



The Brazilian Galapagos

Lizards from the sand dunes of the São Francisco River – some resembling snakes – express their own evolutionary histories

Carlos Fioravanti

Thirty-two years after his first expedition to the sand dunes of the São Francisco River, biologist Miguel Trefaut Urbano Rodrigues, from the University of São Paulo (USP), is still surprised at “that crazy fauna,” as he puts it. In 1980, when he was 27 and even thinner than he is today, he traveled under a merciless sun throughout Santo Inácio, a city then made up of 200 inhabitants in northern Bahia state, and was astonished by the diversity of similar animals: the sister species had only small differences in appearance or genetic make-up. The nearly identical creatures lived in the sand dunes on either side of the river. They had differentiated from a common ancestor and followed their own evolutionary paths from the time that the river – which at that particular point is only 200-300 meters wide – came to separate them.

From the very first trip he brought back a new species of lizard, today called *Eurolophosaurus amathites*, which lives only there. On other expeditions, he and his team found animals that had never been described, like a two-headed snake and a subterranean snake, both with sister species from the other side of the river. In the dunes of the Brazilian Sahara, an area that measures 7,000 km² and stretches along 120 km of river, the team already identified nearly 30 species and eight new exclusive genera of lizard (exclusive to the area), which is more than in all the deserts of North America or Africa. There, and only there, lives a rodent that measures 20 cm, the *rabo-de-facho* (spiny rat), as well as a *bacurau* (nightjar) that measures 20 cm high, similarly adapted to the sand dunes of the São Francisco. These dunes display a biological diversity that is comparable to the Galapagos archipelago, on



whose fauna Darwin based his development of the theory of human evolution.

In recent years, Rodrigues and his team of 20 researchers have been explaining the genetic and evolutionary mechanisms that guided the differentiation of lizards measuring no more than 5 cm in length. The oldest species of the same taxonomic genus of lizards still look like lizards; they have short bodies and, shall we say, normal limbs. The species that began to form over the last thousand years or so, however, started losing digits. Their limbs shrank or disappeared, and their bodies became elongated, to the point where some species of lizards look like snakes except for the small appendages that would have been the front feet of their distant ancestors.

The biologists believed that the loss of complex structures like the limbs was irreversible – it is the so-called law of irreversibility of evolution, or Dollo’s Law, in honor of Belgian naturalist Louis Dollo, who presented the theory in 1890. However, one of the lines of lizards with smaller limbs showed that it is possible to get the limbs back. In collaboration with Rodrigues, Tiana Kohlsdorf, along with her team from USP Ribeirão Preto, studied 15 species of lizards of the genus *Bachia* that live throughout South America. The oldest among them had limbs with five toes and the more recent had four, then three, then two – and then three again. “There is a reversal, since the genetic information was not lost, but rather, it evolved again, because the toes that reappeared are not exactly the same,” says Kohlsdorf.

She, along with Rodrigues and colleagues from Yale University, presented these conclusions in the journal *Evolution* in 2006. They received so much criticism that they had to publish a counterargument in 2010, in an article that also appeared in *Evolution*, as a way to settle the debate. “Based on the tools of statistical analysis we developed,” Kohlsdorf says, “groups from other countries began to show reversion of wings in insects and other animals.”

While the debate raged on, the USP biologists confirmed that in at least two genera of lizards exclusive to the sand dunes, *Calyptommatus* and *Nothobachia*, the loss of limbs is irreversible. The animals had only miniscule appendages of what the limbs had been and were similar in appearance to snakes. Kohlsdorf admits, “We don’t know why only the *Bachias* were able to reverse the loss of limbs.”

Juliana Rossito, a researcher in Rodrigues’ group, examined embryos and adults of the genus *Calyptommatus* at various stages of development to understand how the loss of limbs might have occurred. She observed that in the embryo, the femur begins to form between the fifth and sixteenth days, but then it disappears. Her analyses indicate that this genus of lizards is ready to be born in one month – and not six, as in the case of the closest species – perhaps as a result of the effects of temperature or the scarcity of water. Rodrigues adds, “This species found a way to accelerate its development through natural selection.”

Life in the sands of northern Bahia state, with and without feet (from left to right): *Tropidurus amathites* from Santo Inácio; *Nothobachia ablephara* and *Calyptommatus leirolepis* from Alagoado; *Tropidurus pinima* from Santo Inácio

According to Rodrigues, the reduction in the number of feet and the elongation of the body may have occurred during the evolutionary history of lizards of the family *Gymnophthalmidae*, made up of 45 genera, including *Bachia* and *Calyptommatus*, as a result of adaptations that allowed them to avoid extreme temperatures. This process gave way to species that were able to bury themselves in order to escape predators, as well as the cold and heat, which can reach 50°C in the sand. It is estimated that this process must have occurred at least two dozen times throughout the history of these lizards. This in turn advanced the origin of unique and at times even repugnant lizards as well as a group of animals with its own habits, famous in its own right – the snakes.

In order to understand how and why the lizards are becoming able to live buried in tunnels or caves, Agustín Camacho filmed and analyzed hundreds of tests that compared the locomotion, the ability to escape and the feeding habits of 12 species of lizards of the family *Gymnophthalmidae* using cameras placed in sand inside the laboratory. “The elongated body and absence of feet seem to favor the development of the fossorial lizards that are able to escape predators more quickly, feed more and bury themselves more easily than those with feet,” he concluded. “But it’s still not possible to say which morphotype is better adapted to life in the sand, because even the species with four feet is abundant. They survive well and are seen by the dozens in the sand dunes.” According to him, desert lizards of Australia lived a similar evolutionary life.

Camacho represents the second generation of biologists on the trail of strange creatures in the sands of northern Bahia state. He studied biology in Andalusia, Spain, but “was passionate about coming to the tropics,” he says. He traveled to São Paulo in 2002 for a short course on venomous animals and lizards, returned to Spain to finish his studies, and in late 2003 moved to Salvador, Bahia, to work on his master’s degree at the Federal University of Bahia. His advisor, Pedro Rocha, had completed a doctoral degree on the ecology of the lizards in the São Francisco sand dunes, advised by none other than Rodrigues. In late 2007, Camacho moved to São Paulo and is now pursuing his doctoral degree in an office one floor above that of Miguel Rodrigues.

Rodrigues maintains the same work methodology he had when he first began studying snakes and lizards 40 years ago. First he compares the external features such as the number and shape of the scales, the body length and eye shape. Then he identifies the new species and builds a phylogeny – the history of a species, beginning with the oldest. Kátia Pellegrino, a professor at the Federal University of São Paulo, uses another approach and began to study the blood relationship among liz-

ards by comparing 10 genes. DNA analyses often indicate that the analyses obtained on the basis of external features need to be revised.

Rodrigues and his work group are seeing how restricted environments are able to limit the development of new species with unique characteristics and habits. The creatures that live in the sand dunes are so specialized that they do not survive in – or even venture into – the neighboring *caatinga*. Animals that are typically found in the *caatinga*, in turn, only marginally venture into the sand dunes. Even among the animals themselves, specialization into different environments occurred. Some species of lizards live only on clusters of rocks and do not traverse the *caatinga* areas that separate the rocky outcrops.

FROM THE OTHER SIDE OF THE ATLANTIC

Rodrigues observed the same phenomenon – isolated populations of *Platysaurus*, lizards from another family, but who are very similar in appearance to those of one of the groups of *Tropidurus*, who also have flattened bodies and live only among the stones – in Mozambique, where he went for the first time in 2007. His plan is to go back there in 2013 to conduct a survey of lizards and snakes in the north and south of Mozambique in the company of other Brazilian and Mozambican biologists. They want to see if the genera of this lizard population in Africa originated at the same time as those in Northeastern Brazil. If so, biologists may be able to tell parallel stories of lands that were close together millions of years ago.

The lizards of Bahia





The Santo Inácio sand dunes: a unique space that shelters sister species with distinct evolutionary histories

Similar creatures can have very different stories, however. In 2008, by comparing DNA strands from 10 populations of lizards of the genus *Eurolophosaurus*, José Carlos Passoni, Maria Lúcia Benozzati and Rodrigues, all from USP, showed that one of the species, the *Eurolophosaurus divaricatus*, a lizard that measures 25 cm in length and lives on the left bank of the São Francisco River, would have appeared 5.5 million years ago. The inhabitants of the opposite bank would have appeared more recently: the *E. nanuzae* 3.5 million years ago and the *E. amathites* at least 1.5 million years ago.

Calculations of when these lizard species originated greatly exceed the modest 15,000 years previously concluded using geomorphological data. At that time, the river, as its topography changed, would have diverted its flow from the interior out to sea. The internal lagoons along whose banks the lizards sunned themselves may have disappeared or the river may have absorbed part of the left bank as it began to flow eastward rather than westward. According to Rodrigues, the separation between the northern and southern banks would have occurred some 8 to 10 million years ago.

Now it seems clear to him that rivers – and not just the São Francisco – work as geographical barriers to the development of new species of reptiles and amphibians. A few years ago, Pellegrino, Rodrigues and other biologists demonstrated the validity of this notion with a species of gecko from the Atlantic Forest, the *Gymnodactylus darwini*. Populations of this species that are found to the north and south of the Doce River, which drains areas in the states of Minas Gerais and Espírito Santo, were once thought to be closely related. They no longer appear to be so; one has 38 chromosomes and the other has 40.

Frenchman Antoine Fouquet, who did his post-doctoral studies with Rodrigues, concluded that the forests of Guyana served as an important biological sanctuary for the differentiation of fauna from the Amazon region over the last few thousand years. Interestingly, the first species of snake that Rodrigues identified in 1978, when studying biology in Paris (he completed his undergraduate degree abroad and graduate work in Brazil), came from the Guianas. He identified his first Brazilian species in 1980. How many, in total, has he identified? "I've never stopped to count," he quips, between modesty and impatience with having to do the calculation. In 2010, Peter Uetz of the J. Craig Venter Institute in the U.S. did have the patience and actually did the calculation. The result: in an article published in the journal *Zootaxa* about the world's top 40 most productive biologists in terms of reptile species discovery since the 18th century, Rodrigues, the only Brazilian to make the list, is in 35th place, having described 53 species. Today, that number exceeds 60.

Miguel Trefaut Rodrigues has contracted malaria, dengue fever and many other illnesses from hiking through the forest undergrowth, often at night, in pursuit of the creatures that make him happy. Some of the rigors of travel as well as the intellectual output of this group may be seen on the lab's website: www.ib.usp.br/trefaut. Rodrigues knows that he has accomplished a significant amount, but he is also concerned about what remains to be done and what may have been lost already. "We don't know anything. I'm becoming more and more aware of how completely ignorant we are," he says. "There are sister species of lizards in the Andes and sandbanks of the Atlantic Forest. What happened to the rest of them? They disappeared!" ■

PROJECTS

1. Studies on the ecology and differentiation of the reptile fauna of the mid-São Francisco River sand dunes (Lepidosauromorpha, squamata) – No. 1996/03554-0 (1997-2002)
2. System and evolution of the neotropical herpetofauna – No. 2003/10335-8 (2004-2011)
3. Comparative phylogeography, phylogenesis, paleoclimate modeling and taxonomy of neotropical reptiles and amphibians – No. 2011/50146-6 (2012-2016)

GRANT MECHANISM

1. 2. and 3. Thematic project

COORDINATOR

1. 2. and 3. Miguel Trefaut Urbano Rodrigues – Butantan Institute/USP

INVESTMENT

1. R\$388,398.04
2. R\$975,589.35
3. R\$1,747,802.04

SCIENTIFIC ARTICLES

1. KOHLSDORF T. and WAGNER G. P. Evidence for the reversibility of digit loss: a phylogenetic study of limb evolution in Bachia (Gymnophthalmidae: Squamata). *Evolution*. v. 60, n. 9, p. 1896-912, 2006.
2. FOUQUET, A. *et al.* Molecular phylogeny and morphometric analyses reveal deep divergence between Amazonia and Atlantic Forest species of *Dendrophryniscus*. *Molecular Phylogenetics and Evolution*. v. 62, p. 826-38, 2012.
3. AMARO, R. C. *et al.* Demographic processes in the montane Atlantic rainforest: Molecular and cytogenetic evidence from the endemic frog *Proceratophrysboiei*. *Molecular Phylogenetics and Evolution*. v. 62, p. 880-88, 2012.

FROM OUR ARCHIVES

In the midst of snakes and lizards
Issue No. 169 – Mar. 2010

At the feet of dinosaurs
Issue No. 154 – Dec. 2008

A treasure on the banks of the São Francisco River
Issue No. 57 – Sep. 2000