

Feature

How sloths got their sloth

The evolution of sloths will have to be revised after two independent molecular studies contradicted the traditional view based on morphological evidence. The two surviving genera with their distinctive arboreal lifestyles and specialised diets are also important case studies in ecology and adaptation, while their lost relatives shed light on the recent extinction of megafauna. **Michael Gross** reports.

Considering that they are named after one of the seven deadly sins, sloths are a remarkably popular group of mammals. Perhaps this is because their life in the slow lane reflects a sin that many humans are ready to admit to and might even aspire to.

Nevertheless, scientists studying these unusual creatures have a lot of work to do and no time to be lazy. The upside-down life of sloths typically suspended from tree branches reflects a wider range of unique traits that set them apart from other mammals. Their ecology and evolutionary history are also remarkably complex.

The six extant species grouped in two genera are the survivors of a much larger order (Folivora) that included ground-living and even aquatic relatives. Some years ago, cladistic studies based on anatomical traits, as revealed by comparative anatomy of museum specimens and the fossil record, had reached a consensus on phylogenetic relationships within this group. But now two studies based on different and independent kinds of molecular analyses have shown a very different pattern of family connections.

Shaking the family tree

The numerous extinct relatives of the modern-day sloths comprising Folivora covered a broad range of ecological roles, from elephant-sized giant sloths to smaller forms, most of them ground-living and some even semi-aquatic. Many of these species were widespread across the Americas and only became extinct after the arrival of humans at the end of the Pleistocene. Therefore, fossil and even tissue remains of these animals are abundant and often seen in natural history museums.

Their closest relatives are the anteaters (Vermilingua) within the order Pilosa, which together with the armadillos (Cingulata) complete the

superorder Xenarthra. Morphological analyses of the numerous fossils had suggested a family tree which placed the three-fingered sloths (four species of the genus *Bradypus*) on an early branch diverging from the rest of the sloths, while the two-fingered sloths (two species in the genus *Choloepus*) were considered part of the family Megalonychidae, including giant ground sloths (e.g. *Megalonyx jeffersonii*) and smaller Caribbean endemic sloths (e.g. *Acratocnus* and *Parocnus*). *Bradypus* and *Choloepus* are widely known as three- and two-toed sloths, respectively, even though the distinguishing digits are on their forelimbs, so referring to them as fingers is more accurate.

Preliminary ancient DNA evidence, produced in the early 2000s, appeared irreconcilable with this family tree, although not strong or detailed enough to overthrow it. Now two independent studies, one using mitochondrial

genomes and the other collagen amino acid sequences, have consistently and convincingly suggested an entirely different story of sloth evolution.

The first complete mitochondrial genome obtained from an extinct sloth species was from a 13,000-year-old bone fragment of *Mylodon darwini*, found exceptionally well-preserved in the Mylodon Cave in Chile (named after the numerous fossils found there). The research groups led by Hendrik Poinar at McMaster University in Ontario, Canada and Frédéric Delsuc at Montpellier University in France reported in May 2018 that the mitochondrial genome and preliminary data from the nuclear genome conflicted with the established morphological classification (Proc. R. Soc. B (2018) 285, 20180214). Other initial studies of specific extinct sloth species were also difficult to reconcile with the morphological version of their evolutionary history.

To clarify the issue, Poinar's and Delsuc's teams have now analysed the mitochondrial genomes of ten extinct sloths representing six species in comparison to those of the surviving ones, and came up with a dramatically different family tree, where phylogenetic relationships are drastically rearranged (Curr. Biol. (2019) 29, 2031–2042.e6).

While they were analysing their findings, the researchers were aware



Slothful youth: A young two-fingered sloth feeding on leaves. (Photo: Proyecto Asis/Flickr (CC BY-SA 2.0).)



Digit count: A three-fingered sloth showing its claws. (Photo: Myagi/Pixabay.)

that a separate effort focusing on amino acid sequences of the protein collagen 1, which is more abundant in bones and often survives longer and in more challenging circumstances than the DNA, was underway. The investigation led by Ross MacPhee at the American Museum of Natural History came to very similar conclusions (Nat. Ecol. Evol. (2019) <https://doi.org/10.1038/s41559-019-0909-z>). Considering the unexpected nature of their findings, both teams agreed to publish their papers simultaneously so that each could point to the other as an entirely independent confirmation.

What is still true based on the new molecular analyses is that the two genera of living tree sloths are only very distantly related and independently developed their unique suspensory lifestyle as an adaptation to arboreality. Their kinship links to extinct ground-living species will need to be revised, however. Poinar and Delsuc have suggested a new taxonomy with eight families and three super-families, and they call on paleontologists to test their morphological data against this new molecular framework.

The new studies also cast new light on the likely history of species distribution in South America and the Greater Antilles. The molecular evidence from both studies suggests that the extinct sloths of

the Caribbean, the megalocnids, branched out early, more than 30 million years ago. They may have reached the West Indies across a temporary land bridge known as GAARlandia, where GAAR is an acronym for Greater Antilles – Aves Ridge, which is postulated to have existed between 33 and 35 million years ago. Other phylogenetic trees of other Caribbean mammals have failed to match dispersal times with this hypothesis, so the sloths provide the first such study to confirm this biogeographical land connection.

Further molecular analyses may help to clarify these connections and also to elucidate the origins of some of the peculiar morphological, ecological and physiological adaptations that make the surviving species of tree sloths so unique.

Lazy lifestyle

One of the main reasons to study sloth evolution is in the animals' unique tree-based ecology, which both surviving genera appear to have arrived at by convergent evolution. Living on trees and eating mainly leaves is a lifestyle found in very few mammals, so studies of sloths can give key insights into the limitations of this ecological niche.

The poor nutrient quality of a diet based mainly on tree leaves limits the size range of animals adapting to it. In both genera of sloths it has led to

a range of energy-saving measures, including the slow movements, slow metabolic rate, variable body temperature, as well as a specially adapted digestive system.

Jonathan Pauli and colleagues at the University of Wisconsin at Madison, USA, have studied these adaptations to the low-energy life in the trees in great detail, showing that the three-fingered sloths, which are more narrowly specialised in their diet, are also more radical energy savers than two-fingered sloths (Am. Nat. (2016) 188, 196–204).

Measuring the field metabolic rate for both species in the wild, the researchers found that three-fingered sloths had the lowest value observed for any mammal. They also had more variable body temperature than two-fingered sloths and moved even less. All findings suggest that the three-fingered sloths represent the more extreme examples of the sloth lifestyle.

One might worry that species so extremely specialised that they only eat the leaves of one or two species of tree would be most vulnerable to environmental change. The situation is somewhat more complex for the three-fingered sloths in Costa Rica (*Bradypus variegatus*), however, as their favourite tree, the guarumo tree (*Cecropia obtusifolia*) is a fast-growing plant that is quick to colonise disturbed environments and is also planted in cocoa plantations to provide shade. For sloths the tree has many advantages, including the fact that it grows new leaves all year round, enabling sloths to feed on the new leaves that are more digestible.

To establish just how strongly the welfare of the sloths is linked to this specific tree species, Mario Garcés-Restrepo, with Pauli and Zachariah Peery, all at the University of Wisconsin, has conducted a long-term study on a field site in Costa Rica with a variety of different habitats to establish the fitness of *B. variegatus* as a function of the density of guarumo trees in their range (Proc. R. Soc. B (2019) 286, 20182206).

The researchers found that both the survival of adult sloths and their reproductive success are strongly linked to the density of the guarumo trees. By contrast, juveniles are not that closely attached to guarumo trees and their survival does not correlate with the presence of these trees.

Garcés-Restrepo and colleagues conclude that the young sloths are likely having to make a trade-off between the better security from predation in other, more densely covered trees against the preferred food available in the guarumo trees.

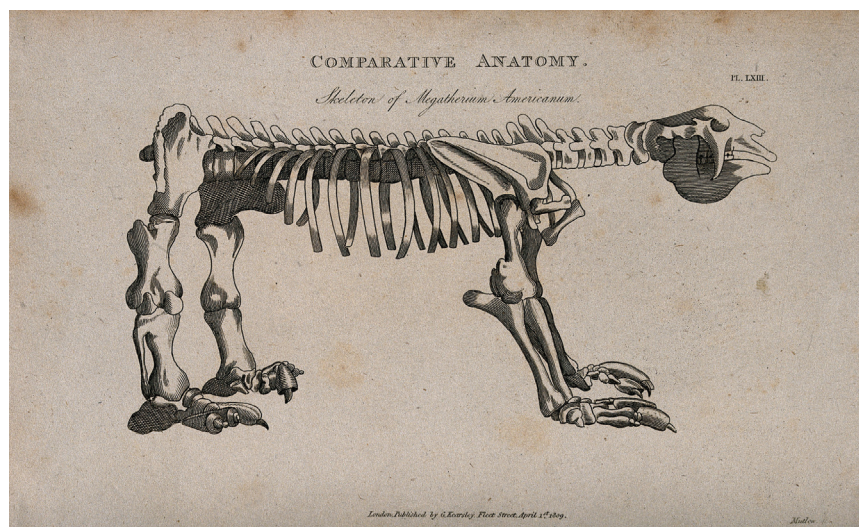
In a separate study using the same field area, the same authors studied the dispersal of the young animals as they become independent, both for three-fingered (*B. variegatus*) and for two-fingered sloths (*Choloepus hoffmanni*). The study showed that juveniles of both species preferred tropical forest habitat and avoided pastures (J. Appl. Ecol. (2018) 55, 2253–2262). The less specialised two-fingered sloths were happy to accept shaded cacao plantations as habitat.

Analysing the survival rates of young sloths, the researchers found that the animals were most at risk immediately after they became independent of their mothers. Survival chances were lower for the three-fingered than for the two-fingered sloths, but in both cases good enough to support a stable population size.

In another field study from the same group, Emily Fountain and colleagues used genomic analyses of kinship between the individual sloths studied in addition to ecological and demographic observations in order to better characterise the quality of the different habitats for the two-fingered sloths, which they found to be strongly dependent on specific local circumstances (Mol. Ecol. (2018) 27, 41–53).

Sloths are in turn ecologically important for a whole range of other species. Some of the complexity came to light when Pauli's group investigated why the lazier and more specialised three-fingered sloth descends from its tree once a week to defecate and bury its faeces underneath its own tree, while two-fingered sloths have been observed just letting their business drop from where they are. For the three-fingered sloth the toilet trip carries both a significant energy cost and an additional risk of becoming prey to jaguars. Thus, the researchers argued, there must be a major fitness benefit to balance the disadvantages caused by this behaviour.

Pauli and colleagues found a plausible explanation in the ecology



Big beast: Skeleton of the extinct ground sloth (*Megatherium americanum*). (Image: Line engraving by Mutlow, 1809. Wellcome Collection (CC BY 4.0).)

of the moths and algae that colonise the fur of the three-fingered sloths to a much greater extent than that of the two-fingered ones (R. Soc. B (2014) 281, 20133006.). The researchers found that the life cycle of the resident moths depends on the toilet behaviour: the moths lay their eggs in the dung the sloth leaves on the ground, and the fledgling moths fly up to find a sloth in the tree above. Enriching the sloth fur with nitrogen, the moths enable the growth of algae. These may help with the camouflage, but the 2014 study by Pauli and colleagues also showed that three-fingered sloths consume some of the algae in their fur and obtain valuable nutrients from them, supplementing their limited diet of guarumo leaves. Thus, by benefit of being too lazy to clean their fur, the sloths manage to support an entire ecosystem that ultimately helps their own survival. And the jaguars win, too.

Although their narrow specialisation and low energy for movement might evoke fears for their survival, only one of the six species is threatened, namely the critically endangered pygmy-three-fingered sloth, *B. pygmaeus*. Found exclusively in mangrove forests on the island Escudo de Veraguas off the Caribbean coast of Panama, this species is threatened by the loss of its mangrove habitat and appears in the EDGE list of the most distinct and endangered mammals (<http://www.edgeofexistence.org/>).

The IUCN Red list assesses the maned three-fingered sloth (*B. torquatus*) as vulnerable, and the remaining four species are of least concern. No tree sloth species are known to have become extinct. Their ground-living relatives were less lucky.

Losing ground

Rich treasures of relatively recent remains and fossils of ground sloths across the Americas bear witness of a range of species that disappeared at the beginning of the Holocene, soon after human hunters arrived. While their extinctions may in part be related to the climate change marking the end of the Pleistocene, the remains found point to severe hunting pressure that is likely to have contributed to their doom.

Until humans arrived in the Americas, there were numerous species of ground sloths, traditionally arranged in three families, although according to the molecular evidence there were at least eight.

Megatherium species, for instance, evolved during the Pleistocene to reach ever larger sizes, culminating in the Late Pleistocene *Megatherium americanum*, which was heavier than today's African elephants. As museum exhibits, the skeletons and reconstructions predate the discovery of dinosaurs, which explains why Georges Cuvier (1769–1832) coined the name *Megatherium*, which simply means 'big beast'. A rib bone from a 19,000-year-old

M. americanum from Argentina was one of the fossils that yielded mitochondrial DNA for the genome study by Delsuc and colleagues discussed above.

Giant ground sloths like *Megatherium americanum* were also herbivores consuming tree leaves among other things, but given their size and ability to stand on their hind legs, they had no need to climb trees to reach the leaves. With their size and powerful claws, they would have had no predators to fear — until human hunters arrived (*Curr. Biol.* (2019) 29, R551–R554).

By contrast, the only aquatic genus of sloth, *Thalassocnus*, died out three million years ago, long before humans arrived. It evolved a number of marine adaptations over time and fed on sea grasses off the Pacific coast of South America. The change to a marine diet may have been triggered by the desertification of its terrestrial habitat. Five species were described with various degrees of aquatic adaptation, but they may all belong to the same lineage representing different stages in its evolution.

Thalassocnus became extinct when South America connected to Central and North America, as the change in ocean currents affected the sea grasses they fed on. At the same time, it gave the South American ground sloths the opportunity to expand into Central and North America.

Among the known extinct sloth relatives, the early extinction of *Thalassocnus* remains an outlier. Most of the ground sloths thrived through the Pleistocene until humans arrived — on some Caribbean Islands they survived until as late as 5,000 years ago.

When hunters with spears started decimating the American megafauna, the sloth lifestyle dangling from the trees became advantageous in unexpected ways. Apart from their smaller sizes, tree sloths also turned out to be unattractive targets for hunting, as their claws may keep them suspended in the tree even if they are killed. Thus, at the beginning of the Holocene, the unusual ecological niche up the trees was the only one where the previously large order of sloths could survive. Being lazy can save lives.

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Q & A

Giorgio Vallortigara

Giorgio Vallortigara is Professor of Neuroscience and Animal Cognition at the Centre for Mind/Brain Sciences at the University of Trento, Italy. He has previously been Director of the Centre and later served as Prorector for Research at the university. He did research at Sussex University, UK, between 1988 and 1991 and was an Adjunct Professor at the University of New England, Australia, from 2007 to 2010. His research has been focused on the development and evolution of brain asymmetry and the mechanisms of object, space, and number representation in the animal nervous system. Giorgio holds a doctorate honoris causa from Ruhr University, Germany, and has received numerous national and international scientific awards.

What turned you on to biology in the first place? Actually, I initially studied experimental psychology. Such a choice represented a sort of detour because my interests were in ethology but at the University of Padua at the time no one was studying behavior within the Biology faculty. Instead, I heard that a professor named Mario Zanforlin — who had just come back from Edinburgh after earning a doctoral degree with Aubrey Manning — was teaching Comparative Animal Psychology in the Institute of Experimental Psychology. I started my training in Mario's lab. It was a continuation of my childhood passion: looking at and wondering about the behavior of animals. While I was an undergraduate student, I kept antlions in my bedroom and convinced Mario to allow me to perform some experiments on them. I was not very successful, but Mario was a fantastic mentor who allowed his students plenty of latitude to pursue their interests, no matter how bizarre those interests might have been.

And what drew you to your specific field of research? A serendipitous finding combined with the intellectual liberality of my mentor. I was involved in experiments on simultaneous visual discrimination learning to investigate innate shape and color preferences in

newborn chicks. In these experiments, the left/right positions of stimuli needed to be counter-balanced to avoid the animals being confounded. But I noticed that my chicks had systematic biases — pecking more to the left or to the right — depending on the visual task (e.g. whether the discrimination was based on position- or object-specific cues). At the time, brain lateralization — the different function of the left and right side of the nervous system — was largely considered a uniquely human attribute associated with language and the so-called higher cognitive functions. I was becoming more and more interested in what was going on in the brain. Mario's interests were mainly focused on behavior instead, and he suggested that I visit Richard Andrew at Sussex University to learn more about the brain. At Sussex there was an 'Ethology and Neurophysiology Group' led by Richard (I think the very term 'neuroscience' was not in use yet) and some fantastic scholars worked there, such as Mike Land, Tom Collett, and Paul Benjamin, to mention a few. It turned out that it was the right place even with respect to my little serendipitous finding: one of the former PhD students of Richard, Lesley Rogers, an Australian neurobiologist, had just published the first evidence showing that the brain of the chicken is lateralized by injecting some drugs (e.g. glutamate) into its left or right hemisphere. I was thrilled: brain asymmetry existed in the brains of non-human animals! Thus, Richard became my co-advisor in the years of my PhD studies and subsequently a friend as well as a great mentor. I also met Lesley soon after, and we started a life-long collaboration and friendship.

What were your other early influences? A very unusual mixture of intellectual traditions affected my career. On one side, my early studies in comparative perception were conducted in the Gestalt research legacy, which had a few remaining advocates after World War II in the northeast of Italy (at the universities in Padua and Trieste in particular) under the tutorship of scientists such as Gaetano Kanizsa, Fabio Metelli, and my own mentor, Mario Zanforlin. This was a tradition infused with rationalism and innatism. Neuroethologists at Sussex were much more on the empiricist side. As a joke,