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Japanese Scientists Identify New Species of Whale

by Yuko Hashimoto, Ph.D., and Michael D. O'Neill

New analyses of the remains of eight whales caught almost 30 years ago, together with analyses of remains of a ninth whale killed accidentally in 1998, have led Japanese scientists to the conclusion that these nine whales constitute members of an entirely new species of living whale. This is the first new species of large, filter-feeding (baleen) whale that has been described in 90 years.

The research effort, which included analysis of whale mitochondrial DNA carried out using Applied Biosystems technology, also revealed that two other particular types of whales previously thought to belong to the same species, are, in fact, separate species.

Together, these results increase from six to eight the number of known species of *Balaenoptera* whales, a genus of filter-feeding (baleen) whales that includes the largest mammal on earth, the blue whale *(Balaenoptera musculus).*

In addition to having important implications for taxonomy and evolution studies, these findings may also be relevant to commercial whaling regulatory efforts that depend in part on statistics as to the populations of different species of whales.

The work supporting the new species classifications was described in Nature (1) and was conducted by Japanese scientists Dr. Shiro Wada (Chief, Cell Biology Section, National Research Institute of Fisheries Science), Dr. Masayuki Oishi (Curator, Iwate Prefectural Museum), and Dr. Tadasu Yamada (Chief, Division of Mammals and Birds, National Science Museum, Tokyo).

The results represent the culmination of decades of effort that began with Dr. Wada's initial analysis of the remains of eight whales harvested in the late 1970s in the Indian Ocean and Solomon Sea. The results also depended on a serendipitous event in 1998 that found Dr. Yamada reading a draft article by Dr. Wada as he journeyed to a remote Japanese island site to examine the remains of a whale that had been killed in a collision with a boat.

Further, the results also depended on the use of DNA sequencing technology from Applied Biosystems to conduct mitochondrial DNA studies to substantiate and support conclusions reached on the basis of more conventional analyses, including allozyme comparisons and skeletal studies.

The scientists have named the new whale species *Balaenoptera omurai* in honor of the late Dr. Hideo Omura, a Japanese scientist much respected for his contributions to the

advancement of whale studies. Dr. Omura did much of his work at the Whale Research Institute, where Dr. Wada had been one of his students.

New Baleen Whale

B. omurai is a new species of baleen whale, a type of whale that has no teeth and uses unusual structures called baleen plates to filter small food (krill and plankton, etc.) from the sea water. Baleen is made from keratin (also found in horns, nails, and hair of other mammals) and hangs down in plates from the roofs of the whales' mouths. Baleen is also called whalebone and, prior to the invention of plastic, was used to provide structural rigidity in products such as corsets, buggy whips, and umbrella ribs.

Whale Taxonomy

Whales belong to the animal order Cetacea, which is divided into two suborders--the Odontoceti and the Mysticeti.

The Odontoceti have teeth, a single blowhole, an asymmetrical skull, and a well-developed melon (acoustical lens) that is used in echolocation. The Odontoceti include killer whales, sperm whales, beluga whales, narwhales, dolphins, and porpoises.

The Mysticeti have baleen instead of teeth. They also have two blowholes, a symmetrical skull, and a melon that is present in the fetal stage, but is absent or poorly developed in the adult. See Figure 1 in the image sidebar for an outline of the taxonomy of the Mysticeti suborder.

The new species belongs to the genus *Balaenoptera* [members of this genus are also called rorqual whales] in the family Balaenopteridae in the suborder Mysteceti. Other members of this *Balaenoptera* genus are the blue whale *(B. musculus),* the fin whale *(B. physalus),* the sei whale *(B. borealis),* the common minke whale *(B. acutorostrata),* the Antarctic minke whale *(B. bonaerensis),* and Bryde's whale *(B. brydei)* [this is the species *(B. brydei)* that, based on their data, the authors suggest should be re-classified as two species—*B. brydei* and *B. edeni].* The humpback whale *(Megaptera novaeangliae)* is in the Balaenopteridae family, but in the genus *Megaptera).*

Rorqual whales are distinguished by longitudinal grooves on their throat (helping them to hold a huge amount of water and then press it through the baleen plates to filter out their food), small pointed dorsal fin, streamlined shape, and pointed head.

In addition to Balaenopteridae, other whales in the Mysticeti suborder include the right whales *(Eubaelaena glacialis, Eubalaena australis, Eubalaena japonica),* the pygmy right whale *(Caperea marginata),* the gray whale *(Eschrichtius robustus),* and the bowhead whale *(Balaena mysticetus).*

[Note: Interestingly, the name "right whale" comes originally from the fact that this whale was deemed the "right" whale to catch by early commercial whalers because of a combination of characteristics, including slow swimming speed, large amounts of oil and blubber, and the fact that these whales would float after being killed.]

First Encounter with New Species

Dr. Shiro Wada's first encounter with the new species dates back to the late 1970s. At that time, in order to investigate the status of Southern Hemisphere Bryde's whales as a precious biological resource, a special harvesting was organized by the Japanese government to determine the number of whales in the Indian Ocean and the tropical sea area of the Pacific Ocean (South Pacific), including the Solomon Sea. Portions of the livers, skeletal muscles, baleen plates, and vertebral bones of these animals were brought back to Japan as samples to be analyzed later.

Allozyme Analysis Suggests New Species

After the harvesting, Dr. Wada conducted allozyme analysis on the collected samples to confirm the species of the whales that had been harvested. Allozymes are allelic forms of an enzyme that can be distinguished by electrophoresis. Allozymes are coded for by allelic variants of the same gene and allozyme differences can be used as indicators of genetic differences within species and between species.

In the case of eight whales (five females and three males) caught as "presumptive" Bryde's whales, alleles for 10 of the 45 allozyme loci tested were found to be different from those of Bryde's whales, and were shared amongst the eight whales. These results strongly suggested that these eight whales belonged to a different species.

Biological Data Supports New Species Hypothesis

To further investigate this possibility, Dr. Wada carefully reexamined the biological data and samples from those eight whales. As a result, he noted additional differences between the eight whales and Bryde's whales.

"Bryde's whales grow up to over 14 meters long," he said. "However, even the largest of those eight whales was relatively small, 11.5 meters in length. Detailed reexamination of the available samples of their vertebral bones revealed that all the individuals but the smallest female (10.1 meters in length) had reached their physical maturity. Therefore [based simply on the size data], it was not possible at all for us to consider that they were Bryde's whales. Furthermore, the color and proportion of their baleen plates (brought back as samples) were distinctly different from those of Bryde's whales."

In addition, photographs (see example in photo sidebar) obtained by chance several years after the whale harvests indicated that these eight whales more closely resembled fin whales than Bryde's whales. All these facts strongly suggested the possibility that the eight whales were members of a new species.

Early Resistance to New Species Classification

It was, however, not a simple matter to conclusively demonstrate that these whales should be recognized as a new species. Dr. Wada spoke about the difficulties.

“The allozyme patterns and biological data showed that these eight whales were not Bryde's whales and that they did not belong to any of the known species of whales in the genus *Balaenoptera.* In 1991, therefore, we prepared a paper (2) describing these results, [and] suggesting that these eight whales were, in fact, members of a new species.”

“However, due to the lack of information on the entire bone framework of these whales and in the absence, in its opinion, of conclusive data, the International Whaling Commission determined to regard these whales as belonging to a special local group of Bryde's whales. Accordingly, we had no other choice but to tentatively call them the ‘small-form Bryde's whale.’”

“I was sure that they were a different species, but this was a period when people didn’t trust comparative enzyme studies for taxonomic studies,” said Dr. Wada.

“To make matters worse, there even appeared some researchers who thought, in my opinion without foundation, that this ‘small-form Bryde's whale’ was actually Eden's whale *(B. edeni).* This threatened to cause more confusion in the classification of whales,” he added.

More Support for the New Species Hypothesis

In 1998, the effort to demonstrate that these eight whales did, in fact, belong to an entirely new species was aided by a serendipitous event that led to the identification of a ninth whale sharing characteristics with the eight—and, most importantly, the entire body and skeleton of this ninth whale was available for study.

The events unfolded as follows. Dr. Wada had written a draft on the putative new species of whale based on his mitochondrial DNA analysis of whale samples. He had asked Dr. Tadasu Yamada, Chief of the Division of Mammals and Birds in the National Science Museum, Tokyo, for help with proofreading the paper.

Dr. Yamada, who was very busy at that time, read the paper as he journeyed to the remote Japanese island of Tsunoshima to identify a whale that had collided with a fishing boat in the Sea of Japan and had died as a result of its injuries. The dead whale had been towed to Tsunoshima Island to await Dr. Yamada’s biological examination.

In fact, this whale turned out to closely resemble Dr. Wada’s putative new species of whale, an actual complete specimen of which Dr. Wada had never seen before. At long last, Dr. Wada had an opportunity to examine the entire bone framework (see image in photo sidebar) of what might be a representative of a new species of whale.

Tsunoshima Island Whale Provides Key Skeletal Data

"The paper I was planning to publish at that time [and which Dr. Yamada had been reviewing] contained no skeletal data because the skeletons of the eight whales caught in the 1970s had not been available for study. That is to say, it was literally a "boneless" paper. But, because we could examine the bone framework of the whale that had been

towed to the coast of Tsunoshima Island and which appeared to be a specimen of the new species, I was able to prepare a paper with 'solid bones' to it," Dr. Wada said with a smile, remembering his lucky encounter with this first complete specimen of the new species of whale.

Genetic Analysis with Applied Biosystems Technology

The current paper published recently in Nature (1) contains the results of sequence analysis of mitochondrial DNA from two of the eight whales caught during the special whaling in the 1970s, and from the collision-victim whale that had been towed to the coast of Tsunoshima Island, as well as analysis of the data on the entire bone framework of this Tsunoshima whale.

"At the time we began the mtDNA analyses of the two harvested whales, which was around 1995-1996, we had only one gel-type ABI Prism 373 DNA Sequencer from Applied Biosystems at the National Research Institute of Fisheries Science. As many researchers queued for the instrument, it was not possible to use it immediately when required.

"Because we wanted to obtain results as soon as possible, we contracted out the sequence analysis to an outside company, though we did the examination of sequence primers, etc., ourselves. Fortunately, the contracted company was also using an Applied Biosystems DNA sequencer (the ABI Prism® 377 DNA Analyzer), so we could leave the analysis to the company without worry."

"At present, our Institute has a number of newer, capillary-type systems from Applied Biosystems—two ABI Prism® 310 Genetic Analyzers and three ABI Prism® 3100 Genetic Analyzers. These systems are fully able to meet the current demand in the Institute, and have been very useful to many research projects on fish and marine products being carried out at present by the Fisheries Research Agency," Dr. Wada said.

Mitochondrial DNA Analysis Supports New Species Classification

With respect to the analyses of mitochondrial DNA from the whale found off Tsunoshima Island and from the two harvested whales, Dr. Wada and his colleagues compared the control region sequences (938 bases) of their mitochondrial DNA with one another and found there were differences of only 4 to 5 bases among those three whales.

By contrast, when the control region sequences of these three whales were compared with the sequences of that region in other species *(B. bonaerensis, B. acutorostrata, B. physalus, B. musculus, B. brydei, B. edeni,* and *B. borealis)* belonging to the same genus, differences of 62 to 97 bases were found. The smallest interspecies difference found among these other species was 31 bases (between *B. borealis* and *B. brydei).*

The authors pointed out that this meant that the difference between the three putative new-species whales and any of the other known species is greater than that between these two acknowledged species. These results strongly supported the hypothesis that the three whales were indeed members of a new species.

Eden's Whale and Bryde's Whale Are Two Different Species

In the course of this mtDNA analysis of the various *Balaenoptera* species, it was also found that Eden's whale, which had been classified as belonging to the same species as Bryde's whale *(B. brydei),* is, in fact, a different species, as it differs from *B. brydei* by 35 bases in its control region sequence. These molecular analysis results corresponded well with the external morphology and skeletal data, Dr. Wada indicated.

Thus, the molecular analysis data have significantly contributed, not only to identifying a new whale species, but also to clearly proving that the genus *Balaenoptera* is comprised of eight species of whales [that is, the original six, and the new species *(B. omurai),* and the reclassified Eden's whale *(B. edeni)].*

People of Tsunoshima Island Honored for Their Generous Help

To obtain the entire bone framework of a whale, what is typically done is to bury the whale in the sand of a beach and wait for nearly one year for the removal of the whale's flesh and fat by natural decomposition. The people of Tsunoshima Island volunteered to carry out this work. The bone framework thus obtained is now stored in the National Science Museum and its replica is exhibited in the Yamaguchi Prefectural Tsunoshima Natural Museum. Dr. Wada and his colleagues gave the new whale species the Japanese vernacular name "Tsunoshima Kujira" in order to honor the contribution of the people of Tsunoshima Island.

The discovery of a new species of whale by scientists in Japan, where whales and people have long had a close relationship, is not only an important scientific advance, but a deeply meaningful event for the people of Japan

Articles on the discovery appeared in the popular news media, including the USA Today (3). A previous BioBeat article provides additional information on DNA analysis of whales (4).

Additional Product Information

Additional information on the Applied Biosystems products mentioned in this article, as well as on other Applied Biosystems products, can be obtained in the Product and Service Literature section of the Applied Biosystems Web site.

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Trademarks

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