



AMERICAN MUSEUM OF NATURAL HISTORY

Media Inquiries: Kendra Snyder, Department of Communications
212-496-3419; ksnyder@amnh.org
www.amnh.org

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EXTINCT SOUTH AMERICAN MAMMAL THAT STUMPED DARWIN TAKES ITS PLACE ON TREE OF LIFE

**NEW ANCIENT DNA STUDY FINDS THAT *MACRAUCHENIA PATACHONICA*
BELONGS TO THE GROUP THAT INCLUDES HORSES, RHINOS, AND TAPIRS**

For the first time, scientists have recovered ancient DNA from one of the more puzzling species to have lived during the last ice age, *Macrauchenia patachonica*, allowing researchers to map its relationships and place it within a group that includes horses, rhinos, and tapirs. One of the last “South American native ungulates,” or SANUs, this animal likely weighed as much as 400-500 kilograms (850-1,100 pounds) and had baffled biologists for more than a century. The new study, led by scientists at the University of Potsdam in Germany and the American Museum of Natural History, was published this week in the journal *Nature Communications*.

Charles Darwin found the first SANU fossils in 1834 at sites in Uruguay and Argentina. He passed them on to the renowned British paleontologist Richard Owen for study and description. Owen was flummoxed by *Macrauchenia*'s unusual combination of features, which made it difficult to ascertain its correct relationships. That problem persisted for the next 180 years, with many further attempts at clarification but none widely accepted.

Owen initially thought *Macrauchenia* was related to the living llama because of its long, camel-like neck, but rejected the idea when more remains were found. One of *Macrauchenia*'s most unusual features turned out to be the position of the opening for the nose. In most mammals, the nasal opening is situated at the front of the skull, just above the front teeth. In *Macrauchenia*, this opening was placed high on the skull, between the eye sockets. Did this feature imply that *Macrauchenia* had a trunk, something like an elephant's?

If *Macrauchenia* was partly aquatic, as some have argued, was the external nose used like a snorkel? Or was it more like a large inflatable balloon, utilized in dominance activities or mating displays of the sort seen in some seals?

Solving the *Macrauchenia* problem ultimately required mitochondrial DNA, extracted from a fossil found in a cave in southern Chile, and a new approach to recovering the extinct mammal's genome. The multinational team that tackled the question was led by Michi Hofreiter, a paleogenomics expert at University of Potsdam in Germany, and Ross MacPhee, a mammalogist and curator at the American Museum of Natural History.

"Mitochondrial DNA is very useful for evaluating the degree of relatedness among species," Hofreiter said. "Our study corroborates and extends the results of another molecular investigation, published two years ago, which utilized the protein collagen to infer relationships. Like that study, ours found that the closest living relatives of *Macrauchenia* are in the placental order known as Perissodactyla, which includes horses, rhinos, and tapirs."

MacPhee added, "Perissodactyls may seem like a very mixed bag of quite different evolutionary lineages, but the fossil record indicates that these and several other now-extinct groups had ancestors that lived very early in the Cenozoic, the most recent era of Earth history."

Because of its damaged condition, ancient DNA generally requires researchers to use material from a species' close evolutionary relatives to fill in gaps, but in this case, the research team had to come up with an alternate approach.

"We had a difficult problem to solve here: *Macrauchenia* doesn't have any really close living relatives," said lead author Mick Westbury from the University of Potsdam. "Because ancient DNA is so degraded and full of unwanted environmental DNA, we rely on being able to use the genomes of close relatives as a kind of scaffold to reconstruct fossil sequences. For this study, we devised a new approach involving iterative mapping that relies on using very strict parameters and the mitochondrial genomes of a number of living species as multiple reference points to more reliably predict the fossil's most likely genetic sequences."

The team eventually recovered almost 80 percent of the predicted mitochondrial genome of *Macrauchenia*. With this information, they were able to place *Macrauchenia* in its

proper phylogenetic position as a member of the larger grouping now called Panperissodactyla. Ancient relatives of the living members of Perissodactyla were already in existence by the beginning of the Eocene epoch, 55 million years ago. The team was able to take advantage of that fact, along with other information, to calibrate or “tune” a molecular clock to determine when evolutionary splitting events occurred.

Using this approach, the researchers determined that *Macrauchenia*'s lineage and that of modern perissodactyls split about 66 million years ago--at approximately the same time as one of the largest extinction events of all time, caused by an extraterrestrial impactor striking the planet close to modern Yucatan. The new calibration is considered to be compatible with, but more accurate than, the one produced by the 2015 collagen study.

“While the coincidence with the extinction of nonavian dinosaurs was not lost on us, molecular clock dating is more like a sundial than a digital watch—it’s just not that precise,” MacPhee said. “While fossils certainly support the idea that the modern orders of placental mammals really began to diversify around this time, molecular evidence suggests that their broader relationships go back into the late Mesozoic, before the big die-off. The deep lineages that make up Panperissodactyla are certainly consistent with this idea, but we need to find the fossils to document it.” Although a large number of samples were processed for this study, in the end only one, from the southern part of Chile, actually yielded verifiable DNA of *Macrauchenia*. The researchers said that was disappointing but not unexpected, as cold conditions, like those found in permafrost regions, are by far the best for DNA preservation. However, the cave locality that produced the successful specimen is actually within the temperate region of southern South America, which suggests that it is worth testing late Pleistocene samples of other SANUs—such as rhino-sized *Toxodon*, which Darwin, who was not usually given to overstatement, considered “the strangest animal ever discovered.”

Other authors on this paper include Sina Baleka, Axel Barlow, Stefanie Hartmann, and Johanna Paijmans from the University of Potsdam; Alejandro Kramarz from Museo Argentino de Ciencias Naturales in Buenos Aires; Analía Forasiepi from Instituto Argentino de Nivología, Glaciología y Ciencias Ambientales in Mendoza; Mariano Bond, Javier Gelfo, and Marcelo Reguero from Museo de La Plata; Patricio López Mendoza and Francisco Mena from Universidad de Chile; Matias Taglioretti and Fernando Scaglia from Museo

Municipal de Ciencias Naturales in Mar del Plata; Andrés Rinderknecht and Washington Jones from Museo Nacional de Historia Natural in Montevideo; Jose Luis Aguilar from Museo Paleontológico in San Pedro; and Guillaume Billet and Christian de Muizon from Muséum National d'Historie Naturelle in Paris.

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