Podcast Interview: Stephanie Marciniak, Logan Kistler, and Ed Louis

PNAS: Welcome to Science Sessions, the podcast of the Proceedings of the National Academy of Sciences, where we connect you with Academy members, researchers, and policymakers. Join us as we explore the stories behind the science. I'm Matthew Hardcastle, and I'm speaking with Stephanie Marciniak of Pennsylvania State University, Logan Kistler of the Smithsonian Institution in Washington, DC, and Ed Louis of Omaha's Henry Doorly Zoo and Aquarium. In a recent PNAS study, they and their colleagues reconstructed the genome of *Megaladapis edwardsi*, a giant extinct species of lemur that lived on the island of Madagascar in the recent past. The researchers used this new genetic information to determine where *Megaladapis* belongs in the evolutionary tree of lemurs and other primates. They also compared its genome to other animals to extrapolate how it might have looked and behaved. The analysis revealed that the extinct lemur likely weighed around 85 kilograms and subsisted on a largely leaf-based diet. Stephanie, how did you become interested in this project?

Marciniak: My background is in ancient DNA, and usually I work with humans and pathogens. But I got interested in this project because it marked sort of an interesting departure from what I usually work on, since I've never worked on extinct giant species before. So I became interested in it in that sense and trying to learn more about them from their genomes.

PNAS: Logan, how did you become involved?

Kistler: Yes, so I got involved in this project as part of a broader set of projects trying to dig more into the genetic history of the subfossil lemurs in Madagascar—these extinct dramatic species that have all disappeared in the last couple of thousand years. And so my involvement in this project started several years ago when we were trying to physically recover DNA from these highly degraded specimens and start to recover whole genomes from very difficult environments.

PNAS: And Ed?

Louis: Well, I've been working in Madagascar for the past 21 years, and I've worked in the past on extant lemurs. We have an amazing collection of samples of every extant lemur at multiple sites, and so that's how my background came in to help with the extant population.

PNAS: How did you reconstruct the genome of *Megaladapis*?

Kistler: So the process of retrieving DNA from ancient bones, or ancient tissue of any kind, has to focus on the fact that DNA degrades over time. So, in living cells, we have repair mechanisms that are always fixing the damage that accumulates, but, as soon as cells die, that starts to break down. And, over time, over really, really short periods of time, it becomes more and more difficult to recover good quality DNA. And that's kind of what we're fighting against, while trying to recover ancient DNA from fossils like the ones that we worked with for this study. The way that we preempt that and get around it is by working in very clean conditions. We did this work at Penn State, in a lab with positive air pressure, with full personal protective equipment, and super stringent protocols to make sure we're keeping everything clean and free of
contamination. These subfossils, in particular, from Madagascar, are particularly difficult to work with because they're from a warm environment, and that makes preservation difficult as well. So it took screening a lot of samples to recover DNA of the quality that we found from this one, and, then from there, we could very carefully take it through several preparations and build up the genome bit by bit.

**PNAS:** How did *Megaladapis* compare in size to living lemurs?

**Louis:** The largest extant lemur right now is an indri, and it's around 8 kilos. And this thing was an estimated 85 kilos, so that's unbelievable. That's like a big jaguar. It's huge. I've handled enough indri and always think they're big. They're more all arms and legs and a big belly. So I can hold their wrist, and their legs and their arms extend; they can touch the ground. This thing was just solid, and I don't know how much it actually got up in the trees.

**Marciniak:** I've heard it compared in size to a female gorilla—if you've ever met a gorilla.

**Louis:** You know, what did this thing sound like? It had to call sometime. They all communicate with calls. They always have lost calls or warning threat calls. You know, I've got great video and sound of indri, and they sound like humpback whales. And you can hear them from miles away.

**Audio clip:** [Indri calls]

**PNAS:** That, precisely, is what an indri sounds like. Stephanie, what information about *Megaladapis* did you reconstruct from its genome?

**Marciniak:** We can speak a little bit to the body size part because we identified positive selection in a gene, the growth hormone receptor, and that's involved in pathways that can affect body size differences. So the evolved gigantism that you see in *Megaladapis* could be something to do with what's happening in this particular pathway because current living lemurs today are a lot smaller.

Because we had the whole genome, we were able to compare the genes that we reconstructed for *Megaladapis* with the genes from other species. So we included other extant lemurs, as well as a bunch of nonvertebrate species that were included in our evolutionary and our dietary analyses. And then we also wanted to compare *Megaladapis* with distantly related species to see if there was some similarity in terms of diet or any other potentially interesting signals.

We found signatures related to dietary adaptation to support the idea that *Megaladapis* was a specialized leaf eater, or folivore. So there was a single positive selection in a gene that's involved in detoxifying potentially harmful metabolites, and these harmful metabolites can be introduced when ingesting or digesting leafy plants. And then we also identified similarities between *Megaladapis* and distantly related species. This included golden snub-nosed monkeys, which are folivores, and horses, which are herbivores. We identified signals in genes that are related to the breakdown of secondary plant compounds, as well as nutrient absorption. So being able to break down potentially toxic metabolites would be important for folivores like *Megaladapis*, given their exposure to these potentially toxic compounds, as well as being able just to break down these hard-to-digest components from leaves in order to harness that energy. So we did
gain that extra little bit of information on a genetic level to be able to speak to some of these
dietary adaptations and provide future avenues of research as well.

**PNAS:** What role did *Megaladapis* play in Madagascar’s ecosystems?

**Kistler:** Mammals in Madagascar mostly got there through a fairly small number of long-
distance dispersal events. There’s a fairly limited number of mammal lineages in Madagascar,
including lemurs, and so they end up sort of diversifying to fill a lot of the niche space on the
island. And, in terms of their relationship to the ecosystems and relationships to other animals,*
*Megaladapis* would have been sort of occupying that large, folivore niche.

**Louis:** That’s a perfect explanation. There's not a lot of big predators in Madagascar. They don't
have big tigers or cats there. What they have is weasels, and there was actually a subfossil giant
foosa that was found there, which, you know, I’ve seen foosa and they can be intimidating when
they come in groups inside your camp, but I can’t imagine. Or even the raptors. There were
raptors that were 45 kilos, supposedly.

**PNAS:** How might this research be extended?

**Marciniak:** I think it’s pretty exciting, being able to recover ancient DNA from environments
where it’s typically really tricky and challenging to do that. So subtropical and tropical
environments are not conducive to DNA preservation, like Logan mentioned, so to be able to
have the fortunate opportunity to have a particular sample that had enough endogenous, or
host, DNA to actually look at some of these important hypotheses that we wanted to test was
really exciting. And then future work can build on this, to demonstrate that you can really delve
into some interesting questions. Even if you have low-coverage genomes, there’s still something
interesting you can learn from them.

**Kistler:** You know, this is sort of still early days in terms of using ancient genomic datasets to
infer this kind of behavioral and ecological traits, especially about extinct species. So there’s
some other examples. We’ve seen it in things like woolly mammoths and sort of their
adaptations to northern latitudes. It's really exciting to see the work being able to move in this
direction.

**PNAS:** What is the significance of this research to lemurs and Madagascar today?

**Louis:** If you look at lemurs today, there’s 112 taxa and 95% of those are in the top three
categories of IUCN status. They're either critically endangered, endangered, or vulnerable. And
this has even been a bigger increase since the revaluation in 2012 to 2018. It's gone from 23
critically endangered lemurs, up to 39. So I think it’s a good lesson that, you know, we've lost so
much already.

I've worked all over Madagascar, been to many places, and every time I get into a forest that's
really remote, I always hope that I’m going to come around a corner one day and see one of
these subfossil lemurs standing in front of me. I think that would be the highlight of my life. It's
sad to know that this is no longer part of the biodiversity of Madagascar. It’s a shame that we've
lost this already. So it's a cautionary tale.
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