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Pinniped Penises in Trade: A Molecular-Genetic Investigation

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Abstract: *This study was prompted by international concern over the expanding global trade in wildlife parts and derivatives. Pinniped (seals, sea lions, fur seals, and walrus) penises purchased in traditional Chinese medicine shops in Asia and North America were examined using molecular genetic techniques. A 261 base pair region of the cytochrome b gene was sequenced for 21 unknown samples, 3 harp seal (*Phoca groenlandica*) reference samples, and 2 hooded seal (*Cystophora cristata*) reference samples. These sequences were compared against published sequences for pinniped species. Eleven samples from Shanghai and 1 from Bangkok were identified as harp seals and 1 Vancouver sample was derived from a hooded seal. One sample (from Hong Kong) was most closely associated with an Australian fur seal (*Arctocephalus pusillus doriferus*), though the possibility of it originating from a Cape fur seal (*A. p. pusillus*) could not be discounted due to the unavailability of a reference sequence. Seven samples were not derived from pinnipeds: 1 from Bangkok was identified as originating from domestic cattle (*Bos taurus*) and the remaining 6 were not identifiable to species due to a lack of relevant published sequences. Of these 6 samples, 2 from Canada were most similar to African wild dog (*Lycaon pictus*), 3 (2 from Toronto and 1 from Bangkok) were most similar to domestic cattle followed by water buffalo (*Bubalis bubalis*), and 1 from San Francisco was most similar to water buffalo. Our results confirm that penises from different pinnipeds are in international trade. The detection of unidentifiable species and, possibly, the Australian fur seal—a species that is not legally hunted—suggests that legal trade in seal products is serving as a cover for illegal trade. These findings are consistent with other recent evidence that the lucrative market for pinniped penises may be encouraging the unregulated hunting of seals, including protected species, and the harvesting of other unidentified mammalian species.*

Venta de Penes de Pinípedos: Una Investigación Genético-Molecular

Resumen: *Este estudio fue impulsado por una preocupación internacional sobre la expansión global de la venta de partes de vida silvestre y sus derivados. Los penes de Pinípedos (focas, leones marinos y morsas) vendidos en tiendas de medicina tradicional China en Asia y Norteamérica fueron examinados usando técnicas de genética molecular. Una región del gen citocromo b de 261 pares de bases fue secuenciada para 21 muestras desconocidas, 3 muestras de referencia de* Phoca groenlándica *y 2 de* Cystophora cristata. *Estas secuencias fueron comparadas contra secuencias publicadas de especies de pinípedos. Once muestras de Shangai y una de Bangkok fueron identificadas como* Phoca groenlándica *y una muetsra de Vancouver se derivó de* Cystophora cristata. *Una muestra (procedente de Hong Kong) estuvo cercanamente asociada con la foca Australiana (*Arctocephalus pusillus doriferus*) aunque la posibilidad de que provenga de una foca del Cabo (*A. p. pusillus*) no puede ser descartada debido a la carencia de una secuencia de referencia. Siete muestras no provenían de pinípedos: Una proveniente de Bangkok se identificó como originaria del toro doméstico (*Bos taurus*) y las seis restantes no fueron identificadas debido a la carencia de secuencias relevantes publicadas. De estas 6 muestras, 2 provenientes de Canada fueron muy similares a las secuencias del perro salvaje africano (*Lycaon pictus*), 3 (2 de Toronto y 1 de Bangkok) fueron muy similares al ganado domestico seguido del Búfalo del agua (*Bubalis bubalis*), una muestra de San Francisco fue muy similar al Búfalo del agua. Nuestros resultados confirman que los penes de diferentes pinípedos son vendidos en el mercado internacional. La*

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detección de especies no identificables y posiblemente la presencia de la foca Australiana—una especie cazada ilegalmente—sugiere que la venta legal de poductos de focas esta sirviendo como cobertura para la venta ilegal. Estos resultados son consistentes con otras evidencias recientes que muestran que el lucrativo mercado de penes de pinípedos podría estar formentando la caza no regulada de focas, incluyendo a especies protegidas, además de la captura de otras especies de mamíferos no identificados.

Introduction

The global trade in wildlife products is a significant one, with estimates of its annual value, both legal and illegal, ranging from US $8 billion (Geist 1994) to about $20 billion (Lavigne et al. 1996). In many cases this trade has resulted in the depletion of species (Ceballos & Brown 1995; Lavigne et al. 1996), including mammals. Since 1600, 49% of mammalian extinctions of known cause are attributable to human hunting for purposes including food; skin and feathers; sport; live trade; and the destruction of animals perceived as pests (Groombridge 1992). In the case of marine mammals the legacy of exploitation, primarily in the form of commercial whaling and sealing, is that a number of species remain at reduced population levels (Gaskin 1982; Mowat 1984; Busch 1985).

Despite evidence that the promotion of large-scale trade in wildlife products is usually incompatible with responsible wildlife management and conservation (Hewitt 1921; Geist 1988, 1989, 1994; Norse 1993; Robinson 1993; Eltringham 1994; Hoyt 1994; Willers 1994; Lavigne et al. 1996), contemporary attempts at the marketing of marine mammal products are often undertaken in the name of "sustainable utilization" (Baker & Palumbi 1994; Lavigne & Smith 1995; Baker et al. 1996). The proponents of this viewpoint argue that if wildlife is to be conserved, it "must pay its own way" (Child & Child 1990; Eltringham 1994) and, therefore, that markets for dead wildlife, including parts and derivatives, must be promoted on a global scale (IUCN 1992; Rasker et al. 1992; Thomsen 1992; UNCED 1992; Robinson 1993).

Recently, some empirical data was brought to bear on the debate surrounding the "sustainable utilization" of whales that, for the first time, confirmed the extent of the problems associated with trade in these species. In their molecular genetic investigation of the origin of whale meat being marketed in Japan, Baker and Palumbi (1994:1539) produced evidence that the "products available currently on the Japanese retail market may include species that have been imported illegally and others that have been hunted or processed illegally." They concluded that the existence of legal commercial whaling serves as a cover for the sale of illegal products, an example of the so-called "look-alike" problem in wildlife trade (Lyster 1985; Geist 1988, 1989; Baker et al. 1996).

Our purpose was to undertake a similar investigation of the international trade in pinniped penises (often with attached testes), which currently comprise the most valuable product derived from these species in the international marketplace (Bräutigam & Thomsen 1993; RT & Associates 1994; Department of Fisheries & Oceans, Canada [DFO] 1995*a*, 1995*b*). Although the deleterious impact of the illegal trade in the penises of some species such as Bengal tiger (*Panthera tigris*), primarily for Asian aphrodisiac markets, has been well-documented (e.g., Thapar 1996), the size and value of the trade in seal penises remains unknown. Those species involved include harp seals (*Phoca groenlandica*) hunted by Norway in the northeast Atlantic (RT & Associates 1994), Cape fur seals (*Arctocephalus pusillus pusillus*) from Namibia, South American fur seals in Uruguay (*A. australis*), and northern fur seals (*Callorhinus ursinus*) in the U.S. (York 1989 in Bräutigam & Thomsen 1993). Harp and hooded (*Cystophora cristata*) seal penises have also been collected by Canadian hunters in the northwest Atlantic for shipment to Asian markets (RT & Associates 1994; Canadian Press 1995; DFO 1995*a*, 1995*c*).

We used molecular genetic techniques similar to those employed by Baker and Palumbi (1994) to identify penises purchased in traditional Chinese medicine shops in Asia and North America. This genetic evidence was combined with a review of available knowledge on the international trade in pinniped penises in order to develop an understanding of the scope and value of this commerce and its potential to impact adversely pinniped populations.

Methods

Selection of DNA Marker

Although Baker and Palumbi (1994) and Baker et al. (1996) analyzed the population-specific variability of the mitochondrial control region to identify the species and geographic origin of the individual whale samples, we selected a region of the cytochrome b gene as the DNA marker to assign unknown samples to species. The reason for this was that our study did not focus on population-specific issues and required a broader survey of mammalian species potentially involved in the penis

trade. Population identification of samples is possible with the control region because of the high mutation rate, which is 3–5 times higher than cytochrome b (Hoelzel & Dover 1991), whereas the characteristics of cytochrome b are more consistent with a species-specific DNA marker: low levels of intra-specific variation and higher levels of inter-specific variation (Kocher et al. 1989; Irwin et al. 1991). Another advantage of this gene for our study was the extensive sequence data base (more than 900 entries in EMBL/Genbank) that already existed for mammals, including pinnipeds (Arnason et al. 1995). These considerations have prompted some authors to support the use of cytochrome b as an informative marker to distinguish between species in wildlife forensic science (Bartlett & Davidson 1992).

Sample Collection

Penis samples, in a variety of forms, were obtained at traditional Chinese medicine shops in the following cities: Bangkok, Hong Kong, Shanghai, Toronto, Calgary, Vancouver, and San Francisco (Table 1). All of the samples purchased were marketed specifically as seal penises.

DNA cytochrome b sequences for harp and hooded seals were obtained from blood (harp 01 and 02; hooded 01 and 02) and muscle (harp 03) from populations in the Northwest Atlantic. Additional cytochrome b sequences were retrieved from previously published studies and EMBL data bases: Antarctic fur seal (*A. gazella*) (EMBL/Genbank accession number X82292); Australian fur seal (*A. pusillus doriferus*) (Lento et al. 1995); Hawaiian monk seal (*Monachus schauinslandi*) (X72209); hooded seal (X82294, L39209); Hooker's sea lion (*Phocarctos hookeri*) (U12851); leopard seal (*Hydrurga leptonyx*) (X82297); New Zealand fur seal (*A. forsteri*) (X82293, Lento et al. 1995); northern fur seal (U12831); ribbon seal (*P. fasciata*) (X82302); ringed seal (*P. hispida*) (X82304); southern elephant seal (*Mirounga leonina*) (X82298); and Weddell seal (*Leptonychotes weddellii*) (X72005). Tiger (X82301) and domestic cattle (*Bos taurus*) (D34635) sequences were included due to the barbed appearance of one penis and the knowledge that bovine species have been used as substitutes in the animal parts trade (R. Wenting, CITES, Canada, personal communication).

DNA Analysis

Where possible approximately 0.2 cm^3 (<0.5 g) of tissue was scraped off penis bone and testes samples. Blood samples were prepared in 1× lysis buffer (4 M urea, 0.2 M NaCl, 0.5% n-lauroyl sarcosine, 10 mM CDTA [1,2-cyclohexanediamine], 0.1 M Tris-HCl pH 8.0). DNA extractions were performed according to Guglich et al. (1994).

Table 1. Purchased tissue samples indicating geographic origin, type, and condition of sample.

Sample	*Purchase location*	*Sample appearance*	*Species most closely resembled*
BK01P	Bangkok	animal parts and plant material in wine	N/A
BK02P	Bangkok	sectioned penis and plant material in wine	domestic cattle
BK03P	Bangkok	baculum with little attached tissue	N/A
BK04P	Bangkok	cross-sectional pieces of baculum	harp seal
BK05P	Bangkok	cross-sectional pieces of baculum	domestic cattle
BK06H	Bangkok	herbal remedy	N/A
BK07C	Bangkok	encapsulated red-brown powder	N/A
TO01P	Toronto	baculum and testes with little attached tissue	domestic cattle
TO02P	Toronto	baculum with little attached tissue	African wild dog
TO03P	Toronto	barbed baculum with attached tissue	domestic cattle
CG01P	Calgary	baculum with little attached tissue	African wild dog
SF01P	San Francisco	baculum with little attached tissue	domestic cattle
SF02P	San Francisco	cross-sectional pieces of baculum	N/A
SF03P	San Francisco	baculum with little attached tissue	N/A
VC01P	Vancouver	baculum with little attached tissue	N/A
VC02P	Vancouver	dried testes	N/A
VC03P	Vancouver	dried testes with part of baculum	N/A
VC04P	Vancouver	baculum with attached tissue	hooded seal
VC05P	Vancouver	cross-sectional pieces of baculum	N/A
SH01P-SH04P	Shanghai	baculum with attached tissue	harp seal
SH05P	Shanghai	baculum with attached tissue	N/A
SH06P-SH08P	Shanghai	baculum with attached tissue	harp seal
SH09T-SH12T	Shanghai	dried testes	harp seal
HK01P	Hong Kong	whole baculum with little attached tissue	Australian fur seal

DNA served as a template for amplification using the polymerase chain reaction (PCR). The PCR primers used were the universal cytochrome b primers (Kocher et al. 1989). These primers amplify a 305 base pair region of the cytochrome b gene corresponding to bases 14842–15148 in the complete human mitochondrial DNA sequence. The DNA was amplified under the following reaction conditions: 10 mM Tris-HCl (pH 8.4), 50 mM potassium chloride (KCl), 0.001% Triton X-100, 2.0 mM magnesium chloride ($MgCl_2$), 0.2 mM dNTP's, 0.2 mM of each primer, 1.5 U of Taq DNA polymerase (Perkin-Elmer-Cetus), and 100 ng of template DNA carried out in a 25 μL volume. Amplification was performed under a temperature regime of 94°C for 30 seconds, 55°C for 1 minute and 72°C for 30 minutes for 30 cycles. The number of cycles for samples that gave a low yield of PCR product was increased to 45. The amplified product was re-amplified in triplicate under the same cycling conditions to generate enough product for DNA sequencing. PCR products were isolated through a 1.5% low-melting point agarose gel and excised and purified using a phenol-chloroform and chloroform extraction.

Amplified product (2–300 ng) was used for cycle sequencing according to the PRISM Ready Reaction Dye Deoxy Terminator Protocol (Applied Biosystems Inc., Foster City, CA). DNA sequences were analyzed by the computer program Image Quantification (Molecular Dynamics). The product was sequenced using both primers to confirm the sequence using both strands.

Analysis of Sequence Variation

DNA sequences from the unknown and reference samples, including published sequences for pinniped species, were reduced to 261 b.p. to align the sequences in the computer program Genetic Data Environment (GDE). Corrected DNA distances of pairwise sequence comparisons were obtained using Kimura's (1980) "two-parameter" model (also used by Baker et al. 1996) assuming a 2-to-1 transition to transversion substitution in the program DNAdist (Phylip 3.5c, Felsenstein 1993). Following the calculation of DNA distances for unknown samples compared to the reference sequences, the pairwise comparisons established the species or group of species closest in sequence similarity to each penis or testes sample. Samples that demonstrated low DNA distance values and clustered with one of the reference seal sequences were determined to represent "putative" seal samples. Samples that demonstrated high distance values to the reference seal species were assumed to represent samples of non-pinniped origin and were considered separately in successive analyses.

A bootstrap analysis was performed to obtain an estimate of support for the pinniped phylogenies. The Phylip program Seqboot resampled the original data 500 times by replacing nucleotides and estimated the variation among the replicates using the DNAdist program. A majority rule consensus tree—a majority rule tree that is constructed according to the groups or clusters that are generated most often in the replicates—was then generated from bootstrap data in the program Consense. Branch points that were generated in greater than 95% of the replicates indicated that an unknown sample had a high probability of originating from the reference pinniped species with which the sample grouped. The generation of a branch point in 95% of the replicates represents a high likelihood of assigning a sample to the species level and is analogous to the significant confidence level applied by Felsenstein (1985). In the absence of any a priori hypothesis as to the species identification of a sample, 95% confidence intervals could not be applied to our analysis (Brown 1994).

Two additional phylogenetic analyses were performed on the putative seal sequences using Phylip 3.5c (Felsenstein 1993): a maximum likelihood estimate using the DNAml program and parsimony analysis using the DNApars program. The maximum likelihood analysis assumes all sites in a sequence evolve independently and each site has a given substitution rate with a 2-to-1 transition to transversion ratio of substitution. A global replacement of the data set was used. Parsimony analysis also assumes that each site and each lineage evolves independently. The most parsimonious trees with the fewest mutational steps were estimated based on the similarities among sequences (i.e., synapomorphies). A bootstrap of 500 replicates, using the programs listed above, was applied to the parsimony analysis.

Results

Samples and DNA Quality

DNA was successfully extracted from the majority of the purchased penis and testes samples. A 305 b.p. region of the cytochrome b gene was amplified and sequenced for 21 unknown samples, 3 reference samples of harp seal, and 2 reference samples of hooded seal (aligned in Fig. 1).

Sample BK01P was not extracted or amplified because it contained several species of fish in addition to the cross-sectional penis samples and the probability of contamination was high (Table 1). Samples BK06H and BK07C were contained in capsules in the form of powder mixed with herbs and the presence of animal tissue was not obvious. The testes samples VC02P and VC03P and samples SH05P and SF02P amplified a small amount of product that did not yield a readable DNA sequence. Samples BK03P, SF02P, SF03P, VC01P, and VC05P were penis bone samples that did not produce an amplified product.

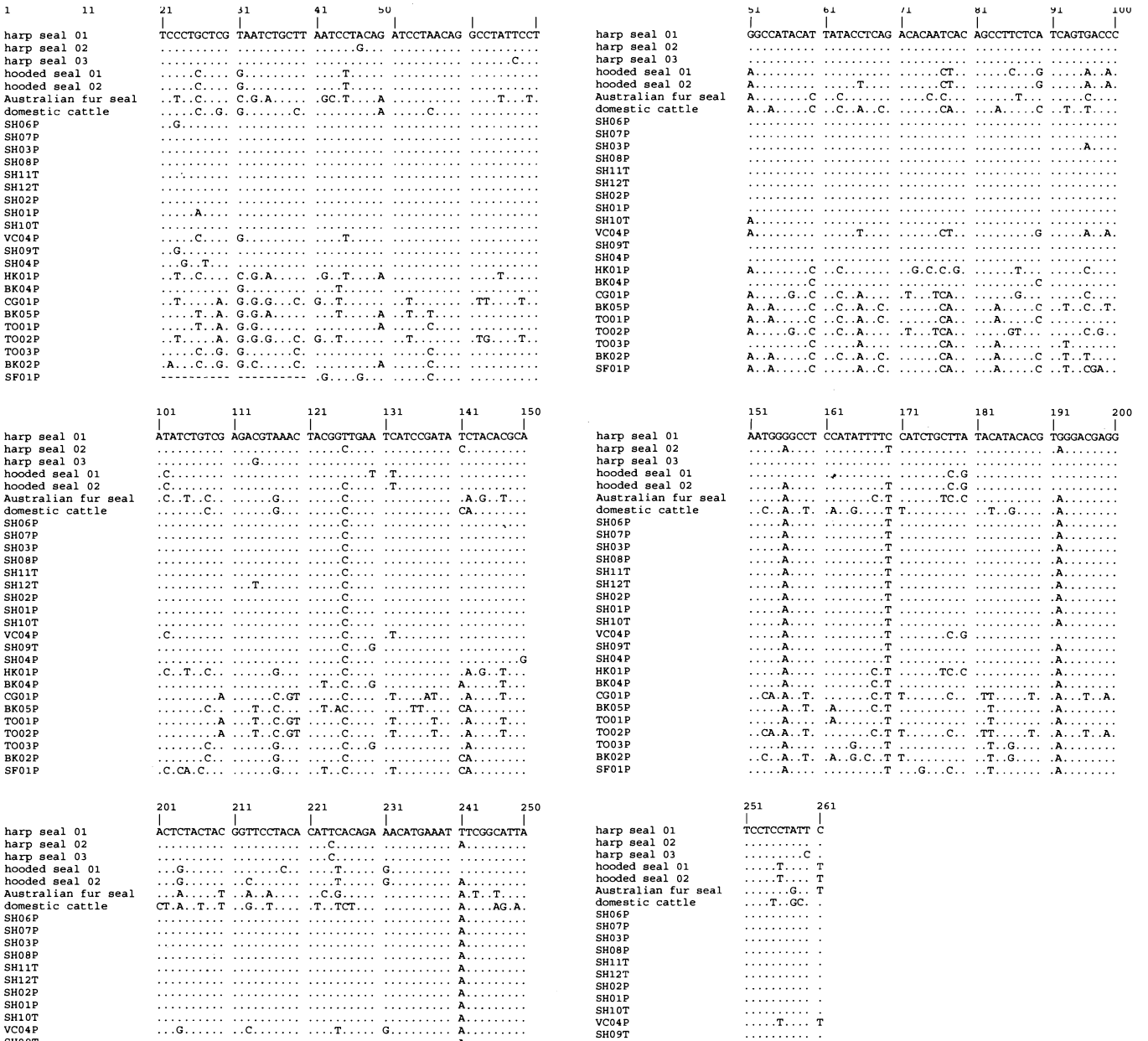

Figure 1. Alignment of 261 b.p. of the cytochrome b gene obtained from reference samples, EMBL sequences, and unknown penis and testes samples. Reference samples include Antarctic fur seal, Australian fur seal, harp seal, Hawaiian monk seal, hooded seal, Hooker's sea lion, leopard seal, New Zealand fur seal, northern fur seal, ribbon seal, ringed seal, southern elephant seal, Weddell seal, domestic cattle and tiger. See Table 1 for definition of sample codes.

Sequence Analysis

Corrected DNA distances for inter-specific comparisons among the reference seal sequences ranged from 0.08–0.20 and were greater than 0.20 for inter-ordinal comparisons to tiger and domestic cattle. Intra-specific DNA distances calculated for harp seal, hooded seal, and New Zealand fur seal were in the range of 0.00–0.04.

Fourteen samples grouped closest to one of the seal species and represented penis or testes of pinniped ori-

gin (Table 1). Eleven Shanghai samples had DNA distance values in the range of 0.02–0.05, and 1 sample (BK04P), demonstrated a value of 0.05–0.08 when compared to the harp seal references. Sample VC04P had an identical sequence to hooded seal 02 and a DNA distance of 0.04 when compared to hooded seal 01. Sample HK01P demonstrated a DNA distance of 0.02 from the Australian fur seal sequence. The pairwise DNA distances observed between the unknown samples and the remaining seal species were in the range of 0.08–0.20.

These 14 samples, which were in the range of intraspecific comparisons estimated for the reference seal samples, were assessed with a bootstrap analysis of 500 replicates to generate a consensus tree (Fig. 2). The phylogenetic relationships of the pinniped species observed in the neighbor-joining tree was consistent with the consensus tree. Twelve of the unknown samples grouped with the harp seal references in a significant number of trees (95.8%). Sample VC04P grouped with the hooded seal references in 99.8% of the replicates. Sample HK01P grouped with the Australian fur seal reference sequence in 97.4% of the generated trees in the bootstrap analysis.

The maximum likelihood estimate of the putative seal samples resulted in the clustering of the same 11 Shanghai samples and 1 Bangkok sample that were grouped with harp seal in greater than 95% of the replicate neighbor-joining trees. In addition, sample VCO4P clustered with hooded seal and sample HKO1P clustered with Australian fur seal, consistent with the groupings in the DNA distance and neighbor-joining analysis. All of these groupings of putative seal samples to their species of origin in the maximum likelihood tree were significant ($p < 0.01$). The most parsimonious trees generated were consistent with the grouping of the putative seal samples in the other two phylogenetic analyses, and the sequences of the penis samples grouped to the species of origin (as described above) in 100% of the 500 replicates that were generated.

Seven samples did not cluster with seal reference sequences (Table 1). The DNA distance of BK02P to domestic cattle was in a range consistent with the intraspecific DNA distances observed for seal species at less than 0.04, and a bootstrap analysis produced a significant clustering of the unknown with domestic cattle in 96.0% of the replicates. Of the remaining six samples, two (CG01P and TO02P) demonstrated DNA distances of 0.24–0.33 from all the reference species and apparently originated from species not present in the DNA sequence data base (Table 2). The observed pairwise DNA distance values for samples BK05P, TO01P, and TO03P indicated that the samples were not domestic cattle, but the range in values is consistent with that of related species (Table 2). Sample SF01P produced a smaller sequence at the 5′ end and the reference sequences were therefore shortened to permit a measurement of DNA distance. The sample grouped with the domestic cattle

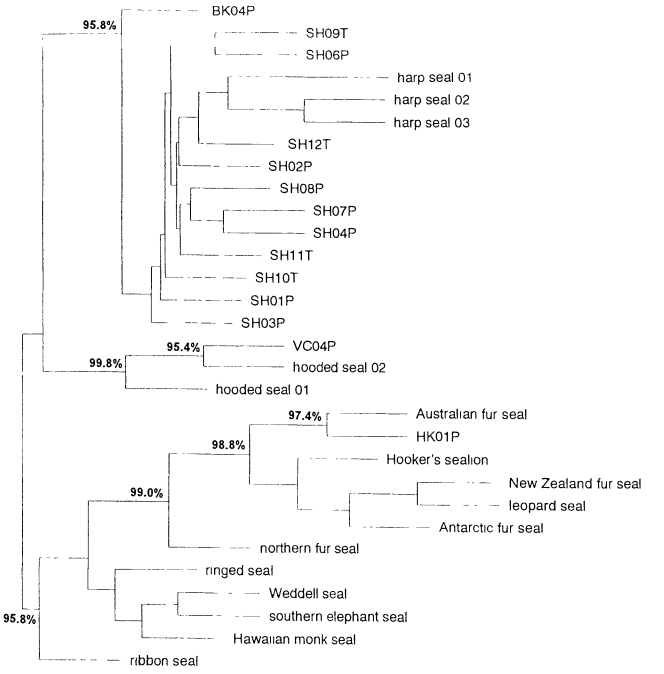

Figure 2. Consensus tree of 500 bootstrap neighbor-joining distance trees. The tree was constructed from the pairwise distance estimates calculated with the computer programs DNAdist, Seqboot, Neighbor, and Consense contained in the Phylip 3.5c (Felsenstein 1993) package. The number located at the nodes indicates the percentage of bootstrap trees that contain this pattern.

reference in a neighbor-joining tree (data not shown) at a DNA distance of 0.16 from the domestic cattle sequence.

The DNA sequences of six samples described above were searched with the BLAST program (NCBI) to identify the cytochrome b sequences of the highest sequence similarity. Samples CG01P and TO02P were most similar to the cytochrome b sequence of an African wild dog (*Lycaon pictus*) (Table 1) and the DNA distances were calculated as 0.18 and 0.21 respectively. Samples TO01P, TO03P, and BK05P demonstrated the closest sequence similarity to domestic cattle followed by the water buffalo (*Bubalis bubalis*) sequence. Sample SF01P demonstrated the closest similarity to the water buffalo sequence, at a DNA distance of 0.10, followed by the domestic cattle sequence.

Discussion

Identification of Species in Trade

This is the first molecular-genetic investigation to confirm the widespread sale of parts from several pinniped species. Our study has also confirmed that the parts of

Table 2. Kimura's DNA distance measures of non-seal penis samples assuming a 2-to-1 transition-transversion substitution ratio.

Samples	*CG01P*	*BK05P*	*TO01P*	*TO02P*	*TO03P*	*BK02P*
harp seal 01	0.2873	0.2612	0.2027	0.2937	0.1410	0.2513
harp seal 02	0.2758	0.2392	0.1922	0.2820	0.1315	0.2295
harp seal 03	0.3101	0.2782	0.2185	0.3111	0.1601	0.2737
ribbon seal	0.2386	0.2385	0.2166	0.2444	0.1357	0.2185
hooded seal 01	0.3111	0.2994	0.2385	0.3121	0.1980	0.2948
hooded seal 02	0.2652	0.2587	0.2009	0.2713	0.1724	0.2542
Australian fur seal	0.2837	0.2422	0.2179	0.2847	0.2153	0.2657
northern fur seal	0.2837	0.2665	0.2628	0.2901	0.2370	0.2745
ringed seal	0.2247	0.2505	0.2115	0.2356	0.1786	0.2407
Weddell seal	0.2437	0.2065	0.2268	0.2444	0.1872	0.2326
leopard seal	0.3091	0.2268	0.2385	0.3101	0.2511	0.2711
Antarctic fur seal	0.3257	0.2370	0.2333	0.3267	0.2291	0.2694
New Zealand fur seal	0.3017	0.2203	0.2217	0.0326	0.2342	0.2765
Hooker's sea lion	0.3091	0.2490	0.2612	0.3046	0.2140	0.2550
Hawaiian monk seal	0.2400	0.2230	0.2179	0.2408	0.2128	0.2550
southern elephant seal	0.2688	0.2059	0.2159	0.2592	0.1867	0.2422
domestic cattle	0.3020	0.1283	0.1246	0.3030	0.0816	0.0274
tiger	0.2548	0.2542	0.2347	0.2527	0.2349	0.2550

other, as yet unidentified, species, are being sold as pinniped parts, making evident the difficulties associated with identifying and monitoring seal parts in trade. As a result, regulated legal trade may create a number of problems, including providing a cover for unregulated, illegal trade; providing opportunities for the substitution of different species (legal or illegal) into trade; and providing for the substitution of illegal for legal parts. One of the Toronto samples (TO01P), which was sold as a seal penis, was fashioned to appear barbed, perhaps to create a tiger penis look-alike. It was subsequently identified as likely to be of ungulate origin. One of the species that we identified in trade, the Australian fur seal, is not legally hunted, which would indicate that the penis was likely derived from illegal activity. Because we were not able to compare the sample in question with a sequence from Cape fur seal, however, another subspecies of *A. pusillus* that is legally hunted, this identification is necessarily tentative.

We are aware of only one other published study that has attempted to identify pinniped penises in trade. It involved the morphological examination of bacula (penis bones) seized by the U.S. Fish and Wildlife Service in various U.S. and Canadian Pacific ports (Espinoza et al. 1994; B. Yates, National Fish and Wildlife Forensics Laboratory, Ashland, Oregon, personal communication). Out of 13 items examined, 10 were found to be from pinnipeds and the remainder were derived from canids (Espinoza et al. 1994). The U.S. Fish and Wildlife Service has continued to conduct serological tests on seized penises labeled as being of pinniped origin and has identified specimens of northern fur seal, canids and ungulates (B. Yates, personal communication).

There are several possible reasons for our inability to successfully amplify DNA from all of the tissues that were sampled. Environmental conditions such as temperature and humidity will increase DNA degradation in post-mortem tissues (Ludes et al. 1993), and bacterial contamination is known to increase nuclease activity (Bar et al. 1988). The lack of amplified product may have been the result of an absence of tissue or the presence of inhibitors in the mixture that prevented amplification; for example, inhibitors to PCR have been identified in human forensic samples (Akane et al. 1994). Finally, some samples may have been contaminated through contact with other tissues before purchase resulting in sequencing error due to the presence of multiple sequences.

The relationships of the seal reference sequences generated in the neighbor-joining trees were different from a previously published phylogeny of pinnipeds using the cytochrome b gene (Arnason et al. 1995) likely because only a portion of the gene was used in the present study. A smaller region of the cytochrome b gene was selected to increase the probability of amplifying a region of cytochrome b from samples possibly containing degraded DNA and, as a result, the accuracy of estimated distance decreased as the size of the sequence decreased (Martin et al. 1990). Despite the minor differences in the arrangement of the phylogenetic relationships, the bootstrap support for both the neighbor-joining (based on Kimura's DNA distance) and the parsimony analysis was high for the deeper branches that separated sequences at the species level in both studies, and this was further supported by the maximum likelihood analysis.

The preliminary identification of certain samples as carnivores or ungulates was based on the inter-generic values estimated in the seal data base. These samples may not represent either of these groups and their identity remains inconclusive. This lack of a positive assign-

ment identifies the need for the establishment of additional data bases to include the range of species involved in the animal-parts trade.

Two types of data bases can be established that are dependent on the available cytochrome b sequences: the first type includes sequences from the species of interest and their closely related species and the second includes sequences from a large number of animals of the species of interest. The two types of data bases correspond to two approaches toward species identification: the determination of the phylogenetic relationships of unknown sequences to reference sequences and a determination of intra-specific DNA distance values. The phylogenetic approach requires a complete set of sequences from related species to allow a positive assignment in an evolutionary context. In the absence of a complete set of species, a sample may be falsely identified as a closely related species simply because that sequence represents the closest association in the context of the available data base. The criteria for species identification using intra-specific DNA distance measures requires sequences from a large number of animals to determine the majority of haplotypes and the appropriate levels of sequence variation within the species. A complete data base of related species is not required for the intra-specific DNA distance criteria because the identification of a species is dependent on the established ranges of within species variation and not the closest relationship to a sequence within a data base. Ideally both approaches should be used to support the identification. This will be limited, however, by the available sequences in a data base.

Conservation Implications

Although the available data on the size of the international trade in penises, bacula, and testes, including those from pinnipeds, are incomplete, they indicate that the trade is lucrative and, apparently, growing (Bräutigam & Thomsen 1993; Guardian News Service 1995). For example, Australia annually exports approximately 5000 tons of domestic cattle penises to the Chinese aphrodisiac market for "$1.10 a kilo plus freight" (Guardian News Service 1995). One kilogram of northern fur seal bacula comprises over 40 subadult males (A. York, cited in Bräutigam & Thomsen 1993), and a single northern fur seal baculum sold for US $35.00 in 1989, and was reported in 1993 to be worth more than the pelt. In 1987, 3000 units of northern fur seal bacula and testes were detained when consigned to a Hong Kong company; in 1988 over 10,000 units were detained (Anonymous 1989, cited in Bräutigam & Thomsen 1993). There are no reports of international trade in walrus (*Odobenus rosmarus*) penises or bacula; however, it is known that they have been sold to tourists in Alaska at US $75.00 a piece (Bräutigam & Thomsen 1993). In addition, throughout the 1980s, large shipments of Cape fur seal derivatives, reported as illegal, were intercepted on import from Hong Kong into the United States and from South Africa into Hong Kong and Taiwan (Bräutigam & Thomsen 1993).

Canada has been attempting to expand its hunt for harp and hooded seals in recent years (RT & Associates 1994; MacKenzie 1996; Smith 1996) and, in 1994, the total number of harp seal penises reported landed was 4547, at a unit price per landed penis of CAN $19.91 (DFO 1995*a*). A recent report prepared for the government of Canada's Northwest Territories concluded that there is a large market for seal penises in Asia, particularly China, and quoted one Canadian company that had received offers of US $130.00 per pound for air dried penises with testes attached (RT & Associates 1994). RT & Associates also reported that Norway supplies almost 50% of Hong Kong's current market demand, shipping approximately 8000 harp seal penises in 1993. The report concluded that "there may also be a sizable market for seal organs within Canada, primarily in ethnic communities of the larger cities" (p. 11), and that out of all the possible seal products examined, the sale of seal organs was the only activity with "excellent" market potential. This assessment is in agreement with an internal government memorandum to Canada's Minister of Fisheries and Oceans, obtained through Access to Information legislation, which is even more emphatic in detailing the benefits of a harvest of seals for their penises: ". . .the demand, hence potential market, for seal sex organs has increased almost beyond comprehension. To be factual and frank, seals can now be harvested on a profitable basis solely for their sex organs; in fact, the entire TAC (Total Allowable Catch) could be harvested on this basis I am told, and those involved (sealers and brokers) would make tremendous profit!" (Rideout 1993). In the summer of 1993 a Chinese syndicate tried to buy 60,000 seals in Newfoundland and Labrador. It offered some native communities up to CAN $50 per seal, just for the penis and gall bladder (Lavigne 1994*b*). More recently, a company by the name of North American Environmental Technologies offered to buy and process 250,000 seals at a price of CAN $25 for a large female seal and CAN $50 for a male (Lavigne 1995).

Because the most valuable part of the seal currently is its penis, hunters may preferentially kill mature males, potentially resulting in an adverse impact on the reproductive potential of the population. Indeed, there is some evidence from the Canadian harp seal hunt that sealers have recently targeted older animals. Between 1972 and 1991, the percentage of pups (young of the year) taken in the annual Northwest Atlantic harp seal hunt remained relatively constant, with a mean of 80.7% (range of 60% to 91%). Data on the age structure of the seal harvest are not available for 1992 and 1993, but for 1994 and 1995, there was a dramatic reduction in the percentage of pups taken in the hunt (35% and 53%, re-

spectively) and a resulting increase in the percentage of older (>1 year old) animals taken (ICES 1992; DFO 1994, 1995*c*). This change in the hunters' preference for older animals coincided with increased publicity over the lucrative nature of the penis trade in Asia (Rideout 1993; RT & Associates 1994). Further evidence of a possible change in an existing hunt to maximize the harvest of penises can be found in Namibia, where the 1994 quota for Cape fur seal bulls was four times higher than the 1993 quota (Anonymous 1994; Lavigne 1994*a*; Eliot 1995).

Consistent with the conclusion of the latest forum on Canadian East coast seal management that "there are no controls with regard to the organ market" and that "the demand for organs might increase which could lead to an increased seal take including illegal activity" (DFO 1995*d*:9), an illegal take of seals specifically for their penises has already been documented. In 1994 fishers on the Galapagos Islands illegally killed an undetermined number of Galapagos sea lions (*Zalophus californianus wollebaeki*), from a small population numbering some 30,000 (Trillmich 1979) to 40,000 (Bonner 1994), and sent a consignment of their penises to Japan, "where they were to be tried out as a new aphrodisiac" (Pearce 1995:29). The Japanese buyers reportedly paid US $50.00 for each penis (Pearce 1995). The large potential market for penises that currently exists in some Asian Pacific countries seems likely to grow in the near future as the populations of these countries continue to increase at the same time that large numbers of their citizens acquire more disposable income (Lavigne et al. 1996).

It has long been recognized that the biological characteristics of pinnipeds make them particularly vulnerable to the effects of the sort of commercial exploitation mentioned above (Ehrenfeld 1970; Lavigne et al. 1996). In the case of many seal populations, the problems of commercial exploitation are exacerbated by the lack of legal protection that currently exists for marine mammals. Unlike the United States, Canada, for example, has no Marine Mammal Protection Act and no federal Endangered Species Act. Furthermore, Canada has successfully defeated attempts to list northern true seals, such as harp and hooded seals, on the Appendices of the Convention on International Trade in Endangered Species (CITES) (Herscovici 1985; Lavigne 1985). As a result, these species currently have no legal protection against the potential impacts of international trade.

To paraphrase Baker and Palumbi (1994), the "sustainable use" of seals is based on the assumption that only abundant species will be killed, that these hunts, and the subsequent trade in the hunted products, will be properly regulated, and that depleted species will continue to have protection. The results of the present study suggest that, with respect to some of the world's seal hunts, the existence of a trade in pinniped penises may render these preconditions for "sustainability" impossible to fulfill.

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