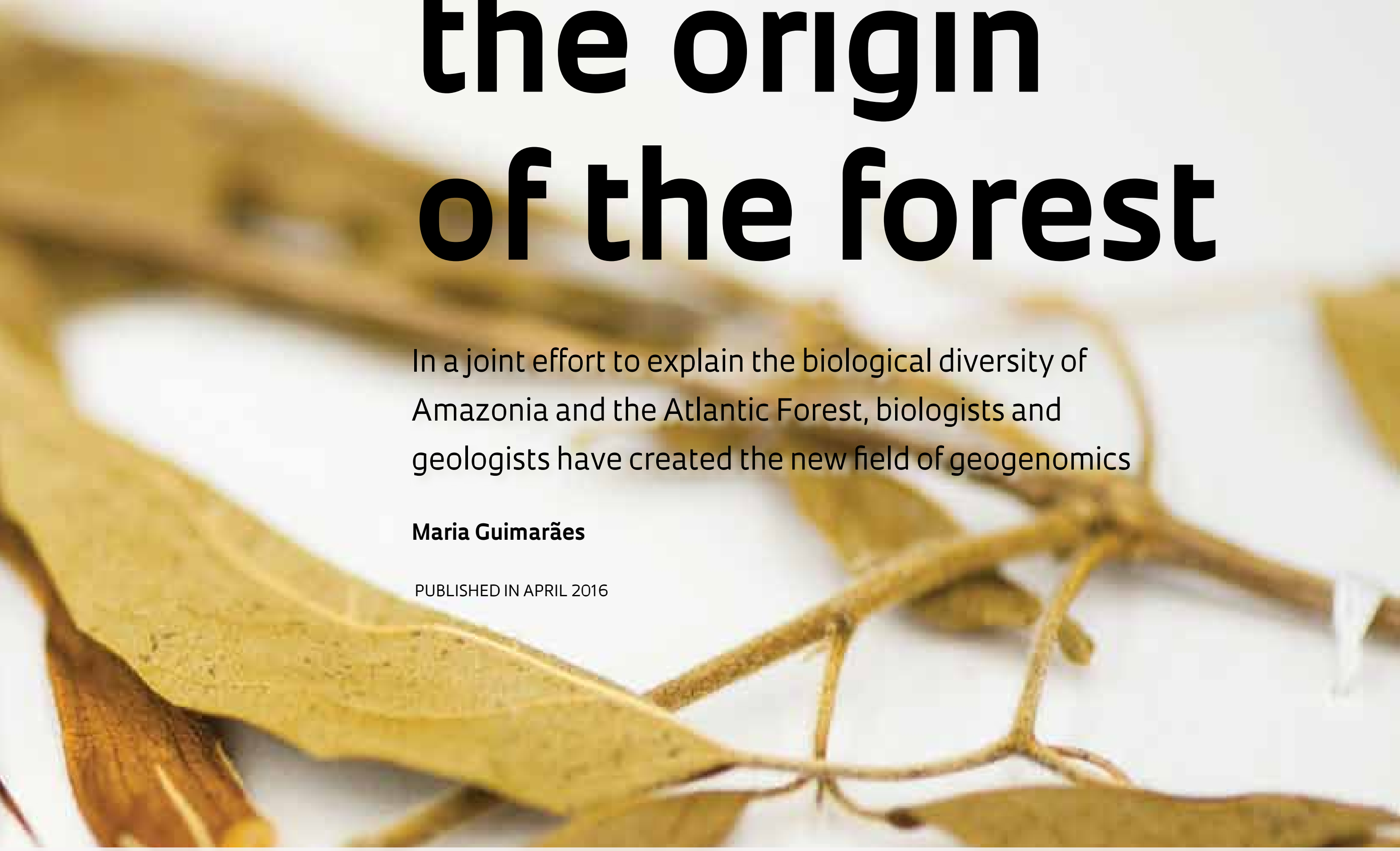
Dried and pressed branches are stored as records of plant species, such as this *Pyrostegia venusta*

Regardless of how thoroughly biologists might study the earth, the trees and bodies of water, they still seem far from gauging and explaining the biodiversity of tropical forests. By the same token, there is no scientific explanation for how or when mountains, rivers and everything that lies beneath the forests actually emerged. Scientists whose projects focus on Amazonia and the Atlantic Forest are now seeking answers: biologists and geologists are joining forces in an attempt to decipher this history, in a field geologist Paul Baker, of Duke University coined geogenomics in 2014. This new field of study has been given significant impetus from the collaboration between the Biota-FAPESP program and the Dimensions of Biodiversity, a National Science Foundation (NSF) program — the United States' leading science funding agency. "Projects of this nature require a participatory approach from the time the questions are first being hammered out," says botanist Lúcia Lohmann of the Biosciences Institute at the University of São Paulo (IB-USP). Lohmann and American ornithologist Joel Cracraft of

Seeking to understand **the origin of the forest**



In a joint effort to explain the biological diversity of Amazonia and the Atlantic Forest, biologists and geologists have created the new field of geogenomics

Maria Guimarães

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the American Museum of Natural History are leading the first project to cement this partnership, focused on Amazonia.

To assemble the teams, they first needed to overcome basic communication barriers. "A geologist would deliver a speech, and the biologists would be at a loss," Lohmann says. And the opposite was also true. "In the first meeting, we spent two hours trying to explain a single slide to the geologists," recalls biologist Cristina Miyaki, also from IB-USP, who heads a similar project, focused on the Atlantic Forest. Once a common vocabulary was established, the exchanges began to take shape. "Now it's clear that projects of this nature need to have researchers from both fields from the outset, but that wasn't the perspective before we started," Lohmann says.

Another nontrivial obstacle to consolidating knowledge is the scarcity of data. "We need to have all the phylogenies dated, with georeferenced databases, in order to produce distribution maps before we can cross-reference them with the geological data," Lohmann notes. Lohmann and her colleagues have planned a trip to the Amazon in 2016. "We're going to collect data from different organisms to assess the extent to which the Negro and Branco rivers present barriers to dispersal."

One can easily imagine that rivers carrying large volumes of water would limit the movement of organisms. But that is not always what is observed when biologists use DNA analyses to retrieve information on the history of a species. "Rivers do not appear to be significant barriers to plants," says Lohmann, who specializes in the family Bignoniaceae. But primate mobility may be limited in such cases, as shown by Brazilian primatologist Jean Philippe Boubli, who is based at the University of Salford in England. Boubli is also affiliated with the National Institute for Amazonian Research (INPA), which allows him access to the institution's large collection of primate samples. "We have an almost complete coverage of Amazonian primate samples, and with genomics we'll be able to investigate the role of the major rivers in the origins of primate diversity," he says, looking ahead. On the basis of a new phylogeny for New World titi monkeys (*Callicebus*), published in March 2016 in the *Frontiers in Zoology* journal,

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Boubli, his doctoral student Hazel Byrne and their colleagues cite deep divergences, which justify the creation of two new genera: *Cheracebus*, for species from the Negro and Orinoco rivers, and *Plecturocebus*, in the southern region of Amazonas State. *Callicebus* would be reserved for species from the Atlantic Forest. "They may be the key to everything," Boubli says. It is a very old, species-rich group and therefore ideal for testing the role of factors such as rivers and climate change in species diversification. "The collaboration with geologists is opening our eyes to things we didn't know about Amazonia," he comments.

"What is becoming clear is that the theories expounded in recent decades have turned out to be overly simplistic, considering the complexity of the Amazon," says biologist Camila Ribas of INPA, who is involved in both Lohmann's and Baker's projects. "The refuge theory holds that the present-day species originated during the glacial cycles, the last of which occurred about 18,000 years ago," she notes. But the different regions of Amazonia appear to have gone through distinct processes, and species respond differently to local conditions. Birds, which are Ribas' specialty, are a good example of organisms that vary markedly in how they cope with the environment: those able to fly long distances, for example, are less affected by barriers. At the opposite extreme, trumpeters (genus *Psophia*)—Amazonian birds that rarely fly—have become the prime example of how major rivers function as the principal barriers between species, according to a study published in 2012 in the *Proceedings of the Royal Society B* by Ribas and colleagues.

One of Ribas' recent projects focuses on the avifauna endemic to the Amazonian white sands, as described in a 2016 paper published in the journal *Biotropica*—the outcome of her student Maysa Matos' Master's research. "These areas feature patches of white sand amid large stretches of forest, with open vegetation more closely related to the Caatinga scrubland or the Cerrado savannah," Ribas explains. What is surprising is that the animals found in distant patches are more alike than one might imagine, even if they are unable to travel through the forest. The findings elicit a number of questions, such as how long that environment has existed and whether the forest was more permeable to those animals in the past.

During his Master's studies, another of Ribas' students, Leandro Moraes, analyzed the role played by the Tapajós and Jamanxim rivers, in the state of Pará, in limiting the distribution of amphibians and reptiles. The findings, to be published soon in the *Journal of Biogeography*, show that rivers limit the movement of one-third of amphibian species; in the case of snakes and lizards, the percentage falls to just 8%. The paper focuses on assessing the importance of these rivers in the configuration of the landscape and the habitats suitable for these animals, and for this reason, Ribas considers it to be an example of how the project is beginning to integrate areas of knowledge.

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The collaboration is opening our eyes to things we didn't know about Amazonia, says Boubli

THE CHANGING LANDSCAPE

In recent years, the notion that the Amazon Basin drainage network evolved predominantly during the past three million years (as opposed to the earlier estimates of 15 million years) has begun to solidify. This timescale appears to agree with indications taken from animal and plant data. The Isthmus of Panama—another structure of major importance to biogeography because it enabled migrations between South and Central America and the North—has also undergone a change in estimated age. A study led by geologist Camilo Montes of the University of the Andes in Colombia, published in *Science* in April 2015, analyzed minerals of Panamanian origin found in South America and estimated the isthmus to be between 13 million and 15 million years old—10 million years older than was previously thought. "The new dating results completely change how we see the past movement of flora and fauna in the region, and this is forcing us to reassess all of the literature," says Lúcia Lohmann.

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This reassessment has proven to be more productive because of the combined efforts of specialists. “Evolutionists and biogeographers need to know the geological history in order to understand why species live where they live and even how species came to exist,” explains Paul Baker, who coined the term “geogenomics.” He has an ambitious plan to drill five holes near the large Amazonian rivers at depths of up to two kilometers, in order to have continuous access to sediment samples of various ages — up to 65 million years old. In a meeting at INPA in 2015, Baker and his colleagues from the Amazonia project reached an agreement on which types of data, gathered from this initiative, might help reconstruct the geological, climatic and biotic history. The challenge is now to obtain funding. “Our budget for drilling alone is $7 million,” he says.

Baker’s project starts from a geological perspective, while in Lohmann’s project, the questions spring chiefly from biology. Geogenomics, however, assumes a two-way street. “The idea is that geologists also use biological data to answer geological questions,” he says. The estimated dates for the emergence of the various trumpeter species studied by Ribas, for example, can help in estimating the age of major rivers such as the Amazon, the Xingu, the Tapajós and the Madeira, according to Baker.

“Biological data provides an order of magnitude that enables us to develop hypotheses, which we can test against the absolute ages derived from geochronological dating,” says sedimentologist Renato Almeida of the USP Geosciences Institute (IGc-USP). He and his colleague André Sawakuchi are investigating the formation of the sedimentary deposits that form the Amazon Basin. “It is a continent-sized area and data on it is absurdly scarce,” he says. The task of reducing this knowledge gap cannot be completed within the timeframe of the current project and most of the data that the group is compiling have yet to be published. One of the team’s missions, in addition to painting a geographic picture of the past, is to help biologists distinguish which hypothesis offers the most firmly-grounded explanation of the biogeographic patterns.

Research efforts have been showing that the Andes Mountains’ uplift has gradually pushed the waters of an immense lake in the region toward the east, forming large-scale drainages in directed towards the Atlantic Ocean. Optically stimulated luminescence is one of the techniques for revealing the past of rivers. It relies on the collection of sediments from the steep banks that line the rivers by using aluminum tubes. “Back in the laboratory, we can determine the date when a grain of quartz was last exposed to sunlight,” explains geographer Fabiano Pupim, a postdoctoral researcher in Sawakuchi’s laboratory. The group is also discovering a wealth of information about the configuration of the sediments on the steep slopes adjacent to the rivers, which rise as high as 20 meters. Their internal structures allow scientists to infer the scale and direction of the river when the sediment was deposited, as well as other information.

Sonar images show that the riverbeds such as that of the Amazon—another unknown territory—have dunes as high as 12 meters. “We need to understand how such an enormous river functions in order to infer what the great rivers of the past were like,” Almeida says. In collaboration with geologist Carlos Grohmann of the Institute of Energy and Environment at USP (IEE-USP), he is also looking into river dynamics using Satellite Image Time Series.

Large rivers limit the distribution of species such as *Psophia crepitans* (*left*) and *Cebus olivaceus* (*below*) but not of plants whose seeds are carried by the wind (*top*)

3

PHOTOS 1 INGRID MACEDO / INPA **2** LÉO RAMOS CHAVES **3** ANSELMO D'AFONSECA

In a laboratory illuminated only by red light, one can find out when samples of sediment last received sunlight

The importance of these rivers goes beyond their function as barriers. The streams and sediments that came from the Andes formed the environmental mosaic typical of Amazonia, which contains some dry areas as well as areas characterized by periodic flooding. Sawakuchi, Pupim and their team (particularly Master's students Dorília Cunha and Diego Souza) have investigated the formation of the Anavilhanas and Tabuleiro do Embaubal Archipelagos in the Amazon River over the last 10,000 years. The emergence of this type of environment and of the rivers themselves signifies distinct timescales, whose significance the geographer hopes to complement with the biological data.

CLIMATE VARIATION

But forests do not only use terrestrial water. Francisco William da Cruz Júnior of IGc-USP, a co-coordinator of the geology component of Brazilian geogenomics, analyzes speleothems—carbonate formations in caves—and stalagmites in particular, to infer past climate. The data obtained by his research group indicates that the Ice Age in South America was not arid, as previously thought by scientists. "Part of the continent was dry, but other areas were moist and may have even been conducive to expansion of the forests, such as in the Peruvian Amazon and the southern Atlantic Forest," he notes.

Based on an analysis of the oxygen isotopes contained in the calcium carbonate in cave material, he observed that different parts of Amazonia and its adjacent regions went through very distinct processes. Evidence of these processes was reported in a 2013 article in *Nature Communications*, coauthored by Cruz and the team of biologists — the lead author being his Chinese colleague, Hai Cheng. The dating results indicate that, in the past 250,000 years, the climate in western Amazonia was more stable than the climate in the area to the east, in the state of Pará, which underwent intensified rainfall during the glacial periods—between 100,000 and 20,000 years ago. The group interprets this relative stability as being responsible for the high level of biodiversity found in the region today, while the less species-rich eastern Amazonia experienced drastic climate variation, which may have led to extinctions. "We are challenging a paradigm," says Cruz. "Climate stability may have been more important than refugia in creating the pattern of high diversity found today in the Amazon forest, particularly near the Andes."

During the glacial period, western Amazonia appears to have been quite moist, much like the Atlantic Forest region in southern and southeastern Brazil. Cruz has found evidence of a climate belt that connects these two regions, and this climate belt has features that are in contrast with those found in the area that includes Pará, in eastern Amazonia, and the Northeast, where the climate varies in cycles of about 23,000 years. "This pattern is being tested in both the Amazonia project and the Atlantic Forest project." He maintains that these correspondences enabled the formation of corridors between the two biomes, which explains the cases where kinship is closer between species of Amazonia and the Atlantic Forest than between species within the same biome. Cruz postulates that in a period during which high moisture is hypothesized to have occurred in eastern Amazonia and northeastern Brazil, the tropical forests are likely to have expanded, forming a forest bridge between these two biomes. Later periods show signs of more abundant rainfall in the region closer to the foot of the Andes, as well as in South and Southeast Brazil, where the forests may also have expanded, to the point where Amazonia and the Atlantic Forest came together. "We are currently testing what these phases might have been."

Evidence of this dynamic comes in the form of fossilized leaves collected by Cruz in the valley of the São Francisco River, a region now covered by Caatinga vegetation. "They indicate that the region was quickly taken over by moist vegetation between 18,000 and 15,000 years ago," he says. Even now, there is a direct climate connection between the two biomes: in the summer, for example, the moisture that travels from Amazonia determines what happens in the Atlantic Forest. "You can't restrict the study to local scenarios; it's not interesting," Cruz says.

The Atlantic Forest project, began a year after the Amazonia project and is led by biologists Cristina Miyaki of USP and Ana Carolina Carnaval of

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the City University of New York. It is still at an earlier stage of integration between research specialties. "During this third year, several papers we are working on include the angle or hypothesis that the team of paleoclimatologists (or the remote sensing team) has offered our team," Carnaval says. A paper with genomic data that tests theories formulated by Cruz and other members of the geology team—such as palynologist Marie-Pierre Ledru of the Institute of Evolutionary Sciences of Montpellier, France—is being finalized for publication. "It's really cool because paleoclimatology points to a path, and genomics then tests it and sees what agrees with it and what doesn't," she says. "Then, we bring the discussion back to the paleoclimatologists and they refine the ideas."

These findings are now coming to light, and they promise to be very fruitful in the next few years, when the current funding has been replaced by funding for other projects. Firming up the partnership is, it seems, the biggest victory. "We're beginning to delineate what is not yet understood," says Miyaki. Her work has always involved assumptions from the field of geology in order to understand the diversification of birds in the Atlantic Forest. But now, with the new lessons learned, comes the feeling that the earlier analyses were too superficial and that the interpretations, though they were the best ones possible at the time, were naive.

Geogenomics is an example of the best of modern science. "In a way, we're going back to the natural history of old, when researchers had an understanding of both biology and geology," Miyaki jokes. But, with ever more specialized techniques, increasingly massive databases and a growing level of detail, the only way to bring this knowledge together is to assemble large groups. Now that the researchers have moved past the initial rocky years, when each specialty continuously produced papers that were very similar to their earlier ones, truly integrated findings should begin to appear. ■

Layers of a stalagmite (*top*) and fossilized leaves (*below*) are indicators of past climate

Projects

1. Structure and evolution of the Amazonian biota and its environment: an integrative approach (No. 2012/50260-6); **Grant Mechanism:** Thematic Project; **Principal Investigators:** Lúcia Lohmann (IB-USP) and Joel Cracraft (AMNH); **Investment:** R$3,752,671.77.
2. Dimensions US-Biota São Paulo: a multidisciplinary framework for biodiversity prediction in the Brazilian Atlantic Forest hotspot (No. 2013/50297-0); **Grant Mechanism:** Thematic Project; **Principal Investigators:** Cristina Miyaki (IB-USP) and Ana Carolina Carnaval (CUNY); **Investment:** R$3,781,927.16.

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