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HUNTING HOFSTENIA

TWO INTREPID SCIENTISTS AND ONE ELUSIVE FLATWORM INTRODUCE A NEW MODEL ORGANISM TO THE FIELD OF REGENERATIVE BIOLOGY

BY DUSTIN GRINNELL

When Whitehead Member Peter Reddien first presented research on the three-banded panther worm to his faculty colleagues a few years ago, he left out the rather intriguing backstory.

As he described the attributes of the new flatworm now bolstering the regenerative biology research in his lab, he neglected to mention the harrowing Bermuda excursion with postdoc Mansi Srivastava during which the pair spent two days scouring the bottom of a saltwater pond in search of a tiny creature that may or may not have resided there. He hadn't divulged the headaches associated with bringing more than one hundred worms back to Cambridge, or the struggle to keep them alive upon arrival, or even the challenges faced in running productive experiments with them.

Perhaps that's why Whitehead Member Harvey Lodish—apparently connecting the dots on his own—interrupted the presentation with a touch of incredulity: "Wait a minute," Harvey said. "You actually *went* to Bermuda?"

Admits Reddien: "It was a bit crazy, but we just went down there. We were okay with the possibility of being unlucky."

Long before Reddien and Srivastava headed for Bermuda, they grappled with one central question: Why bother with a new model organism? Modern molecular genetics already boasted a distinguished list of such organisms, including yeast, fruit flies, nematodes, zebrafish, and mice. Each of these scientifically tractable, easy-to-breed models is supported by strong communities sharing expertise, tools, and techniques to further exploit their utility. Roughly a decade earlier, Reddien added the flatworm *Schmidtea mediterranea* (also referred to as "planarians") to the roster of model organisms, developing a platform for planarians as a system for the study of regeneration. Reddien has referred to the planarian as an "up-and-comer" in the world of model systems—and Reddien's impressive body of work has made that a certainty—but he'd always been intrigued by another group of flatworms known as acoels, which, like planarians, were reputed to have the ability to regrow severed body parts.

During the hunt for Hofstenia, *Whitehead Member Peter Reddien set up shop in a laboratory on the third floor of the Bermuda Natural History Museum. (Right) Museum Director Wolfgang Sterrer, with Whitehead postdoctoral scientist Mansi Srivastava, pointed the Whitehead team in the right direction. Photos: Mansi Srivastava, Peter Reddien*

Planarians and acoels are similar in size and shape. They're both flat, with heads, tails, backs, and bellies. It had long been thought that the two should be classified in the same subtype of the same phylum, although molecular data had suggested that perhaps they weren't so closely related after all. Still, both were found to rely on stem cell-like cells known as neoblasts to drive the regenerative process at wound sites. Sufficiently curious, Reddien labeled a manila folder "Acoels and Friends" and tucked it into his filing cabinet. For 10 years, he studied the field, filling the folder with published papers and a few ideas of his own.

One paper piqued his interest. It suggested that acoels and planarians were in fact separated by hundreds of millions of years of evolution, and that perhaps acoels were among the first animals evolving in the lineage of organisms with bilateral symmetry.

"Given the broad morphological similarities between acoels and planarians, and the proposed evolutionary distance from one another, we knew that if we compared their regenerative properties, and there were common features, these features would likely have also been present in our common ancestors," Reddien says. It's pretty heady stuff for evolutionary and regenerative biologists alike.

In the months before Srivastava started her postdoctoral work with Reddien, the two chatted about the little-studied acoels.

"Some labs were using species of acoels to study regeneration, but there were tradeoffs," says Srivastava. One species, she notes, regenerated poorly but allowed for the study of gene function with RNA interference (RNAi). Another species regenerated well but wasn't amenable to RNAi use. "We wanted a species that could do both, so we approached it as a blank slate problem."

Thinking it might be helpful to consult with taxonomists specializing in acoels, Reddien decided to attend a flatworm meeting in Belgium. There he met the Swedish Museum of Natural History's Ulf Jondelius, an expert in the phylogenetic relationships and geographic distributions of acoels. Reddien asked directly which species of acoel would serve as the best model organism. Jondelius recommended *Hofstenia miamia*, a brown and white flatworm about the size of a grain of rice. *Hofstenia*, known to live in Japan, the Red Sea, the northern Bahamian islands, and Bermuda, had already been on Reddien's and Srivastava's radar, in part because of a 2007 paper describing *Hofstenia*'s habitat in Bermuda and methods used to collect the tiny worms for study.

Emboldened, Reddien and Srivastava set their sights on Bermuda, determined to find *Hofstenia* lurking among decaying mangrove leaves at the bottom of saltwater ponds. However, merely knowing where these organisms lived *did not* a new model system make. Even *if* they could find the worms in the wild, and if they could get them back to Whitehead safely, they still didn't know whether the worms could be cultured in a lab or be amenable to sophisticated molecular-genetic techniques. Of course, there was also the matter of money. Knowing that federal funding for this fishing expedition was a pipedream, Reddien scraped together just enough cash for a whirlwind two-day excursion. There would be no time for pink sand beaches. This was strictly a business trip.

Before the pair left Cambridge, they obtained a United States Fish and Wildlife Service permit that would allow them to bring live animals back from Bermuda. An officer asked how many worms they expected to bring into the country. Not knowing if they'd find any, they picked a number: 120.

Needing a base of operations, our two researchers contacted Wolfgang Sterrer, an invertebrate zoologist and Director of the Bermuda Natural History Museum. Sterrer offered up the museum's third-floor lab, with all the microscopes and benches they'd need. At last, they were ready.

Carrying wetsuits and snorkeling gear, they arrived at Walsingham Pond, a 20-minute drive from the capital city of Hamilton. Crowded by mangrove trees, the saltwater pond has no discernible shore and is, in fact, a sink-hole formed long ago when the roof of a cave collapsed. It wasn't until they were waist-deep in the pond's shallow end that they realized neither one of them knew how to snorkel. By the time they had figured that out, Sterrer pulled up in a red convertible. He pointed casually to a sloped edge of the pond, and said, "I think that's where the worms were found." Before hopping back into his car, he issued a warning: "Keep your eyes open. There's a barracuda in the pond."

Cautiously, they snorkeled to the back of the pond with buckets and re-sealable plastic bags. Visible through the clear-blue water, the pond's mushy bottom pulsed with upside-down jellyfish. Diving proved difficult immediately. Even with weight belts, neither Reddien nor Srivastava was heavy enough to sink to the bottom. Srivastava handed over her weight belt. With it, Reddien descended about 12 feet, returning to surface intermittently with handfuls of old mangrove leaves. Srivastava bagged and bucketed the detritus and swam it to shore. After hours of work, they called it a day and made their way back to the museum's lab. They never did encounter the barracuda.

Srivastava alongside Bermuda's Walsingham Pond, where she and Reddien would scrounge for hundreds of Hofstenia *flatworms. Photo: Peter Reddien*

Back in the lab, the two began the standard method for collecting marine invertebrates—pour organic material into bins and wait. Without circulating water, the organisms consume the remaining oxygen and are then forced crawl to the surface. After a lunch break came a tiny taste of success.

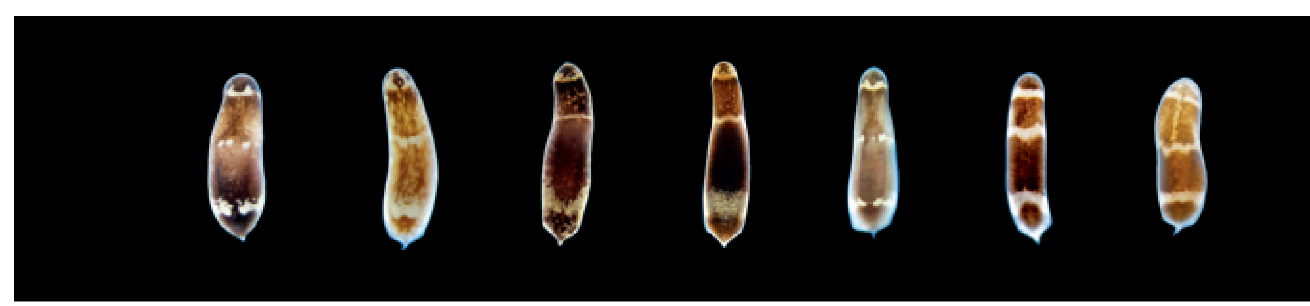
"We saw the first worm," says Srivastava. "It was so exciting!"

The flatworm was unmistakable. It was brown with the three cream-colored stripes that have earned it the name three-banded panther worm. Each worm has a unique striping pattern, much like fingerprints—some stripes are solid, others mottled.

"I think marine invertebrates are extraordinarily beautiful," says Srivastava. "*Hofstenia* have these gorgeous pigmentation patterns, and they just glide along gracefully. It gets me thinking, 'What's life like for these organisms?' "

Enthusiasm waned quickly, however, when, moving from bin to bin, Reddien and Srivastava found just two more worms. The good news was they had found the worms in the wild. The bad news, well, as Reddien saw it: "We were going to have to take a lot more trips to Bermuda. You can't start a model system with three animals. It was hard to be upset, since we didn't know whether this was going to succeed. Still, we had one more day."

Throughout the animal kingdom, many species can regrow missing tissues and body parts. Lizards can regrow tails, sharks can replace teeth, starfish can regenerate arms, and if you cut a planarian into pieces *each* piece will make a new worm. Even a planarian's brain will regrow, if severed. This ability was lost in humans. No one yet knows why.



Looking at this lineup of Hofstenia, *it's easy to understand how the flatworm earned its more common name: Three-banded panther worm. Photo: Mansi Srivastava*

The wetsuits and snorkel gear that Reddien and Srivastava carried with them on their bus ride to Walsingham Pond earned them strange looks from fellow passengers. Photos: Mansi Srivastava

When Reddien and Srivastava left for Bermuda, it had been a chilly, gray April day in Cambridge. When they returned just a few days later, the flowers were in full bloom and there wasn't a cloud in the sky.

"I remember it being a beautiful day," says Srivastava. "But the long journey had just started. We had this new organism and nobody knew how to take care of it."

Problems surfaced quite quickly. In an unexpected twist, the worms started eating each other. That's when Reddien and Srivastava realized they had approached the care and feeding of their new subjects from the planarian perspective. As scavengers, planarians eat dead food and flourish when fed liquefied calf liver—a special "frozen liver smoothie" the Reddien lab makes three times a year. *Hofstenia*, it turns out, require an entirely different diet. *Hofstenia* don't eat dead material, so when fed calf liver the worms became tiny cannibals. So, what do *Hofstenia* eat in the wild? The biggest clue could be found in their name. The "panther" in three-banded panther worm reflects *Hofstenia*'s predatory ways. Recognizing the worms' need for living food, Reddien and Srivastava introduced a diet of brine shrimp, also known as "sea monkeys". The population began to thrive.

"So we overcame the first hurdle, which was keeping them alive," says Reddien, "But it was still unclear whether *Hofstenia* would regenerate like planarians."

Published research suggested they could regenerate tails, but the researchers knew they'd need evidence of greater regenerative capacity if they were going to invest in developing the worm as a new model organism. So Srivastava did what any regenerative biologist would do: she put a worm under the microscope and cut off its head.

"And just like a planarian," she says, "the head regenerated beautifully."

With the second problem seemingly solved, they still needed to find a way of producing a worm population large enough to sustain experiments for years. After all, frequent trips to Bermuda were out of the question. With planarians, the demand for more animals can be satisfied quite literally by cutting the worms in half or in quarters, as each fragment can generate another entire worm with breathtaking efficiency. The process is far from equivalent in *Hofstenia*, but Reddien and Srivastava were surprised and delighted to discover that *Hofstenia* lay fertilized eggs that are easy to handle and hatch in just nine days.

The celebration was short-lived, however. Not long after hatching, the baby worms began dying. The problem was that the infant worms were much smaller than the brine shrimp they were being fed.

"I needed an organism smaller than the babies, so I called my undergraduate advisor," says Srivastava. "He suggested rotifers, these tiny critters that you can culture in the lab."

Sure enough, a diet of rotifers propelled the newborns into adulthood, keeping hopes for a new model system alive.

While basic scientists hesitate to discuss clinical applications, knowing exactly how regeneration occurs could unlock some exciting medical possibilities, perhaps one day offering the ability to regrow new muscle, skin, intestine, even a nervous system. Reddien pays little thought to such possibilities. His sole focus is to understand the genetic, molecular, and cellular players active at a wound site and, ultimately, to create a model of all the mechanisms involved. In his words, he wants to "to crack regeneration." Such a model is the proverbial Holy Grail in regenerative biology.

"It would be like landing on the moon," he adds. "But executing a 'lunar landing' would only be the beginning. Even if we had a model with explanatory power, there would be many more layers of resolution that we could investigate."

On their last day in Bermuda, Reddien and Srivastava mustered their wetsuits, buckets, and snorkeling gear and hopped a bus back to Walsingham Pond. ("We got some funny looks from passengers," recalls Srivastava.) As they reprised their actions from the previous day, Reddien surfacing periodically with sunken mangrove leaves, Srivastava noticed green filamentous algae covering the roots of the mangrove trees. On a whim, she peeled off clumps of the algae, stuffing it in plastic bags for the trip back to the lab.

"The paper had said the worms were collected at the bottom of the pond," says Srivastava. "But that didn't necessarily mean that's where they lived. Anyway, this was our last day. We had nothing to lose."

Back at the museum, they let the containers sit and went to lunch. When they returned, they were able to retrieve 12 worms from a container filled with mangrove leaves. Significant progress to be sure, but not nearly enough to meet their goals. Concerned, they then flipped the top off the bin containing the filamentous algae.

"The surface of the water was filled with *Hofstenia*," says Peter. They spent the next several hours collecting, sorting, and counting until they reached the permitted 120-worm limit.

"We still didn't know if they would survive in the lab, or even regenerate, for that matter," he notes. "But we had accomplished what we had come to accomplish."

"What's amazing about doing science, is just when it looks the bleakest, things work out," she notes. "I've had tragic losses, but lots of success, too."

Next on the checklist: would the molecular and genetic toolkit Reddien had developed for his planarians work in *Hofstenia*? Perhaps the most critical question was whether they would be able to study gene function with RNAi, which allows the scientists to silence specific genes and assess the consequences. Srivastava began with a classic experiment in the field—attempting to produce a worm with two heads. In planarians, inhibition of the gene *beta-catenin* results in production of a head (complete with a new set of eyes) where a tail should be in animals cut in half.

Hofstenia, however, don't have eyes, but rather a mouth where the eyes should be. Reddien and Srivastava hypothesized that silencing *beta-catenin* in *Hofstenia* would likely result in death. Still, they had to try.

"I remember shouting to everyone in the lab: 'Come here, come here, I have a two-headed worm!" Srivastava recalls. Although it didn't have eyes, the worm had grown a new mouth where its tail should be. It was exactly what they would have predicted from their planarian data.

"In that moment, I knew this was going to work, and it was going to be great," Reddien says. "Until you have a couple moments like that, you're unsure how it's going to play out."

They continued to try all of the molecular techniques in their toolbox. Almost all of them worked in *Hofstenia*. Now the basic science discoveries could unfold. Like planarians, *Hofstenia* deploy neoblast-like cells at wound sites as part of the regenerative process. *Hofstenia* also employ the same signaling pathways planarians rely on to regenerate their head-tail and back-belly axes. The scientists also spent more than a year focused on *Hofstenia*'s phylogenetics, producing strong evidence that acoels were indeed the earliest branch of bilaterally symmetrical animals and were separated from planarians and humans by approximately 550 million years of evolution.

"Even though these two organisms are distantly related," says Reddien, "they share a large set of common regenerative features, which suggests that these features were probably present at the dawn of the Bilateria. This is exciting because we can continue to use this comparison to find those common threads of regenerative biology that might be found widely in the animal kingdom."

In May of 2014, Reddien and Srivastava were finally ready to unveil their findings to the scientific community. In a *Current Biology* paper on which Srivastava was first author, *Hofstenia miamia* debuted on the world stage.

"We decided to just lay it all out there," says Srivastava. "We introduced *Hofstenia* as a new model organism, how to grow them in the lab, their advantages as a system, and also explained the conserved regenerative pathways and phylogenetic relationships between planarians and *Hofstenia*."

The scientific community embraced the worm (and the data it helped generate) with open arms. Those who study regeneration recognized *Hofstenia*'s promise in the quest to decode the regenerative processes, and evolutionary biologists updated their understanding of acoels' place on the tree of life.

Hofstenia in hand, Srivastava is headed to Harvard University in the summer of 2015 to launch a lab of her own as an assistant professor in organismic and evolutionary biology. Not surprisingly, her plans include developing more tools, including transgenic methods, with which to study regeneration in *Hofstenia*. She refers to her new model as the "new kid on the block," but one that could figure prominently in answering the myriad remaining questions about how these tiny creatures are so adept at tissue replacement and whether any of their seemingly mystical powers might one day be applicable in humans.

For his part, Reddien will maintain a population of *Hofstenia* as a complement to his ongoing planarian studies. With new findings and continued success, he hopes more molecular biologists will embrace *Hofstenia* as a model system that may one day give rise to its own research community. He says it's only the beginning of a long and interesting tale, one that began in completely unexpected fashion.

"As a molecular geneticist, you spend most of your life in the lab with pipettes and thinking about data," Reddien says. "When we were in Bermuda with snorkeling gear in the middle of the pond, I remember thinking, 'What on earth are we doing here?' But it was a great adventure, and it couldn't have worked out any better."

The lab at the Bermuda Natural History Museum had the bench space and microscopes Srivastava and Reddien needed to comb through bags and buckets of pond detritus in search of three-banded panther worms. Photo: Peter Reddien