Phylogenetic relationships within the family Otariidae were investigated using two regions of the mitochondrial genome. A 360-bp region of the cytochrome b gene was employed for the primary phylogenetic analysis, while a 356-bp segment of the control region was used to enhance resolution of the terminal nodes. Traditional classification of the family into the subfamilies Arctocephalinae (fur seals) and Otariinae (sea lions) is not supported, with the fur seal Callorhinus ursinus having a basal relationship relative to the rest of the family. This is consistent with the fossil record which suggests that this genus diverged from the line leading to the remaining fur seals and sea lions about 6 million years ago (mya). There is also little evidence to support or refute the monophyly of sea lions. Four sea lion clades and five fur seal clades were observed, but relationships among these clades are unclear. Similar genetic divergences between the sea lion clades ($D_a = 0.054$–$0.078$), as well as between the major Arctocephalus fur seal clades ($D_a = 0.040$–$0.069$) suggest that these groups underwent periods of rapid radiation at about the time they diverged from each other. Rapid radiations of this type make the resolution of relationships between the resulting species difficult and indicate the requirement for additional molecular data from both nuclear and mitochondrial genes. The phylogenetic relationships within the family and the genetic distances among some taxa highlight inconsistencies in the current taxonomic classification of the family.

Key Words: Otariidae; mtDNA; phylogeny; fur seal; sea lion; cytochrome b; control region.

INTRODUCTION

The family Otariidae (Order Carnivora) contains the 14 extant species of fur seals and sea lions. The most recent classification of the family (Rice, 1998) is based upon skull morphology, dentition, and geographic distribution as proposed in the historic assessments of both fur seal and sea lion species (Scheffer, 1958; King, 1960, 1969; Repenning et al., 1971). However, in the past, there has been some confusion with otariid taxonomy, primarily because the broad geographic distribution of the family (Fig. 1) made it difficult to obtain sufficient skeletal material from all species, and because there appears to be convergence of some morphological characters noted for some taxa (Berta and Deermere, 1986). While a more comprehensive selection of material and a greater understanding of the distribution and ecology of species have served to reduce this confusion, there are still some elements of the current taxonomy that require further study.

The division of the Otariidae into two subfamilies, Arctocephalinae (fur seals) and Otariinae (sea lions), has been commonly recognized in the literature (e.g., Riedman, 1990; Reynolds et al., 1999). The fur seals are represented by two genera (Callorhinus and Arctocephalus), while the sea lions are represented by five (Phocarctos, Neophoca, Zalophus, Eumetopias, and Otaria). However, two lines of evidence suggest such a subfamilial classification is misleading. Only a few diagnostic characters separate the groups (e.g., the pres-
ence/absence of underfur, the presence of five or six upper canines), and these may not be not sufficient to warrant the division (Repenning et al., 1971; King, 1983). Further, a number of recent studies have suggested that the subdivision into two subfamilies is ambivalent. This includes a taxonomic review based on anatomical descriptions (Brunner, 2000) and a comprehensive phylogenetic analysis based primarily on fossil and morphological evidence (Bininda-Emonds et al., 1999). The latter suggests that the sea lions and the genera *Arctocephalus* and *Callorhinus* form a polytomy, indicating that the monophyly of the Arctocephalinae could not be assured.

Such a relationship between Callorhinus, Arctocephalus, and the sea lions is also proposed in the fossil record (e.g., Repenning et al., 1979; Miyazaki et al., 1994). The modern fur seals and sea lions evolved from the ancestral family Enaliarctidae about 11 million years ago (mya) (Repenning, 1976; Repenning et al., 1979; Miyazaki et al., 1994). Arctocephalus is characterized by ancestral character states such as dense underfur and the presence of double rooted cheek teeth and is thus thought to represent the most “primitive” line (Kim et al., 1975). It was from this basal line that both the sea lions and the remaining fur seal genus, Callorhinus, are thought to have diverged. The fossil record from the western coast of North America presents evidence for the divergence of Callorhinus (~6 mya), the fur seals disperse south, before 5 mya and after the closure of the Central American Seaway. Sea lions followed about 3 mya. (3) Fur seals disperse to both coasts of South America. Sea lions follow later. (4) Fur seals disperse to Africa and the subantarctic with the assistance of the West Wind Drift. (5) *A. pusillus* colonizes Australia from South Africa. (6) Sea lions move south from the northwestern Pacific into Australia and New Zealand about 3 mya.
Goldsworthy et al., 1997). C.A. Repenning even suggests that the subfamilial classification of the Otariidae could be better upheld if *A. pusillus* was removed from the equation, as these subfamilies could then be separated on the basis of skull morphology (as cited in Stirling and Warneke, 1971). Furthermore, the status of the two subspecies of *A. pusillus*, the Cape fur seal from South Africa (*A. p. pusillus*) and the Australian fur seal (*A. p. doriferus*), is also questionable. The skulls of both taxa are indistinguishable from each other, yet Repenning et al. (1971) tentatively maintained the subspecific classification based on only one character. The reports of interspecific and intergeneric hybrids within the Otariidae (e.g., Goldsworthy et al., 1999; Rice, 1998, and references therein) also suggest questions about the closeness of the putative species and genera. Thus, further evidence is required to better investigate taxonomic relationships within the Otariidae.

Molecular genetic techniques are useful for providing evidence for taxonomic studies, as shown by those that have investigated the phylogenetic relationships of the Pinnipedia within the Carnivora (e.g., Sarich, 1969; Slade et al., 1994; Arnason et al., 1995). Only two studies have sought to address the question of intrafamilial relationships within the Otariidae using molecular techniques (Lento et al., 1995, 1997). However, these studies contained only representative taxa of the family. A more comprehensive approach was adopted by Bininda-Emonds et al. (1999) who compiled data from a range of studies pertaining to all species within the Carnivora for phylogenetic analysis. These data included morphological, molecular, and fossil data. While it included the work of Lento et al. (1995), molecular information for all species in the family were not available. Here we present additional molecular evidence of the phylogenetic relationships of the Otariidae through the screening of all species within the family using two regions of mitochondrial DNA.

### MATERIALS AND METHODS

#### Sample Collection

All extant fur seal and sea lion species within the family Otariidae are represented in the phylogenetic analysis. Details of the sampling of each species are presented in Tables 1 and 2. Skin biopsies or blood were obtained from most species for extraction of genomic DNA and sequencing. For the remaining species, either genomic DNA was supplied, or the sequence data for the relevant genes were obtained from Genbank.

#### Laboratory Analysis

Total genomic DNA was extracted from blood and skin biopsies using the modified CTAB/protease K extraction protocol outlined in Wynen et al. (2000). The polymerase chain reaction (PCR) was used to amplify a region of the mitochondrial tRNA$_{thr}$-control region using the primers Thr/Pro and Cent (Wynen et al., 2000). PCR was also used to amplify the first 429 bp of the

<table>
<thead>
<tr>
<th>Species Label</th>
<th>Common name</th>
<th>Populations represented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arctocephalus gazella</td>
<td>AGZ</td>
<td>Antarctic fur seal</td>
</tr>
<tr>
<td>A. tropicalis</td>
<td>ATR</td>
<td>Subantarctic fur seal</td>
</tr>
<tr>
<td>A. forsteri</td>
<td>AF</td>
<td>New Zealand fur seal</td>
</tr>
<tr>
<td>A. philippi</td>
<td>AHP</td>
<td>Juan Fernandez fur seal</td>
</tr>
<tr>
<td>A. galapagoensis</td>
<td>AGL</td>
<td>Galapagos fur seal</td>
</tr>
<tr>
<td>A. australis</td>
<td>AA</td>
<td>South American fur seal</td>
</tr>
<tr>
<td>A. townsendi</td>
<td>ATO</td>
<td>Guadalupe fur seal</td>
</tr>
<tr>
<td>A. pusillus pusillus</td>
<td>APP</td>
<td>Cape fur seal</td>
</tr>
<tr>
<td>A. p. doriferus</td>
<td>APD</td>
<td>Australian fur seal</td>
</tr>
<tr>
<td>Callorhinus ursinus</td>
<td>CU</td>
<td>Northern fur seal</td>
</tr>
<tr>
<td>Eumetopias jubatus</td>
<td>EJ</td>
<td>Steller’s sea lion</td>
</tr>
<tr>
<td>Zalophus californianus</td>
<td>ZC</td>
<td>Californian sea lion</td>
</tr>
<tr>
<td>Otaria byronia</td>
<td>OB</td>
<td>Southern sea lion</td>
</tr>
<tr>
<td>Neophoca cinerea</td>
<td>NC</td>
<td>Australian sea lion</td>
</tr>
<tr>
<td>Phocarctos hookeri</td>
<td>PH</td>
<td>Hooker’s sea lion</td>
</tr>
<tr>
<td>Phoca vitulina</td>
<td>PV</td>
<td>Harbor seal</td>
</tr>
</tbody>
</table>

$^a$ Haplotypes were selected from these species to exhibit the range of variation known to occur within that species based on previous studies (Lento, 1995; Lento et al., 1994; Wynen et al., 2000; Goldsworthy et al., 2000).
A 25-μl reaction volume consisted of 17.775 μl milliQ water, 0.125 μl 10 mM dNTPs, 1.5 μl 25 mM MgCl₂, 2.5 μl 10× buffer (500 mM KCl, 100 mM Tris pH 9.0, 1% Triton X), 1.0 μl each of 10 μM primers, 0.1 μl Taq polymerase (5–10 units), 1.0 μl extracted DNA, and was overlaid with oil. Amplifications were conducted under the following conditions: 1 cycle of 94°C for 2 min; 8 cycles of 94°C for 30 s, 48°C for 15 s, 72°C for 40 s; 25 cycles of 94°C for 15 s, 52°C for 15 s, 72°C for 40 s; and 1 cycle of 25°C for 1 min. The primers used were adapted from Lento et al. (1994): Cyb2 and B-Glu-L (but without 5′-biotinylation).

### Table 2
Details of Sequences Used for Phylogenetic Analysis and Intraspecific Variation for Partial Cytochrome b (360 bp) and Control Region (356 bp) Sequences

#### A. Cytochrome b

<table>
<thead>
<tr>
<th>Taxa</th>
<th>ID</th>
<th>OTU</th>
<th>Hap.</th>
<th>π</th>
<th>Var.</th>
<th>Ts 1</th>
<th>2</th>
<th>3</th>
<th>Tv 1</th>
<th>2</th>
<th>3</th>
<th>Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGZ 1-5×</td>
<td>5</td>
<td>5</td>
<td>0.012</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0 AF38078-882</td>
<td></td>
</tr>
<tr>
<td>ATR 1-5×</td>
<td>5</td>
<td>5</td>
<td>0.007</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0 AF38083-887</td>
<td></td>
</tr>
<tr>
<td>AF 1-5×</td>
<td>5</td>
<td>5</td>
<td>0.023</td>
<td>15</td>
<td>12</td>
<td>0</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>2 U12837, U12839, U12841, U18537-38</td>
<td></td>
</tr>
<tr>
<td>APH 2-3, 5×</td>
<td>3</td>
<td>3</td>
<td>0.009</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0 AF38093-895</td>
<td></td>
</tr>
<tr>
<td>AGL 1-3×</td>
<td>3</td>
<td>1</td>
<td>0.000</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 AF380898-900</td>
<td></td>
</tr>
<tr>
<td>AA 1-5×</td>
<td>5</td>
<td>2</td>
<td>0.015</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0 AF380901-905</td>
<td></td>
</tr>
<tr>
<td>ATO 5-6×</td>
<td>2</td>
<td>1</td>
<td>0.000</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 AF380906-897</td>
<td></td>
</tr>
<tr>
<td>APP 1-5×</td>
<td>5</td>
<td>5</td>
<td>0.006</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0 U18448-52</td>
<td></td>
</tr>
<tr>
<td>APD 2-4×</td>
<td>3</td>
<td>2</td>
<td>0.002</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0 AF380916-918</td>
<td></td>
</tr>
<tr>
<td>CU 1-5×</td>
<td>5</td>
<td>3</td>
<td>0.006</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1 AF380888-92</td>
</tr>
<tr>
<td>Ej 1-5×</td>
<td>5</td>
<td>2</td>
<td>0.001</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 AF380920-924</td>
</tr>
<tr>
<td>ZC 10-11×</td>
<td>2</td>
<td>1</td>
<td>0.000</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 D26524, X82310</td>
<td></td>
</tr>
<tr>
<td>OB 1-5×</td>
<td>5</td>
<td>2</td>
<td>0.000</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 AF380906-910</td>
<td></td>
</tr>
<tr>
<td>NC 1-5×</td>
<td>5</td>
<td>1</td>
<td>0.000</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 AF380901-915</td>
<td></td>
</tr>
<tr>
<td>PH 6, 7×</td>
<td>2</td>
<td>2</td>
<td>0.003</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0 U12851, AF380919</td>
<td></td>
</tr>
<tr>
<td>PV PV×</td>
<td>1</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>— X63726</td>
<td></td>
</tr>
<tr>
<td>Overall×</td>
<td>60</td>
<td>38</td>
<td>93</td>
<td>76</td>
<td>8</td>
<td>8</td>
<td>60</td>
<td>17</td>
<td>5</td>
<td>0</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

Note. The identifying (ID) number of each individual sequence and its origin are presented, along with the total number of operational taxonomic units (OTUs), haplotypes (Hap.), and the nucleotide diversity (π) for each species and both regions. The number of variable sites (Var.) are also presented, including the number of transition (Ts) or transversion (Tv) mutations. For cytochrome b, the numbers of these mutations occurring at each codon position (1, 2, or 3) are also given. For the control region, the range of sequence lengths prior to alignment are also presented. Taxa are abbreviated according to their scientific names and are as described in Table 1.

* Sequenced as part of this study; † Sequenced as part of Wynen et al., 2000; ♦ Genbank (Lento et al., 1997); ♦ Lento, 1995; ♦ Goldsworthy et al., 2000; † Sequenced as part of this study by Rus Hoelzel; ♦ Genbank (Maldonado et al., 1995); ♦ Genbank (Masuda and Yoshida, 1994); ♦ Genbank (Arnason et al., 1995); ♦ Genbank (Arnason and Johnsson, 1992). Genbank accession numbers are listed where relevant; * Overall values presented for ingroup taxa only.
conducted using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Palo Alto, CA). The products were run through a 6 M urea/5% acrylamide gel (FMC Long Ranger Singel) on an ABI Prism 377 DNA sequencer (PE Applied Biosystems). The primers employed for sequencing the regions were the same as used for amplification. Each fragment was sequenced initially from the 5’ end and only sequenced from the 3’ end if the first sequence was too short and/or contained too many ambiguous sites. The sequenced product of the trNA\(^{\text{mcr}}\)-control region corresponds to sites 16342–16680 of the Gen Bank sequence for Phoca vitulina (accession number X63726; Arnason and J ohnsson, 1992). The cytochrome b region employed corresponds to sites 15094–15454 of the same P. vitulina sequence.

Data Analysis

Sequences were examined using Seqed (version 1.0.3; Applied Biosystems, Foster, CA) to ascertain quality and to verify scoring. Sequences were aligned using CLUSTAL W (Thompson et al., 1994) and the resulting alignments were evaluated by eye and corrected where required. Intra- and interspecific sequence statistics were calculated using DnaSP version 2.2 (Rozas and Rozas, 1997). These data included the number of polymorphic sites, nucleotide diversities, and divergences. Nucleotide diversities (\(\pi\)) and nucleotide divergences (\(D_i\)) were calculated from Nei (1987; Eqs. 10.5 and 10.21, respectively).

The phylogenetic relationships of the family were examined primarily using cytochrome b sequence data, as it has been shown to be useful for the inference of intraspecific to intergeneric relationships (Kocher et al., 1989). Prior to analysis, the presence of a hierarchical structure in the data was determined using PAUP* version 4.0ba (Swofford, 1999). A frequency distribution of 500,000 randomly generated, equiprobable trees was plotted and the g\(_2\) score determined (Hillis and Huelsenbeck, 1992). This sample statistic (an estimate of the population parameter \(\gamma_1\)) was used to test for asymmetry by comparing it with the critical values in Table B.22 in Zar, (1996).

Subsequently, three methods of reconstructing phylogenetic relationships were employed: the maximum parsimony (MP), maximum likelihood (ML), and neighbor-joining (NJ) methods. All tree reconstructions proceeded without a specified root, with P. vitulina nominated as the outgroup post priori. This species was chosen as it is from the Phocidae, a sister family to the Otariidae (Vrana et al., 1994).

MP analysis was conducted in PAUP*. A full heuristic search was conducted with 100 random stepwise additions (five trees held over at each replicate) and accelerated character transformation (ACCTRAN), and by employing the tree bisection-reconnection (TBR) branch-swapping algorithm. Where a consensus tree needed to be generated, it was either by strict consensus or in accordance to the 50% majority rule criteria. Bootstrap analysis employing the full heuristic search conditions as described above was conducted with 2000 replications. This analysis was repeated for a range of weighting schemes that were employed to investigate whether different mutational models might better reflect the relationships. These include (1) all mutations unweighted; (2) transversion (Tv) mutations weighted as the reciprocal of the transition (Ts)/transversion ratio (Ts/Tv), which in this case equalled 7; (3) using the same weighting scheme as described in (2) but only at the 3rd codon position; (4) excluding all Ts mutations; or (5) excluding all 3rd codon positions.

NJ trees were reconstructed in MEGA version 1.01 (Kumar et al., 1993) using the algorithm of Saitou and Nei (1987), and based on distances calculated using Kimura’s two-parameter model (Kimura, 1980). A bootstrap analysis was performed on each of the resulting trees, and values were obtained after 2000 replications. A standard error test was also performed (Rzhetsky and Nei, 1992, 1993) to examine the significance of the interior branch lengths. Sequences for reconstructing NJ trees include all nucleotides or Tv changes only.

The ML analyses were conducted in PHYLIP 3.57c (Felsenstein, 1993) using DNAML. All trees were reconstructed with global rearrangements and 10 randomized additions, and without a specified outgroup. The Ts/Tv ratio was varied until the maximum log likelihood value was obtained. The analysis was also conducted with three independent rates of mutation specified, 0, 1, and 2. Trees were also reconstructed using DNAMLK, where the ML method is employed as in DNAML, but under the constraint of a molecular clock. Evidence for a molecular clock is then examined by comparing trees from DNAML and DNAMLK using the likelihood ratio test as described in the DNAMLK notes (Felsenstein, 1993). A relative rate test was also employed to test for molecular clock-like sequence evolution (the two-cluster test as implemented in LINTRE, Takezaki et al., 1995).

The control region data were also used to examine the phylogenetic relationships within the Otariidae. However, the rapid mutation rate of this region suggests that it is more suitable for the inference of relationships at the terminal nodes, rather than at the internal nodes. Therefore, these data were employed to examine those major clades observed in the phylogenies based on cytochrome b in greater detail. Phylogenetic analysis proceeded using the same methods as described above. Characters were treated the same as specified in (1) and (4) above, as well as in (2) but with Ts/Tv = 4. In all cases, alignment gaps were removed prior to analysis, except for one MP analysis where gaps were treated as a 5th character state.

Phylogenetic analysis using all three above-men-
tioned methods with unweighted characters was conducted with both the cytochrome b and control region combined. A partition homogeneity test was conducted using PAUP* prior to further analysis to ascertain phylogenetic congruence. A heuristic search was employed, with 100 random stepwise additions and accelerated character transformation (ACCTRAN), and with the tree bisection–reconnection (TBR) branch swapping algorithm. Results were obtained after 1000 homogeneity replicates.

RESULTS

A total of 61 cytochrome b sequences were obtained for analysis, and a further 64 from the control region. All taxa were represented in both data sets, with the exception of A. p. pusillus for which there were no control region sequences. There were large variations in sequence length observed in the control region (276 to 298 bp for the ingroup taxa; up to 339 bp for the outgroup taxon; Table 2B) making sequence alignment problematic. The alignment between A. forsteri and P. vitulina in Slade et al. (1994) was used as a guide. The final aligned “array” was 356 bp. There were no alignment gaps required for the 360 bp of the cytochrome b region used for analysis.

Details regarding intraspecific genetic variation for both cytochrome b and control region, and interspecific nucleotide divergences for cytochrome b only, are presented in Tables 2 and 3, respectively. There is considerable variability in nucleotide diversities within species. Neophoca cinerea showed no variation in either cytochrome b or the control region, while the maximum nucleotide diversities for each region were 2.3% (A. forsteri, Table 2A) and 7.8% (A. australis, Table 2B), respectively. The two species that showed very high nucleotide divergences for the more conserved cytochrome b region (A. forsteri 2.3%, A. australis 1.5%) each contained two highly divergent clades. The pairwise divergence between these intraspecific clades (0.031 and 0.025, respectively) were larger than those observed between A. forsteri, A. australis, and A. ga
apagoensis (0.008–0.017, mean 0.011; Table 3). These intraspecific divergences are also much larger than observed between the subspecies A. p. pusillus and A. p. doriferus (0.002).

The above results and some preliminary phylogenetic analyses based on cytochrome b revealed a number of relationships requiring further attention. A two-tiered approach to analysis was thus adopted to investigate these relationships. First, the more conserved cytochrome b region was employed to investigate all relationships within the family. The control region data were then included to try and improve the resolution of these relationships. The inclusion of the two regions together necessitated a rationalization of the dataset, as only individuals for which both regions had been sequenced could be included. Further, to adequately investigate the phylogenetic relationships of the family based on the cytochrome b data, such a rationalization was required to ensure the successful completion of the more demanding analyses, such as the bootstrap analyses. Second, a more focused approach was undertaken with a number of taxa where intraspecific relationships were obscuring the interspecific relationships. Therefore, further phylogenetic analyses were conducted on these and closely related taxa, encompassing as many individuals as possible.

### TABLE 3

Cytochrome b Data—Pairwise Comparisons of the Number of Substitutions (above Diagonal) and the Nucleotide Divergence (D'c) (below Diagonal)

|    | AGZ | AA | AF | AGL | APH | ATO | ATR | APD | APP | CU | EJ | ZC | OB | NC | PH |
|----|-----|----|----|-----|-----|-----|-----|-----|-----|-----|----|----|----|----|----|----|
| AGZ | 11  | 9  | 16 | 17  | 18  | 15  | 20  | 18  | 35  | 23  | 23 | 23 | 17 | 21 | 17 |
| AA  | 0.038 | 0  | 2  | 13  | 14  | 12  | 17  | 17  | 35  | 19  | 21 | 17 | 21 | 13 |  |
| AF  | 0.034 | 0.008 | 4  | 13  | 14  | 13  | 18  | 17  | 35  | 20  | 20 | 16 | 20 | 13 |  |
| AGL | 0.049 | 0.009 | 0.017 | 16 | 18  | 19  | 25  | 24  | 42  | 22  | 24 | 23 | 25 | 18 |  |
| APH | 0.055 | 0.041 | 0.045 | 0.046 | 1  | 21  | 26  | 24  | 34  | 24  | 25 | 24 | 23 | 16 |  |
| ATO | 0.057 | 0.044 | 0.046 | 0.050 | 0.004 | 22  | 27  | 25  | 38  | 26  | 29 | 25 | 25 | 17 |  |
| ATR | 0.049 | 0.039 | 0.040 | 0.054 | 0.062 | 0.063 | 5  | 4  | 29  | 18  | 20 | 17 | 21 | 17 |  |
| APD | 0.063 | 0.052 | 0.056 | 0.070 | 0.074 | 0.076 | 0.017 | 0  | 33  | 23  | 26 | 19 | 25 | 20 |  |
| APP | 0.059 | 0.050 | 0.052 | 0.067 | 0.070 | 0.071 | 0.014 | 0.022 | 31  | 22  | 25 | 19 | 23 | 20 |  |
| CU  | 0.105 | 0.107 | 0.105 | 0.121 | 0.100 | 0.110 | 0.089 | 0.094 | 0.092 | 34  | 33 | 36 | 33 | 37 |  |
| EJ  | 0.066 | 0.058 | 0.062 | 0.061 | 0.070 | 0.072 | 0.052 | 0.065 | 0.061 | 0.099 | 19  | 27 | 27 | 24 |  |
| ZC  | 0.069 | 0.063 | 0.061 | 0.067 | 0.071 | 0.081 | 0.058 | 0.073 | 0.070 | 0.096 | 0.054 | 22 | 28 | 23 |  |
| OB  | 0.055 | 0.054 | 0.049 | 0.066 | 0.069 | 0.071 | 0.051 | 0.055 | 0.055 | 0.104 | 0.077 | 0.063 | 25 | 20 |  |
| NC  | 0.065 | 0.063 | 0.064 | 0.069 | 0.066 | 0.060 | 0.061 | 0.069 | 0.066 | 0.096 | 0.075 | 0.078 | 0.073 | 20 |  |
| PH  | 0.056 | 0.040 | 0.041 | 0.050 | 0.045 | 0.047 | 0.048 | 0.056 | 0.055 | 0.107 | 0.067 | 0.064 | 0.059 | 0.056 |  |
| PV  | 0.157 | 0.150 | 0.146 | 0.156 | 0.163 | 0.172 | 0.148 | 0.150 | 0.152 | 0.148 | 0.163 | 0.156 | 0.149 | 0.150 | 156 |

Note. Taxa are abbreviated according to their scientific names and are as described in Table 1.
The following criteria were used when removing individuals prior to more in-depth phylogenetic analysis. Where no intraspecific variation was observed, only one individual was included for the species for the phylogenetic analysis (e.g., *Neophoca cinerea*). Where highly divergent lineages were evident within some species (e.g., *A. forsteri, A. australis*), the two most divergent individuals for these species was included for analysis. This ensured that the range of detected genetic variation for that species as sampled in this study was encompassed in the analyses. A total of 27 and 28 operational taxonomic units (OTUs) were used for analyses based on cytochrome *b* and control region, respectively.

It has been demonstrated that the addition of taxa improves the accuracy of the phylogenetic tree (Wheeler, 1992; Graybeal, 1998). The removal of the above taxa in this case could be interpreted as a reduction in the accuracy of the resulting phylogenies. However, Graybeal (1998) also suggested that the addition of taxa is particularly beneficial if these taxa “break up long branches” and with the only taxa removed in this study being those that contain little or no additional phylogenetic information, the reduction in phylogenetic accuracy is likely to be minimal. Where the problems arise with the highly divergent taxa, the more focused analysis serves to overcome these.

Only individuals for which both cytochrome *b* and control region sequences had been obtained were used for the combined region analyses. There are two exceptions: one OTU each for *Z. californianus* and *A. townsendi*. Because Maldonado et al. (1995) found no variation in 368 bp of the same cytochrome *b* region of 40 *Z. californianus* individuals, the combination of the regions from unknown individuals was not considered problematic in this case. The *A. townsendi* OTU (*ATO45*) resulted from the control region sequence of *ATO4* and cytochrome *b* sequence of *ATO5*. While this is not ideal, this combined sequence was used only for subsequent phylogenetic analyses aimed at better resolving relationships of some fur seal clades and not for any intraspecific analysis. A total of 26 OTUs were used for these analyses where both control region and cytochrome *b* sequences were combined.

Phylogenetic Analysis—Cytochrome *b*

Of the 360 bp of the cytochrome *b* gene available for analysis, 112 sites were variable and 86 were parsimony informative where the outgroup taxon is included. Excluding this taxon, these figures change to 93 and 86, respectively (Table 2A). The frequency histogram of tree lengths from the 500,000 randomly generated trees was significantly skewed ($q_l = 0.75634$; 112 variable sites; $P < 0.01$), indicating the presence of hierarchical structure within the data.

The phylogenetic relationships of the Otariidae as
inferred by the cytochrome b region employing the MP and ML methods are displayed in Fig. 2. The tree in Fig. 2A was obtained using the MP method using unweighted characters and is the 50% majority rule consensus representation of the 260 most parsimonious trees. The tree length (TL) was 221 steps, with a consistency index (CI) of 0.593, and a retention index (RI) of 0.720. A series of major fur seal and sea lion clades with medium to high levels of bootstrap support were identified, and these are labeled 1–5 for fur seals and a–d for sea lions (Fig. 2). A strict consensus representation is also presented (Fig. 2B) and shows how these major clades were maintained in all of the 260 MP trees, as well as the maintenance of the basal positions of both C. ursinus and N. cinerea.

Further MP analyses proved less revealing with an overall reduction in resolution. When Tv and Ts were weighted as 7:1 (Ts/Tv = 7), both overall and at 3rd codon positions only, 12 most parsimonious trees were obtained in both cases (TL = 413; CI = 0.680; RI = 0.740 and TL = 377; CI = 0.650; RI = 0.737). The two consensus topologies were identical to each other and were very similar to that shown in Fig. 2A, with the same major clades (as described above) being recognized in all cases. The differences between these MP trees and that in Fig. 2A lay in how the major clades were related. This reflects the poor resolution between these major clades that was consistently observed throughout our analyses. Even less support was observed for these relationships when the phylogeny was reconstructed based on the sequences excluding 3rd codon positions (no. sites = 240, no. parsimony sites/variables sites = 19/31, and 19/21 excluding the outgroup). Eighteen most parsimonious trees were obtained (TL = 46, CI = 0.696, RI = 0.791), but with about 79% of Ts and 71% of Tv occurring at 3rd codon positions, there was insufficient variation remaining to resolve relationships (frequency histogram of 400,000 randomly generated trees was not significantly skewed, $g_s = 0.453965$, 31 variable sites, $P > 0.2$). While our overall sequence was quite short, Kallersjo et al., (1999) also noted that despite popular belief, the 3rd codon positions contain most of the phylogenetic structure in the data set, and does not recommend their exclusion. Three most parsimonious trees were obtained using only Tv mutations (TL = 30; CI = 0.833; RI = 0.839), but again, there was insufficient variation in this dataset to be of use in resolving relationships of the taxa (no. sites = 25 or 16 excluding the outgroup; frequency histogram of 400,000 randomly generated trees was not significantly skewed, $g_s = 0.648180$, 25 variable sites, $P > 0.1$).

The reconstruction of phylogenetic relationships employing the ML method produced a range of topologies that were obtained using different weighting schemes. The tree that had the highest ML value (Ts/Tv ratio = 6, In likelihood = −1633.10) is presented in Fig. 2C. The major fur seal and sea lion clades identified in the MP analyses were also identified here. All topologies obtained through ML analysis contained all of these major clades, with the differences between topologies lying in the relationships of these clades to each other.

Similarly, NJ topologies showed all of the major fur seal and sea lion clades noted in Fig. 2. Some differences were observed in the relationships between these clades, but these differences occurred where there was very poor bootstrap support (ranging from 20 to 41%). The standard error test showed that the interior branch lengths that were significant (i.e., where the confidence probability, CP > 0.95 or 0.99) corresponded to those nodes where the bootstrap support was 92 or greater. These nodes are the same nodes in Fig. 2A with bootstrap support greater than 91. As found for MP analysis, the NJ tree based on the exclusion of 3rd codon positions resulted in very poor resolution.

Overall, the best resolved topologies showed the highly supported basal position of C. ursinus, and the maintenance of the major fur seal and sea lion clades identified in Fig. 2. The position of N. cinerea basal to the rest of the fur seal and sea lion taxa was also maintained, albeit with less bootstrap support. The very high bootstrap support for many of the major clades is significant, because it was noted in a study by Cummings et al. (1995), that while phylogenies obtained from data sets with small numbers of nucleotides (such as is the case in this study) infrequently reflect the true phylogeny of the genome, clades with bootstrap support >95% in the former are usually present in the latter. The major differences observed between topologies came in the relationships between the remaining major clades. There were also discrepancies in the internal arrangements of both the A. australis/f forsteri/ galapagoensis clade (clade 3) and the A. philippi/townsendi clade (clade 2), which were investigated further as described below.

The topologies created by DNAML and DNAMLK for Ts/Tv = 6 were not identical, and therefore the likelihood ratio test as described by Felsenstein (1993) could not be used. Instead, the relative rate test of Takezaki et al. (1995) was employed, and no significant differences in the relative mutation rates of all the sequences was observed ($Q = 22.23; 0.5 < P < 0.75$). A linear regression analysis was conducted of genetic divergence (calculated using the cytochrome b data) vs estimated time of divergence based on the fossil record. The dates used were of the proposed divergences: Phoca vitulina (representing Phocidae) from Callorhinus (23 mya); Phoca vitulina from the sea lion and Arctocephalus taxa (23 mya); Callorhinus from the sea lion and Arctocephalus taxa (6 mya); and sea lion taxa from Arctocephalus taxa (3 mya) (Kim et al., 1975; Repenning et al., 1979; Miyazaki et al., 1994; Slade et al., 1994). While the analysis showed a significant relationship ($R^2 = 0.868, P < 0.000$), the 95% confidence
limits were very large, and little faith could be placed in estimating the time of divergence using the line of regression, especially for those divergences close to the time of interest (i.e., 3 mya). Further, these data assume the monophyly of the sea lion and Arctocephalus groups, and our analysis has shown that this assumption is not valid. Therefore, estimating times of divergence from this regression would not be valid.

Phylogenetic Analysis—Control Region

A 356-bp “array” of the 5’ end of the control region was used for analysis, with 162 variable sites and 117 of these being informative (outgroup taxon included). The removal of alignment gaps and missing information left 253 bp, with 118 variable sites and 89 parsimony informative sites (outgroup taxon included). These figures changed to 106 and 89, respectively, when the outgroup was excluded (Table 2B). Evidence of hierarchical structure was observed as the histogram of the 500,000 random trees was skewed (g5 = 0.7937; 158 variable sites; P < 0.001).

MP analysis of the control region data returned a single most parsimonious tree of 442 steps (CI = 0.500; RI = 0.589). A single most parsimonious tree was also found when alignment gaps were treated as a fifth character (TL = 613; CI = 0.520; RI = 0.584). The two topologies differed greatly from those obtained using cytochrome b, as the bootstrap support for internal nodes were extremely low (3–37), with most being below 10 (trees not shown). The two least supported of the major clades observed in Fig. 2A (bootstrap values <70%) were split: E. jubatus from Z. californianus; and A. galapagoensis from A. forsteri/A. australis. Similar results were observed in the topologies that were created using the NJ algorithm. The phylogeny with the highest ML value was obtained for a Ts/Tv ratio of 3 (ln likelihood = −2221.45). This topology retained all the major clades except that of E. jubatus/Z. californianus.

Phylogenetic Analysis—Cytochrome b and Control Region

A partition–homogeneity test was performed on the combined cytochrome b–control region data and it was found that the null hypothesis of homogeneity within the data set could not be rejected (P = 0.482). As expected, there was evidence of structure within these data (g5 = −1.034; 265 variable sites; P < 0.001).

Three most parsimonious trees were found (TL = 654; CI = 0.53; RI = 0.602), and the consensus topology (obtained through 50% majority rule criteria) is presented in Fig. 3. All of the major fur seal and sea lion clades are recognized with medium to high level of bootstrap support, and all of the branch lengths leading to these major clades in the NJ tree were significant when tested with the standard error test. However, while the combining of data sets has been reported to increase internal support in topologies (Sol-tis et al., 1998), as well as increasing the probability of reflecting the true whole genome tree (Cummings et al., 1995), there are still difficulties in resolving the internal relationships of these major clades for our data.

The results from Figs. 2 and 3 reveal some interesting and inconclusive relationships between fur seal taxa, e.g., between A. australis, A forsteri, and A. galapagoensis or A. philippii and A. townsendi (clades 3 and 2, respectively). To better investigate these relationships, all of the individuals available for these taxa and those of A. gazella (clade 1) were incorporated into an analysis based on combined control region/cytochrome b sequence data. All three methods of tree reconstruction were used, and the results are presented in Fig. 4. Greater resolution is observed for relationships between species with the inclusion of the control region data and additional individuals. However, the relationships of the divergent lineages within A. forsteri and A. australis to each other and to A. galapagoensis remain poorly resolved. In all cases, A. gazella is shown to be a sister taxon to these three species. The relationships of A. philippii and A. townsendi are better resolved apart from the position of one A. philippii lineage (APH2). Either the lineage is included with the remaining A. philippii lineages (as loosely supported in the ML and NJ trees: Figs. 4A and C), or as a sister taxon to both species (see MP tree: Fig. 4B). Those topologies reconstructed from the control region data alone (not presented here), give greater support to A. philippii being distinct from A. townsendi, while still being retained in the same clade (bootstrap support to each monophyletic group of 60 and 96, respectively, after 2000 replicates using MP).

Although further investigation into the close relationship of A. p. doriferus and A. p. pusillus is required, this was not pursued in this study due to the absence of control region sequences for the latter species. No further improvement in resolution of sea lion taxa (clades a–d, Figs. 2 and 3) was possible with the data currently in hand. Additional, more conserved markers, e.g., ND2, ND5, and/or a nuclear gene are required to adequately investigate these relationships.

DISCUSSION

Phylogenetic Relationships

This study presents molecular data for investigation into the phylogenetic relationships within the family Otaridae. We found no support for the recognition of two subfamilies containing the fur seals (Arctocephalinae) and sea lions (Otarinae). The fur seal Callorhinus ursinus was found to be basal to the remaining fur seal and sea lion taxa in the family, a relationship that received high bootstrap support across all analytical methods employed. This supports evidence from the fossil record that suggests Callorhinus diverged from
the line leading to extant sea lion and Arctocephalus fur seal species about 6 mya (Miyazaki et al., 1994). While some studies argue that morphological data support our findings by suggesting that Callorhinus is distinct compared to the rest of the family (Brunner, 2000; Repenning et al., 1971), others show findings to the contrary (Berta and Demere, 1986). A further study, based on a compilation of available morphological, fossil, and molecular data indicated that neither the basal position of Callorhinus nor the monophyly of Arctocephalinae could be assured (Bininda-Emonds et al., 1999).

This study presented some evidence to refute the monophyly of sea lions, a relationships that has been claimed elsewhere (Kim et al., 1975; Morejohn, 1975; Berta and Demere, 1986; Bininda-Emonds et al., 1999). We identified four major sea lion clades and four southern fur seal clades, all of which received high levels of bootstrap support. The relationships between these clades consistently infer polyphyly for sea lions and fur seals, although there was very little support for the deep internal nodes. We found that divergences between sea lion taxa are large (Da = 0.054–0.078, mean = 0.067; Table 3), with the average being similar to that between the sea lion group and Arctocephalus (Da = 0.061). Therefore, if there was a single sea lion

---

**FIG. 3.** Results of phylogenetic analysis of 15 species of the family Otariidae, inferred from combined cytochrome b/control region sequences and using the MP algorithm. The MP tree was obtained through 50% majority rule consensus from 3 MP trees (TL = 654; CI = 0.53, RI = 0.602). Figures at the nodes indicate bootstrap values obtained after 2000 replications, and those in brackets show the percent agreement for that node by the 3 MP trees. * indicates where branch lengths are significant based on the NJ tree. As for Fig. 2, major fur seal clades are numbered from 1 to 5, and major sea lion clades are marked a–d.
ancestor as proposed by other studies, then our data imply that at the time of divergence from the Arctocephalus line about 3 mya, there was a rapid radiation within the sea lion group that resulted in the five extant monotypic genera. Such a rapid radiation and/or population expansion has made the resolution of relationships at these deep nodes difficult using the markers employed in this study. Nonetheless, the phylogeny presented here (with respect to the sea lion taxa) is very similar to that presented by Barnes et al. (1985), with the exception of the relatively close relationship between the northern sea lions, Eumetopias and Zalophus, identified here. Neither was this relationship evident in other phylogenies (Morejohn, 1975; Bininda-Emonds et al., 1999). Rather, it was between Neophoca and Phocarctos that the closest intergeneric relationship was observed (Morejohn, 1975; Barnes et al., 1985; Bininda-Emonds et al., 1999). Although the pairwise divergence between these species is similar to that between Eumetopias and Zalophus (0.056 and 0.054, respectively), this is not reflected in the phylogenies presented here (Figs. 2 and 3).

A sister–taxon relationships for Arctocephalus pusillus and A. tropicalis was consistently observed in our analyses. This is interesting because A. pusillus has often been regarded as phenotypically intermediate between the southern fur seals and sea lions on the basis of its behavior, size, vocalization, and morphology (Repenning et al., 1971; Stirling and Warneke, 1971; Trillmich and Majluf, 1981; Goldsworthy et al., 1997). There is no evidence in the molecular data that the phenotypic intermediacy of A. pusillus reflects a close phylogenetic affinity with any sea lion lineage. Berta and Demere (1986) shows a similar close relationship, although A. gazella was also found to make up the third taxon of a trichotomy. In our study, however, A. gazella appeared to be more closely related to the A. australis/A. galapagoensis/A. forsteri clade (Fig. 4).

The relationship between A. pusillus and A. tropicalis is inconsistent with the study by Lento et al. (1997) which showed reciprocal paraphyly of haplotypes between A. tropicalis and A. gazella. The samples used in that study were obtained from the small population at Macquarie Island, where hybridization has been reported between these species (Shaughnessy et al., 1988; Goldsworthy et al., 1999). In the current study, we found a large number of species-specific nucleotide differences in cytochrome b between A. gazella and A.

**FIG. 4.** Phylogenetic relationships of six species of the genus Arctocephalus from clades 1, 2, and 3 in Figs. 2 and 3 with Callorhinus ursinus as outgroup. All trees were reconstructed using combined cytochrome b/control region sequence data. Details for each are as follows: (A) ML phenogram obtained where the Ts:Tv ratio was 8, (B) MP tree obtained through 50% majority rule from 40 MP trees (TL = 355; CI = 0.586; RI = 0.825) (C) NJ tree; figures at the nodes of trees B and C indicate bootstrap values obtained after 2000 replications. Labels as listed in Table 1.
tropicalis (n = 15). When we compare the sequences from Lento et al. (1997) with those in this study, their A. tropicalis samples show haplotypes identical to our A. gazella samples. Further, these Macquarie Island samples were also used by Lento (1995) to obtain control region sequences. These sequences were subsequently compared with a larger study of 248 individuals of both species (Wynen et al., 2000). Species-specific haplotypes were observed in the control region, and again, the A. tropicalis samples of Lento (1995) were found to have haplotypes identical to those of A. gazella. It is therefore probable that these A. tropicalis samples from Macquarie Island were from hybrid individuals. As such, this interspecific hybridization might have affected the composite phylogeny produced by Bininda-Emonds et al. (1999) whose study incorporated the phylogeny of Lento et al. (1995).

The phylogenetic relationships as inferred in Figs. 2, 3, and 4 suggest a close relationship between A. philippii and A. townsendi (bootstrap values of 100). The interspecific divergence between these species was extremely low (D_s = 0.004, Table 3), and is similar to that observed between the subspecies A. pusillus pusillus and A. p. doriferus (D_s = 0.002, Table 3). However, these results differ from those of some other studies which propose that A. philippii is more closely related to the A. australis/A. forsteri/A. galapagoensis group than to A. townsendi (Berta and Demere, 1986; Bininda-Emonds et al., 1999). Prior to these studies, the close relationship between A. townsendi and A. philippii had been recognized to the extent where it had been proposed that they be classed as subspecies (Scheffer, 1958) or placed together into the separate genus Arctophoca (Sivertsen, 1954). This view is supported by a recent taxonomic review of the family by Brunner (2000). However, Repenning et al. (1971) retained them as separate species based on skull morphology, but conceded that this conclusion was based on a small sample size. Our results are also from a small sample size, and thus a reassessment of the taxonomic position of A. philippii and A. townsendi would require a greater sampling effort encompassing all populations of both species.

Highly divergent lineages were observed within both A. forsteri (D_s = 0.031; 11 fixed differences) and A. australis (D_s = 0.025; nine fixed differences). The relationships of these lineages are poorly resolved, and while the inclusion of control region data and additional individuals provided greater support for the intraclade relationships (Fig. 4), there is still no evidence of monophyly of both A. australis and A. forsteri. Regional differences in A. forsteri had been previously reported by Shaughnessy (1970) based on transferrin types, and the divergent lineages within this species had also been reported by Lento et al. (1997), based on cytochrome b. The latter study suggested these lineages might be a result of two presealing populations: one extending from southern and western Australia to the east coast of New Zealand (which might be called A. forsteri forsteri), and the other primarily occurring in the subantarctic islands of New Zealand (A. f. snareensis). Lento et al. (1997) proposed that the current distribution and geographic overlap of haplotypes from each clade may be explained by secondary contact as the latter moved north and west, colonizing empty rookeries that were a result of sealing. A similar scenario might explain the two divergent lineages observed in A. australis. King (1983) notes that there is some evidence for the presence of two subspecies occurring within A. australis, with those occurring on mainland South America (A. australis gracilis) being smaller than those occurring at the Falkland Islands (A. a. australis). It is possible that the two subspecies are represented by the two divergent lineages found in this study. However, all of the A. australis skin samples obtained for this study came from Punta San Juan in Peru. So, if the lineages are representative of the subspecies, then like A. forsteri, it is possible that there is some secondary contact between these two populations. However, more extensive sampling is required for both species to better investigate these intraspecific questions. This is achieved not only through increasing the number of individuals sampled, but also by ensuring all populations throughout the entire geographic range are represented.

Repenning et al. (1971) had noted that the skull characters of many of the Arctocephalus species suggest a relationship to, and possible descent from, A. australis or an A. australis-like ancestor. The authors also suggest that there is a gradational series from A. australis to A. forsteri to A. gazella, although it was noted that it was unknown how this applied to phylogeny. Our phylogenetic analysis found no evidence that A. pusillus, A. tropicalis, A. philippii, or A. townsendi were direct descendants or close relatives of A. australis, but rather evolved from lineages that diverged at more or less the same time from the ancestral Arctocephalus line. However, Fig. 4 shows a relatively close relationship between A. australis, A. forsteri, and A. galapagoensis despite the poor resolution of these relationships. Further, it was also shown that A. gazella shared a common ancestor with this clade, suggesting that the gradational series above may be in some way indicative of the phylogenetic relationships of these taxa. The relatively close relationship of A. australis, A. forsteri, and A. galapagoensis was also noted through anatomical similarities (Brunner, 2000).

Biogeography—Current and Historical

While many relationships in the presented phylogenies remain unresolved, those that are supported are consistent with the proposed dispersal patterns of the Otariidae (Repenning et al., 1979) (Fig. 1). The northeast Pacific region is considered the center of origin of
the family, which evolved under temperate climatic conditions (Repenning et al., 1979; Miyazaki et al., 1994). However, two equatorial crossings and subsequent dispersal and radiation have led to a much broader geographical distribution of this family in recent times (Fig. 1). Callorhinus stayed in the Northern Hemisphere, but both the sea lion and Arctocephalus fur seal groups dispersed further afield.

Fossils found in Peru of seals ancestral to Arctocephalus suggest that this line had dispersed south along the coast of North and South America some time prior to 5 mya (Repenning, 1976). The average genetic divergence between the major fur seal clades (excluding Callorhinus) tends to be lower than observed between the sea lions (D_s = 0.040–0.069, mean = 0.047, cf. 0.054–0.078, mean = 0.067). Assuming a clock-like rate of mutation, as demonstrated with the cytochrome b data in this study, these results suggest that a similar radiation occurred in the fur seals as in the sea lions, but at a slightly later time. Such a radiation probably accompanied the far-reaching dispersals of fur seals throughout the southern ocean. The resulting broad distribution of the Arctocephalus fur seals (Fig. 1) is a reflection of their remarkable dispersal abilities as has been reported in the literature (e.g., Torres and Aguayo, 1984; Shaughnessy and Burton, 1986).

The common ancestor to A. tropicalis and A. pusillus probably moved around Cape Horn, and dispersed to the east facilitated by the West Wind Drift. Arctocephalus tropicalis colonized islands in the north subantarctic, while A. pusillus colonized southwest Africa. Recent migration events from Africa to Australia led to the subspecies, A. pusillus doriferus. The large geographical separation combined with the close genetic relationship of A. townsendi and A. philippii, make it difficult to speculate on the dispersal of their ancestral line. This is especially so given that the range of A. galapagoensis lies between the ranges of these two species. While it is likely that the Peru Current sweeping up the west coast of South America, bypassing the Juan Fernández Islands, would facilitate immigration to the Galapagos Islands from the south, it is difficult to hypothesize on the mechanisms leading to current distribution of A. philippii and A. townsendi, straddling that of a less related species. The remaining fur seal species, A. gazella, A. australis, A. galapagoensis, and A. forsteri appear to share a common ancestor (Fig. 4). The line leading to A. gazella dispersed into the subantarctic, probably colonizing islands to the south of South America and eventually throughout the southern ocean. A. forsteri and A. galapagoensis diverged later, as the former probably dispersed east or west to Australia and New Zealand.

Fossil evidence also suggests a major dispersal south by the sea lions about 3 mya, but with separate dispersals occurring on both the east and west sides of the Pacific (Repenning et al., 1979) (Fig. 1). Aside from suggesting that all sea lion genera diverged at approximately the same time, there is little our data can add with regards to dispersal patterns. Although the geographic proximity of Neophoca cinerea and Phocarctos hookeri would suggest a close relationship, there is no evidence of this in the data presented. The similar distributions of Eumetopias jubatus and Zalophus californianus might be a reflection of a more recent common ancestry, but this is speculative at best. While our molecular data allow some speculation as to the dispersal patterns of both the fur seals and sea lions, additional evidence from fossil deposits, for example, are required to substantiate these speculations. However, there are only a few such deposits from the appropriate time period documented at this stage.

**Taxonomic Considerations**

Some of the relationships inferred in this study through the use of molecular markers are inconsistent with current classification. This study raises concern as to how great the genetic distance should be between species, and between subspecies. For example, the divergence between A. pusillus pusillus and A. p. doriferus (D_s = 0.002) is very low, and yet subspecific classification for these taxa was only tentatively maintained by Repenning et al. (1971) based on one slight difference in skull morphology, and on geographic separation. The divergence between A. townsendi and A. philippii is comparable (D_s = 0.004), yet the retention of these groups as separate species on the basis of skull morphology is supported (Repenning et al., 1971). A contrasting scenario is evident within A. forsteri and A. australis, where genetic divergences between clades within each species are much larger (D_s = 0.031 and 0.025, respectively), even larger than observed between some species.

Clearly, such discussion regarding taxonomic classification and phylogenetic relationships can only proceed in light of all evidence presented. Such an attempt was made by Bininda-Emonds et al. (1999), but there was an absence of comprehensive molecular data for the family Otariidae for inclusion in the study. While the current study has presented the first comprehensive molecular study based primarily on one mitochondrial gene, the inference of phylogenetic relationships on the basis of a single locus is to be regarded with caution (Harpending et al., 1998). Further, the number of nucleotides used in the study were very low when compared to more extensive phylogenetic analyses in the literature. It has been demonstrated that increasing the number of characters improves phylogenetic accuracy and resolution (Wheeler, 1992; Graybeal, 1998; Soltis et al., 1998). This is especially so when many different loci are included from the genome (in this case, the mitochondrial genome) instead of using large blocks of sequence from the same gene (Cummins et al., 1995). Additional loci incorporated from
the nuclear genome would also provide further resolution.

The addition of sequence data from multiple loci is especially required for interpreting the phylogenetic relationships of the Otariidae given the rapid radiation events that appear to have occurred about 3 mya. While it has been suggested that the addition of taxa leads to greater improvements in phylogenetic accuracy relative to the addition of characters (Graybeal, 1998), this could be arguable in the case of the otariid data set because all extant species within the family are currently represented. What is likely to be more important for future analyses is the incorporation of all extant genetic variation for all of these species. While only a small sample size is sufficient to investigate interspecific relationships (Tajima, 1983), this is only on the proviso that the samples are randomly collected. With the broad geographic distribution of this family, and of some species in particular, a random sample is difficult to collect. It is imperative, however, that future studies make efforts to sample all populations within a species to cover all the intraspecific genetic variation, a necessary step for the evaluation of phylogenetic relationships within the family Otariidae.

ACKNOWLEDGMENTS

The authors thank all those people who assisted in the collection of samples required for the completion of this study, especially Giacomo Bernardi and Richard Campbell. Special thanks to Carl McIntosh at the National Zoological Park—Smithsonian Institution (NZP—SI) who was incredibly generous with his time coordinating samples, as well as Chris Burridge, Jesús Maldonado, Beth Slikas, Jack Dumbacher, Olaf Bininda-Emonds, and an anonymous reviewer for their valuable comments on the manuscript. This work was partially funded by the Antarctic Science Advisory Committee.

REFERENCES


