Estimates of evolutionary divergence and phylogenetic relationships within Cracidae and among other basal birds

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Abstract

Because of convergence in morphology and fragmented fossil records of avian taxa phylogeny reconstruction has been difficult for some groups. Estimated ages of appearance of bird orders may vary widely depending on the type of evidence used (e.g. fossil versus molecular). The K/T boundary marks the extinction of many organisms, but the number of taxa affected and the degree of speciation before and after this time period are debated. This study analysed sequences of mitochondrial cytochrome *b* and 12S rRNA genes to determine whether the Order Craciformes (formerly in Galliformes), basal to Neornithes, appeared and radiated before or after the K/T boundary. Phylogenetic analyses revealed associations between species formerly classified in distinct tribes within the family Cracidae. The basal position of cracids to the rest of the Galloanserae was confirmed. Estimated ages of divergence from common ancestors are consistent with fossil evidence for most of the lineages examined although some large differences in ages were found among studies. Although estimated times of divergence should be interpreted with caution, 12S mtDNA data suggest two 'proto-Orders' survived the K/T extinctions (a cracid ancestor and a galloanseriform ancestor) and gave rise to Craciformes, Galliformes, and Anseriformes subsequently. Cracidae was estimated to have diverged from other Galliformes and Anseriformes in the early Eocene.

Introduction

All modern birds, the Neornithes, are thought to be monophyletic based on morphological (Cracraft 1981 and 1988) and molecular data (Sibley & Ahlquist 1990). There are two main clades within this group: the paleognaths (ratites and tinamous) and the neognaths (all other modern birds). The orders Galliformes (pheasants, guineafowl, quails, and according to some classifications, cracids) and Anseriformes (screamers, ducks, swans and geese) are placed at the base of the neognaths (Cracraft 1988, Sibley & Ahlquist 1990, Lee *et al.* 1997). Although primitive avian lineages are known from the Jurassic (*Archaeopteryx,* Feduccia 1996) and even the Triassic (*Protoavis*, Chatterjee 1997), modern orders are thought to have arisen either in the late Cretaceous or early Tertiary. Avian taxa share a large portion of characters in part due to the constraints of flight on the basic body plan (Feduccia 1996, Chatterjee 1997). Because of convergence and evolutionary constraints that have led to similar morphology, few synapomorphies are available that are informative for phylogeny reconstruction (e.g., Cracraft 1988). Fossil evidence can be useful to differentiate between homologous versus derived characters but several problems exist. Firstly, an ancestor may posses characters that arose independently in later lineages, or may be neotenous (e.g., the palate and soft, curly feathers in ratites; Swinton 1975). Secondly, the fossil record may be incomplete for some groups. Fossil data are fragmentary and poor for some avian taxa during the early evolution of birds in

the late Cretaceous. The use of molecular data has been valuable in identifying phylogenetic relationships in some problematic taxa with poor fossil records or with highly convergent morphology. For example, the hoatzin, a cracid-like South American bird, is now known to be most closely related to cuckoos, based on DNA sequence of two mitochondrial rRNA genes (Hedges et al. 1995). Moas (an extinct group) and kiwis, both flightless ratites from New Zealand, are less closely related to each other than to ratites on other continents, suggesting two separate invasions of ratite ancestors on the islands (Cooper et al. 1992).

Molecular data has been used to obtain rough estimates of times of divergence among taxa, particularly when estimates can be compared with other groups having better fossil records (Kornegay *et al.* 1993, Shields & Wilson 1987, Irwin *et al.* 1991, Sibley & Alquist 1990). Recent molecular evidence suggests that more avian lineages survived extinction across the Cretaceous-Tertiary (K/T) boundary (~65 MYA) than previously thought, and that speciation was gradual beginning in the Cretaceous (perhaps linked to continental breakup) rather than explosive, at the start of the Tertiary (Cooper & Penny 1997, Hedges et al. 1996). Previous estimates of divergence for some lineages based on fossil evidence appear to be 50 to 90% later than biogeographic and molecular data suggest in part due to the paucity of informative fossils of early ages (Hedges et al. 1996).

Members of the family Cracidae (chachalacas, guans, and curassows) are generally considered to be 'primitive' and are traditionally placed within the order Galliformes (Cracraft 1981, Feduccia 1996, Kornegay *et al.* 1992). Based on molecular evidence, some authors have drawn attention to the high degree of divergence of cracids from other Galliformes (Jollès *et al.*1976, Prager & Wilson 1976, and Prager & Wilson 1980), even placing this family within its own order (Order Craciformes: Sibley & Ahlquist 1990). Because of the presumed antiquity of this group, estimating the time since divergence from ratites and other basal lineages (other Galliformes and basal Anseriformes) may be useful to determine patterns of speciation of Aves and other taxa, and in particular how the K-T events affected these speciation patterns. Molecular data can be used to: 1) estimate the time since divergence of cracids from other birds; 2) determine whether cracids survived the K-T extinctions, and 3) determine whether speciation within the family occurred *before* or *after* the K-T boundary.

The present study estimates the time and order of divergence of three representative taxa

within cracids (chachalacas, guans, and curassows), the radiation of the group relative to the K-T boundary, and the degree of divergence relative to other gallinaceous birds. Mitochondrial DNA sequence data from two genes, cytochrome *b* and 12S rRNA, are compared to determine evolutionary distance among taxa. Published information on rates of evolution and fossil evidence is used in conjunction to obtain conservative temporal estimates of divergence within Cracidae and among other primitive birds.

Materials and Methods

The distributions of the cracids considered are shown in Figure 1; the family is currently restricted to the Neotropics. The species compared in this study are listed in Table 1. Attempts were made to obtain sequences for both cytochrome *b* and 12S for the same species within Galliformes and Anseriformes. *Anhima* and *Chauna* sequences were only found for 12S; these taxa were of interest because they are considered basal within the Anseriformes, and resemble Galliformes more than they resemble the other members of their order. It was not possible to find published sequence for both genes for the same species of Anseriformes other than the chosen taxa. The mallard *Anas platyrhynchos* was initially included but sequence for 12S could not be aligned (GenBank accession no. L22477 and L08260), a possible indication of a 12S homolog, or extreme divergence from all the other taxa considered. Sequence for either or both genes was obtained for a total of four cracids, three ratites, five Galliformes and four Anseriformes.

The mitochondrial genes used were chosen for various reasons. When gene trees are used for phylogeny reconstruction there is a possibility that the gene tree is not the same as the species tree and incorrect topologies may result. However, mitochondrial gene trees have a higher probability of recovering the true species tree (see Moore 1995). Estimates of 12S rRNA evolution appear to be approximately the same as the rate of transversion substitutions in protein-coding genes, 0.2% per MY (Irwin et al. 1991). At this rate this gene is highly conserved among species, especially the regions that form stems in the secondary structure (see Houde et al. 1997 and comments on the evolution of stems verus loops; Mindell et al. 1997, Kocher et al. 1989). A slower rate of evolution is necessary to resolve deeper branches and avoid 'multiple hits' (homoplasies). Cytochrome *b* has been calibrated for some mammals (Irwin et al. 1991) and for geese (Shield & Wilson 1987) to evolve at the same rate as the rest of the mtDNA molecule, 2% per MY. Transversion substitutions should be considered for analyses at higher taxonomic

levels (Moore & DeFilippis 1997). This gene is also highly conserved among species (Kocher et al. 1989). Because mtDNA does not undergo recombination when there is hybridization, an offspring will have mtDNA from either one species or the other (the female), not a mixture of the two (although heteroplasmy occurs in rare cases). *Ortalis poliocephala wagleri*, whether it is considered a true subspecies or a hybrid (see below), was therefore included in the analyses; inclusion of the possible hybridizing 'parental' species was not possible at this point due to lack of samples.

Specimen sources. Blood, brain and liver samples were collected opportunistically from a fresh carcass of great curassow (*Crax rubra rubra*) (Calakmul Biosphere Reserve, Campeche, Mexico) now in the collection of the museum of the Universidad Autónoma de Campeche. Skin and feather samples of black-fronted piping guan (*Pipile jacutinga*) and of a hybrid chachalaca (*Ortalis poliocephala wagleri*, considered by some a subspecies; e.g. Vaurie 1968, and see Howell & Webb 1995) were obtained from the Bird Collection of the Canadian Museum of Nature. These samples were placed in 95% ethanol and at 4° C after collection. All other DNA sequences were obtained from GenBank.

DNA extractions. DNA was obtained by digesting samples overnight at 55° C in lysis buffer (~15 µl of blood or tissue, 100 mM Tris-Cl pH 8.0, 10 mM EDTA pH 8.0, 100 mM NaCl, 0.1% SDS, ~0.2 mg protease K) (Kocher et al. 1989) followed by phenol/chloroform extractions (two times with phenol, once with 24:1 chloroform to isoamyl alcohol). For fresh samples, DNA was precipitated by further addition of one-tenth the volume of 2.5 M NaAc and an equal volume of cold isopropanol, looping out the DNA with a glass pipette, air-drying for 2-3 min., and resuspending in 50 µl ddH20. For museum samples, due to the viscous nature of the solution, DNA had to be precipitated by addition of 7 M NaAc, addition of 95% ethanol at -20° C, and cooling overnight at -20° C. Centrifugation @ 14, 000g for 20 min. produced a pellet; the supernatant was removed and 75% ethanol at -20° C was then added to wash the pellet by inverting. After cooling for 10 min and centrifuging again, the supernatant was removed, the pellet was air-dried and resuspended in 30 µl ddH_20.

PCR and DNA sequencing. Mitochondrial DNA sequences were amplified by the PCR using the primers 12S 'a' (light strand) and 'b' (heavy strand) and cytochrome *b* 'b1' (light) and 'b2' (heavy) (modified from Kocher *et al.* 1989 by truncating 8-9 bp from the 5' end). PCR reaction mixes consisted of ~1X PCR buffer (Boehringer Mannheim; 10 mM Tris pH 8.5, 1.5 mM $MgCl_2$, 50 mM KCl), 0.04 µM of each primer, 0.199 µM stock of dNTPs., 0.02 U *Taq* polymerase (Boehringer Mannheim), and 2.5 µl of template DNA (1:10 dilutions for fresh samples and undiluted for museum samples, using the above protocol) and addition of ddH_20 for a final volume of 25.0 µL. The cycle was as follows (the same for 12S and cytochrome *b*): 94° C for 1:30 min, 35 cycles of denaturing at 94° C for 30 sec, annealing at 57° C for 30 sec, and extending at 72° C for 45 sec, and a final extension step of 72° C for 3 min. Products were visualized on a 0.8% agarose gel stained with 1.5 µl ethidium bromide. The observed bands were

harvested and the DNA was purified using the GeneClean Kit III (Bio 101, Inc.). Sequencing of 12S and cytochrome *b* was carried out using the ThermoSequenase radio labeled terminator cycle sequencing kit (Amersham Life Science Inc.) and the manufacturer's recommendations. α-P^{33}-ddNTPs (0.45 μCi/μl, Amersham Life Science Inc.) were used in these reactions. The cycle for sequencing was as above and was conducted for both light and heavy strands. Products were subjected to electrophoresis through 6% polyacrylamide gels (in 1X TBE buffer). Gels (40 cm wide) were run for 2 hrs at 60 W and exposed to X-ray film (Kodak BioMax) for ~24hrs.

Analyses of Data. Sequences were aligned by eye (where indels occurred alignment was conservative, taking into consideration stem regions for 12S and codon alignment for cytochrome *b*) and entered into PAUP v. 3.1.1 (Swofford & Begle 1993) for conducting maximum parsimony analyses. Midpoint rooting was used, with the rhea as an outgroup. The branch-and-bound algorithm was initially used but due to computer time constraints and since identical topologies were obtained for the first 3 analyses, the heuristic algorithm was then used for all analyses. Indices of support were obtained by conducting bootstrap replications (100) using the heuristic algorithm. Various weighing schemes were tested. Cytochrome *b* analyses were conducted by weighing positions as follows: 1) all positions equally; 2) weighing transversions 4 times transitions; and 3) weighing transversions 4 times transitions in addition to weighing 1st and 2nd positions four times 3rd positions. For 12S, positions were weighed as follows: 1) all positions weighed equally; 2) stem regions weighed four times loop regions; 3) all regions given equal weight but transversions weighed four times transversions; and 4) weighing transversions to transitions 4 to1 and stems to loops 4 to1.

Genetic distance measures (Kimura's 2-parameter model, since it allows different rates of substitutions for transitions and transversions) were obtained by entering sequences into PHYLIP (Felsenstein 1993) and selecting the DNADIST program. A matrix was generated for 12S and mean genetic distances within and among groups (cracids, anserids and galliforms) were estimated using the full matrix. These means were then translated into crude times of divergence from a common ancestor using the known rate of evolution (0.2%/MY). 12S was chosen because it evolves more slowly than cytochrome *b* (see above) and may be better for analyses of deep branches (Houde et al. 1997, Moore & DeFilippis 1997).

Results

The cracid sequences obtained for 12S rRNA (258 bp) and for cytochrome *b* (289 bp) are shown on Figs. 2 and 3, respectively. Unusual regions of insertions in 12S in *Crax* and *Ortalis poliocephala wagleri* (following stem region 39, Fig 2) were also found in the published sequence of *Ortalis vetula*. In general, sequences were highly conserved for these taxa and alignment was straightforward. All cracid samples in this study were sequenced at least twice. The missing bases in *Crax* (cytochrome. *b*, Fig. 2) may be due to poor resolution rather than actual deletions since this was a region for which sequence was obtained only once.

An interesting finding was the number of 12S homologs found in the cracids. A homolog was found in *Crax* (it ran faster in agarose gels and this allowed isolation and individual sequencing). A second product (perhaps a fast-evolving homolog) was found in *Ortalis*, but only when sequencing the heavy strand. This may simply be due to the decreased homology of 12S 'b' primers (found partly on a loop and partly on stem 38) when compared to the notable homology of 12S 'a' (found on stem 27) in gallinaceous birds (see Desjardins & Morains 1990 and Kocher et al. 1989 to compare sequences). Three homologs were found in Pipile, two fast (one similar to 12S but so divergent it could not even be aligned) and one slow. (As mentioned before, a *Anas platyrhynchos* 12S sequence could not be used due to this problem). Mitochondrial gene homologs have often be found (e.g. Kidd 1997), and their discrimination from the true genes is imperative for use in genetic analyses. To determine which of these sequences were homologs and which were likely to be the true 12S genes, three separate tests were carried out. Firstly, sequence from *Gallus gallus* was used for comparison. Secondly, the stem and loop regions were identified and the most conserved sequences were chosen as the true 12S genes (this was the most reliable test as some sequences abruptly terminated or had large indels in stem regions). Thirdly, more conserved sequences clustered together (in the same cluster as published sequences of *O. vetula* and *Meleagris gallopavo*) while divergent sequences were in separate clusters (trees not shown). These results were consistent with analyses of stem regions. Thus homologs were identified and discarded from all analyses. No homologs were found for cytochrome *b*.

In three cases when sequencing both strands, differences of one base pair were clearly detected when comparing gels. At first these were interpreted as 'errors' during PCR amplification. However, other authors have discovered a sequencing artifact that commonly occurs in 12S, stem 32; 'compression' occurs in one strand but not the other resulting in a missing base (Houde et al. 1997).

Cytochrome b. Trees resulting from maximum parsimony analyses are shown in Fig. 4 and 5. The only difference in topology using three different weighing schemes for characters was the resolved branch for *Casuarius* in Fig. 5. Bootstrap values at the nodes also changed somewhat depending on the weight but remained high for resolved branches.

Three main results were obtained. Firstly, branching order could not be resolved in

consensus trees for Anseriformes and Galliformes with this limited data set (289 bp). The information obtained for cracids was surprising. *Crax rubra* (in tribe Cracini, Vaurie 1968) appears to be more closely related to *Ortalis poliocephala wagleri* (in tribe Penelopini) than the two congeneric chachalacas are to each other. The *Ortalis vetula* lineage is basal for this clade. These cracids form a separate clade from the Anseriformes and Galliformes (and in all consensus trees this was maintained although the order of branching within Anseriformes and Galliformes varied). The other surprise was the placement of *Pipile jacutinga* as basal to all the Galloanserae including the other cracids, although the bootstrap index is borderline significant (~70%). Unfortunately only two Anseriformes were available for comparison, one of them primitive (*Anseranas semipalmata*).

12S rRNA. Consensus trees from maximum parsimony analyses for this gene are shown on Figs. 6 to 8. Branches were resolved only for the primitive Anseriformes and for most of the cracids, and for two Galliformes in Fig. 6. *Crax* again clusters with *O. p. wagleri* , the cracids form a separate clade (they are sister taxa to Anseriformes) and in Fig. 8 *Pipile jacutinga* is possibly basal to the other Galloanserae, but this branch may actually be unresolved. In Fig. 8, the branch for *Cygnus atratus* remained unresolved although the resolution for the other taxa improved slightly.

Consistent patterns can be seen from both data sets: the great curassow is a close relative to the chachalacas, and the lineage of the plain chachalaca is ancestral. The cracids (except for *Pipile jacutinga*, a guan) form a separate cluster from Anseriformes and Galliformes. Although support for the basal placement of *Pipile jacutinga* is very weak, the pattern is not inconsistent in both analyses.

Genetic distance and estimates of divergence from a common ancestor. A matrix of genetic distance for 12S can be found in Appendix A. Mean genetic distances calculated form this matrix for each group and among groups are shown on Table 2. When distances were calculated it was noted that *Pipile* is highly divergent from the other cracids (compare the two mean distances) and that the possible patterns of evolution of cracids differ somewhat when *Pipile* is excluded: cracids as a group are equally distant from Galliformes and Anseriformes but *Pipile* is

more closely related to chicken-like birds. *Pipile* is also more closely related to the rhea than the rest of the cracids are, as a group. *Cygnus* was also found to diverge greatly from the primitive Anseriformes, and a separate estimate is shown when this group is excluded. Table 2 provides indices of the degree of divergence within major clades. The order Anseriformes includes highly divergent members (mean distance is 0.1038), even when only basal lineages are compared. Cracids show similar genetic distance within the group as Galliformes (~0.06). The estimates in MYA since a shared common ancestor for this gene are also shown. The branching order is important in interpreting these ages. For example, *Pipile* appears basal to the other groups but the estimated age of divergence from the rhea is shorter than for the other cracids. This may be an effect of unequal rates of evolution relative to other cracids (for example a slow rate would translate into short genetic distance from the ancestor of cracids and ratites, and the divergence would be estimated as a more recent event). The split between ratites (rhea) and cracids as a group is estimated to have occurred ~80 MYA. The divergence of Anseriformes and Galliformes from cracids appears to have occurred ~54-36 MYA. Divergence of the taxa within cracids was estimated at ~32 MYA. The split between *O. vetula* and *O. p. wagleri* is estimated at ~33 MYA, and between *O. p. wagleri* and *Crax rubra* at ~2 MYA (Appendix A).

Discussion

The use of molecular data to reconstruct phylogenies, especially when other evidence is lacking (fossils, biogeographic, informative traits) can be very effective, but may also be misleading in some cases due to various assumptions. Gene trees are used to trace species trees, but unless many genes are used the two may be inconsistent and an erroneous phylogeny may result (Moore 1995). Multiple hits can be a concern due to homoplasy and convergence, but using appropriate genes with a rate of evolution useful for the taxonomic level and the desired degree of resolution reduces this problem (e.g. Moore & DeFilippis 1997). Estimating ages since divergence (requiring assumptions of a molecular clock) from a common ancestor is particularly difficult as these may be biased by unequal rates of evolution among groups. If no fossil evidence is available to calibrate the rates of change with time, one has to compare with other groups with better records, or look for parallel evidence (geological, paleoclimatic, or for similar trends in other taxa). In this study these potential pitfalls are taken into consideration. Although

using two genes, these are a part of the same linkage group (mitochondrial DNA) (Moore 1995, Wilson et al. 1985) and are not truly independent. However, by choosing cytochrome *b* (a protein coding gene) and 12S rRNA (a 'structural' gene), and their differences in the rate and mode of evolution, somewhat different ways of measuring divergence were possible.

Phylogenetic relationships within Cracidae and other primitive birds. Figure 9 shows the traditional classification of this family based on morphology. *Crax* and the genus *Ortalis* belong to separate tribes, Cracini (basal to the subfamily) and Penelopini, respectively. According to molecular data in this study *Crax rubra* is closely related to *Ortalis* and the *Ortalis vetula* lineage is ancestral. The discrepancy may be explained by various possibilities. The genus *Crax* may not be monophyletic but convergence in morphology could have led to their placement in a unique tribe. The species in this study, *C. rubra*, may be the only species that is 'misclassified'. Another possibility is reticulate evolution; hybridization (and introgression) between a male *Crax rubra* and female *Ortalis p. wagleri* in the past could have resulted in offspring with maternal (mitochondrial) *Ortalis* genes with *Crax* nuclear background. Cracids are generally similar in morphology and if *Crax* and *Ortalis p. wagleri* are compared one of the most obvious differences is the plumes on the head of *Crax* (a characteristic of curassows) (e.g. see Howell & Webb 1995). During speciation and secondary contact exaggerated traits may evolve (character displacement and reinforcement) (e.g Sætre 1997); if *Crax rubra* in fact evolved from the *Ortalis p. wagleri* lineage, some of these characters may have evolved for species-recognition. It is also possible that the genes analysed do not show the true species trees; however Sibley and Ahlquist (1990) found a similar pattern using DNA-DNA hybridization techniques. Fig. 10 is a simplified phylogeny and shows *Crax* and *Ortalis* on the same branch (as shown in their Fig. 357, they do not use species names for these two taxa). This finding suggests that more *Crax* species should be genetically analysed to determine their true relationship to the *Ortalis* genus.

Although estimates of divergence should be interpreted with caution, it is possible to look for paleoclimatic or geological events that coincide with the estimated dates of divergence for cracids. Figure 1 shows that *Pipile jacutinga* is geographically separate from the other cracids in this study. The other taxa are currently found in Mexico and Central America; *O. p. wagleri* is separated from *O. vetula* and *Crax rubra* (both found in eastern Mexico and the Yucatan Peninsula) by the Sierra Madre ranges and the Volcanic Belt. An estimate of 32 MYA since the

last common ancestor corresponds to the mid-Oligocene. North American forests were undergoing changes due to climate; subtropical forests were being replaced by deciduous conifer and hardwood forests in places (see Cracraft 1973 for review). By the end of the Miocene (<22 MYA) the climate was cooler and eventually led to the Pleistocene glaciations (Cracraft 1973). Cracids are forest and secondary-forest species (Vaurie 1968, Howell & Webb 1995, Cox et al. 1997, Gonzáles-García 1995). If the Sierra Madre ranges and descending treeline split an ancestral *Ortalis* lineage, this could have resulted in the divergence between *O. vetula* and *O. poliocephala* (and eventually *O. p. wagleri*), restricting the later to western Mexico. *Crax rubra* and *O. vetula* currently overlap over a great portion of their ranges. *Crax* overlaps with *O. poliocephala* in pockets along the Isthmus of Tehuantepec and northern Central America. An estimated split from a common ancestor ~2 MYA corresponds to the uplift of the Yucatan Peninsula (late Pleistocene, Schuchert 1968), the final closure of the Strait of Panama (3.5 to 1.7 MYA, Coates et al. 1992), and the uplifting of the Andes in the late Pliocene and early Quaternary (van der Hammen 1982). Such geographic and geological changes may have affected species ranges (e.g. Tordoff & McDonald 1957), brought new species into contact (increasing competition among them), or may have had effects on climate and habitat. The cooler climates of the Pleistocene may have resulted in fragmented distributions leading to speciation.

The placement of *Pipile* as basal to other Cracids, Galliformes and to Anseriformes must be interpreted with caution (*Pipile* and *Cygnus* were mostly unresolved in the 12S phylogenetic tree). Fig. 10 (Sibley & Ahlquist 1990) shows one species of *Pipile* which clustered with other members of the tribe Penelopini (Fig 9). In Fig. 10 cracids are basal to the other Galloanserae, but *Pipile* is not the basal lineage to the rest of the cracids. Sampling other members of *Pipile* may help clarify the position of the genus, or at least of *Pipile jacutinga*. Although it would be surprising that a highly divergent group has gone undetected as being basal, this cannot be ruled out, particularly when morphology is highly conserved in this family, and when the species are relatively not well known (e.g. Cox et al. 1997, Gonzáles-García 1995). The data of this study are consistent with the placement of cracids as basal to the Parvclass Galloanserae.

The degree of divergence of cracids from both Anseriformes and Galliformes (Table 2) and the formation of a separate clade would suggest their placement in a unique Order, consistent with the classification of Sibley and Ahlquist (1990) (Order Craciformes, including the families Cracidae and Megapodiidae, the brush turkeys). In this study a closer relationship to

Galliformes was only detected for *Pipile*, although the data does not disagree with previous suggestions of this relationship (Cracraft 1981 and 1988, Sibley and Ahlquist 1990, Tordoff & McDonald 1957).

Estimates of divergence and the K/T boundary. The fossil record for Galliformes is fragmentary but some informative fossils are known. A fossil cracid, *Procrax brevipes*, is known from the lower Oligocene (~30 MYA) from South Dakota and most resembles *Pipile*, although it is unique in many characters (Tordoff & MacDonald 1957). *Gallinuloides* (a fossil genus) classified in a subfamily of Cracidae (Fig. 9, Vaurie 1968; Tordoff & MacDonald 1957) is known from the middle Eocene of Wyoming (~54 MYA). Helm-Bychowski and Wilson (1986) review gallinaceous fossils. Numida may have originated >35-40 MYA, Phasianoidea <33-41 MYA, and turkeys may have split from pheasants 16-20 MYA. Ducks and geese (e.g. *Romainvillea*, a goose) are known from the upper and middle Eocene (~40 MYA) (Swinton 1975). Rhea fossils are available from the lower Pleistocene (~3 MYA) and other ratites from the Pliocene (Swinton 1975). The recent age of ratite fossils point out the scarcity of fossils of early times for some groups.

The estimated ages since divergence from common ancestors were compared with known fossils and with evidence from other molecular studies. The estimated split between Galliformes and Craciformes is 53 MYA (Table 2, using 12S), which corresponds to the age of *Gallinuloides*. The age obtained for the split between Anseriformes and cracids is similar, ~54 MYA; since anseriform fossils are know which are distinct in form (for example, *Romainvillea*) the common ancestor is probably of earlier age and roughly corresponds to the estimate obtained from 12S in this study. When the genetic distance estimates in Appendix A are converted to times since divergence, the splits between the *Gallus* lineage and other members are as follows: *Gallus-Numida*, ~34 MYA; *Gallus-Phasianus*, ~34 MYA; *Meleagris-Phasianus*, ~34 MYA; *Gallus-Meleagris*, ~41 MYA. The fossil evidence above therefore agrees with the age estimates for *Numida* and *Phasianus*. However, *Meleagris* estimates obtained from 12S sequence are much higher than the fossil ages. This group may have an accelerated rate of evolution relative to other Galliformes, also noted by Mindell et al. 1997. Overall, the estimates from molecular data agree closely with actual fossil dates, but unequal rates of evolution give inaccurate ages; data from multiple genes would be useful in detecting this and improving estimates. An advantage of the molecular data is that although early fossils of rhea are not available, the time since shared

ancestry between ratites and cracids can be estimated at ~79 to 80 MYA (if rates of evolution are roughly equal).

Sibley and Ahlquist (1990) estimated $\Delta T_{50}H$ values of 22.9 for the split between the Gallomorphae (including Craciformes) and Anserimorphae, of 21.6 for the split between Craciformes (Megapodiidae and Cracidae) and Galliformes, of 19.8 for the split between megapodes and cracids, and of 12.8 for the split of *Numida* from other Galliformes. Calibrating this constant can be difficult: based on fossils for a particular group, this is estimated at δ 1.0= 4.2 MYA to 2.3 MYA (Sibley & Ahlquist 1990). Respectively, these dates would be ~96, 90, 83, and 54 MYA if the higher factor is used and ~53, 50, 46, and 29 MYA for the lower factor. The 12S data from this study agree more closely with the more recent ages (the first 2 and the last figures are the only ones that can be compared), although these seem somewhat low. Because fossils found are not necessarily the earliest for a group, and because rates of evolution can vary among taxa, these figures have to serve as rough guides only. Although a range of ~96 to ~53 MYA (almost a 40 MY difference) is very unsatisfactory, this information is valuable and data collected independently (for example, Sibley and Ahlquist (1990), this study, and other studies that use other genes to estimate these dates) can help determine where the true ages lies. Based on this study, on the estimates of Sibley and Ahlquist (1990), and on the fossil evidence, the above dates seem to correspond more with the low-range ages, perhaps within 5 MY (a more acceptable range). The real times of divergence from common ancestors may become more evident as independent estimates are obtained and compared in this manner.

The Cretaceous-Tertiary boundary (~65 MYA) is associated with massive extinctions, but the effects on species and the radiation of taxa before and after this period are not well known (Cooper and Penny 1997). At ~65 MYA South America was separate from North America, the final split of Africa and South America was taking place (Rand & Mabesoone 1982), North America was still detaching from Eurasia at high latitudes (where Greenland is now), Africa was rotating westward, India was moving north towards Eurasia, and Australia had begun to split from Antarctica and move north (Dietz & Holden 1970). The estimated times since divergence from 12S suggest that cracids had split from the ratite lineage (~79 MYA) before the K/T boundary, but that the split of Anseriformes and Galliformes from cracids had not

yet occurred (~53-53 MYA). The split of Anseriformes from ratites (~73 MYA) and of Galliformes from ratites (~73 MYA) suggest that 2 primitive lineages had appeared before the K/T boundary (a 'proto-craciform', and a 'proto-galloanseriform'), probably closely related. The family Cracidae is believed to have radiated in North America (Tordoff & MacDonald 1957). Megapodiidae fossils are scarce or have not been found but the modern distribution is Oceania (Australia, New Guinea, and the Philippines). The cracid-like ancestors then must have survived the K/T extinctions and radiated shortly after, splitting into the Megapodiidae lineage in Eurasia, the Cracidae lineage in North America, and ancestors of the other Galloanserae (see Table 1 for the species ranges in this study). In Anseriformes, the two branches of the basal clade are now in separate continents: screamers in America and the magpie goose in New Guinea and Australia (the split is estimated ~44-40 MYA from Appendix A). Movement between North America and Eurasia could in theory continue until the final split of North America, Greenland and northern Europe (~30-40 MYA?) and/or until glaciations restricted species ranges and movement. Data from 12S in this study suggest a post-K/T boundary radiation for Galliformes (~33 MYA), Anseriformes (~52 MYA), and Craciformes (~32 MYA). It would be informative to obtain 12S information for a Megapodiidae representative and estimate the split of this lineage from Cracidae to determine whether this event took place before the K/T boundary. The data for 12S support the appearance of two primitive lineages before the K/T boundary ('proto-Orders'), but divergence and radiation into the Orders Craciformes, Galliformes and Anseriformes probably occurred subsequently.

Some authors have suggested the ancestor from which some modern bird orders originated was chicken-like in features (e.g. Tordoff & MacDonald 1957). Within some modern bird orders there are members with gallinaceous features; although in most cases this is probably a result of convergent evolution, these features may indicate ancestral lineages within the order. For example, the hoatzin (mentioned above) appears to have split at the base of cuculiform lineage (Hedges et al. 1995). The genera *Chauna* and *Anhima* in Anseriformes are basal, and resemble Galliformes. Within Gruiformes, some families have remarkably chicken-like features. Among the early lineages in this Order (Houde et al. 1997), the seriemas have an overall gallinaceous form. Specimens of female *Chunga burmeisteri* (black-legged seriema) have been found with the usual single ovary, but also with the double ovary (Boyle 1917). This may be due to a mutation within the species, or may be a relict from an early ancestor. The presence of a

single ovary in birds is considered an adaptation for light body weight for flight (Feduccia 1995). Detection of primitive lineages and estimates of divergence from ratites and other basal lineages may help determine how many orders (or proto-orders) may have survived the K/T extinctions. This information can then be used to determine whether radiation was gradual in the Cretaceous or explosive in the Tertiary. This study supports speciation linked to continental breakup and isolation of ancestral lineages based on estimated ages of divergence from common ancestors, but in the more recent past.

The taxonomy of Cracidae needs to be reviewed and inclusion of more species within each tribe is desirable to determine relationships among taxa. The basal position of *Pipile jacutinga* needs to be analysed more closely. Analysis of sequence from Megapodiidae may provide an estimate of divergence from Cracidae. Addition of sequence, and perhaps inclusion of nuclear genes that have slow rates of evolution could clarify the relationships among the three Orders (the amount of sequence in this study could not resolve relationships among Galliformes and of some Anseriformes). Without fossil evidence, molecular data from various independent sources (e.g. different genes) can be used to estimate the times of divergence of taxa from common ancestors.

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TABLE 1: Species considered in this study, their approximate range, and GenBank accession numbers for mtDNA sequences (dashes indicate that these were not used in a particular comparison).

SPECIES	COMMON NAME	NATIVE RANGE	GENBANK ACC #	
			CYTOCHROME *B*	**12S rRNA**
CRACIFORMES				
Pipile jacutinga (Aburria jacutinga)	Black-fronted piping guan	Brazil, Argentina, Paraguay	this study	this study
Crax rubra rubra	Great curassow	Mexico, Central America, Columbia	this study	this study
Ortalis poliocephala wagleri	Rufous-bellied West Mexican chachalaca	Western Mexico	this study	this study
Ortalis vetula	Plain chachalaca	Eastern Mexico to North Central America	L08384	U88017
RATITES				
Rhea americana	Greater Rhea	South America	Z49101 (written as complementary strand in GenBank)	U59664
Struthio camelus	Common ostrich	Africa	-----	U76048
Casuarius bennetti	Dwarf cassowary	New Guinea	-----	U76044
GALLIFORMES				
Coturnix coturnix	Common quail	Eurasia, India, Africa	L08377	X57245
Meleagris gallopavo	Common turkey	US, Mexico	L08381	U83741
Phasianus colchicus	Ring-necked pheasant	Asia	N/A	U83742
Numida meleagris	Helmet guineafowl	Africa	L08383	U88016
Gallus gallus domesticus	White leghorn chicken	(originally) Malaysia, India	Desjardins & Morais 1990	
ANSERIFORMES				
Anhima cornuta	Horned screamer	Northern South America	N/A	U83728
Chauna chavaria	Northern screamer	Columbia, Venezuela	N/A	U83729
Anseranas semipalmata	Magpie goose	South New Guinea, Australia	U46466	U83730
Cygnus atratus	Black swan	Australia, Tasmania	U46468	U83731

TABLE 2: Mean genetic distances for each group or taxon generated from 12S sequences, calculated from pairwise distances among species (Appendix A). The ages in brackets are rough estimates of time since divergence from a common ancestor (see text). *Pipile jucutinga* is also shown in a separate column for comparison with other taxa, and the other cracids in particular. Note that when *Pipile* is excluded when the mean distance is calculated for Cracidae the mean decreases by ~20%. In the Anseriform column, the mean distance is also calculated excluding *Cygnus*, a highly divergent taxon relative to the other basal Anseriformes.

	Cracidae	**Rhea**	**Anseriformes**	**Galliformes**	***(Pipile jacutinga)***
Cracidae	**0.0647** (~32 MYA) Excluding *Pipile*: 0.0433 (~22 MYA)	_____	_____	_____	*0.0861* *(~43 MYA)*
Rhea	0.1575 (~79 MYA)	_____	_____	_____	*0.1330* *(~67 MYA)*
Anseriformes	0.1086 (~54 MYA)	0.1462 (~73 MYA)	**0.1038** (~52 MYA) Excluding *Cygnus*: .0713 (~36 MYA)	_____	*0.1007* *(~50 MYA)*
Galliformes	0.1066 (53 MYA)	0.1466 (~73 MYA)	0.1078 (~54 MYA)	**0.0661** (~33 MYA)	*0.0720* *(~36 MYA)*

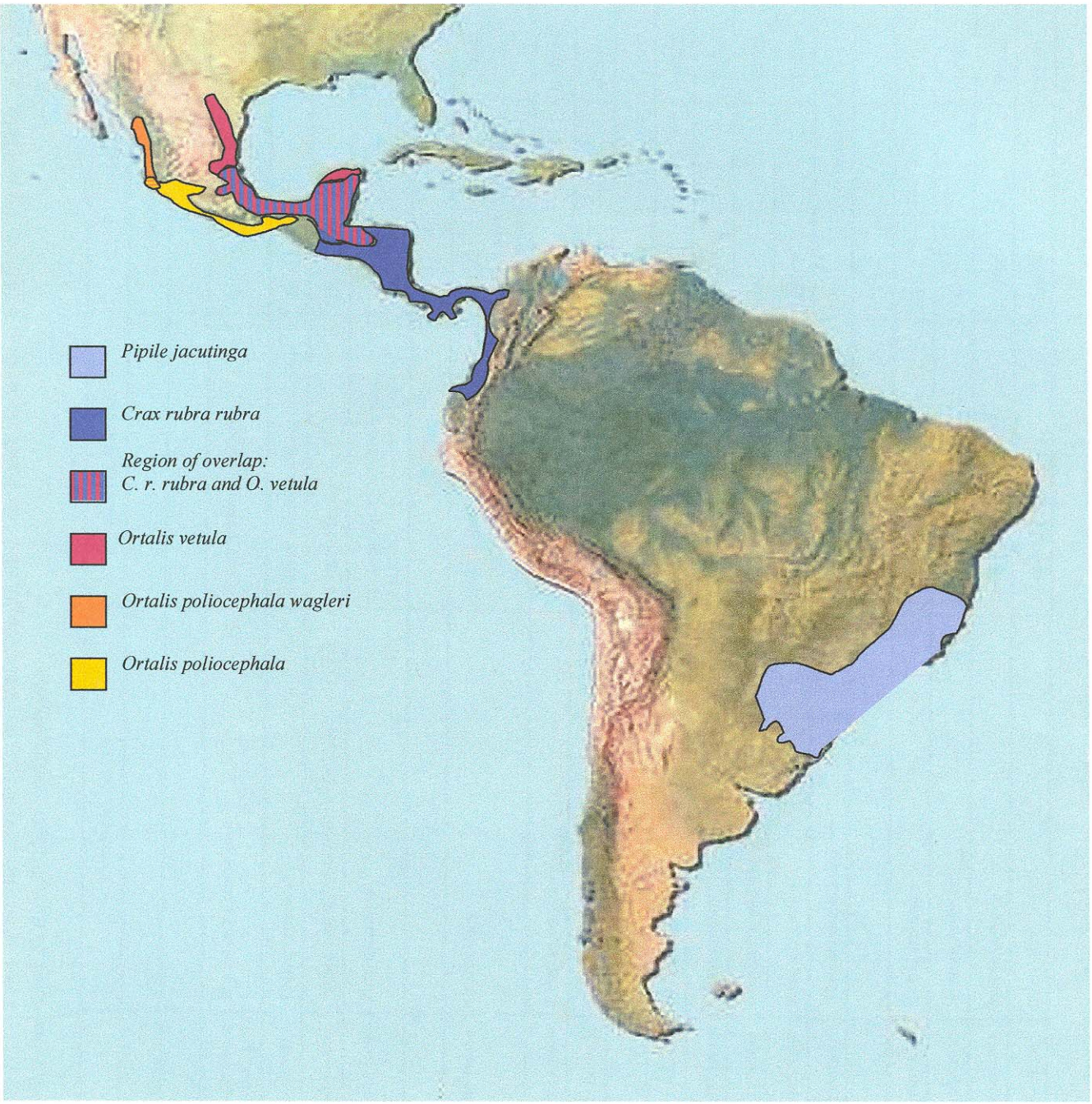

Figure 1. Distribution of cracid species in the present study.

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          27             28             28     22             31                2     32                  32          32     35          35          36                38
        xxxxxxxxxx       xxxxxxx        xxxxxxx xxxxxxxxxx   xxxxx xxxxxxxxx     xxx  xxxx xxx xxxxxxxxxx       xxxxx xxxxxxxxx xxx xx xxx       xx x       xxxxxxxx          xxxxxxxxxxxx
Pipile   GCC-TAGCCCTAAATCTTGATACTTCCTC-CACCAAAGTATTCGCCCGAGAACTACGAGC ACAAACGCTTAAAACTCTAAGGACTTGGCGGTG-CCCAAACCCACCTAGAGGAGCCTGTT CTATAATCGATAATCCACGATTCACCCAACCACCCCTTGCCAAGCACAGCCTACATACCG
Crax     GCC-TAGCCCTAAATCTAGATACTTCCTC-CACCAAAGTATTCGCCCGAGAACTACGAGC ACAAACGCTTAAAACTCTAAGGACTTGGCGGTGTCCCAAACCCACCTAGAGGAGCCTGTT CTATAACCGATAACCCACGATGCACCCAACCACCCCTCGCCAA-CACAGCCTACATACCG
Ortal pw GCC-TAGCCCTAAATCTAGATACTTCCCC-TACCAAAGTACTCGCCCGAGAACTACGAGC ACAAACGCTTAAAACTCTAAGGACTTGGCGGTGTCCCAAACCCACCTAGAGGAGCCTGTT CTATAACCGATAACCCACGATGCACCCAACCACCCTCTTGCTAA-TGCAGCCTACATACCG
Rhea     GCT-TAGCCCTAAATCCCGATACTTACCC-CACCCAAGTATCCGCCCGAGAACTACGAGC ACAAACGCTTAAAACTCTAAGGACTTGGCGGTG-CCTAAACCCACCTAGAGGAGCCTGTT CTATAATCGATAACCCACGATACACCCGACCATCTCTTGCCCA-TGCAGCCTACATACCG
ortal ve GCC-TAGCCCTAAATCTAGATACTTCCTC-CACCAAAGTATTCGCCCGAGAACTACGAGC ACAAACGCTTAAAACTCTAAGGACTTGGCGGTGTCCCAAACCCACCTAGAGGAGCCTGTT CTATAACCGATAACCCACGATGCACCCAACCACCCCTCGCCAA-CACAGCCTACATACCG
Cygnus   GCC-TGGCCCTAAATCTTGATACTTACTTT-ACCGAAGTATCCGCCAGAGAACTACGAGC ACAAACGCTTAAAACTCTAAGGACTTGGCGGTGCCCCAAACCCACCTAGAGGAGCCTGTT CTACAATCGATAATCCACGATTAACCCAACCACCCCTTGCCAA-CACAGCCTACATACCG
Anhima   GCC-TAGCCCTAAATCAAAGTACTTACCAA-ACCCAAGTACTCGCCCGAGAACTACGAGC ACAAACGCTTAAAACTCTAAGGACTTGGCGGTGCCCCAAACCCACCTAGAGGAGCCTGTT CTATAATCGATAACCCACGATTCACCCAACCACCCCTCGCCAA-CACAGCCTACATACCG
Chauna   GCC-TAGCCCTAAATCCAGGTACCTACCAA-ACCCAAGTACCCGCCCGAGAACTACGAGC ACAAACGCTTAAAACTCTAAGGACTTGGCGGTGCCCCAAACCCACCTAGAGGAGCCTGTT CTATAATCGATAATCCACGATTAACCCAACCACCCCTTGCCAA-CACAGCCTACATACCG
Anseran  GCC-TGGCCCTAAATCTAGATGCTTACCCC-ACCCAAGCATCCGCCCGAGAACTACGAGC ACAAACGCTTAAAACTCTAAGGACTTGGCGGTGCCTAAACCCACCTAGAGGAGCCTGTT CTATAATCGATAATCCACGATTCACCCGACCACCCCTTGCCAA-CACAGCCTACATACCG
Meleag   GCCCTGGCCCTAAATCTTGATACTAA-TAT-ACTCACGTATCCGCCTGAGAACTACGAGC ACAAACGCTTAAAACTCTAAGGACTTGGCGGTGCCCTAAACCCACCTAGAGGAGCCTGTT CTGTAATCGATAATCCACGATCCACCCAACCACCTCTTGCCAA-CACAGCCTACATACCG
Phasian  GCC-TGGCCCTAAATCTAGATGCCTAC-AT-ACCCATGCATCCGCCCGAGAACTACGAGC ACAAACGCTTAAAACTCTAAGGACTTGGCGGTGCCCCAAACCCACCTAGAGGAGCCTGTT CTATAATCGATAATCCACGATCCACCCAACCACCCCTTGCCAA-CACAGCCTACATACCG
Numida   GCC-TAGCCCTAAATCTAGATACTT-CAAT-ACCTAAGTATCCGCCTGAGAACTACGAGC ACAAACGCTTAAAACTCTAAGGACTTGGCGGTGCCCCAAACCCACCTAGAGGAGCCTGTT CTATAACCGATAATCCACGATCCACCCAACCACCTCTTGCCAA-CACAGCCTACATACCG
Coturnix GCC-TAGCCCTAAATCTAGATACCCAC-AT-ACTTATGTATCCGCCTGAGAACTACGAGC ACAAACGCTTAAAACTCTAAGGACTTGGCGGTGCCCCAAACCCACCTAGAGGAGCCTGTT CTATAACCGATAATCCACGATCTACCCAACCACCCCTTGCCAA-CACAGCCTACATACCG
Gallus   GCC-TAGCCCTAAATCTAGATACCTCCCATCACACATGTATCCGCCTGAGAACTACGAGC ACAAACGCTTAAAACTCTAAGGACTTGGCGGTGCCCCAAACCCACCTAGAGGAGCCTGTT CTATAATCGATAATCCACGATTCACCCAACCACCCCTTGCCAA-CACAGCCTACATACCG
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             39, 40                    39
           xxxxxxxxxxxxxxx       xx       xxxxxx
Pipile   CCGTCGCCAGCCCACCTCTAATGAAAGAACAACAGTGAGCTCAATAGCCCT------GCT AATAAGACAGGTCAAGGT
Crax     CCGTCGCCAGCTCACCTCCCTTGAGAGTCCCACAGTGAGCCCAATAGTCCTCTTTCCACT AGCAAGACAGGTCAAGGT
Ortal pw CCGTCGCCAGCTCACCTCCCCTGAGAGTTCCACAGTGAGCTCAATAGTCCCCTCCCCACT AACAAGACAGGTCAAGGT
Rhea     CCGTCCCCAGCCCGCCTTAAATGAGAGAACAACAGCAAGCATAATAGCTAC----CCGCT AGCAAGACAGGTCAAGGT
ortal ve CCGTCGACAGCTCACCTCCCTTGAGAGTCCCACAGTGAGCCCAATAGTCCTCTTTCCACT AGCAAGACAGGTCAAGGT
Cygnus   CCGTCGCCAGCCCACCTCGAATGAGAGCACAGCAGTGGACACAATAGCATA----CCGCT AATAAGACAGGTCAAGGT
Anhima   CCGTCGCCAGCTCACCTCCCCTGAGAGCCCAACAGTGAGCTAAGCAGTTACC---CCACT AGCAAGACAGGTCAAGGT
Chauna   CCGTCGCCAGCTCACCTCCACTGAGAGTCCAACAGTGAGCTAAATAGTTACC---CCACT AGCAAGACAGGTCAAGGT
Anseran  CCGTCGCCAGCTCACCTTCCCTGAAAGCCCAACAGTGAGCTCAATAGTCAT----CCACT AACAAGACAGGTCAAGGT
Meleag   CCGTCGCCAGCCCACCTAAAATGAAAGATCAATAGTGAGCTCAATAGTC------CCACT AACAAGACAGGTCAAGGT
Phasian  CCGTCGCCAGCCCACCT-AAATGAAAGGCCAACAGTGAGCTCAACAGTCCA----CCACT AGCAAGACAGGTCAAGGT
Numida   CCGTCGCCAGCCCACCT-ACATGAAAGCGCAACAGTGAGCTCAACAGTCC-----CCACT AACAAGACAGGTCAAGGT
Coturnix CCGTCGCCAGCCCACTT--AATGAAAGAACAACAGTGAGCTCAATAGCCG-----CCACT AATAAGACAGGTCAAGGT
Gallus   CCGTCGCCAGCCCACCTCTAATGAAAGAACAACAGTGAGCTCAATAGCCCC----TCGCT AATAAGACAGGTCAAGGT
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FIGURE 2: 12S rRNA mitochondrial gene (258 bp) sequences for Galliformes, Anseriformes, cracids, and a ratite. The markers above the sequences correspond to stems of the secondary structure and the numbers are the stem numbers according to Houde *et al*. (1997) and Mindell *et al*. (1997). Species names are abbreviated from Table1.

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Pipile    CTAGCGATCT GCCTACTAAC ACAAATCCTC ACAGGACTAC TCCTAGCTAC GCACTACACT GCAGACACAA CACTAGCCTT TTCATCCGTC GCCCACACAT GTCGAAACGT ACAATATGGC TGACTAATTC GAAACCTCCA TGCAAACGGA GCATCCTTCT TCTTCATCTG CATTTACCTC CACATCGGAC GAGGATTCTA
Crax      CT-GC-ATCT GCCT-ATAAC C-AAAT-CTC ACTGGCCTCC TACTAGCCAT ACACTACACC GCAGACACTA CCCTCGCCTT CTCCTCCGTA GCTCACACAT GCCGGAACGT CCAGTACGGC TGACTAATCC GCAACCTACA CGCAAACGGC GCCTCATTCT TCTTCATCTG CATCTACCTC CACATTGGCC GCGGCCTCTA
Ortal pw  CTAGCAATCT GCCTTATAAC CCAAATCCTC ACTGGCCTCC TACTAGCCAT ACACTACACC GCAGACACTA CCCTCGCCTT CTCCTCCGTA GCTCACACAT GCCGGAACGT CCAGTACGGC TGACTAATCC GCAACCTACA CGCAAACGGC GCCTCATTCT TCTTCATCTG CATCTACCTC CACATTGGCC GCGGCCTCTA
Ortal ve  CTAGCAATCT GCCTTACAAC CCAAATCCTC ACTGGCCTCC TACTAGCCAT ACACTACACT GCAGACACTA CCCTTGCTTT CTCCTCCGTA GCCCACACAT GCCGAAACGT CCAGTACGGC TGACTAATTC GCAACCTACA TGCAAACGGC GCCTCTTTCT TCTTCATCTG CATCTACCTC CACATTGGTC GCGGTTTTTA
Cygnus    CTGGCCATCT GTTTAGCCAC ACAAATCTTA ACAGGCCTCC TGCTAGCCAT GCACTACACC GCAGACACCT CACTCGCCTT CTCCTCAGTG GCCCATACAT GCCGAGACGT CCAATATGGA TGACTCATCC GCAACCTTCA TGCTAACGGC GCCTCATTCT TCTTTATCTG CATCTACCTG CACATCGGAC GAGGCCTCTA
Anseran   CTAGCGATCT GCCTCATAAC ACAAATCCTT ACAGGACTAC TGCTAGCCAT ACACTATACC GCAGACACCA CCCTCGCCTT CTCCTCCGTG GCCCACACAT GCCGAAACGT ACAATACGGC TGACTCATTC GCAACCTACA TGCAAACGGT GCTTCCTTCT TCTTCATTTG CATCTACCTC CACATTGGAC GAGGCCTCTA
Meleag    CTAGCAGTAT GCCTCATCAC TCAAATCTTA ACCGGCCTCC TACTAGCCAT ACATTACACT GCAGACACCA CTCTTGCATT CTCTTCTGTG GCCTACACAT GCCGAAACGT ACAATACGGT TGACTCCTCC ATAACCTCCA TGCGAATGGG GCCTCATTCT TCTTCATCTG CATCTTCCTA CACATTGGAC GCGGCCTATA
Numida    CTAGCAGTCT GCTTCATGAC CCAAATTATC ACCGGCCTAC TACTAGCTAT ACACTACACT GCAGATACCT CCCTAGCCTT CTCATCCGTA GCCCACACAT GTCGAAATGT CCAATACGGA TGACTAATCC GAAACCTACA TGCAAACGGA GCCTCATTCT TCTTCATCTG CATCTACCTC CACATTGGCC GAGGCCTATA
Coturnix  CTAGCAATAT GCCTCATCAC CCAAATCCTC ACCGGCCTTC TACTAGCCAT ACACTACACC GCAGACACCT CCCTAGCCTT CTCTTCCGTA GCCCACACAT GTCGAAACGT ACAGTACGGC TGACTCATTC GCAATCTCCA TGCAAACGGC GCATCATTCT TCTTCATCTG CATCTTCCTC CACATCGGAC GAGGCCTATA
Struthio  CTAGGAATTT GCCTAATTAC CCAAATTCTA ACAGGGCTCC TACTAGCCAT ACATTACACA GCCGACACTA CACTAGCATT CTCATCCGTC GCCCACACAT GCCGGAACGT ACAGTACGGA TGATTTATCC GCAATCTCCA TGCAAACGGC GCATCCTTCT TCTTCATCTG TATTTACCTA CACATCGGCC GAGGACTCTA
Casuar    CTAGGGATTT GCCTAATTAC CCAAATCCTC ACAGGACTAC TACTAGCTAT GCACTACACA GCTGACACCT CACTAGCCTT CTCATCCGTA GCCCACACCT GCCGAAACGT ACAGTATGGC TGACTAATTC GTAACCTCCA TGCAAATGGA GCATCATTCT TCTTCATCTG TATCTACCTT CACATCGGAC GAGGGTTCTA
Rhea      CTAGGAATCT GCCTCATCA- CCAAATCCTA ACAGGCCTCC TCCTAGCTAT ACATTACACA G-GGACACCT CATTAGCCTT CTCATCCGTA GCCCACACCT GCCGCAACGT CCAATATGGT TGACTGATCC GCAATCTCCA TGCAA-CGGT GCATCCTTCT TCTTCATCTG CATCTACCTT CACATCGGCC GAGGATTCTA
Gallus    TTAGCAGTCT GCCTCATGAC CCAAATCCTC ACCGGCCTAC TACTAGCCAT GCACTACACA GCAGACACAT CCCTAGCCTT CTCCTCCGTA GCCCACACTT GCCGGAACGT ACAATACGGC TGACTCATCC GGAATCTCCA CGCAAACGGC GCCTCATTCT TCTTCATCTG TATCTTCCTT CACATCGGAC GAGGCCTATA


Pipile     CTACGGCTCA TACCTGAACA AAGAAACCTG AAACACAGGT GTCATCCTCC TACTTACCCT AATTGCAACC ?CCTTCGTAC CATCAATCC
Crax       CTACGGCTCA TACCTTTATA AAGAAACCTG AAACACAGGA ATTATCCTCC TA-TAGTGCT TATACGAACT GCTTTCGTAG GGTATGTCT
Ortal pw   CTACGGCTCA TACCTTTATA AAGAAACCTG AAACACAGGA ATTATCCTCC TACTAGTGCT TATAGCAACT GCTTTCGTAG GGTATGTTC
Ortal ve   CTATGGCTCA TACCTCTACA AAGAAACCTG AAACACAGGG GTAATCCTCC TGCTAGTACT CATAGCAACT GCTTTCGTAG GATACGTCC
Cygnus     CTACGGCTCC TATCTGTACA AAGAAACCTG AAACACAGGG GTAGTCCTCC TGCTCACCCT CATAGCAACT GCCTTCGTAG GATACGTCC
Anseran    TTACGGCTCT TATCTATACA AAGAAACGTG AAACACAGGA GTAATCCTTC TCCTAACACT CATAGCAACT GCCTTCGTAG GCTACGTAC
Meleag     TTATGGTTCG TACCTATATA AAGAAACCTG AAATACAGGA GTAGTCTTAC TTCTCACCCT CATAGCAACA GCCTTTGTAG GCTATGTCC
Numida     CTACGGCTCC TACCTATATA AAGAAACCTG AAACACAGGA GTAATTCTCC TCCTCACACT AATAGCAACC GCTTTCGTAG GCTACGTTC
Coturnix   TTACGGCTCC TACCTTTACA AAGAAACCTG AAACACAGGA GTAATCCTGC TTCTCACACT AATAGCCACT GCTTTCGTAG GATACGTCT
Struthio   CTATGGCTCT TACCTCTATA AAGAAACCTG AAACACCGGC GTTATCCTCC TACTAACATT AATAGCAACT GCATTTGTAG GTTATGTCC
Casuar     CTATGGCTCC TATCTTTATA AAGAAACCTG GAACACCGGA GTAATCCTCC TACTGACATT AATAGCAACC GCCTTCGTAG GCTATGTCC
Rhea       CTACGGCTCA TATCTCTACA AAGAGACCTG AAACACCGGA GTCGTCCTCC TACTAACCTT AATGGCAACT GCCTTTGT-G GTTATGTCC
Gallus     CTACGGCTCC TACCTCTACA AGGAAACCTG AAACACAGGA GTAATCCTCC TCCTCACACT CATAGCCACC GCCTTTGTGG GCTATGTTC
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FIGURE 3: Cytochrome *b* gene (289 bp) sequence for Galliformes, Anseriformes, cracids and ratites. The start of the fragment corresponds to codon number 38 (L) of the published sequence of *Gallus gallus* (Desjardins & Morais 1990). Species names are abbreviated from Table 1.

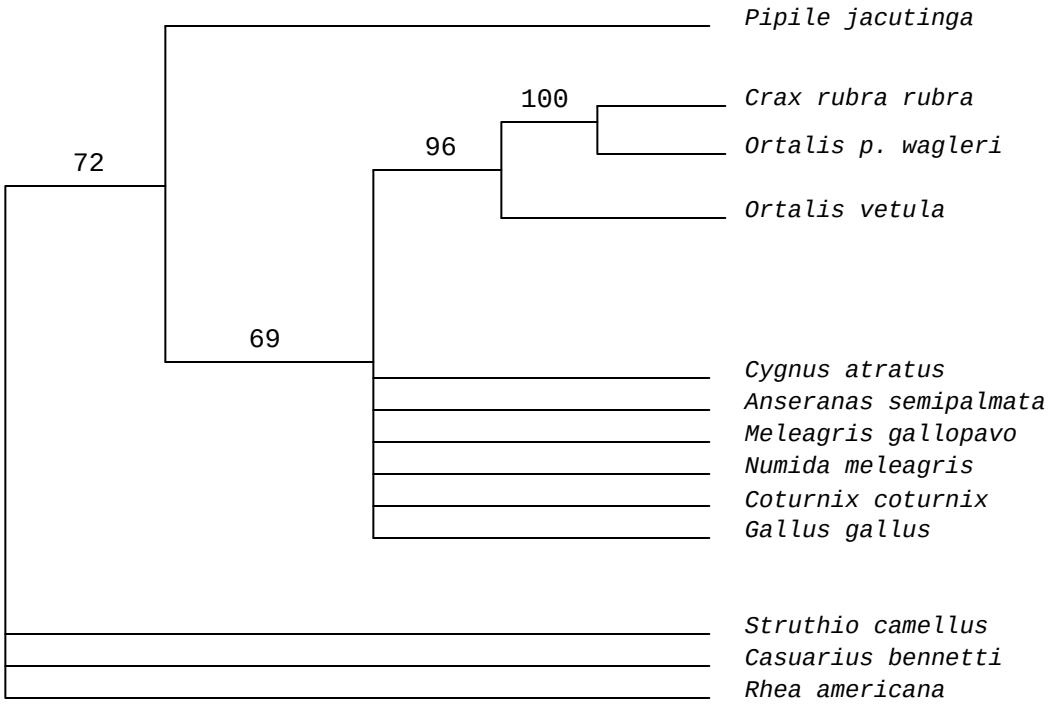

FIGURE 4: Phylogenetic tree obtained by maximum parsimony analysis (PAUP) of cytochrome *b* (289 bp), weighing transversions four times transitions, and 1st and 2nd positions four times third positions. The tree was rooted with sequence from the rhea. Indices of support from bootstrap analyses are shown for resolved nodes.

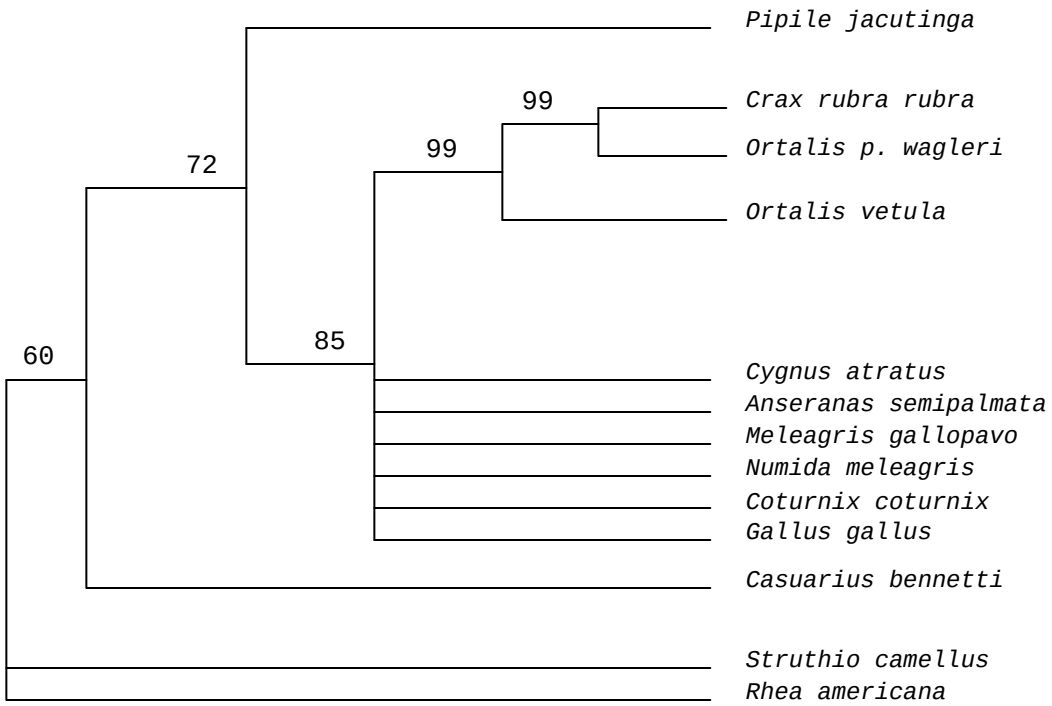

FIGURE 5: Phylogenetic tree obtained by maximum parsimony analysis (PAUP) of cytochrome *b*, weighing transversions four times transitions, and weighing 1st, 2nd, and 3rd positions equally. The tree was rooted with sequence from the rhea. Indices of support from bootstrap analyses are shown. The same topology was obtained by weighing transitions and transversions equally except bootstrap values decreased 5 to 20% (tree not shown).

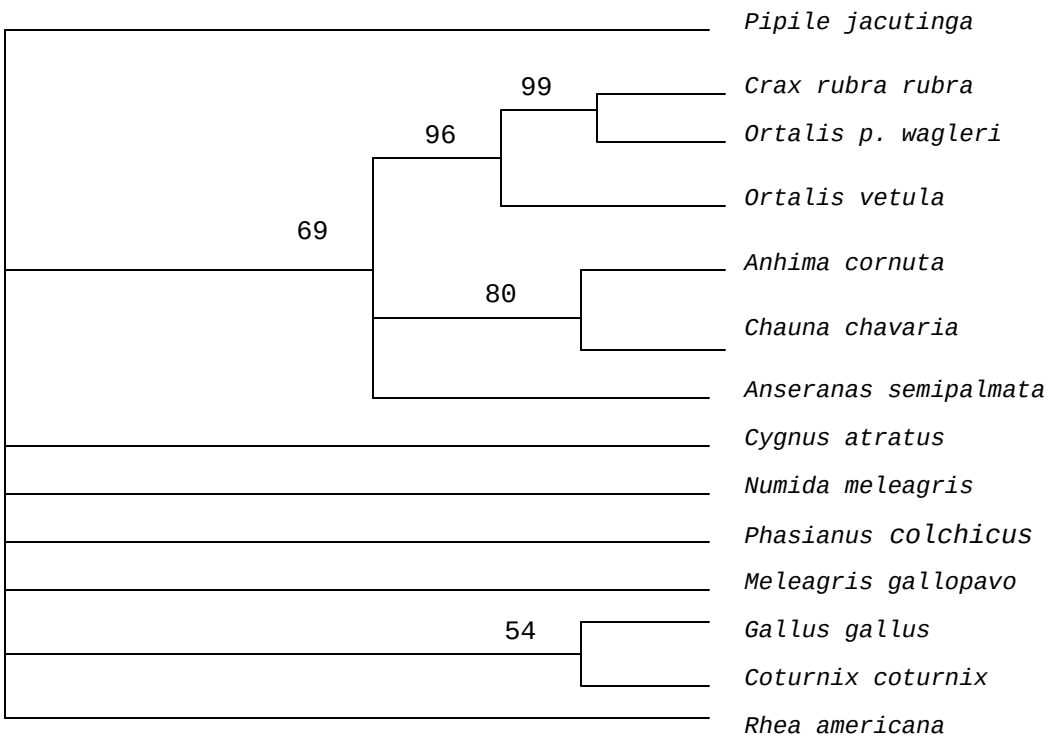

FIGURE 6 : Concensus of two trees obtained by maximum parsimony analysis (PAUP) of 12S (258 bp), weighing transversions four times transitions and weighing stem regions four times loops. The tree was rooted with sequence from the rhea. Indices of support from bootstrap analyses are shown for resolved nodes. The same topology for a concensus of six trees was obtained by weighing all positions and regions equally except bootstrap values decreased 1 to 14% and the node for *Gallus* and *Coturnix* is unresolved (tree not shown).

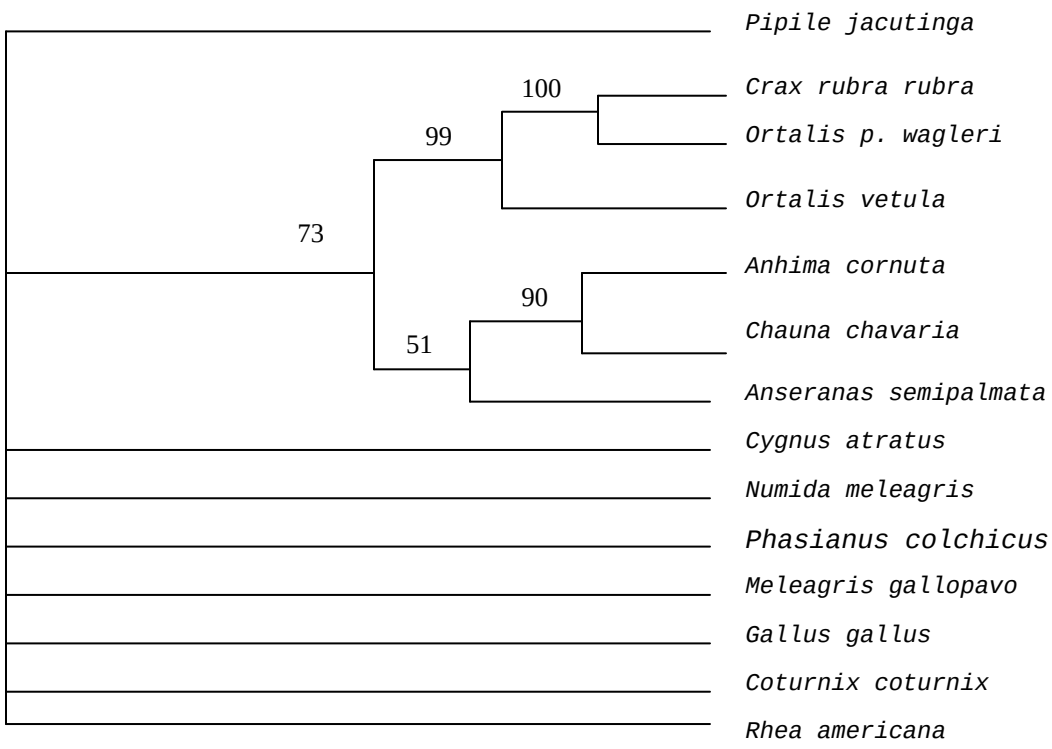

FIGURE 7 : Concensus of two trees obtained by maximum parsimony analysis (PAUP) of 12S weighing transversions four times transitions and weighing stem regions and loops equally. The rhea was used as the outgroup.

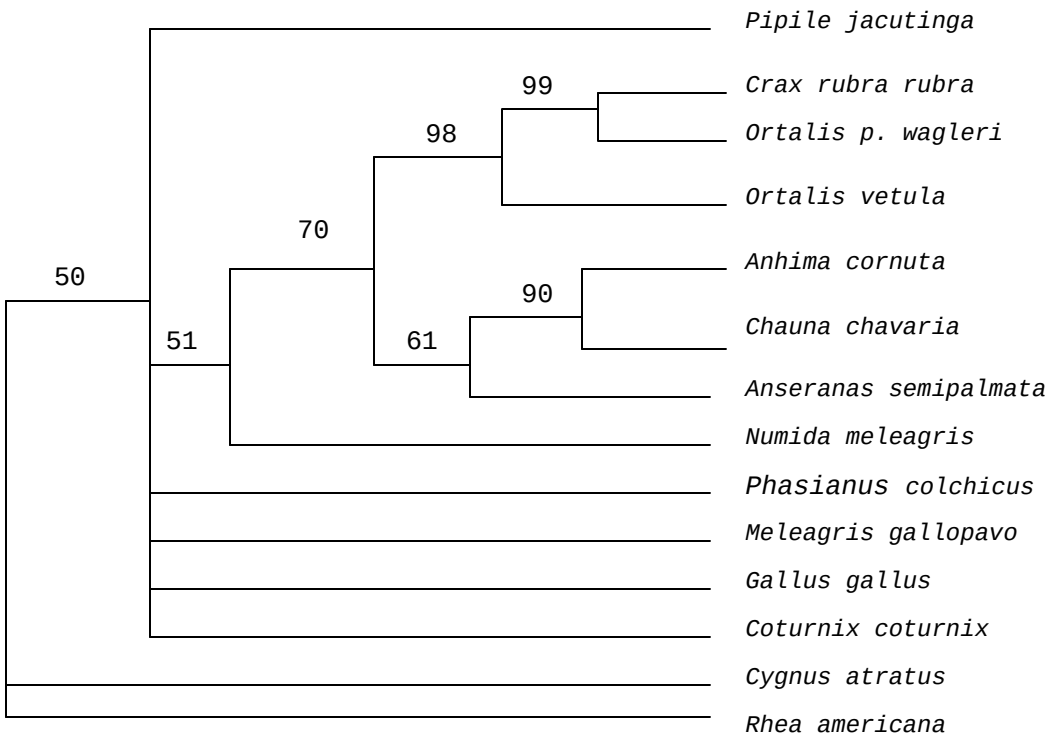

FIGURE 8: Phylogenetic tree obtained by maximum parsimony analysis (PAUP) of 12S weighing transversions and transitions equally and weighing stem regions and loops 4:1 respectively. Boostrap indices are shown for resolved nodes.

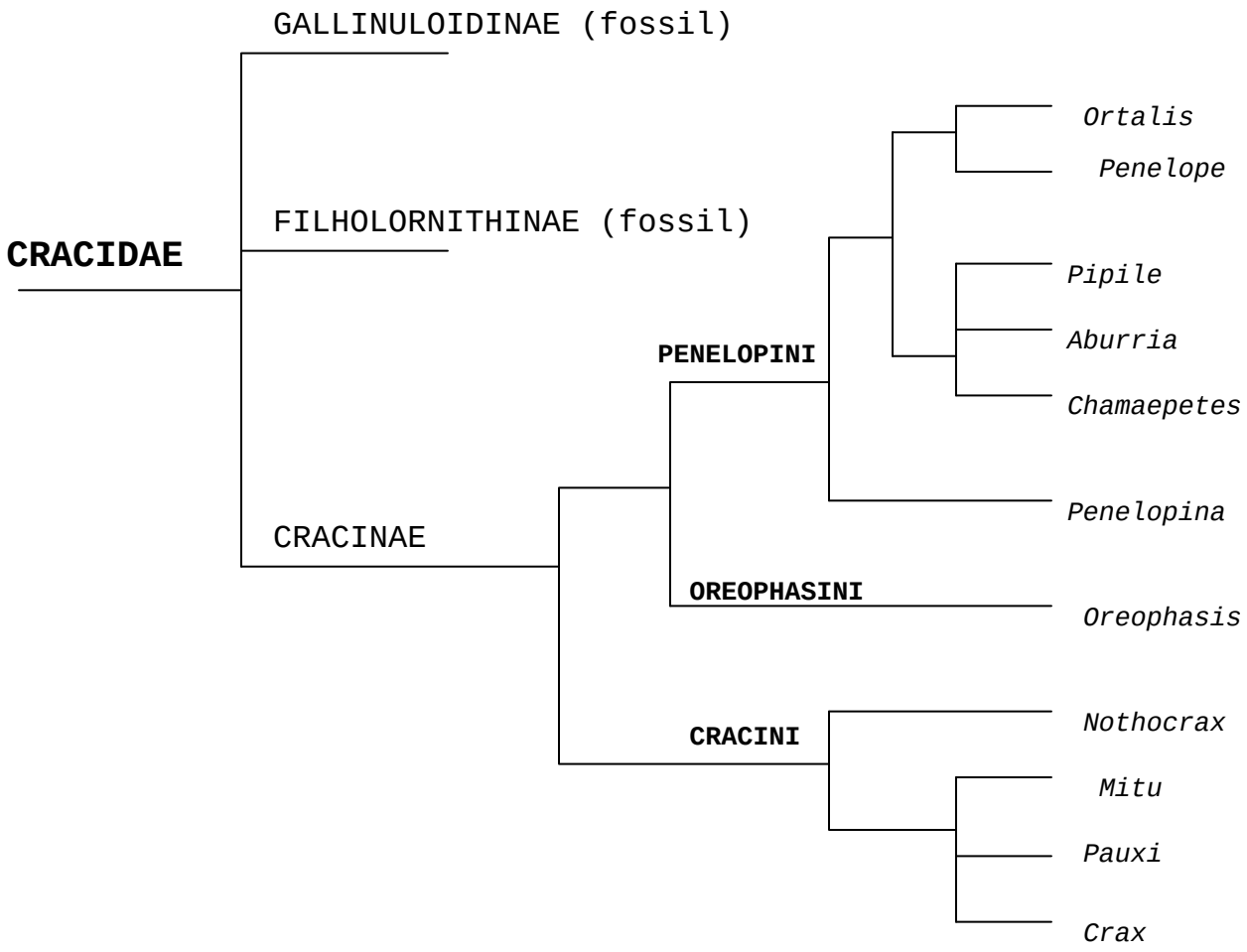

FIGURE 9: Phylogeny of the family Cracidae (Vaurie 1968). The subfamilies including fossil groups, the tribes (in bold), and the genera in each tribe, are shown. Note the placement of *Crax* and *Ortalis* in separate tribes.

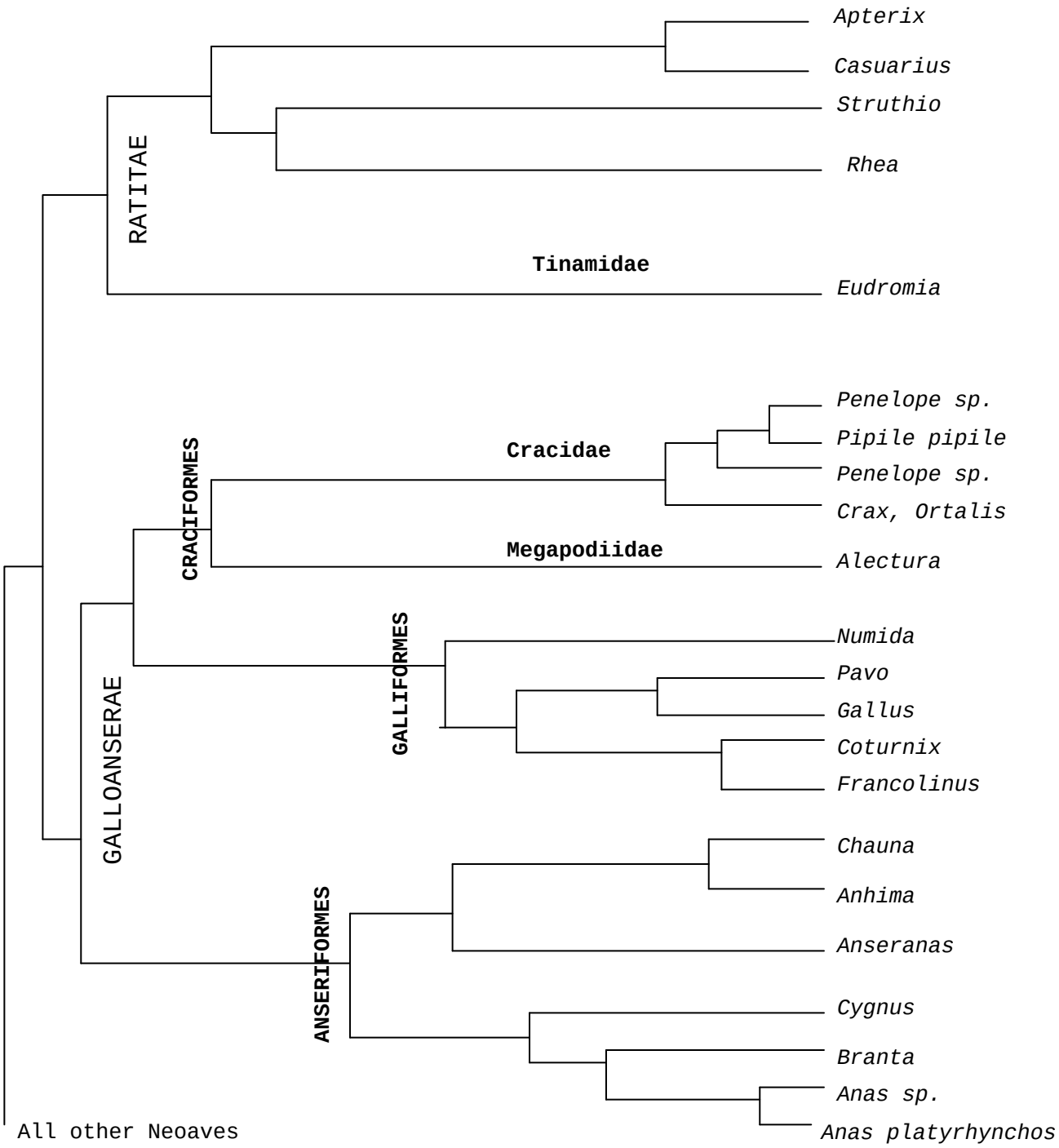

FIGURE 10: Classification of basal bird orders (in bold) according to Sibley and Alquist 1990. (Modified from Fig. 357, Sibley and Alquist 1990).

APPENDIX A

Distance matrix generated from 12S sequences by the DNADIST program in PHYLIP v.3.5 (Felstein, 1993), using Kimura's 2-parameter genetic distance (assumes a higher rate of transition substitutions than transversions).

	Pipile	***Crax***	***Ortalis v***	***Rhea***	***Ortalis pw***	***Cygnus***	***Anhima***	***Chauna***	***Anseranas***	***Meleagris***	***Phasianus***	***Nimida***	***Coturnix***	***Gallus***
Pipile	*													
Crax	0.0770	*												
Ortalis v	0.0996	0.0608	*											
Rhea	0.1330	0.1671	0.1573	*										
Ortalis pw	0.0817	0.0039	0.0651	0.1726	*									
Cygnus	0.0775	0.1428	0.1622	0.1566	0.1480	*								
Anhima	0.1246	0.0896	0.0986	0.1531	0.0943	0.1580	*							
Chauna	0.1101	0.0988	0.0988	0.1334	0.1037	0.1278	0.0451	*						
Anseranas	0.0907	0.0933	0.1068	0.1415	0.0980	0.1233	0.0887	0.0801	*					
Meleagris	0.0877	0.1350	0.1350	0.1437	0.1402	0.1146	0.1481	0.1237	0.1006	*				
Phasianus	0.0873	0.1053	0.1288	0.1483	0.1103	0.1138	0.1092	0.0861	0.0639	0.0678	*			
Numida	0.0738	0.0866	0.0866	0.1494	0.0914	0.1143	0.1094	0.1097	0.0818	0.0686	0.0593	*		
Coturnix	0.0694	0.1204	0.1300	0.1532	0.1255	0.1008	0.1426	0.1046	0.1048	0.0683	0.0719	0.0639	*	
Gallus	0.0418	0.1226	0.1273	0.1384	0.1277	0.0962	0.1324	0.0995	0.0997	0.0822	0.0680	0.0685	0.0421	*