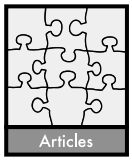

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Phylogenetic relationships of *Amazonetta, Speculanas, Lophonetta,* and *Tachyeres*: four morphologically divergent duck genera endemic to South America

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We studied the phylogenetic relationships of four duck genera endemic to South America: Brazilian teal *Amazonetta brasiliensis*, spectacled duck *Speculanas specularis*, crested duck *Lophonetta specularioides*, and four species of steamer ducks *Tachyeres patachonicus*, *T. leucocephalus*, *T. pteneres*, *T. brachypterus*. Genetic divergence within and among species was compared using population-level sampling of the mitochondrial DNA (mtDNA) control region, supplemented with three additional mtDNA genes and six independent nuclear loci from one individual of each species and a variety of outgroup taxa. The monophyly of these four morphologically divergent South American genera was strongly supported. Within this clade, *Amazonetta* and *Speculanas* were supported as sister species in all analyses, but different gene regions yielded conflicting or ambiguous results for *Lophonetta* and *Tachyeres*. This lack of resolution resulted from little informative variation in nuclear loci and high levels of homoplasy in the mtDNA control region. Control region sequences from the four *Tachyeres* species fell into two distinct clades. In one clade, *T. patachonicus* and *T. leucocephalus* share a set of closely related haplotypes ($\leq$0.6% sequence divergence); while no identical haplotypes were shared between species, the control region phylogeny was insufficiently resolved to either support or reject reciprocal monophyly. The second clade, $\sim$1.7% divergent from the first, comprised haplotypes of the Falkland Islands species *T. brachypterus* and a captive individual of *T. pteneres*. These distinctive South American ducks likely experienced two bouts of rapid diversification, thus making analysis of their phylogenetic relationships difficult. Incomplete lineage sorting, founder effects, and perhaps introgression likely have contributed to obscuring the relationships among steamer ducks.

Multiple independent loci are often required to confidently resolve species-level phylogenies, but different genes may support different relationships among taxa. Several phenomena can lead to incongruent gene trees. The topology of a given gene tree may differ from that of the species tree because of evolutionary rate heterogeneity, convergent base composition bias, stochastic lineage sorting, introgressive hybridization, or simple sampling error in the presence of homoplasy (Funk and Omland 2003, McCracken and Sorenson 2005, Avise 2007). These obstacles to phylogenetic reconstruction are exacerbated when taxa diverge rapidly, potentially leading to an unresolved polytomy among three or more taxa (Hoelzer and Melnick 1994). In some cases, a polytomy can be resolved by analyzing additional DNA sequence data or different types of character data (i.e a soft polytomy). In contrast, a hard polytomy, resistant to additional data and analyses, may reflect the nearly simultaneous divergence of three or more lineages (Hoelzer and Melnick 1994).

Improving phylogenetic resolution typically relies on collecting more DNA sequence data from multiple loci, but phylogeneticists face a difficult empirical problem. On one hand, mitochondrial DNA (mtDNA) seems ideal for species-level phylogenies, given its high mutation rate, low effective population size, and lack of recombination (Avise et al. 1987, Moritz et al. 1987, Moore 1995). Like any other single locus, however, mtDNA provides only one estimate of the species tree (Avise 2006), and the probability that it accurately reflects the species tree may be low if the time between successive speciation events is short (Nei 1987, Pamilo and Nei 1988, Wu 1991). Thus, one would like to infer phylogenies based on data from several independent loci (Pamilo and Nei 1988, Wu 1991, Peters et al. 2005), but the only source of additional loci, the nuclear genome, offers none of the advantages of mtDNA. Nuclear gene trees may be poorly resolved due to lower mutation rates and are substantially less likely than mtDNA to track the species tree through a short internode (Moore 1995). In addition to the stochasticity of mutation and lineage sorting, individual gene trees may be subjected to the misleading effects of introgressive hybridization, making it

essential to consider multiple independent loci (Page and Charleston 1998).

South American duck genera

Several unresolved questions about the phylogenetic relationships of dabbling ducks (tribe Anatini) persist despite several comprehensive morphological and molecular analyses (Livezey 1986a, 1991, Johnson and Sorenson 1998, 1999). Livezey (1991) published the first modern dabbling duck phylogeny, based on cladistic analysis of 120 morphological characters. He included all dabbling ducks and many of the 'perching ducks' in the tribe Anatini, classifying *Anas* and the genera *Mareca* (comprising six species more often included in *Anas*), *Amazonetta*, *Callonetta*, *Lophonetta* and *Speculanas* within the subtribe Anateae. However, molecular analyses based on the mitochondrial ND2 and cytochrome *b* genes (Johnson and Sorenson 1998, 1999) supported the monophyly of a 'dabbling duck' clade comprising all *Anas* species (including *Mareca*) and four additional genera endemic to South America: Brazilian teal (*Amazonetta brasiliensis*), crested duck (*Lophonetta specularioides*), spectacled duck (*Speculanas specularis*), and steamer ducks (*Tachyeres* spp.). Molecular data also provided strong support for the monophyly of these four South American genera, but left basal relationships within this clade and the position of this South American clade in relation to three other *Anas* clades poorly resolved (Johnson and Sorenson 1999). These findings are noteworthy because Livezey (1986a, 1996) grouped steamer ducks, together with torrent ducks (*Merganetta*), and blue duck (*Hymenolaimus*) in a clade near the shelducks and sheldgeese (subfamily Tadorninae). *Amazonetta* was grouped with *Callonetta* outside the Anatini, whereas *Lophonetta* and *Speculanas* were placed as a sister clade to *Anas* dabbling ducks (Livezey 1991). The shared ancestry of *Amazonetta*, *Speculanas*, *Lophonetta*, and *Tachyeres* had thus not been previously recognized before Johnson and Sorenson's studies (1998, 1999), and phylogenetic relationships among these genera have received little study to date.

Brazilian teal is found in tropical wetlands of northeastern South America (Narosky and Yzurieta 2003). Spectacled duck is restricted to freshwater lakes, ponds and rivers of the southern Andes in Patagonia, north to southern Mendoza (Harris 1998). Crested duck is widely distributed in the Andes from Perú south to Tierra del Fuego, throughout coastal and steppe regions of southern Patagonia, and east to the Falkland Islands (Kear 2005). In contrast to spectacled duck, which predominantly occurs throughout the forested regions of the Patagonian Andes, crested ducks inhabit the treeless steppe regions of Patagonia. *Tachyeres* includes four species. The flying steamer duck *Tachyeres patachonicus* inhabits freshwater and marine habitats throughout southern Argentina, Chile and the Falkland Islands. The other three species are flightless. The flightless steamer duck *T. pteneres* is endemic to coastal habitats of Tierra del Fuego and southern Chile. The white-headed steamer duck *T. leucocephalus* is endemic to the coast of Chubut, Argentina. The Falkland steamer duck *T. brachypterus* is endemic to the Falkland Islands (Fig. 1). Livezey (1986b) and Corbin et al. (1988), using morphological and allozyme data, respectively, concluded that *T. patachonicus* is the sister species to a clade comprising the three flightless species, and that *T. leucocephalus* and *T. brachypterus* are each other's closest relatives.

To further test the monophyly of this clade of South American ducks and to resolve relationships among the four genera as well as the species of steamer ducks, we completed phylogenetic analyses at two different hierarchical levels. First, we sequenced the mtDNA control region from multiple individuals of each of the four genera and seven species to compare genetic divergence within and between species, and specifically test whether a lack of reciprocal monophyly contributes to poorly resolved relationships among the four species of steamer ducks. Second, to better resolve relationships among genera, we sequenced three additional mtDNA genes and six independent nuclear loci from one individual of each of the South American species and a variety of outgroup taxa included in previous morphological analyses (Livezey 1986a, 1991, 1996).

Material and methods

Sampling, PCR, and DNA sequencing

We collected Brazilian teal (n = 7), spectacled ducks (n = 2), crested ducks (n = 23), and steamer ducks (*T. patachonicus* n = 7, *T. leucocephalus* n = 3, *T. brachypterus* n = 4) in Argentina and the Falkland Islands between 2001 and 2003 (Appendix 1). The identifications of all coastal specimens of *T. patachonicus* and *T. lecucocephalus* were verified with wing-loading criteria published by Humphrey and Livezey (1982), as shown in Table 1 of Wilson et al. (2007). One *T. pteneres* sample was obtained from a captive bird in the United States, and the other from a bird on Navarino Island, Chile. Five representative dabbling ducks (*Anas crecca crecca*, *A. c. carolinensis*, *A. acuta*, *A. americana*, *A. clypeata*) and 18 other waterfowl genera shown in Appendix 1 were selected as outgroups.

DNA sequences were obtained using standard protocols for DNA extraction, PCR, and sequencing (McCracken and Sorenson 2005). We amplified and sequenced most of the mtDNA control region, tRNA-Phe, and the 5′ end of the 12S rRNA gene (corresponding to bp 82 to 1529 in the chicken *Gallus gallus* mitochondrial genome; Desjardins and Morais 1990) from each of the 53 sampled *Amazonetta*, *Speculanas*, *Lophonetta*, and *Tachyeres* specimens. We also sequenced 12S rRNA (12S), NADH dehydrogenase subunit 2 (ND2), part of ND5, cytochrome *b*, and adjacent t-RNA genes (chicken mtDNA genome positions 1268 to 2293, 5217 to 6312, and 14 771 to 16 063; Desjardins and Morais 1990) from one individual of each species included in the control region data set and each of the outgroup species. Each region was amplified and sequenced using two or more overlapping primer pairs (Appendix 2). Finally, we sequenced six nuclear loci located on five different chromosomal linkage groups in the chicken genome (Hillier et al. 2004): the complete coding region and intron sequences of the αA and βA hemoglobin subunits (HBA2, HBB, respectively),

Figure 1. Geographic ranges of crested duck, Brazilian teal, spectacled duck, and the four species of steamer ducks. The range for flying steamer duck includes the coastal and inland habitats delimited by the bold line.

lecithin-cholesterol acyltransferase introns 2, 3 and 4 (LCAT), T-cell surface glycoprotein CD4 precursor intron 4 (CD4), and phosphoenolpyruvate carboxykinase introns 3 and 9 (PCK1). The HBA and HBB sequences each included three exons and two introns; LCAT included two complete exons and three introns; the other nuclear sequences were primarily introns with limited sequence of the flanking exons. We designed primers for each locus (Appendix 2) based on GenBank sequences for chicken and other vertebrates. Sequences from opposite strands were reconciled and verified for accuracy using Sequencher 4.7 (Gene Codes, Ann Arbor, Michigan). Sequences are archived in GenBank (see Appendix 1).

Phylogenetic analysis of the mtDNA control region

The mtDNA control region sequences varied in length due to insertions and deletions of nucleotides (Baker and Marshall 1997). We aligned control region sequences for the four South American genera and five representative species of *Anas* dabbling ducks using direct optimization as implemented in POY 3.0.11 (Wheeler et al. 2003). The analysis included all 53 control region haplotypes and used 10 random addition replicates (each limited to five trees), equal weights for all changes, tree bisection and reconnection (TBR) branch-swapping, and an insertion-deletion cost equal to one. The resulting implied alignment was used for subsequent analyses.

We used AIC (Akaike 1973) as implemented in modeltest 3.7 (Posada and Crandall 1998) to determine the model of sequence evolution that best fit the mtDNA control region data. With these parameter values fixed, we completed 100 replicate maximum likelihood searches in PAUP* 4.0b10 (Swofford 2002), each with random addition of taxa. We also used equal weights parsimony and 1000 heuristic tree searches to find all equally parsimonious trees for the optimized alignment. Multiple base indels were coded as missing data, and new binary characters for each unique gap (0 = absent, 1 = present)

were added to the end of the data matrix. Single-base indels were included in the parsimony analysis by treating gaps as a fifth character state. We identified mtDNA control region characters supporting alternative branching patterns among the four South American duck genera, and compared these characters in terms of sequence position, substitution types, number of steps, and consistency indices. Finally, trees with alternative basal relationships found in maximum likelihood and parsimony analyses, respectively, were compared under both optimality criteria using a Shimodaira-Hasegawa test (Shimodaira and Hasegawa 1999).

Additional mtDNA data

Additional mtDNA sequence data from three gene regions (12S rRNA, ND2, and ND5/cytochrome *b*) for a single representative of each species was assembled with the control region data. The combined mtDNA data set comprised 4468 characters. We conducted maximum likelihood and Bayesian analyses on the combined mitochondrial data set and individual data partitions using a single, best-fit model of sequence evolution for each partition. Data were partitioned by gene region (CR, 12S, ND2, ND5/cytochrome *b*) or by codon position, combining data for ND2 and ND5/cytochrome *b* (Table 3). We also evaluated two mixed models with separate parameters estimated for each partition. Mixed model 1 partitioned the data into gene regions: CR, 12S, ND2, and ND5/cytochrome *b*, whereas mixed model 2 included five data partitions: CR, 12S, and 1st, 2nd, and 3rd codon positions combined across ND2 and ND5/cytochrome *b*. Best-fit models were assessed using AIC, and alternative topologies were compared using the approximately unbiased test of Shimodaira (2002) (Table 3).

Clade probabilities for the combined mtDNA data were obtained from the posterior distribution using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2005). Bayesian analyses were replicated twice, each with four Markov chains of 2.5 million generations. Trees were sampled every 1000 generations, of which the first 0.5 million generations were discarded as burnin.

Analysis of nuclear DNA

Alignments for each of the six nuclear gene regions were unambiguous. Multiple base indels were coded as missing data, and new binary characters for each unique gap (0 = absent, 1 = present) were added to the end of the data matrix. Double peaks in nuclear sequences, reflecting heterozygous positions, were coded with IUPAC degeneracy codes and treated as polymorphisms. We performed 1000 heuristic tree searches using parsimony with random addition of taxa to find the most parsimonious tree(s) for each nuclear locus.

We also concatenated the six nuclear loci and conducted maximum likelihood analysis using the best fit model of sequence evolution for the entire nuclear data set, as selected by AIC. Statistical support for clades was evaluated by nonparametric bootstrapping (Felsenstein 1985) in PAUP* 4.0b10 (Swofford 2002). We completed full heuristic searches for 1000 or 100 pseudoreplicate data sets for parsimony and maximum likelihood analyses, respectively. Trees were rooted with *Anas americana*, *A. c. crecca*, *A. c. carolinensis*, *A. acuta*, and *A. clypeata* as a paraphyletic outgroup to the South American clade, based on the results of Johnson and Sorenson (1998, 1999) as well as additional analyses presented below.

To more thoroughly test the monophyly of the four South American genera, we conducted a Bayesian phylogenetic analysis of eight concatenated mtDNA and nuclear loci for one individual each of *Amazonetta*, *Speculanas*, *Lophonetta*, *Tachyeres*, five representative *Anas* dabbling duck species, and 18 other waterfowl genera. Three mtDNA genes (ND2, 12S, cytochrome *b*) and five nuclear loci (HBA2, LCAT, CD4, PCK1-3, and PCK1-9) were used for this analysis, and the alignment for each locus was made by eye. One true goose and a swan (subfamily Anserinae) were included as outgroups. Clade probabilities for the combined data set were obtained from the posterior distribution with MrBayes 3.1.2, using the same Markov chain protocols described above and the GTR+I+G model, as determined in Modeltest 3.7. Support for each node was also measured using nonparametric boostrapping (Felsenstein 1985), with equal weights parsimony and full heuristic tree searches for 1000 pseudoreplicate data sets using PAUP* 4.0b10.

Results

Monophyly of the South American duck genera

The monophyly of the four South American duck genera and their relationship to dabbling ducks were both strongly supported in analyses with an expanded set of outgroup taxa. Bayesian posterior probability and maximum parsimony bootstrap support for a clade comprising *Amazonetta*, *Speculanas*, *Lophonetta*, *Tachyeres* was 100%, and equally strong support also was observed for a clade comprising these four South American genera and *Anas* (Fig. 2). There was no evidence that *Amazonetta*, *Speculanas*, *Lophonetta*, or *Tachyeres* are closely related to any other waterfowl genera outside *Anas*. Therefore, the selection of five representative *Anas* species as the outgroup for all subsequent analyses was fully justified.

mtDNA control region

The mtDNA control region alignment comprised 1314 characters, of which 169 (13%) were variable and 157 (12%) were parsimony informative (Table 1). An 11 bp deletion at positions 879–889 was a synapomorphy for *Tachyeres*. Optimization alignment and unweighted parsimony analysis of 53 sequences produced 36 equally parsimonious trees (length = 489, CI = 0.681). Genus-level relationships were (*Tachyeres* (*Lophonetta* (*Amazonetta, Speculanas*))) (Fig. 3). Four trees with equal likelihood (−lnL = 3905.07) were obtained in the ML analysis, all with identical relationships among genera: ((*Tachyeres*, *Lophonetta*) (*Amazonetta*, *Speculanas*)). The best-fit model was TVM+I+G (Fig. 3). While a sister relationship between *Amazonetta* and *Speculanas* was supported in both analyses, parsimony and maximum likelihood produced

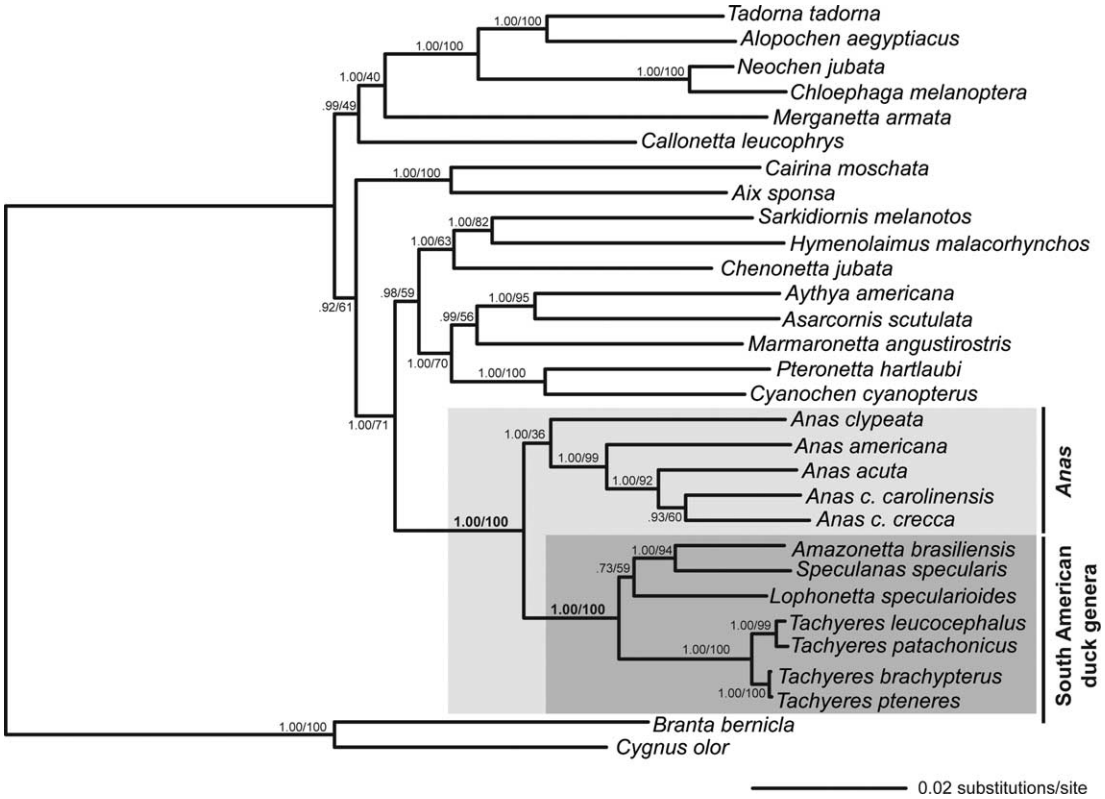

Figure 2. Bayesian 50% majority-rule tree showing the monophyly of *Amazonetta*, *Speculanas*, *Lophonetta*, and *Tachyeres* in relation to *Anas* and 18 other duck genera based on concatenated analysis of three mtDNA gene regions and five nuclear loci. Support for clades is indicated by the posterior probabilities/maximum parsimony bootstrap values. The best-fit model was GTR+I+G with I = 0.59 and $\alpha = 0.52$.

conflicting results for basal relationships in the South American clade. In the ML analysis, *Lophonetta* and *Tachyeres* were sister taxa, albeit with only moderate bootstrap support (80%). In contrast, the parsimony analysis indicated moderate support (90% bootstrap value) for *Lophonetta* as the sister species to *Amazonetta–Speculanas*.

These alternative topologies, however, did not differ significantly under either parsimony or maximum likelihood criteria. Table 2 lists individual control region characters that support each of the two resolutions of basal relationships in the South American clade. Ten characters (8 transitions and 2 indels) with a mean CI of 0.78 have fewer steps on the maximum parsimony topology in which *Lophonetta* is sister to *Amazonetta–Speculanas*, whereas constraining the analysis to find a tree with *Lophonetta* sister to *Tachyeres* (the maximum likelihood topology) results in fewer steps for only 5 characters (4 transitions and 1 transversion), but with a slightly higher overall mean CI (0.87). Likelihood scores associated with these two alternative topologies did not differ significantly using a Shimodaira-Hasegawa test (P = 0.19, Shimodaira and Hasegawa 1999).

Relationships within *Tachyeres*

All of the *Tachyeres* we examined fell into one of two distinct mtDNA clades with sequence divergence between clades of 1.4 to 2.1%. One clade included all *T. leucocephalus* and *T. patachonicus* individuals plus a *T. pteneres* from Navarino Island, Chile. The second clade included all four *T. brachypterus* from the Falkland Islands and a second *T. pteneres* obtained from a private avicultural

Table 1. Number of positions, variable and informative positions, %GC, consistency index, rescaled consistency index, best-fit models, uncorrected percent of sequence divergence, and transition:transversion ratio for four mtDNA gene regions. Values reported are based on the one sequence per species dataset. The sequence divergence values exclude comparisons among *Tachyeres* species. [a]estimates for the HKY85 model.

mtDNA locus	Positions	No. variable/informative positions	%GC	CI	RC	Best fit model (AIC)	Uncorrected % sequence divergence	Ti/tv ratio[a]
Control region	1032	137/79	46.1	0.87	0.72	TVM+I+G	4.9–7.8	6.4
12S rRNA	1051	41/23	49.3	0.89	0.76	TVM+I	1.6–2.5	6.9
ND2	1095	127/54	50.1	0.93	0.80	TrN+I+G	4.7–6.9	15.6
ND5/cytochrome *b*	1290	147/75	50.0	0.86	0.66	TIM+G	5.2–7.5	21.7

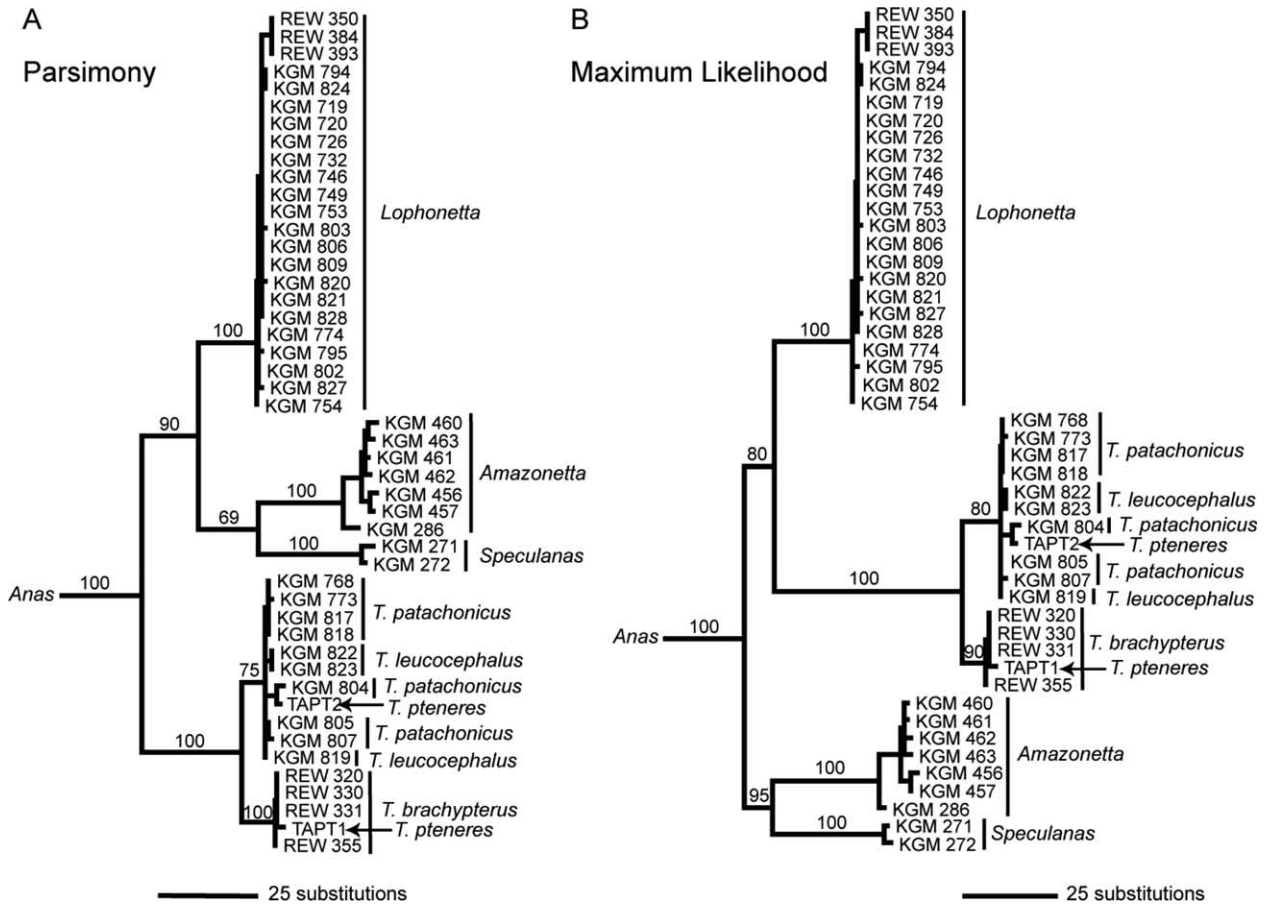

Figure 3. (A) one of 36 equally parsimonious trees derived based on 1314 characters from the mtDNA control region, tRNA-Phe, and 12S rRNA (length = 489, CI = 0.68), (B) one of four most likely trees derived for the same data set ($-\ln L = 3905.07$). In both analyses, multiple trees differed only in intraspecific relationships. The best-fit model was TVM + I + G with I = 0.73 and $\alpha = 1.47$. Bootstrap values above branches indicate support for clades.

collection. Although none of the *Tachyeres* species were strictly monophyletic, it is interesting to note that no identical haplotypes were shared between species (Fig. 3). Unfortunately, we cannot rule out the possibility that one or both of the *T. pteneres* used in our analysis were from misidentified individuals; the complete history of the

Table 2. Character state changes in the control region data supporting two alternative branching patterns for *Tachyeres* (TA), *Lophonetta* (LO), *Amazonetta* (AM), and *Speculanas* (SP).

Position	Substitution	Type	Steps	Consistency index
(TA (LO (AM, SP)))				
85	A → G	TI	4	0.25
113	T → C	TI	2	1.0
175	T → C	TI	4	0.5
176	C → T	TI	2	0.5
678	T → C	TI	1	1.0
762	G → A	TI	1	1.0
812	- → A	insertion	1	1.0
831	- → C	insertion	1	1.0
844	T → C	TI	2	0.5
1306	C → T	TI	1	1.0
Mean ± SD				0.78 ± 0.30
((TA, LO) (AM, SP))				
52	T → C	TI	3	0.33
259	C → A	TV	2	1.0
769	T → C	TI	1	1.0
777	A → G	TI	1	1.0
1252	G → A	TI	1	1.0
Mean ± SD				0.87 ± 0.30

captive individual is not known and no voucher specimen is available for the sample from Navarino Island, Chile, which lies within a region where *T. patachonicus* and *T. pteneres* are broadly sympatric.

Combined analyses of additional mtDNA

Sequence variation in ND2 and ND5/cytyochrome *b* was comparable to the control region in terms of number of informative and variable sites, but 12S rRNA had lower levels of variation (Table 1). Consistency and rescaled consistency indices were similar for the four mtDNA gene regions, although ND2 showed slightly higher values for both indices (Table 1).

The combined mtDNA data set was 4468 characters, of which 460 (10.3%) were variable and 233 (5.2%) were parsimony informative. A single most parsimonious tree was obtained (length = 1343, CI = 0.675). This combined data set yielded the same grouping of the taxa as in the parsimony analysis of control region data only, albeit with higher bootstrap support at most nodes (Fig. 4A, Table 3). *Lophonetta* was supported as the sister species to *Amazonetta–Speculanas* in the parsimony tree with moderate bootstrap support (88%, Fig. 4A). The most likely tree for the combined mtDNA data set (−lnL = 12 040.71) supported a sister relationship between *Lophonetta* and *Tachyeres* with weak bootstrap support (60%) and low posterior probability (0.24, Fig. 4B). Similar to results from the control region, maximum likelihood and parsimony produced different relationships for *Lophonetta* and *Tachyeres* despite including additional mtDNA data in the analysis. Likelihood scores for the two alternative topologies were not significantly different (P = 0.25; Shimodaira-Hasegawa test).

Mixed model analyses with parameters estimated independently for the different mtDNA data partitions provided a better fit as measured by AIC but produced the same phylogenetic inferences as single-model ML and Bayesian analyses. Notably, excluding control region sequences from the analysis resulted in the same grouping of taxa in both maximum likelihood and parsimony analyses (*Tachyeres* (*Lophonetta* (*Amazonetta*, *Speculanas*)); unpubl. data).

Nuclear DNA data

Concatenating the six nuclear loci resulted in 5559 characters, of which 107 (2%) were variable but only 33 (<1%) were parsimony informative. Each locus had from one to twelve informative characters: HBA2, nine; HBB, five; LCAT, twelve; CD4, two; PCK1-3, one; PCK1-9, four.

Parsimony analysis of the six concatenated sequences produced two equally parsimonious trees (length = 640, CI = 0.959). Both of these trees included *Lophonetta* as the sister group to the other three genera in the South American clade (*Lophonetta* (*Tachyeres* (*Amazonetta*, *Speculanas*))), although this resolution of basal relationships was weakly supported (Fig. 4C). The model of sequence evolution for the concatenated nuclear loci was HKY+I. Two equally likely trees (−lnL = 9466.43; Fig. 4D) supported the same relationships (*Tachyeres* (*Lophonetta* (*Amazonetta*, *Speculanas*))) as found in all parsimony analyses of the mtDNA data, and in maximum likelihood analyses of three mtDNA genes (i.e. excluding the control

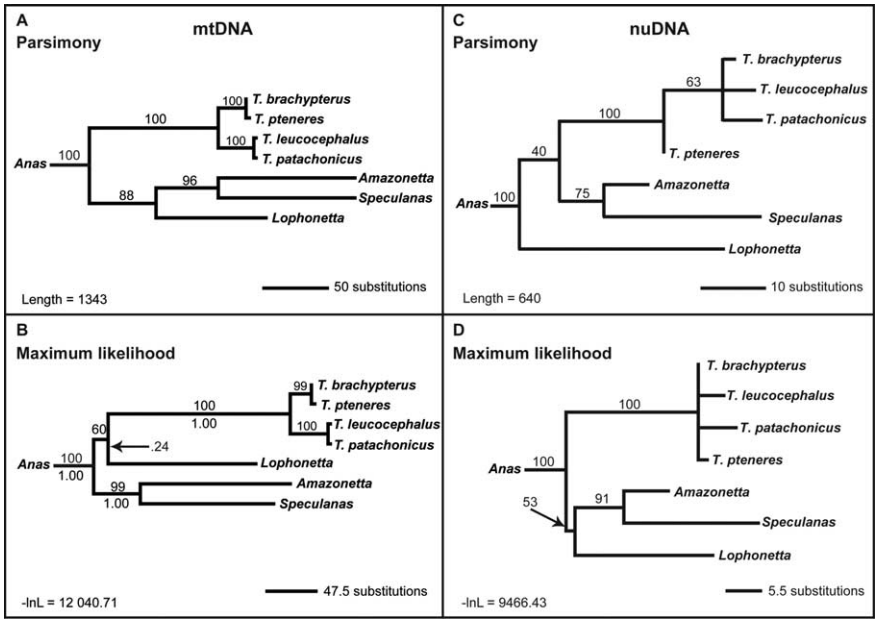

Figure 4. (A) top left: most parsimonious tree derived from 4468 characters of the combined data set from four mtDNA gene regions (tree length = 1343), (B) bottom left: maximum likelihood tree obtained for the same combined mitochondrial data set (−lnL = 12 040.71). The best-fit model for the combined mitochondrial data was GTR+I+G, (C) top right: one of two parsimony trees (length = 640) for the concatenated six nuclear loci (the two trees differed in relationships among the four *Tachyeres* species), (D) bottom right: most likely tree (−lnL = 9466.43) for the nuclear data. The best-fit model for the concatenated nuclear loci was HKY+I with I = 0.89. Support values above and below branches correspond to nonparametric bootstrap, and Bayesian posterior probabilities, respectively.

Table 3. Log-likelihood for best-fit models selected using AIC and partitioned by codon position and locus for three possible resolutions of *Tachyeres* (TA), *Lophonetta* (LO), *Amazonetta* (AM), and *Speculanas* (SP). *Minimum P-values for the approximately unbiased test of Shimodaira (2002).

	Best-fit model	(TA (LO (AM, SP)))	((TA, LO) (AM, SP))	(LO (TA (AM, SP)))	Parameters	AIC	P-values*
1st position	HKY+I	1541.02	1540.04	1541.02	5	3086.9	0.20
2nd position	HKY+I	1118.05	1118.05	1118.05	5	2234.14	0.98
3rd position	GTR+G	3264.41	3266.03	3266.03	9	6546.37	0.19
Control region (CR)	TVM+I+G	2418.41	2415.72	2418.41	9	6097.54	0.15
12S/tRNAs	TVM+I	2141.80	2141.96	2142.27	8	4297.68	0.43
ND2	TrN+I+G	3142.97	3143.09	3143.09	7	6295.84	0.33
ND5/cytb	TIM+G	3552.04	3552.30	3552.30	6	7113.87	0.27
Total mtDNA combined	GTR+I+G	10092.88	10092.89	10096.33	10	24104.44	0.34
Mixed 1 (CR, 12S, ND2, ND5/cyt *b*)		11255.22	11253.07	11256.07	30	23804.93	0.29
Mixed 2 (1, 2, 3, CR, 12S)		10483.69	10481.8	10485.78	36	22262.63	0.17

region), albeit with weak bootstrap support (53%). There was no difference in likelihood scores between the two possible topologies as determined by the Shimodaira-Hasegawa test ($P = 1.00$).

Discussion

Rapid cladogenesis generates phylogenies with short internal branches, thus limiting the accumulation of informative genetic variation needed to resolve phylogenetic relationships (Hoelzer and Melnick 1994, Rokas and Carroll 2006). Even with somewhat longer internodes, relationships may be obscured by the stochastic effects of homoplasy and lineage sorting. Finally, a broadly distributed ancestral species may give rise to two or more new species through founder events or peripatric speciation, without going extinct, thus yielding non-dichotomous phylogenetic patterns. All of these processes may have contributed to the difficulty of resolving relationships in our study.

Johnson and Sorenson (1999) found that *Amazonetta*, *Speculanas*, *Lophonetta* and *Tachyeres* formed a strongly supported clade, and our analysis of additional loci and outgroup taxa strongly corroborates this finding. However, relationships within this well-supported clade were not well resolved. In our study, both mtDNA and nuclear DNA supported *Amazonetta* and *Speculanas* as sister-species, but the relationships of *Lophonetta* and *Tachyeres* were less certain. Three different resolutions of a basal trichotomy were found depending on the data set (mtDNA versus nuclear) and method of analysis (parsimony versus maximum likelihood). However, both parsimony analysis of mtDNA and maximum likelihood analysis of nuclear sequences placed *Lophonetta* as the sister group of *Amazonetta–Speculanas*. In contrast, maximum likelihood analysis of the mtDNA data placed *Lophonetta* sister to the morphologically divergent *Tachyeres*, although this result was obtained only when control region data were included. Sequence data from the six nuclear loci contributed relatively little additional phylogenetic information, such that combined analysis of both mtDNA and nuclear data yielded results similar to that for mtDNA alone. For most nuclear loci, gene trees were poorly resolved or unresolved due to a general lack of informative variation, owing to their slower evolutionary rates. This contrast in evolutionary rates between mtDNA and nuclear loci is a general challenge for phylogeneticists seeking to test species level relationships with multiple loci and suggests the need for larger data sets and new methods of analysis (Maddison and Knowles 2006, Edwards et al. 2007).

Uncertain basal relationships among the four South American genera primarily reflect the difficulty of resolving a short internal node in the face of stochastic effects (e.g. homoplasy in mtDNA, limited informative variation in nuclear loci), rather than any significant conflict in our data set. Different topologies found in parsimony and maximum likelihood analyses of the control region data, for example, reflect the relative influence of a small number of characters. Two transversions uniting *Lophonetta* and *Tachyeres* at positions 259 (Table 2) and 269 (where *Lophonetta* has T, *Tachyeres* has C, and other taxa have A) strongly influence the likelihood analysis, whereas two indels that help unite *Lophonetta* with *Amazonetta* and *Speculanas* in the parsimony analysis (Table 2) are effectively ignored because gaps are treated as missing data in the likelihood analysis. All four of these characters lie within highly variable regions with numerous insertions and deletions across dabbling ducks. Peters et al. (2005) encountered a similar discrepancy when analyzing control region data in wigeons and their allies (*Anas* spp.); the most likely topology for the control region disagreed with the placement of *Anas penelope* in other analyses. Taken together, our analyses suggest somewhat greater support for a sister relationship between *Lophonetta* and *Amazonetta–Speculanas*, but this conclusion requires additional testing.

Based on morphology, Livezey (1991) placed *Amazonetta* as the sister genus to *Callonetta*, and basal in the subtribe Anateae. In the same study, *Lophonetta* and *Speculanas* were inferred as sister species, comprising a clade sister to the 'true' dabbling ducks (Livezey 1991). While *Speculanas* and *Lophonetta* are members of the same South American clade, our analysis indicates that these partially sympatric species are not sister taxa. The speculum is similar in both species, being bronze-colored with a posterior black and terminal white border, and lacking any anterior border differentiation (Johnsgard 1965). Johnsgard (1978) also found strong similarities in the displays of *Lophonetta* and *Speculanas*, as well as similarities in their tracheal structure. Both mitochondrial and nuclear sequence data, however, indicate that *Speculanas* is more closely related to *Amazonetta*, which is substantially smaller in size. This and other morphological differences might have arisen as these species diverged in distinct habitats in distant parts of South America. The larger bodied *Speculanas* inhabits the southern Andes, whereas the

smaller *Amazonetta* inhabits tropical regions, including the Amazon basin (Fig. 1).

The placement of *Tachyeres* with these three genera by Johnson and Sorenson (1999) was surprising given the substantial morphological differences among them; morphologically, steamer ducks had been grouped with the shelducks (Livezey 1986a). Based on genetic data, however, it appears that morphology in steamer ducks is highly derived and divergent from other dabbling ducks. These large-bodied ducks likely evolved sympatrically in Patagonia with *Lophonetta* and *Speculanas*, although present day distribution patterns may not reflect their ancestral distribution.

Within *Tachyeres*, mtDNA suggests that *T. leucocephalus* and *T. patachonicus* are more closely related to each other than to *T. brachypterus*. These results conflict with phylogenies based on morphology (Livezey 1986b) and electrophoretic data (Corbin et al. 1988), which placed *T. patachonicus* as the sister group of all three flightless species, suggesting a single loss of flight. Our mtDNA data suggest that either 1) *Tachyeres* lost the capability of flight twice, or 2) flight was lost in ancestral *Tachyeres* and subsequently regained by *T. patachonicus*. MtDNA is just one locus, however, and nuclear loci provided little resolution of these relationships. Furthermore, the ability to fly in steamer ducks is dependent on a number of anatomical, behavioral and environmental conditions (Livezey and Humphrey 1992). For example, Humphrey and Livezey (1982) found that up to 25% of male *T. patachonicus* from marine localities are permanently flightless. Flightlessness in steamer ducks probably evolved because of the year-around habitability of the southern South American marine littoral, making migration unnecessary (Livezey and Humphrey 1986).

The mtDNA data suggest a recent divergence of *T. leucocephalus* and *T. patachonicus*. After diverging from the two southernmost species (*T. pteneres*, *T. brachypterus*), the ancestral *T. patachonicus* population may have given rise to *T. leucocephalus* through a founder event at the periphery of its coastal range north of the Gulf of San Jorge in Chubut. The possibility that flightlessness evolved three times independently from a flying ancestor also should be considered. Large-bodied *T. patachonicus* can be found year-around in marine habitats (Wilson et al. 2007), and some larger individuals are effectively flightless (Humphrey and Livezey 1982). Thus, a single, broadly distributed and flighted ancestor may have given rise to flightless descendents in three disjunct geographic areas (Fig. 1; but see Livezey 1986b). While perhaps less parsimonious in terms of morphological evolution, this hypothesis provides a simple explanation for current distributions.

In addition to incomplete lineage sorting, interspecific hybridization also might contribute to the lack of mtDNA monophyly among *Tachyeres* species (Peters et al. 2007). Waterfowl are well known for their capacity to hybridize and produce fertile offspring (Johnsgard 1960, Tubaro and Lijtmaer 2002). Indeed, it can be difficult to definitively identify steamer ducks using field marks or external characters in regions where they co-occur. Furthermore, there is evidence that maritime populations of *T. patachonicus* show 'hybrid' characteristics of high genetic heterozygosity (Corbin 1983) and increased morphometric variability (Livezey 1986c). This highlights the importance of sampling multiple individuals for species-level phylogenetics (Peters et al. 2005). Further studies with population-level sampling of each of the four *Tachyeres* species are needed.

In conclusion, relationships within this distinctive clade of South American ducks were not well resolved despite sequencing more than 10 000 characters from six independent linkage groups. This lack of resolution likely resulted from high levels of homoplasy and a lack of informative characters (i.e. soft polytomies), rapid divergence times among genera and species (i.e. hard polytomies), or a combination of these factors. In the case of soft polytomies, it may be possible to resolve relationships by sequencing additional loci and applying phylogenetic methods that incorporate random lineage sorting and mutation (Edwards et al. 2007). In either case, it is clear that this group underwent at least two periods of rapid diversification, one producing the four genera and a more recent radiation among species of *Tachyeres*. This clade provides a striking example of closely related taxa that have radiated into morphologically and behaviorally divergent forms.

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Appendix 1. Locality and specimen information for South American ducks included in this study.

Museum catalog no.	Field catalog no.	Species	Date	Country	Province	Locality	Longitude	Latitude	Elevation (m)
UAM 17533	KGM 271	*Speculanas specularis*	19 Apr 01	Argentina	Neuquén	Río Chimehuin	−71.07170	−39.91610	1267
UAM 17532	KGM 272	*Speculanas specularis*	19 Apr 01	Argentina	Neuquén	Río Chimehuin	−71.07170	−39.91610	1267
UAM 14628	KGM 456	*Amazonetta brasiliensis*	15 Oct 01	Argentina	Salta	NE La Caldera	−65.37080	−24.55030	1468
UAM 14644	KGM 457	*Amazonetta brasiliensis*	15 Oct 01	Argentina	Salta	NE La Caldera	−65.37080	−24.55030	1468
UAM 14862	KGM 460	*Amazonetta brasiliensis*	15 Oct 01	Argentina	Corrientes	S Pedro Fernández	−58.99810	−28.72360	64
–	KGM 461	*Amazonetta brasiliensis*	18 Oct 01	Argentina	Corrientes	N Santa Lucia	−59.02690	−28.77250	45
UAM 14627	KGM 462	*Amazonetta brasiliensis*	18 Oct 01	Argentina	Corrientes	N Santa Lucia	−59.04780	−28.81170	45
UAM 14851	KGM 463	*Amazonetta brasiliensis*	18 Oct 01	Argentina	Corrientes	N Santa Lucia	−59.04780	−28.81170	45
UAM 17972	REW 320	*Tachyeres brachypterus*	15 Nov 02	Falkland Islands	East Falkland	Stanley Harbour	−57.86770	−51.69130	2
–	REW 330	*Tachyeres brachypterus*	23 Nov 02	Falkland Islands	East Falkland	Stanley Harbour	−57.86770	−51.69130	2
–	REW 331	*Tachyeres brachypterus*	23 Nov 02	Falkland Islands	East Falkland	Stanley Harbour	−57.86770	−51.69130	2
–	REW 355	*Tachyeres brachypterus*	16 Dec 02	Falkland Islands	East Falkland	Bertha's Beach, Fitzroy Farm	−58.38380	−51.89090	3
UAM 22621	KGM 768	*Tachyeres patachonicus*	29 Oct 03	Argentina	Santa Cruz	Estancia La Angostura	−70.69680	−48.62020	408
UAM 22625	KGM 773	*Tachyeres patachonicus*	30 Oct 03	Argentina	Santa Cruz	Laguna del Pescado	−72.92810	−49.12530	466
UAM 20715	KGM 804	*Tachyeres patachonicus*	7 Nov 03	Argentina	Santa Cruz	Puerto Santa Cruz	−68.50030	−50.06430	0
UAM 22624	KGM 805	*Tachyeres patachonicus*	7 Nov 03	Argentina	Santa Cruz	Puerto Santa Cruz	−68.50030	−50.06430	0
UAM 20714	KGM 807	*Tachyeres patachonicus*	9 Nov 03	Argentina	Santa Cruz	Puerto Deseado	−65.88510	−47.75500	0
UAM 20799	KGM 817	*Tachyeres patachonicus*	11 Nov 03	Argentina	Chubut	N Caleta Córdova	−67.36170	−45.72610	0
UAM 22623	KGM 818	*Tachyeres patachonicus*	11 Nov 03	Argentina	Chubut	N Caleta Córdova	−67.36170	−45.72610	0
UAM 22622	KGM 819	*Tachyeres leucocephalus*	11 Nov 03	Argentina	Chubut	Bahía Bustamante	−66.53500	−45.13480	0
UAM 20801	KGM 822	*Tachyeres leucocephalus*	12 Nov 03	Argentina	Chubut	N Camarones	−65.69530	−44.75720	0
UAM 20800	KGM 823	*Tachyeres leucocephalus*	12 Nov 03	Argentina	Chubut	N Camarones	−65.69530	−44.75720	0
MDS	TAPT2	*Tachyeres pteneres*	1995	Chile	–	Navarino Island	–	–	–
MDS	TAPT1	*Tachyeres pteneres*	–	Captive	–	Sylvan Heights Waterfowl, NC	–	–	–
UAM 19628	KGM 719	*Lophonetta specularioides*	20 Oct 03	Argentina	Chubut	RP 17, W Tecka	−71.06760	−43.60620	804
UAM 19632	KGM 720	*Lophonetta specularioides*	20 Oct 03	Argentina	Chubut	RN 40, S Tecka	−70.87550	−43.71010	934
UAM 19626	KGM 726	*Lophonetta specularioides*	22 Oct 03	Argentina	Chubut	RN 40, W Shaman	−70.67430	−44.38960	655
UAM 19629	KGM 732	*Lophonetta specularioides*	23 Oct 03	Argentina	Chubut	RN 40, N Río Mayo	−70.43980	−45.42210	578
UAM 22747	KGM 746	*Lophonetta specularioides*	26 Oct 03	Argentina	Santa Cruz	RP 41, Estancia La Frontera	−71.86200	−46.84210	783
UAM 19636	KGM 749	*Lophonetta specularioides*	26 Oct 03	Argentina	Santa Cruz	RN 40, ca Estancia Telken & La Paloma	−70.74550	−46.87610	618
UAM 20781	KGM 753	*Lophonetta specularioides*	28 Oct 03	Argentina	Santa Cruz	RN 40, N Las Horquetas	−70.97490	−48.30230	540
UAM 19627	KGM 754	*Lophonetta specularioides*	28 Oct 03	Argentina	Santa Cruz	RN 40, N Las Horquetas	−70.97490	−48.30230	540
UAM 19630	KGM 774	*Lophonetta specularioides*	31 Oct 03	Argentina	Santa Cruz	Estancia Santa Margarita	−72.41400	−49.55810	246
UAM 19625	KGM 794	*Lophonetta specularioides*	3 Nov 03	Argentina	Santa Cruz	RN 40, ca El Zurdo	−71.22580	−51.99600	122
UAM 19640	KGM 795	*Lophonetta specularioides*	5 Nov 03	Argentina	Santa Cruz	RN 40, ca Estancia Monte Dinero	−68.66560	−52.26760	72
UAM 19631	KGM 802	*Lophonetta specularioides*	6 Nov 03	Argentina	Santa Cruz	RN 3, ca Paraje Lemarchand	−69.48180	−50.75020	281
UAM 19633	KGM 803	*Lophonetta specularioides*	6 Nov 03	Argentina	Santa Cruz	RP 288, ca Puerto Punta Quilla	−68.48800	−50.08890	3
UAM 19635	KGM 806	*Lophonetta specularioides*	8 Nov 03	Argentina	Santa Cruz	Bahía Río Deseado	−65.97270	−47.74210	0
UAM 19747	KGM 809	*Lophonetta specularioides*	10 Nov 03	Argentina	Chubut	S Lago Colhué Huapí	−68.94000	−45.65240	267
UAM 19637	KGM 820	*Lophonetta specularioides*	11 Nov 03	Argentina	Chubut	Bahía Bustamante	−66.53500	−45.13480	0
UAM 19634	KGM 821	*Lophonetta specularioides*	11 Nov 03	Argentina	Chubut	Bahía Bustamante	−66.52120	−45.14930	0
UAM 19639	KGM 824	*Lophonetta specularioides*	12 Nov 03	Argentina	Chubut	S Camarones	−65.71630	−44.80330	0
UAM 19624	KGM 827	*Lophonetta specularioides*	13 Nov 03	Argentina	Chubut	Cabo Raso	−65.23010	−44.33410	0
UAM 19638	KGM 828	*Lophonetta specularioides*	13 Nov 03	Argentina	Chubut	Playa Bonita, S Rawson	−65.04820	−43.36090	0
–	REW 350	*Lophonetta specularioides*	9 Dec 02	Falkland Islands	East Falkland	Bertha's Beach, Fitzroy Farm	−58.38380	−51.89090	3
–	REW 384	*Lophonetta specularioides*	28 Dec 02	Falkland Islands	East Falkland	Bertha's Beach, Fitzroy Farm	−58.38380	−51.89090	3
–	REW 393	*Lophonetta specularioides*	31 Dec 02	Falkland Islands	East Falkland	Fitzroy, Fox Point	−58.38380	−51.89090	3

Appendix 1 (*Continued*)

Museum catalog no.	Field catalog no.	Species	Date	Country	Province	Locality	Longitude	Latitude	Elevation (m)
MDS	–	*Anas crecca crecca*	–	Captive	–	Sylvan Heights Waterfowl, NC	–	–	–
MDS	–	*Anas crecca carolinensis*	1994	USA	California	Solano County	–	–	–
MDS	–	*Anas americana*	1994	USA	Texas	Brazoria County	–	–	–
MDS	–	*Anas acuta*	–	Captive	–	National Zoological Park, DC	–	–	–
UWBM 43948	–	*Anas acuta*	11 Jul 92	Russia	Chukotskiy Avtonomnyy Okrug	–	–	–	–
MDS	–	*Anas clypeata*	1994	USA	Texas	Henderson County	–	–	–
UWBM 71262	–	*Anas clypeata*	26 May 94	Russia	Magadanskaya Oblast'	–	–	–	–
MDS	–	*Sarkidiornis melanotos*	–	Captive	–	Sylvan Heights Waterfowl, NC	–	–	–
MDS	–	*Cairina moschata*	–	Captive	–	Sylvan Heights Waterfowl, NC	–	–	–
MDS	–	*Aix sponsa*	–	Captive	–	Sylvan Heights Waterfowl, NC	–	–	–
MDS	–	*Tadorna tadorna*	–	Captive	–	Sylvan Heights Waterfowl, NC	–	–	–
MDS		*Chenonetta jubata*	–	Captive	–	Sylvan Heights Waterfowl, NC	–	–	–
MDS	–	*Callonetta leucophrys*	–	Captive	–	Cedar Creek Natural History Area, MN	–	–	–
MDS LMTX R654	–	*Aythya americana*	–	United States	Texas	Laguna Madre	–	–	–
MDS	–	*Asarcornis scutulata*	–	Captive	–	Sylvan Heights Waterfowl, NC	–	–	–
MDS	–	*Pteronetta hartlaubi*	–	Captive	–	Sylvan Heights Waterfowl, NC	–	–	–
MDS	–	*Cyanochen cyanopterus*	–	Captive	–	Sylvan Heights Waterfowl, NC	–	–	–
MDS	–	*Marmaronetta angustirostris*	–	Captive	–	Sylvan Heights Waterfowl, NC	–	–	–
MDS	–	*Alopochen aegyptiacus*	–	Captive	–	Sylvan Heights Waterfowl, NC	–	–	–
MDS	–	*Neochen jubata*	–	Captive	–	Sylvan Heights Waterfowl, NC	–	–	–
MDS	–	*Chloephaga melanoptera*	–	Captive	–	Sylvan Heights Waterfowl, NC	–	–	–
UWBM 54417	–	*Merganetta armata*	5 Oct 1995	Argentina	Tucumán	Río Los Sosa, Route 307, km 20	–	–	650
MDS	–	*Hymenolaimus malacorhynchos*	–	New Zealand	–	Manganuiateao River	–	–	–
JFBM 38648	–	*Branta bernicla*	1990	United States	Alaska	Cape Pierce	–	–	–
MDS	–	*Cygnus olor*	–	Captive	–	Sylvan Heights Waterfowl, NC	–	–	–

Genbank accession numbers:
mtDNA control region HM063476–HM063528; 12S ribosomal RNA HM063529–HM063558; ND2 AF059114, AF059115, AF059116, AF059123, AF059124, AF059150, AF059157, AF059158, AF059159, AF059160, AF059161, AF059162, AF059163, AF059164, AF059170, AF059171, AF059172, AF059173, AF059174, AF090337, HM063559–HM063568; ND5/cytochrome b AF059053, AF059055, AF059062, AF059063, AF059064, AF059097, AF059098, AF059099, AF059100, AF059101, AF059103, AF059104, AF059110, AF059111, AF059112, AF059113, AF090337, HM063569–HM063573, HM063574–HM063581; LCAT HM063582–HM063611; CD4 HM063612–HM063640; PCK1-3 HM063641–HM063670; PCK1-9 HM063671–HM063699; HBA2 GQ271019, GQ271050, GQ271051, GQ271063, GQ271064, GQ271492, GQ271510, GQ271536, GQ271537, GQ271538, GQ271540, GQ271544, GQ271545, GQ271548, GQ271550, GQ271551, GQ271552, GQ271558, GQ271608, GQ271609, GQ271612, GQ271613, GQ271616, GQ271619, GQ271624, GQ271711, GQ271714, GQ271740, GQ271741; HBB GQ271807, GQ272272, GQ272273, GQ272276, GQ272277, GQ272280, GQ272283, GQ272310, GQ272312, GQ272318, GQ272319.

Appendix 2. Primers used to amplify and sequence mtDNA and nuclear DNA from the South American clade of ducks.

Locus	Primer	5′ to 3′ primer sequence	Reference
Control region	Duck.L81	TATTTGGYTATGYAYRTCGTGCAT	Muñoz-Fuentes et al. 2008
	H774	CCATATACGCCAACCGTCTC	Sorenson et al. 1999b
	L736	ATCTAAGCCTGGACACACCTG	Sorenson et al. 1999a
	H1530	GTGGCTGGCACARGATTTACC	
12S rRNA	L1263	YAAAGCATGRCACTGAA	Revised from Sorenson et al. 1999b
	H1859	TCGDTTRYAGRACAGGCTCCTCTA	Revised from Sorenson et al. 1999b
	L1754	TGGGATTAGATACCCCACTATG	Revised from Sorenson et al. 1999b
	H2294	TYTCAGGYGTARGCTGARTGCTT	Revised from Sorenson et al. 1999b
ND5/Cytochrome *b*	L14770	TAGGNCCNGARGGNYTNGC	
	H15295	CCTCAGAAKGATATYTGNCCTCAKGG	Sorenson et al. 1999b
	L14996	AAYATYTCWGYHTGATGAAAYTTYGG	McCracken & Sorenson 2005
	H15646	GGNGTRAAGTTTTCTGGGTCNCC	McCracken & Sorenson 2005
	L15413	GGGGGWTTYTCMGTNGAYAAYCC	McCracken & Sorenson 2005
	H16064	CTTCANTYTTTGGYTTACAAGRCC	Sorenson et al. 1999b
ND2	L5216	GGCCCATACCCCGRAAATG	McCracken & Sorenson 2005
	H5766	RGAKGAGAARGCYAGGATYTTKCG	McCracken & Sorenson 2005
	L5758	GGCTGAATRGGMCTNAAYCARAC	Sorenson et al. 1999b
	H6313	CTCTTATTTAAGGCTTTGAAGGC	Sorenson et al. 1999b
T-cell surface glycoprotein CD precursor (CD4)	CD4.4F	CTCCATCGATTAATNAGAACATCTCC	This study
	CD4.5R	TTCCKGAAGTTCAGAYGCCATGAC	This study
Lecithin cholesterol acyltransferase (LCAT) introns 2, 3, 4	LCAT2F	GTGGTGAACTGGATGTGCTACCG	This study
	LCAT3R	ACCTGCCAGTTTGCTCTGGTCCAG	This study
	LCAT3F	GTACCTGGCTTYGGCAAGACC	This study
	LCAT5Rb	CCCGATGTACTGATCTTTCCAGG	This study
Phosphoenolpyruvate carboxykinase (PCK1-3)	PCK1-3.F	GGTCGCTGGATGTCAGAAGAGG	McCracken & Sorenson 2005
	PCK1-3.RI	GYAGTAAAGGTGGGYGGAGG	
	PCK1-3.FI	GCAGCAGATAGCAARTGAGGTG	
	PCK1-3R	CCATGCTGAAGGGGATGACATAC	McCracken & Sorenson 2005
Phosphoenolpyruvate carboxykinase (PCK1-9)	PCK1-9.F	GGAGCAGCCATGAGATCTGAAGC	Sorenson et al. 2003
	PCK1-9.RI	CTTGAGAGCTGGCTTTCATTG	
	PCK1-9.FI	CTTACATTTTCTGTTCTGCTAGAGC	
	PCK1-9.R	GTGCCATGCTAAGCCAGTGGG	Sorenson et al. 2003
αA hemoglobin subunit (HBA2)	HBA2.14a.F	GGGCACCCGTGCTGGGGGCTGCCAAC	McCracken et al. 2009
	HBA2.373a.R	GCAGCCGCCACCTTCTTGCC	McCracken et al. 2009
	HBA2.342.F	GACCTACTTCCCCCACTTTGACC	McCracken et al. 2009
	HBA2.756.R	CTGGCAACAGGGTGGGTCCAGCTCTAGCC	McCracken et al. 2009
βA hemoglobin subunit (HBB)	HBB.1.F	GCCACACGCTACCCTCCACCCGACACC	McCracken et al. 2009
	HBB.646a.R	CCTGCCTSTCCTCSTGGTTCTKCC	McCracken et al. 2009
	HBB.482.F	GCTGCACGTGGACCCCGAGAACTTCAGG	McCracken et al. 2009
	HBB.1251a.R	TTTTTCTCCCTTCTGHCTTCATTTGG	McCracken et al. 2009
	HBB.1173.F	GCCAGTRGGAGCTTGCCCTTGGTGCC	McCracken et al. 2009
	HBB.1761.R	GGATGTTCTGGGAGCGTTGCTGCC	McCracken et al. 2009