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Microscopic, Biochemical, and Molecular Characteristics of the Chilean Blob and a Comparison With the Remains of Other Sea Monsters: Nothing but Whales

SIDNEY K. PIERCE[1,*], STEVEN E. MASSEY[1], NICHOLAS E. CURTIS[1], GERALD N. SMITH, JR.[2], CARLOS OLAVARRÍA[3], AND TIMOTHY K. MAUGEL[4]

[1] *Department of Biology, University of South Florida, Tampa, Florida 33620;* [2] *Department of Medicine, Division of Rheumatology, Indiana University School of Medicine, Indianapolis, Indiana 46202;* [3] *Centro de Estudios del Cuaternario Fuego-Patagonia y Antártica Punta Arenas, Chile, and School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand; and* [4] *Department of Biology, University of Maryland, College Park, Maryland 20742*

Abstract. We have employed electron microscopic, biochemical, and molecular techniques to clarify the species of origin of the "Chilean Blob," the remains of a large sea creature that beached on the Chilean coast in July 2003. Electron microscopy revealed that the remains are largely composed of an acellular, fibrous network reminiscent of the collagen fiber network in whale blubber. Amino acid analyses of an acid hydrolysate indicated that the fibers are composed of 31% glycine residues and also contain hydroxyproline and hydroxylysine, all diagnostic of collagen. Using primers designed to the mitochondrial gene *nad2,* an 800-bp product of the polymerase chain reaction (PCR) was amplified from DNA that had been purified from the carcass. The DNA sequence of the PCR product was 100% identical to *nad2* of sperm whale (*Physeter catadon*). These results unequivocally demonstrate that the Chilean Blob is the almost completely decomposed remains of the blubber layer of a sperm whale. This identification is the same as those we have obtained before from other relics such as the so-called giant octopus of St. Augustine (Florida), the Tasmanian West Coast Monster, two Bermuda Blobs, and the Nantucket Blob. It is clear now that all of these blobs of popular and cryptozoological interest are, in fact, the decomposed remains of large cetaceans.

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* To whom correspondence should be addressed. E-mail: pierce@cas.usf.edu

Introduction

Sea monsters have been reported since ancient times. For instance, Homer described the sea monsters *Scylla* and *Charybdis*; the Bible spoke of *Leviathan*; and St. Brendan encountered the beast *Jasconius.* Later on, world-roving mariners such as Columbus, Magellan, and Cook described encounters with sea monsters. Many of these accounts have been variously attributed to early descriptions of cetaceans or other large aquatic mammals, to misidentification of natural phenomena, or simply to overactive imaginations. Because the deep sea is still difficult to explore, tales of large marine creatures, new to science, are rarely substantiated through direct field observations. However, a few monsters, like the Nordic tale of the *Kraken*—a large and ferocious squid-like animal—may have a basis in reality, as shown by the recovery last year of an intact colossal squid *Mesonychoteuthis hamiltoni* (http://news.nationalgeographic.com/news/2003/04/0423_030423_seamonsters.html), complete with hooklike tentacles and eyes the size of dinner plates.

For over a century the amorphous, decomposed remains of large animals have washed onto beaches around the world. Lacking a skeleton, or other identifiable morphology, a positive identification of the remains is problematic, especially by untrained observers. Wild claims, especially in the nonscientific literature, are regularly made that the blobs are the remains of sea monsters. For example, the Tasmanian West Coast Monster is still referred to as a monster,

although an Australian scientific team, led by W. Bryden, visited the carcass 2 years after it beached and identified it as a whale (Wall, 1981). Other relics such as the St. Augustine (Florida) Sea Monster and the Bermuda Blob are still described by some as the remains of a gigantic octopus (*Octopus giganteus*), even though A. E. Verrill—who named the St. Augustine specimen sight unseen—recanted his identification in favor of whale remains (Verrill, 1897a, b, c), and in spite of microscopic and biochemical analyses showing that they were nothing more than the collagenous matrix of whale blubber (Pierce *et al.,* 1995)

Last summer another blob washed ashore, this time on a beach in Chile (Fig. 1). The Chilean Blob rapidly generated a large amount of media interest around the world, and several immediate, and varied, identifications were made (including *O. giganteus*), almost all by novices with no more evidence than images of the carcass on the beach displayed on the Internet. Yet Chilean scientists, including G. P. Sanino of the Centre for Marine Mammals Research Leviathan in Santiago, had visited the grounding site and had identified the remains as that of a whale (pers. comm.).

To augment the gross anatomical observations of the carcass, we have obtained samples of the Chilean relic and have used a variety of techniques—including polymerase chain reaction (PCR) on recovered DNA—to establish its true identity. In addition, we have compared the results with those we have obtained from several other blobs, including some that have previously been reported (Pierce *et al.,* 1995).

Materials and Methods

Samples of carcasses

All of the carcasses were sampled by others and sent to us in a variety of states of preservation. The Chilean Blob (Fig. 1) was sampled from its location on Pinuno Beach, Los Muermos, Chile, within a few days after it was discovered on 26 July 2003, by Elsa Cabrera of the Chilean Centro de Conservación Cetacea. Some of the tissue was preserved in ethanol, and some was fresh frozen. The material was shipped to Tampa by overnight express, and the frozen tissue had thawed by the time it reached us. The St. Augustine carcass was originally sampled by Dewitt Webb, M.D., in 1896. Apparently it was initially preserved in formalin, which solution it was in when given to us by Professor Eugenie Clark in 1995 (Pierce *et al.,* 1995). Bermuda Blob 1, also provided by Professor Clark, washed onto Bermuda in 1995 and was also preserved in formalin when it was sampled (Pierce *et al.,* 1995). Bermuda Blob 2 beached in January 1997. Professor Wolfgang Sterrer of the Bermuda Biological Laboratory provided us with both formalin-fixed and fresh-frozen samples. The Tasmanian West Coast monster arrived on the beach in northwestern Tasmania in 1960, where it sat, mostly buried in sand, until it was sampled in

Figure 1. The Chilean carcass as it was found on Pinuno Beach. Photo by Elsa Cabrera (© E. Cabrera, 2003).

1962. After the existence of the monster was called to our attention by Leonard Wall—a member of the scientific party that sampled it—Curator A. P. Andrews of the Tasmanian Museum and Art Gallery in Hobart provided us with a sample in an unknown fixative which, by its odor, contained ethanol. Finally, the Nantucket Blob washed onto Nantucket Island, Massachusetts, sometime during November 1996. A sample was collected, frozen, and sent to us by personnel in the Nantucket Shellfish Warden's office.

Microscopy

The original conditions of preservation of the relics were unsatisfactory for electron microscopy. So, small pieces were cut off of each and soaked, at least overnight, in several changes of filtered (0.2 μm) artificial seawater. They were then placed into 2% glutaraldehyde and taken through the same fixation, embedding, and sectioning procedures that were described previously for the St. Augustine and Bermuda Blob 1 carcasses (Pierce *et al.,* 1995). The sections were viewed and photographed with a transmission electron microscope (Zeiss EM 10 or Phillips Morgagni).

Hydrolysis

Preliminary examination of the samples prepared for microscopy suggested strongly that all of the remains were almost exclusively composed of collagen fibers, as we had found before with the St. Augustine and Bermuda Blob 1 carcasses (Pierce *et al.,* 1995). To confirm the collagen identification, the amino acid compositions of hydrolysates of the carcass samples was determined as follows. Small pieces were cut off and soaked in seawater as above. Each piece was placed into 5N HCl and heated overnight at 100 °C. The hydrolysate was neutralized with concentrated NaOH, mixed 1:1 with ethanol, brought to a boil, and finally centrifuged at 20,000 $\times$ *g* for 20 min. The supernatant was lyophilized, and the residue was taken up in an appropriate volume of lithium citrate buffer. The amino acid composition of this solution was determined with a ninhydrin-based, HPLC analysis (Pierce *et al.,* 1995). Amino acid composition was calculated as residues/1000 amino acids.

Molecular analysis

The Chilean carcass was subjected to two independent molecular analyses. First, in Tampa (done by authors SEM and NEC), DNA was obtained from the frozen-thawed, unfixed tissue by phenol/chloroform extraction, followed by ethanol precipitation. The DNA was amplified in PCR using the temperature profile described previously (Carr *et al.,* 2002). The sequence of the universal primers corresponded to the vertebrate mitochondrial *nad2* gene—the same sequence used to identify *Physeter catadon* (= *macrocephalus*) (sperm whale) as the source of the Newfoundland Blob (Carr *et al.,* 2002). A single, 800-bp PCR product was obtained, then cloned into the pPCR-Script Amp SK (+) plasmid (Stratagene) and sequenced (model CEQ 8000, Beckman-Coulter) using the CEQ DTCS Quick Start Kit (Beckman-Coulter) and T3 sequencing primer.

The second independent analysis of the Chilean Blob was carried out in Auckland, New Zealand (by author CO). Genomic DNA was extracted with phenol/chloroform from three subsamples taken from an original 10-g, ethanol-preserved piece of tissue which was shipped to New Zealand by Ms. Cabrera. An 800-bp portion of the mtDNA control region, proximal to the Pro-tRNA gene, was amplified by PCR from two of the subsamples, using primer sequences Dlp-1.5 (Dalebout *et al.,* 1998) and Dlp-8G (Lento *et al.,* 1998; Pichler *et al.,* 2001). The temperature profile consisted of a 2-min preliminary denaturing period at 94 °C, followed by 35 cycles of 30-s denaturing at 94 °C, 40 s of annealing at 54 °C, and 40 s extension at 72 °C. Amplification and subsequent cycle sequencing were improved by the addition of an M13 tag to the 5′ end of the Dlp-1.5 primer. The PCR products were sequenced (model ABI3100, Applied Biosystems) in both directions, using the BigDye cycle sequencing kit, with M13Dlp-1.5 and Dlp-8G as the sequencing primers.

In addition to the Chilean Blob, we attempted, in Tampa, to extract DNA from samples of all the other remains. However, either because the samples of the other blobs were too small or because their preservation was wrong, only the Nantucket Blob yielded amplifiable DNA. A single, 800-bp PCR product was obtained from the Nantucket Blob, using the temperature profile of Carr *et al.* (2002) and the sequencing procedure that we described above. Subsequently, primers designed to the D-loop region of whale mitochondrial DNA (Wada *et al.,* 2003) were also used to amplify a single 1100-bp PCR product from the Nantucket Blob, which was sequenced as described above using T3 and T7 primers. The amplification conditions were an initial 90-s denaturation at 94 °C, 30 cycles of a 30-s denaturation at 94 °C, a 30-s annealing at 55 °C, and a 45-s extension at 72 °C, followed by a final 240-s extension at 72 °C.

Results

Fine structure

The microscopic anatomy of all the carcasses, including the Chilean Blob, is virtually identical (Figs. 2, 3). These large masses consist almost entirely of pure collagen fibers arranged in cross-hatched layers, often perpendicular to each other. This arrangement is exactly that of the collagen fiber infrastructure of freshly preserved humpback whale blubber (Fig. 2) (see also Pierce *et al.,* 1995) and is totally unlike the fine structure of octopus or squid mantle, which consists mostly of muscle fibers with only a few collagen fibers (Pierce *et al.,* 1995). Furthermore, al-

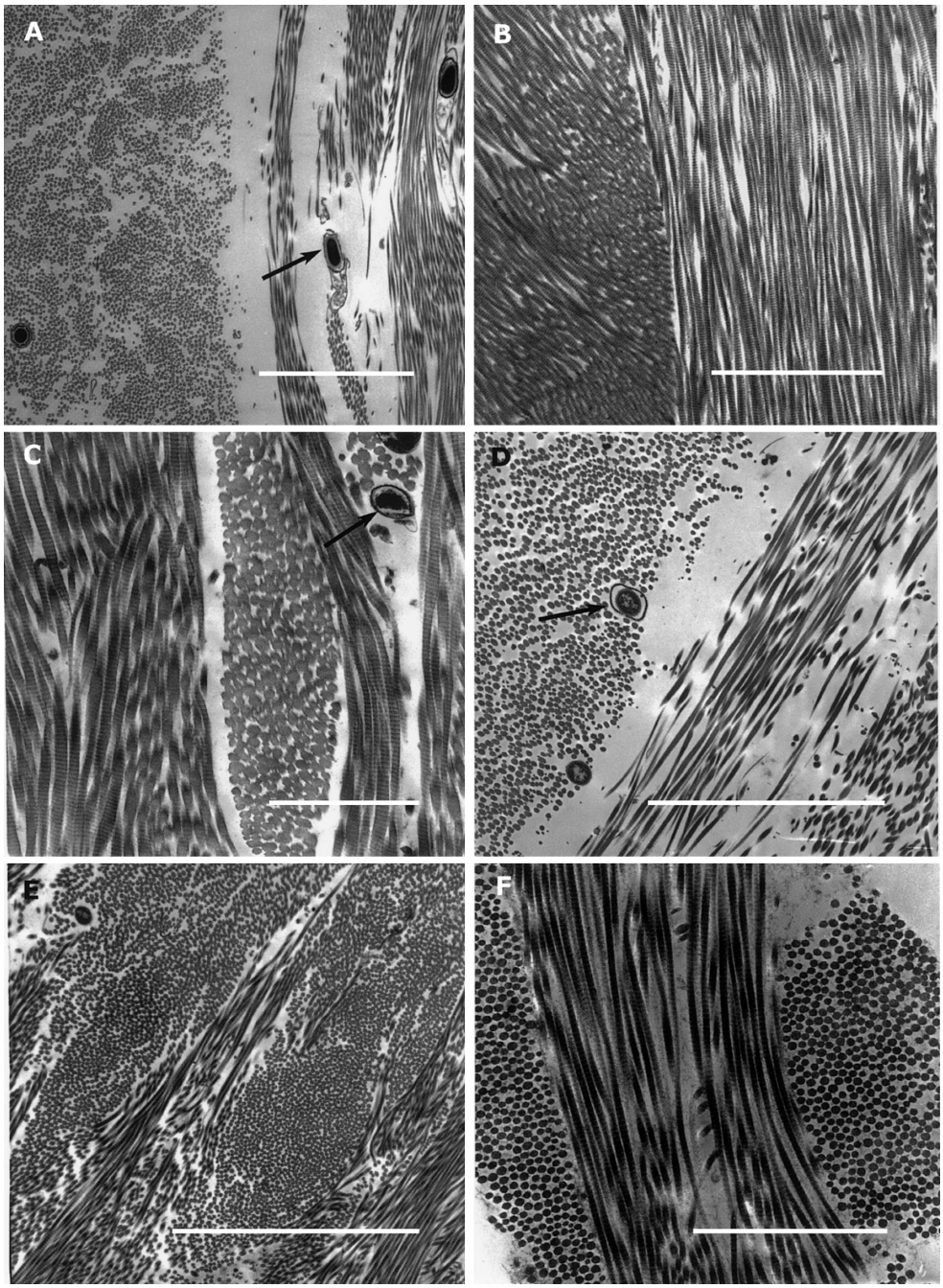

Figure 2. Electron micrographs of sections of tissue from various monsters. (A) St. Augustine carcass (from Pierce *et al.,* 1995); scale bar = 5 μm. (B) Bermuda Blob 1 (from Pierce *et al.,* 1995); scale bar = 5 μm. (C) Tasmanian West Coast Monster; scale bar = 2 μm. (D) Bermuda Blob 2; scale bar = 5 μm. (E) Nantucket Blob; scale bar = 5 μm. (F) Humpback whale blubber (from Pierce *et al.,* 1995); scale bar = 2 μm. In all cases, the tissues are composed entirely of collagen fibers arranged in layers of perpendicularly running fiber bundles. No cellular elements were found. Bacteria were often present amidst the fibers in the carcasses and can be seen in A, C, and D (arrows).

though the fiber layers in the blobs are much thicker than those in vertebrate skin, the arrangement of the collagen fibers in the two sites are similar (See Discussion). Virtually no cellular remnants, other than bacteria and bacterial cysts, were found in any of the carcasses, reflecting their advanced state of decay.

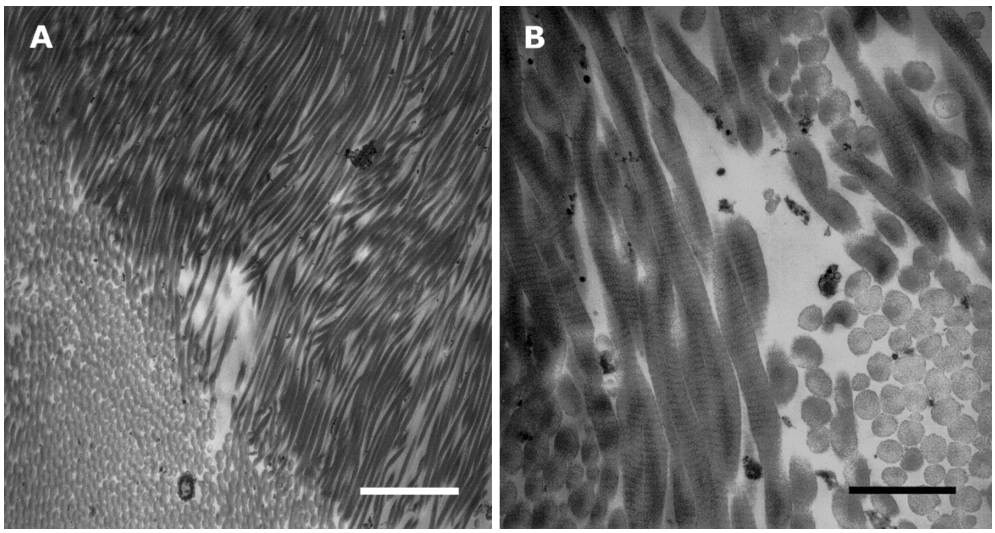

Figure 3. Electron micrographs of tissue sections from the Chilean Blob. (A) Lower magnification. Scale bar = 2 μm. (B) The banding pattern on the fibers is evident. As with the other carcasses, no cellular structures were present, but bacteria (bottom center of A) were often seen. Scale bar = 1 μm.

Amino acid composition

The amino acid compositions of the hydrolysates of all the carcasses were very similar, and they were also diagnostic of collagen. The amino acids in each blob hydrolysate consisted of about 30% glycine residues, and all contained residues of hydroxyproline and hydroxylysine (Table 1).

DNA sequences

The 587-bp consensus sequence (Genbank accession number AY582746) obtained from four sequencing runs on the DNA extracted in Tampa from the Chilean carcass was 100% identical to the mitochondrial *nad2* gene sequence of *P. catadon* (Genbank accession numbers AJ277029, AF414121) (Fig. 4). Sequencing of the PCR product ob-

Table 1

Comparative amino acid compositions of the blob tissue samples following acid hydrolysis (values are amino acid residues/1000 residues)

Amino acid	Chilean	St Augustine[a]	Bermuda 1[a]	Bermuda 2	Tasmanian	Nantucket
Asp	28	50	52	42	31	45
Thr	22	28	27	19	19	23
Ser	40	45	47	36	50	35
OH-Pro	90	54	79	113	84	146
Pro	213	169	88	182	92	136
Glu	63	82	83	62	78	63
Gly	314	330	339	298	363	280
Ala	96	106	113	94	133	94
Val	13	18	25	21	22	22
Cys	0	0	0	0	0	0
Met	4	0	0	3	1	3
Ile	8	11	14	10	11	11
Leu	25	28	32	23	30	25
Tyr	3	0	0	0	0	6
Phe	12	14	16	12	15	14
OH-Lys	11	15	13	26	7	20
Lys	21	0.4	10	18	12	25
His	6	4	6	0	0	8
Arg	29	48	55	42	51	45

[a] Data taken from Pierce *et al.,* 1995.

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                 1                                                          60
Physeter catadon TAATACTAACTATATCCCTACTCTCCATTCTCATCGGGGGTTGAGGAGGACTAAACCAGA
Chilean Blob     TAATACTAACTATATCCCTACTCTCCATTCTCATCGGGGGTTGAGGAGGACTAAACCAGA

                 61                                                         120
Physeter catadon CTCAACTCCGAAAAATTATAGCTTACTCATCAATCGCCCACATAGGATGAATAACCACAA
Chilean Blob     CTCAACTCCGAAAAATTATAGCTTACTCATCAATCGCCCACATAGGATGAATAACCACAA

                 121                                                        180
Physeter catadon TCCTACCCTACAATACAACCATAACCCTACTAAACCTACTAATCTATGTCACAATAACCT
Chilean Blob     TCCTACCCTACAATACAACCATAACCCTACTAAACCTACTAATCTATGTCACAATAACCT

                 181                                                        240
Physeter catadon TCACCATATTCATACTATTTATCCAAAACTCAACCACAACCACACTATCTCTGTCCCAGA
Chilean Blob     TCACCATATTCATACTATTTATCCAAAACTCAACCACAACCACACTATCTCTGTCCCAGA

                 241                                                        300
Physeter catadon CATGAAACAAAACACCCATTACCACAACCCTTACCATACTTACCCTACTTTCCATAGGGG
Chilean Blob     CATGAAACAAAACACCCATTACCACAACCCTTACCATACTTACCCTACTTTCCATAGGGG

                 301                                                        360
Physeter catadon GCCTCCCACCACTCTCGGGCTTTATCCCCAAATGAATAATTATTCAAGAACTAACAAAAA
Chilean Blob     GCCTCCCACCACTCTCGGGCTTTATCCCCAAATGAATAATTATTCAAGAACTAACAAAAA

                 361                                                        420
Physeter catadon ACGAAACCCTCATCATACCAACCTTCATAGCCACCACAGCATTACTCAACCTCTACTTCT
Chilean Blob     ACGAAACCCTCATCATACCAACCTTCATAGCCACCACAGCATTACTCAACCTCTACTTCT

                 421                                                        480
Physeter catadon ATATACGCCTCACCTACTCAACAGCACTAACCCTATTCCCCTCCACAAATAACATAAAAA
Chilean Blob     ATATACGCCTCACCTACTCAACAGCACTAACCCTATTCCCCTCCACAAATAACATAAAAA

                 481                                                        540
Physeter catadon TAAAATGACAATTCTACCCCACAAAACGAATAACCCTCCTGCCAACAGCAATTGTAATAT
Chilean Blob     TAAAATGACAATTCTACCCCACAAAACGAATAACCCTCCTGCCAACAGCAATTGTAATAT

                 541                                          587
Physeter catadon CAACAATACTCCTACCCCTTACACCAATACTCTCCACCCTATTATAG
Chilean Blob     CAACAATACTCCTACCCCTTACACCAATACTCTCCACCCTATTATAG
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Figure 4. Alignment of sperm whale *nad2* nucleotide sequence with that of the PCR product from the Chilean Blob DNA. The sequences are identical.

tained from the Chilean Blob in the Auckland extraction had a 552-bp consensus sequence (Genbank accession number AY 582747) that was 99% identical to the mitochondrial control region sequence of *P. catadon* (Genbank accession numbers AJ277029, X72203, M93154). The sequence obtained in Auckland for the Chilean Blob differed by a single nucleotide from the three *P. catadon* sequences in the database (Fig. 5). The first 429-bp consensus sequence obtained from the Nantucket Blob DNA was 99% identical with the mitochondrial *nad2* gene sequence of *Balaenoptera physalus* (finback whale) (Genbank accession number X61145); only a single nucleotide was different (data not shown). The subsequent 1055-bp consensus sequence (Genbank accession number AY58748) obtained from 2–4 sequencing runs on the Nantucket Blob DNA was 99% identical to the control region of *B. physalus* mitochondrial DNA (Genbank accession number X61145), with only six nucleotide differences (Fig. 6).

Discussion

The molecular results reported here provide irrefutable evidence that the Chilean carcass was the highly decomposed remains of a sperm whale. The nearly 100% match between the two gene sequences obtained in our PCR experiments and the *Physeter catadon* gene sequences leaves no other possibility. The match between the Nantucket Blob DNA and the control region mitochondrial DNA of *Balaenoptera physalus* is equally robust, leaving no doubt about the specific identity of that relic. The six nucleotide differences observed were consistent with variation within the fin whale species and may indicate a different subpopulation from the previously published sequence (Arnason *et al.,* 1991), although even if this is case, both sequences were from specimens of North Atlantic origin. Unfortunately, our attempts to extract usable DNA from the other monsters were not successful, due most likely to some combination of

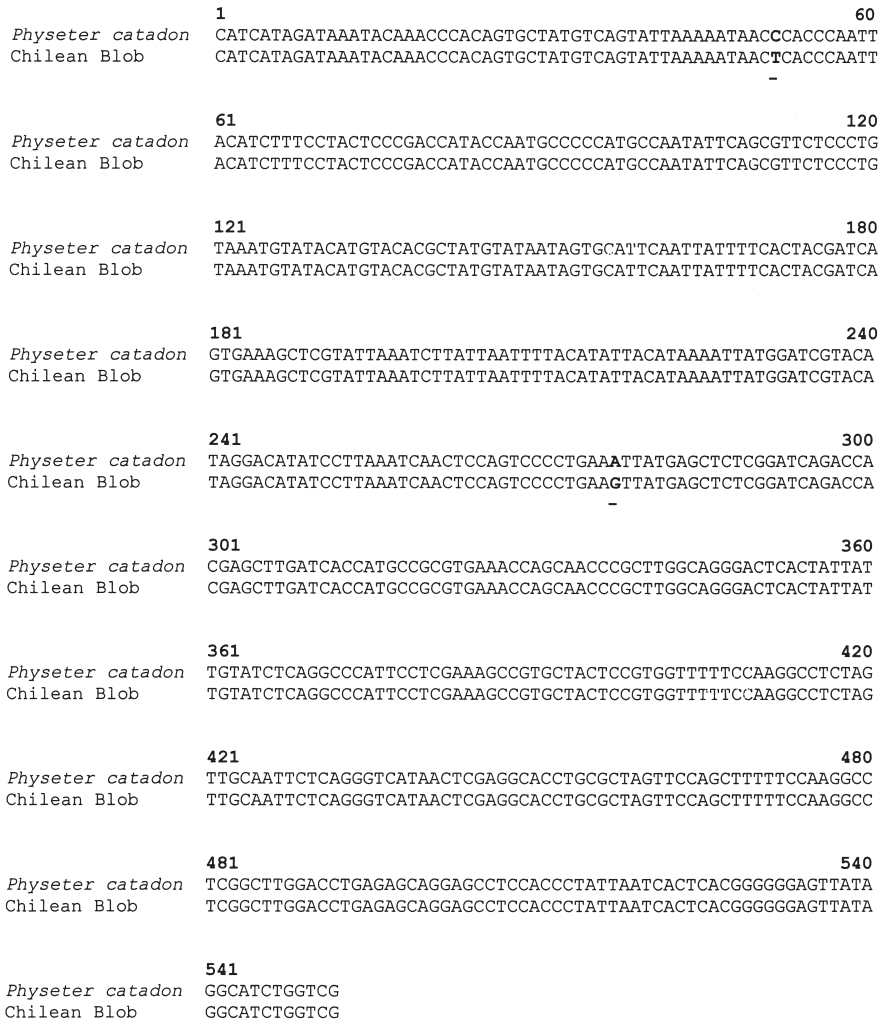

Figure 5. Alignment of sperm whale mtDNA control region nucleotide sequence with that of the PCR product from the Chilean Blob DNA. Nucleotide differences are indicated in **boldface** and underlined.

method of preservation, small sample size, or advanced stage of decomposition. However, when the microscopic anatomy and biochemical composition of the Chilean and Nantucket Blobs are compared with those of the other remains, similarities are manifest. Thus, there is no doubt that they are all derived from the same type of organism.

The amino acid composition of the hydrolysates of all the blobs consists of about 30% glycine residues along with some hydroxyproline and hydroxylysine residues. Only collagen has such an amino acid composition (Eastoe, 1955; Kimura *et al.,* 1969). While there are some differences among the amino acid compositions of the blob hydrolysates—likely resulting from differences in preservation as well as species—the results indicate that all the blobs, including the Chilean and Nantucket, are large masses of collagen.

The collagenous matrix of the blobs is confirmed by their fine structure. They are all composed of bundles of long, banded fibers that are similar in their dimensions, not only to each other, but also to the collagen fibers in rat tail tendon (see Pierce *et al.,* 1995). The bundles of fibers are arranged parallel to each other in layers, and each layer is sandwiched between perpendicularly oriented layers of other fiber bundles. The fiber layering pattern is similar to the arrangement of collagen fibers in vertebrate dermis (Moss, 1972), and identical to the collagen fiber pattern in humpback whale blubber and in all the other blobs. In addition, the unimodal fiber diameter and the tight packaging of the fibers in the Chilean Blob and the others is characteristic of mammalian dermis, including pygmy sperm whale blubber (Craig *et al.,* 1987) and our humpback blubber control. Collagen is much less abundant in octopus and squid mantle, which are composed primarily of muscle; and the few collagen fibers present in these molluscan species are not arranged in the network (Pierce *et al.,* 1995) so obvious in the Chilean Blob and the other blob tissue samples. Thus, both the biochemical and microscopic analyses show clearly that the Chilean

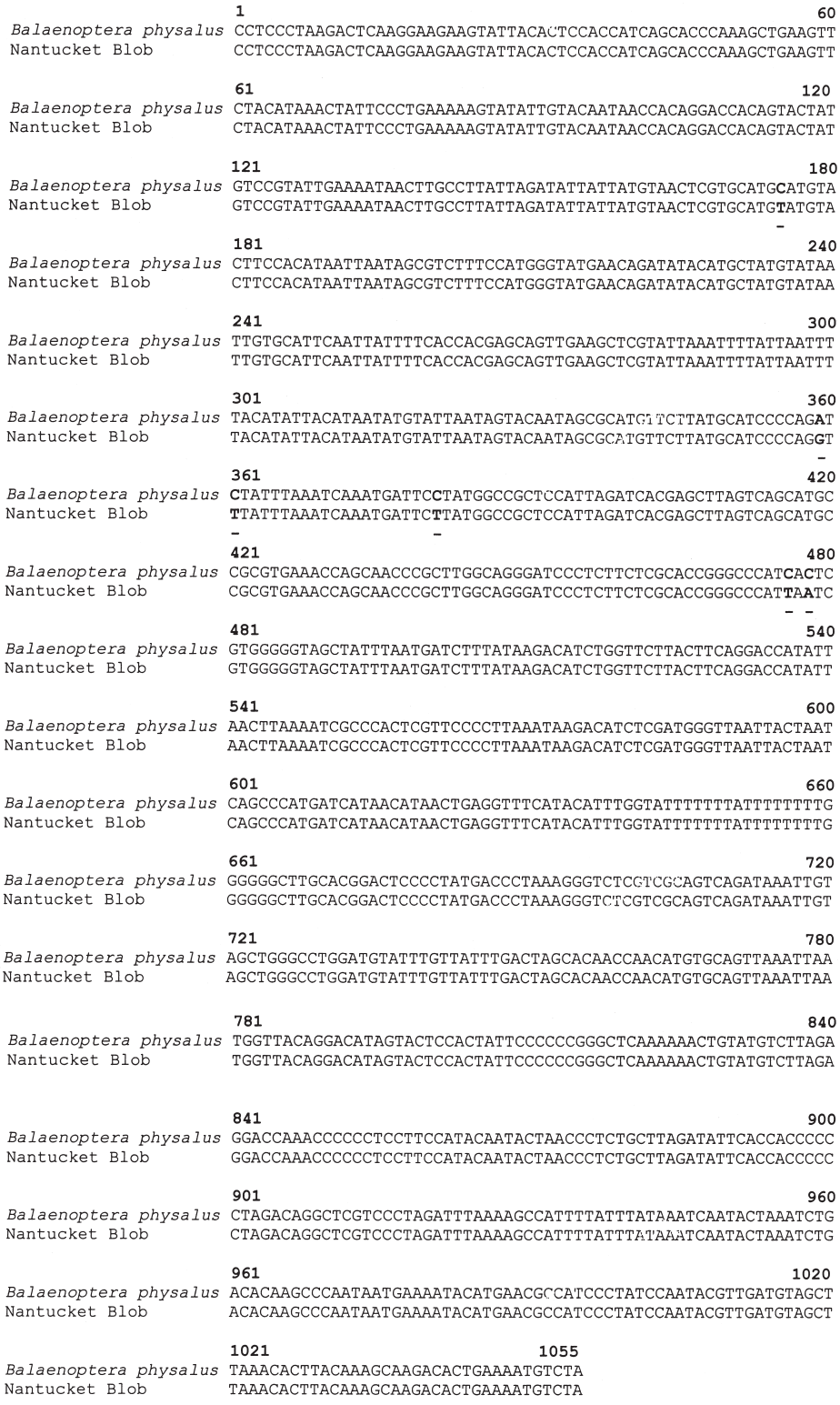

Figure 6. Alignment of fin whale mitochondrial control region nucleotide sequence with that of the PCR product from the Nantucket Blob DNA. Nucleotide differences are indicated in **boldface** and underlined.

Blob has the characteristics of all the other blobs and is the remains of the collagen matrix of whale blubber—as are they all.

The results, taken together, leave no doubt that all of the blobs examined here—St. Augustine, Bermuda 1, Bermuda 2, Tasmanian West Coast, Nantucket, and Chilean—represent the decomposed remains of great whales of varying species. Once again, to our disappointment, we have not found any evidence that any of the blobs are the remains of gigantic octopods, or sea monsters of unknown species.

Acknowledgments

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