The Origin and Genetic Relationships of the Baikal Seal, Phoca sibirica, by Restriction Analysis of Mitochondrial DNA

Authors: Sasaki, Hiroyuki, Numachi, Ken-ichi, and Grachev, Mikhail A.

Source: Zoological Science, 20(11) : 1417-1422

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.20.1417

The BioOne Digital Library (https://bioone.org/) provides worldwide distribution for more than 580 journals and eBooks from BioOne's community of over 150 nonprofit societies, research institutions, and university presses in the biological, ecological, and environmental sciences. The BioOne Digital Library encompasses the flagship aggregation BioOne Complete (https://bioone.org/subscribe), the BioOne Complete Archive (https://bioone.org/archive), and the BioOne eBooks program offerings ESA eBook Collection (https://bioone.org/esa-ebooks) and CSIRO Publishing BioSelect Collection (https://bioone.org/csiro-ebooks).

Your use of this PDF, the BioOne Digital Library, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Digital Library content is strictly limited to personal, educational, and non-commmercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne is an innovative nonprofit that sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://complete.bioone.org/journals/Zoological-Science on 04 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use

ZOOLOGICAL SCIENCE **20**: 1417–1422 (2003)

The Origin and Genetic Relationships of the Baikal Seal, *Phoca sibirica*, by Restriction Analysis of Mitochondrial DNA

Hiroyuki Sasaki[1*], Ken-ichi Numachi[2] and Mikhail A. Grachev[3]

[1]*Graduate School of Fisheries, Hokkaido University, 3-1-1 Minato, Hakodate, Hokkaido 041-8611, Japan*
[2]*School of Marine Science and Technology, Tokai University, 3-20-1 Orido, Shimizu, Shizuoka 424-8610, Japan*
[3]*Limnological Institute, Russian Academy of Science, Irukutsk, Russia*

ABSTRACT—The origin and genetic relationships of the Baikal seal, *Phoca sibirica*, were studied by restriction fragment length polymorphism analysis of mitochondrial DNA (mtDNA). Using 17 different six-base recognition restriction endonucleases, we examined 98 Baikal seals, and two other related species, the ringed seal, *P. hispida*, (n=87), and the Caspian seal, *P. caspica*, (n=94). Analysis revealed the existence of 87 mtDNA haplotypes in the total of 279 specimens. The haplotypes of each species were divided into different clusters on a dendrogram obtained by UPGMA based on haplotype frequency and mtDNA base substitution. No common haplotypes were found among the species examined. The Baikal seal is much more closely related to the ringed seal than the Caspian seal. The amount of divergence suggested that an ancestor of the Baikal seal came down to the lake approximately 0.4 million years ago as was previously indicated by paleontological studies. The seals examined here showed lower variabilities.

Key words: Baikal seal, Caspian seal, ringed seal, molecular phylogeny, mitochondrial DNA polymorphisms

INTRODUCTION

The Baikal seal, *Phoca sibirica*, is the only species of phocids (true seals) distributed in a freshwater region. This species is endemic to Lake Baikal, located in the midst of Siberia far from the habitat of their marine relatives. The origin of this species has therefore received substantial attention. This species is considered a close relative of the Caspian seal, *Phoca caspica*, and the ringed seal, *Phoca hispida*, as classified as the subgenus *Pusa* (Bonner, 1989) or into a separate genus *Pusa* (Rice, 1998). The Baikal seal differs externally from the latter two species by its unspotted or rarely spotted appearance (Reeves *et al*., 1992). The Caspian seal, endemic to the Caspian Sea, is very similar externally to the ringed seal. However, its rings are sparser than on ringed seals, although a few specimens lack rings completely as the Baikal seal. The ringed seal is widely distributed in the circumpolar Arctic and subarctic coasts, and is classified into five subspecies – *P. h. hispida* in the Arctic basin, *P. h. ochotensis* in the Seas of Okhotsk and Japan, *P. h. saimensis* in Lake Saimaa, *P. h. ladogensis* in Lake Ladoga, and *P. h. botnica* in the Baltic Sea (Jefferson *et al*., 1993).

Several reports based on fossil records have been published to date on the origins of the Baikal seal (Kozhov, 1963; Ray, 1976; Repenning *et al*., 1979; Davies, 1958; MacLaren, 1960). The results of these studies present two primary hypotheses on the origins of the Baikal seal. Ray (1976) and Repenning *et al*. (1979) suggested that about 2.5 to 3 million years ago, when the Arctic Ocean extended below 61° north latitude, an ancestor of *Pusa* gained access to the Arctic Ocean from the Paratethys of southeastern Europe, and then about 300,000 years ago migrated to Lake Baikal via the Yenisey River. Alternatively, the *Pusa* group may have evolved along with the Baikal, Caspian and ringed seals from a common ancestor in the trace of the Paratethyan Basin, and then moved eastward through canals and lakes formed during glaciation. Some of these groups may have eventually settled in Lake Baikal, while the ancestor of the ringed seal continued northward and distributed over a wide range in the Arctic Ocean (Davies, 1958; MacLaren, 1960). The latter hypothesis suggests that the ancestor of the Baikal seal reached Lake Baikal much earlier than suggested by the former hypothesis.

Studies on the biochemical or molecular evolution of the Baikal seal have been rather scarce to date. There is no report on the differences between the closely related spe-

---

* Corresponding Author: Tel: +81-543-34-0411;
Fax: +81-543-34-9764.
E-mail: hhssk@hotmail.com

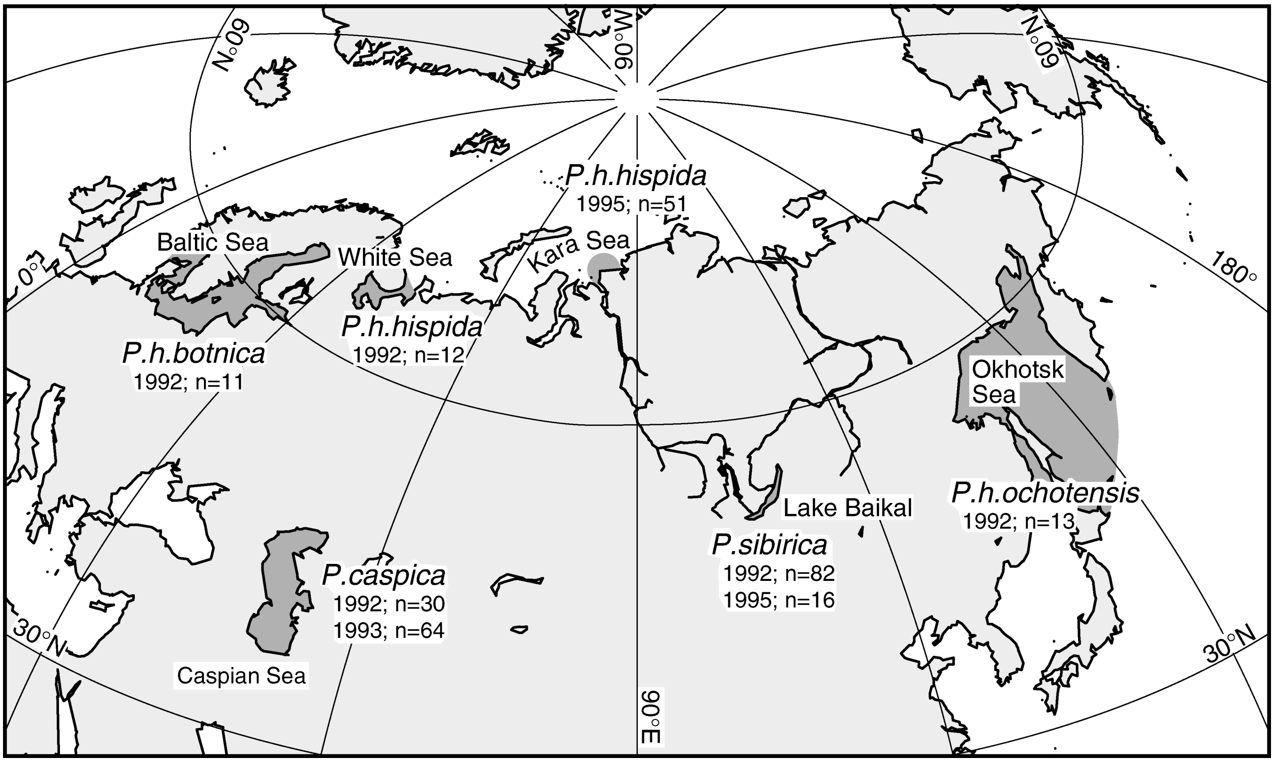

Fig. 1. Individual number and sampling localities of specimens examined of three species of subgenus *Pusa*. Of the ringed seal, *Phoca hispida* three subspecies are included.

cies of the subgenus *Pusa*, but for Malikov *et al.* (1997) examining nucleotide sequence divergence of the Baikal seal with more distantly related harbor and spotted seals.

In the present report, the origin and differentiation time of the Baikal seal are examined by analyzing restriction fragment length polymorphisms (RFLP) of mtDNA in 279 specimens representing three species of the subgenus *Pusa*; the Baikal seal, Caspian seal, and the ringed seal. Intra-and interspecific genetic variability are compared with among the *Pusa* species. We present and discuss data on the differentiation time of the Baikal seal ancestor from the other two species ancestor.

MATERIALS AND METHODS

Samples and Restriction Analysis

A total of 279 specimens, consisting of three related species (98 Baikal seals, 94 Caspian seals, and 85 ringed seals), were sampled from a wide range of localities (Fig. 1). Crude total DNAs isolated by SDS / phenol extraction from muscle or liver tissues of each animal were digested with 17 different six-base sequence recognition endonucleases: *Acc* I, *Apa* I, *Ava* I, *Bam H* I, *Bgl* I, *Bgl* II, *Cla* I, *Dra* I, *EcoR* I, *EcoR* V, *Hind* III, *Hpa* I, *Kpn* I, *Nco* I, *Pst* I, *Pvu* II, and *Ssp* I. The resulting mtDNA fragments were electrophoresed in a horizontal 0.8% agarose gel, transferred to nylon membranes. MtDNA fragments were visualized with digoxigenin labeled closed circular mtDNA probes from chum salmon, *Oncorhynchus keta*, or Caspian seals. The probes were purified from liver tissue by CsCl-ethidium bromide density gradient ultracentrifugation (Numachi *et al.*, 1990). The salmon mtDNA probe exhibited the same fragmental patterns as the seal probe (Sasaki *et al.*, 2001).

Data Analysis

Specimens were classified into the composite genotypes (haplotypes) assigned by the arrangement of fragmental patterns produced by each restriction enzyme. Sequence diversity D, nucleotide diversity π, nucleon diversity H and net nucleotide difference between populations were calculated (Nei and Li, 1979; Nei and Tajima, 1981; Nei, 1987). Quantification of the genetic differentiation of mtDNA was completed by analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992). Phylogenetic relationships of haplotypes and populations were assessed using the unweighted paired group method with arithmetic average (UPGMA) (Nei, 1987) and neighbor-joining method (Saitou and Nei, 1987) included in PHYLIP (version 3.572, Felsenstein, 1993). Phylogenetic relationship among haplotypes was caluculated on the basis of a matrix of the number of base substitutions per nucleotide. When comparing among populations, the net difference among them was estimated by subtracing the average level of variation within populations from the level of variation among them (Nei, 1987). Haplotypes of the harbor seal and gray seal deduced from the complete mtDNA sequences were used as an outgroup (Arnason and Johnsson, 1992; Arnason and Gullberg, 1993).

RESULTS AND DISCUSSION

Haplotypes Demonstrated in *Pusa*

A total of 98 restriction sites was identified in the 279 samples examined. Of these sites, 67 (68.37%) were polymorphic. Specimens were classified into the haplotypes assigned by the arrangement of fragmental patterns produced with each of the enzymes. In total, 87 different haplotypes were discriminated from the 279 specimens. Fragment patterns data for these haplotypes are available from one of the authors (HS) upon request.

Relationships Among Haplotypes and Species

Haplotypes of each species were clearly divided into their own distinct clusters, with no common haplotype among the species (Fig. 2). The results clearly show that each species is polymorphic, represented by 12 to 47 haplotypes, and the haplotypes found within a species are genetically similar to each other and distinct from those of other species.

Population Structure of the Examined Species

Heterogeneity of population samples from different localities and different sampling years was examined in the Baikal and Caspian seals by AMOVA, using the haplotypic frequencies and differences between haplotypes. Neither the Baikal or Caspian seal exhibited a heterogeneous population structure ($P > 0.05$). Results of the Baikal seal agreed with a previous report which revealed that the Baikal seal moves throughout the entire basin of Lake Baikal (Stewart *et al*., 1996). Three sample sets of the Caspian seals examined, n=18 from a frozen area in the northeastern quadrant in February 1993, and n=30 and n=46 from a more southern area of the quadrant in November 1992 and November 1993 respectively showed no genetic heterogeneity ($P > 0.05$). Jefferson *et al.* (1993) reported that Caspian seals are prompted to move to the northeastern quadrant by ice formation and south into deeper and cooler regions after the pupping season. Average value of haplotype frequency presented here might represent the genetic composition of this species.

Genetic Differentiation of the Ringed Seal Subspecies

The haplotypes discriminated in the ringed seals collected from various locations were all assigned to one group (Fig. 2). The ringed seals examined in the present study were previously classified into three subspecies according to their distribution range (Nishiwaki, 1965; Jefferson *et al.*, 1993): the arctic ringed seal, which is distributed in the Arctic Ocean including the Kara Sea and White Sea, the Baltic ringed seal, distributed in the Baltic Sea, and the Okhotsk ringed seal, distributed in the Okhotsk Sea. However, the haplotype distribution in four population samples belonging to these subspecies from different localities was complex, as shown in Fig. 3. AMOVA indicated that the common ringed seal from the White Sea differed from the other populations ($P < 0.05$). In addition, the common ringed seal from the Kara Sea differed from the Okhotsk ringed seal ($P < 0.05$).

Intraspecies Genetic Variability

Both H and π values were lowest in the Baikal seal, and highest in the ringed seal inclusively (Table 1). The Caspian seal showed intermediate values. All of the ringed seal subspecies also showed higher values, although samples from the White Sea showed monomorphism. These lower values

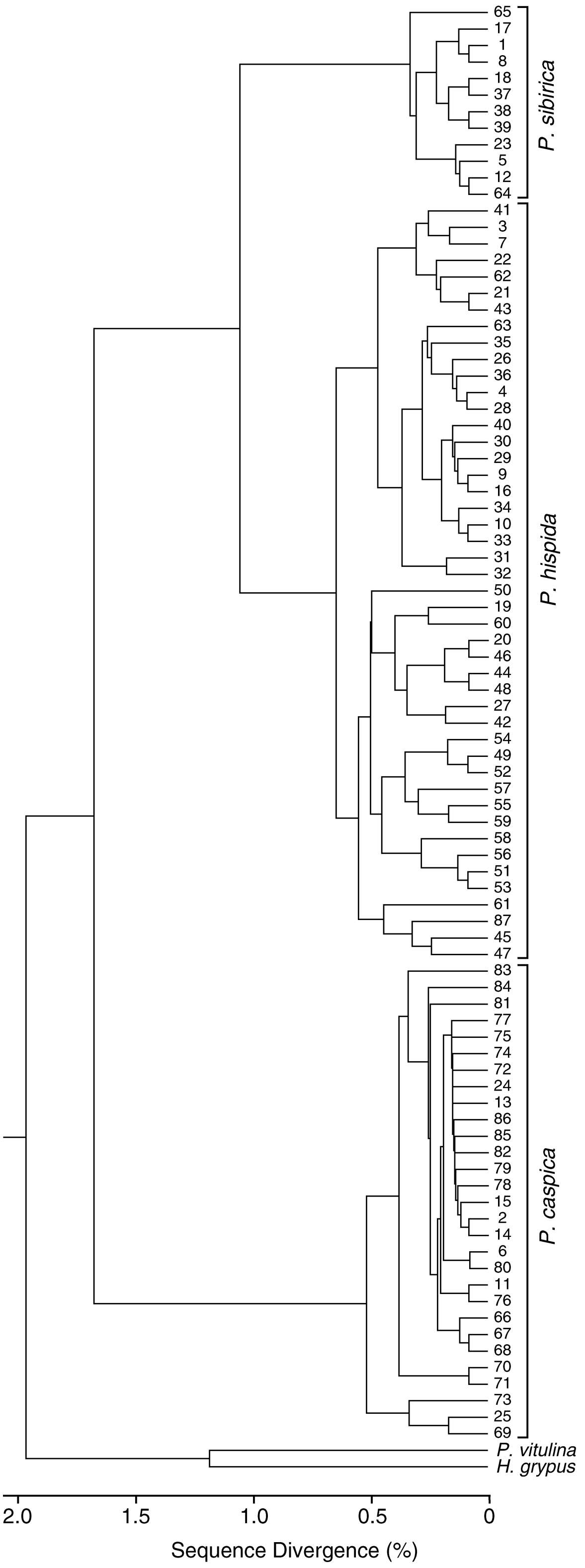

Fig. 2. Genetic relationships of the 87 haplotypes of mtDNA demonstrated in all the total of 279 specimens examined of the three species of subgenus *Pusa.* The harbor seal, *Phoca vitulina* and gray seal, *Halichoeus grypus* were added as the outgroup. The UPGMA method based on a matrix of the number of base substitutions per nucleotide was used (Nei, 1987). Numeral on right side of the dendrogram represents haplotype ID number.

Fig. 3. Comparison of haplotype frequency among the four populations of the ringed seal and dendrogram of the mtDNA haplotypes of the ringed seal obtained by the UPGMA. Numeral on right side of dendrogram represents haplotype ID number. PHB; *Phoca hispida botnica* in Baltic Sea, PHW; *P. h. hispida* in White Sea, PHK; *P. h. hispida* in Kara Sea, and PHO; *P. h. ochotensis* in Okhotsk Sea.

in the Baikal and Caspian seals may be caused by the smaller population sizes in the lake. The seal population in Lake Baikal was estimated to be 58,000 in 1986 (Reeves *et al.*, 1992). In the Caspian Sea, the seal population rewrite according to Ridgeway and Harrison (1981) estimated at approximately 450,000 – 600,000. Monomorphism in the ringed seal from the White Sea remains to the further studies.

Table 1. Genetic variability within the *Pusa* species by nucleon diversity (H) and nucleotide diversity (π)

	H	π	Number of individuals
P. sibirica	0.481	0.0010	98
P. caspica	0.722	0.0011	94
P. hispida	0.923	0.0039	87
P. h. ochotensis	0.982	0.0040	13
P. h. hispida	0.950	0.0039	63
P. h. botnica	0.836	0.0037	11

Genetic Relationships Among *Pusa* Species and Origin of the Baikal Seal

The UPGMA dendrogram constructed from a matrix of net nucleotide divergence revealed that the Baikal seal is much more closely related to the ringed seal, and the Cas-

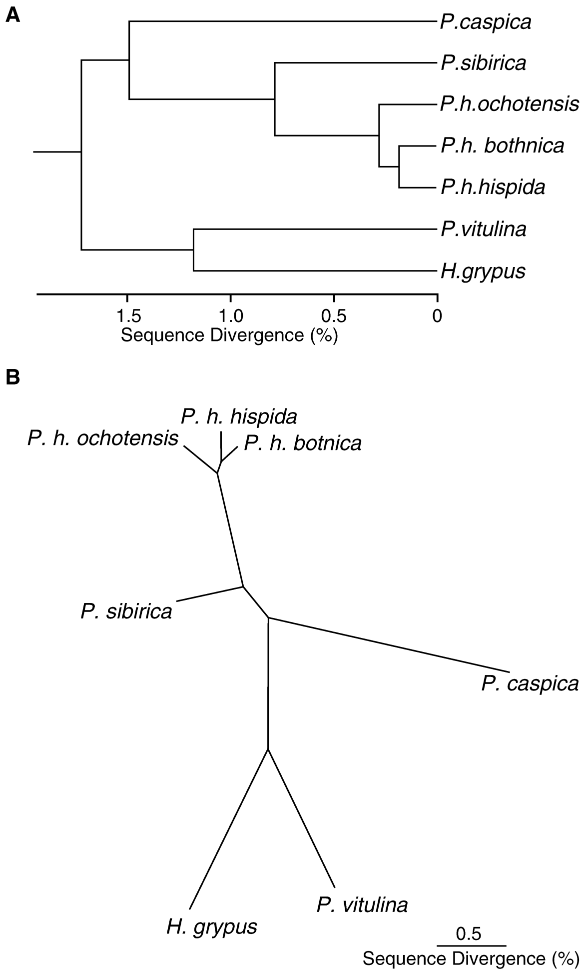

Fig. 4. Dendrogram (A) and (B) of the four species of subgenus *Pusa* including harbor seal, *Phoca vitulina* and gray seal, *Halichoeus grypus*, as the outgroup, respectively obtained by the UPGMA method and the Neighbor-joining method which was based on the net difference among them was estimated by subtracing the average level of variation within populations from the level of variation among them (Nei, 1987).

pian seal was positioned far from the other two (Fig. 4a). The dendrogram obtained by the neighbor-joining method also showed a similar result (Fig. 4b). Skull morphometry based on the same specimens as the present study supports our results (Koyama *et al.*, 1997). The Baikal seal presumably came from the north and not directly from the area west of the old Paratethyan basin, which was located in the present Caspian Sea. Our results support the paleontological and geographical hypotheses presented by Ray (1976) and Repenning *et al.* (1979).

Differentiation Time

The period of seal immigration into Lake Baikal via the Yenisey River appears to have occurred approximately 0.4 million years ago from estimates of genetic distance between these seal species (Table 2), assuming that the rate of nucleotide substitution in mtDNA is 2% per one million years, as is suggested for animal species (Hoelzel and Dover, 1991). This strongly supports the immigration course and time suggested by Ray (1976) and Repenning *et al.* (1979). On the other hand, the common ancestor of the Baikal and ringed seals was estimated to have diverged from the Caspian seal approximately 0.7 million years ago. This figure is much more recent than the 2.5 to 3 million years estimated by the above paleontological studies. The close relationship between the *Pusa* species and harbor seals shown in the present study suggests that differentiation time of the *Pusa* species and harbor seals is more recent than previously suggested.

Table 2. Estimates of percent of net nucleotide substitution between the seal species examined.

	PS	PC	PHO	PHB	PHH	PV
PC	2.4546					
PHO	1.5311	3.0786				
PHB	1.4818	2.9759	0.5074			
PHH	1.5273	3.0471	0.5680	0.3466		
PV	2.8 162	4.0088	3.1255	3.1656	3.1753	
HG	2.7460	3.4946	3.6533	3.6228	3.5972	2.2786

The net difference among them was estimated by subtracing the average level of variation within populations from the level of variation among them (Nei, 1987). PS; *Phoca sibirica*, PC; *P. caspica*, PHO; *P. h. ochotensis*, PHB; *P. hispida botnica*, PHH; *P. h. hispida*, PHO; *P. h. ochotensis* in, PV; *Phoca vitulina*, and HG; *Halichoeus grypus*..

ACKNOWLEDGMENTS

The authors thank Dr. E. Petrov of the Limnological Institute, Russian Academy of Science, Irukutsk, Russia, Dr. K. Sergeevich of the Caspian Scientific Research Institute, Astrahan, Russia, Dr. O. Minnibaeva, Sev PINRO, Russia, and Dr. V. A. Vladimirov, Marine Mammal Laboratory, VINIRO, Russia, for collecting samples and their technical support in the field. We are also grateful to Dr. M. Amano and Mr. Y. Koyama, Ocean Research Institute of University of Tokyo, Japan, Dr. M. Olsson, Swedish Museum of Natural History, Sweden, the staff of Otaru Aquarium in Japan and Dr. T. Oike D.V.M. and the staff of Minami Chita Beachland Aquarium in Japan for their kind donations of the Baikal, Caspian and ringed seal samples.

REFERENCES

Arnason U, Gullberg A (1993) Comparison between the complete mtDNA sequences of the blue and the fin whale, two species that can hybridize in nature. J Mol Evol 37: 312–322

Arnason U, Johnsson E (1992) The complete mitochondrial DNA sequence of the harbor seal, *Phoca vitulina*. J Mol Evol 34: 493–505

Bonner WN (1989) The natural history of seals. Christopher Helm, London

Davies JL (1958) Pleistocene geography and the distribution of northern pinnipeds. Ecology 39: 97–113

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479–491

Felsenstein J (1993) PHYLIP (Phylogeny Inference Package) version 3.572. Distributed by the author. Department of Genetics, University of Washington, Seattle

Hoelzel AR, Dover GA (1991) Molecular Genetic Ecology. Oxford University Press, Oxford

Jefferson TA, Leatherwood S, Webber MA (1993) FAO Species Identification Guide, Marine Mammals of the World. FAO, Rome

Koyama Y, Amano M, Miyazaki N, Petrov EA, Grachev MA (1997) Age composition, growth and skull morphology of three species in the subgenus *Pusa* (*Phoca sibirica*, *Phoca caspica* and *Phoca hispida*). In “Report on the animal community, Environment and phylogeny in Lake Baikal, Monbusho Grant-in-Aid for International Scientific Research (Project Number: 07041130)” Ed by N Miyazaki, Ocean Research Institute, University of Tokyo, Tokyo, pp 79–90

Kozhov M (1963) Lake Baikal and its life. Dr. W. Junk Publishers, Hague

MacLaren IA (1960) On the origin of the Caspian and Baikal seals: the paleoclimatological implication. Am J Sci 258: 47–65

Malikov NG, Grachev MA, Mertvetsov NP (1997) An estimation of the time of divergence of the seals *Phoca vitulina*, *Phoca largha* and *Phoca sibirica* from a common ancestral species. J Evol Biochem Physiol 33: 252–254

Nei M (1987) Molecular Evolutionary Genetics. Columbia University Press, New York

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76: 5269–5273

Nei M, Tajima F (1981) DNA polymorphism detectable by restriction endonucleases. Genetics 97: 145–163

Nishiwaki M (1965) Whales and Pinnipeds. University of Tokyo Press, Tokyo

Numachi K, Kobayashi T, Chang K, Lin Y (1990) Genetic identification and differentiation of the Formosan landlocked salmon, *Oncorhynchus formosanus*, by restriction analysis of mitochondrial DNA. Bull Inst Zool Acad Sin 29: 61–72

Ray RE (1976) Geography of phocid evolution. Syst Zool 25: 391–406

Reeves R, Stewart B, Leatherwood S (1992) The Sierra Club Handbook of Seals and Sirenians. Sierra Club Books, San Francisco

Repenning CA, Ray CE, Grigorescu D (1979) Historical biogeography, plate tectonics and the changing environment. In “Pinniped Biogeography” Ed by J Gray, AJ Boucot, Oregon State University Press, Corvallis

Ridgeway SH, Harrison RJ, (1981) Hand book of marine mammals. vol. 2. Academic Press, New York. pp 29–53

Rice DW (1998) Marine mammals of the world. Systematics and distribution. Special publication number 4. The society for marine mammalogy, Lawrence

Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406–425

Sasaki H, Shimazaki K, Numachi K (2001) Universal chemical probe and biopsy samples for restriction analysis of mitochondrial DNA in seals and dolphins. Bull Inst Oceanic Res & Develop Tokai Univ 22: 101–105

Stewart BS, Petrov EA, Baranov EA, Timonin A, Ivanov M (1996) Seasonal movements and dive patterns of juvenile Baikal seals, *Phoca sibirica*. Mar Mamm Sci 12: 528–542

(Received July 7, 2003 / Accepted August 10, 2003)