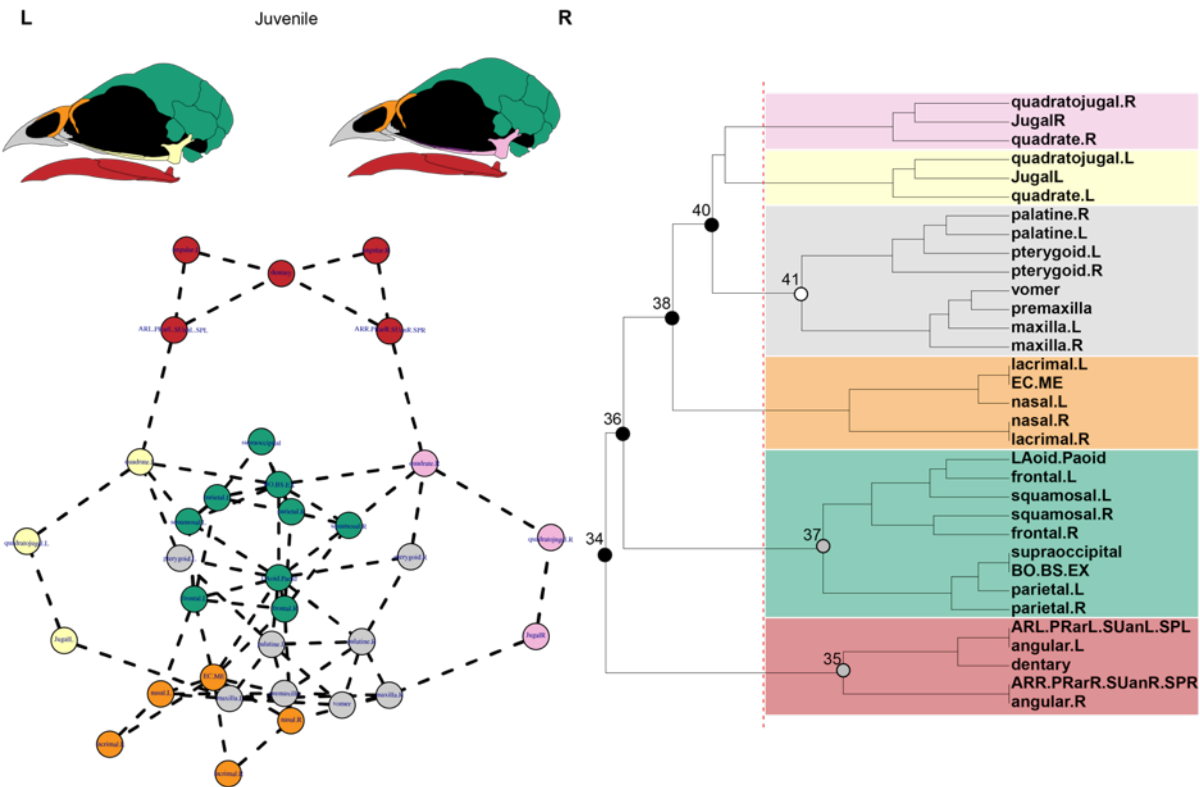
Aythya ferina



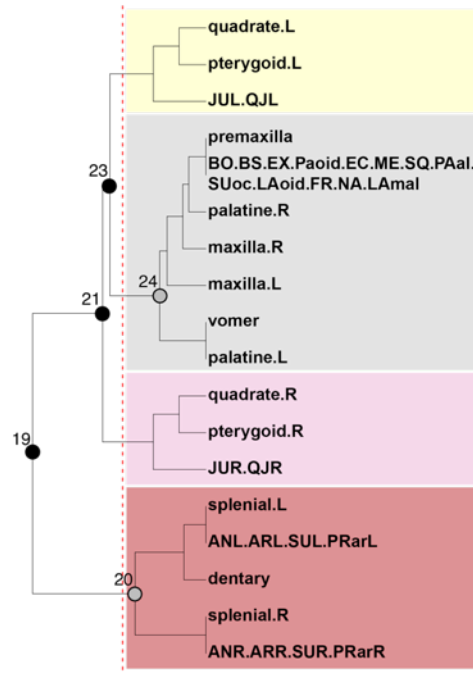
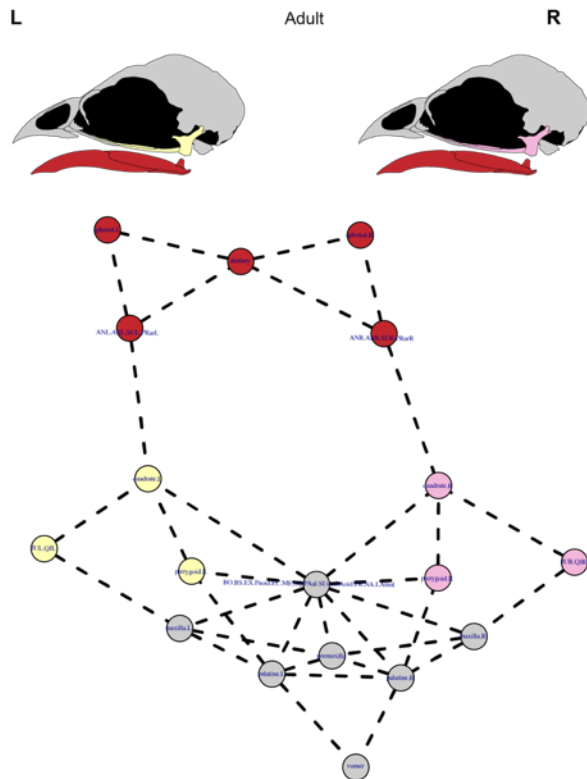
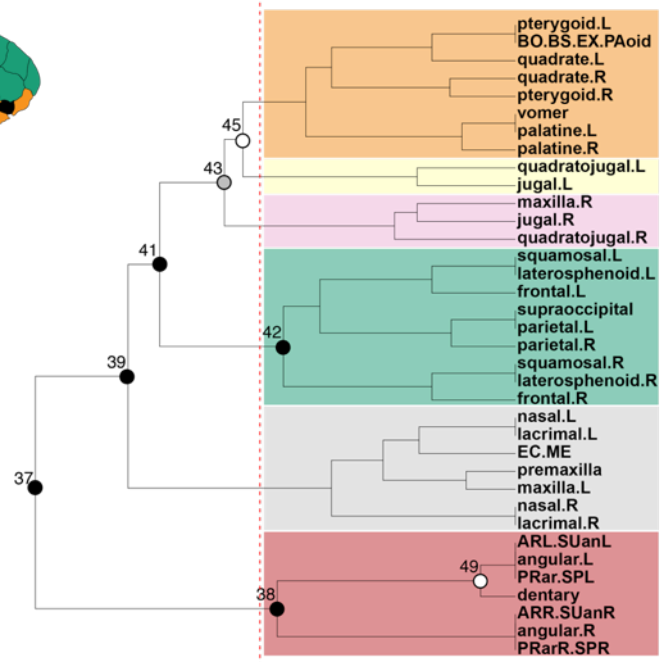
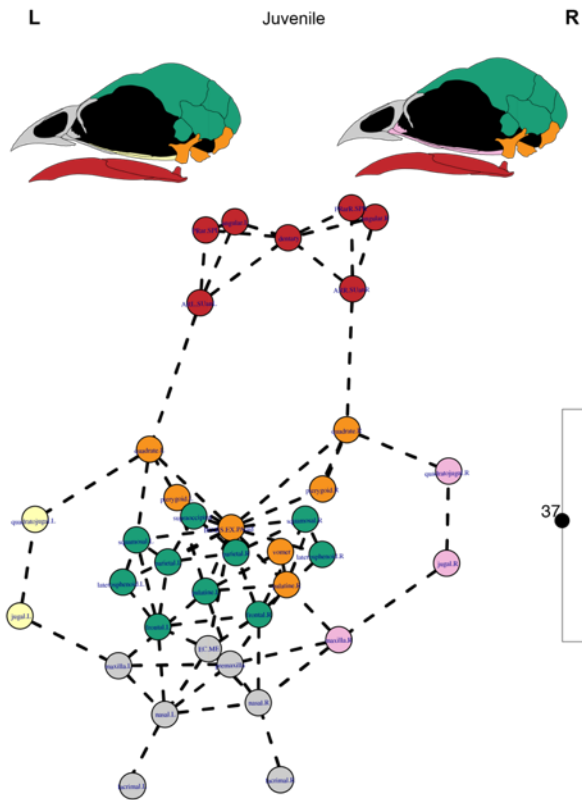
Mergus merganser



Cygnus olor



Fratercula arctica

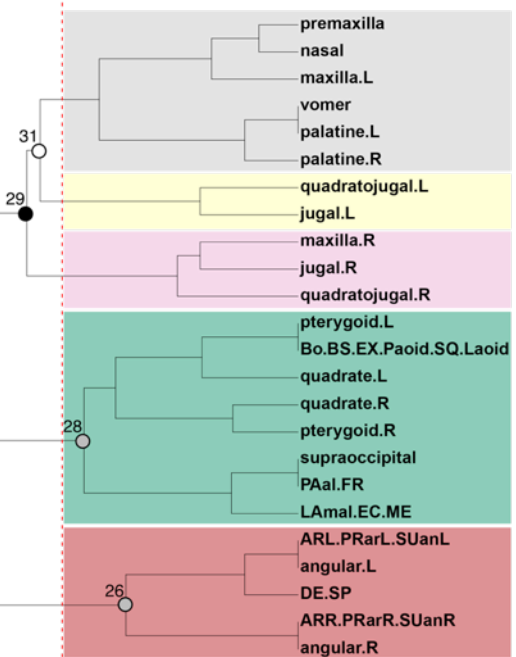
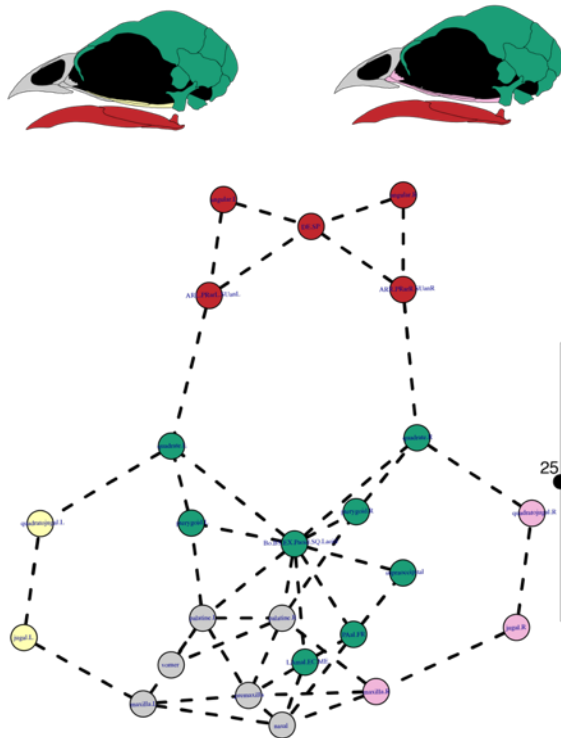


Ptychoramphus aleuticus

L

Juvenile

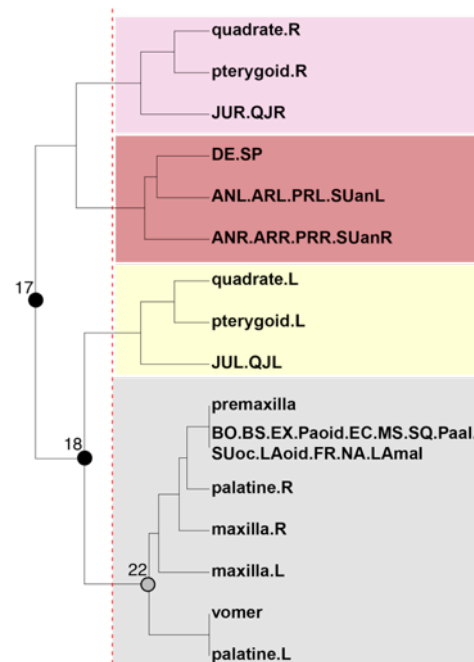
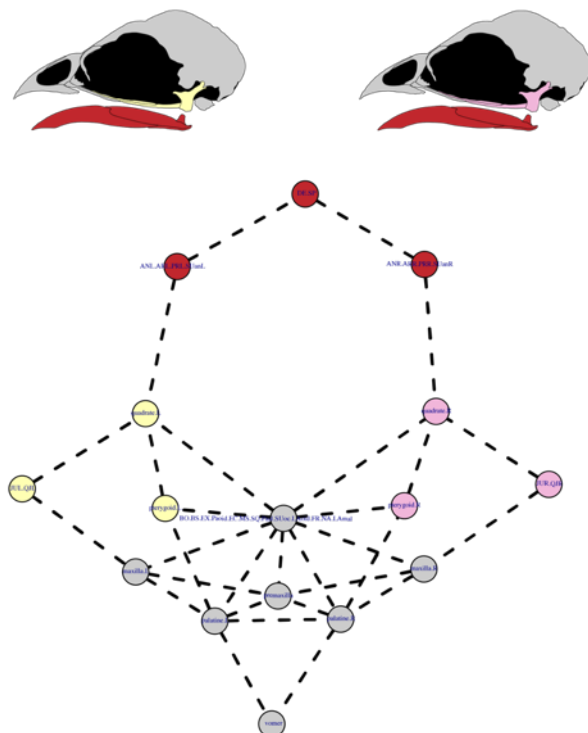
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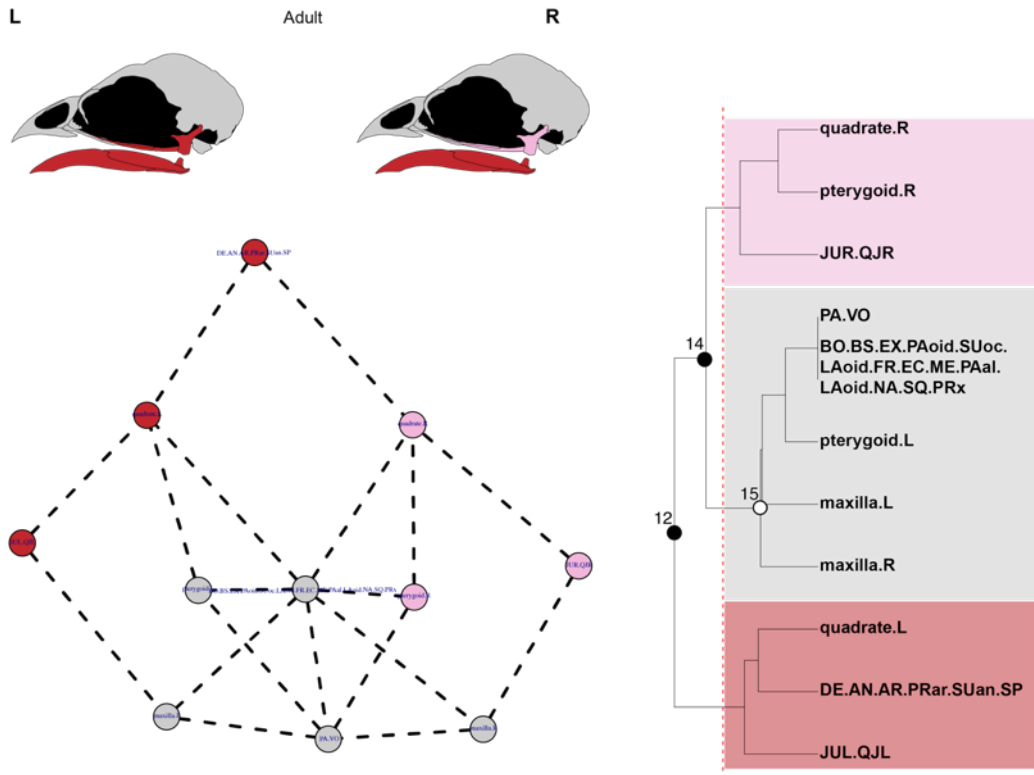
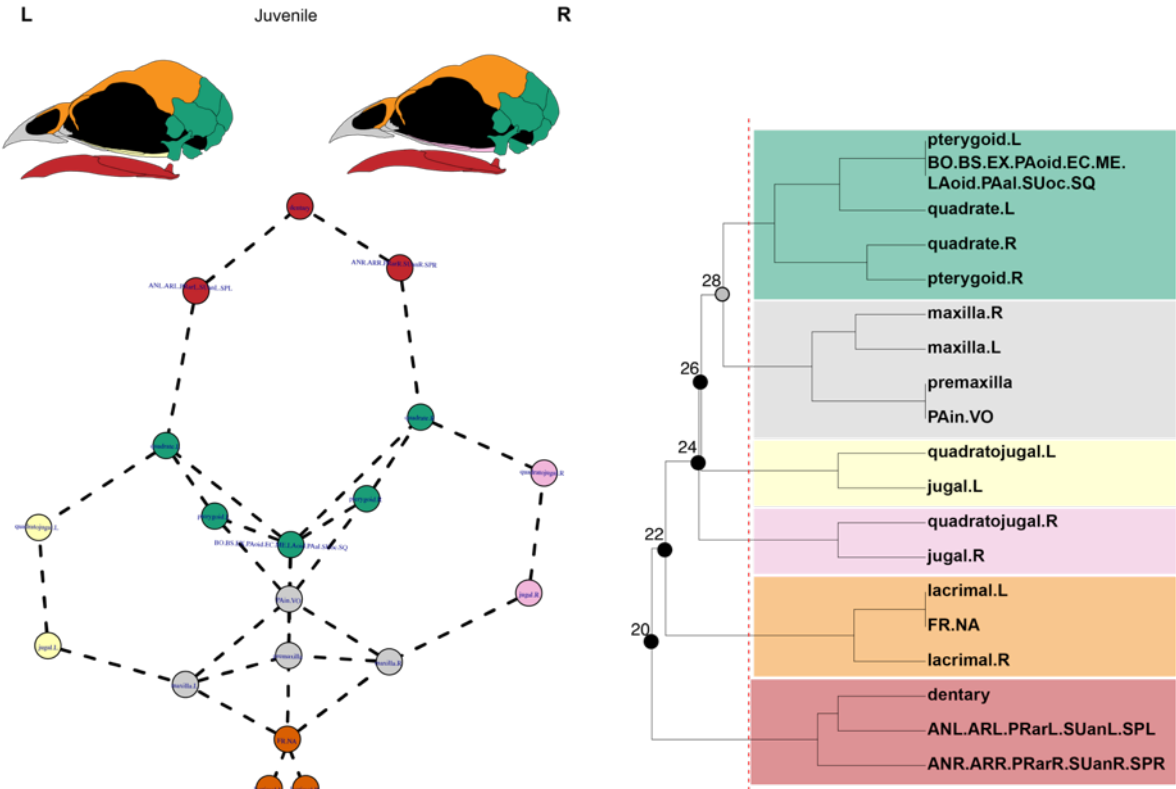
L

Adult

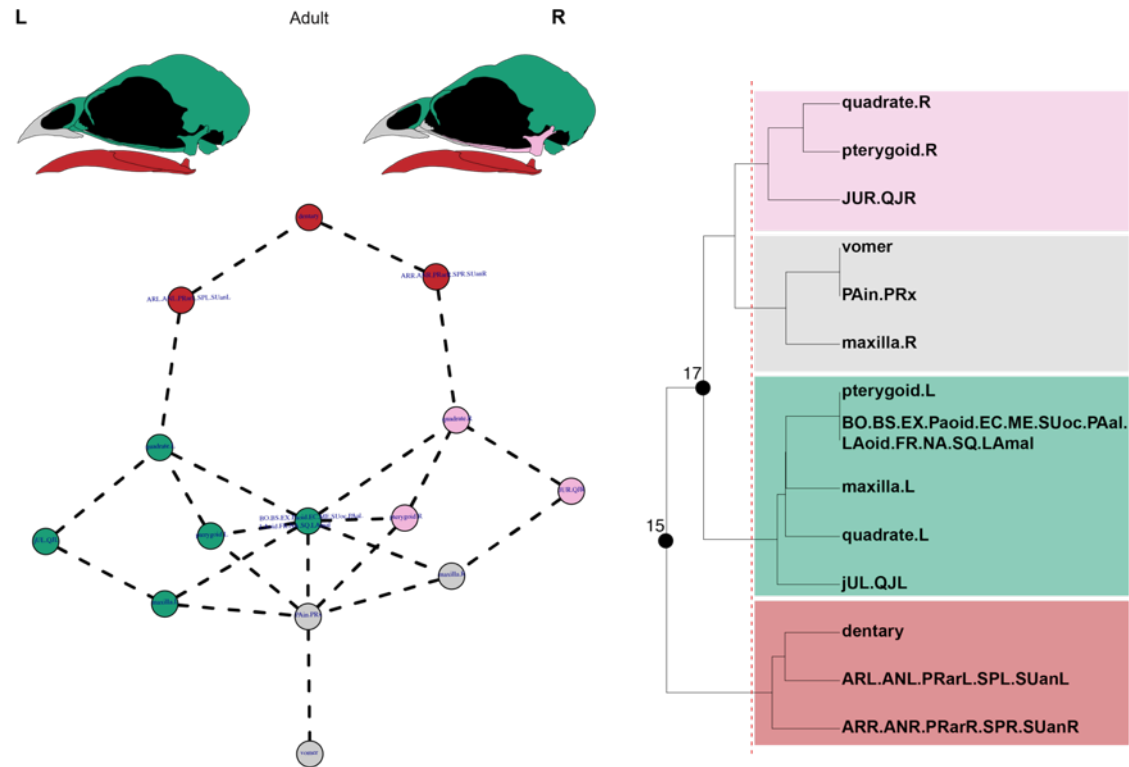
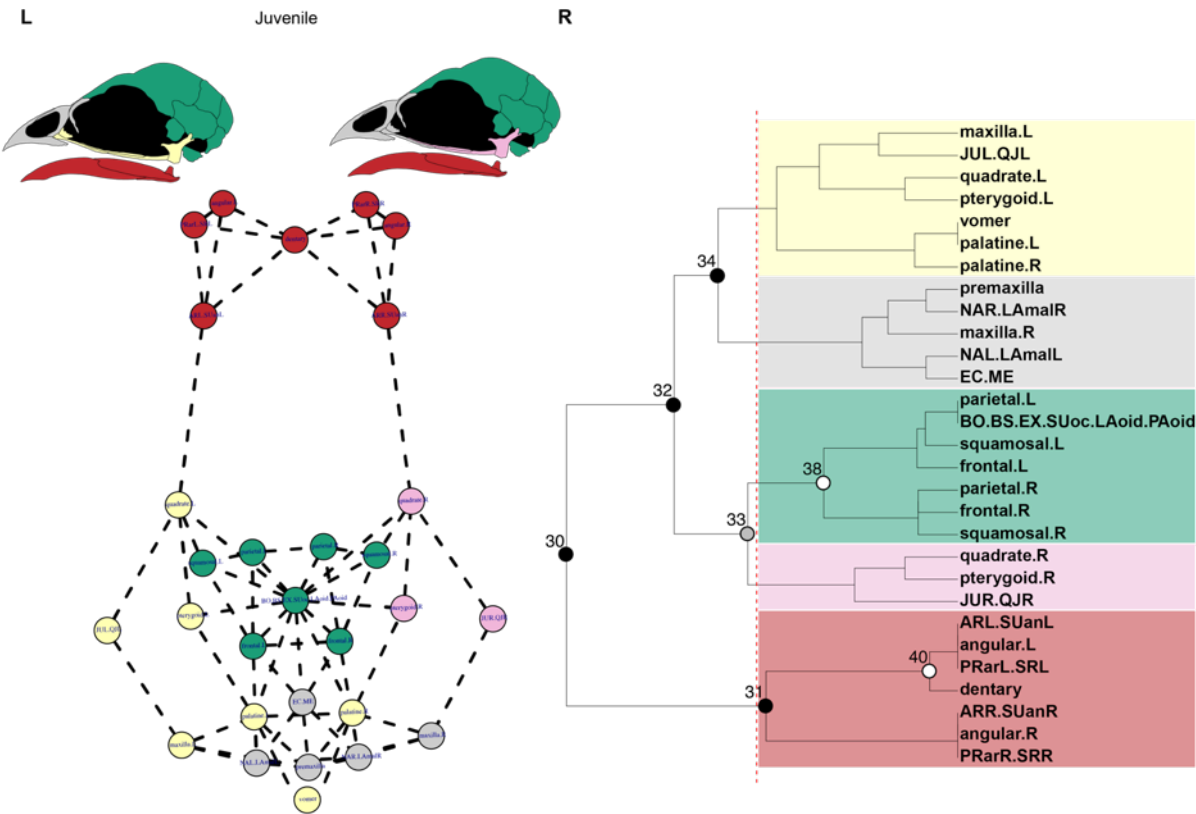
R



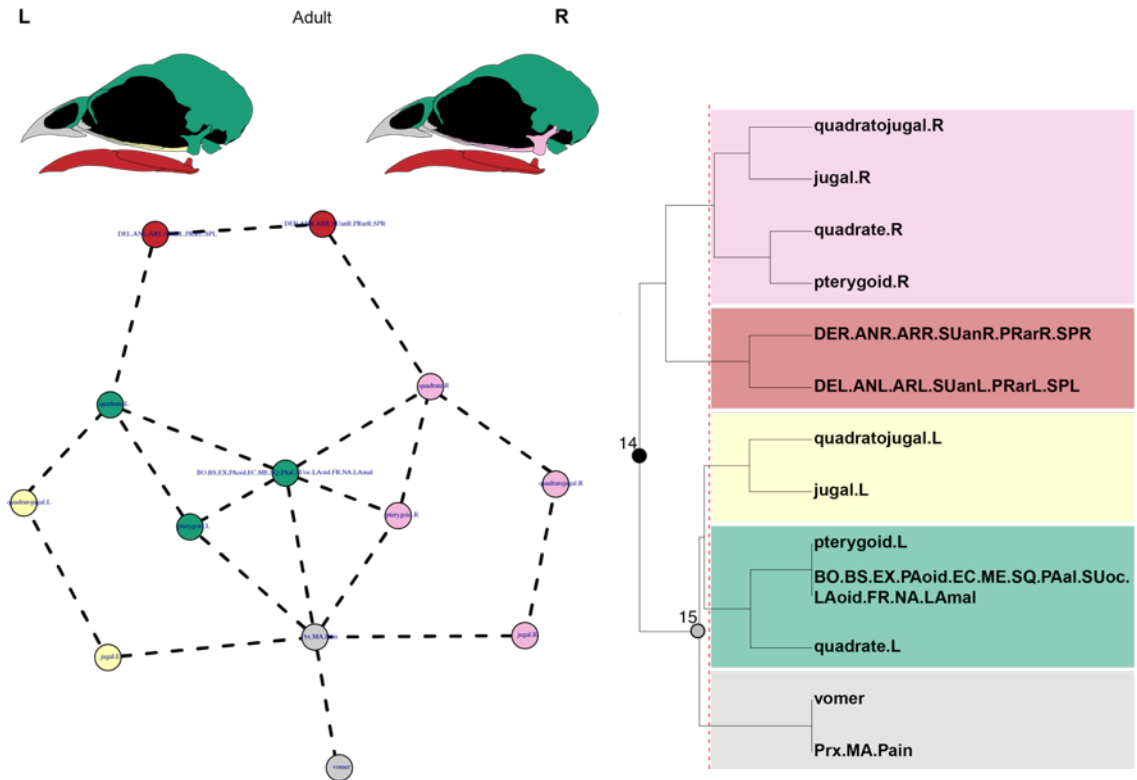
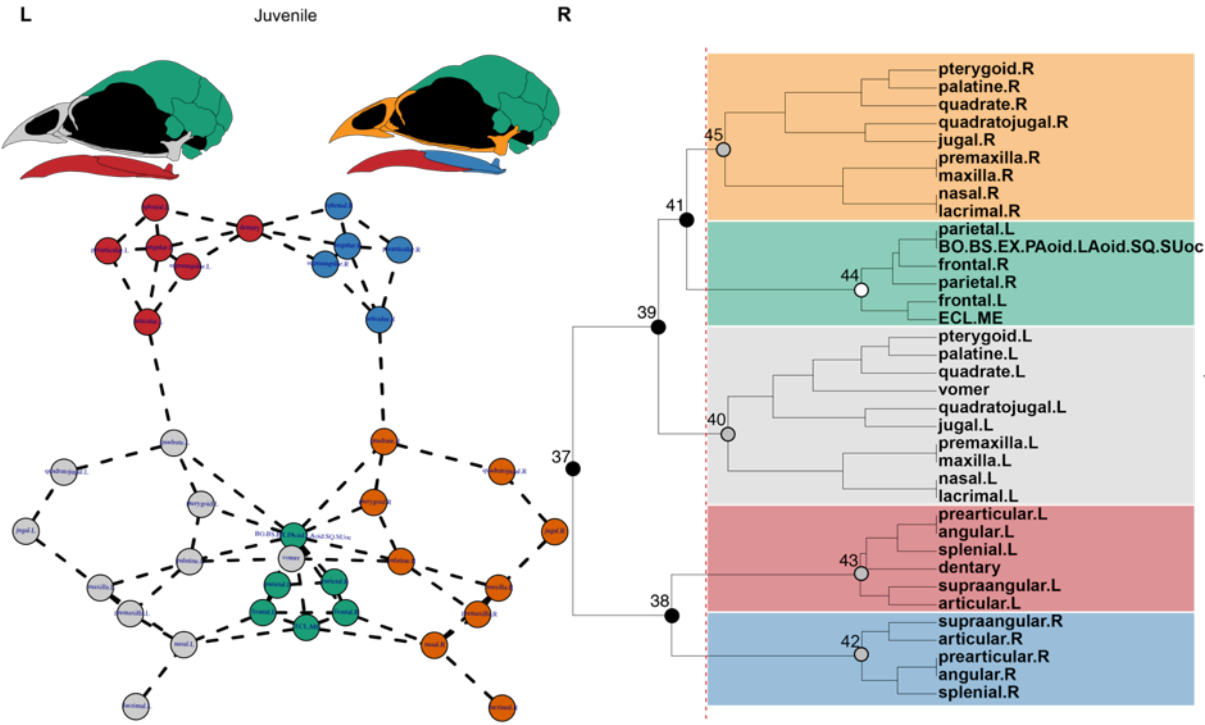
Recurvirostra avosetta



Larus ridubundus



Ciconia ciconia

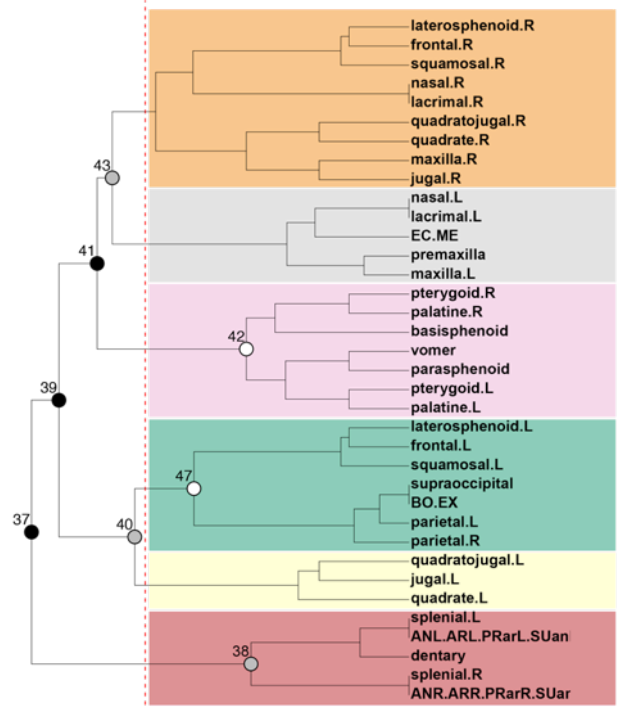
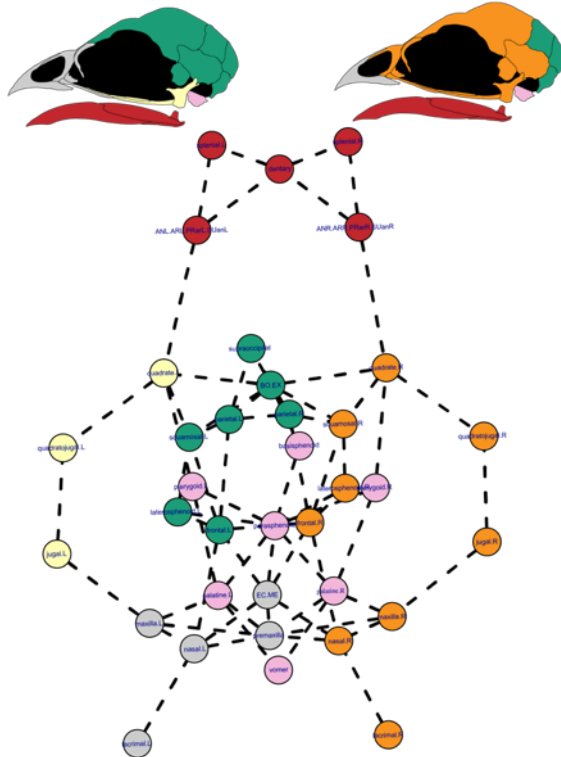


Gallus gallus

L

Juvenile

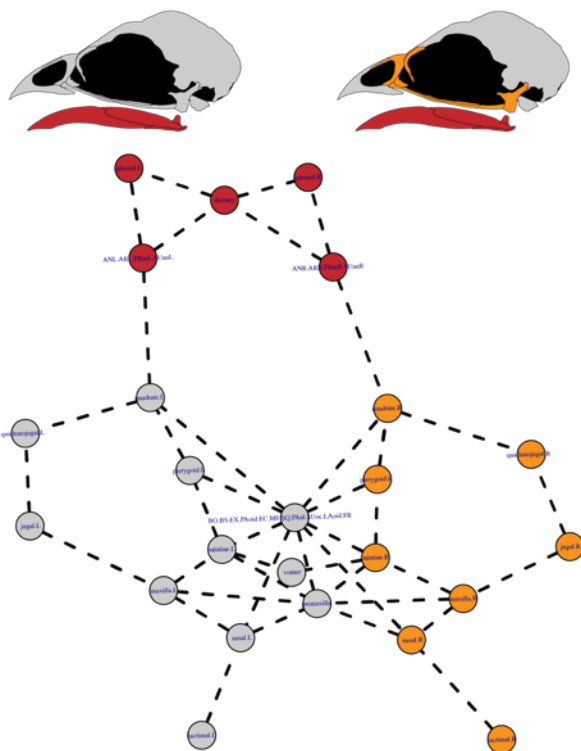
R



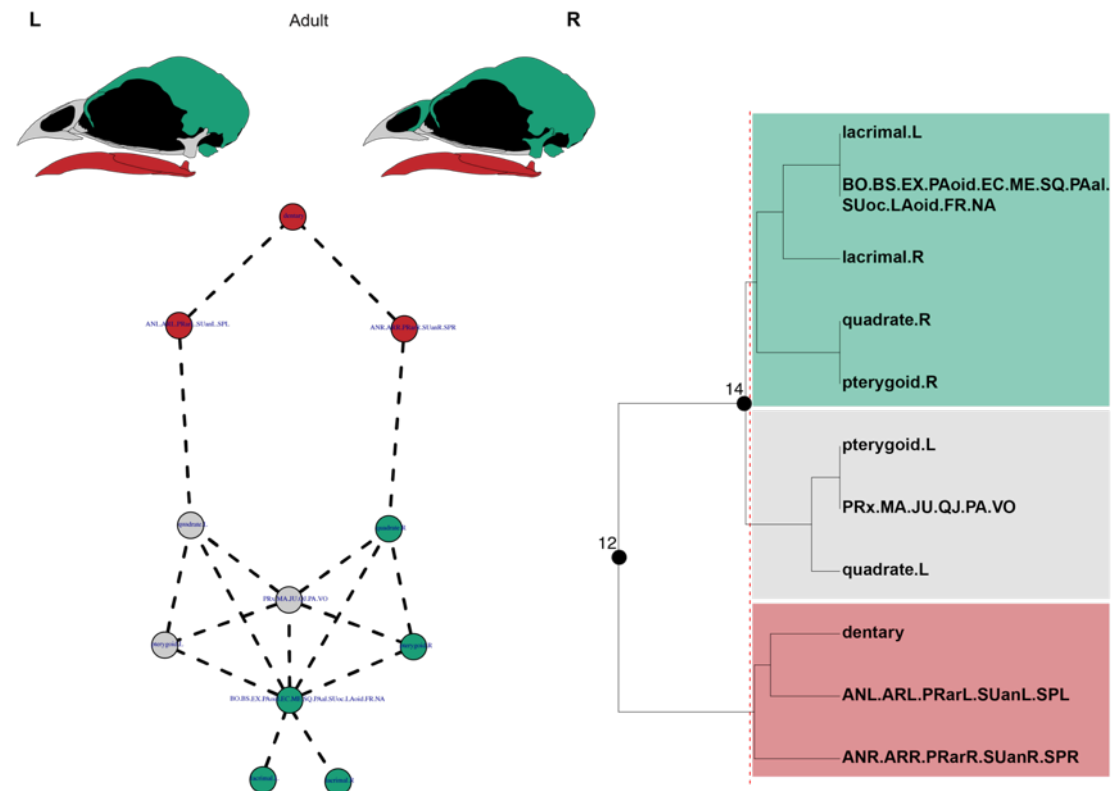
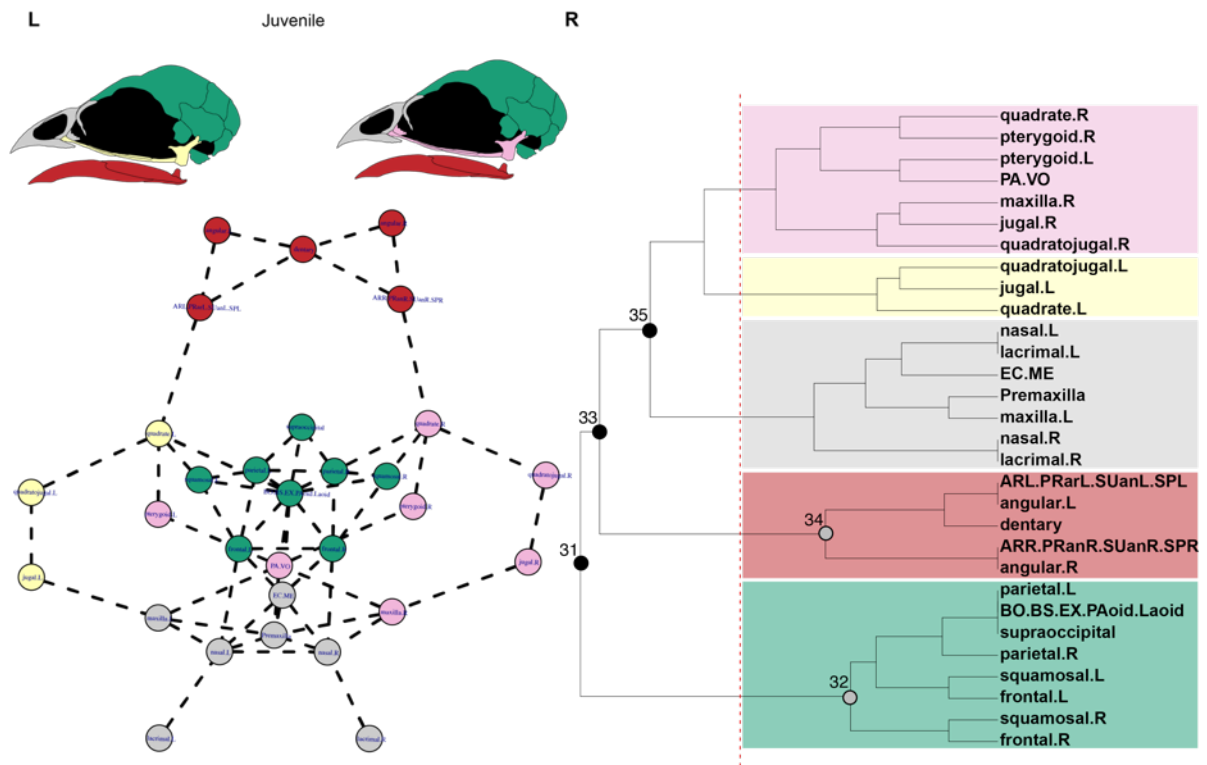
L

Adult

R



Grus paradisea



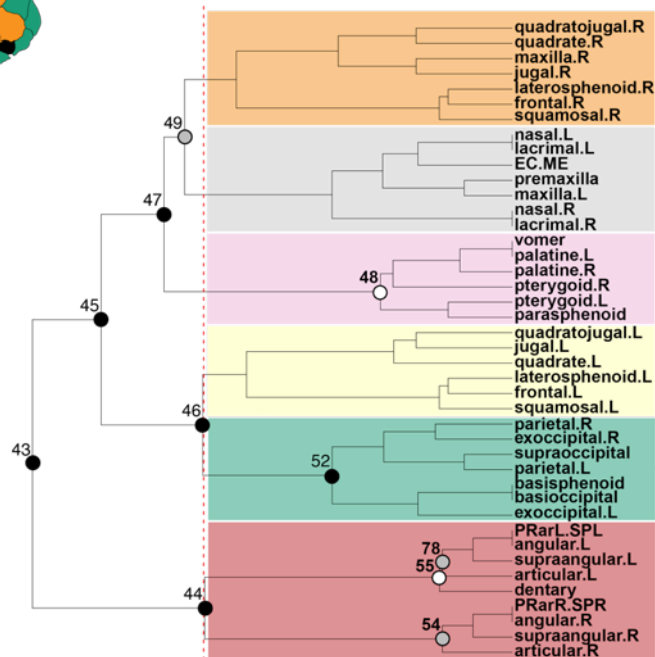
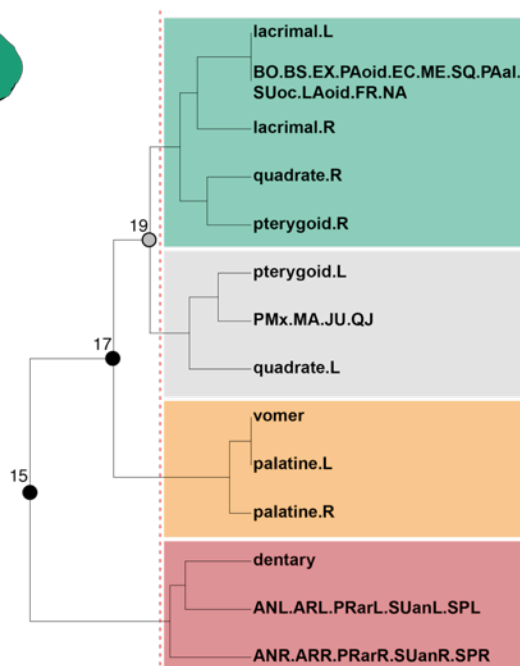


Figure 1 consists of two parts. The top part shows two lateral views of an adult brain, labeled 'L' (left) and 'R' (right). The brain is colored green and red, with a red line indicating the midline. The bottom part shows a gene regulatory network. The network is a directed graph with nodes and edges. The nodes are colored green, red, orange, and grey. The edges are dashed lines. The nodes are labeled with gene names: *ANR-1*, *ANR-2*, *ANR-3*, *ANR-4*, *ANR-5*, *ANR-6*, *ANR-7*, *ANR-8*, *ANR-9*, *ANR-10*, *ANR-11*, *ANR-12*, *ANR-13*, *ANR-14*, *ANR-15*, *ANR-16*, *ANR-17*, *ANR-18*, *ANR-19*, *ANR-20*, *ANR-21*, *ANR-22*, *ANR-23*, *ANR-24*, *ANR-25*, *ANR-26*, *ANR-27*, *ANR-28*, *ANR-29*, *ANR-30*, *ANR-31*, *ANR-32*, *ANR-33*, *ANR-34*, *ANR-35*, *ANR-36*, *ANR-37*, *ANR-38*, *ANR-39*, *ANR-40*, *ANR-41*, *ANR-42*, *ANR-43*, *ANR-44*, *ANR-45*, *ANR-46*, *ANR-47*, *ANR-48*, *ANR-49*, *ANR-50*, *ANR-51*, *ANR-52*, *ANR-53*, *ANR-54*, *ANR-55*, *ANR-56*, *ANR-57*, *ANR-58*, *ANR-59*, *ANR-60*, *ANR-61*, *ANR-62*, *ANR-63*, *ANR-64*, *ANR-65*, *ANR-66*, *ANR-67*, *ANR-68*, *ANR-69*, *ANR-70*, *ANR-71*, *ANR-72*, *ANR-73*, *ANR-74*, *ANR-75*, *ANR-76*, *ANR-77*, *ANR-78*, *ANR-79*, *ANR-80*, *ANR-81*, *ANR-82*, *ANR-83*, *ANR-84*, *ANR-85*, *ANR-86*, *ANR-87*, *ANR-88*, *ANR-89*, *ANR-90*, *ANR-91*, *ANR-92*, *ANR-93*, *ANR-94*, *ANR-95*, *ANR-96*, *ANR-97*, *ANR-98*, *ANR-99*, *ANR-100*. The network is a complex web of interactions, with many nodes having multiple incoming and outgoing edges. The nodes are arranged in a hierarchical manner, with some nodes at the top and others at the bottom. The edges are dashed lines, indicating that the interactions are not direct but rather mediated by other factors. The network is a representation of the gene regulatory network in the adult brain, showing the complex interactions between different genes and their products.

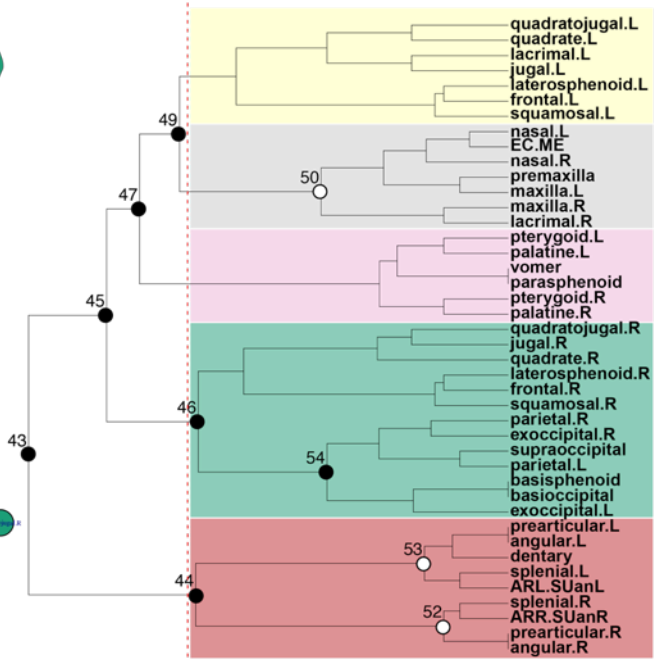
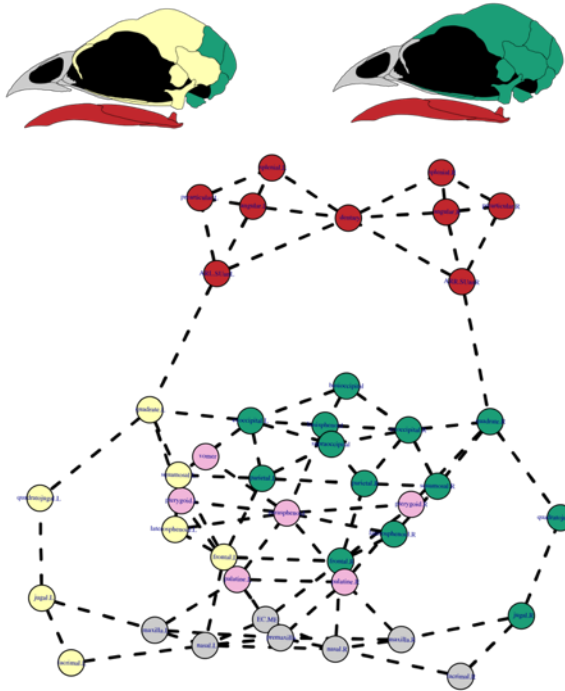


Otis tardas

L

Juvenile

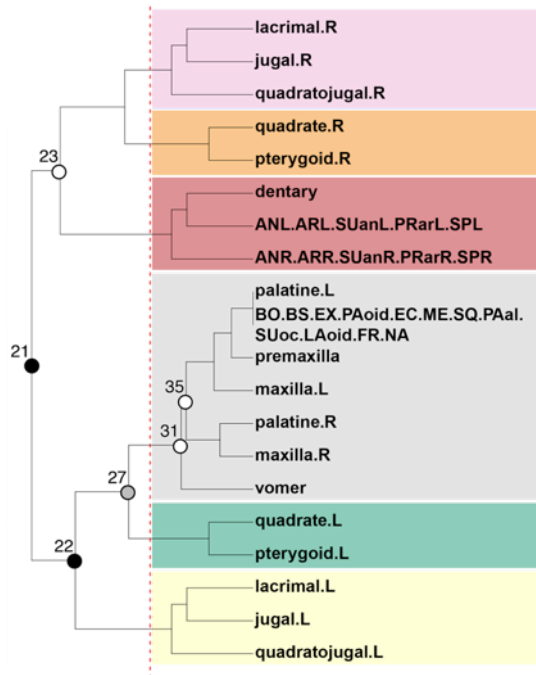
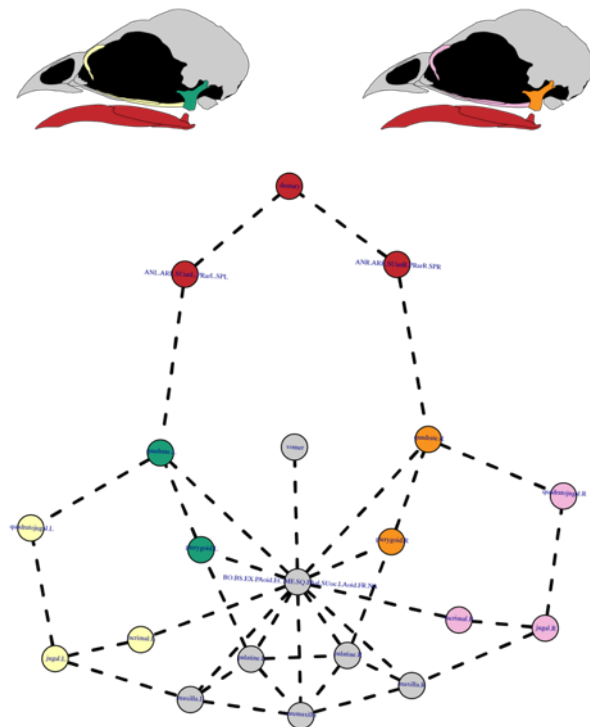
R



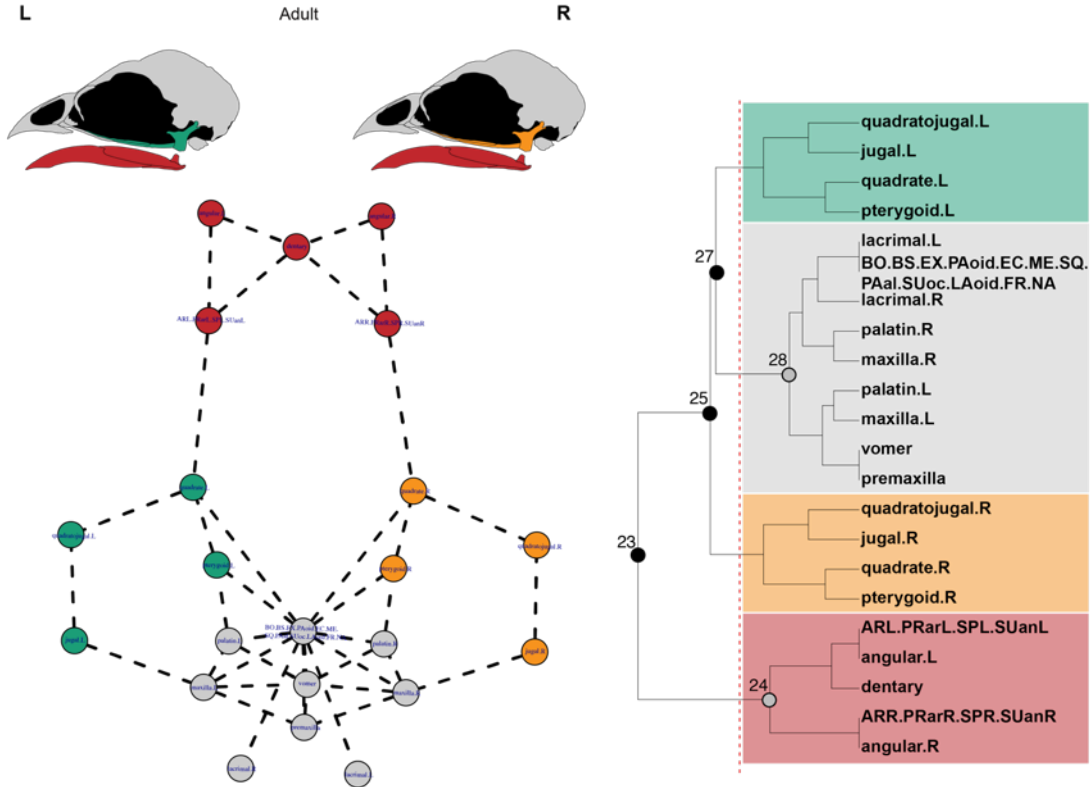
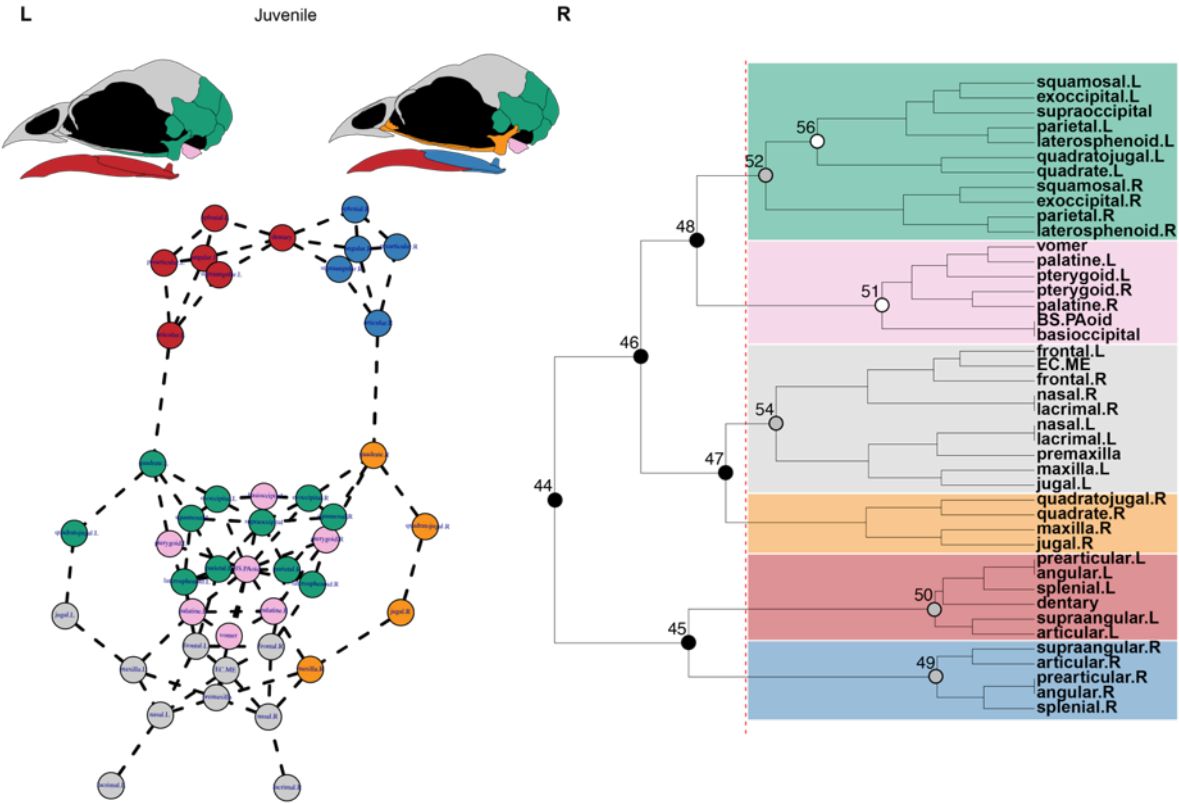
L

Adult

R



Rhea americana

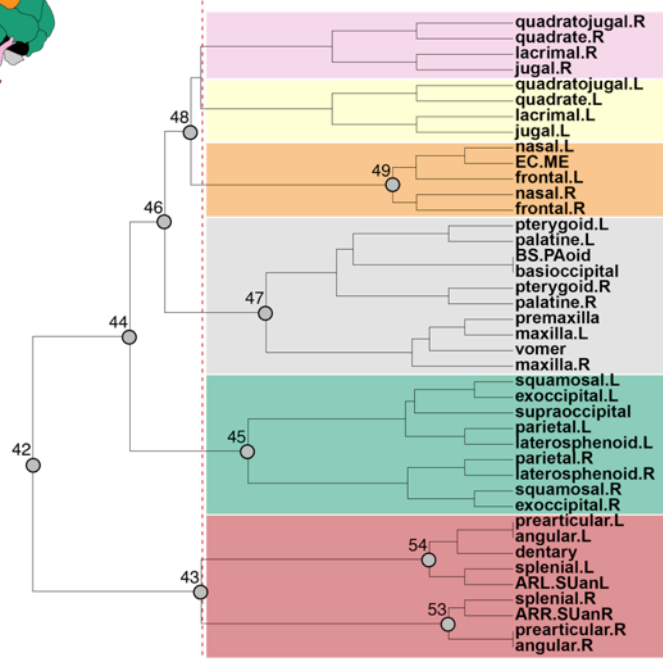
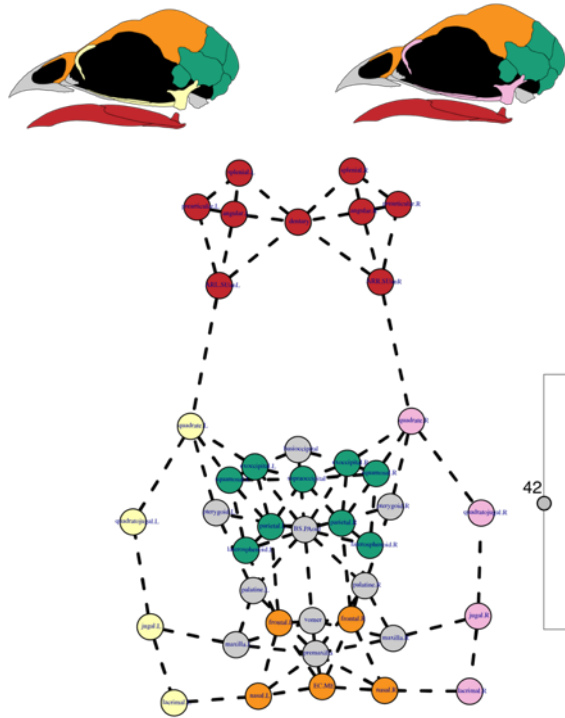


Struthio camelus

L

Juvenile

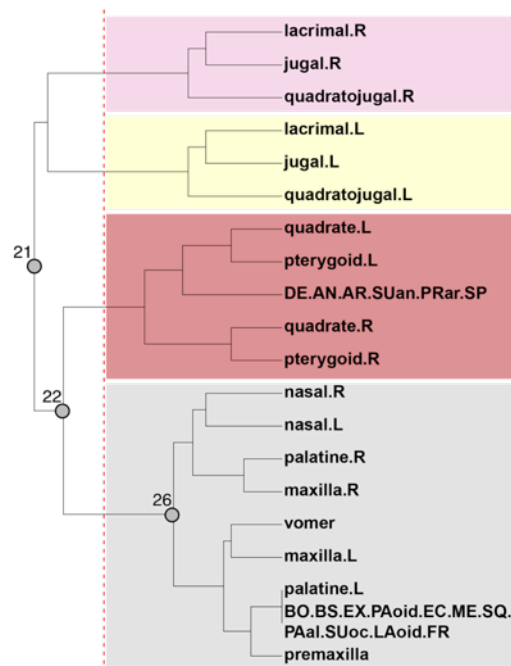
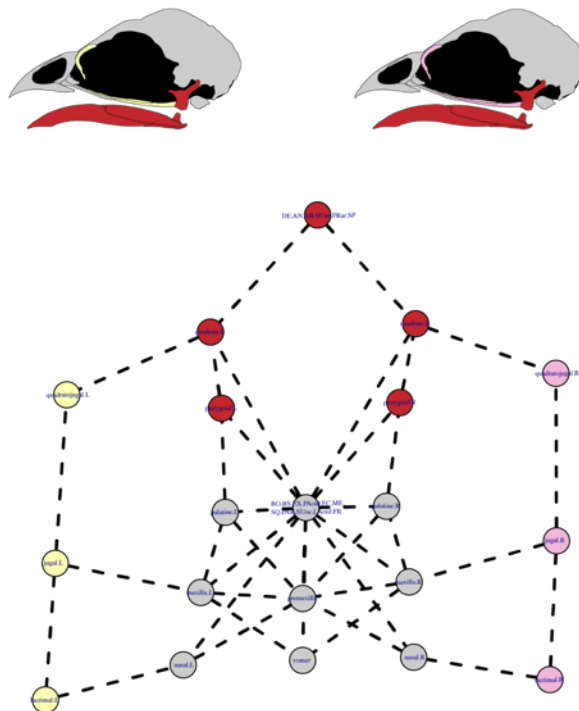
R



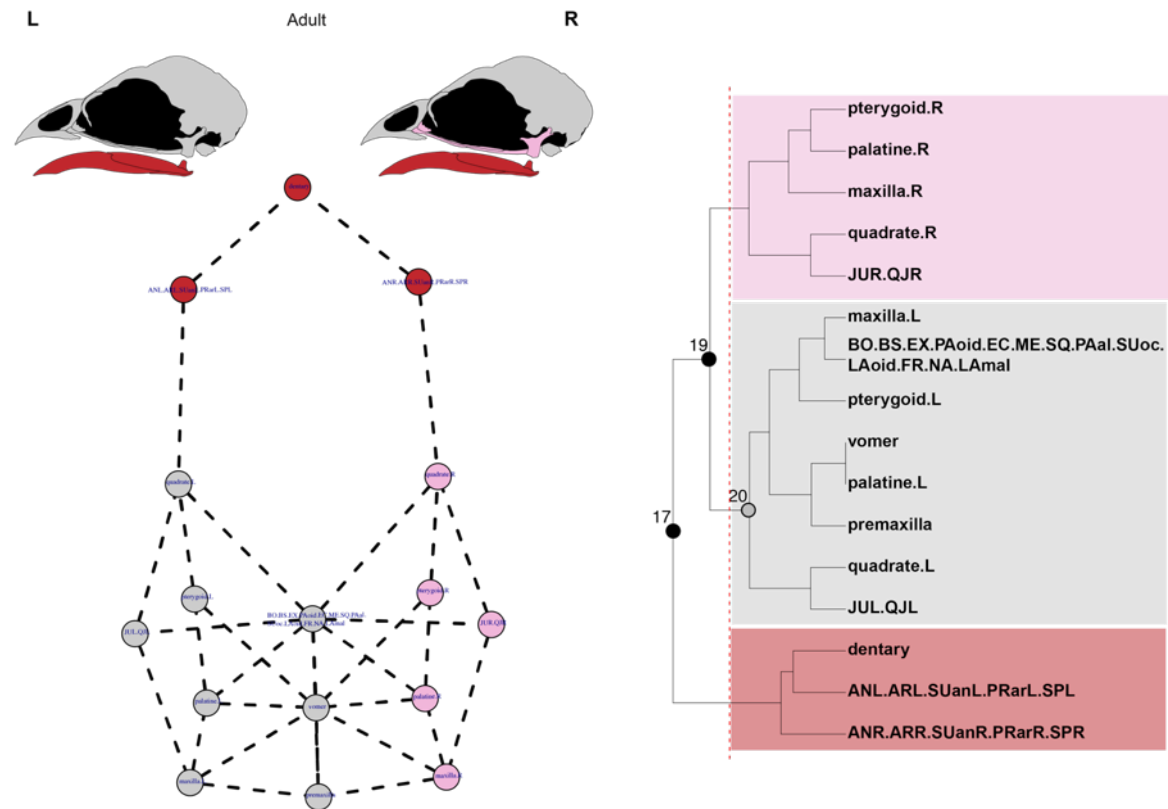
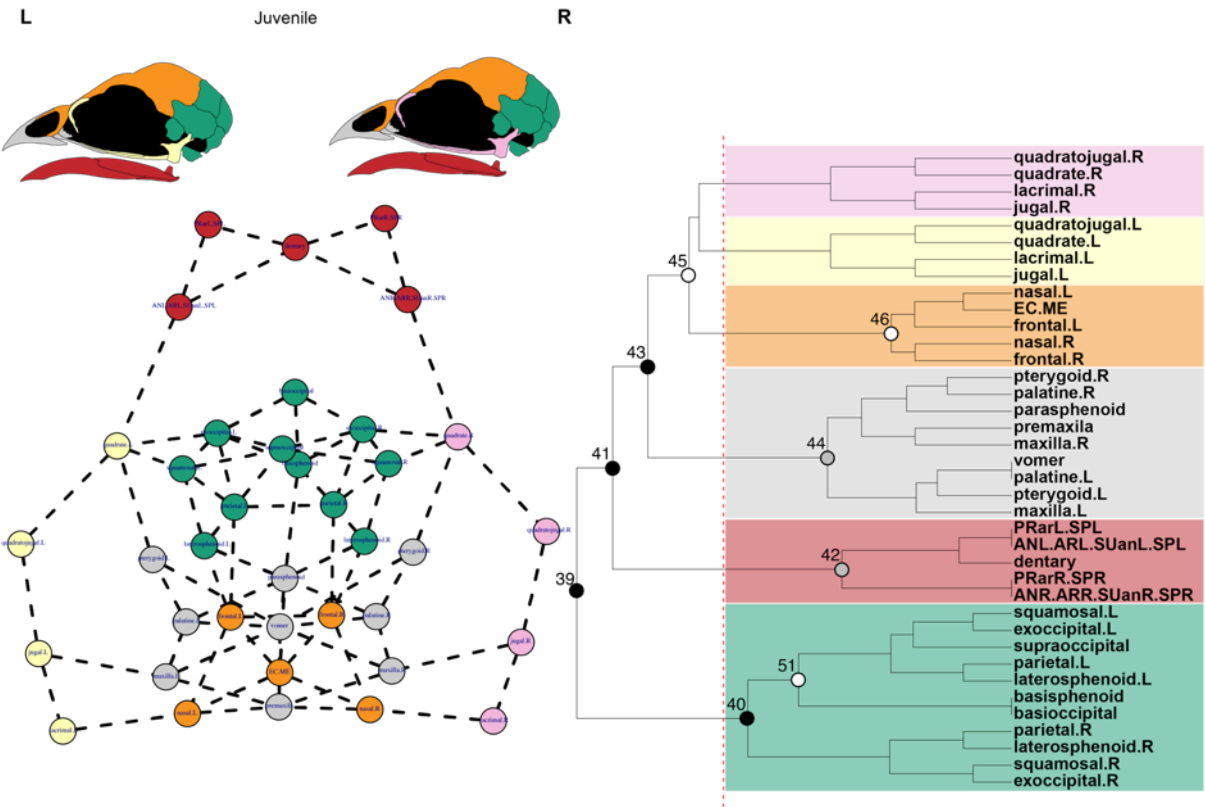
L

Adult

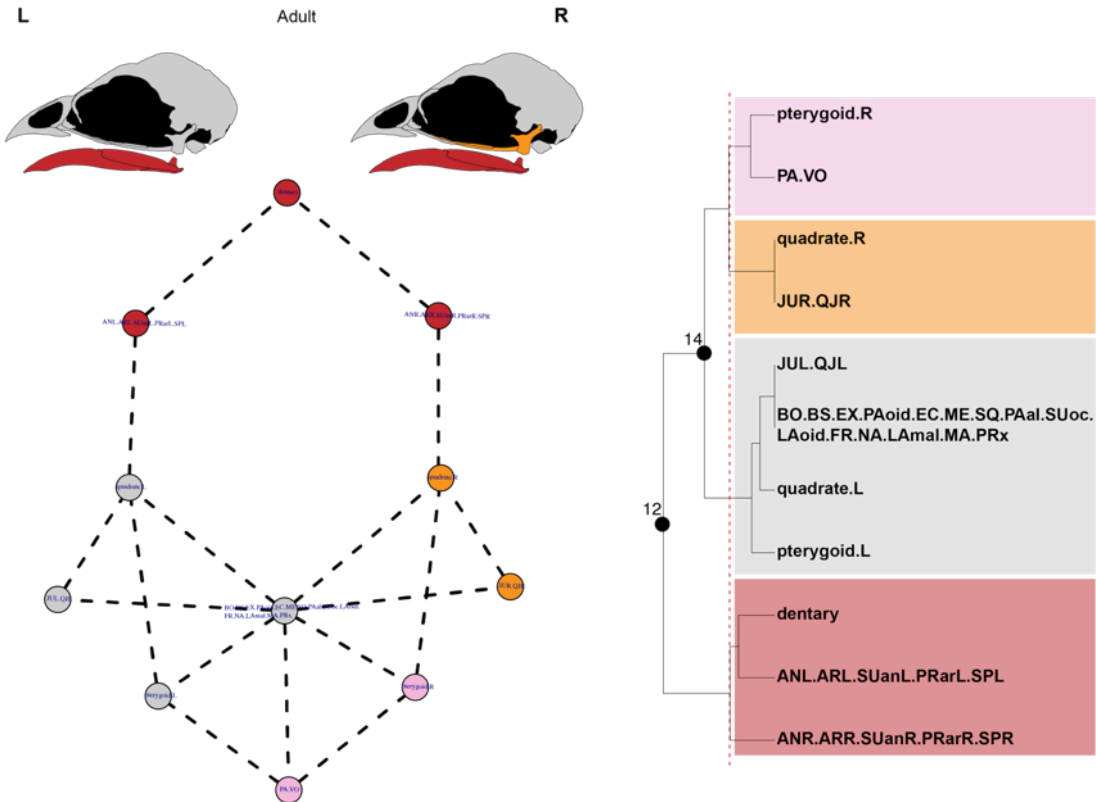
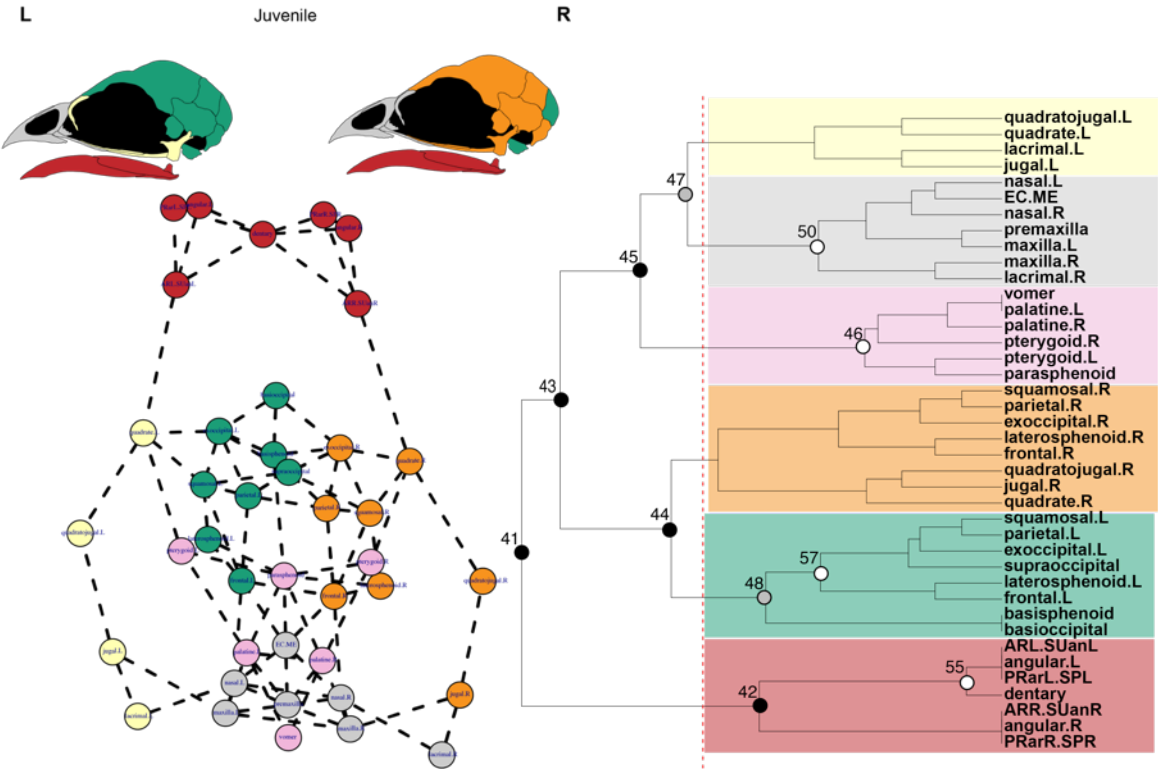
R



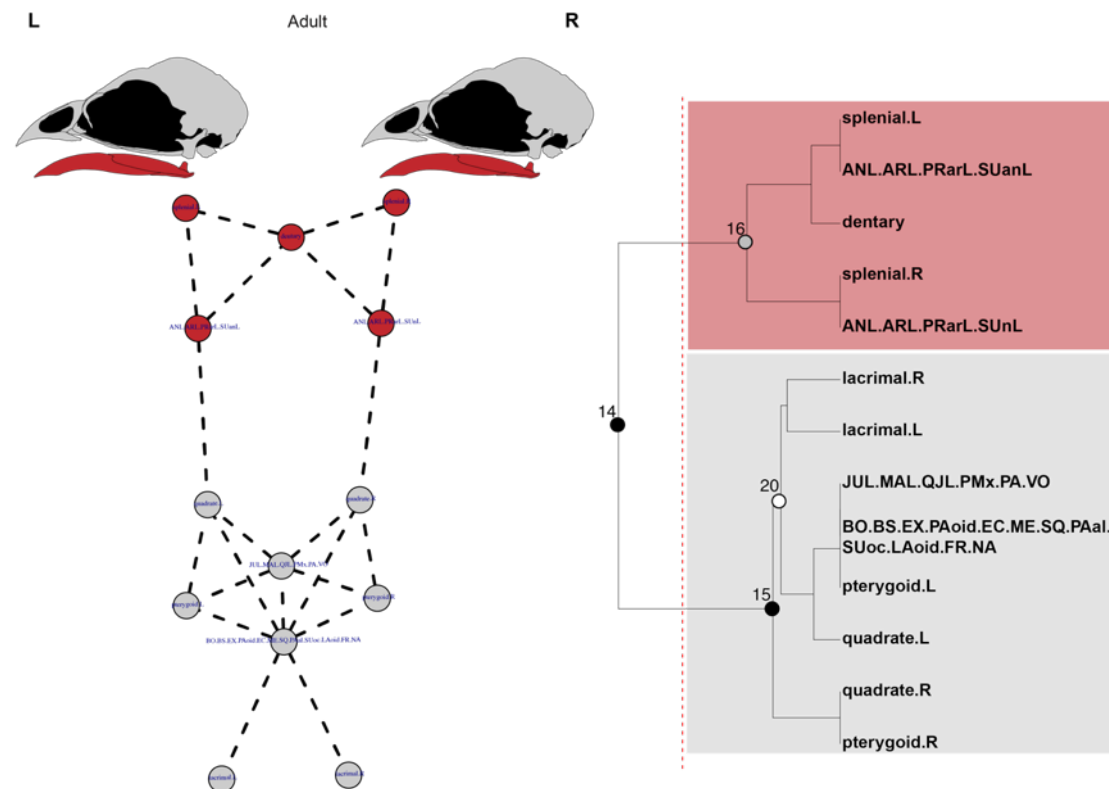
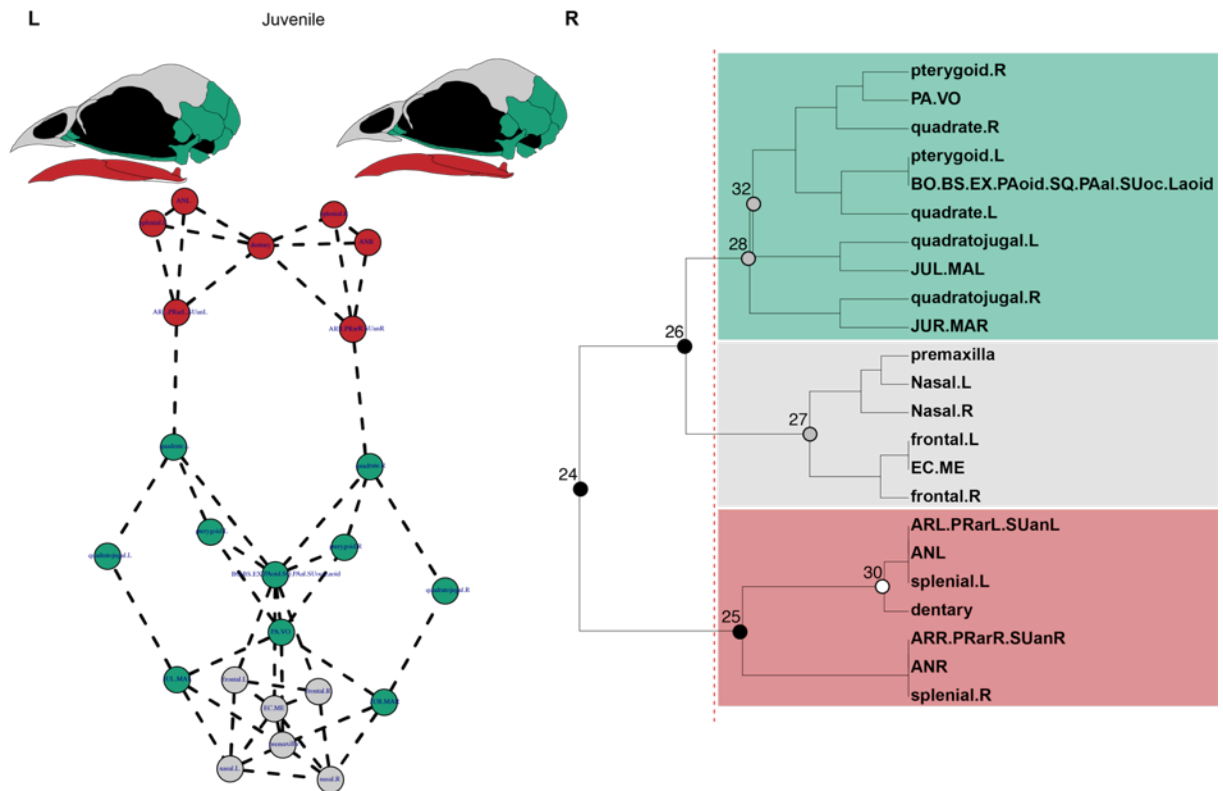
Dromaius novaehollandiae



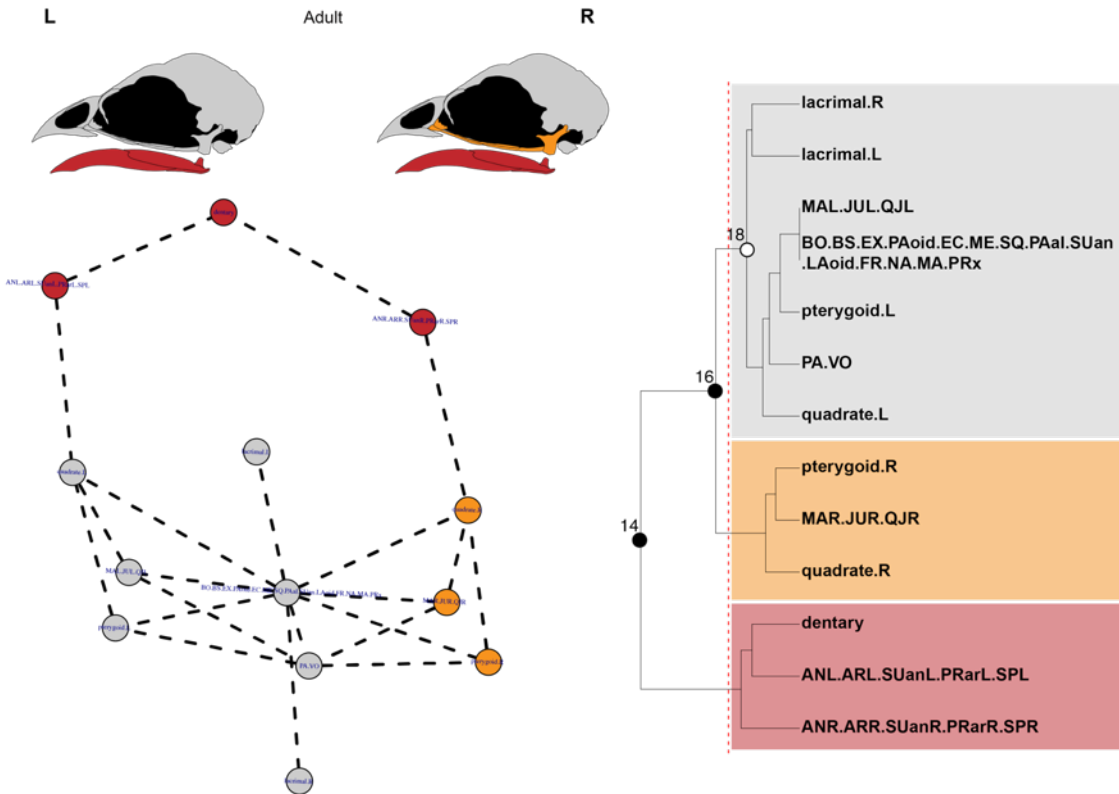
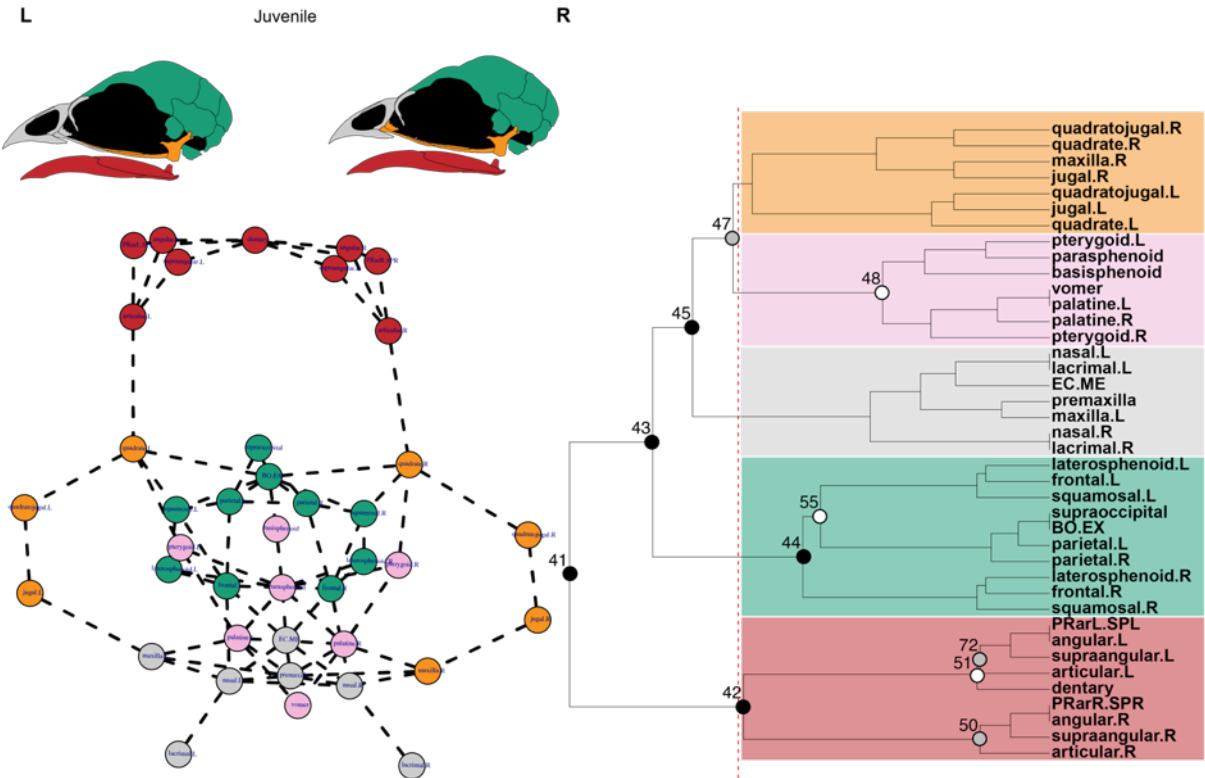
Pelecanus occidentalis



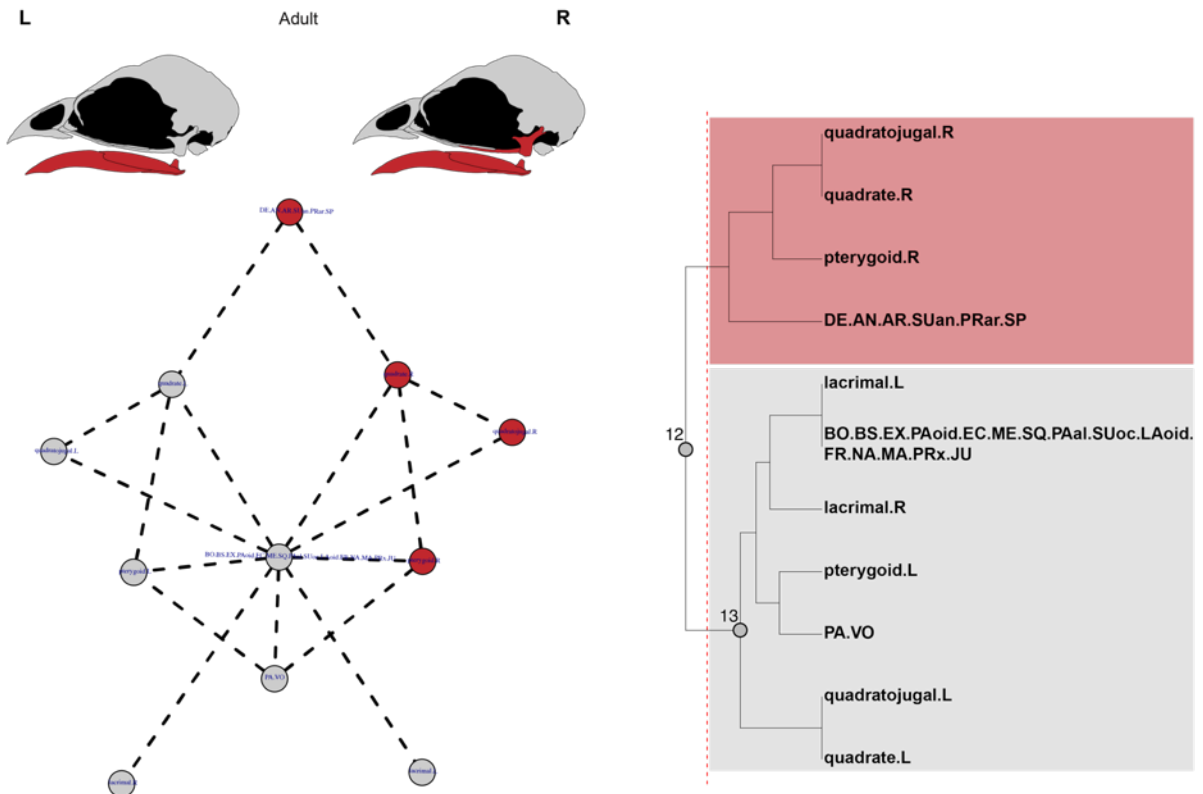
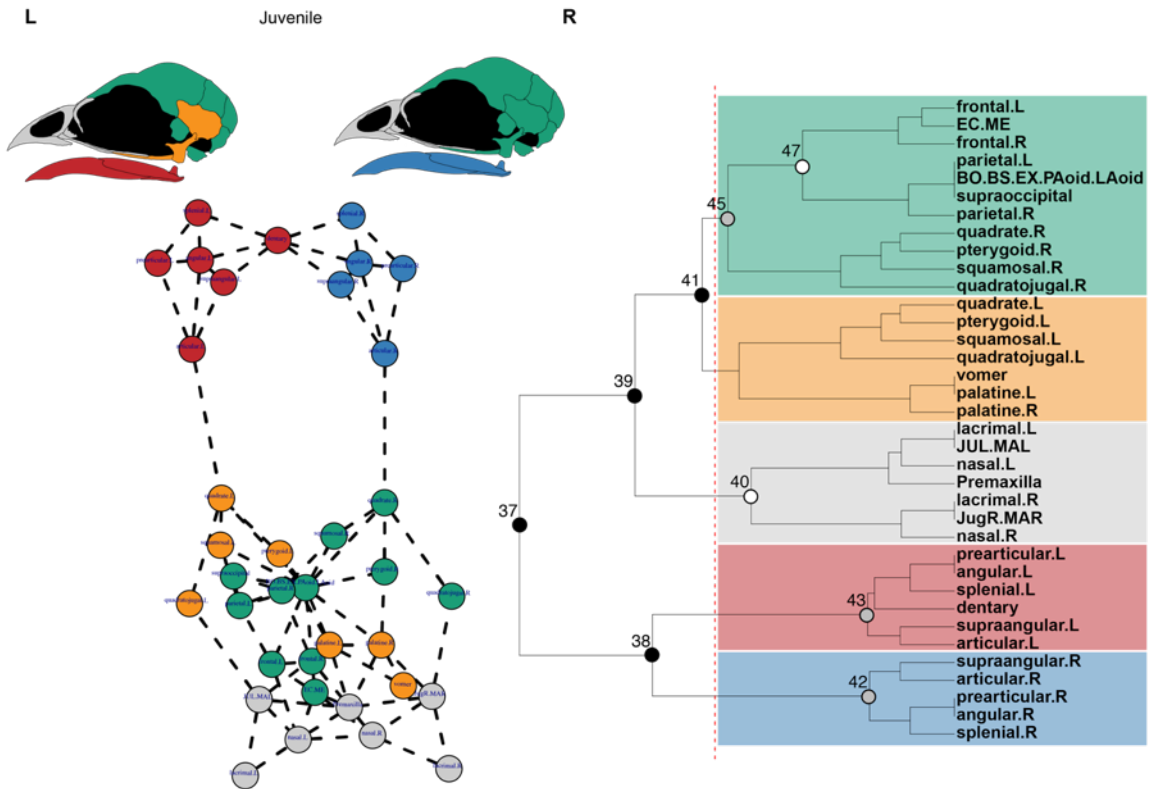
Theristicus caudatus



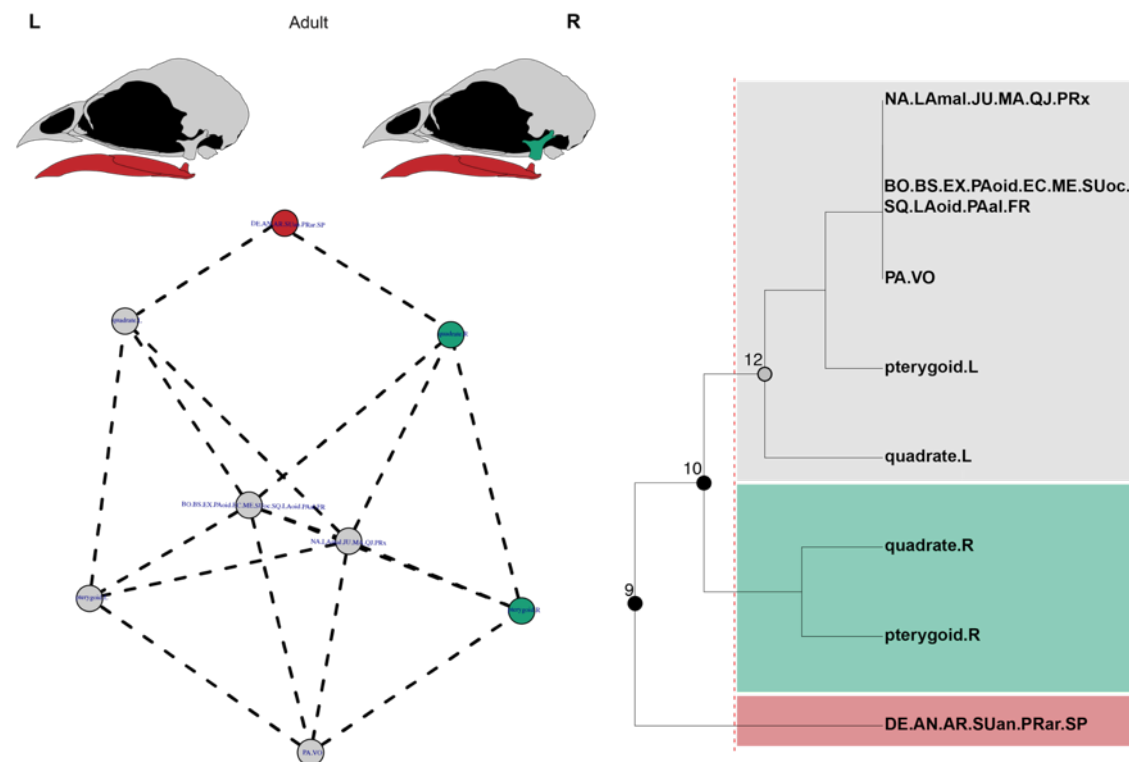
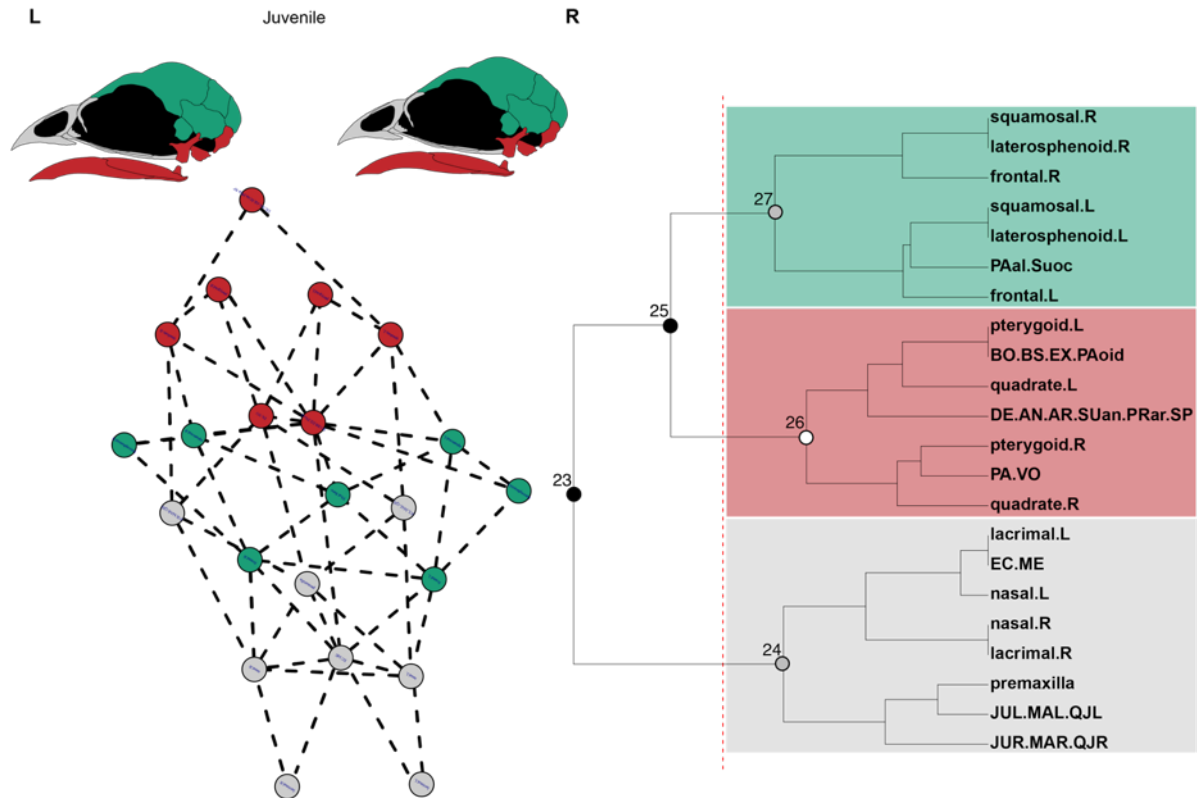
Ardea purpurea



Platalea leucorodia



Platycercus elegans

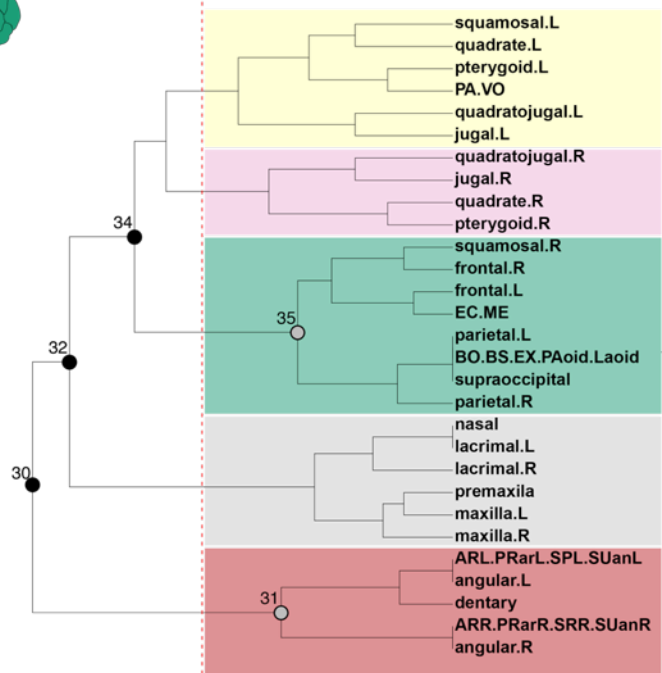
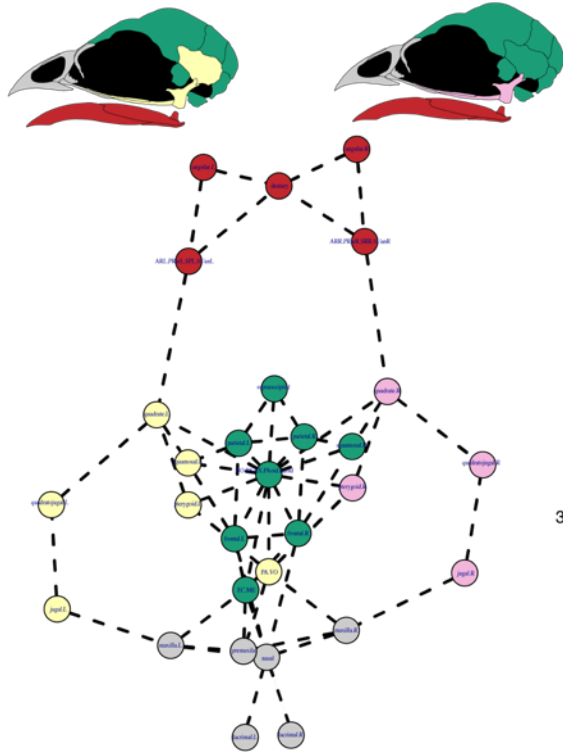


Upupa epos

L

Juvenile

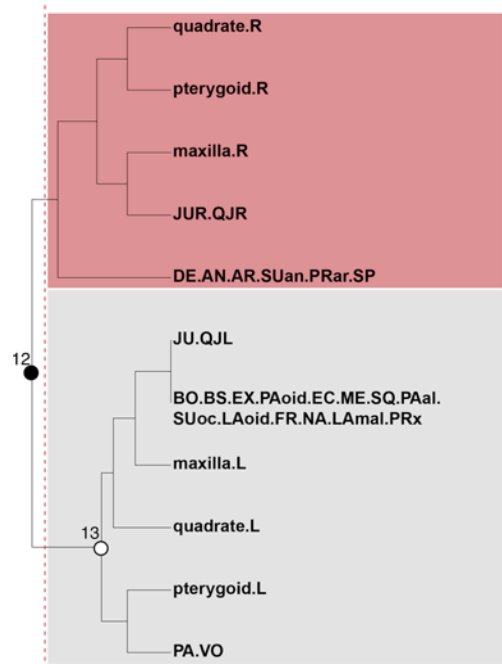
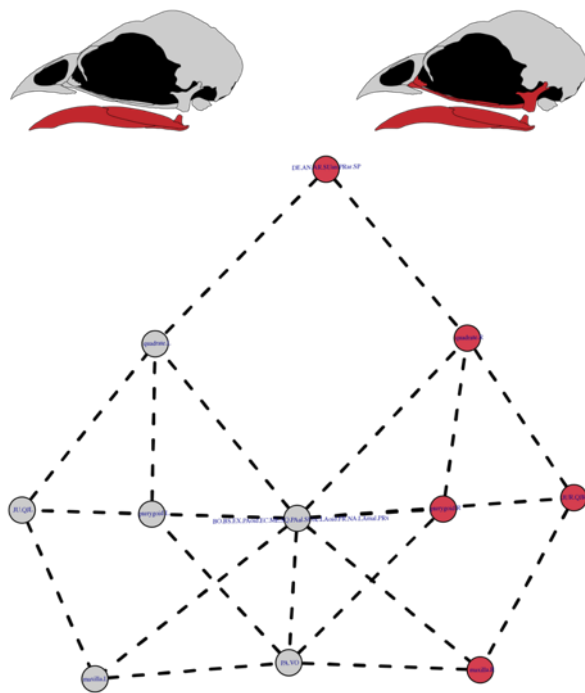
R



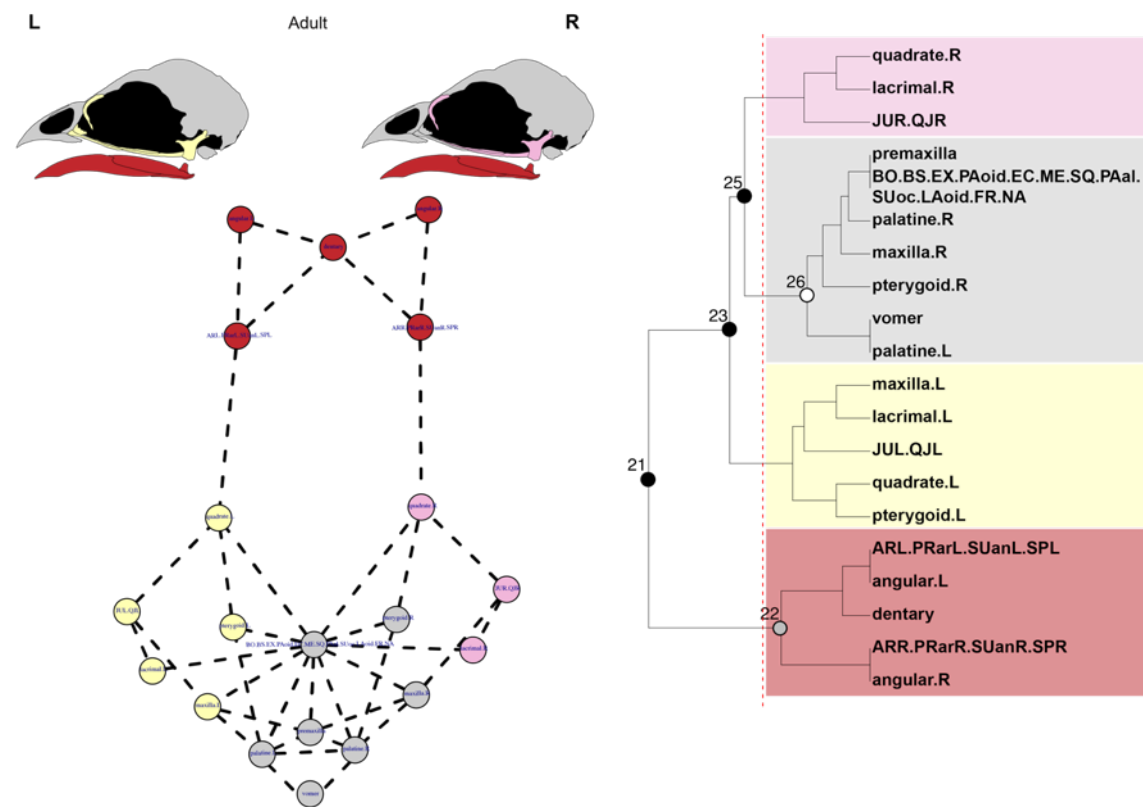
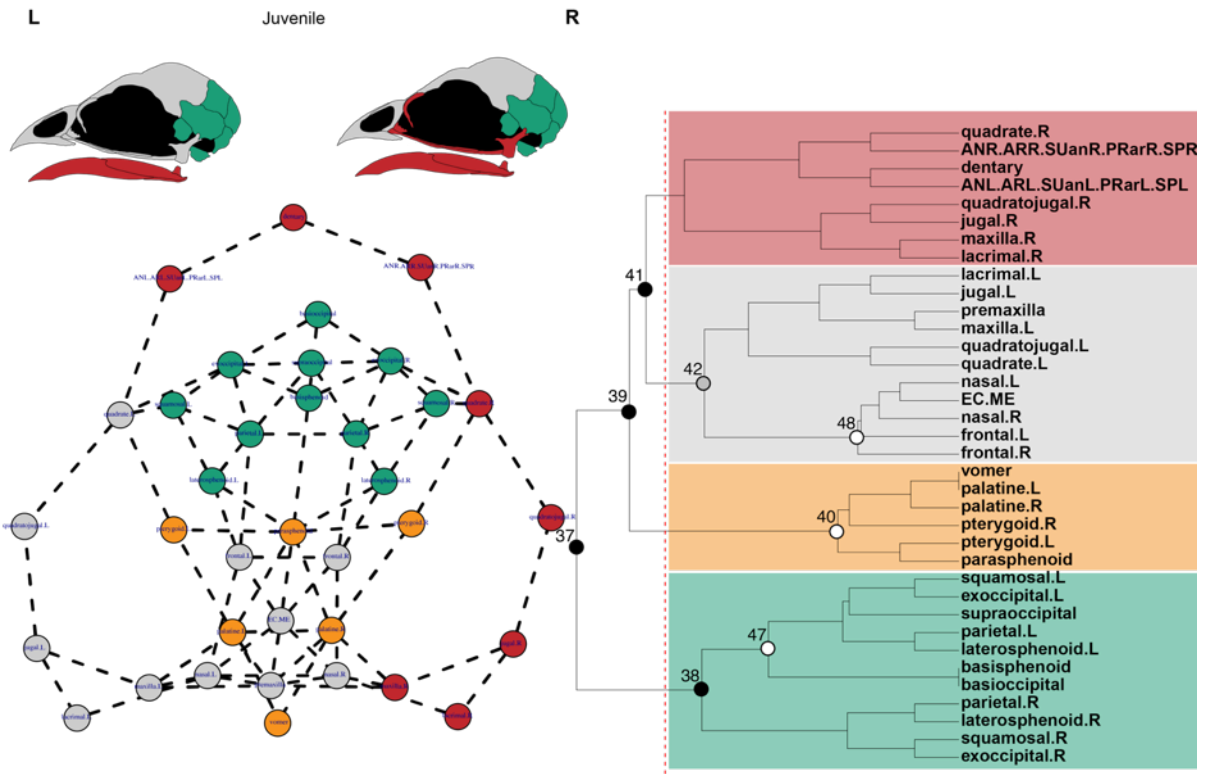
L

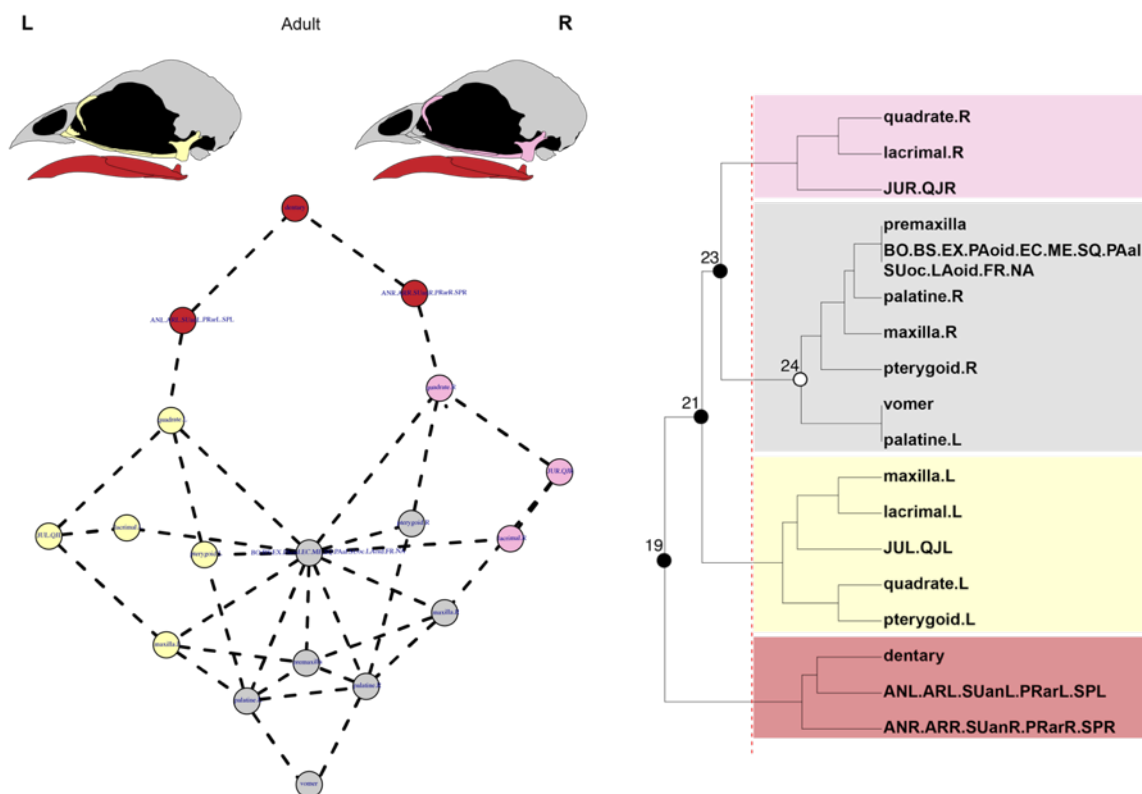
Adult

R

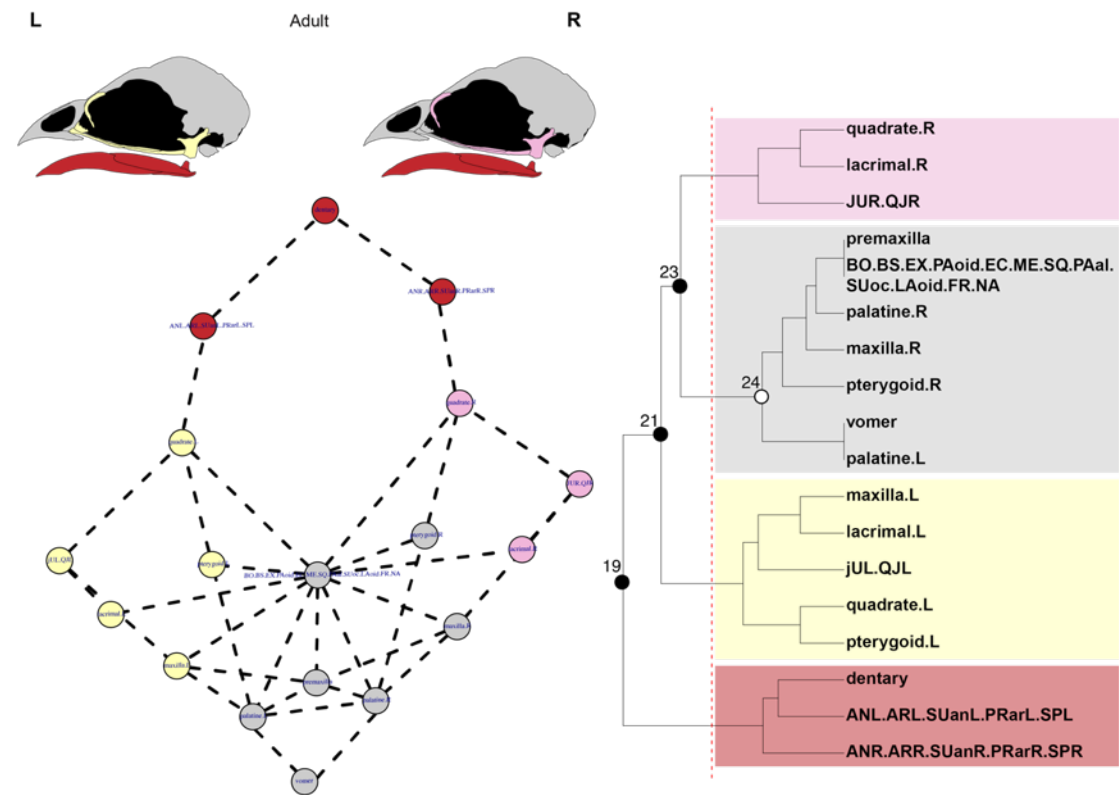
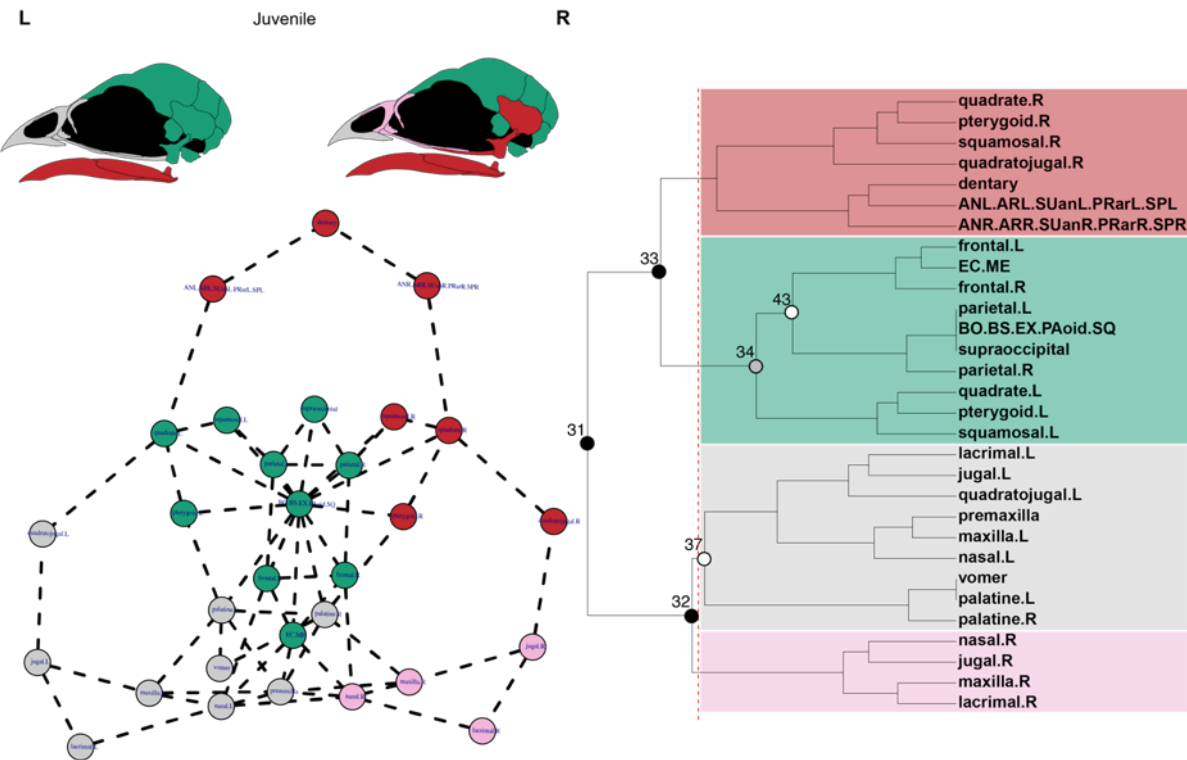


Pygoscelis papua





Spheniscus magellanicus

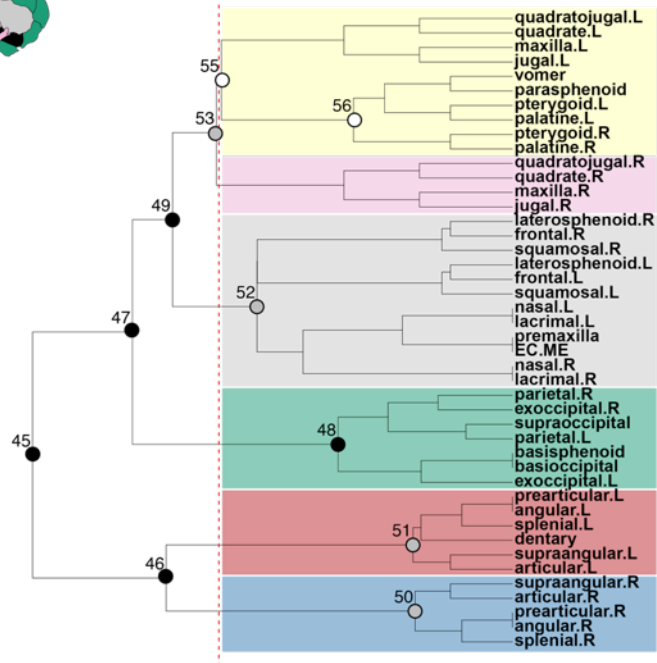
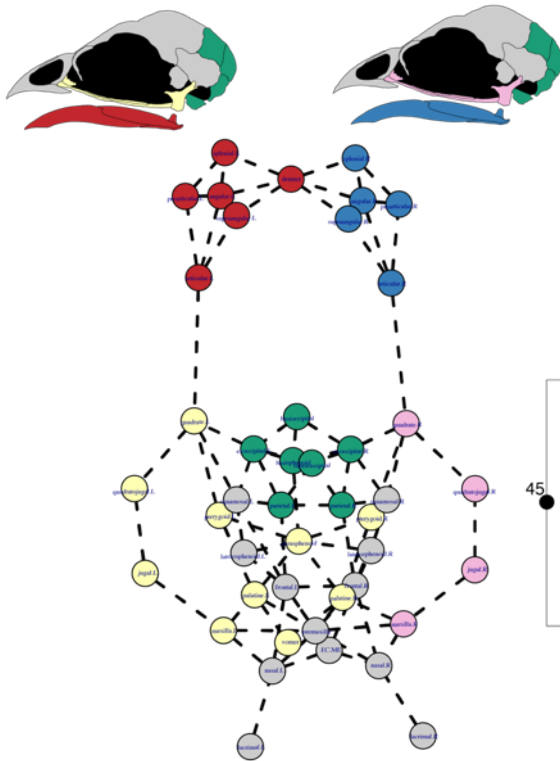


Corvus corax

L

Juvenile

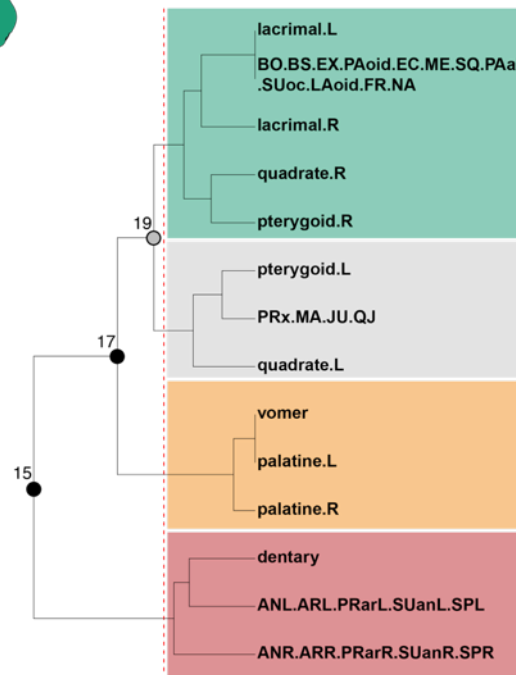
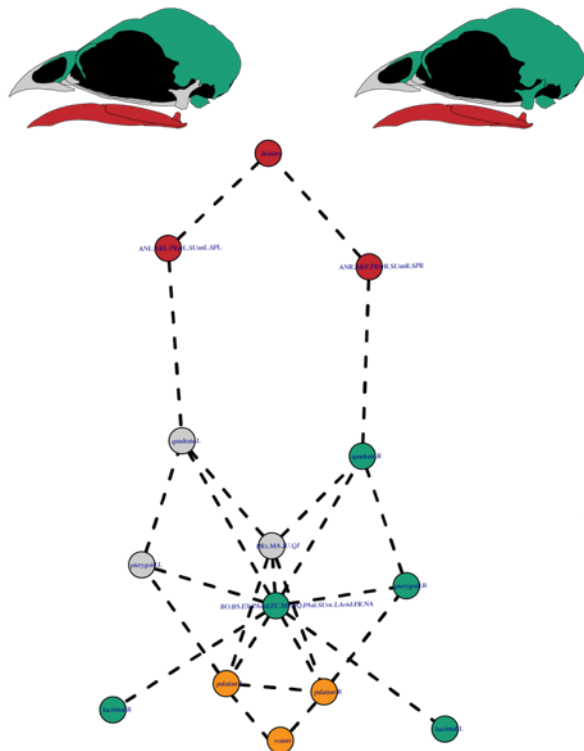
R



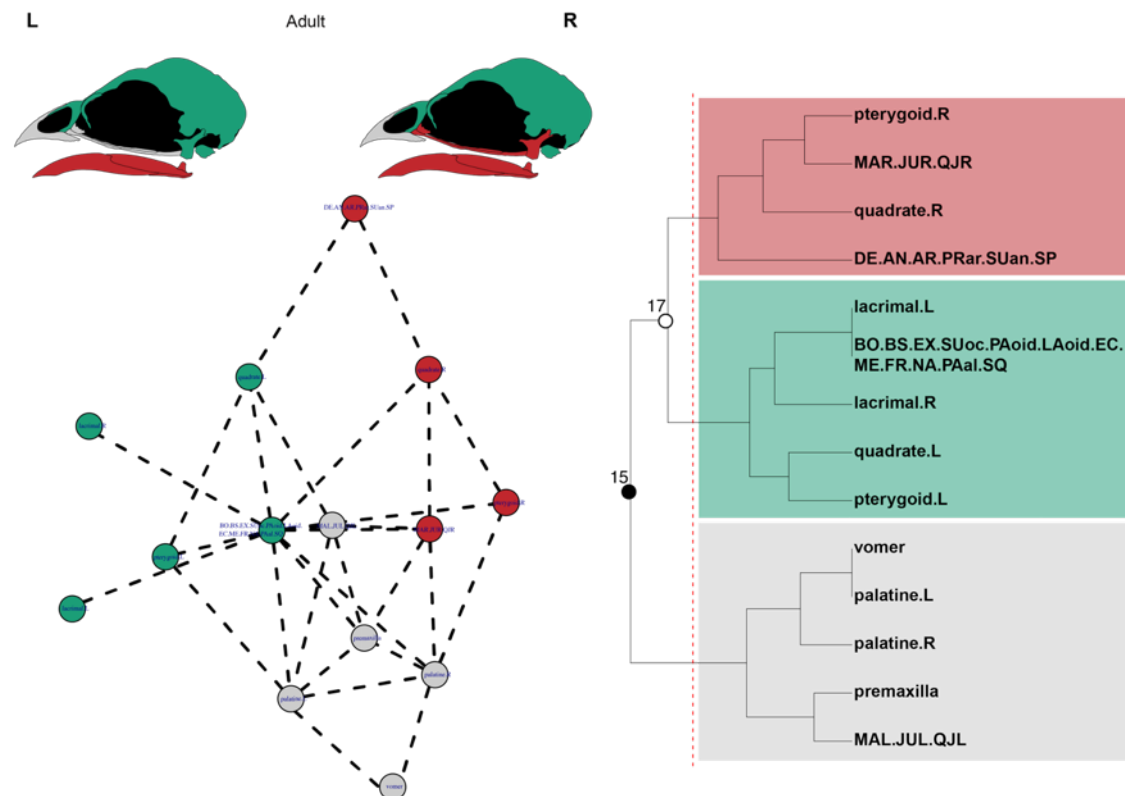
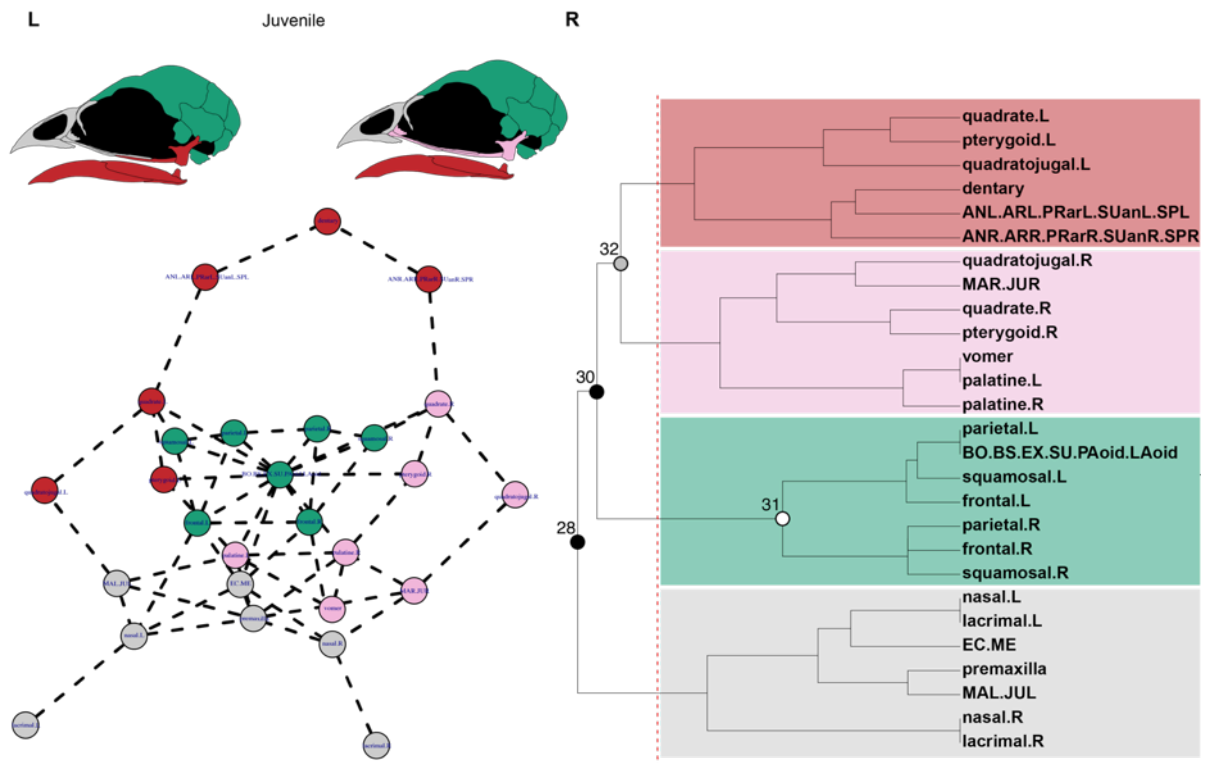
L

Adult

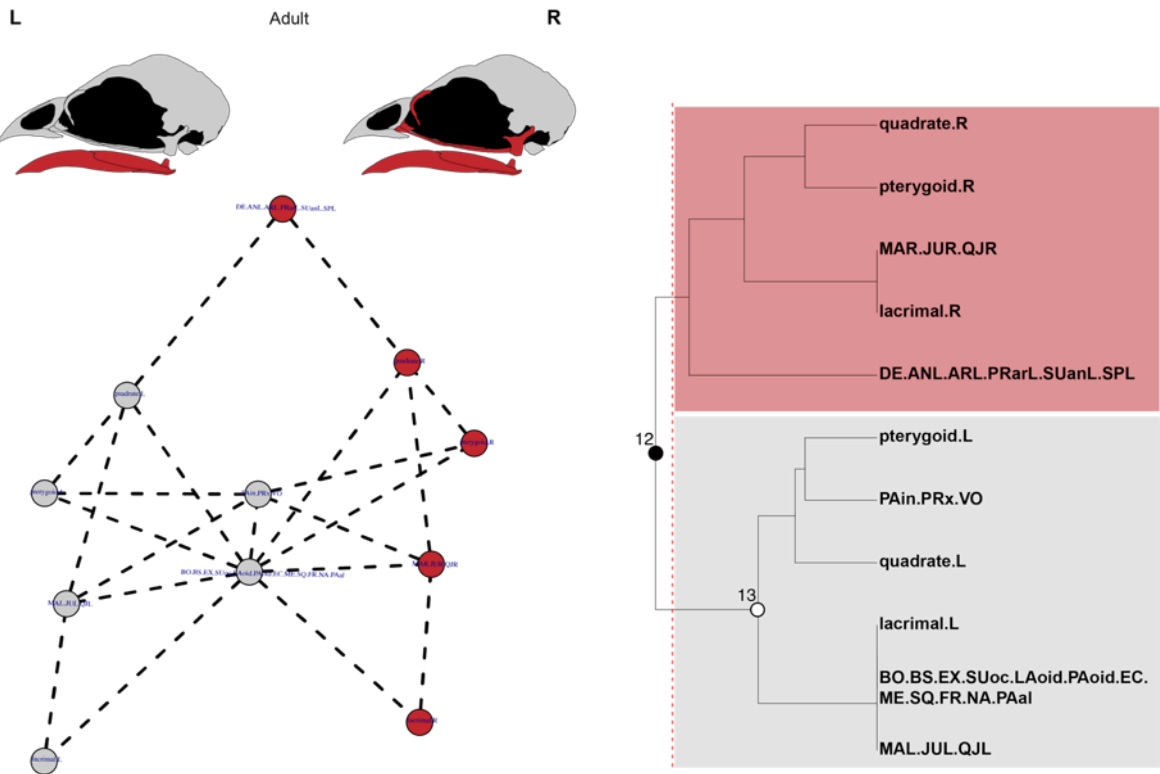
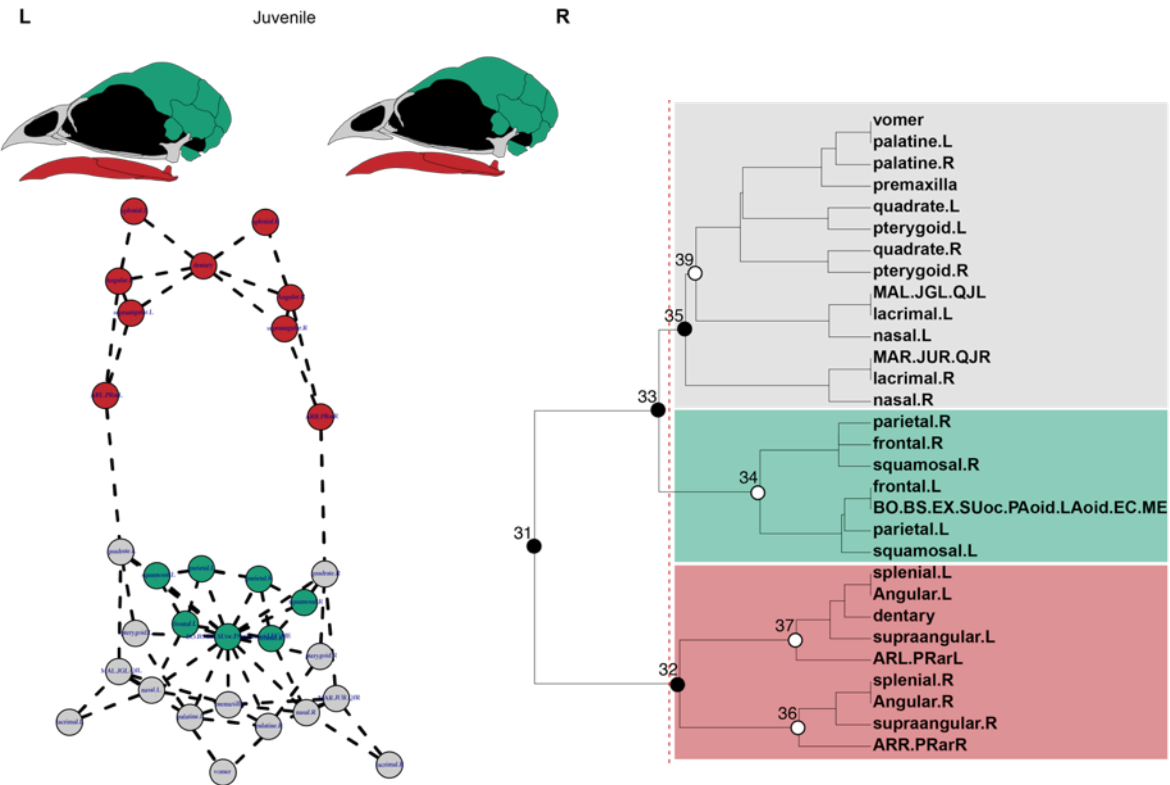
R



Corvus coronae



Corvus monedula

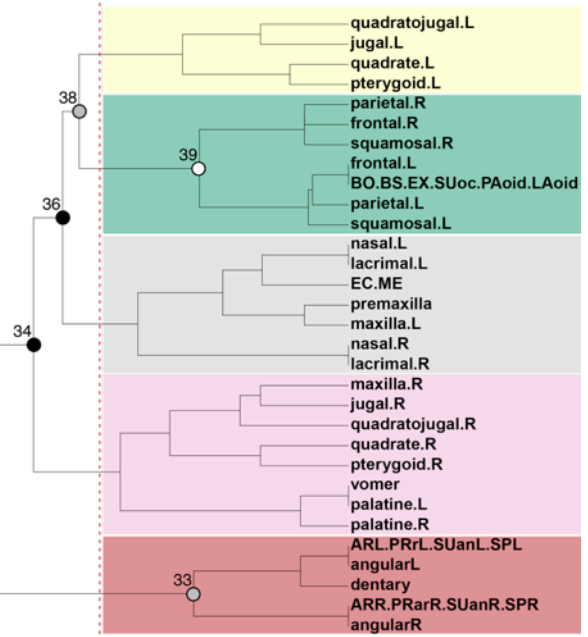
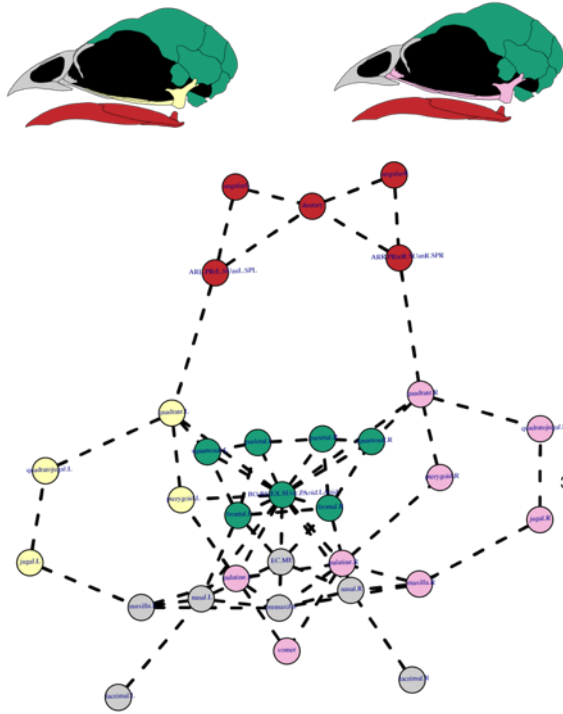


Pica pica

L

Juvenile

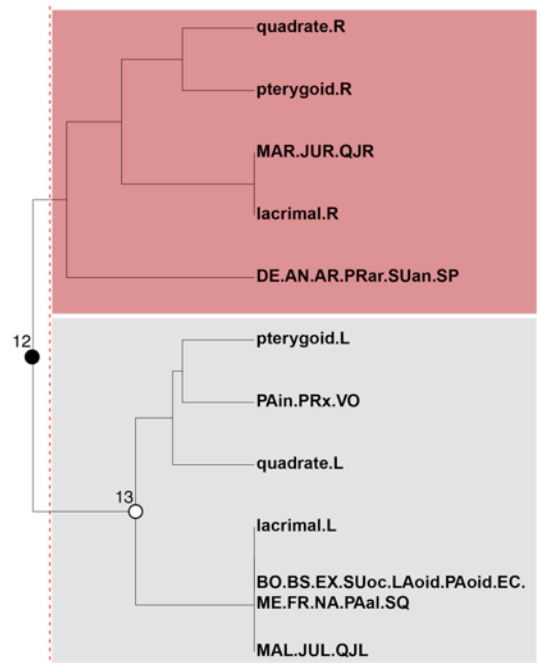
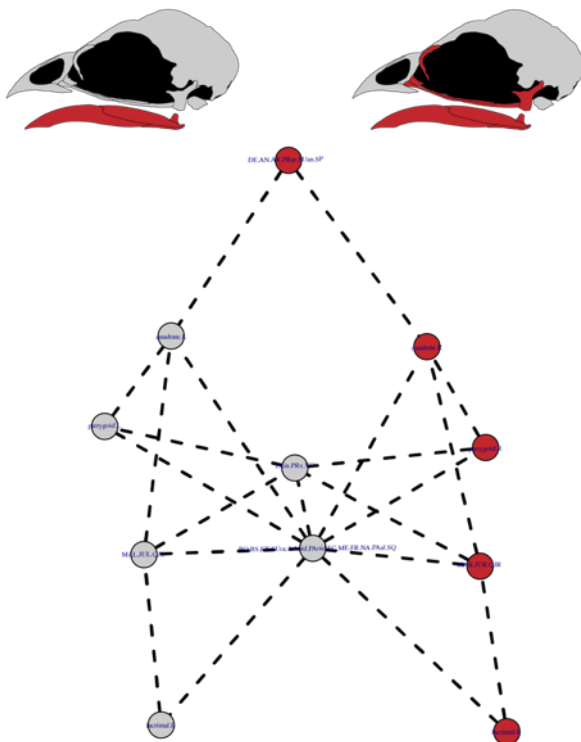
R



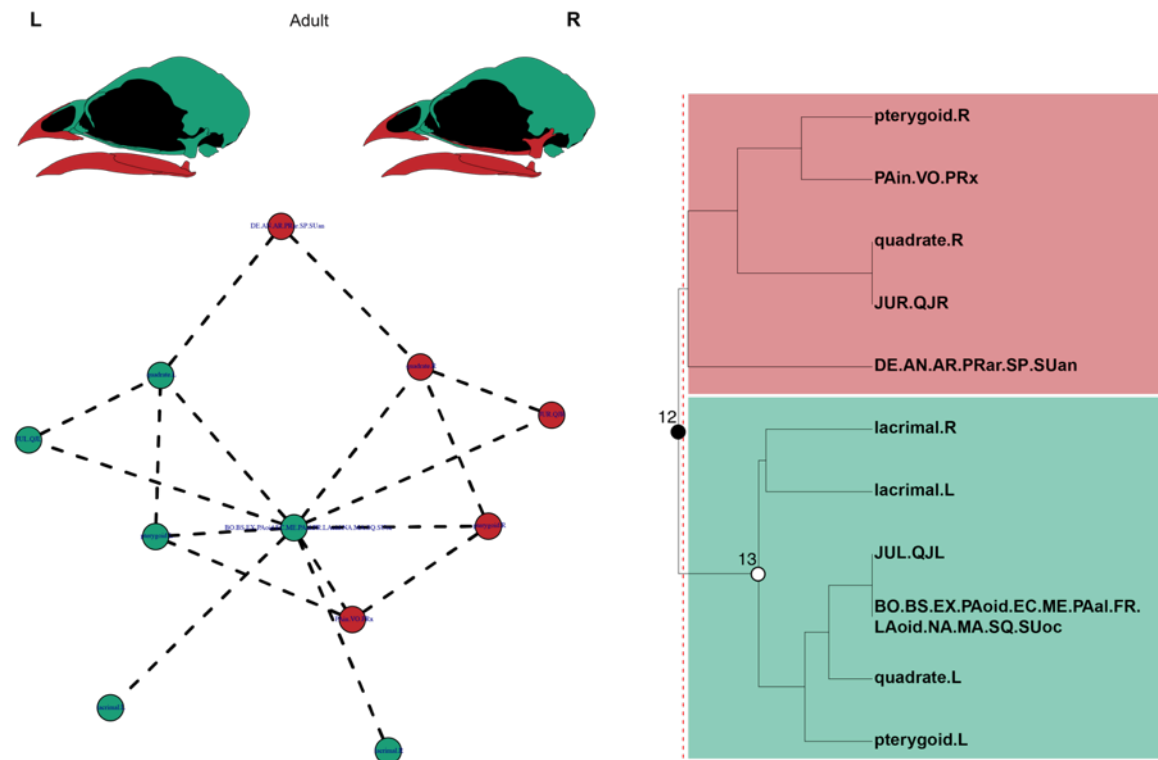
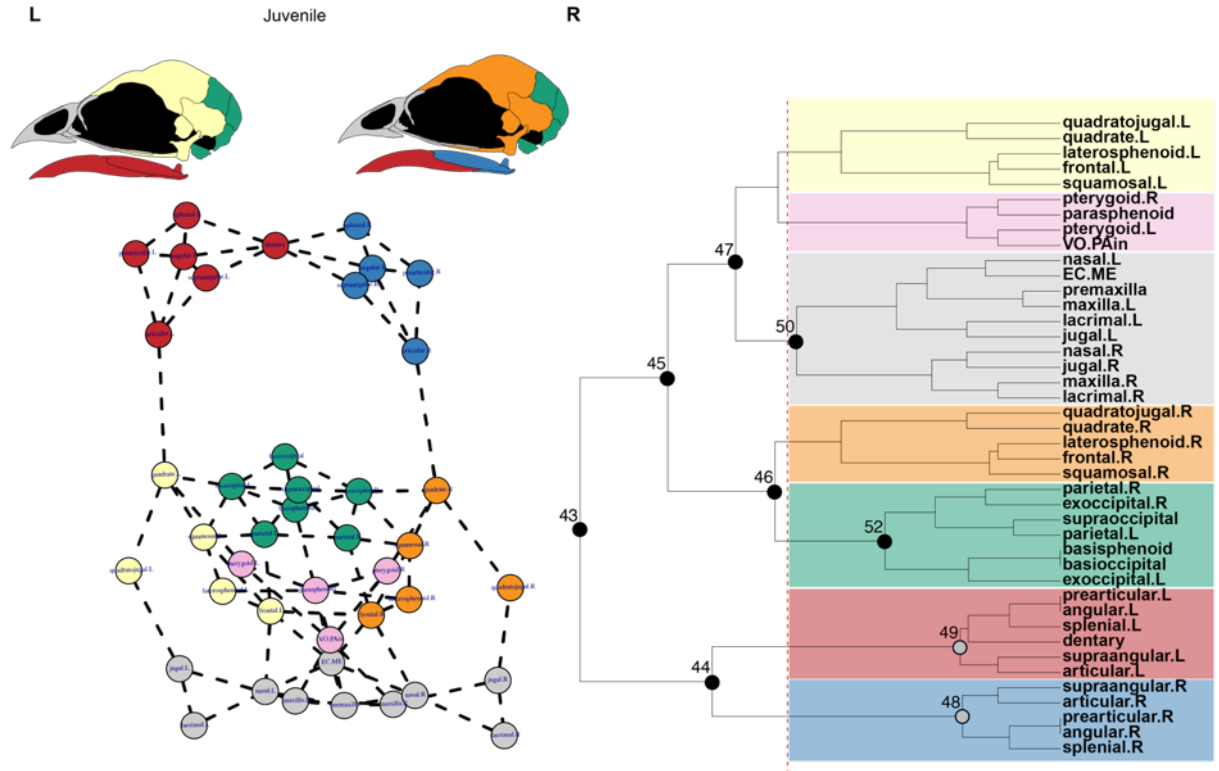
L

Adult

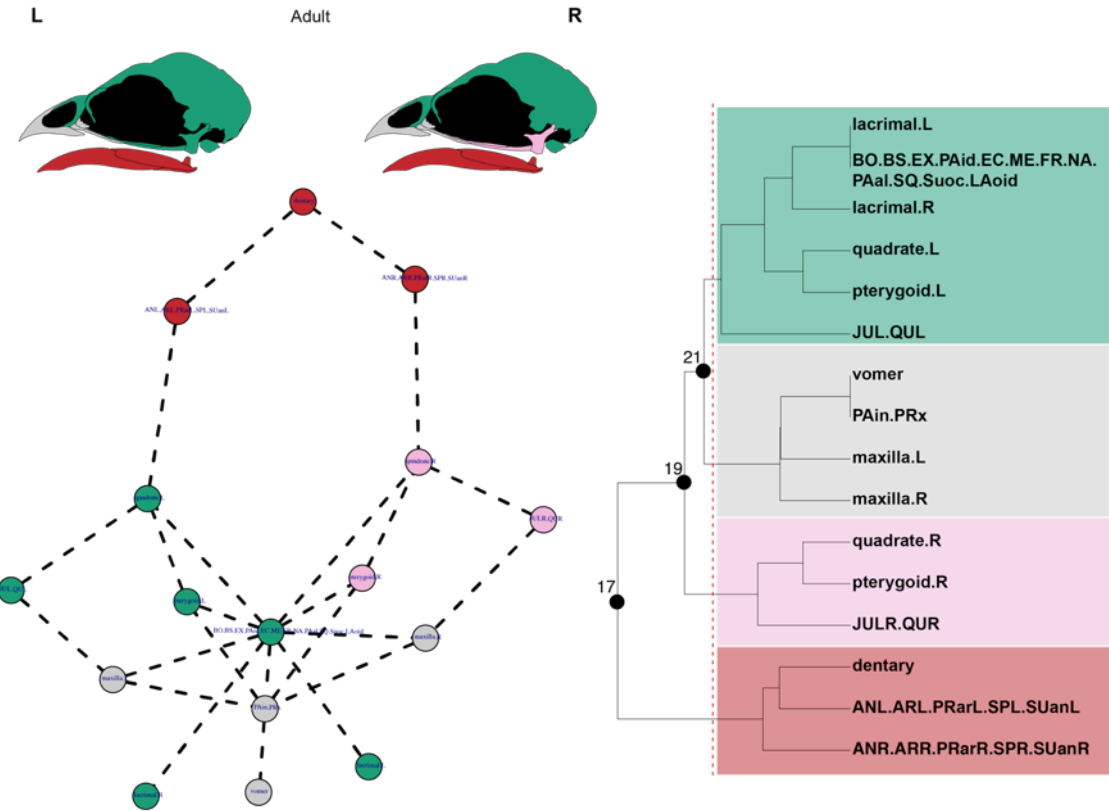
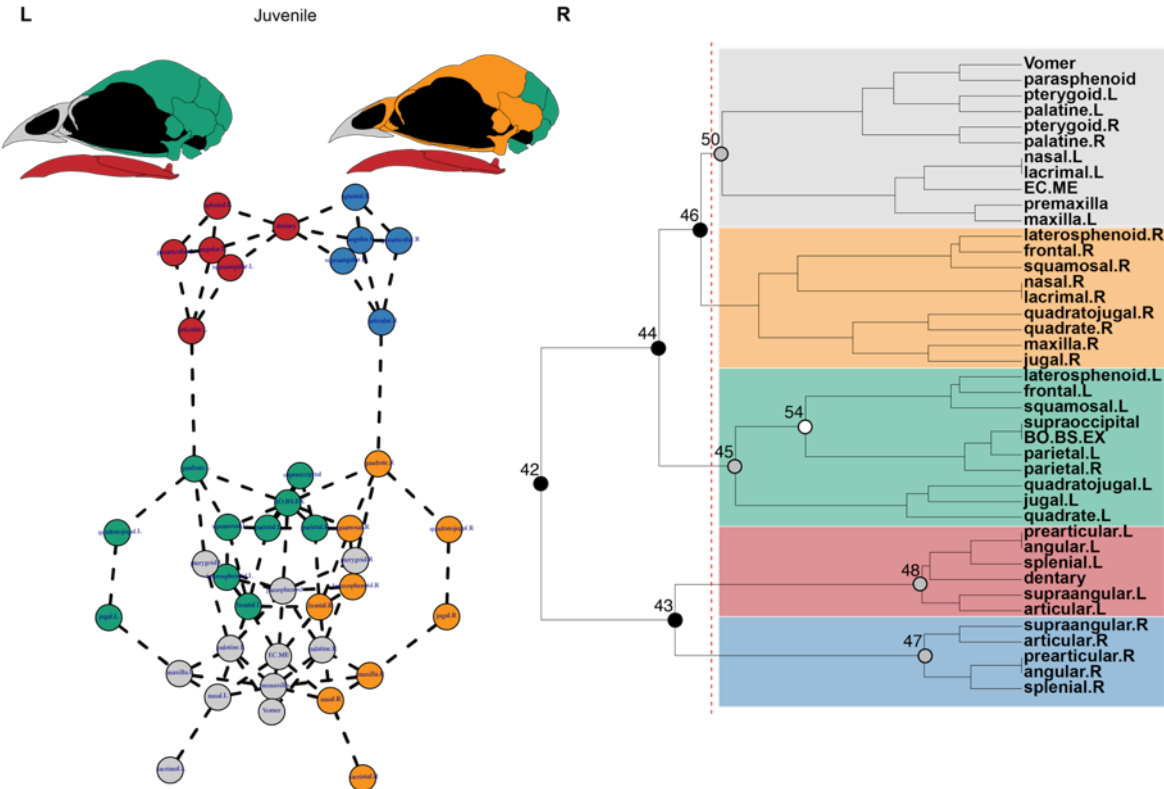
R



Phoenicopterus ruber



Podiceps cristatus

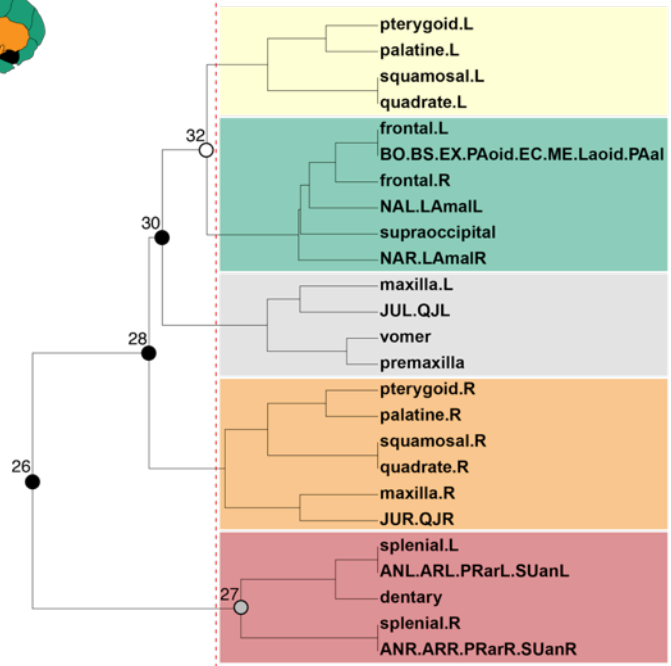
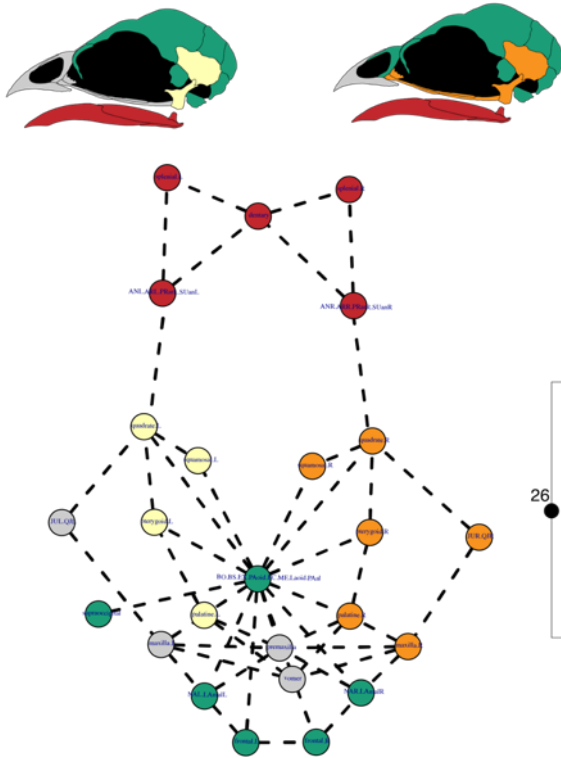


Apus apus

L

Juvenile

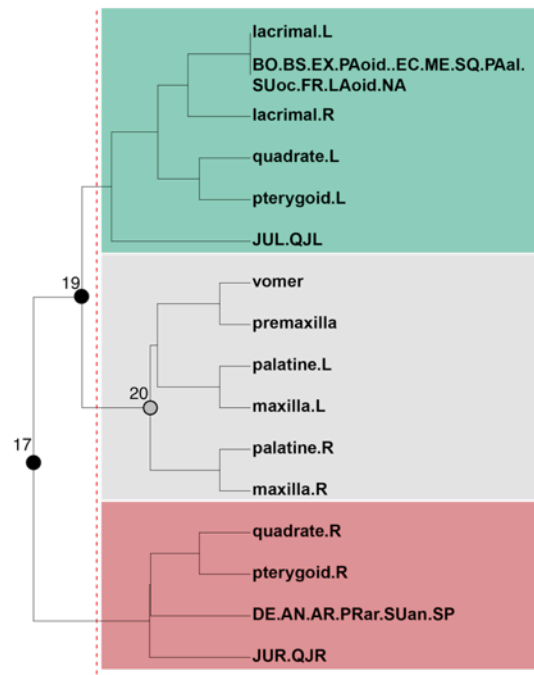
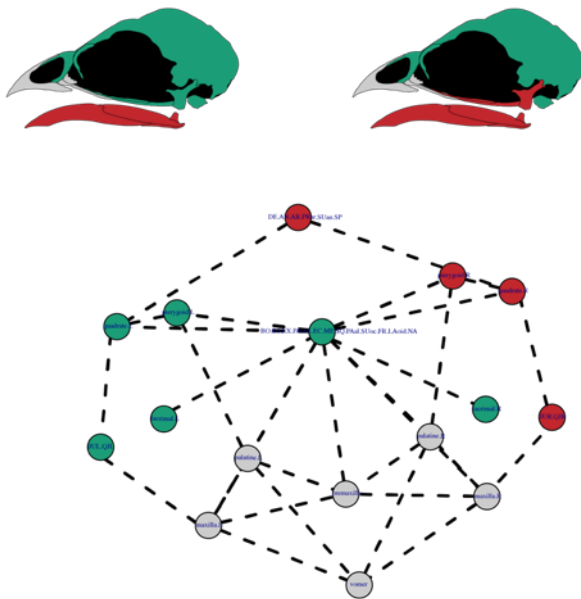
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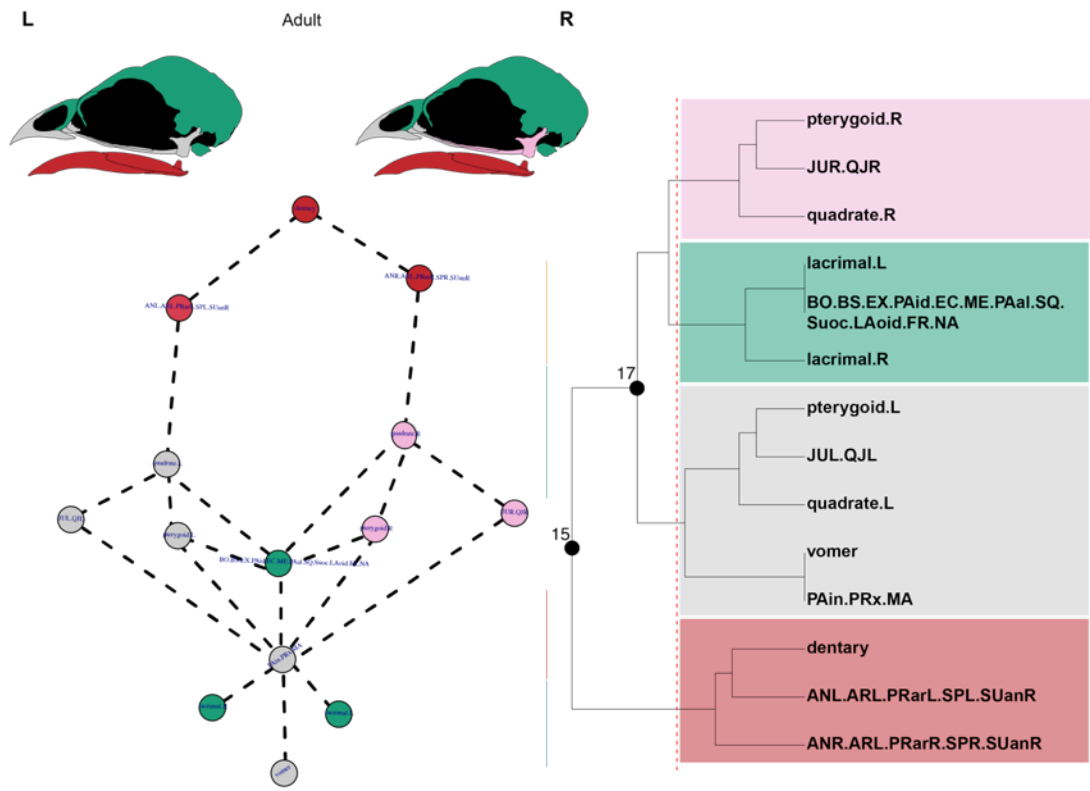
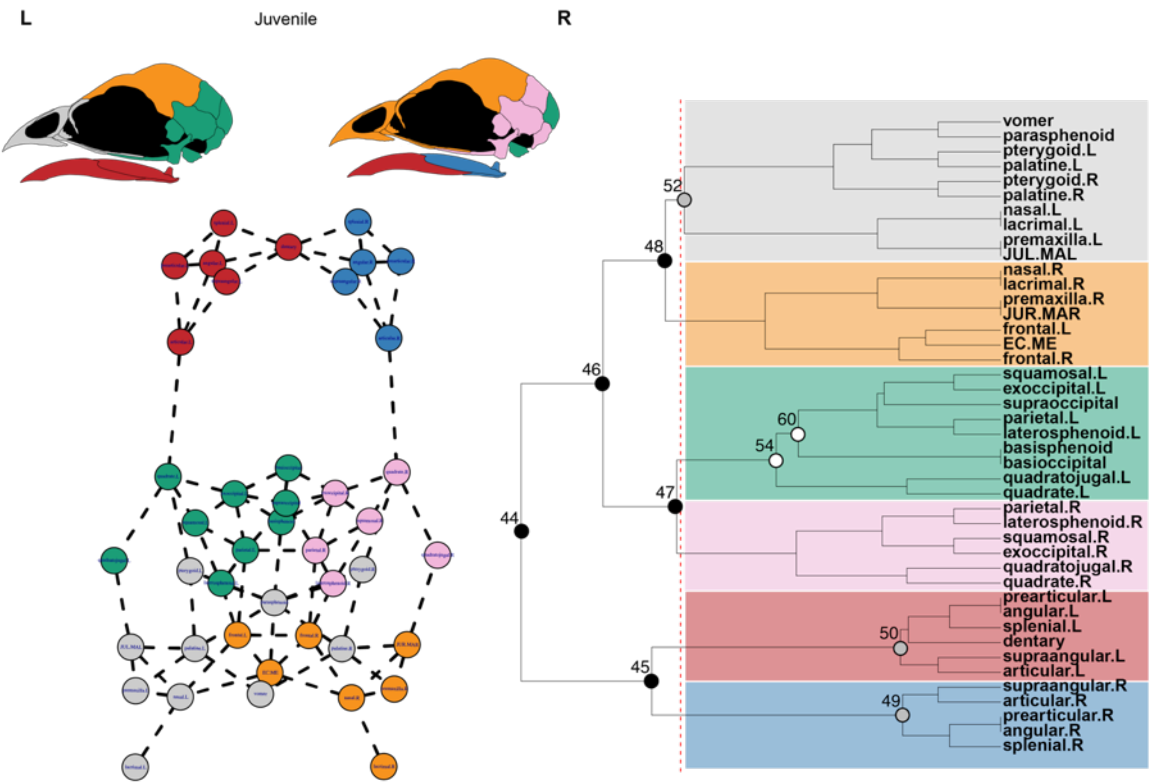
L

Adult

R



Egretta garzetta

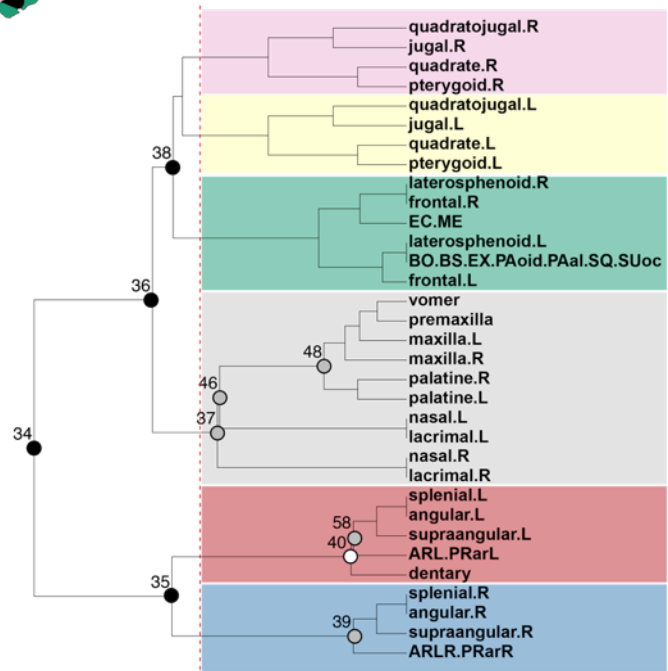
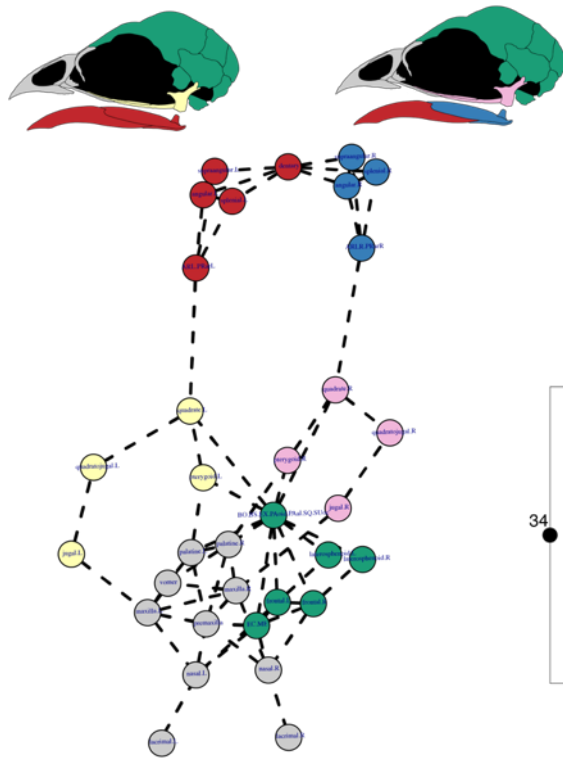


Bubulcus ibis

L

Juvenile

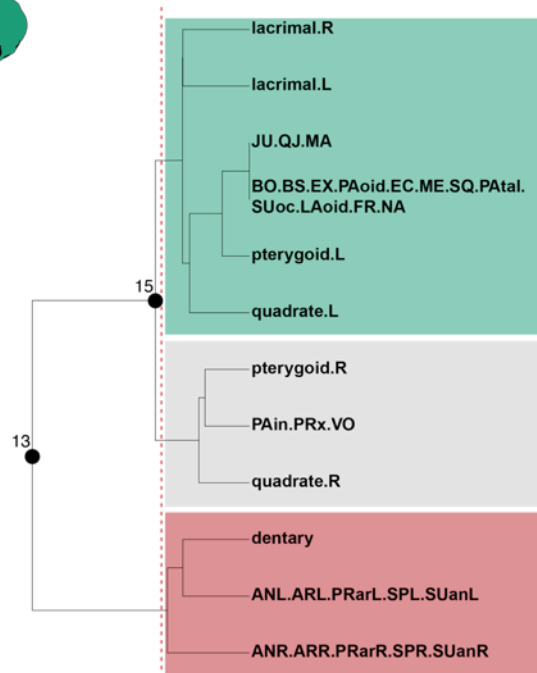
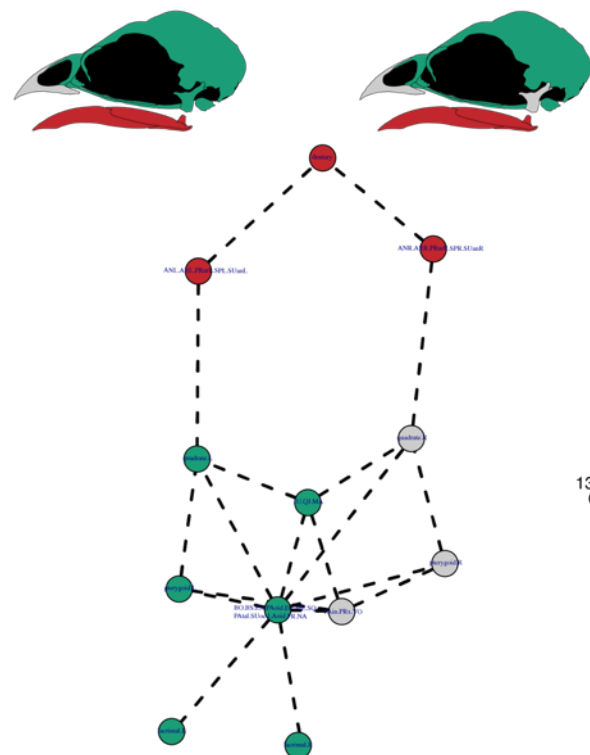
R



L

Adult

R



Nycticorax nycticorax

