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Fern Matters

"How do I know thee? Let me count the ways." I am paraphrasing Elizabeth Browning on the matter of ferns. Until recently, there is only one way — conventional taxonomy — to classify plants, by appearance, reproduction and other physical traits.

Ferns are globally distributed, diverse and ancient. Fossil records show that they were already around 320 millions ago, thus predating dinosaurs and other vascular plants. While they are widely found on the planet, they are most abundant in the tropics. More than 900 species occur in Costa Rica.

The classic fern is fairly easy to recognize. They generally have feathery leaves, with a main stem, and roots in the form of rhizomes. Their size range widely, from a few mm (less than 1/8 in) to 20 m (65 ft) in height. At Nectandra, they grow everywhere, on the ground, on trees (photo), on rocks and even on other ferns. It seems like every non-macro photograph I have taken at the reserve have at least one background fern peeking through.

The dominance and sheer number of different looking ferns at the reserve suggested that the ferns were a good place to

start phase two of the Nectandra botanical survey. Phase one, begun in 2002, covered mainly the larger vascular plants on the property. The results gave us a general picture of the diversity and population of the trees. With that basic information, we wanted to initiate surveys of at least the less studied dominant flora — ferns and bryophytes — although we realized that they were big items for a small organization budget.

From the perspective of a curator, the high biodiversity at Nectandra is nine parts blessing and one part curse. The blessing comes with the density and richness of rare and interesting plants ready to be catalogued. The curse comes with the need for expertise on each plant group. Deep botanical knowledge of tropical plants is hard to come by, but expertise in tropical ferns is even rarer. We did not have a practical strategy in place to proceed, but unbeknownst to us, the solution was right around the corner.

As with so many key events in the history of Nectandra, the solution walked in on two feet, in 2008, in the form of an undergraduate student, Joel Nitta, from UC Berkeley attending a course on Tropical Plant Systematics at OTS

(Organization for Tropical Studies) in Costa Rica. As his course was finishing, his UC professor suggested Nectandra Cloud Forest Reserve as a place to visit before returning to California. Joel was particularly interested in the filmy ferns (family Hymenophyllaceae, previous photo), a group of largely epiphytic ferns that only grows in continuously humid environments. Unique among ferns, filmy ferns do not have true roots and are extra sensitive to humidity changes. Nectandra's location on the Atlantic mid-elevation slope was optimal for these ferns. Their thin fronds, just 1-2 layers of cells, are translucent, hence the moniker.

Joel wrote to ask for permission to specifically collect filmy ferns at Nectandra. He spent 2-3 days collecting and left happy with 25 or so species, a relatively high number for a small reserve. While the impetus for collecting was for Joel's undergrad thesis, he left a duplicate set of the specimens and shared all his data with us. Thus serendipitously, the Nectandra fern survey was off, but not exactly running.

Two years later, we saw an opportunity to recruit Joel during his gap year before graduate studies. We were delighted when he accepted our proposal for a longer, more extensive general survey of Nectandra ferns. During his prior trip, Joel's total focus was on the filmies. He barely paid attention to the other ferns. He estimated needing two solid weeks for a reasonably complete survey. His plan was to spend two weeks collecting and one week touring parts of the country he missed on his first visit.

We explained to Joel that conservation and protection of the fauna and flora are the main priorities for our daily existence, but as conservators, it made sense to document what we were conserving. In addition, it is essential that we make available collected specimens and gathered information to other fellow scientists and future scholars. The objective for the survey, we jointly concluded, was to collect triplicate copies of each specimen, one for Nectandra, one to be deposited at INBio (Instituto Nacional de Biodiversidad, Costa Rica), and one at the Jepson Herbarium (UC Berkeley). Joel had an additional request. He wanted to preserve some of the collected material for future molecular genetic studies.

Three months later, all the necessary collecting permits, paraphernalia, reference books and chemicals were duly assembled and ready for Joel's arrival. Freddy, our ground staff with knowledge of the local flora, was assigned to Joel as field assistant for the duration. Not one to waste time, Joel hit the trails early the day after arrival with Freddy in tow. On day three, Joel asked to meet me. "Evelyne, I have a slight problem," he said anxiously. "I just realized there are a lot of ferns here, many more than I had thought. I don't think I can finish the survey in the time I allotted." I assured him that it was not a problem. It was a long term project with no deadline and no specific goalposts. Privately, I was overjoyed with his "bad" news. It implied that there are a *lot* of ferns at Nectandra, more than expected, good news indeed.

For the next few weeks, Joel and Freddy could be seen combing the reserve, at times lugging long distance, a 16ft extension ladder to get hard-to-reach epiphytic ferns. This was in January during the wettest period of the year. The two would return from the trails many afternoons drenched, muddy, hauling an enormous number of small and large plastic bags laden with specimens. Every afternoon, the specimens were sorted, cleaned, catalogued, pressed and prepared for drying. Tiny samples of each were also stored in suitable preservatives for later nucleic acid sequencing. Anxious to do all he thought he had agreed to do, Joel decided to spend the entire three weeks at Nectandra, foregoing his original plan to play tourist. His only down time was spent long distance running in the nearby area. The local residents would watch from their houses the energetic young runner loping the many dozen kilometers between Nectandra and the nearby small villages.

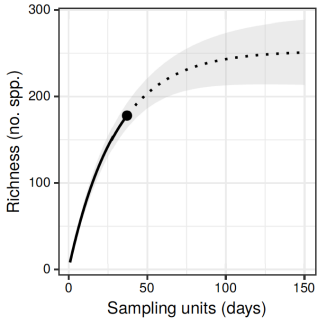
Probably feeling that he left his task only half done, Joel made another 3-week collecting trip two years later, between his first and second year at Harvard working toward his PhD. After Nectandra, he returned to his studies, eventually married, became a father, graduated with PhD in Organismic and Evolutionary Biology, and is now an assistant professor at the University of Tokyo after two postdoctoral stints, one in Japan and one at the Smithsonian Institute.

The most unusual part of this arrangement between Joel and Nectandra is the informality of it. There was no written agreement, no rigid agenda, no long discussion over any aspect of the arrangements — logistic or financial. Both parties just took care of the necessities as they come up. As previously mentioned, there were no fixed schedule, only mutual commitment to the general goals we agreed on at the outset. By the time of his graduation (2016), Joel had identified almost all of the samples down to the species level. Dr. Alan Smith at UC Berkeley helped with the more difficult identifications. The earlier collection was deposited at the Jepson Herbarium in California, and the second at the Harvard University Herbaria. There was simply no opportunity for Joel to analyze and publish his Nectandra findings, nor to do the sequencing work on the samples while he was at Harvard.

Knowing Joel's working style and his doggedness, we quietly waited. We were not disappointed. Joel was able to persuade his postdoctoral mentor in Japan to support work on the Nectandra collection. In 2018, Joel sent us a poster presented at the annual Japan Society for Plant Systematics, with summary data on the Nectandra ferns, comparing their gene markers with ferns from Japan and Tahiti. This past month, Joel submitted a formal manuscript to his selected journal for publication. Joel made a number of observations. They are milestones because of the significance of the data.

What do we know about the Nectandra ferns that we didn't know before?

First, Nectandra (158 ha/390 A) is remarkably diverse. Joel collected a total of 176 species representing 69 genera and 22 families. In field surveys using random sampling, it is possible to estimate mathematically the probable richness of species for the location. Applying the program to Nectandra, the resulting curve (left) showed that the final count could have reached 253 species had Joel put in a total of >100 collecting days. The solid line is the actual collection rate. The dotted line is the expected rate. This suggests that there are yet many more species still out there to be collected.



What about the fern diversity among biological reserves in Costa Rica?

Biological Reserve	Elev (m)	Area (Ha)	spp	spp/Ha
Atlantic slope				
Nectandra	1000-1200	158	176	1.11
OTS La Selva	35-130	1533	197	0.13
Alberto Brenes	550 - 1520	7800	281	0.04
Pacific slope				
San Luis	540 - 855	251	39	0.16
Monteverde	1500 - 1640	3800	147	0.04
Los Santos (Savegre)	2000-3491	10000	123	0.01

The table shows the density of fern species at Nectandra (spp/Ha) is very high, relative to other sites. It is a tabulation of what has been published. There are many other factors that are inapparent and unclear, for example the sampling methods, height of sampling, thoroughness and duration of sampling etc. — variables that can greatly affect the numbers. The table gives only a very rough trend of the six surveys. It is not meant to be a direct comparison.

What did we learn from the gene marker sequencing?

Joel used an approach known as biocoding, sometimes known as barcoding. It is a method used to distinguish one species from another based on the gene sequence difference of a specific gene. This would be analogous to identifying fingerprints between individuals. Instead of fingerprints, the recognition in this case is of a short DNA segment of a gene found in plant chloroplasts. This general approach works well for speciation of fauna using a single universal mitochondrial DNA marker. However, biocoding plants turned out to be technically more difficult and less definitive than barcoding animals. There are not many molecular studies comparing large number of ferns groups or comparing those from different geographic locations.

Joel wanted to combine traditional taxonomy with barcoding to gain insights into the biodiversity of the 176 Nectandra ferns. His results using the chloroplast *rbcL* marker confirmed what others have found, that this sequence is not distinct enough to tell 100% of the species apart, but it can speciate 82% of the Nectandra 176 ferns. Interestingly, using the same approach with ferns from elsewhere, it could discriminate 95% of Moorean (Tahiti) ferns but only 72% of Japanese ferns.

Using the same fingerprint analogy, it would be equivalent to saying 95% of Mooreans fingerprints are distinct enough to tell individuals apart, whereas that is true only for 72% of Japanese persons. Nectandra is somewhere in between.

These barcoding results have direct implication on the evolutionary relationships among the ferns at each location. Japanese ferns are closer to each other genetically. Moorean ferns are relatively further apart from each other. Nectandra ferns fall somewhere in between. One can infer from these results that the degree of evolutionary pressures exerted on ferns at each location were different and likely distinct.

Joel's third significant result involved three specimens that were unidentifiable by conventional taxonomy. The sequences on two suggest they are inter-species hybrids. The third unidentifiable specimen had an unresolvable sequence and may be part of a species complex (morphologically similar but genetically distinct).

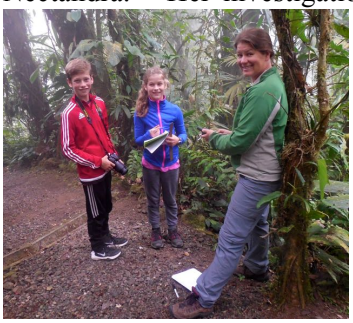
To come full circle on the opening query, there are now *two* ways, both imperfect but complementary, to classify ferns. Together they provide much more detailed and intriguing information. We hope Joel's investigations will continue and will trigger more data gathering and learning about our wonderful world.

— Evelyne T. Lennette —

News Highlight

Sabbatical

Dr Nicole Fenton, professor from Institut de recherche sur les forêts (IRF), Université du Québec en Abitibi-Témiscamingue) spent four months sabbatical at Nectandra. Her investigation was on: *The Influence of epiphyllous bryophytes on the water cycle in a tropical submontane cloud forest in Costa Rica*. Dr. Fenton came to Nectandra for the first time in 2010 to participate in a bryophyte workshop for international attendees organized by the Jepson Herbarium UC Berkeley. This time, her family accompanied her on her sabbatical. Her two children are pictured working as her field assistants. At other times, they enjoyed learning Spanish from their soccer teammates.



Internships

Ben Camper, from the San Ramon campus of USAC (Universities Studies Abroad Consortium) and a third year student from Clemson University in S Carolina, teamed up with fellow student Evan Klumpp in the fall of 2018 to do a general survey of reptiles and amphibians at Nectandra .

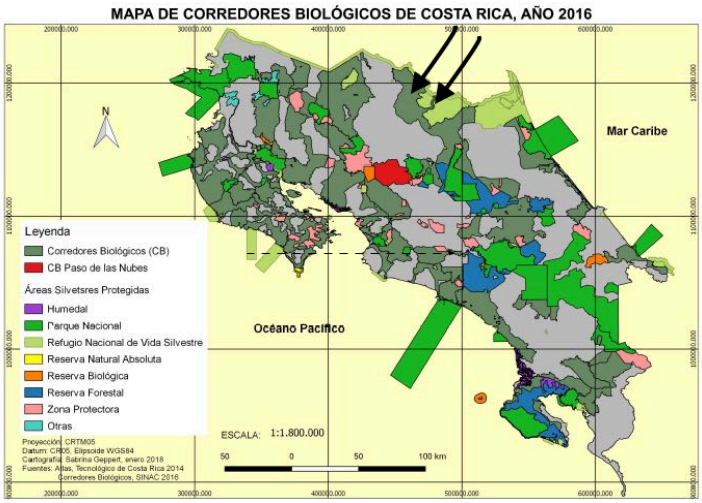
Their field research was done mostly late evening and at night in our wet forest. They used the catch-photograph-release approach to document all their collection. Ben is shown here with his camera set up ready to photograph his subjects. The specimens were often tiny frogs no more than 2 cm large. Cataloguing and identification of these small frogs often involved differentiation of microscopic morphologic features and macrophotography for documentation.

Ben extended his internship a second term. For the Spring term, Ben marked off study plots located within mature or secondary forest. He compared the diversity and density of amphibians and reptiles observed in the various plots. He is in the process of analyzing his collection and writing up his findings.

Biological Corredor Paso de las Nubes
(Corridor of the Clouds)

The Nectandra Cloud Forest Reserve is located within the Biological Corredor Paso de las Nubes.

In 2006, the Costa Rican government designated a 2646 Km^2 area (1020 sq miles) marked in red in the map below as the Biological Corridor of the Clouds. This corridor is flanked on the east by the Juan Castro Blanco National Park (green) and on the west the Alberto Brenes Biological Reserve (orange), see inset below.



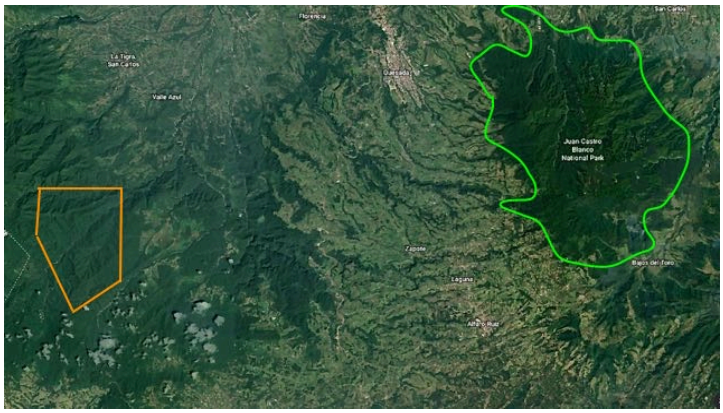
The Juan Castro Blanco NP is the headwater region for 18 rivers and 8 streams, and include two large watersheds that supply much of the water for the surrounding region. The biological corridor designation allows for active participation from multi-level sectors — federal, municipal, commercial, agricultural, private reserves, and non-profit organizations — to develop management plans for this hydrologic important region. It is the source of much of the potable water and as well as water for hydroelectric generation.

The inset at left is an enlarged map of the area.
Almost daily, the moisture laden trade wind from the north

east (arrows in map above) blows across the vast plain of San Carlos (grey). The wet air mass is forced upward when it eventually meets the Tilarán volcanic range. As the air rises, it cools, and condenses into clouds. Hence, the daily clouds that bathes the entire corridor (in red).

Nectandra is located halfway up the Tilarán's slope. Its approximate location within the corridor is marked by the yellow star.

The government development program for the region simmered until 2016, when various organizations were invited to participate. In the last four years, the Committee of the BC Paso de las Nubes have been busily organizing programs to monitor, conserve and develop green zones in the corridor.



It is difficult to appreciate physical conditions within the corridor from the two dimensional maps. The aerial view of the region shows the corridor cleanly bisected at midline. The fairly intact forested region is on the left and the denuded area on the right. On the ground, the deforested areas are dominated by ranchland and farmland, pockmarked by small farming hamlets, with no major urban areas.

The shift from forest to other land uses occurred mostly within the last five decades. It accelerated with the construction of the national route 702. The highway is visible as a thin north-south midline in the map. The highway is used by 70% of all the country's tourists. It is the main route to the town of La Fortuna, used as the basecamp for visitors to the Arenal Volcano National Park. The highway also is one of the two accesses to the agricultural products grown in the San Carlos region to the north (the grey area just below the arrows in the first map).

Nectandra Institute (NI), is represented on the Committee of the BC Paso de las Nubes by Manrique Esquivel, our staff biologist, who is in charge of NI's reforestation program, the community plant nursery, and the macroinvertebrate monitoring of the many streams in the Balsa watershed.

We will report more on Manrique's participation and experiences in the corridor's program in our future issues of the newsletter.

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