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PHYLOGENY OF CRANES (GRUIFORMES: GRUIDAE) BASED ON CYTOCHROME-*B* DNA SEQUENCES

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ABSTRACT.—DNA sequences spanning 1,042 nucleotide bases of the mitochondrial cytochrome-*b* gene are reported for all 15 species and selected subspecies of cranes and an outgroup, the Limpkin (*Aramus guarauna*). Levels of sequence divergence coincide approximately with current taxonomic ranks at the subspecies, species, and subfamilial level, but not at the generic level within Gruinae. In particular, the two putative species of *Balearica* (*B. pavonina* and *B. regulorum*) are as distinct as most pairs of gruine species. Phylogenetic analysis of the sequences produced results that are strikingly congruent with previous DNA-DNA hybridization and behavior studies. Among gruine cranes, five major lineages are identified. Two of these comprise single species (*Grus leucogeranus, G. canadensis*), while the others are species groups: *Anthropoides* and *Bugeranus; G. antigone, G. rubicunda,* and *G. vipio;* and *G. grus, G. monachus, G. nigricollis, G. americana,* and *G. japonensis.* Within the latter group, *G. monachus* and *G. nigricollis* are sister species, and *G. japonensis* appears to be the sister group to the other four species. The data provide no resolution of branching order for major groups, but suggest a rapid evolutionary diversification of these lineages. *Received 19 March 1993, accepted 19 August 1993.*

THE 15 EXTANT SPECIES of cranes comprise the nominate family (Gruidae) of the order Gruiformes, and are currently divided into two subfamilies, Balearicinae and Gruinae (Brodkorb 1967). Balearicine cranes are anatomically unspecialized relative to gruines and are represented by only two extant species in the genus *Balearica* (the crowned cranes of Africa). Gruines share derived anatomical features such as an anteriorly sculpted sternum (often associated with tracheal coiling inside keel) in which the furcular process is fused to the anteroventral tip of the keel. Three extant gruine genera are recognized: *Grus* (10 species), *Anthropoides* (2 species), and *Bugeranus* (1 species). These genera are defined on the basis of soft anatomical features, although their monophyly has not been addressed by phylogenetic analysis. Fossil balearicines are known from the lower Eocene and later deposits in Eurasia, whereas Gruines date from the late Miocene (Brodkorb 1967).

Evolutionary relationships among cranes have been addressed with a variety of different approaches during the past two decades. Archibald (1976) derived the species groups shown in Table 1 on the basis of similarities in unison calls. These behavior patterns include both stereotyped movements and vocalizations, and are most elaborately developed in gruines. Notable among Archibald's conclusions is the postulated close relationship between the Siberian (*Grus leucogeranus*) and Wattled (*Bugeranus carunculatus*) cranes. Both species lack the elaborate tracheal coiling found in other gruines, although both show the characteristic sculpted keel. Archibald's findings were generally supported by the anatomical-phenetic study of Wood (1979) and the recent allozyme survey of Dessauer et al. (1992).

Ingold et al. (1987b) examined allozyme variation among cranes, and Ingold et al. (1989) reported the results of selected comparisons using microcomplement fixation and DNA-DNA hybridization. Both these studies, however, were inadequately designed for phylogenetic reconstruction (Krajewski 1989).

Crane phylogeny was estimated from a complete matrix of DNA-DNA hybridization comparisons by Krajewski (1989; for a refinement of the analysis, see Krajewski and Dickerman 1990). DNA-DNA hybridization (Fig. 1) confirmed the distinctness of balearicine and gruine cranes, and supported Archibald's (1976) gruine species groups. An exception is the placement of *G. leucogeranus* as an isolated lineage among gruines, perhaps representing the oldest phylogenetic branch within the subfamily. Among

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TABLE 1. Crane species and subspecies studied. Species groups are those identified by Archibald (1976) on basis of unison-call similarities.

Family Gruidae (Cranes)
- Subfamily Balearicinae
  - Genus *Balearica*
    - *B. pavonina* (Black Crowned Crane)
    - *B. regulorum* (Gray Crowned Crane)
- Subfamily Gruinae
  - Genus *Bugeranus*
    - *B. carunculatus* (Wattled Crane)
  - Genus *Anthropoides*
    - *A. virgo* (Demoiselle Crane)
    - *A. paradisea* (Stanley Crane)
  - Genus *Grus*
    - Species Group Leucogeranus[a]
      - *G. leucogeranus* (Siberian Crane)
    - Species Group Canadensis
      - *G. canadensis* (Sandhill Crane)[b]
        - *G. c. canadensis* (Lesser Sandhill Crane)
        - *G. c. tabida* (Greater Sandhill Crane)
        - *G. c. rowani* (Canadian Sandhill Crane)
        - *G. c. pratensis* (Florida Sandhill Crane)
    - Species Group Antigone
      - *G. antigone* (Sarus Crane)
        - *G. a. antigone* (Western Sarus Crane)
        - *G. a. sharpei* (Eastern Sarus Crane)
      - *G. rubicunda* (Brolga)
      - *G. vipio* (White-naped Crane)
    - Species Group Americana[c]
      - *G. monachus* (Hooded Crane)
      - *G. grus* (Common Crane)
      - *G. americana* (Whooping Crane)
      - *G. japonensis* (Japanese Crane)
      - *G. nigricollis* (Black-necked Crane)

[a] Archibald (1976) placed Siberian Crane in *Bugeranus*. Species Group Leucogeranus was suggested by Krajewski (1989).

[b] Mississippi (*G. c. pulla*) and Cuban (*G. c. nesiotes*) Sandhill Cranes were not included in study.

[c] Krajewski (1989) suggested that this group be renamed Species Group Grus.

other gruines, *Anthropoides* and *Bugeranus* form a clade, as do three Australasian species of *Grus* (*G. antigone, G. rubicunda,* and *G. vipio*). The largest assemblage of *Grus* includes four Eurasian species (*G. grus, G. japonensis, G. monachus,* and *G. nigricollis*), as well as the North American Whooping Crane (*G. americana*). The Sandhill Crane (*G. canadensis*) shows no close affinities with other gruine groups.

DNA-DNA hybridization, however, was unable to resolve relationships either within or between major clades. Within groups, small interspecific genetic distances (<1% divergence)

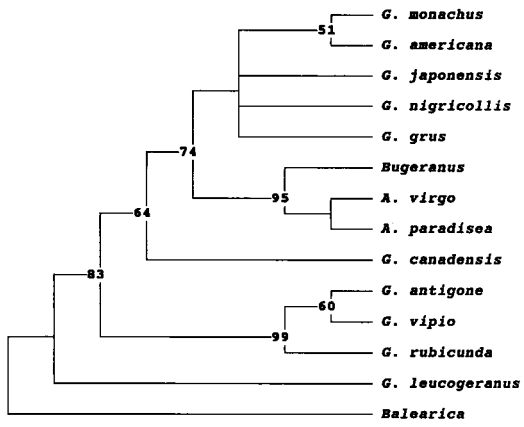

Fig. 1. Bootstrap consensus tree for DNA-DNA hybridization distances among cranes (redrawn from Krajewski and Dickerman 1990). Nodal value shows frequency each putative clade represented among 100 bootstrap pseudoreplicates. Bifurcating nodes without bootstrap values appear in 100% of pseudoreplicates; polytomies have bootstrap values less than 50%.

prevented phylogenetic resolution. Poor resolution between groups may indicate a relatively rapid evolutionary radiation of the major gruine lineages (Krajewski 1989, 1990). Further resolution of crane relationships, as well as testing the DNA-DNA hybridization results, requires an additional and independent type of comparative genetic data.

In this study we report sequences of the mitochondrial cytochrome-*b* gene for all crane species and selected subspecies. A mitochondrial sequence was chosen to complement the nuclear data derived from DNA-DNA hybridization, as well as to exploit the advantages of mitochondrial DNA (mtDNA) for phylogenetic inference (Moritz et al. 1987). We chose the cytochrome-*b* gene because preliminary comparisons indicated that divergences were in the range of 1 to 10%. Such divergences are sufficient to distinguish species, but not so large as to be distorted by multiple substitutions (Moritz et al. 1987).

METHODS

Laboratory procedures.—DNA was extracted from whole blood samples using standard methods of cell lysis, Proteinase K and RNase A digestion, extraction with phenol and chloroform, and ethanol precipitation (Sambrook et al. 1989). Voucher information for the specimens employed is given in Appendix A. We included subspecies representatives for the polytypic

TABLE 2. Cytochrome-*b* primer sequences and sources.

Primer name[a]	Sequence	Source
L14841	5′-CCATCCAACATCTCAGCCATGATGAAA-3′	Kocher et al. (1989)
L15087	5′-TACTTAAACAAAGAAACCTGAAA-3′	Edwards et al. (1991)
L15136	5′-ATAGCAACAGCATTTGTAGG-3′	Krajewski et al. (1992)
L15418	5′-GATAAAATCCCATTCCACCCCTA-3′	This study
L15615	5′-GTTCAATCCCAAACAAACTAGGA-3′	This study
H15149	5′-TGCAGCCCCTCAGAATGATATTTGTCCTCA-3′	Kocher et al. (1989)
H15498	5′-GGAATAAGTTATCTGGGTCTC-3′	Krajewski et al. (1992)
H15767	5′-ATGAAGGGATGTTCTACTGGTTG-3′	Edwards et al. (1991)
H15915	5′-AACTGCAGTCATCTCCGGTTTACAAGAC-3′	Edwards et al. (1991)

[a] H and L refer to heavy and light strands of mtDNA, respectively, and numbers correspond to position of each primer's 3′ base in human mitochondrial genome (Anderson et al. 1981). Corresponding positions in chicken mitochondrial genome (Desjardins and Morais 1990) can be obtained by adding 149 to human position number. Primer H15915 lies in threonine tRNA gene 3′ to cytochrome-*b*.

Sarus (*G. antigone*) and Sandhill (*G. canadensis*) cranes in order to assess the effect, if any, of intraspecific polymorphism on phylogenetic inferences (Smouse et al. 1991). A Limpkin (*Aramus guarauna*, Aramidae) served as an outgroup.

Portions of the cytochrome-*b* gene were isolated and amplified via the polymerase chain reaction (PCR; Innis and Gelfand 1990). PCR reactions were done in 100 μl volumes using 1.5 mM Mg^{2+}, 1.0 μM concentrations of each primer, and 1.0 U of Taq polymerase (Promega Corp.). A thermal cycle began with 2.5 min at 94°C for initial denaturation, followed by 35 cycles of denaturation (94°C, 40 s), primer annealing (48°C, 1 min), and polymerase extension (68–72°C, 3.5 min). A final extension for 7 min was included to minimize the number of partial strands. Primers and their sources are given in Table 2. The primers span 1,042 base pairs (bp) of cytochrome *b*, covering all but the first (5′) 98 bp of the gene sequence.

Balanced-primer reaction products were purified by electrophoresis through a 2.5% low-melting agarose gel, stained with ethidium bromide, excised, and stored in 250–1,000 μl of deionized water. Gel slices were melted at 65°C for 3 to 5 min and 5 to 10 μl were removed for asymmetric PCR (McCabe 1990). Reaction mixtures and thermal-cycle parameters for asymmetric amplification were identical to those given above, except that primer amounts were set to 50 pmol (excess primer) and 1 pmol (limiting primer). An 8 μl portion of each asymmetric reaction product was electrophoresed through 2.5% agarose and visualized by ethidium bromide staining to check for the presence of a visible single-stranded DNA band. Successful reactions were precipitated in 2.5 M ammonium acetate and two volumes of cold 95% ethanol, dried, and rehydrated in 10 μl of deionized water for sequencing. Dideoxy sequencing followed the protocol for the Sequenase enzyme system (United States Biochemical; Sambrook et al. 1989) using [^{35}S]dATP. Gel drying and autoradiography followed standard protocols.

DNA sequences obtained from each autoradiograph were aligned manually with previous overlapping sequences from the same DNA sample to ensure accuracy. After resolution of base-calling errors and sequencing artifacts, sequences for each species were aligned with one another. Manual alignment presented no difficulties because the sequences are highly similar, and no gaps are required to maintain the reading frame.

Data analysis.—Methods of phylogenetic inference have been the subject of considerable debate in recent years, particularly as regards DNA sequence data (Felsenstein 1988, Penny et al. 1992). For data sets of more than a few species, analytical options are largely restricted to parsimony methods and methods that employ genetic distances (Swofford and Olsen 1990). We used both strategies in analysis of the crane sequences and contrast the results of each below.

Genetic distances were computed using Kimura's (1980) two-parameter model to correct for multiple substitutions at individual sites. Because observed dissimilarities between sequences were generally small (<10%), distance estimates should not be grossly distorted by multiple substitutions. However, the crane sequences do show a substantial transition bias and Kimura's (1980) correction is clearly appropriate. Phylogenetic relationships from distance matrices were estimated using the method of Fitch and Margoliash (1967). Levels of resolution in the estimated phylogeny were assayed by bootstrap resampling of sites (Felsenstein 1985, 1988). Pseudoreplicate distance matrices and associated best-fit trees were generated and summarized as a majority-rule consensus tree, on which node values give the frequency with which each putative clade is observed among the pseudoreplicates. Distance analyses were carried out using programs SEQBOOT, DNADIST, FITCH, and CONSENSE in Felsenstein's PHYLIP package (1991, version 3.4).

In parsimony analyses, equal weight was given to all codon position sites. Although the sequences show a strong bias in favor of third-codon-position substitutions, divergence is sufficiently low that few first

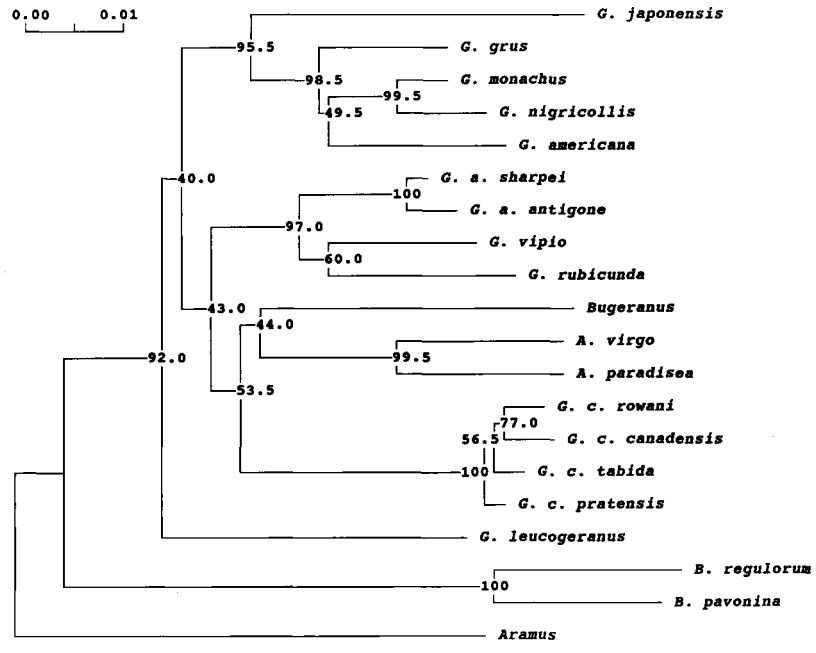

Fig. 2. Bootstrapped distance tree based on 1,042 bp of crane cytochrome-*b* DNA sequence. Distances calculated with Kimura's (1980) two-parameter method for 200 random samples of sites in crane alignment, and trees constructed using Fitch-Margoliash (1967) method as implemented by SEQBOOT, DNADIST, FITCH, and CONSENSE programs of PHYLIP 3.4. Branch-length units are substitutions per site. Nodal values show percentage of pseudoreplicate trees in which each putative clade occurs. Best-fit tree has an associated sum-of-squares value of 0.8966 (average percent standard deviation = 4.8702, global search option in effect).

and second positions are represented in the set of phylogenetically informative sites (see below). We reasoned that differential weighting of these few sites would only exaggerate the stochastic element in sequence variation and would not substantially improve the phylogenetic estimate.

Similarly, we treated transition and transversion substitutions as equally informative, because transversion parsimony would be forced to operate on only a small fraction of the data (see below). Moreover, the low divergence observed between most sequences indicates that transition differences still carry substantial phylogenetic information (i.e. most have not been "concealed" by superimposed transversions). As for distance analyses, parsimony estimates were assayed for resolution by bootstrapping. These analyses employed the DNAPARS and DNABOOT programs of PHYLIP 3.4.

RESULTS

The aligned cytochrome-*b* sequences for all taxa employed in this study are given in Appendix B. Table 3 shows pairwise distances among sequences calculated with Kimura's (1980) method. Divergences between cranes and the Limpkin are in the range of 8.93 to 12.28%, those between gruine and balearicine cranes in the range 9.16 to 12.25%. The two species of *Balearica* show a divergence of 3.54%, while among gruine species distances range from 1.36% (*G. monachus* and *G. nigricollis*) to 8.15% (*G. japonensis* and *Bugeranus*). At the subspecies level, distances are 0.58 to 0.97% among Sandhill Crane (*G. canadensis*) sequences and 0.77% between the two Sarus Crane (*G. antigone*) sequences.

As expected, third-position substitutions are the most common among crane cytochrome-*b* sequences (71.9% of polymorphic sites are in third position), followed by first (18.9% of polymorphic sites) and second positions (9.2%). The ratio of transition to transversion differences between sequences ranges from approximately 5.0 (*G. grus* vs. *G. monachus*) to 2.5 (gruines vs. balearicines).

Best-fit and bootstrapped distance analyses of the data in Table 3 yielded the tree shown in Figure 2. Bootstrap values on nodes of the tree

indicate the relative amounts of resolution implied by the sequence-distance data, but should not be interpreted as statistical confidence intervals (Krajewski and Dickerman 1990). Balearicines are separated from gruines at the base of the distance tree as expected from all previous studies. Among gruines, there are three major species groups, two of which appear highly resolved by the data. One of these is the Antigone group of Archibald (1976), in which the Brolga (*G. rubicunda*) and White-naped Crane (*G. vipio*) appear as sister species. The two species of *Anthropoides* are clearly sisters, although the association of these with the Wattled Crane (*Bugeranus carunculatus*) is less well resolved. The third putative clade includes all members of the Grus species group. Within this group some relationships are highly resolved, including Black-necked (*G. nigricollis*) and Hooded (*G. monachus*) cranes as sister species, and the monophyly of these with the Common (*G. grus*) and Whooping (*G. americana*) cranes apart from the Japanese Crane (*G. japonensis*). A cluster containing all Sandhill Crane (*G. canadensis*) subspecies occurs with perfect consistency among bootstrap replicates. The Siberian Crane (*G. leucogeranus*) appears as the sister group to all other gruines. Bootstrap values, however, indicate that relationships among these five major lineages cannot be resolved by the sequence distances.

The crane cytochrome-*b* sequences contain 171 phylogenetically informative sites for parsimony analysis. Most of these (137 sites, 80.1%) are at third positions, although some informative variation is also found at first (28 sites, 16.4%) and second (6 sites, 3.5%) positions. If only transversions are considered, the number of informative sites drops to 26, only 15.2% of the total set of informative sites. Given these values, we chose to include all informative positions in our parsimony analysis rather than using only conservative sites (first and second positions) or changes (transversions).

Bootstrapped parsimony analysis of the crane cytochrome-*b* sequences yields the topology shown in Figure 3. This tree is nearly identical to that obtained by distance analysis (Fig. 2), the only differences being minor rearrangements in the Grus species group and among *G. canadensis* subspecies. Levels of bootstrap resolution on the parsimony tree are also similar to those from distance analysis and indicate that relationships among species groups are not resolved.

TABLE 3. Sequence distances (substitutions per site) × 1,000 among crane taxa computed using Kimura's (1980) two-parameter method.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. *G. vipio*	00.0																			
2. *G. leucogeranus*	53.6	00.0																		
3. *G. americana*	66.0	66.1	00.0																	
4. *G. a. sharpei*	34.3	53.7	55.7	00.0																
5. *G. japonensis*	71.1	80.9	56.7	59.7	00.0															
6. *G. c. pratensis*	52.7	69.5	64.1	55.7	73.3	00.0														
7. *G. rubicunda*	34.4	67.4	74.5	32.3	74.4	63.1	00.0													
8. *B. regulorum*	107.1	102.8	107.7	104.8	122.5	106.3	111.8	00.0												
9. *A. paradisea*	57.8	76.8	78.6	53.6	78.4	57.8	55.7	114.9	00.0											
10. *A. virgo*	59.9	74.7	72.3	55.6	76.4	61.0	68.2	112.7	33.4	00.0										
11. *G. grus*	61.9	60.0	33.4	51.5	53.6	55.8	63.0	110.2	68.0	61.9	00.0									
12. *Bugeranus*	62.8	80.8	69.1	56.6	81.5	63.0	67.2	120.3	64.9	59.8	63.9	00.0								
13. *G. monachus*	59.7	56.8	30.3	53.5	55.6	57.8	61.9	100.1	72.2	64.9	24.4	64.8	00.0							
14. *Aramus*	98.0	91.7	93.7	89.3	103.6	99.4	102.6	109.3	107.0	105.9	92.7	104.7	91.5	00.0						
15. *G. nigricollis*	59.7	64.0	34.3	54.6	57.7	63.0	65.0	104.6	74.2	70.2	30.3	65.9	13.6	94.8	00.0					
16. *G. a. antigone*	34.4	54.9	56.9	7.7	66.1	59.0	37.5	111.8	57.9	58.9	54.8	58.7	56.8	95.0	55.7	00.0				
17. *B. pavonina*	104.7	106.2	103.3	91.6	114.4	103.8	110.3	35.4	111.2	106.9	106.5	118.9	104.3	122.8	105.5	99.6	00.0			
18. *G. c. tabida*	56.5	71.3	66.9	59.7	78.4	5.8	65.0	104.8	59.7	63.9	61.8	63.7	60.6	102.3	63.7	61.8	105.7	00.0		
19. *G. c. canadensis*	56.5	71.3	69.0	59.7	76.3	9.7	65.0	103.7	59.7	61.8	61.8	63.7	62.7	101.2	65.8	61.8	102.4	7.7	00.0	
20. *G. c. rowani*	58.6	72.3	69.0	61.7	80.4	6.8	67.1	104.8	61.8	66.0	63.8	65.8	62.7	104.5	65.8	63.9	105.7	6.7	8.7	00.0

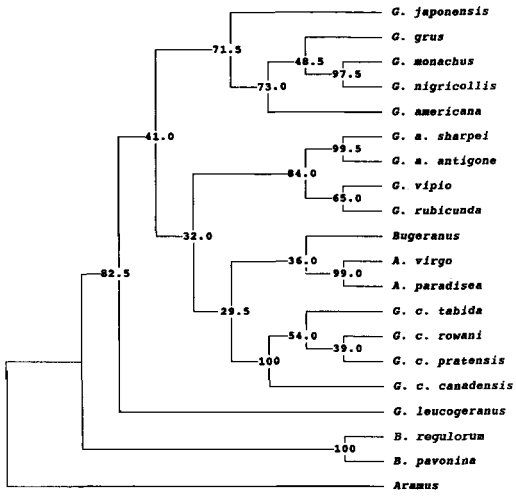

Fig. 3. Bootstrapped parsimony tree for crane cytochrome-*b* DNA sequences based on 200 pseudoreplicates and carried out by DNABOOT program of PHYLIP 3.4 (For labelling conventions, see Fig. 2). The most-parsimonious tree found by DNAPARS differed from bootstrap consensus in placement of *Bugeranus* (which appeared as sister to Grus species group).

DISCUSSION

Levels of divergence.—Observed levels of cytochrome-*b* sequence divergence among cranes correlate well with some aspects of their current taxonomy. All subspecific distances are less than 1%, whereas interspecific distances within subfamilies are from 1 to 9%. Haplotypes of Sarus (*G. antigone*) and Sandhill (*G. canadensis*) crane subspecies form separate monophyletic groups, suggesting that ancestral mtDNA polymorphism does not complicate phylogenetic estimation for these species. Divergence at the genus level within gruines, however, does not show a clear pattern; the range within Grus (1 to 8%) encompasses all distances between *Anthropoides, Bugeranus,* and *Grus* (5 to 8%). This pattern reflects the uncertain taxonomic status of gruine genera as discussed above and by Krajewski (1989).

In the case of crowned cranes (*Balearica*), however, cytochrome-*b* divergences support recognition of *B. pavonina* and *B. regulorum* as distinct species. Although genetic divergence alone provides no basis for species recognition, the relatively large divergence (3.5%) between these two taxa supports the arguments of Walkinshaw (1964) in favor of species status. Ingold et al. (1987a) found a similarly large genetic divergence between crowned cranes using allozyme techniques.

Relationships.—The most striking feature of the trees in Figures 2 and 3 is their congruence with the DNA-DNA hybridization results of Krajewski (1989) shown in Figure 1. Both DNA-DNA hybridization and cytochrome-*b* sequence studies identify the same species groups within Gruinae: the Anthropoides group, the Antigone group, the Grus group, and isolated branches bearing the Siberian (*G. leucogeranus*) and Sandhill (*G. canadensis*) cranes. Bootstrap values on the cytochrome-*b* trees indicate that relationships between these groups are unresolved, a pattern also observed in DNA-DNA hybridization analyses.

DNA-DNA hybridization provided no resolution of branching order within species groups. Cytochrome *b*, however, offers more resolution at this level. In the Grus group, the Hooded (*G. monachus*) and Black-necked (*G. nigricollis*) cranes are sister species. There is virtually no literature on the evolutionary relationships of *G. nigricollis*, beyond the general conclusions of Archibald (cited in Johnsgard, 1983) and Wood (1979) that it falls in the Grus species group. Peters (1934) placed *G. nigricollis* in sequence between *G. grus* and *G. monachus*. Black-necked and Hooded cranes are allopatric, with the former occurring in southwestern China, and the latter in easternmost Siberia and Korea. The low level of genetic divergence (1.36%) between them suggests a relatively recent speciation, probably in the Pleistocene.

Also within the Grus group, *G. grus, G. americana, G. monachus,* and *G. nigricollis* form a monophyletic lineage apart from *G. japonensis*. Love and Deninger (1992) reached the same conclusion on the basis of similarities among these species for a short satellite DNA sequence. The affinities of *G. grus* and *G. americana* within this subgroup are not resolved by cytochrome *b*, though Love and Deninger (1992) argued that these two are sister species. The branching order in Figures 2 and 3 is not consistent with the scenario of Archibald (cited in Krajewski 1989) that *G. grus* represents a widespread, ancestral lineage from which the remaining four species in the group were derived by successive habitat specializations.

Although bootstrap resolution is low, the Brolga (*G. rubicunda*) and White-naped Crane (*G. vipio*) appear to be sister species. This contradicts the conclusion of Archibald (1976) that the

Brolga is most closely related to the Sarus Crane (*G. antigone*). The latter two species are sympatric in northern Australia, may hybridize (although no hybrid specimens exist in museums), and have quite similar unison calls. Arguments from distributional, reproductive, and phenotypic similarity to phylogeny, however, always beg the question of distinguishing primitive from derived resemblance. Given the genetic and other data currently available, it is probably best to suspend judgement on the Brolga's sister group.

Patterns of cladogenesis.—Unison call characteristics (Archibald 1976), DNA-DNA hybridization (Krajewski 1989), and cytochrome-*b* mtDNA sequences (this study) suggest nearly identical phylogenetic relationships of cranes. The similarity among these independent data sets is remarkable not only for those relationships they elucidate, but also for those they do not. In particular, no data set has shown any indication of resolving the branching order of the five major gruine lineages (an exception may be *G. leucogeranus*, but behavior and genetic data show no congruence relative to this enigmatic species). This fact and the branch lengths on the tree of Figure 2 (see also Krajewski 1989:fig. 5) indicate a rapid diversification of gruine lineages. Because the subfamily seems to have originated in the late Miocene (Krajewski 1990) and Pleistocene fossils are known from most extant species (Brodkorb 1967), this radiation most likely occurred during the early Pliocene or late Miocene. Given that a putative fossil humerus of *G. canadensis* has been found in North American Pliocene deposits (Brodkorb 1967), an earlier date seems more likely, perhaps around five million years ago near the Miocene-Pliocene boundary. Lacking additional fossils, however, such attempts at dating are speculative.

Classification.—We suggest no modifications of the crane classification scheme of Krajewski (1989). This reflects the lack of a well-resolved branching order among gruine species groups, as noted above. Indeed, the suggestion by Krajewski (1989) that *Anthropoides* and *Bugeranus* be merged into *Grus* may have been premature in light of the cytochrome-*b* results.

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APPENDIX A. Voucher specimens with institution and identification number. DNA samples derived mostly from captive cranes, with blood or tissue samples provided by institutions listed. Canadian Sandhill Crane (*G. canadensis rowani*) specimen was a wild bird shot by sport hunters in Manitoba. Subspecific identification of this individual based on skull morphometric data in Aldrich (1979). Abbreviations: ICF, International Crane Foundation; PWRC, Patuxent Wildlife Research Center; AZ, Akron Zoo; UWZM, University of Wisconsin Zoological Museum.

Aramus guarauna, UWZM 198; *Balearica pavonina*, ICF 1-09; *B. regulorum*, ICF 2-18; *Anthropoides virgo*, ICF 3-12; *A. paradisea*, ICF 4-07; *Bugeranus carunculatus*, ICF 5-07; *Grus leucogeranus*, ICF 6-06; *G. canadensis canadensis*, AZ; *G. c. rowani*, Manitoba (wild); *G. c. tabida*, ICF 7-79; *G. c. pratensis*, ICF 7-31; *G. antigone antigone*, ICF 8-45; *G. a. sharpei*, ICF 8-28; *G. rubicunda*, ICF 9-08; *G. vipio*, ICF 10-02; *G. grus*, ICF 11-16; *G. monachus*, ICF 12-21; *G. americana*, PWRC 83004; *G. nigricollis*, ICF 14-02; *G. japonensis*, ICF 15-38.

APPENDIX B. Crane cytochrome-*b* DNA sequences. Sequences below show light-strand (L) nucleotides of each taxon used in study. Numbers above each line refer to positions in chicken mtDNA sequence (Desjardins and Morais 1990). Dots indicate match to *G. vipio* reference sequence. Position 14991 is a third-codon position; reading frame can be translated from position 14992. Phylogenetic analyses did not include terminal stop codon (TAA).

	1	1	1	1	1	1	1	1
	5	5	5	5	5	5	5	5
	0	0	0	0	0	0	0	0
	0	1	2	3	4	5	6	7
	0	0	0	0	0	0	0	0
G. vipio	CTTCGGATCTCTCCTAGGCATCTGCCTGGCAACACAAATCCTAACCGGCCTACTACTAGCCGCACACTACACCGCAGACA							
G. leucogeranus	...T.....T.....A.T.....T.....T.....T.....							
G. americana	...T.....T.....T.....A.....T.....T..C.....T.....							
G. a. sharpei	.....A.....G....							
G. japonensis	...T.....G.....T.....A.....T.....T.....							
G. c. pratensis	.....T...T.A.....T.....							
G. rubicunda	.....							
B. regulorum	.....C.....A.C.....T...T.....T.....T.....							
A. paradisea	.....T.....AA.....T.....							
A. virgo	.....T.....A.....T.....							
G. grus	...T.....A.....T.....T.....T.....							
Bugeranus	.....A.....G.....G.....T.....T.....							
G. monachus	...T.....T.....A.....T.....T.....T.....							
Aramus	.....C.....A.T.....T.....T.....T.....							
G. nigricollis	.....T.....A.....T.....T.....T.....							
G. a. antigone	.....A.....G....							
B. pavonina	.....C.....T.....AA.C.....T.....T.....							
G. c. tabida	.....T...T.A.....T.....							
G. c. canadensis	.....T...T.A.....T.....							
G. c. rowani	.....T...T.A.....T.....							

	1	1	1	1	1	1	1	1
	5	5	5	5	5	5	5	5
	0	0	1	1	1	1	1	1
	8	9	0	1	2	3	4	5
	0	0	0	0	0	0	0	0
G. vipio	CAACCCTAGCCTTCTCATCCGTTGCCCACACATGCCGAAACGTACAACACGGCTGACTAATCCGCAACCTACATGCAAAT							
G. leucogeranus	.....T.....GT.T.....C							
G. americana	.....T.....T.....GT.T.....C							
G. a. sharpei	.....C.....T.....C							
G. japonensis	..G..T.G.....C..T..T.....T....T.....C							
G. c. pratensis	.....T..T.....T..T.....C							
G. rubicunda	.....T.....T.....C							
B. regulorum	.....T.....T.....C							
A. paradisea	.....T..T.....T.....C							
A. virgo	.....T..T.....C							
G. grus	.....T.....T.....GT.T.....C							
Bugeranus	.....T..T.....T.....C							
G. monachus	.....T.....T.....GT.T.....C							
Aramus	.....A.....T.....G.....T.....C							
G. nigricollis	.....T.....T.....GT.T.....C							
G. a. antigone	.....C.....T.....C							
B. pavonina	.....T.....T.....C							
G. c. tabida	.....T..T.....T..T.....C							
G. c. canadensis	.....T..T.....T..T.....C							
G. c. rowani	.....T..T.....T..T.....C							

APPENDIX B. Continued.

	1 5 1 6 0	1 5 1 7 0	1 5 1 8 0	1 5 1 9 0	1 5 2 0 0	1 5 2 1 0	1 5 2 2 0	1 5 2 3 0
G. vipio	GGAGCATCATTCTTCTTTATCTGCATCTACCTCCACATTGGACGAGGCCTATACTACGGCTCATATCTGTACAAAGAAAC							
G. leucogeranus	.....C.....C.....G.....							
G. americana	.....C.....C.....A.....							
G. a. sharpei	.....C.....							
G. japonensis	.....C.....T..C.....C.....G..							
G. c. pratensis	.....C.....C.....T.....CT.A.....							
G. rubicunda	.....T.....							
B. regulorum	.....T.....C.....A.G.....C.....T..C.....T.....C..A.....							
A. paradisea	.....C.....C.....C.....TT.....T.....C.....							
A. virgo	.....C.....C.....T..C.....T....T.....C..A.....							
G. grus	.....C.....C.....G.....T.....							
Bugeranus	.....C.....C..T.....C.....							
G. monachus	.....C.....C.....G.....T.....							
Aramus	.....C.....C.....C.....							
G. nigricollis	.....C.....C.....G.....T.....							
G. a. antigone	.....C.....							
B. pavonina	.....T.....C.....A.G...G.C.....T..C.....T.....C..A.....							
G. c. tabida	.....C.....C.....T.....CT.A.....							
G. c. canadensis	.....C.....C.....T.....CT.A.....							
G. c. rowani	.....C.....C.....T.....CT.A.....							

	1 5 2 4 0	1 5 2 5 0	1 5 2 6 0	1 5 2 7 0	1 5 2 8 0	1 5 2 9 0	1 5 3 0 0	1 5 3 1 0
G. vipio	CTGAAATACAGGAGTTATCCTCCTACTTACCCTCATAGCTACCGCCTTCGTAGGCTATGTCCTACCATGAGGACAAATAT							
G. leucogeranus	.....C.....C.....C.....							
G. americana	.....C.....C.....A.....C.....							
G. a. sharpei	.....C.....C.....C.....							
G. japonensis	.....C.....C.....C.....G.....							
G. c. pratensis	.....T.....C.....G.....							
G. rubicunda	.....C.....C.....							
B. regulorum	.....C..T..T.....A.....C.....							
A. paradisea	.....C.....T.....C.....G.							
A. virgo	.....C.....T.....C.....G.....G.							
G. grus	.....C.....C.....A.....C.....G.....G.							
Bugeranus	.....C.....T.....C.....G.....							
G. monachus	.....C.....C.....G.....A.....C.....							
Aramus	.....C.....C.....C.....C.....							
G. nigricollis	.....C.....C.....G.....A.....C.....							
G. a. antigone	.....C.....C.....C.....							
B. pavonina	.....C.....C..T..T.....A.....G.....							
G. c. tabida	.....T.....CA.....							
G. c. canadensis	.....G.....T.....C.....G.....							
G. c. rowani	.....G.....T.....C.....G.....							

APPENDIX B. Continued.

	1 5 3 2 0	1 5 3 3 0	1 5 3 4 0	1 5 3 5 0	1 5 3 6 0	1 5 3 7 0	1 5 3 8 0	1 5 3 9 0
G. vipio	CATTTTGAGGGGCTACAGTCATCACCAATCTCTTCTCAGCCGTCCCCTACATCGGCCAAACCCTTGTAGAATGAGCTTGA							
G. leucogeranus	.							
G. americana	.	T	.	.	A	.	A.C	.
G. a. sharpei	.	.	.	.	.	.	.	.
G. japonensis	.C	T	.	.	A	.	C	.
G. c. pratensis	C	.	T....C	.	A	.	C	....G.....
G. rubicunda	.....A	.	.	.	.	.	.	.
B. regulorum	....C....A..C	.	C..A	....A	....G	.	A.C	....G....C...
A. paradisea	.	.	.	.	A..T	.	.	.
A. virgo	.	T	.	.	A	.	.	.
G. grus	.	T	.	.	A	.	C	.
Bugeranus	.C	.	.	.	A	.	.	G.....
G. monachus	.	T	C	.	A	.	C	.
Aramus	.....A	....T	C	.	A	.	C..G..G	.
G. nigricollis	.	T	C	.	A	.	C	.
G. a. antigone	.	.	.	.	.	.	.	.
B. pavonina	....C....A..C	.	C..A.G	....A	....G	.	A	....G....C...
G. c. tabida	C	....T	C	.	A	.	C	....G.....
G. c. canadensis	C	....T	C	.	A	.	C	....G.....
G. c. rowani	C	....T	C	.	A	.	C	....G.....

	1 5 4 0 0	1 5 4 1 0	1 5 4 2 0	1 5 4 3 0	1 5 4 4 0	1 5 4 5 0	1 5 4 6 0	1 5 4 7 0
G. vipio	GGGGGCTTCTCAGTAGACAATCCCACATTAACTCGATTCTTCACTTTACACTTCCTCCTCCCATTCATAATCATAGGCCT							
G. leucogeranus	.	C	.	.	.	T	.	.
G. americana	.....T	C	C	.	.	.	.	.
G. a. sharpei	.	.	.	.	.	T	.	.
G. japonensis	.	C	.	....T..C	.	.	.	.
G. c. pratensis	.	.	C	.	.	.	T	.
G. rubicunda	.	.	.	.	.	T	.	.
B. regulorum	..A..T	C	C	C	C	C	.	.
A. paradisea	.....T	.	.	.	.	T	....T..G	.
A. virgo	.....T..T	.....T	.	.	....G	....T	....T..G	.
G. grus	.	C	.	C	C	.	.	.
Bugeranus	.	....T	.	C	.	....T	.	T
G. monachus	..A	.	C	.	C	.	.	.
Aramus	..A..G	.	C	C	C	.	.	.
G. nigricollis	..A	.	C	.	C	.	.	.
G. a. antigone	.	.	.	.	.	T	.	.
B. pavonina	.....T	.	C	C	C	.	C	.
G. c. tabida	.	.	.	.	C	.	.	T
G. c. canadensis	.	.	.	.	C	.	.	T
G. c. rowani	.	.	.	.	C	.	.	T

APPENDIX B. Continued.

	1 5 4 8 0	1 5 5 9 0	1 5 5 0 0	1 5 5 1 0	1 5 5 2 0	1 5 5 3 0	1 5 5 4 0	1 5 5 5 0
G. vipio	CACCCTAATCCACCTCACCTTCCTTCACGAATCCGGCTCAAACAACCCCCTAGGCATTGTATCAAACTGCGATAAAATTC							
G. leucogeranus	.....					C.....		C.
G. americana	.....		C.....			C.....		C.
G. a. sharpei	.....					C.....		C.
G. japonensis	.....T.....T.....C.....					C.....	T.....	C.
G. c. pratensis	.....							C.
G. rubicunda	.....T.....		G.....					C.
B. regulorum	.....T.....A.....		T.....		T.....	T..C.....	C.....	C.
A. paradisea	.....		G.....					C.
A. virgo	.....					C.....		C.
G. grus	.....					C.....		C.
Bugeranus	.....T.....		T.....			C.....		C.
G. monachus	.....	C.....				C.....		
Aramus	.....T.A.....		T.....			C.....		C.
G. nigricollis	.....	C.....				C.....		
G. a. antigone	.....					C.....		C.
B. pavonina	.....T.....A.....		T.....		T.....	T..C.....	C.....	C.
G. c. tabida	.....							C.
G. c. canadensis	.....							C.
G. c. rowani	.....				T.....			C.

	1 5 5 6 0	1 5 5 7 0	1 5 5 8 0	1 5 5 8 0	1 5 6 0 0	1 5 6 1 0	1 5 6 2 0	1 5 6 3 0
G. vipio	CATTCCACCCCTATTTTTCCTTAAAAGATATCCTAGGATTCATACTCATACTATTTCCACTCATAACCCTAGCTCTATTC							
G. leucogeranus	.....C.....			G.....		C.....	T....C.....	
G. americana	.....		C.....		C.....	T..C.....		T
G. a. sharpei	.....C.....				C.....	C.....	C.....	
G. japonensis	.....			T.....	T.C.....	T..C.....	C.....	
G. c. pratensis	.....			G.....		C.....		T
G. rubicunda	.....					C.....		
B. regulorum	.....C.....C.....C.C.....				T.CC.C.....A.....		C..C.....	
A. paradisea	.....					C.C.....		
A. virgo	.....					C.C.....		
G. grus	.....			G.....	T..C.....			T
Bugeranus	.....					C.C.....		
G. monachus	.....				T.....T..C.....			T
Aramus	.....		T.....	GC.....	T.CC.C.....A.....		C.....	
G. nigricollis	.....		C.....		T.....T..C.....			T
G. a. antigone	.....C.....			C.....		C.....		
B. pavonina	.....C.....C.....C.....				T.CC.....A.....		C..C.....	
G. c. tabida	.....			G.....		C.....		T
G. c. canadensis	.....			G.....		C.....T.....		
G. c. rowani	.....			G.....		C.....		T

APPENDIX B. Continued.

	1 5 6 4 0	1 5 6 5 0	1 5 6 6 0	1 5 6 7 0	1 5 6 8 0	1 5 6 9 0	1 5 7 0 0	1 5 7 1 0
G. vipio	TCACCAAACCTACTAGGAGACCCAGAAAACTTCACCCCAGCAAACCCCCTAGTCACACCTCCCCATATCAAACCAGAATG							
G. leucogeranus	.....T.....			T.....		A.....C..A.....T.....C...C		
G. americana	.....T.....					G.....T.GG..G.....		
G. a. sharpei	.....T.....					T.....G.....		
G. japonensis	.....T.....			G.....	T.....	T..G..G.....		
G. c. pratensis	.....T.....			G.....				
G. rubicunda	.....G.....		TGG.....	G.....		A.T.....		
B. regulorum	.....T.....				AT....A.....C..A..C.....			
A. paradisea	.....T.....					A.....		
A. virgo	.....T.....		C.....	T.....		T....T.....		
G. grus	.....T.....				T.....	T..G..G.....		
Bugeranus	.....T.G.....	G.....		T.....		TT....T..G.....		
G. monachus	.....T.....					T....T....G.....		
Aramus	.....T.....	T.....T.....			A.....C..A....G.....			
G. nigricollis	.....T.....					T..G..G.....		
G. a. antigone	.....	C.....				T.....G.....		
B. pavonina	.....T.....	G.....		T.T....T..C.....G.....				
G. c. tabida	.....T.....							
G. c. canadensis	.....T.....							
G. c. rowani	.....T.....							A

	1 5 7 2 0	1 5 7 3 0	1 5 7 4 0	1 5 7 5 0	1 5 7 6 0	1 5 7 7 0	1 5 7 8 0	1 5 7 9 0
G. vipio	ATACCTCTTATTTGCATACGCCATCCGACGTTCAATCCCAAACAAACTAGGAGGCGTACTAGCCTTAGCCGCCTCCGTAC							
G. leucogeranus	....T..C.....						C.....T....	
G. americana	....T.T.....		T.....				C.G.....	
G. a. sharpei	....T.T.....		T.....			T.....	T.....	
G. japonensis	....T.....T..T.TG.....					T.....		
G. c. pratensis	....T.T.....C.....TT...C.....					T.....C.....G.		
G. rubicunda	....T..C.....		T.....			T..G.....		
B. regulorum	....T..C....C.....G.T...T...C.....T.....G.....C.....TA...							
A. paradisea	...TT.....G..T.....T.....T.....T.....T.....							
A. virgo	...TT.....		T.....T.....G.....C....T.....					
G. grus	....T.T.....		TC.....			T...T.....C.....		
Bugeranus	....T.T.....G.....G...T.....G.....T.....C....T.....							
G. monachus	....T.TC.....A.G...T.....						C.....	
Aramus	....T.....G.....T....TG.TA.....					A.....C.....		
G. nigricollis	....T.TC.....T....T.....						A.....	
G. a. antigone	....T.T.....		T.T.....			T.....	T.....	
B. pavonina	....T..C....C.....T....T...C.....T.....C.G.....A...							
G. c. tabida	....T.T.....C...T.....TT...C.....					T.....C.....G.		
G. c. canadensis	....T.T.....C.....TT...C.....					T.....C.....G.		
G. c. rowani	....T.T.....C.....TT...C.....					T.....T.C.....G.		

APPENDIX B. Continued.

	1 5 8 0 0	1 5 8 1 0	1 5 8 2 0	1 5 8 3 0	1 5 8 4 0	1 5 8 5 0	1 5 8 6 0	1 5 8 7 0
G. vipio	TAATCCTCTTTCTAGCTCCACTCCTCCATAAATCTAAACAACGTACAATAACCTTCCGCCCATTCTCCCAACTCCTATTC							
G. leucogeranus	.....C..G....T..T.....G.....C.....T.....C.....T.....							
G. americana	.....C.....C.....CC.....							
G. a. sharpei	.....C.....C.....							
G. japonensis	.....C.....C..G.....C.....							
G. c. pratensis	.G.....C.....T..CC.....							
G. rubicunda	.....C.....T.....C.....							
B. regulorum	.....C.....C.....TC.....T.....							
A. paradisea	.....G..C.....C.....CC.....							
A. virgo	.....C.....CC.....							
G. grus	.....C.....CC.....							
Bugeranus	.....C.....C.....C.....							
G. monachus	.....C.....CC.....							
Aramus	.....C.C...ATC..C.....T..C.....C.....T..T..TC.A..T.....							
G. nigricollis	.....C.....C.....CC...T.....							
G. a. antigone	.....C.....C.....							
B. pavonina	..G.....C.....C..G.....CC.....T.....							
G. c. tabida	.G.....C.....T..CC.....							
G. c. canadensis	.....C.....T..CC.....T.....							
G. c. rowani	.G.....C.....T..CC.....							

	1 5 8 8 0	1 5 8 9 0	1 5 9 0 0	1 5 9 1 0	1 5 9 2 0	1 5 9 3 0	1 5 9 4 0	1 5 9 5 0
G. vipio	TGAACCCTAGCCGCCAACCTCCTTATCCTAACATGAGTTGGCAGCCAACCAGTAGAACACCCCTTTATCATTATCGGCCA							
G. leucogeranus	.....T.....A.....C.....C.....							
G. americana	.....G.....T..C.....G.....T.....G.....A..C.....C.....							
G. a. sharpei	.....A.....T.....A..C..A..C.....							
G. japonensis	.....A.....C.....G.....A..G..G..C.....							
G. c. pratensis	.....T...A.T.....T..C.....T.....C.....C.....							
G. rubicunda	.....A.....T.....A.....G..C.....							
B. regulorum	.....A.T.....C..T.....C.....A..C.....C.....							
A. paradisea	.....A.....T.....G.....TA..C..G..C.....							
A. virgo	.....A.....T.....G.....T..A..C..G..C.....							
G. grus	.....A.....T.....T....G.....A..C..G..A...A....							
Bugeranus	.....A.....G.T..C...T.....C.....GT.....C.....C.....							
G. monachus	.....A.....T..C.....G.....A..C.....C.....							
Aramus	.....T.A.....A.....T.....G..T..A..C.....C.....							
G. nigricollis	.....A.....T..C.....G.....C.....C.....							
G. a. antigone	.....A.T.....C.....C...C....							
B. pavonina	.....A.T.....C..T.....G..C.....A..C..A..C.....							
G. c. tabida	.....T...A.T.....T..C.....T.....C.....C.....							
G. c. canadensis	.....T...A.T.....T..C.....T.....C.....C.....							
G. c. rowani	.....T...A.T.....T..C.....T.....C.....C.....							

APPENDIX B. Continued.

	1 5 9 6 0	1 5 9 7 0	1 5 9 8 0	1 5 9 9 0	1 6 0 0 0	1 6 0 1 0	1 6 0 2 0	1 6 0 3 0
G. vipio	ACTAGCTTCCCTTACCTACTTCACTATTCTCCTAATCCTTTTCCCCATCATCGGGGCCCTAGAAAACAAAATACTAAACTACTAA							
G. leucogeranus	.....C.....C.....T.....							
G. americana	.....C.....C.....C.....A.....TC.....							
G. a. sharpei	.....C.....C.....C.....A.....							
G. japonensis	.....C.....C.....C.....A.....T.....							
G. c. pratensis	.....C.....							
G. rubicunda	.....C.....A.....C.....A.....							
B. regulorum	.....C.....T..T..C..C.....T..C.....A.C...A.....							
A. paradisea	.....A.....A..C.....G.....G....C.....							
A. virgo	.....A..C.....G....C.....C.....							
G. grus	.....C.....C.....C.....A.....T.....							
Bugeranus	.....C.....A.....T.....							
G. monachus	.....C.....C.....C.....A.....T.....							
Aramus	.T.G..A.....A.....C.....A..T.....TGC...A.....T.....							
G. nigricollis	.....C.....G.....C.....A.....T.....							
G. a. antigone	.....C.....C.....C.....A.....							
B. pavonina	.....C.....T..T.....C.....T..C.....C...C...A.....							
G. c. tabida	.....T.....C..C.....							
G. c. canadensis	.....T.....C..C.....							
G. c. rowani	.....T.....C..C.....							