MITOCHONDRIAL DNA VARIATION AMONG PHALAROPES AND ALLIES

DONNA L. DITTMANN AND ROBERT M. ZINK
Museum of Natural Science, Louisiana State University, Baton Rouge, Louisiana 70803 USA

ABSTRACT.—We used patterns of variation among mitochondrial DNA (mtDNA) restriction fragments to infer phylogenetic relationships among Red (*Phalaropus fulicaria*), Red-necked (*P. lobatus*), and Wilson's (*P. tricolor*) phalaropes, and seven other shorebird species. Digestion of mtDNA with 18 restriction endonucleases yielded 393 fragments. Differentiation was considerable and nucleotide divergence estimates ranged from 5.46% to 20.02%. Previous allozyme analysis (Dittmann et al. 1989) suggested that Red and Red-necked phalaropes were sister taxa, and Wilson's was the basal member of the group; mtDNA data from this study supported this grouping. No mtDNA fragments united the phalaropes as a monophyletic group, but most phylogenetic and phenetic analyses depicted them as such. We suggest that Wilson's Phalarope evolved shortly after the phalarope lineage itself arose. Although we were unable to resolve with certainty the relationships of phalaropes and other shorebirds in related genera, our data were reasonably consistent with the ordering of tribal level taxa in the 1983 American Ornithologists' Union Check-list (AOU 1983). In general, the higher level taxa surveyed were at the limits of resolution of mtDNA restriction endonuclease analysis. *Received 24 September 1990, Accepted 24 February 1991.*

THE SYSTEMATIC relationships among the three species of phalaropes and their relationship to other shorebirds were addressed by Dittmann et al. (1989). They used protein electrophoresis to show that the Red Phalarope (*Phalaropus fulicaria*) and Red-necked Phalarope (*P. lobatus*) are sister taxa. Although Wilson's Phalarope (*P. tricolor*) possesses general phalarope traits (spinning behavior, lobed toes, reversed sexual dimorphism in plumage coloration, largely aquatic, polyandry, dense plumage), it differs consistently in the expression of these characters from the other two species, and allozyme evidence reveals that it is probably a sister taxon to them. However, Wilson's Phalarope was so genetically distinct from the other phalaropes that Dittmann et al. (1989) could not distinguish between alternative hypotheses: that the phalaropes are not monophyletic, which conflicts with all existing classifications, or that the Wilson's Phalarope evolved shortly after the phalarope lineage itself arose, which makes it difficult to corroborate monophyly of phalaropes. The lack of strong allozyme support for the monophyly of a seemingly well-defined lineage such as phalaropes deserves testing with independent genetic information to clarify the evolutionary history of the group. Allozyme analyses also were unable to document the closest living relative to any of the phalaropes among the Long-billed Dowitcher (*Limnodromus scolopaceus*), Greater Yellowlegs (*Tringa melanoleuca*), Red Knot (*Calidris canutus*), Sanderling (*C. alba*), and Stilt Sandpiper (*C. himantopus*). Although genetic differentiation was considerable, there were no clear phylogenetic patterns.

We studied patterns of mitochondrial DNA (mtDNA) variation among phalaropes and other shorebirds. Mitochondrial DNA evolves rapidly and can provide considerable discrimination among avian species surveyed (Avise and Zink 1988, Shields and Helm-Bychowski 1988). In the avian genus *Ammodramus*, Zink and Avise (1990) found that both mtDNA and allozymes yielded similar phylogenetic conclusions. The same result occurred in similar studies of towhees (*Pipilo*; Zink and Dittmann 1991) and crowned sparrows (*Zonotrichia*; Zink et al. 1991). We analyzed patterns of mtDNA restriction fragment variation to determine (1) if there is evidence for the monophyly of the phalaropes, (2) the relationships among the three phalarope species, (3) the nearest relative of the phalaropes, and (4) if phylogenetic estimates were congruent with those based on allozymic variation. To determine if mtDNA restriction fragment analyses would be phylogenetically informative at higher taxonomic levels, we included taxa from different subfamilies.

TABLE 1. MtDNA haplotypes for shorebirds. Each letter refers to the common mtDNA restriction fragment profile for the following restriction endonucleases: *Ava* I, *Ava* II, *Bam*H I, *Bcl* I, *Bgl* I, *Bgl* II, *Eco*R I, *Hind* III, *Kpn* I, *Cla* I, *Nci* I, *Pvu* II, *Stu* I, *Sst* II, *Xba* I, *Nde* I, *Pst* I, and *Nco* I. Proximity of letters in alphabet does not imply number of restriction site differences. An underlined letter denotes polymorphism in and unique to the species. The size of the mtDNA molecule (±SD) is given in kilobases.

Taxon	Haplotype																	Size
Red Phalarope	B	B	C	B	D	C	B	C	B	C	D	B	D	B	C	C	A	C	19.0 ± 0.44
Red-necked Phalarope	C	C	B	B	C	B	B	B	A	B	C	C	C	B	B	B	A	B	18.8 ± 0.27
Wilson's Phalarope	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	19.3 ± 0.66
Western Sandpiper	H	I	E	I	K	F	D	F	A	I	J	J	J	C	H	I	C	D	18.2 ± 0.78
Sanderling	F	F	G	G	F	G	D	E	A	D	I	F	I	C	F	E	E	E	19.1 ± 0.33
Stilt Sandpiper	I	H	H	C	G	D	F	G	A	D	E	H	E	B	I	E	A	C	18.5 ± 0.20
Red Knot	D	D	D	E	I	E	C	H	A	F	G	D	G	C	E	H	C	F	18.9 ± 0.81
Greater Yellowlegs	J	J	F	H	J	H	H	I	A	H	K	I	K	B	G	F	F	C	19.0 ± 0.73
Short-billed Dowitcher	E	E	I	D	H	D	G	J	A	E	F	E	F	C	D	G	B	D	18.5 ± 0.68
American Avocet	G	G	J	F	E	A	E	D	A	G	H	G	H	C	G	D	D	C	18.4 ± 0.35

METHODS

The following specimens were used: 4 Wilson's Phalaropes, 4 Red Phalaropes, 4 Red-necked Phalaropes, plus one each of Greater Yellowlegs, Stilt Sandpiper, Sanderling, Western Sandpiper (*Calidris mauri*), Short-billed Dowitcher (*Limnodromus griseus*), Red Knot, and American Avocet (*Recurvirostra americana*). All specimens were collected in Louisiana except for Red and Red-necked phalaropes, which were collected offshore in North Carolina. Voucher specimens are preserved in the Museum of Natural Science, Louisiana State University. Within 1 h of collection of specimens, tissue samples were either preserved in liquid nitrogen or placed in MSB-EDTA buffer (Lansman et al. 1981). Mitochondria were isolated from homogenized tissue following Lansman et al. (1981), and mtDNA was isolated in cesium chloride equilibrium density gradients. After dialysis, mtDNA was digested with one of 18 restriction endonucleases (see below). Restriction fragments were end-labeled with ^{32}P or ^{35}S radionuclides, and the fragments were separated on agarose gels ranging in concentration from 0.7% to 1.5%. Fragments were visualized by autoradiography. Fragment size was determined by reference to a molecular size standard purchased from Bethesda Research Laboratories.

Each fragment profile for each enzyme was assigned a unique letter, yielding a composite mtDNA haplotype for each individual. Each restriction fragment was assigned a number and was scored as present or absent for each individual. We lacked funds to map restriction sites; Zink and Avise (1990) suggest that although sites are more informative than fragments, the latter contain phylogenetic information. The fragment data set was used to estimate p, the percentage of nucleotides that differ between each pair of mtDNA haplotypes (program courtesy of R. M. Ball, University of Georgia). Because of polymorphism in Wilson's Phalarope, a consensus haplotype was based on the most common patterns. The fragment data were entered into the computer program HENNIG86 (Ver. 1.5; Farris 1988), which infers a tree according to the principle of maximum parsimony. All possible topologies were examined (option "IE"). We report the consistency index, a measure of homoplasy commonly used (Archie 1989, Farris 1990), and the retention index ("ri"), also a measure of homoplasy, where 1.0 indicates no homoplasy for both indices. We used PHYLIP (ver. 3.0; Felsenstein 1987) to infer a phylogenetic tree that was based on the principle of Dollo parsimony; this procedure favors gains over losses, which is useful because restriction sites are easier to "lose" than to "gain" (Dowling et al. 1990). We used PHYLIP to infer trees from the matrix of p-values, with both the FITCH (allowing variable rates of change) and KITSCH (uniform rates of change between sister taxa) options. We also performed bootstrap analyses (Felsenstein 1985) with Wagner (PHYLIP:BOOT) and Dollo (PHYLIP:DOLBOOT) parsimony. Each bootstrap analysis involved 100 subsamples of the data. We coded each restriction fragment by the restriction endonuclease that produced it, and randomly resampled fragments with respect to endonuclease. This procedure limits the bias due to endonucleases with large numbers of fragments. Bootstrapping requires independent characters to be interpreted as a statistical statement about nodes. Restriction fragments are often not independent and we view these analyses as only descriptions of the fragment data.

RESULTS

A total of 393 fragments was observed (Appendix), of which 128 were phylogenetically informative (found in two or more but not all taxa). We scored each species for 60–70 fragments, which represents approximately 2% of

TABLE 2. Matrix of *p*-values among phalaropes and other shorebirds.

	1	2	3	4	5	6	7	8	9
1. American Avocet	0.0000								
2. Wilson's Phalarope	0.1135								
3. Red-necked Phalarope	0.1442	0.0827							
4. Red Phalarope	0.1199	0.0770	0.0546						
5. Western Sandpiper	0.1663	0.1180	0.1303	0.1441					
6. Sanderling	0.0939	0.1205	0.1350	0.1289	0.1092				
7. Stilt Sandpiper	0.1756	0.1450	0.1442	0.1219	0.0903	0.0623			
8. Red Knot	0.1759	0.1344	0.1378	0.1263	0.0928	0.1237	0.1143		
9. Greater Yellowlegs	0.1156	0.1314	0.1606	0.1256	0.1441	0.1620	0.1436	0.1471	
10. Short-billed Dowitcher	0.1402	0.2002	0.1969	0.1934	0.1105	0.1251	0.1163	0.1623	0.1485

the mtDNA genome. The size of the mtDNA genomes ranged from 18.2 to 19.3 kilobases (kb; Table 1), two to three kb larger than most passerine birds (Shields and Helm-Bychowski 1988). Each restriction endonuclease generated at least three patterns (except *Kpn* I), and for most endonucleases, each species had a unique pattern (Table 1). Intertaxon *p*-values (Table 2) ranged from 5.46% (Red-necked vs. Red phalaropes) to 20.02% (Wilson's Phalarope vs. Short-billed Dowitcher). The percentage of shared fragments was low (approximately 10–25%).

Fragment patterns that unite various subsets of species were of special interest. For example, the Red and Red-necked phalaropes had the same restriction profile for *Bcl* I (Fig. 1). For the enzyme *Sst* II, most bird species exhibit two bands; one is a 1.7 kb fragment, and the other comprises the remainder of the mtDNA genome (Zink unpubl. data). No phalarope had the "typical" avian pattern, although the Short-billed Dowitcher, Red Knot, American Avocet, Western Sandpiper, and Sanderling did. At *Sst* II, the Wilson's Phalarope was unique, and the Red and Red-necked phalaropes, Stilt Sandpiper, and Greater Yellowlegs shared a unique pattern. We observed no fragment out of 393 that united the phalaropes as a monophyletic group relative to any other outgroup taxon (or combination of outgroups).

To summarize the remaining information on phylogenetically informative fragments, we inferred phylogenetic trees and distance trees. All trees were rooted at the American Avocet (which does not alter conclusions presented below). The maximum parsimony analysis yielded three equally parsimonious trees (Fig. 2) of length 281, consistency index of 0.45, and retention index of 0.31; the consistency index and retention index indicate considerable homoplasy. The phalaropes occurred as a clade in two of these, with Red and Red-necked phalaropes as sister species in all three. The remaining taxa showed considerable variation in placement. A strict consensus tree (not shown) retained only the following three pairs of sister taxa: Red and Red-necked phalaropes, Short-billed Dowitcher and Red Knot, and Stilt Sandpiper and Sanderling. The bootstrapped Wagner parsimony tree (Fig. 3) was similar to the maximum parsimony trees, although no node occurred in >89% of the replicates. The Dollo parsimony tree (not shown) depicted the phalaropes as a clade, with the members of *Calidris* plus Greater Yellowlegs as a sister taxon, and the Short-billed Dowitcher and American Avocet outside these groups. The bootstrapped Dollo tree (not shown) grouped the phalaropes, but no nodes occurred in more than 81% of the 100 replicates. Both distance analyses (Fitch and Kitsch; Fig. 4) grouped the phalaropes, but only the Kitsch tree depicted the members of *Calidris* as a clade.

DISCUSSION

Level of differentiation among species.—The phalaropes exhibited an average nucleotide divergence of 7.1%, typical of well-differentiated avian congeners, including Long-billed and Short-billed dowitchers (Avise and Zink 1988). Other values among shorebirds included in this study exceed those generally reported for birds. In fact, the level of nucleotide divergence equaled or exceeded the upper limit generally accepted for phylogenetic analysis of mtDNA restriction fragments because of the likelihood

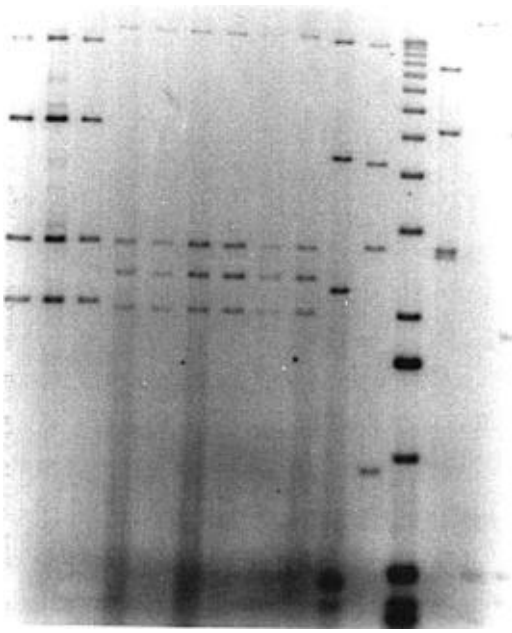

Fig. 1. Patterns of mtDNA restriction fragments produced by digestion with *Bcl* I. From left to right are three Wilson's Phalaropes, three Red-necked Phalaropes, three Red Phalaropes, Stilt Sandpiper, Short-billed Dowitcher, molecular size standard, Red Knot, American Avocet, and Sanderling. The sizes of fragments produced by the standard (lane 12) are (from bottom to top, in base pairs): 506, 516, 1018, 1635, 2036, 3054, 4072, 5090, 6108, 7126, 8144, 9162, 10180, 11198, and 12216.

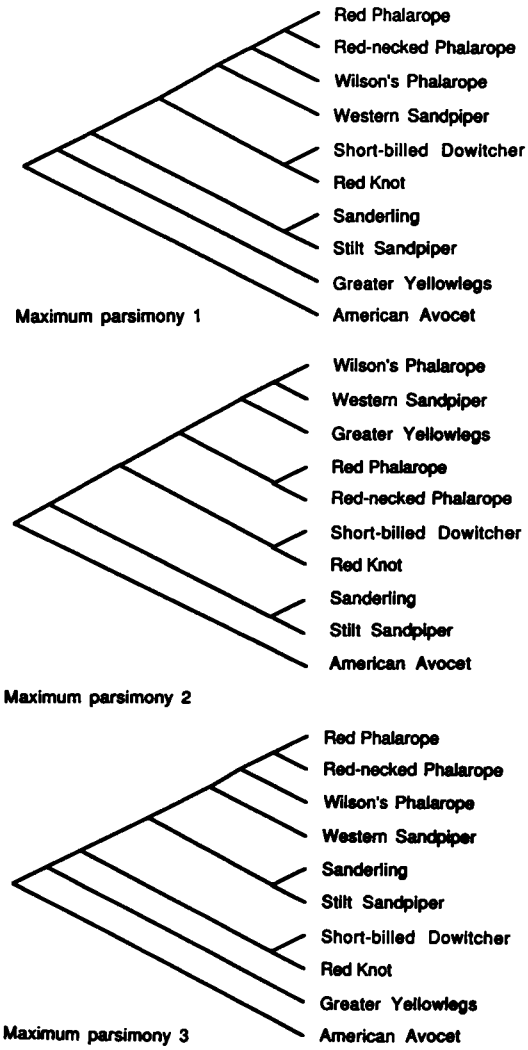

Fig. 2. Three equally parsimonious trees generated by Wagner parsimony analysis of mtDNA fragment data.

of comigration of nonhomologous fragments (Kessler and Avise 1985, Moritz et al. 1987, Dowling et al. 1990). The *p*-values reported here exceeding 0.10 to 0.15 should be compared with other studies only approximately.

Monophyly and relationships of phalaropes.—Our mtDNA data supported the conclusion—derived from allozyme comparisons, morphology, and behavior—that Red and Red-necked phalaropes were sister species, and Wilson's Phalarope was more distant. We used Shields and Wilson's (1987) calibration of mtDNA evolution in geese, 2% sequence evolution per million years, to estimate that Wilson's Phalarope evolved 4 million years ago (MYA). This divergence data is lower than that (11 MYA) based on allozyme evidence, and the discrepancy is unexplained.

Allozyme evidence led Dittmann et al. (1989) to suggest that if phalaropes were monophyletic, there must be a short interval between the origin of phalaropes and the evolution of Wilson's Phalarope. Most of our analyses (Figs. 1–4) were consistent with phalarope monophyly. However, mtDNA evidence for monophyly of phalaropes was indirect, because not one mtDNA fragment (out of 393) united the phalaropes unambiguously as a monophyletic group. Some fragments united phalaropes and one or two other taxa, but phylogenetic analysis of all fragments revealed that the other taxa were not closely related to phalaropes. Because of this, and fragments shared by various pairs of phalaropes, phalaropes were united as a clade. The only apparent invariant morphological character to support phalarope monophyly is a fea-

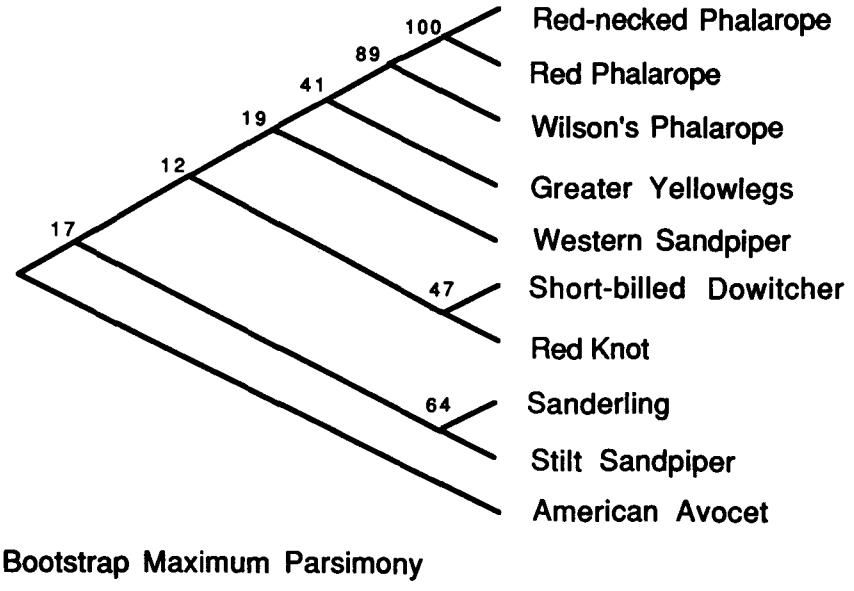

Fig. 3. Bootstrapped analysis using Wagner parsimony.

ture of the skull (Strauch 1978). However, phalaropes share many general traits that seem, relative to other shorebirds, to be synapomorphies. Although the morphological and behavioral traits of phalaropes might be correlates of only two aspects of phalarope biology (namely their aquatic habits and reversed sexual dimorphism), monophyly of the phalarope lineage seems more likely than convergence in Wilson's Phalarope.

The lack of unambiguous mtDNA synapomorphies for the phalaropes could result from a short interval between the origin of phalaropes and the divergence of Wilson's Phalarope. Only rapidly evolving characters have a significant probability of evolving into a new state on a short-lived branch, and such evidence of homology (synapomorphy) will be subsequently erased (i.e. evolve into autapomorphies) on long branches of an evolutionary tree because of the rapid and uniform rate of molecular change (Lanyon 1988). Lanyon (1988) also suggested that synapomorphies for such groups might be key innovations that rapidly become "locked" into a lineage instead of continuing to evolve at a uniform rate. The behavioral and morphological traits that seem to make phalaropes a clade might be examples of such synapomorphies. At the least, the lack of clear evidence for phalarope monophyly in allozymes and mtDNA was consistent with this hypothesis. Analysis of genomic regions with slower rates of change might resolve monophyly of the phalaropes. As an alternative, we used the successive approximation approach to character weighting (Farris 1969), which emphasizes characters with greatest congruence. The tree (not shown) is very similar to the middle one in Figure 1, and the phalaropes are not a clade. Apparently, there is no conservative group of fragments most consistent with phalarope monophyly.

Phylogenetic affinities of phalaropes and allies.— Variation in the placement of taxa in the minimal-length and distance trees is a result of a high level of homoplasy. Homoplasy is revealed in the low values of the consistency and retention indices (Farris 1989, Archie 1989). Homoplasy is likely due to the rapid rate of mtDNA evolution, which at these taxonomic levels results in few shared derived fragments (Moritz et al. 1987). Thus, phylogenetic and phenetic analyses (Figs. 1–4) of our mtDNA data did not support a consistent picture of evolutionary relationships among the shorebird taxa we studied. Either mtDNA and allozymes provide inappropriate resolution for these taxonomic levels or there is a biological reason (i.e. bursts of diversification) for the difficulty in resolving phylogenetic patterns, as suggested above for phalarope monophyly.

Others (e.g. Jehl 1968, Strauch 1978) have been

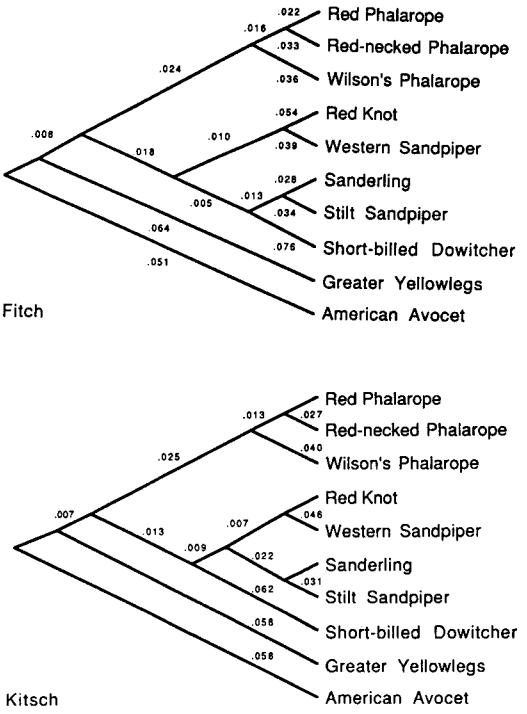

Fig. 4. Upper: Distance analysis (Fitch) allowing for variable rates of evolutionary change. Lower: Distance analysis constraining branch tips to be contemporaneous (Kitsch).

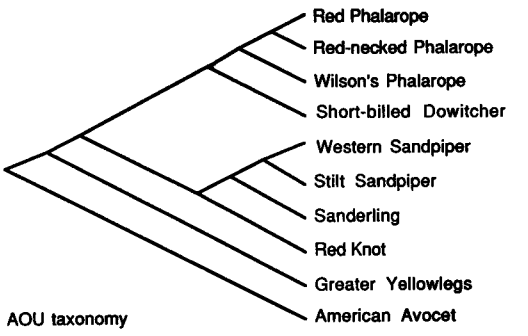

Fig. 5. Branching diagram obtained from linear sequence in AOU classification by assuming sequence is arranged from primitive (basal) to derived within each level.

unable to demonstrate unambiguously the sister group to phalaropes, although Jehl (1968) suggested that phalaropes were "close" to tringines, a relationship supported by the bootstrapped maximum parsimony analysis (Fig. 3). Comparison of classifications (e.g. Jehl 1968, AOU 1983) reflected uncertainty concerning phylogenetic relationships among shorebird taxa. To evaluate the AOU classification with our mtDNA data, we followed the AOU Check-list Committee's discussion (1983: xvi–xvii) and converted the AOU Check-list sequence into a phylogeny (Fig. 5). The AOU tree required 297 steps to "explain" the mtDNA data, compared with 281 steps for the most parsimonious trees. One of our distance analyses (Kitsch; Fig. 4) was consistent with the AOU branching order at the tribal level, with the exception of the dowitcher. The uncertainty over the validity of distance analysis for phylogenetic inference (Farris 1986) makes the last result difficult to interpret. Nonetheless, the similarity of our Kitsch analysis to the AOU classification suggested that homoplasy has not compromised all phylogenetic information in our mtDNA restriction fragment data.

The allozyme data (Dittmann et al. 1989) were consistent with the idea that the phalaropes were closest relatives of scolopacines. However, no representative of the tribe Scolopacini (woodcocks) was present in the allozyme survey. Thus, Dittmann et al.'s (1989) statement that their data were consistent with Lowe's (1931) belief that the phalaropes were close to scolopacines applies only at the level of "Scolopacidae" and not the woodcock tribe (Scolopacini).

Mitochondrial DNA vs. allozymes.—To compare the allozyme and mtDNA results directly, we constructed a maximum parsimony tree from the mtDNA data for the taxa included in Dittmann et al. (1989), except that the Short-billed Dowitcher was used. We obtained four equally parsimonious trees of 228 steps with this reduced data set. These trees and the consensus tree (not shown) were similar to those derived from parsimony analysis of the full mtDNA data set. We evaluated the two trees published by Dittmann et al. (1989) and found them to be 238 and 236 steps, a difference of 10 and 8 steps, respectively. These two trees differed considerably from those derived from the mtDNA data, except that Red and Red-necked phalaropes were sister taxa. Although relatively few steps separated these topologically different trees, which suggests congruence between allozyme and mtDNA results, neither the allozyme nor reduced mtDNA data sets supported a particular order of taxa. To explore further the relationship between the two data sets, we computed a correlation coefficient of 0.36 ($P > 0.05$), which suggests low congruence. Inspection of the plot

(not shown) revealed that the mtDNA *p*-values were "saturated" around 0.08, which probably contributed to instability of systematic affinities of the higher-level taxa studied. Because the mtDNA genome represents a single genetic lineage (Dowling et al. 1990), studies of other genomic regions should be used to clarify shorebird relationships.

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APPENDIX. Presence/absence of mtDNA fragments in phalaropes and other shorebirds. Sequence of enzymes follows that in Table 1.

American Avocet											
0000	00000	00000	00001	10000	00001	00000	00000	00001	00000	11110	00000
00101	00000	00000	00001	10000	00000	00000	00001	10000	00000	01110	00000
00000	00000	01000	00000	00000	00000	01000	00100	10000	00011	00010	00000
00001	00000	10000	01100	00100	01000	01110	00000	00000	00000	01100	00100
00000	00000	00000	00100	00100	00000	11100	00000	00000	00000	01000	00000
01001	00001	00010	00000	00000	10001	00010	00001	00000	10010	11000	00010
00010	11110	00000	00000	00000	00000	1010					
Wilson's Phalarope											
1110	00000	00000	00000	00000	00111	00000	00000	00000	00000	00001	00000
00011	00000	00000	00000	00011	11000	00000	00000	00000	01110	00000	00000
00000	00001	01000	00000	00000	00000	11100	01000	00000	00000	00011	01111
11000	00000	00000	00011	11111	00000	00000	00000	00000	00010	00100	11111
00000	00000	00000	00000	00011	10000	00000	00000	00011	11100	00000	00100
00000	00000	11110	00000	00000	00011	10001	11111	00000	00000	00000	00000
00000	00000	00000	11110	00000	00000	0000					
Red-necked Phalarope											
0000	11100	00000	00000	00000	00001	11011	00000	00000	00000	00000	00000
00000	11110	00000	00000	00000	10110	00000	00000	01000	00001	10000	00000
00000	00000	00110	00000	00000	00100	01011	10000	00000	00000	00000	00000
00100	00000	00000	00000	11111	11100	00000	00000	00100	00000	00100	01011
11110	00000	00000	00000	00001	01111	00000	00000	00000	00011	10000	00100
00000	00000	01000	11000	00000	00001	01100	00110	00111	10000	00000	00000
00000	00000	00000	00001	11000	00000	0000					
Red Phalarope											
0111	10010	00000	00000	00000	00001	11100	00000	00000	00000	00000	00000
00010	01001	00000	00000	00000	10110	00000	00000	01000	00010	00000	00000
00000	10000	00001	10000	00000	00000	11000	00100	00000	00000	00000	00000
00100	00000	00000	00010	00110	00010	00000	00000	00000	00000	00011	00110
00011	11000	00000	00000	01001	00000	00001	10000	00000	00000	01000	00100
00000	00000	00101	10000	00000	00001	01100	00100	00001	11110	00000	00000
00000	00000	00000	00001	00100	00000	0000					
Western Sandpiper											
0000	10000	10000	00000	00000	00000	00000	10010	00000	00000	00000	00100
00000	00010	01111	00000	00001	10000	00000	00000	00010	00010	10000	00000
00001	00000	00000	00000	00001	11000	01000	00000	10000	00100	01100	00000
00011	00000	00000	00011	00110	00000	00000	01110	00000	00001	00100	00000
00000	00101	00000	01011	00000	00000	00001	01000	00000	00000	10110	00000
00110	00000	01000	01000	00010	01001	00011	01111	00000	00000	10000	00000
00000	00000	00000	00000	00000	00000	0100					
Sanderling											
0000	00000	00001	11110	01000	00001	00000	00000	00100	10001	00000	00000
00000	00000	00001	00010	00001	00001	00000	00000	00100	00010	10001	10000
00000	00000	00000	00000	11100	00000	01000	00111	00000	00000	00000	00000
00011	00000	00000	00000	00100	00000	00001	11111	00000	00000	00100	00000
00000	00100	00111	10000	00000	00000	00010	00000	00000	00001	00001	00000
00010	11000	00110	00000	01100	00001	00010	01000	00000	10001	11000	00000
00011	10000	00000	00000	00000	00011	0000					
Stilt Sandpiper											
0010	00010	00000	00000	10111	00000	00000	10000	00100	10000	00000	11000
00000	00000	00000	00000	01000	00001	11110	00000	00000	00010	00000	01000
00000	00100	00000	01000	00010	00000	01000	00111	00000	00000	00001	00010
00010	00000	00000	00000	00110	00000	00001	10101	00000	00000	00100	00000
00000	10110	00100	00000	01000	00000	00010	00000	00000	00000	01000	00100
00000	00000	00000	11000	00000	00101	01101	00100	00010	00000	11100	00001
00000	00000	00000	00001	00000	00101	0000					
Red Knot											
0010	00011	01000	00000	00000	01000	00000	11000	00000	00000	00000	00000
01010	00010	10000	00000	00101	10000	00000	00110	00000	00000	00001	10001
10000	00010	00000	00111	10000	00000	00000	00000	00001	11000	00000	00000
00000	00000	01011	00000	10100	10000	00000	00010	00011	11100	00100	00101

APPENDIX. Continued.

00001	00110	00000	00000	00001	00100	00101	00000	00000	00000	00000	11000
00110	00000	00100	10111	00000	00001	00010	01000	00000	11000	10000	00000
11100	00000	00001	00000	01000	11100	0001					
Greater Yellowlegs											
0110	00010	00000	00000	00000	01000	00000	00000	00000	00010	00100	00011
10000	01000	00001	11100	00001	00000	00000	00000	00000	10000	00000	00000
01110	00000	01000	00000	00000	00011	01000	00000	00000	00000	10000	00010
00000	11100	00000	00011	00100	00001	00000	00000	10000	00000	00100	00010
00000	10100	00001	00000	10001	00000	00100	00000	11100	00000	01000	00000
00000	00110	00001	00000	00001	00001	01100	01100	00000	00000	00000	00000
00000	00001	11110	00000	00000	00000	1010					
Short-billed Dowitcher											
0010	00000	01110	00000	00000	10000	00000	00111	11111	11110	00000	00000
00001	00000	00000	00000	00000	10000	00001	10000	00000	00000	00000	00111
00000	00000	00000	01000	00010	00000	00000	00100	11110	00000	00000	00000
00000	00011	00000	00000	01000	00000	00000	01010	11110	00000	10100	00010
10000	10011	11000	00000	00000	00000	00000	00111	00000	00000	10110	00011
11000	00000	00100	10011	10000	00001	00010	00000	00000	00101	00011	11100
00000	00000	00000	00000	00011	00000	0000					