TABLE 1.—*Currently accepted taxonomy of northern hair seals, subfamily Phocinae (Honacki et al., 1982; King, 1983; Wilson and Reeder, 1993).*

Genus	Subgenus	Species	Authority	Common name
Erignathus[a]		*barbatus*	Erxleben, 1777	Bearded seal
Cystophora[b]		*cristata*	Erxleben, 1777	Hooded seal
Halichoerus[c]		*grypus*	Fabricius, 1791	Gray seal
Phoca[c]	*Pusa*	*hispida*	Schreber, 1775	Ringed seal
Phoca[c]	*Pusa*	*caspica*	Gmelin, 1788	Caspian seal
Phoca[c]	*Pusa*	*sibirica*	Gmelin, 1788	Baikal seal
Phoca[c]	*Phoca*	*vitulina*	Linnaeus, 1758	Harbor seal
Phoca[c]	*Phoca*	*largha*	Pallas, 1811	Spotted seal
Phoca[c]	*Histriophoca*	*fasciata*	Zimmermann, 1783	Ribbon seal
Phoca[c]	*Pagophilus*	*groenlandica*	Erxleben, 1777	Harp seal

[a] Tribe Erignathini.
[b] Tribe Cystophorini.
[c] Tribe Phocini.

Chromosomal studies have not resolved the relationships within the Phocini because karyotypes are nearly identical (Anbinder, 1985; Arnason, 1974, 1977; Fay et al., 1967). Genetic variation revealed by allozyme electrophoresis is too low for phylogenetic analysis (McDermid and Bonner, 1975; Shaughnessy, 1975; Simonsen et al., 1982; Zasypkin, 1989).

In this study, DNA sequences of the mitochondrial cytochrome *b* gene of eight phocine and two monachine species were compared. We chose the cytochrome *b* gene because the relationship of its structure and function is understood and the gene has been well characterized in mammals (Arnason and Johnsson, 1992; Arnason et al., 1993; Irwin and Arnason, 1994; Irwin et al., 1991).

MATERIALS AND METHODS

Laboratory methods.—Twenty-eight muscle samples were obtained from the University of Alaska Museum Frozen Tissue Collection (Appendix I). DNA of *Phoca groenlandica* was provided by U. Arnason, Institute of Genetics, University of Lund, Sweden. DNA was extracted from <20 mg of tissue following the method of Medrano et al. (1990) from *Phoca hispida* (n = 8), *P. largha* (n = 6), *Phoca vitulina richardsi* (n = 5), *P. fasciata* (n = 5), *Erignathus barbatus* (n = 2), and *C. cristata* (n = 1). Two regions of the mitochondrial cytochrome *b* gene were amplified in asymmetric polymerase chain reactions (PCR—Gyllensten and Erlich, 1988). We used primer pairs L14841/15149 (Kocher et al., 1989) and L15513/H15915 (Irwin et al., 1991) and Kocher buffer mix (Kocher et al., 1989) in 25-μl reactions. Thermocycling parameters were 1 min each, 94°C, 50–54°C, 72°C, for 30 cycles. The hot-start protocol of Nuovo (1992) was used for samples on which this procedure failed. Primers were washed from amplified DNA by centrifugation dialysis. Nucleotide sequences of both strands were determined following the Sanger method (Sanger et al., 1977) using a sequencing kit (Sequenase version 2.0; US Biochemical, Cleveland, OH). Sequences were manually scored. In addition, the cytochrome *b* gene of one sample of each of the seven taxa was cloned and sequenced following methods described by Arnason et al. (1991).

Cytochrome *b* sequences were aligned by eye to sequences of the Atlantic harbor seal, *P. vitulina vitulina* (Arnason and Johnsson, 1992) and the gray seal, *H. grypus* (Arnason et al., 1993). Nucleotide sequences were translated to amino-acid sequences using the computer software program GCG (Devereux et al., 1984). The positions of variable nucleotides and amino acids were compared with respect to the outermembrane, transmembrane, and innermembrane regions of the cytochrome *b* protein (Irwin et al., 1991). Significance level of Chi-square tests was set at 0.05.

Phylogenetic analysis.—Phylogenies were determined using maximum-parsimony and neighbor-joining methods. Ancestral and derived character states were determined by comparison

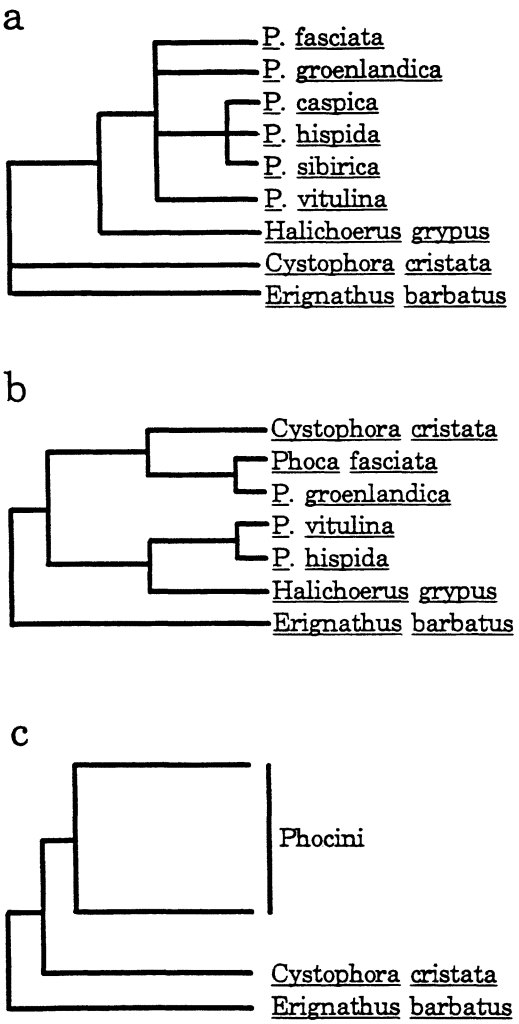

FIG. 1.—Phylogenies of the Phocinae based on comparative studies of cranial and skeletal morphology of: a, Burns and Fay (1970); b, de Muizon (1982); and c, Wyss (1988).

with monachine outgroups, the Weddell seal, *Leptonychotes weddelli,* and the Hawaiian monk seal, *Monachus schauinslandi* (U. Arnason, in litt.)

Maximum-parsimony phylogenies were determined using the computer program Phylogenetic Analysis Using Parsimony (PAUP version 3.1—Swofford, 1993). No constraint was placed on character transformation (unordered and reversible). Intraspecific variation was coded as polymorphic. We used an exhaustive search to determine the tree-length distribution of all possible trees. The branch-and-bound option of PAUP was used for subsequent maximum-parsimony searches in which we investigated the effects of character weighting and designated outgroups on tree topologies.

The neighbor-joining option of the computer program, PHYLIP (Phylogenetic Inference Package—Felsenstein, 1990) was used to calculate a phylogeny from nucleotide data. We used neighbor-joining in addition to maximum parsimony because computer simulations have shown that it performs better than maximum parsimony when <600 nucleotides are analyzed, and it is less sensitive to parallel or backward mutations than maximum-parsimony methods (Nei, 1991). *Monachus schauinslandi* was the designated outgroup.

Intraspecific variation was examined in a 203-base pair sequence of *E. barbatus* (n = 2), *P. fasciata* (n = 5), *P. hispida* (n = 8), *P. largha* (n = 6), and *P. vitulina richardsi* (n = 5). We completed a branch-and-bound search that included all eight sequences of *P. hispida* and included *P. largha* as the designated outgroup.

RESULTS

Nucleotide sequences.—A 458-base pair sequence from position 146–363 and position 841–1,080 of the cytochrome *b* gene was obtained for one sample of each taxon in the study and a 203-base pair sequence from position 146–348 was obtained for all samples. Additional sequences of *P. hispida* and *C. cristata* were made available for comparison by U. Arnason (in litt.).

One hundred forty-five substitutions were detected at 129 of the 458 nucleotide sites (Fig. 2). About one-half (68) of the 129 variable sites were phylogenetically informative (synapomorphies). Eighty-five percent of the substitutions were transitions, and 15% were transversions (ratio 5.7:1). The ratio of nucleotide substitutions at first, second, and third codon positions was 2.3:1:13.7.

Twenty-two unique replacements were detected at 19 of the 152 amino-acid sites, but only four were synapomorphies. All amino-acid replacements were at sites known to be highly variable in mammals (hypervariable sites—Irwin et al., 1991; Irwin and Arnason, 1994). The proportions

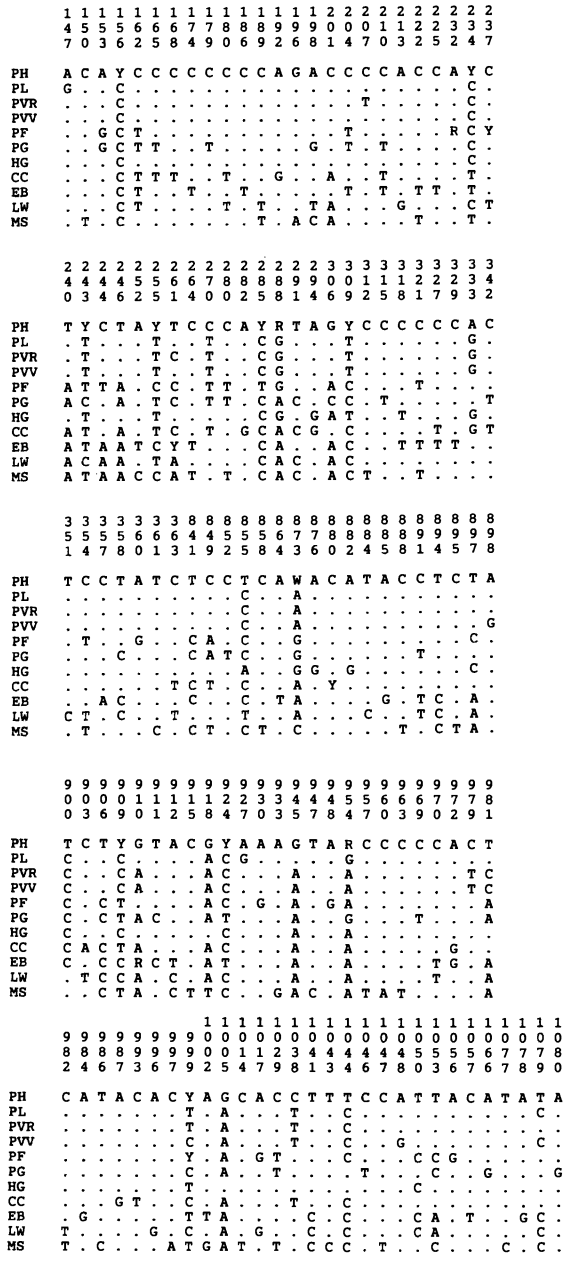

FIG. 2.—Nucleotide sequences of the cytochrome *b* gene of northern hair seals and monachine outgroups: *Phoca hispida* (PH); *Phoca largha* (PL); *Phoca vitulina richardsi* (PVR); *P. v. vitulina* (PVV; data from Arnason and Johnsson, 1992); *Phoca fasciata* (PF); *Phoca groenlandica* (PG); *Halichoerus grypus* (HG; data from Arnason et al., 1993); *Cystophora cristata* (CC), *Erignathus barbatus* (EB); *Leptonychotes weddelli* (LW; data provided by U. Arnason); *Monachus schauinslandi* (MS; data provided by U. Arnason). A 218-base region spanning nucleotides 146–363, and a 240-base region spanning nucleotides 841–1,080 were sequenced. Substitutions were detected at 129 sites. Positions of nucleotides in the gene are indicated vertically above the first sequence (PH). Dots indicate that nucleotides are identical to the first sequence. Symbols R and Y within nucleotide sequences indicate intraspecific polymorphisms of purines and pyrimidines, respectively. W indicates a polymorphism of adenine and thymine.

of replacements at hypervariable sites in the outermembrane, transmembrane, and innermembrane regions were not significantly different ($\chi^2 = 0.52$).

Intraspecific variation.—Variation was detected in four taxa. The nucleotide sequence of *C. cristata* differed from the sequence obtained by U. Arnason (in litt.) at one position (position 880). One of the two substitutions in the samples of *E. barbatus* coded for an amino-acid replacement (position 910), as did one of the three substitutions observed in the six samples of *P. fasciata* (position 232). All 11 transitions and one transversion observed in the eight samples of *P. hispida* were at third codon positions, and none coded for amino-acid replacements.

Phylogenetic analysis.—Sequence data were examined to detect variation in rates of evolution that could affect phylogenetic inference. The difference in rate of evolution between the two portions of the cytochrome *b* gene spanning nucleotides 146–363 and 841–1,080 was not significant ($\chi^2 = 0.93$). Differences in the rate of evolution of nucleotides corresponding to the outermembrane, transmembrane, and innermembrane regions of the cytochrome *b* protein were not significant (transitions, $\chi^2 = 0.57$; transversions, $\chi^2 = 3.13$).

The tree-length distribution of an exhaustive search using PAUP was skewed to the right ($g^1 = -0.538310$), indicating the presence of phylogenetic signal in the dataset (Hillis, 1991). The three minimal trees obtained from the exhaustive search were 223 steps long. Topologies of 50%-Majority-Rule consensus trees calculated for minimal trees (223 steps) and trees ≤228 steps were identical with one exception; the harbor and spotted seal taxa formed a polytomy in consensus trees 224–228 steps in length.

Most branch-and-bound searches produced trees identical to consensus trees that resulted from the exhaustive search. Weighting transversions more heavily than transitions (5:1) produced no effect on either the consensus of minimal trees or the bootstrap tree topologies. Applying weight to the second codon position caused the Atlantic harbor seal, *P. vitulina vitulina,* and the spotted seal, *P. largha,* to be grouped as sister taxa because of a single second-position substitution at position 1,079. Deletion of the outgroup, *Monachus,* had no effect on either the consensus of minimal trees or the bootstrap tree topologies. Three groups were found in most of the replicates of bootstrap trees (Fig. 3): *Halichoerus* and *P. hispida*; *Halichoerus* and all members of *Phoca* except *P. fasciata* and *P. groenlandica*; all phocines except *Erignathus.* Neighbor-joining grouped *P. largha* with *P. vitulina,* and partially resolved the *Erignathus-Leptonychotes-Monachus* polytomy by grouping *Erignathus* with the Phocinae. Parsimony analysis grouped *P. hispida* monophyletically, but relationships among the eight individuals were unresolved.

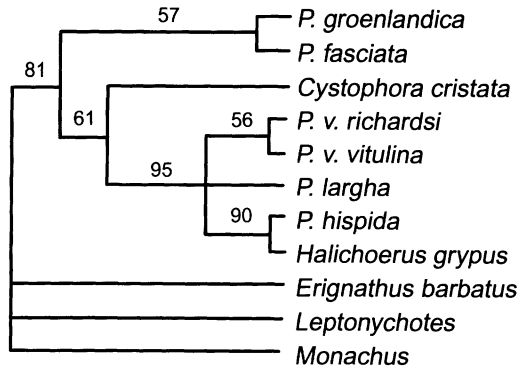

FIG. 3.—Parsimony tree based on an unweighted analysis of 458 base pairs of the cytochrome *b* gene. The percentage of 100 Bootstrap trees in which a node was found is indicated on the tree. Tree length = 230 steps; consistency index (CI) = 0.700, CI excluding uninformative characters = 0.522.

DISCUSSION

The monophyly of Phocidae is widely accepted (Wyss, 1988), but subfamilial relationships are unclear. De Muizon (1982: 198, fig. 8) considers monk seals, *Monachus,* to be more closely related to phocines

than are other Monachinae. Other studies suggest that *Monachus* diverged from the phocid lineage before the monachine and phocine subfamilies (Repenning and Ray, 1977; Repenning et al., 1979; Wyss, 1988). The cytochrome *b* sequence of *Leptonychotes,* a monachine, was more similar to phocine sequences than was the sequence of *Monachus*; however, data from other pinnipeds are necessary to determine the phylogenetic position of *Monachus.*

Several relationships among members of the Phocinae indicated by cytochrome *b* (Fig. 3) are consistent with earlier studies. Most authors have noted the close relationship between *P. groenlandica* and *P. fasciata* (Burns and Fay, 1970; Davies, 1958*b*; de Muizon, 1982; McLaren, 1975). The closer relationship between the harbor seals of the Atlantic and Pacific, *P. vitulina vitulina* and *P. v. richardsi,* than between harbor and spotted seals, *P. largha,* agrees with the most recent review of the harbor seal group (Burns et al., 1984).

The phylogeny of the Phocinae based on cytochrome *b* differs from phylogenies based on cranial and skeletal morphology (Fig. 1). Most striking is the placement of the gray seal, *H. grypus,* with the ringed seal, *P. hispida,* an arrangement found in both neighbor-joining and parsimony analyses and one that invalidates currently accepted taxonomy by making *Phoca* paraphyletic. Although no previous study has proposed *P. hispida* and *H. grypus* as sister taxa, de Muizon (1982) indicates their proximity (Fig. 1b). Introgression of the mitochondrial genome (Ferris et al., 1983) seems an unlikely explanation for this apparent close relationship between the gray and ringed seals because interspecific hybridization is thought to be rare in populations of hair seals (Burns et al., 1984). Variation within ringed seals was greater than variation between harbor and spotted seals; however, this did not pose a problem for reconstruction of phylogeny. Individuals of *P. hispida* formed a monophyletic group in the parsimony analysis.

We do not suggest taxonomic revision based on sequences of a single gene, but we point out that the phylogeny of the Phocinae remains unsettled. The close relationship between gray and ringed seals merits further study. These taxa come into contact in the North Atlantic and extensive overlap in their distributions may have occurred during Pleistocene glacial periods. Moreover, a sister-taxon relationship between such morphologically and ecologically distinct taxa as gray and ringed seals would alter our understanding of the historical biogeography of the Phocinae (Davies, 1958*b*; Ray, 1976; Repenning et al., 1979).

Acknowledgments

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APPENDIX I

Specimens examined.—All samples are curated in the University of Alaska Museum Frozen Tissue Collection (AF).

Cystophora cristata. Greenland, Angmagssalik (AF1420).

Erignathus barbatus. Bering Sea, near St. Lawrence Island, Alaska (AF1417). Bering Sea, near St. Matthew Island, Alaska (AF1419).

Phoca fasciata. Bering Sea, near St. Lawrence Island, Alaska (AF1413, AF1414). Bering Sea, Norton Sound (AF1415, AF1416). Bering Sea, near St. Matthew Island, Alaska (AF1446).

Phoca hispida. Arctic Ocean, near Barrow, Alaska (AF1401, AF1402, AF1403, AF1404, AF1423, AF1452). Chukchi Sea, near Shishmaref, Alaska (AF 1426). Bering Sea, Norton Sound (AF 1425).

Phoca largha. Chukchi Sea, near Point Hope, Alaska (AF1405, AF1406, AF1407, AF1408). Bering Sea, near St. Matthew Island, Alaska (AF1435, AF1436).

Phoca vitulina richardsi. Gulf of Alaska (AF1410, AF1412). Gulf of Alaska, near Kodiak Island, Alaska (AF1422, AF1428, AF1429).


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