Final Report:

# Conservation efforts for federally endangered ferns of Puerto Rico's Cordillera Central

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*View near the summit of Cerro Punta by Jimmy Lange*

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## Related reports

This final report follows a series of reports listed below. The first (2015) report includes greater detail on methods developed to propagate these ferns from spore and/or from proliferous buds.

- Possley, J. and J. Maschinski. 2015. Determining Propagation and Spore Storage Potential of Three Rare Puerto Rican Ferns. Report from Fairchild Tropical Botanic Garden to USFWS Caribbean Field office and Puerto Rico Departmento de Recursos Naturales y Ambiente under Cooperative Agreement F14AC01201.
- Possley, J. and J. Lange. 2016. Interim Report: Update on conservation efforts for federally endangered ferns of Puerto Rico's Cordillera Central. Report from Fairchild Tropical Botanic Garden to USFWS Caribbean Field office and Puerto Rico Departmento de Recursos Naturales y Ambiente under Cooperative Agreement F14AC01201,
- Possley, J. and J. Lange. 2017. Interim Report: Update on conservation efforts for federally endangered ferns of Puerto Rico's Cordillera Central. Report from Fairchild Tropical Botanic Garden to USFWS Caribbean Field office and Puerto Rico Departmento de Recursos Naturales y Ambiente, under Cooperative Agreement F14AC01201, Amendment No. 1.

## Executive summary

The ferns of Puerto Rico include eight federally listed endemic taxa which are found within the island's Cordillera Central: *Adiantum vivesii, Cyathea dryopteroides, Elaphoglossum serpens, Polystichum calderonense, Tectaria estremerana, Thelypteris inaboensis, Thelypteris verecunda* and *Thelypteris yaucoensis.* Despite extreme rarity and acute threats, these ferns have been poorly studied since their initial description. They are very difficult to access as well as difficult to identify. In many cases, there is uncertainty as to whether these populations still exist and as to whether they represent valid taxa. Together with partners from USFWS-CESFO, DNER, and University of Puerto Rico, the authors of this report sought out these elusive fern populations, documented their occurrence when found, attempted propagation to develop ex situ conservation collections and conducted genetic analyses to determine the phylogenic status of questionable taxa.

In 2014, 2016 and 2017, we made three field surveys to visit or search for seven of these eight taxa. We did not attempt to visit *Thelypteris inaboensis* due to the remote locations of this species (reaching the area would require several hours of hiking and overnight camping).

Field surveys were relatively simple for *Cyathea dryopteroides* and *Polystichum calderonense*, owing to the fact that local botanists (Monsegur, Velez and Sustache) were already familiar with them. Genetic work was not a priority for either of these species. As a result of this program, both species have been successfully cultivated at Fairchild Tropical Botanic Garden, with spores cryogenically stored at the USDA NLGRP. Living material has been shared among several botanical institutions, further safeguarding germplasm. Some potential reintroduction habitat has been evaluated and described. Recovery actions such as reintroductions or augmentations are very possible in the next decade for both *C. dryopteroides* and *P. calderonense*.

For three taxa, *Adiantum vivesii*, *Tectaria estremerana* and *Thelypteris verecunda*, field surveys followed by genetic studies at the University of Florida have advanced our understanding of the very complicated relationships between these rare taxa and their more common congeners. Results suggest that each of these taxa are of hybrid origin. When combined with the MS research of Sepúlveda-Orengo (2000), we suggest that there is strong evidence to support the delisting of *A. vivesii*, though other Puerto Rican *Adiantum* species of hybrid origin may be capable of reproduction and, in turn, deserving of protection. Further studies into the spore morphology and viability are needed to determine whether *Tectaria estremerana* and *Thelypteris verecunda* are true species and therefore in need of legal protection.

Conservation efforts for the remaining two taxa, *Elaphoglossum serpens* and *Thelypteris yaucoensis*, were less successful, yet some progress was made. Two surveys that failed to find *E. serpens* in its last known locality (Cerro Punta) further support the idea that this occurrence (like the type locality) was extirpated when the cellular tower was constructed. A survey of Pico Rodadero that failed to obtain material of *T. yaucoensis* highlighted the difficulty in identifying separate taxa in the *Goniopteris* group of Thelypteridaceae; this area should be re-surveyed.

This highly productive collaboration has made strides toward recovering Puerto Rico's federally listed ferns, while laying the groundwork for future efforts. We highly recommend additional field surveys by knowledgeable botanists, spore collection for banking and ex situ conservation collections, and genetic research for each of the taxa discussed here as well as other rare fern taxa that may not be federally listed.

## Introduction

Puerto Rico has a rich fern flora that includes 22 endemic taxa, many of which are exceedingly rare (Proctor 1989). Despite this, there are little to no ex situ conservation collections that can act as safeguards for wild populations. Additionally, taxonomic uncertainty in several of the described species has made conservation planning difficult. The collaborative efforts reported herein are intended to reduce the extinction risk and increase information about the taxonomic status of the federally listed endemic fern taxa of Puerto Rico's Cordillera Central, including: *Adiantum vivesii* Proctor*, Cyathea dryopteroides* Maxon*, Elaphoglossum serpens* Maxon & C.V. Morton*, Polystichum calderonense* Proctor*, Tectaria estremerana* Proctor & A.M. Evans*, Thelypteris verecunda* Proctor and *Thelypteris yaucoensis* Proctor*.* Project objectives included locating new or historical populations of these rare ferns, monitoring known populations, collecting leaf tissue for DNA extraction and phylogenetic studies, collecting spores for propagation and ex situ conservation, and collecting environmental data to quantify microhabitat requirements. The latter two objectives are necessary steps toward identifying potential reintroduction sites and conducting reintroductions to the wild.

The following report describes activities carried out in Puerto Rico over three trips, each approximately one week long, in November 2014, January-February 2016, and February 2017. Additionally, we report on follow-up work at Fairchild and the University of Florida. Information in this report is organized by species, with each section containing relevant background information, methods, results, and discussion.

# *Adiantum vivesii* Proctor

## Background

*Adiantum vivesii* is a federally endangered fern known from a single locality in the municipality of Quebradillas, in Puerto Rico's karst belt. It was first described by George Proctor (1989), who reported that the species formed a clonal stand. The taxonomic status of *A. vivesii* is uncertain, as it has been speculated to be of hybrid origin, with *A. tetraphyllum* as one potential parent (Sepulveda-Orengo 2000). This is supported by Sepulveda-Orengo's findings that *A. vivesii* spores were abortive. However, no molecular studies had been conducted to confirm this possibility. Prior to this work, the wild population had not (to our knowledge) been monitored by biologists in well over a decade. Our objectives regarding *A. vivesii* were threefold: find *A. vivesii* at its type locality, collect tissue samples, and facilitate genetic work in the Sessa Lab at University of Florida to definitively determine whether *A. vivesii* is a valid taxon.

## 2016 Quebradillas survey

In January 2016, Possley and Lange traveled to the type locality described in Proctor (1989) as Barrio San Antonio (Mo. Quebradillas) Road 119, km 16, 0.4 km north. Proceeding north from the "16 km" marker, they searched for *A. vivesii* for approximately 3 hours (**Map 1**). Several *Adiantum* leaf samples were collected during the survey, along with accompanying herbarium specimens. All herbarium specimens were deposited at MAPR. Tissue samples were shipped to University of Florida for genetic analysis.

## 2017 Quebradillas survey

On February 20, 2017, a larger group led by Monsegur revisited the *A. vivesii* type locality, beginning the survey at a private home just west of kilometer marker 16 on Road 119. Monsegur spoke to the homeowner, who remembered Papo Vives and Proctor visiting in the 1980s. He permitted access to the valley from his property. Access was possible by hiking north between mogotes and descending a steep cliff into a ravine (**Map 1**). This ravine, which was immediately west of the area surveyed by Possley and Lange in 2016, had very little disturbance, and high diversity of native ferns and other vascular plants (**Fig. 1**). *Adiantum* species were plentiful in the area, and several individuals looked very similar to the type specimen of *A. vivesii*. However, during the approximately 3-hour survey, the group did not locate any clonal stands of *Adiantum* in a hollow at the base of a cliff as Proctor described. A variety of morphotypes were collected for genetic sequencing at University of Florida; these were accompanied by herbarium specimens which were deposited at MAPR (see **Appendix 2**).

Other fern species observed during the survey included *Adiantum obliquum, A. pyramidale*, *A. tenerum*, *A. wilsonii*, *Asplenium dentatum*, *Thelypteris poiteana*, *T. hildae*, *Blechnum occidentale,Tectaria incisa, T. heracleifolia, T. cicutaria, Bolbitis pergamentacea*, *Lomariopsis* sp., and many other *Asplenium* spp. which we did not identify to species.

**Figure 1**. Botanists working on the side of the ravine that was the source of 2017 *Adiantum* collections

## Genetic studies

### *Materials & Methods*

<u>DNA Extraction and Sequencing</u> - Leaf material for DNA extraction was collected in the field in Puerto Rico in February 2017 and from herbarium specimens, including the isotype for *Adiantum vivesii* from SJ (Proctor 41389). A total of 28 individuals were sampled: five identified as *Adiantum latifolium*, two as *A. obliquum*, three as *A. petiolatum*, four as *A. pyrimadale*, five as

*A. tetraphyllum*, four as *A. vivesii*, and four unidentified *Adiantum* individuals. We extracted DNA from the field-collected, silica-dried leaf material and the herbarium samples and amplified one protein-coding region (IBR3-1) of the nuclear genome using polymerase chain reaction (PCR) and the F2 and R2 primers of Rothfels et al. (2013). We then ran the PCR products on agarose gel, cut out the resulting bands, and re-extracted the DNA. Using the pGEM-T Easy Vector System, we cloned the re-extracted DNA. At least 12 and up to 16 transformed colonies were chosen for each individual, after an incubation period of 16 hours, and amplified using the initial IBR3-1 primers. The final PCR products were purified and sent to Eurofins with F2 and R2 primers for sequencing.

Alignment and Phylogenetic Analyses - The IBR3-1 sequences were assembled using the sequence analysis software, Geneious Prime (version 2019.2.1). Chromatograms of all sequences were visually inspected and trimmed by hand. We also downloaded IBR3-1 sequences from Genbank for *Adiantum pedatum* and *Cryptogramma acrostichoides* (as the outgroup) and aligned these sequences along with the Puerto Rico samples using the MAFFT plugin for Geneious (voucher information for the Genbank accessions available in Apendix 3). We used the software IQ-TREE to perform Maximum Likelihood analysis and reconstruct the phylogenetic tree.

### *Results*

Of the 29 individuals sampled for the molecular analysis, we were unsuccessful in acquiring ample PCR product for cloning of 7 individuals, resulting in a total of 22 individuals in the phylogenetic analysis. Several of the individuals were sampled more than once, resulting in a total of 27 samples for the analysis (see Appendix 3 for voucher information). The final alignment was 872 base pairs in length with 233 variant sites (25%).

The phylogenetic analysis resolved five main groups that included Puerto Rican samples, which we will refer to as the “pyramidale group”, the “pulverulentum group”, the “petiolatum group”, the “latifolium group”, and the “tetraphyllum group” (**Fig. 2**). The pyramidale and pulverulentum groups each form a well-supported clade (99% and 99%) that are successively sister to the rest of the Puerto Rican individuals. Each of these two groups is further divided into sub-clades (four sub-clades for pyramidale, two sub-clades for pulverulentum) with strong support (100%) indicating the presence of allelic variation which may be associated with multiple copies of the IBR3-1 gene. However, ploidy level (number of chromosome sets) cannot be conclusively determined from sequence data alone. The petiolatum and latifolium groups together form a clade with relatively strong support (94%), but support for them as individual clades is low (69% and 52%). Nevertheless, we will treat the petiolatum and latifolium groups as separate entities for the purposes of this report. Similar to the pulverulentum group, the latifolium group is further divided into two sub-clades indicating allelic variation that may be associated with multiple copies of the IBR3-1 gene. Finally, the tetraphyllum group appears to be non-monophyletic, with samples resolving into separate clades labeled “tetraphyllum group A” and “tetraphyllum group B”. Group A is strongly supported (96%) as sister to one accession of *A. obliquum*, while Group B is sister to the type specimen of *A. vivesii* but with poor support (less than 50%).

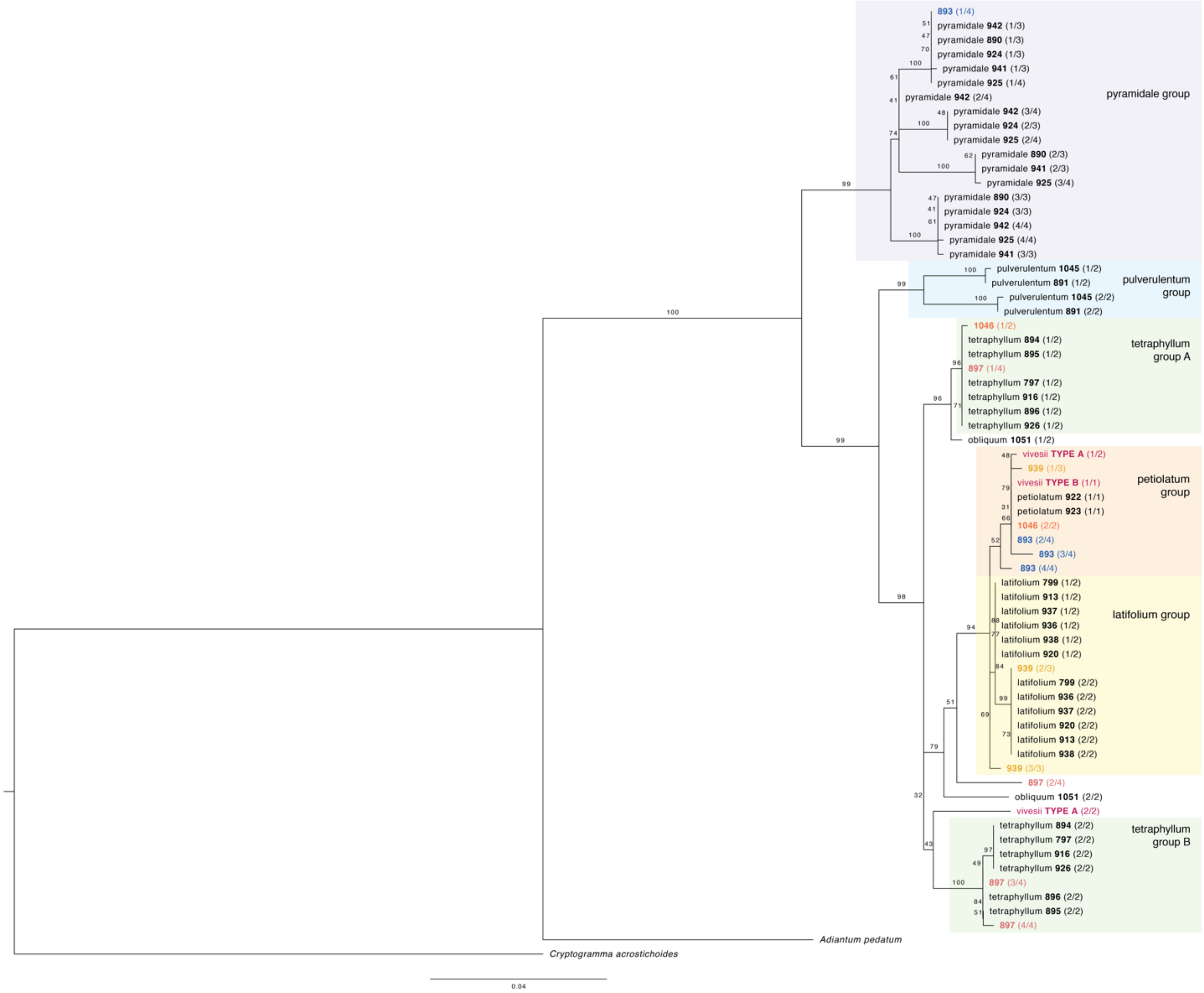


**Figure 2. (See enlarged version on following page).** Maximum clade credibility chronogram from the IQ-TREE analysis of Puerto Rican *Adiantum*. Numbers on the branches of the tree are the Maximum Likelihood bootstrap support values. The colored boxes highlight five main groups, with the pyramidale group in purple, pulverulentum group in blue, the petiolatum group in orange, the latifolum group in yellow, and two distinct tetraphyllum groups, A & B, in green. The bold number in the tip label is the unique accession number for each individual sequenced. The numbers in parentheses after the accession number indicates the number of sequence variants, or copies of the IBR3-1 gene, recovered for each (e.g., the accession *tetraphyllum 894* has 2 sequence variants – (1/2) in tetraphyllum group A and (2/2) in tetraphyllum group B). Those individuals inferred to be hybrids are each highlighted by a different color text. Accessions 1046 and vivesii TYPE A (red and pink text) are inferred to be hybrids between *A. tetraphyllum* and *A. petiolatum*. Accession 897 is also likely a hybrid between *A. tetraphyllum* and *A. petiolatum* though the placement of 897 (2/4) is not well resolved. Accession 893 (blue) is inferred to be a hybrid between *A. pyramidale* and *A. petiolatum*, and accession 939 (yellow) is inferred to be a hybrid between *A. petiolatum* and *A. latifolium*.

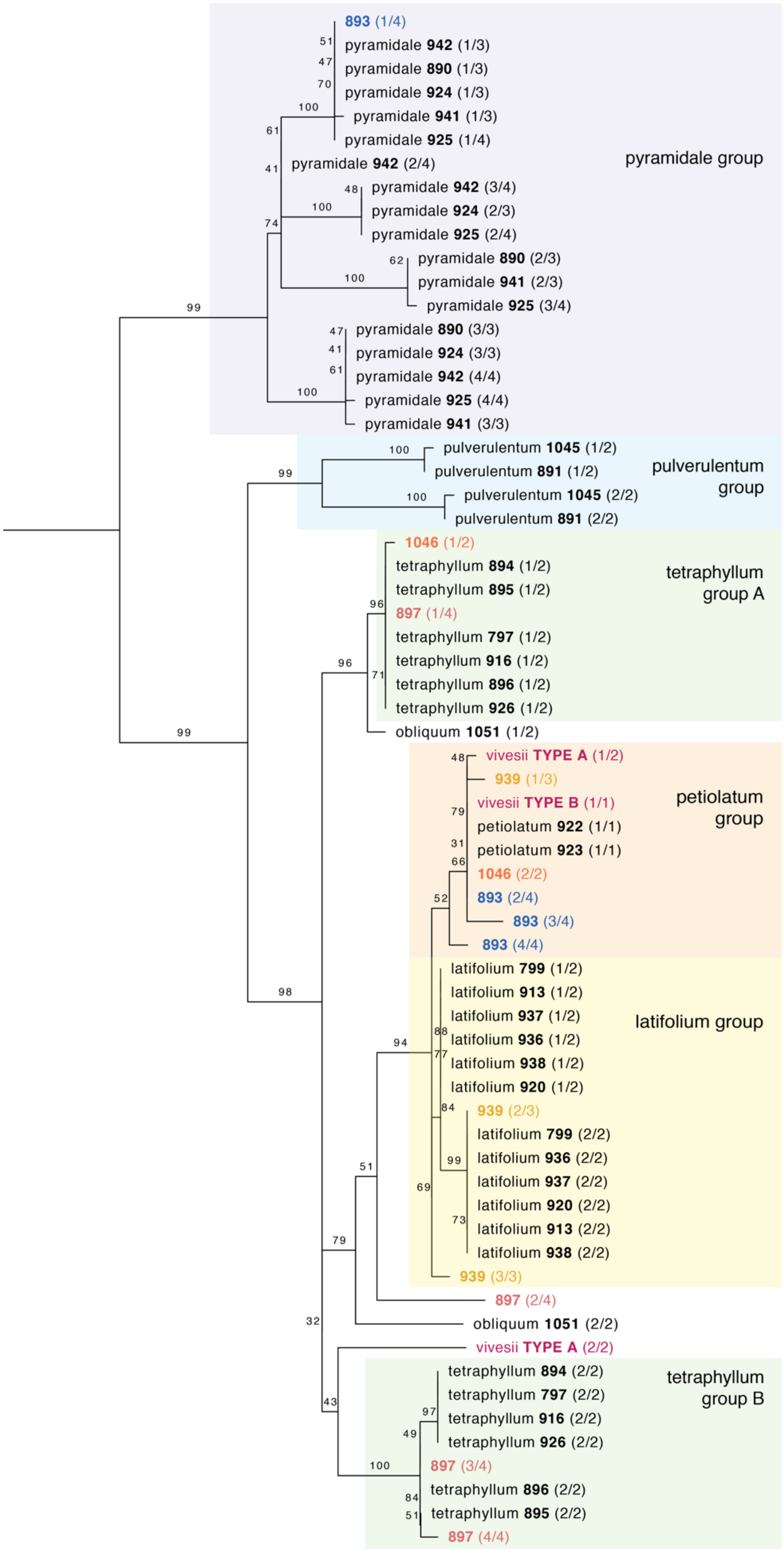


**Figure 2**, enlarged

Five samples, including the *A. vivesii* isotype, have sequence variants that fall in different groups, indicating that they are of hybrid origin. The isotype of *A. vivesii* (vivesii TYPE A and vivesii TYPE B in **Fig. 2**) has one sequence variant nested in the petiolatum group and a second sequence variant sister to tetraphyllum group B, strongly suggesting that *A. vivesii* is of hybrid origin. Sample 1046 displays a similar pattern, but with one sequence variant nested in tetraphyllum group A rather than sister to tetraphyllum group B. Sample 897 has three different sequence variants nested in tetraphyllum groups A and B with a fourth sequence variant sister to the latifolium-petiolatum clade. Sample 893 (blue in **Fig. 2**), which was originally identified as *A. vivesii*, is also a hybrid but with different parentage than the vivesii TYPE and sample 1046, having one sequence variant nested in the pyramidale group, indicating an *A. petiolatum x A. pyramidale* hybrid which does not involve *A. tetraphyllum*. Finally, sample 939 has sequence variants nested in the petiolatum group and the latifolium group, indicating it is a *A. latifolium x A. petiolatum* hybrid.

## *Discussion*

*Adiantum pulverulentum, A. tetraphyllum, A. obliquum, A. petiolatum, A. latifolium* and *A. vivesii* all belong to a monophyletic group of ca. 25 tropical species (e.g., Huiet, et al. 2018, Regalado et al, 2017), but the relationships within this clade have been difficult to resolve, perhaps because of hybridization and introgression. Numerous different cytotypes (n = 29, 30, 57, 58, 60, 90, 114, 116, 150, and ca. 180) have been documented throughout the genus and polyploid species ranging from triploids up to dodecaploids (12 sets of chromosomes) have been reported (Tryon & Tryon, 1982). Of the taxa included in this study, there are published chromosome counts only of *A. tetraphyllum*, with diploid individuals (two sets of chromosomes) reported from Paraguay and tetraploid individuals (four sets of chromosomes) reported from Jamaica (Smith & Foster 1984, Walker 1966).

The sequence data presented here suggests *A. vivesii* is in fact of hybrid origin with *A. petiolatum* as one parent and the other parent likely being *A. tetraphyllum*. However, our phylogenetic analysis resolved the vivesii TYPE A (2/2) sequence variant as sister to the tetraphyllum group (with very weak support) rather than nested within it, indicating another species not included in the phylogeny could be the second parent. There are several closely related taxa from the Antilles and continental areas of the Caribbean which we did not include in this study (see Regalado 2017), but *A. tetraphyllum* is the only one of these species known from Puerto Rico (Proctor 1989). Additionally, although the sequence data are not sufficient to separate *A. latifolium* and *A. petiolatum*, the two species are morphologically distinct *with A. petiolatum* being once-pinnate while *A. latifolium* is fully twice-pinnate (**Fig. 3**). The morphology of *A. vivesii* is intermediate in its leaf division (**Fig. 3**). Since *A. tetraphyllum*, another fully twice-pinnate species, is likely one progenitor of *A. vivesii*, this further supports *A. petiolatum* as being distinct from *A. latifolium*, as the second parent of *A. vivesii* should be once-pinnate.

Examination of the spores of both the isotype and a clonal individual at the type locality indicate *A. vivesii* is a sterile hybrid and not reproductively competent as the spores examined were malformed and abortive (Sepúlveda-Orengo 2000). As such the results of this study do not support continued protections for *A. vivesii*, but additional work is necessary to fully understand its parentage. Furthermore, based on the molecular analysis, hybrid individuals involving *A. petiolatum* appear to be relatively common and of multiple origins. While *A. vivesii* as described by Proctor is a sterile hybrid, the other hybrid individuals included in this study (893, 897, 939, and 1046) may in fact be valid polyploid species in need of formal descriptions and subsequent evaluation of conservation status.

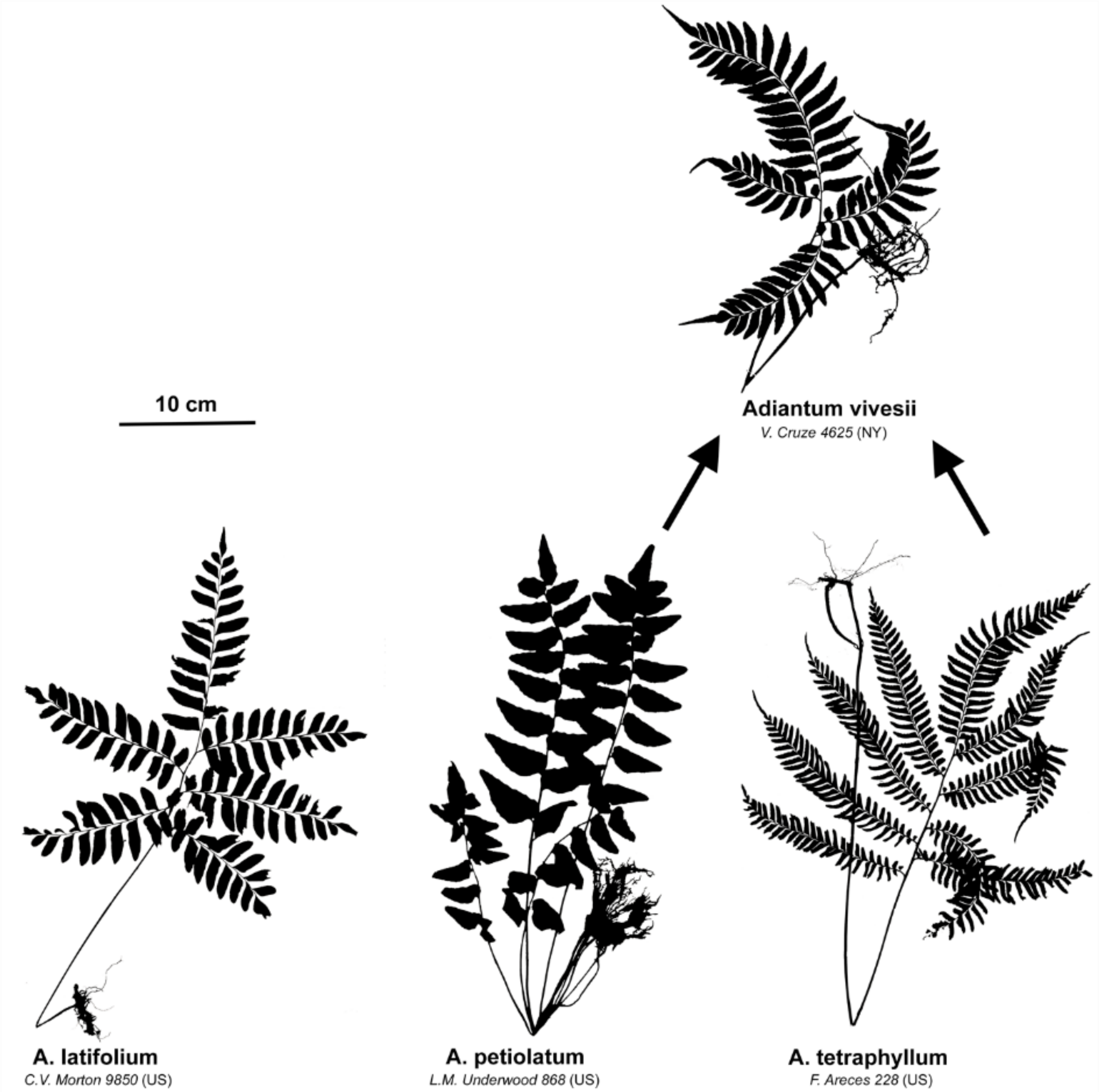


**Figure 3.** Silhouettes of four *Adiantum* taxa with arrows representing the hypothesized origin of *A. vivesii*.

# *Cyathea dryopteroides* Maxon

## Background

*Cyathea dryopteroides* is a federally endangered fern, endemic to elfin forest in the municipalities of Adjuntas, Jayuya, and Ciales in Puerto Rico's karst belt. The taxonomic status of *C. dryopteroides* is a confusing topic (at least to the authors of this report). At the generic level, the species was described as belonging to the genus *Cyathea* by Maxon (1925). Many taxonomists assign the taxon to the genus *Alsophila* (Large & Braggins 2004). Conant (1983) proposed the synonym *Alsophila amintae,* due to confusion between the subject of this discussion and a hybrid between *A. amintae* and *A. bryophila* that was also given the epithet *dryopteroides* (explanation provided by Y. Camacho, pers. comm.). Our objectives regarding *C. dryopteroides* were to: collect spores from a genetically representative sample of wild populations, test germination rates, store spores long-term at NLGRP, and grow plants for ex situ conservation and possible future reintroductions.

## 2014 Monte Jayuya survey

On 11/8/14, University of Puerto Rico graduate student Yahaida Camacho led the group to plants near the summit of Monte Jayuya in Toro Negro Forest (**Fig. 4**, **Map 2**). We collected fertile fronds from 21 individuals and successfully retrieved spores from 16. We assessed soral development using microphotography, conducted germination testing in the laboratory, propagated plants from spore, and sent excess spores to long-term cryogenic storage at the USDA National Laboratory for Genetic Resources Preservation (NLGRP). Full methods for these processes were described and illustrated in Possley and Maschinski (2015).

**Figure 4**. Possley and Camacho with *C. dryopteroides* at Monte Jayuya, 2014

## 2016 Cerro Punta survey

We observed some *C. dryopteroides* at Cerro Punta on 1/30/16 while searching for *Elaphoglossum serpens* (**Map 3**). During the search, the group found 3 previously undocumented *C. dryopteroides*, bringing the known population of *C. dryopteroides* at Cerro Punta at that time from 4 to 7 individuals. None of the 3 “new” plants encountered were sporulating.

## 2017 Monte Guilarte survey

On 2/16/17, the team hiked to the summit of Monte Guilarte and then descended to the area where Camacho had previously mapped *Cyathea dryopteroides*. We encountered the species growing in pockets of humus, usually near rock, on very steep, slippery red clay hillside. We covered approximately 3 acres looking for *C. dryopteroides*, finding 15 individuals (**Map 4**). When we encountered an individual, we mapped it, collected environmental data, and collected a fertile frond if one was present that appeared to be mature. We attempted to proceed with care, but we often slid in the mud, causing small-scale impacts to surrounding vegetation. Much of the survey area appeared to be high quality and relatively undisturbed. Other vascular plants associated with the *Cyathea dryopteroides* included: *Cyathea arborea*, *Thelypteris deltoidea*, *Prestoea acuminata* var. *montana* (Sierra palm), *Guzmania* sp., *Oplismenus* sp., *Pilea* sp., at least 3 species of *Danaea*, numerous Hymenophyllaceae (including a healthy population of *Trichomanes capillaceium*), and *Elaphoglossum crinitum*.

We recorded seven different categories of data at each individual *C. dryopteroides* we encountered. These included:

1. Number of fronds,
2. Presence of fertile fronds,
3. Photosynthetically active radiation (PAR) using a Hydrofarm digital light meter at four equally spaced points above the fern,
4. Temperature and relative humidity using a thermohygrochron,
5. Soil depth, in centimeters,
6. The species of plant which was the “nearest neighbor,” and
7. Elevation, as reported in Garmin eTrex GPS units.

For *C. dryopteroides* at Monte Guilarte, we found that 8 out of 14 individuals observed (57%) were sporulating (we did not record sporulation for one of the 15 individuals). Four of the 8 sporulating individuals had near-mature fertile fronds, which we collected. The most common neighboring plant was an herb in the genus *Pilea* (at 6 of 15 points) (**Fig. 5**). Less common neighbors included *Danaea* sp. (at 4 of 15 points), *Begonia* sp. (at 2 points), and *Oplismenus* sp., *Selaginella* sp., and *Prestoea acuminata* var. *montea*. These and other results are summarized in **Table 1**.

**Figure 5**. *Cyathea dryopteroides* habitat and associated plants at Monte Guilarte. Letters indicate common associates: D=*Danaea* sp., C=*Cyathea dryopteroides*, P=*Pilea* sp., O=*Oplismenus* sp., S=*Prestoea acuminata* var. *montea* (Sierra palm).

**Table 1**. Environmental data associated with ***C. dryopteroides*** at Monte Guilarte, 2/16/17.

| | **Minimum** | **Average** | **Maximum** | **N** |
|---|---|---|---|---|
| **# of Green Fronds** | 2.5 | 4.9 | 11 | 14 |
| **PAR (umol)** | 1.1 | 7.8 | 16.1 | 14x5 |
| **Temp(F)** | 64 | 70.3 | 78 | 15 |
| **RH(%)** | 65 | 71.2 | 82 | 15 |
| **Soil depth (cm)** | 0 | 6.6 | >20 | 15x3 |
| **Litter depth (cm)** | 0 | 1.8 | 2.5 | 13 |
| **Elevation (m)** | 1156 | 1170 | 1198 | 15 |
| **Most common neighbor** | *Pilea* sp. | | | |

### 2017 Cerro Punta survey

On 2/17/17, we drove to the summit of Cerro Punta, revisited known *C. dryopteroides* (with coordinates for 7 individuals provided by Yahaida Camacho), and fanned out to locate additional plants (**Map 3**). We mapped every *C. dryopteroides* we encountered, collected fertile fronds that appeared to be mature, and collected environmental data at 12 individuals. In total, we mapped 60 individuals, however it is possible that the true number of individuals we encountered was closer to 55; teams of two unknowingly mapped plants in the same area and GPS accuracy is not high enough to enable us to discern whether or not we mapped the same individuals. However, at most, there were only 5 plants that were mapped twice. We had to cut the survey short due to time, but we were still finding unmapped plants in the ravine that Lange and Flickinger had surveyed (**Map 3**). This ravine contained excellent fern habitat and at least 30 *C. dryopteroides* that were previously undocumented.

Soil here was primarily red clay. *Cyathea dryopteroides* tended to be growing in thin, blackish humus over clay or over rock, often with long-creeping rhizomes (**Fig. 6**). In a visual estimate, canopy cover over the *C. dryopteroides* averaged around 75%. Despite the presence of trash, disturbed soil, and weeds at the summit of Cerro Punta, there were still pockets of relatively undisturbed forest with high plant diversity, including high fern diversity. Some of the more abundant species we noted in *C. dryopteroides* habitat included *Cyathea arborea*, *Diplazium* sp., *Begonia* sp., *Prestoea acuminata*, *Pilea* sp., and *Marratia laevis*.

We recorded six different categories of data at each individual *C. dryopteroides* we encountered, as described above (**Table 2**). For *C. dryopteroides* at Cerro Punta, we found that 36 out of 44 individuals (82%) we checked were sporulating. The most common neighboring plant species of 12 *C. dryopteroides* sampled was *Pilea* sp. (4X), followed by unknown Rubiaceae (2X) and Begonia sp. (2X) and one time each for *Blechnum fragile*, *Oplismenus* sp., *Marratia laevis*, and *Diplazium* sp. Other results are summarized in the table below.

**Figure 6.** Two *C. dryopteroides* rhizomes at Cerro Punta. In the left image, some of the red clay soil is visible next to the completely horizontal rhizome. In the right image, the most common neighboring taxa, *Pilea sp*., can be seen.

**Table 2**. Environmental data associated with ***C. dryopteroides*** at Cerro Punta, 2/17/17.

| | **Minimum** | **Average** | **Maximum** | **N** |
|---|---|---|---|---|
| **# of Green Fronds** | 3.5 | 8.0 | 21 | 12 |
| **PAR (umol)** | .91 | 42.3 | 1013 | 5x12 |
| **Temp(F)** | 61.5 | 68.9 | 81.8 | 12 |
| **RH(%)** | 60.8 | 69.1 | 78.2 | 12 |
| **Soil depth(cm)** | 0 | 9.5 | >20 | 3x12 |
| **Litter depth (cm)** | 0 | 1.8 | 3.5 | 12 |

| | | | | |
|---|---|---|---|---|
| **Elevation (m)** | 1290 | 1303 | 1322 | 11* |
| **Most common neighbor** | *Pilea* sp. | | | |

*outlier of 200m removed

### Update on ex situ collections

We continue to maintain a healthy ex situ collection of 23 *C. dryopteroides* Fairchild's nursery from our 2014 spore collections at Monte Jayuya. These plants are in 1-gallon pots. Most show healthy vigorous growth, and most produce sporangia in Fairchild's nursery in winter months (Feb/March). In addition to sporophytes, ex situ conservation material of *C. dryopteroides* includes spores from 13 genetic lines at Monte Jayuya in cryogenic storage at the NLGRP.

We collected fertile fronds from 10 *C. dryopteroides* individuals in February 2017 (6 from Cerro Punta, and 4 from Monte Guilarte). We brought fronds to MAPR on the day of collection to dehisce. Some fronds appeared to make healthy spore prints. However, when we inspected material more closely, we found that in every case but one, material we collected was not spores, but rather crumbled pieces of sporangia walls. A single collection (made by Jonathan Flickinger at Cerro Punta) was comprised mostly of sporangia walls, but it also appeared to contain a few spores. We attempted to isolate the spores from the sporangia and sowed this material a few weeks after collection, on March 28, 2017. On October 4, 2017, we noted that tiny gametophytes were present. Gametophytes did not form until 2019. By late 2019, we can confirm that this material is indeed *C. dryopteroides* (**Fig. 7**).

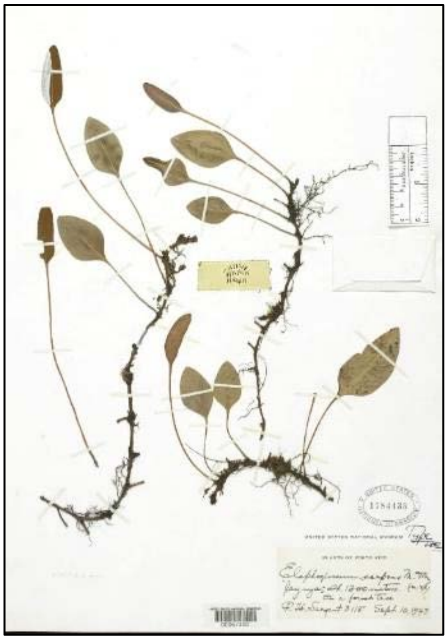
**Figure 7.** Young *Cyathea dryopteroides* sporophytes from Cerro Punta, photographed at Fairchild in December 2019.

It is unfortunate that we did not succeed in collecting many mature spores from *C. dryopteroides* in 2017. It is possible that *C. dryopteroides* from Monte Guilarte and Cerro Punta do not produce viable spores. But a more likely explanation for our lack of success is that it is exceedingly difficult to assess soral development of *Cyathea* (sect. *Alsophila*) in the field. We were inexperienced with anatomy of *Cyathea* sporangia, and now believe that everything we collected was underdeveloped. It is possible that, with this particular species, fronds are only in the "perfect" condition to collect (i.e. with viable spores) for a day or even only a few hours. For tips on how to make better collections of *C. dryopteroides* in the future, see **Figure 8**.

### Discussion

Overall, this cooperative effort has made some important initial steps toward recovering *Cyathea dryopteroides*, but there is still much work to be done to acquire information on the status of the wild populations. More extensive surveys are needed at Cerro Punta and Cerrote de Penuelas, and we highly recommend that spores be obtained from Cerro Punta for propagation and long-term cryogenic storage. It is also of critical importance to revisit the known populations in order to learn whether they were impacted by 2017's Hurricane Maria.

**Figure 8. Above:** various stages of development in sori of *C. dryopteroides*. In each photo, the cup-shaped indusia are visible, protecting the developing sporangia. Both indusia and sporangia are green while developing and brown at maturity. In the upper right-most photo, the sori and sporangia are both brown, yet may still be a few days away from maturity. Below: After having released their spores, sporangia have a ragged appearance (visible with a 10x hand lens) and hang out of the sori. Photos by Jack Hahn, Fairchild Tropical Botanic Garden.



## *Elaphoglossum serpens* Maxon & C.V. Morton

### Background

*Elaphoglossum serpens* is a federally endangered fern that is endemic to the Jayuya municipality in Puerto Rico's karst belt. Extirpated from the type locality at Monte Jayuya, it was also known from the summit area of Cerro Punta, where it was last verified as present over two decades ago by Proctor (USFWS 2010). Our first objective regarding *E. serpens* was simply to try to find any living plants.

### 2016 Cerro Punta survey

We surveyed for *E. serpens* at Cerro Punta on 1/30/16. We did not find *E. serpens*, but we did find two other *Elaphoglossum* species: *E. latifolium (*syn*: E. longifolium,* per Axelrod (2011)) and *E. maxonii* (**Fig. 9**)*.* This latter species, which was epiphytic on *Cyathea* trunks west of the peak's summit, looks very similar to herbarium specimens of *E. serpens,* but, after comparing specimens at MAPR (with much help from Velez), it was apparent that the species we observed was in fact *E. maxonii*, with its much stouter, less hairy (i.e., less scaly) rhizome. We found *Elaphoglossum latifolium* growing on the base of *Lyonia stahlii* at the peak's summit. We collected tissue and herbarium specimens from both species, sending the former to University of Florida and the latter to MAPR.

### 2017 Cerro Punta survey

As reported above for *C. dryopteroides*, our team of six biologists surveyed for rare ferns, including *Elaphoglossum serpens*, near the summit of Cerro Punta on 2/16/17 (**Map 3**). As in previous surveys, we did not locate *E. serpens*. Monsegur and Velez navigated to an area described in handwritten notes in USFWS files. However, they reported that the area was very disturbed, and they did not find any *Elaphoglossum* species.

**Figure. 9**. Left: *Elaphoglossum latifolum* (=*E. longifolium*) growing on the base of *Lyonia stahlii* at Cerro Punta. Right: *Elaphoglossum maxonii* looks very similar to specimens of *E. serpens*, but the rhizome is much stouter. Both species are epiphytic.

## Discussion

*Elaphoglossum serpens* may be extirpated, since its last known location was clearly destroyed from construction of the cell phone tower at the summit of Cerro Punta. However, additional surveys in the least disturbed areas surrounding the summit are needed. This fern is small and could be easily overlooked, even by experienced biologists.

# *Polystichum calderonense* Proctor

## Background

*Polystichum calderonense* is a federally endangered fern that is endemic to the Adjuntas and Penuelas municipalities in Puerto Rico's karst belt. This species was first described by George Proctor (1989), from the type locality at the summit of Silla de Calderón in the Guilarte State Forest. Proctor (1989) noted that this species is very similar to *P. emarginatum* of Hispaniola, and that these populations belong to a complex "much in need of further study." Other locations of *P. calderonense* have been reported within Guilarte State Forest (J. Velez, pers. comm.) and on private property at Cerrote de Penuelas, where Proctor (1991, as cited in USFWS 2010) reported 12 plants. Our objectives regarding *P. calderonense* were to: collect spores from a genetically-representative sample of wild populations, test germination rates, store spores long-term at NLGRP, and grow plant for ex situ conservation and possible future reintroductions.

## 2014 Silla de Calderón survey

On 11/6/14, the team visited *Polystichum calderonense* in the Guilarte Forest, near the summit of Silla de Calderón (**Map 5**). There was evidence of a recent lightning strike and small fire which appeared to have killed two *P. calderonense* and a handful of nearby trees. In total, we counted 12 living plants, one of which was small and not yet reproductive. We collected spores from 4 genetic lines and 11 buds from 7 lines. We assessed soral development using microphotography, conducted germination testing in the laboratory, propagated plants from spore, and sent excess spores to long-term cryogenic storage at NLGRP. Full methods of these processes were described and illustrated in Possley & Maschinski (2015).

## 2016 Silla de Calderón survey

We revisited the Silla de Calderón population of *P. calderonense* on 2/2/16. We counted 15 individuals, of which two were tiny new plantlets, one still connected to the parent. There were no fronds present that had sporangia at the ideal stage for collection. We collected 2 fronds from the largest individual to try to propagate additional material of this species from spore at Fairchild. We also collected 12 proliferous buds. Many of the largest individuals were making new plantlets from the proliferous buds on the ends of their fronds. Many of the ferns were very dry with multiple brown fronds. The invasive bamboo grass at the site (*Arthrostylidium* sp.) seemed to have significantly spread since our November 2014 visit (**Fig. 10**).

**Figure 10.** USFWS CESFO biologist JP Segarra at the summit of Silla de Calderón.
A *large P. calderonense* is visible in the upper left portion of the photo.
The invasive bamboo grass *Arthrostylidium* sp.is just below the endangered fern.

### 2017 Silla de Calderón survey

On February 15, 2017, the team revisited Silla de Calderón population of *P. calderonense* again, with the objectives of monitoring the plants, collecting environmental data at each individual, and seeking potential introduction habitat.

On the ascent, most of the habitat we passed was disturbed and dominated by weedy species like *Urena lobata* and *Odontosoria aculeata.* However, there were a few higher-quality areas, especially one location along a riverbed at ~1035 meters where Monsegur spotted several orchids (one in the genus *Beloglottis*) and two *Tectaria trifoliata* (**Map 5**). This location (18.15014N, 66.80880W) supported several fern species and was relatively cool and humid when we were there (68 degrees F, 77% RH), but it may not be an appropriate introduction site for *P. calderonense* due to the soil, which was deep red clay, with little/no humus layer. Other ferns noted in this area included *Danaea* sp., *Blechnum fragile*, *Asplenium radicans*, and *Cyathea* spp.

At the summit of Silla de Calderón, we found 14 individual *P. calderonense.* Whereas in January 2016, most of the *P. calderonense* had one or more dried brown fronds and the humus substrate was extremely dry, in February 2017 rainfall appeared to have returned to normal levels. The ferns did not have browning, and the humus layer was moist. All *P. calderonense* were in 80-100% shade, and all were within 0-2 meters of rock outcropping.

Associated species in shade near the *Polystichum calderonense* included: *Arthrostylidium* sp. *Scleria* sp., *Pilea* sp., *Pecluma* sp., *Elaphoglossum eggersii*, *Guzmania berteroniana*, *Chionanthus domingensis*, *Sapium laurocerasus*, *Cordia sweata*, *Coccoloba pyrifolia, Chrysophyllum oliviforme*, *Cunonia comocladifolia*, *Nectandra coriacea*, *Nephrolepis exaltata*, *Hillia parasitica*, and *Guarea glabra*. Near the summit, in full or near-full sun, the non-native fern *Nephrolepis brownii* was abundant. We also noted two other species of *Polystichum* near the summit. The rarer, larger one keyed out to *P. platyphyllum*. The smaller, more common species keyed to *P*. *polystichiforme*.

We recorded microhabitat data at each individual *P. calderonense* (**Table 3**). We found that 6 out of 14 individuals were sporulating, the most common neighboring plant species was *Arthrostylidium* sp. (6X), and less common neighbors included *Guzmania* sp. (2X), and one time each *Coccoloba pyrifolia*, *Nephrolepis exaltata*, *Pilea* sp., and *Polystichum calderonense*. These and other results are summarized in **Table 4**, below.

Before descending the mountain, we explored the ridge to the south of the summit (**Map 5**). This area appeared to have pockets of high-quality habitat that would be ideal recipient sites for introduction of *P. calderonense*. Areas that were flatter and easier to access were more disturbed and often dominated by *Oplismenus* sp*.* However, areas near steep rock outcroppings often had deeper shade (estimated 80-100%) and fewer weeds. We observed both of the other *Polystichum* species here. Trees included a large Magnolia (*M. portoricensis*) and many small myrtaceous trees including *Eugenia stewardsonii* and *Siphoneugena dussii*.

**Table 3**. Environmental data associated with ***P. calderonense*** at Silla de Calderon, 2/15/17.

| | Minimum | Average | Maximum | N |
|---|---|---|---|---|
| **# of Green Fronds** | 3.5 | 7 | 14 | 13 |
| **PAR (umol)** | 2 | 18 | 261 | 5x12 |
| **Temp (F)** | 68 | 69 | 72 | 13 |
| **RH (%)** | 58 | 61.5 | 68 | 13 |
| **Soil depth (cm)** | 0 | 10.4 | >20 | 3x13 |
| **Litter depth (cm)** | 0 | 2.3 | 13 | 5 |
| **Elevation (m)** | 1149 | 1163 | 1171 | 13 |
| **Most common neighbor** | *Arthrostylidium* sp | | | |

## 2017 Monte Guilarte survey

On February 16, 2017, as reported above in the *C. dryopteroides* section, we hiked to the summit of Monte Guilarte to monitor *C. dryopteroides* and survey for *Polystichum calderonense* and/or potential habitat. We did not relocate *Polystichum calderonense*, but we did find and map what we determined to be suitable habitat near the summit (**Map 4**). This area, which only covered a few hundred square meters, had abundant rock outcropping, higher fern diversity, and notably several species of *Asplenium* (which weren't present downslope). We also mapped one smaller area closer to the *C. dryopteroides*, which we believed could potentially support *Polystichum calderonense*.

## Update on ex situ collections

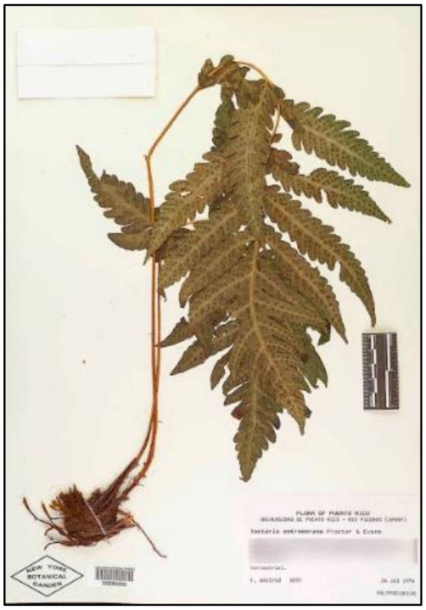
We continue to maintain a healthy ex situ collection of *Polystichum calderonense* in Fairchild's nursery from our 2014 spore collections at Silla de Calderon. There are approximately 90 individuals in pots ranging from pint- to 1-gallon-sized. In addition, there are hundreds of small sporophytes in ten germination boxes. The number of individuals we have in germination boxes far exceeded the amount of space available to grow ferns in Fairchild's nursery, thus we sent spore boxes to cooperating institutions to grow (**Appendix 4**). Of these, 5 institutions are now cultivating *P. calderonense* in their greenhouses.

*Polystichum calderonense* germinates quite readily, but we have found that growing healthy plants can sometimes be difficult. Small young plants will wither and die in the Miami heat or in direct sunlight. Acclimation of young plants is particularly difficult in the summer. Larger plants will periodically brown in the nursery from prolonged direct sunlight and/or desiccation. The plants are also susceptible to scale insect and mealy bugs, which can cause frond browning but usually does not cause mortality.

In addition to sporophytes, ex situ conservation material includes spores in cryogenic storage at the NLGRP. In 2014 we sent *Polystichum calderonense* spores from 4 genetic lines at Silla de Calderón to the NLGRP for long-term storage. In 2017 we sent spores from 2 genetic lines in Fairchild's nursery (descended from Silla de Calderón plants) to NLGRP.

## Discussion

Overall, this program has made important initial steps toward recovering *Polystichum calderonense*, but more field surveys are required at reported localities (especially Cerrote de Penuelas). It is also of critical importance to revisit the only known population at the summit of Silla de Calderón, in order to learn whether it survived September 2017's Hurricane Maria. Regarding ex situ germplasm, Fairchild has shared plants with other institutions but we still hope to identify a facility in Puerto Rico that might also house some of these ferns, which could bring them closer to reintroduction.



# *Tectaria estremerana* Proctor & A.M. Evans

## Background

*Tectaria estremerana* is a federally endangered fern that is endemic to Puerto Rico's karst belt. It was first described by George Proctor (1989), from the type locality at the Arecibo Observatory, where he reported seeing "many plants." Other locations of this fern have been reported in the municipalities of Florida and Utuado (USFWS 2010). The taxonomic status of *T. estremerana* is uncertain, as it has been speculated to be a hybrid between *T. incisa* and *T. cicutaria* (Axelrod 2011). Axelrod (2011) mentioned that *T. estremerana* is possibly a fertile tetraploid, but our unsuccessful attempts to germinate spores have suggested it may not be fertile. Our objectives regarding *T. estremerana* were to find living plants, collect tissue samples, and facilitate genetic work in the Sessa Lab at University of Florida to definitively determine whether *T. estremerana* is a valid taxon.

## 2014 Rio Abajo survey

We first searched for *T. estremerana* on 11/5/14 in Rio Abajo Forest, near Highway 10 in an area reported by Axelrod in an herbarium specimen at MAPR (**Map 6**). To access a ravine formed between mogotes, we descended a cement drainage feature alongside a bridge on Highway 10. Total survey time was approximately 3 hours, with 6 people searching for plants. While portions of the area were disturbed for fence building, etc., the majority of the habitat appeared to be healthy forest (**Fig. 11**) with full shade and very high humidity, with bryophytes and Hymenophyllaceae growing abundantly on rocks and tree trunks, and yellow and red-flowered gesneriads present on cliff faces. Ferns we observed at this location included *Tectaria heracleifolia*, *T. cicutaria*, *T. incisa*, *Odontosoria clavata*, and *Lomariopsis kunzeana*. Additionally, we found three *Tectaria estremerana*. Plants appeared healthy and robust, though all had a few scale insects present on the lower surface of the fronds. We collected the best frond from each, although two seemed to have already dispersed most of their spores and one appeared to be too young.

**Figure 11.** Jennifer Possley examines the fern habitat in 2014 where Axelrod vouchered *Tectaria estremerana* alongside Highway 10 in Rio Abajo Forest.

The second potential *T. estremerana* we surveyed was also along Highway 10, very near the first collection site. We again descended a drainage feature but after taking an overview of the area we determined that the habitat was not appropriate for our target species. There were many bryophytes and ferns present, but karst outcroppings were few and the light levels were higher. We did not locate any *T. estremerana* at this location.

The third location where we searched for *T. estremerana* was in undisturbed forest, near “Finca Opiola” (**Map 7**). Total survey time was approximately 2 hours, with 3 people searching for plants. The habitat appeared to be ideal for native ferns. The karst limestone was quite dramatic in places, with large holes, caves, and towers carved into the rock. Bryophytes and Hymenophyllaceae coated rocks (*Trichomanes punctatum* was especially abundant) and tree trunks, both red- and yellow-flowered gesneriads were present. Other species we noted included *Tectaria heracleifolia*, *Tectaria cicutaria*, *Lomariopsis kunzeana*, and *Asplenium dentatum*. We noted a single *Tectaria incisa* and a single *Tectaria estremerana*; we collected one fertile frond from each.

The last location where we surveyed for *T. estremerana* was on private property near El Jobo, approximate coordinates 18.329° N, 66.696° W (**Map 8**). Total survey time was approximately 45 minutes, with three people searching for plants. After obtaining verbal permission from the property resident, we passed through a pasture behind the house toward the base of a mogote. From the road, it wasn’t apparent whether there would be any fern habitat. But after wading through thick brush at the pasture’s edge and proceeding west along the mogote edge, we arrived in a small area of high-quality habitat. We noted bryophytes, Hymenophyllaceae, and gesneriads. Other ferns we noted included *Tectaria heracleifolia* and *T. cicutaria*, as well as an unusual *Vittaria* that may have been *V. costata*. We did not, however, locate any *T. estremerana* at this location.

## 2016 Rio Abajo survey

In January of 2016, we visited previously documented and suspected locations of *T. estremerana* with staff from CESFO and/or MAPR within the Rio Abajo Forest: in a ravine near Highway 10, near Finca Opiola, and near the village of El Jobo. On each visit we found 1-3 ferns that appeared to be *T. estremerana* (most did not have well-developed indusia, thus we could not use Proctor's taxonomic key). We attempted to collect spores, but never succeeded in this effort. We did however collect tissue samples from *T. estremerana* and its putative parents in several locations, for genetic analysis at the Sessa Lab at University of Florida.

Because we felt we had collected sufficient genetic samples to allow genetic testing to determine whether or not *T. estremerana* is a hybrid of *T. incisa* and *T. heracleifolia*, we did not include any objectives related to this taxon in our 2017 work. However, we noted that both parents are present in the area of Quebradillas where we surveyed for *A. vivesii*, and this may be another potential locality for *T. estremerana*.

## Ex situ propagation studies

We made spore collections from four fertile fronds of what we believed to be *Tectaria estremerana* in 2014 (**Appendix 2**). As reported in Possley and Maschinski (2015), these spores were lumpy and asymmetrical and did not produce gametophytes.

## Genetic studies

### *Materials & Methods*

<u>DNA Extraction and Sequencing</u> - Leaf material for DNA extraction was collected in the field in Puerto Rico in February 2017. A total of 14 individuals were sampled: three identified in the field as *Tectaria estremerana*, three as *T. incisa*, two as *T. heracleifolia*, and six as *T. cicutaria* (see Appendix 3 for voucher information). We extracted DNA from the field-collected, silica-dried leaf material and amplified one protein-coding region (pgiC) of the nuclear genome using polymerase chain reaction (PCR) and the 14F and 16R primers of Ishikawa et al. (2002). We selected this nuclear region because of its utility in previous studies on hybridization in ferns (Sessa et al., 2012). We then ran the PCR products on agarose gel, cut out the resulting bands, and re-extracted the DNA. Using the pGEM-T Easy Vector System, we cloned the re-extracted DNA. At least 12 and up to 16 transformed colonies were chosen for each individual, after an incubation period of 16 hours, and amplified using the initial pgiC primers. The final PCR products were purified and sent to Eurofins with 14F and 16R primers for sequencing.

<u>Alignment and Phylogenetic Analyses</u> - The pgiC sequences were assembled using the sequence analysis software, Geneious Prime (version 2019.2.1). We also downloaded pgiC sequences from Genbank for 13 additional Tectaria taxa and <u>*Triplophyllum securidiforme*</u> (as the outgroup) and aligned these sequences along with the Puerto Rico samples using the MAFFT plugin for Geneious (voucher information for the Genbank accessions available in Appendix 3). We used the software IQ-TREE to perform Maximum Likelihood analysis and reconstruct the phylogenetic tree.

### *Results*

The final alignment was 791 base pairs in length with 193 variant sites (12.8%).

The phylogenetic analysis resolved three main groups, or clades, for the Puerto Rico samples which we will refer to as the "heracleifolia group", the "incisa group", and the "cicutaria group" (**Fig. 12**). The heracleifolia group is further divided into two well-supported clades (100% and 98%) indicating the presence of allelic variation which may be associated with multiple copies of the pgiC gene, but ploidy level (number of chromosome sets) cannot be determined by sequence data alone. The incisa clade is also divided into two clades, but on shorter branches with moderate support (85%, 87%). The structure of the incisa group also indicates allelic variation that may be due to multiple copies of the pgiC gene. The cicutaria group includes a few short branches, but not much structure, basically forming a single polytomy, indicating little to no allelic variation was recovered from *T. cicutaria*.

Seven samples have sequence variants in different groups indicating they are of hybrid origin. Two sequence variants were recovered for samples 928, 943 and 946, with one nested in the incisa group and the other nested in the cicutaria group, indicating they are of hybrid origin (*T. incisa x T. cicutaria*). Similarly, samples 930, 933 and 947 are also of hybrid origin but with additional allelic variation, having two sequence variants nested in the incisa group and one nested in the cicutaria group (*T. incisa x T. cicutaria*). Finally, four sequence variants were recovered for sample 929 with two nested in the incisa group and two nested in the heracleifolia group (*T. incisa x T. heracleifolia*).

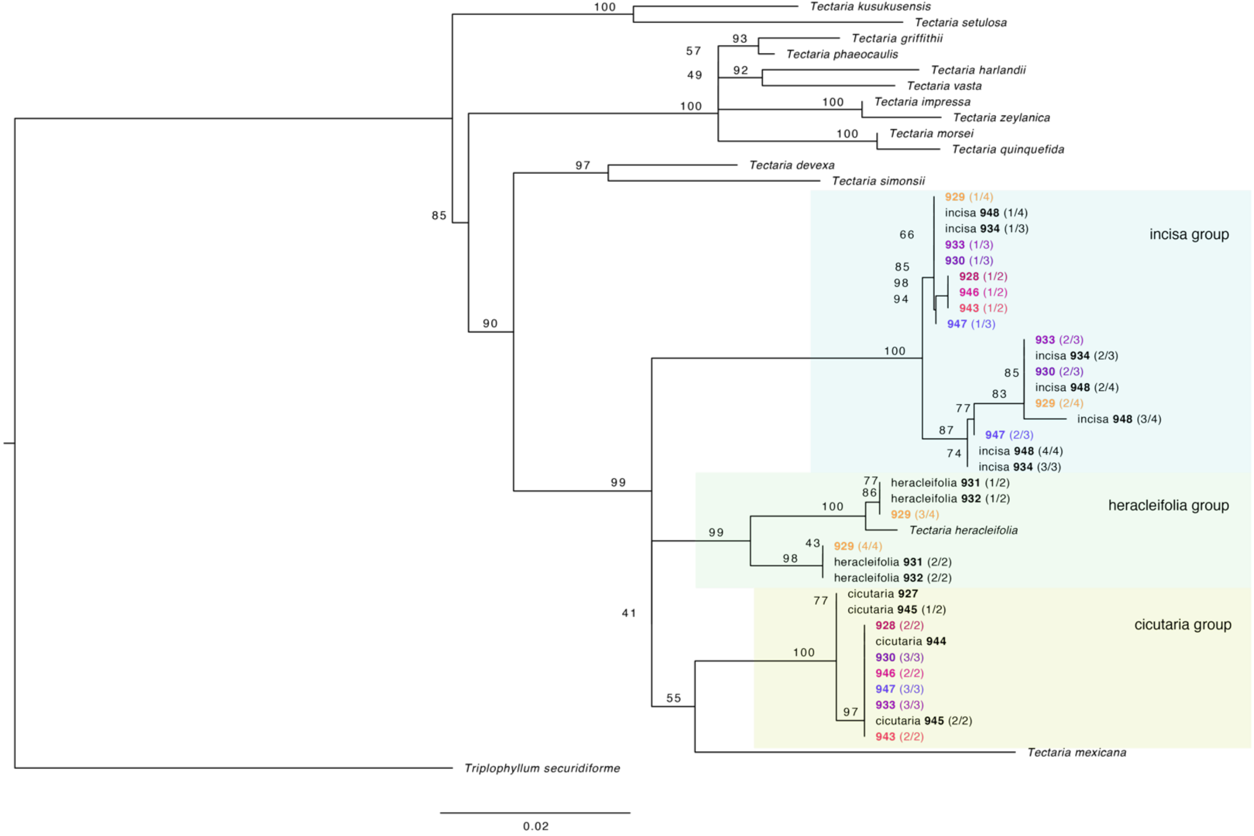


**Figure 12. (See enlarged version on following page).** Maximum likelihood phylogeny from the IQ-TREE analysis. Numbers on the branches are bootstrap support values. Colored boxes highlight three main groups, with the incisa group in blue, heracleifolia group in green, and cicutaria group in yellow. The colored numbers in bold are the accession numbers for hybrid individuals with the numbers in parentheses indicating the number of sequence variants, or different alleles of the pgiC gene, recovered for each (e.g., 1 of 3, 2 of 3, 3 of 3). Accession 929 (yellow) is inferred to be a hybrid between *T. heracleifolia* and *T. incisa*, the purple and pink accession numbers are inferred to be hybrids between *T. ciucutaria* and *T. incisa*.

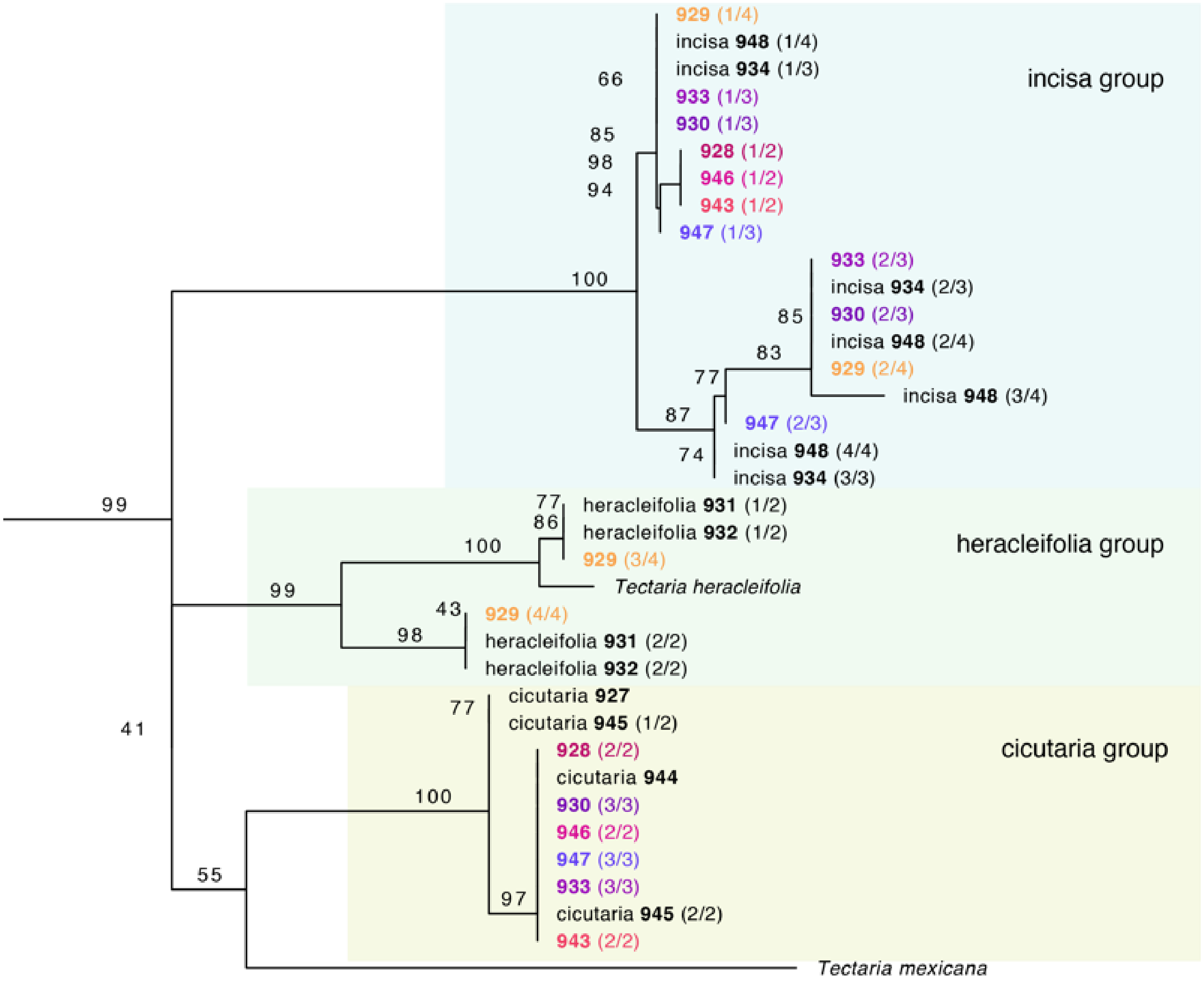


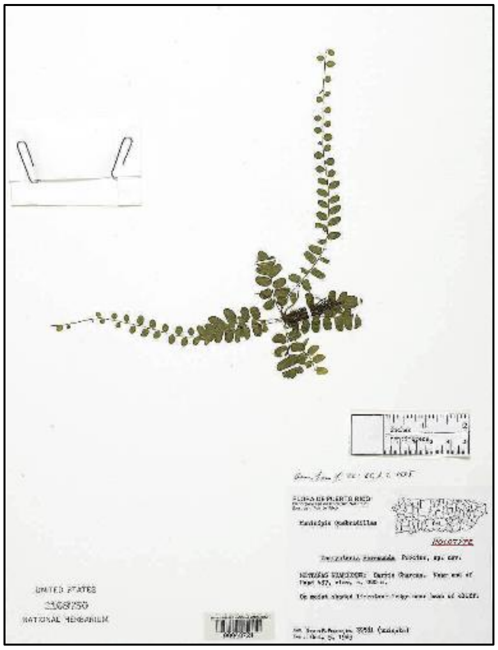
**Figure 12, enlarged**

*Discussion*

Neotropical species of *Tectaria* have a complicated evolutionary history with both diploid and tetraploid individuals found within what has historically been considered a single species. For example, cytological studies of *Tectaria incisa* from Jamaica (Walker 1966, Walker 1973) found that both diploid (n=40) and tetraploid (n=80) individuals occur in different populations on the island. This variation in ploidy level has probably contributed to the taxonomic confusion associated with this species. In Proctor's *Ferns of Puerto Rico and the Virgin Islands* (1989), he lists 17 synonyms of *T. incisa* – a testament to taxonomic confusion due to morphological variability. Furthermore, *Tectaria heracleifolia* also includes both diploid and polyploid populations, with tetraploid individuals in Jamaica and Florida and diploid individuals in Mexico and Central America (Tryon & Tryon 1982). In the treatment of *Tectaria* in Flora Mesoamericana (Moran, 1995), *T. heracleifolia* and *T. incisa* of Central America are together considered a species complex in need of further work due to multiple intermediate forms likely the result of hybridization.

Proctor (1989) described *T. estremerana* as being a tetraploid (n=80) and *T. cicutaria* as a diploid (n=40) when comparing the two taxa, but did not cite a publication for said chromosome counts. Nevertheless, following this description by Proctor, samples 927, 944, 945 would be “diploid” *T. cicutaria* individuals, while the hybrids 928, 930, 943, 946, and 947 would be "tetraploid" *T. estremerana* individuals. If these hybrid *T. estremerana* samples are in fact tetraploids they would most likely be reproductively competent and deserving of species-level protections, but ploidy level (number of chromosome sets) cannot be determined by sequence data alone. Both diploid and tetraploid individuals of *T. incisa*, a putative parent of *T. estremerana*, have been documented in the Antilles (Jamaica); therefore, it is unclear if these “estremerana” individuals are sterile diploids, sterile or apogamous triploids, or possibly fertile tetraploids. Based on previous visual assessment of the spores and unsuccessful germination attempts at Fairchild, *T. estremerana* is most likely a sterile F1 hybrid and not reproductively competent.

Additional work is needed to fully understand this species complex, including careful morphological analyses of the voucher specimens of the samples included in this molecular analysis. Four samples labeled as “cicutaria” and one sample labeled as “incisa” were found to be of hybrid origin (*T. cicutaria x T. incisa*) in this analysis and one sample labeled as “estremerana” was indeed of hybrid origin, but a cross between different parents (*T. heracleifolia x T. incisa*). While it is possible that samples for DNA extraction were mislabeled, it is more likely that the characters used in Proctor’s key are not sufficient for deciphering between diploid “cicutaria” and hybrid “estremerana” forms. While these molecular analyses can be helpful in determining if an individual is of hybrid origin and the parents of hybrids, they tell us nothing about the capacity of that hybrid to reproduce and evolve as a unique genetic lineage. Examining the spores of hybrid taxa has long been a reliable method for determining if a fern is a sterile F1 hybrid or a fertile allopolyploid deserving of species status following criteria outlined in the conservation literature (e.g., O'Brien and Mayr 1991, Haig and Allendorf 2006). Additionally, chromosome counts or flow cytometry (a measurement of the relative genome size as a proxy for chromosome number) are critical for fully understanding the biology and life history of the species.



# *Thelypteris verecunda* Proctor

## Background

*Thelypteris verecunda* is a federally endangered fern that is endemic to the Camuy, Hatillo, San Sebastian, and Quebradillas municipalities in the north portion of Puerto Rico’s karst belt. It was first described by Proctor in 1985. Proctor (1989) noted that it is very rare, and that “there is a possibility that this plant represents a hybrid between *Thelypteris abdita* and *T. reptans*”. Each of these taxa is now placed in the genus *Goniopteris*, along with several other similar and potentially co-occurring taxa.

Our objectives regarding *T. verecunda* were to find living plants, assess the population status, collect spores for long-term storage and propagation, and collect tissue samples for genetic work.

## 2017 Collazo Falls survey

On February 20, 2017 we traveled to Collazo Falls, as it was a reported location for *T. verecunda* (**Map 9**). We did not locate any *Thelypteris* by the waterfall, but there were several plants beneath the adjacent bridge that, on initial assessment, appeared consistent with *T. abdita, T. reptans*, and/or *T. verecunda*. Other ferns present in the immediate area included *Thelypteris grandis* (which resembled *T. augescens*), *Thelypteris sancta, Macrothelypteris torresiana*, and *Pteris grandifolia*.

We collected multiple samples of each *Thelpyteris* for DNA and for ex situ conservation. To transport plants, we “planted” small bare-root plants into a ball of moist sphagnum moss pressed into the corner of a Ziploc bag, and then pressed the air out of the bag before sealing it, so that the plant was held in place.

### 2017 Barrio Charcas survey (=*T. verecunda* type locality)

On February 21, 2017, we visited this location (**Map 10**) following detailed directions from Susan Fawcett, a doctoral student at the University of Vermont who is investigating the taxonomy of *Thelypteris* subgenus *Goniopteris* (including *T. verecunda*). Susan stated:

> *I visited the type locality for G. verecunda on rd. 137. It should be easy to find. When 137 splits, keep right -- plants will be on the left-hand side, low on rocks around bend, about ~1/4 mile beyond mermaid and pirate sculptures, and ~1/4 mile from where the road ends. The coordinates are: 18.41021 -66.93397.*

With these instructions, plants resembling *T. verecunda* were indeed easy to find. We collected live material (as described above), for ex situ collection, genetic work at U-VT and UF, and to voucher at MAPR. After we finished surveying the roadside cliffs (**Fig. 13**), the team obtained landowner permission to explore adjacent mogotes (**Map 10**). Lange collected one potential *T. verecunda.* Possley and Sessa found several large, proliferating *Goniopteris* that keyed out to *T. reptans* but which looked very different from south Florida specimens of *T. reptans* with which we are familiar.

**Figure 13.** A roadside cliff in Barrio Charcas with high native fern diversity.

### 2017 Camuy Caverns survey

On February 21, 2017, we visited Camuy Caverns to conduct surveys along interior park roads for one hour (**Map 11**). On the road toward the sinkhole (Sumidero Tres Pueblos), intermittent roadside cliffs supported populations of what appeared to be *T. verecunda* and/or *T. reptans*. We collected several plants as described above.

### Update on ex situ conservation collections

Collections of small plantlets of *T. verecunda* and similar taxa made from several localities in February 2017 (**Table 4**) had 100% survivorship at Fairchild Tropical Botanic Garden. The largest plants (4-5 individuals) began to produce sporangia by August 2017.

**Table 4**. Summary of *Thelypteris* (subgenus *Goniopteris*) wild collections, February 2017. Plants recorded here as "*Thelypteris* cf. *verecunda*" appeared to span a range of morphology between that described for *T. verecunda, T. reptans*, and/or *T. abdita*.

| **Taxon** | **Location** | **Quantity** |
|---|---|---|
| *Thelypteris* cf. *verecunda* | Collazo Falls | 6 |
| *Thelypteris* cf. *verecunda* | Barrio Charcas | 4 |
| *Thelypteris* cf. *verecunda* | Camuy Caverns | 8 |
| *Thelypteris hildae* | Camuy Caverns | 2 |

### Genetic studies

*Materials & Methods*

DNA Extraction and Sequencing - Leaf material for DNA extraction was collected in the field in Puerto Rico. A total of 17 individuals were sampled: two identified in the field as *Thelypteris reptans*, 13 as *T. verecunda*, and two as *T. abdita* (see Apendix 3 for voucher information). We extracted DNA from the field-collected, silica-dried leaf material and amplified one protein-coding region (pgiC) of the nuclear genome using polymerase chain reaction (PCR) and the 14F and 16R primers of Ishikawa et al. (2002). We selected this nuclear region because of its utility in previous studies on hybridization in ferns (Sessa et. al, 2012). We then ran the PCR products on agarose gel, cut out the resulting bands, and re-extracted the DNA. Vector cloning is an expensive and labor-intensive process; due to these constraints, we selected four of the 13 *T. verecunda* samples (two from each of the two localities) for the remainder of the analyses. Using the pGEM-T Easy Vector System, we cloned the re-extracted DNA. At least 12 and up to 16 transformed colonies were chosen for each individual, after an incubation period of 16 hours, and amplified using the initial pgiC primers. The final PCR products were purified and sent to Eurofins with 14F and 16R primers for sequencing.

Alignment and Phylogenetic Analyses - The pgiC sequences were assembled using the sequence analysis software Geneious Prime (version 2019.2.1). We also downloaded pgiC sequence data from Genbank for *Parathelypteris noveboracensis* as the outgroup and aligned this sequence along with the Puerto Rico samples using the MAFFT plugin for Geneious (voucher information for the Genbank accession is available in Appendix 3). We used the software IQ-TREE to perform maximum likelihood analysis and reconstruct the phylogenetic tree.

*Results*

The final alignment was 676 base pairs in length with 111 variant sites (16.4%).

The two *T. reptans* samples (numbered 909 and 910 in Fig. 13) included in this analysis each have four sequence variants. These different sequence variants, or alleles, form four well supported subclades, a structure indicative of polyploidy although ploidy level (number of chromosome sets) cannot be determined by sequence data alone. All four *T. verecunda* samples (898, 900, 904, 907 in Fig. 3) have two sequence variants each, both of which have little to no sequence divergence compared to two of the *T. reptans* sequence variants. One of these shared sequence variants (labeled (2/2) for all *T. verecunda* samples and (4/4) for all *T. reptans* samples) is sister to all of the sequences from the two *T. abdita* individuals, causing both *T. verecunda* and *T. reptans* to be polyphyletic (see **Fig. 14**).

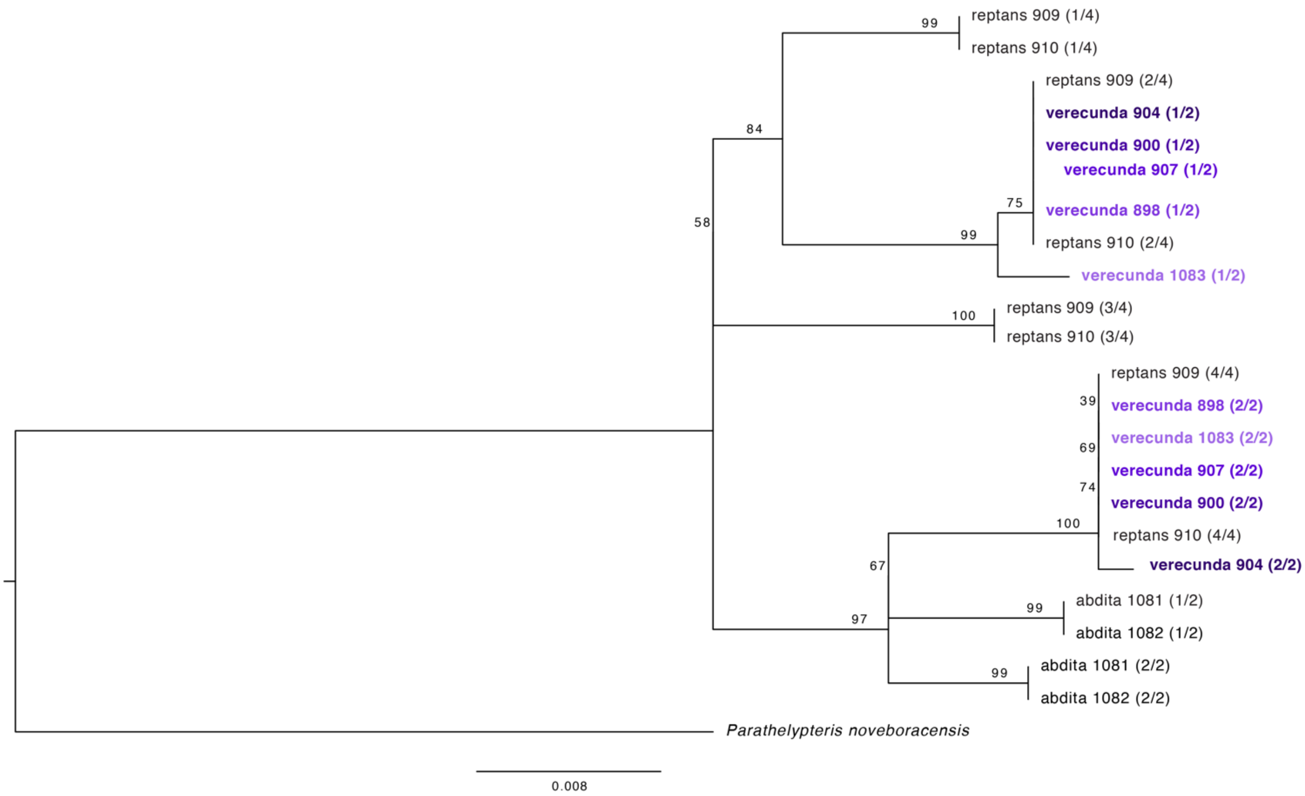


**Figure 14.** Maximum likelihood phylogeny from the IQ-TREE analysis of *Thelypteris verecunda* and relatives. Numbers on the branches are maximum likelihood bootstrap support values, numbers following the taxon name are the DNA accession numbers and the numbers in parentheses indicate the number of sequence variants, or different alleles of the pgiC gene, recovered for each (e.g., 1 of 2, 2 of 2). *T. reptans* samples each have four sequence variants while *T. verecunda* samples (in purple) and *T. abdita* each have two sequence variants.

### *Discussion*

The results of the genetic analyses support the hypothesis that *T. verecunda* is of hybrid origin, sharing parts of its genome with *T. abdita* and *T. reptans*; however, these results also support *T. abdita*, a fern endemic to Puerto Rico, as a progenitor of *T. reptans*, a common and wide-spread species in the Caribbean.

Cytological studies report both diploid and tetraploid individuals of *T. reptans* with a Jamaican individual reported as diploid (n = 36) and individuals from Florida as tetraploid (n = 72) (Smith

1971, Wagner 1963, Walker 1966). If Puerto Rican *T. reptans* is an allopolyploid species derived from a cross between *T. abdita* and another, unknown *Thelypteris* sp. (=*Goniopteris* sp.), then *T. verecunda* (being derived from a limited amount of the variability in *T. reptans)* would be either a sterile F1 hybrid between the progenitors of *T. reptans* (*T. abdita* x unknown *Goniopteris* sp.), a tetraploid due to a backcross between tetraploid *T. reptans* and diploid *T. abdita*, or there may have been multiple origins of *T. reptans* that sampled different variation in both of its progenitors and *T. verecunda* is derived from a different origin than the *T. reptans* included in this analysis.

*Thelypteris verecunda* is a taxon that may be undeserving of species status. *Goniopteris* is a very morphologically plastic group, and plants that have been described as *T. verecunda* may simply be within the natural range of variation within *T. reptans*. It is fortunate that University of Vermont PhD candidate Susan Fawcett is studying the taxonomy of Caribbean *Goniopteris*, and has herself collected material from each of the above populations for morphological and genetic studies. We are hopeful that her work will shed light on the status of *T. verecunda* as a species.

## *Thelypteris yaucoensis* Proctor

### Background

*Thelypteris yaucoensis* is a federally endangered fern that is endemic to the Yauco and Ciales municipalities in the southern portion of Puerto Rico's karst belt. It was first described by Proctor (1989), who noted it as "very rare" and present in just three stations. The taxon is closely related to and thus very similar in appearance to *Thelypteris sclerophylla.* The program objectives regarding *T. yaucoensis* were to find living plants, assess the population status, collect spores for long-term storage and propagation, and collect tissue samples for genetic work.

### 2016 Pico Rodadero survey

On 1/29/16, Monsegur, Labiosa, Santiago, Possley and Lange traveled to the summit of Pico Rodadero near Yauco to search for *T. yaucoensis* (**Map 12**)*.* We believed that succeeded in locating the population, collecting tissue samples for DNA, and collecting spores. We sowed spores from two different genetic lines into spore boxes at Fairchild. We sent DNA to the Sessa Lab at University of Florida.

Later, with the help of Susan Fawcett (University of Vermont), we determined that our 2016 collections of *Thelypteris yaucoensis* from Pico Rodadero may in fact be *Thelypteris asplenioides*. Like many of the species in *Thelypteris* subgenus *Goniopteris*, it is very difficult to tell these species apart. We revisited our 2016 Pico Rodadero specimen in February 2017, after Monsegur collected a similar looking plant on Cerro Punta which keyed out to *T. asplenioides.* Indeed, our specimen from Pico Rodadero did key out to *T. asplenioides,* due to the fact that the upper surface of the frond was glabrous (as opposed to having "few to many" minute stellate hairs, per Proctor's key). Velez suggested that examination of specimens of both species at UPRRP might shed some light on the issue, and that she can annotate our 2016 specimens if necessary. Unfortunately, it now seems likely that our 2016 collections at Pico Rodadero in 2016 were *T. asplenioides,* not *T. yaucoensis*. We have DNA from this population at both the

Sessa Lab at University of Florida and also with Fawcett at University of Vermont. Fairchild also maintains several dozen young sporophytes which are available for further study if needed.

## Update on ex situ conservation collections

In early 2016 we sowed spores from two genetic lines of *Thelypteris* from Pico Rodadero into several germination containers at Fairchild. Plants were allowed to grow until sporophytes formed that reached the top of the spore container. On 10/5/17, we separated young sporophytes from the germination containers into individual plugs. Currently, we have two individuals from this propagation effort, which key out to *Thelypteris* (=*Goniopteris*) *asplenioides*.

## Genetic studies

### *Materials & Methods*

DNA Extraction and Sequencing - Leaf material for DNA extraction was collected in the field in Puerto Rico in 2016. Samples from four individuals identified as *Thelypteris yaucoensis* were sent to University of Florida for genetic studies including material from a collection made by G. R. Proctor in 1987 (see Appendix 3 for voucher information). We extracted DNA from the leaf material and amplified one protein-coding region (pgiC) of the nuclear genome using polymerase chain reaction (PCR) and the 14F and 16R primers of Ishikawa et al. (2002). We selected this nuclear region because of its utility in previous studies on hybridization in ferns (Sessa et. al, 2012). We then ran the PCR products on agarose gel, cut out the resulting bands, and re-extracted the DNA. Using the pGEM-T Easy Vector System, we cloned the re-extracted DNA. At least 12 and up to 16 transformed colonies were chosen for each individual, after an incubation period of 16 hours, and amplified using the initial pgiC primers. The final PCR products were purified and sent to Eurofins with 14F and 16R primers for sequencing.

Alignment and Analysis - The pgiC sequences were assembled using the sequence analysis software, Geneious Prime (version 2019.2.1), and aligned using the MAFFT plugin for Geneious. The alignment was assessed by eye for evidence of variation between samples.

### *Results*

We were unsuccessful in acquiring sufficient PCR product for cloning from one of the individuals sampled in 2016, resulting in data for only three individuals in the final analysis – two samples collected in 2016 and the Proctor specimen collected from the type locality. Phylogenetic analyses are uninformative with such a small number of samples, hence these were not done.

The final alignment was 707 base pairs in length with 205 variant sites (29%). The two samples from 2016 (samples 1032 and 1033) are identical, sharing multiple single nucleotide polymorphisms with one another that are not shared with any of the alleles present in the 1987 type-locality collection by Proctor (sample 1039). This provides strong evidence that the two new collections (samples 1032 and 1033) are not the same entity as *Thelypteris yaucoensis* identified by Proctor.

## Discussion

As with many of the fern taxa described here, the taxonomic status of *Thelypteris yaucoensis* is in question, as it may simply be a morphovariant of a similar taxon in the subgenus *Goniopteris*. Susan Fawcett's work will be crucial in determining the status of this taxon, but further field surveys to attempt to collect material that keys out to *T. yaucoensis* is needed.

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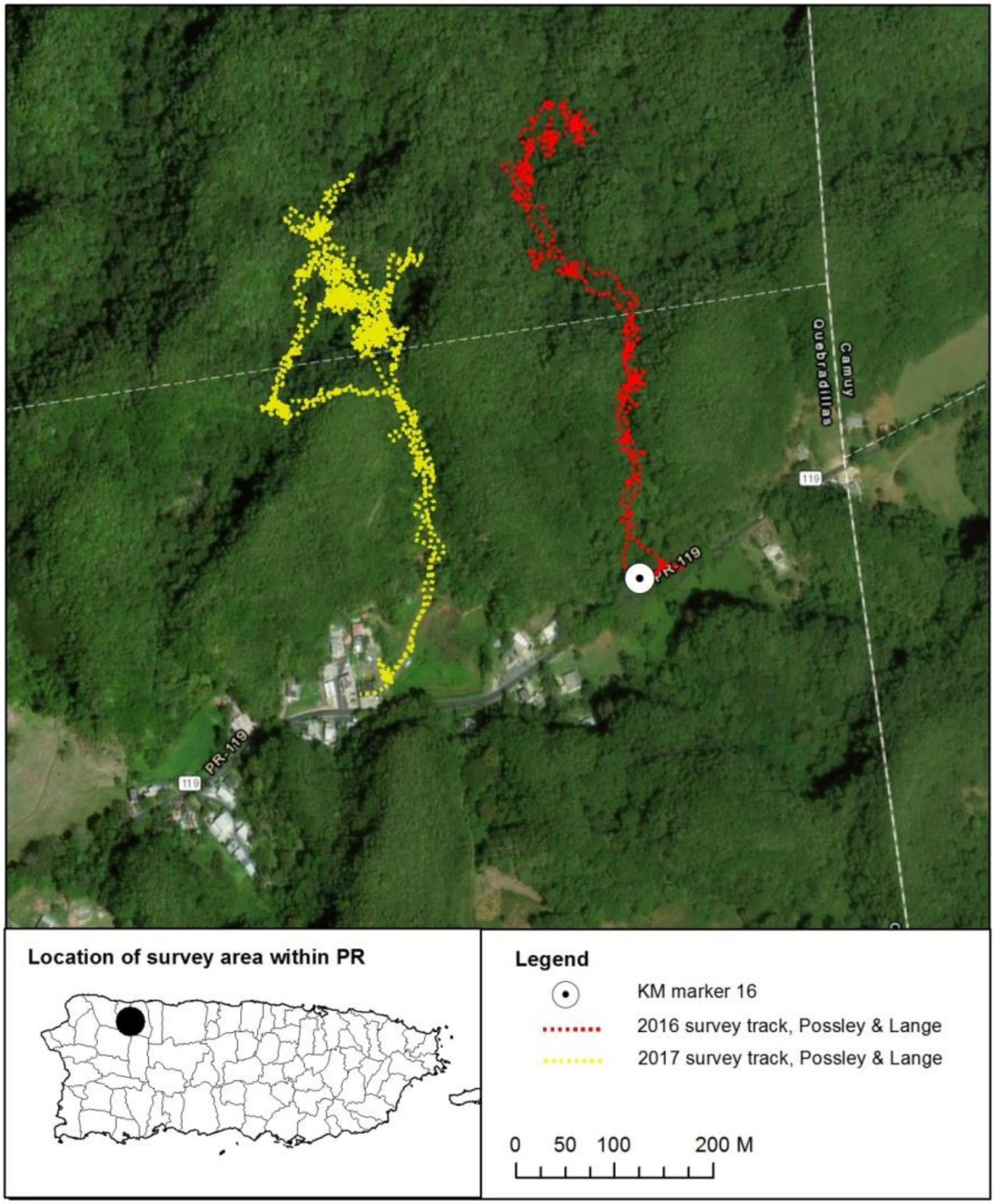
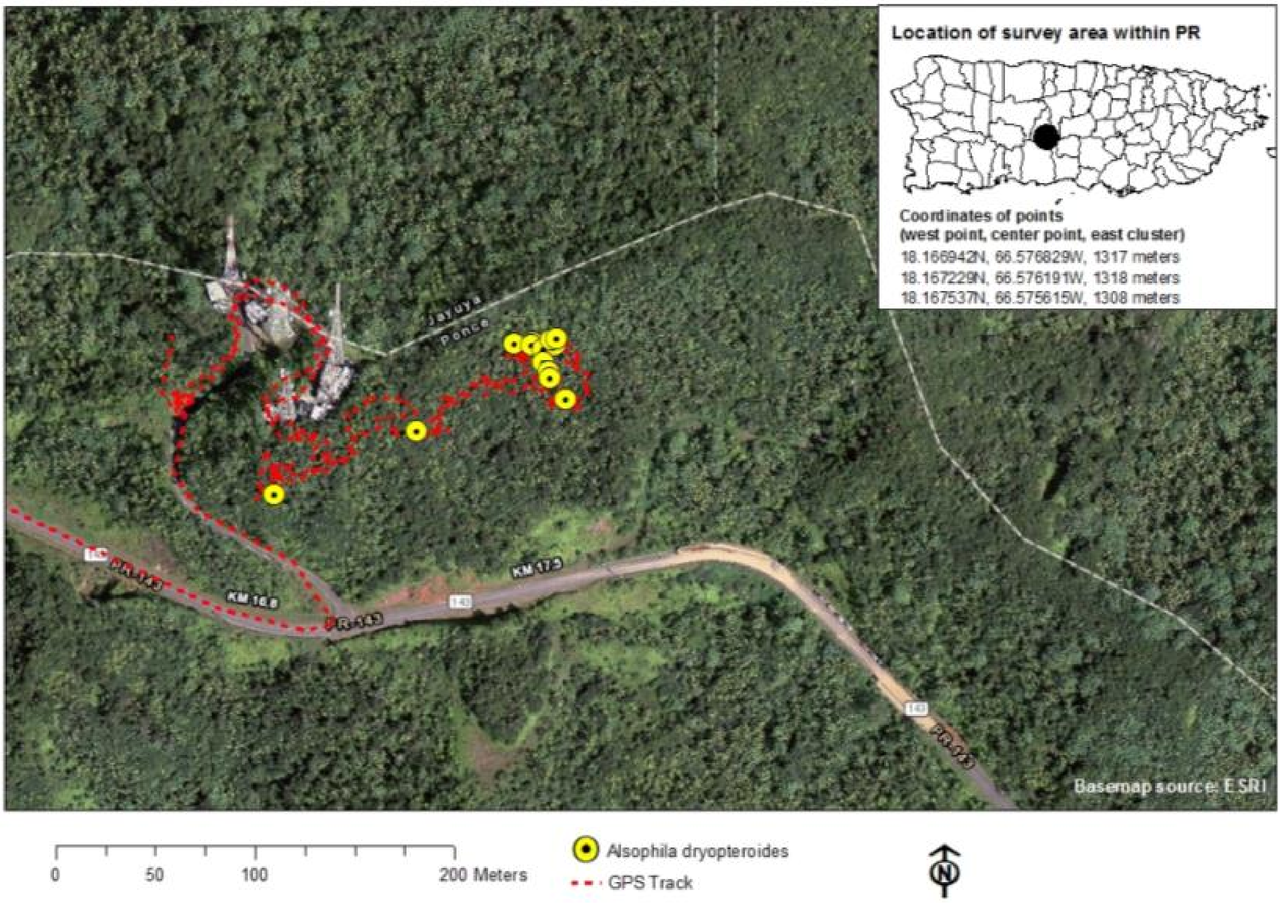
Appendix 1

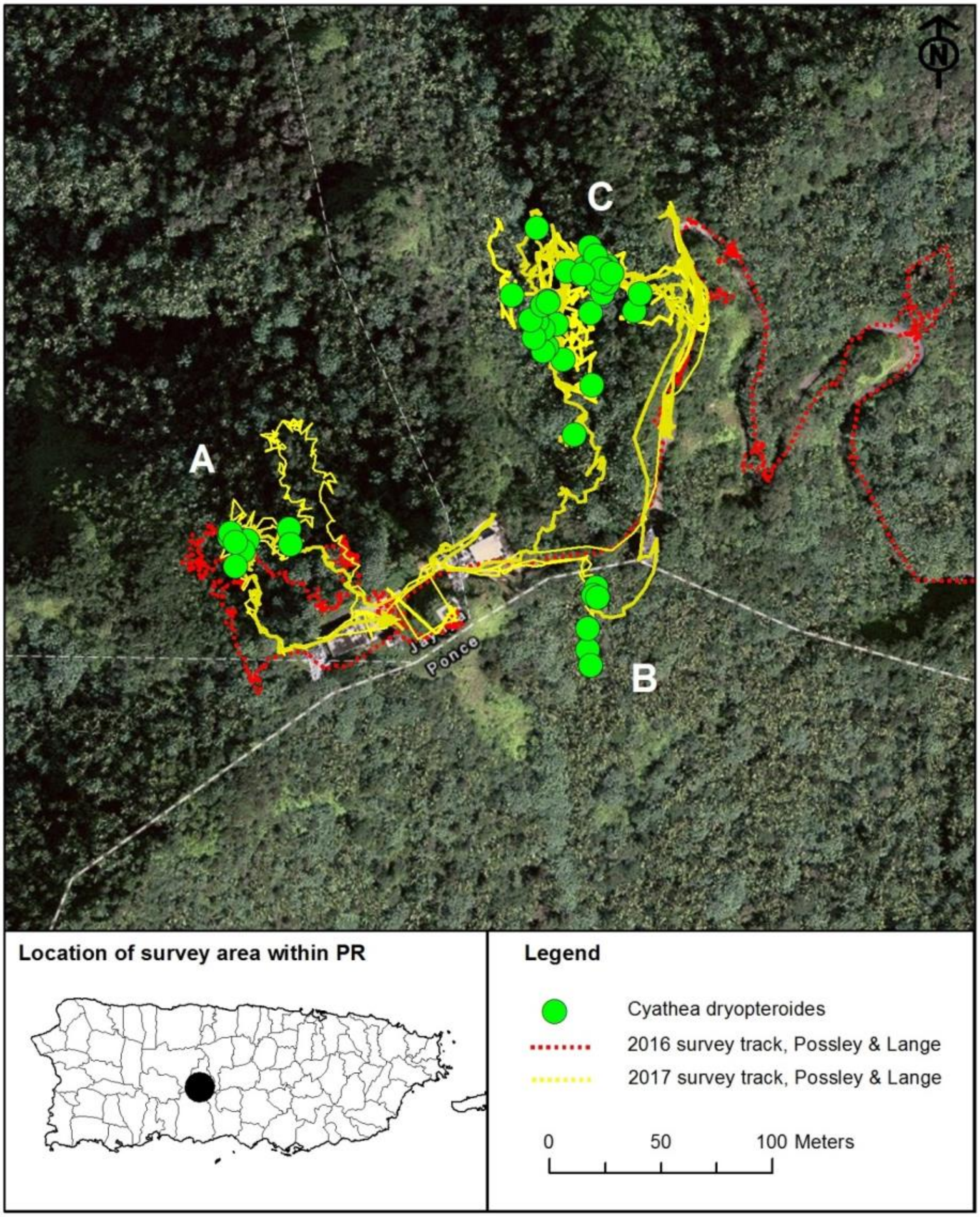
## Field Maps



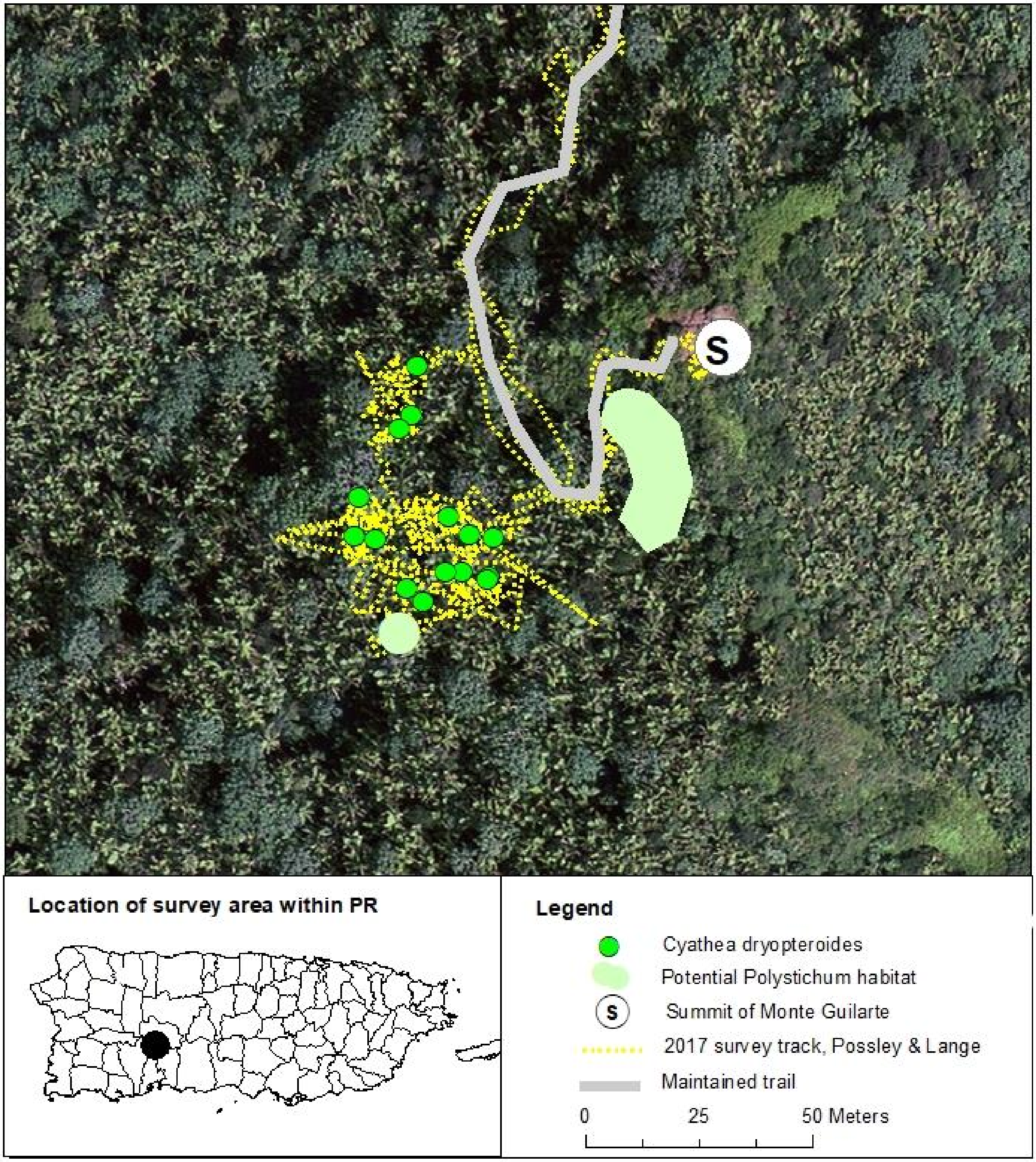
**Map 1.** *Adiantum vivesii* survey areas in 2016 and 2017. Ravines were very steep-sided. Sampling was along elevational gradients that are not well represented here.



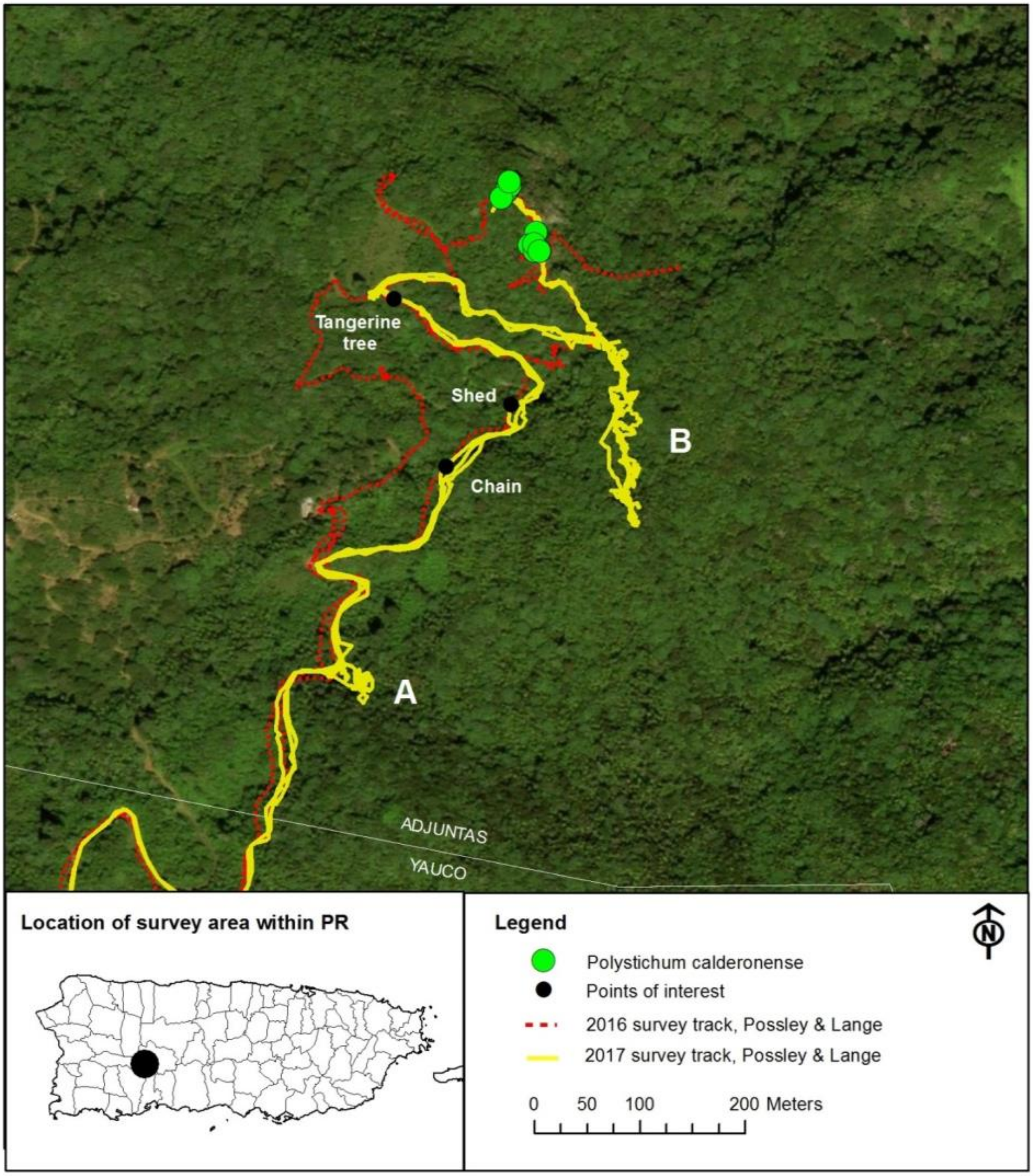
**Map 2.** Survey area and location of *Cyathea dryopteroides* at Monte Jayuya in 2014.



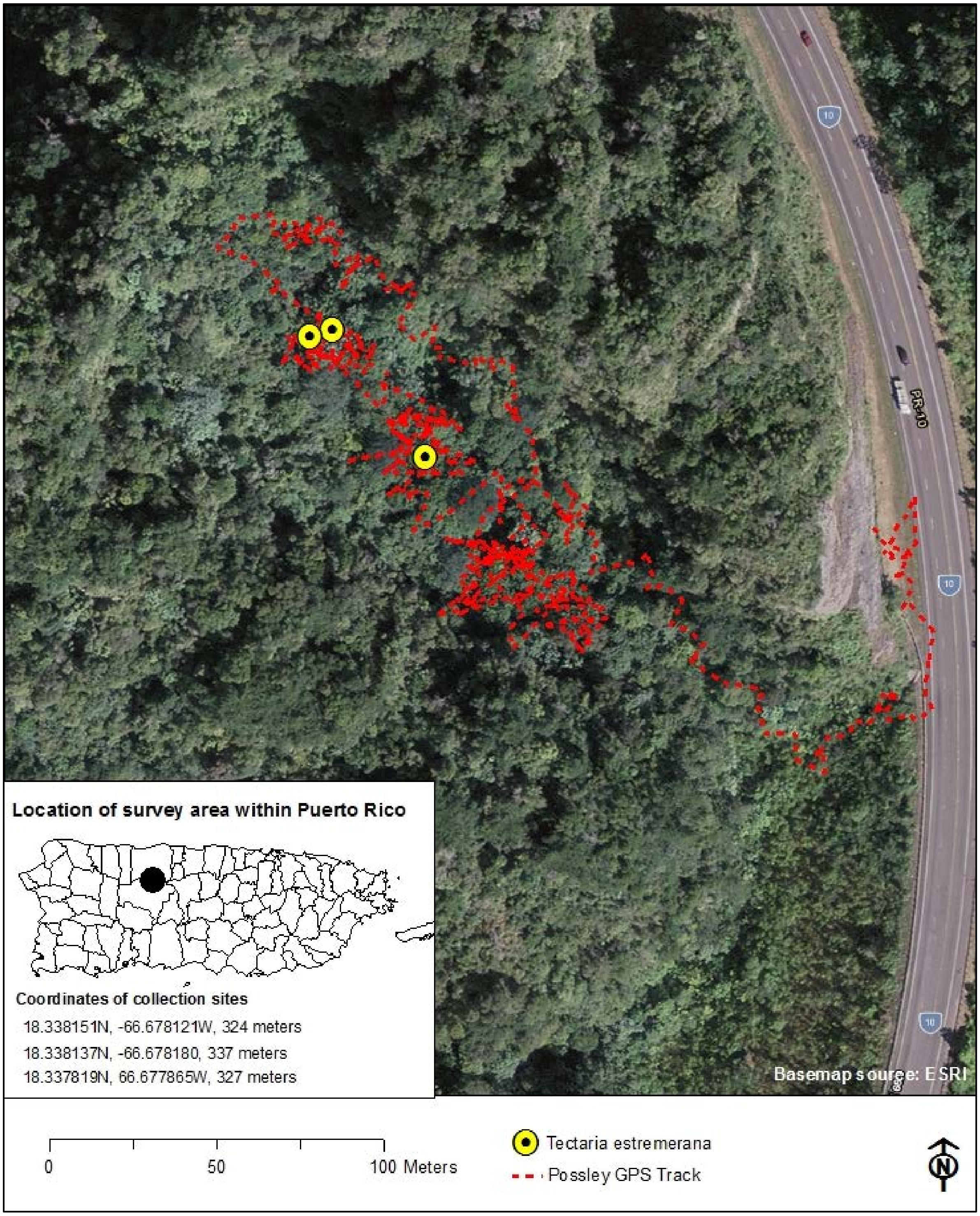
**Map 3.** Area surveyed for *Cyathea dryopteroides* and *Elaphoglossum serpens* at Cerro Punta in 2016 and 2017. Areas **A** and **B** were previously known to have plants present; these were under study by UPR student Yahaida Camacho. Area **C** was found by Lange and Flickinger during the February 2017 survey.



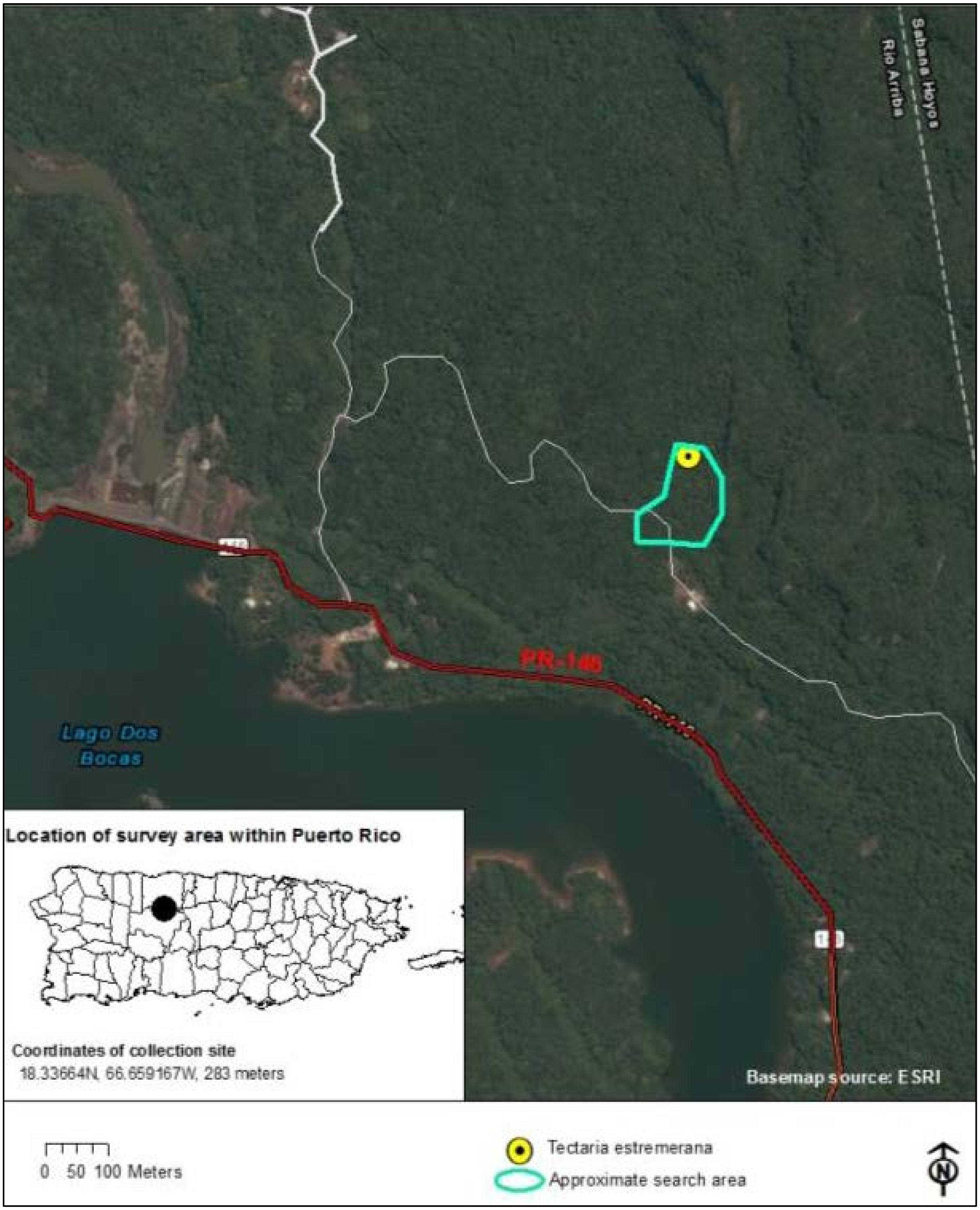
**Map 4.** Survey area and location of *Cyathea dryopteroides* at Monte Guilarte in 2017.



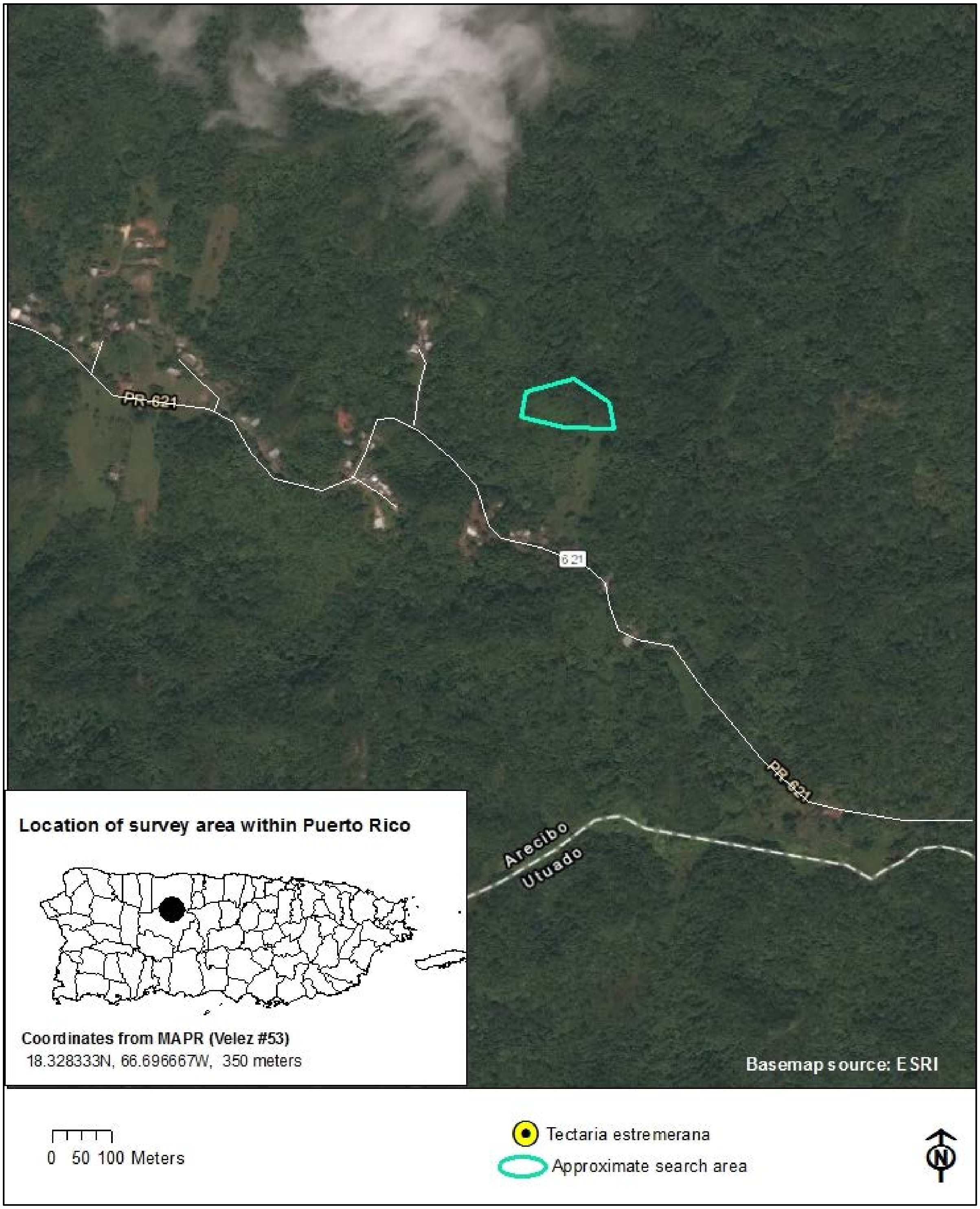
**Map 5.** *Polystichum calderonense* survey area at Silla de Calderón (Guilarte Forest) in 2014, 2016, and 2017. "**A**" indicates the location where a stream meets the unimproved road, where high fern diversity was observed. "**B**" indicates an area we judged to be a potential *P. calderonense* introduction site; other *Polystichum* species were present along this ridge. These and other landmarks are included to aid future navigation.



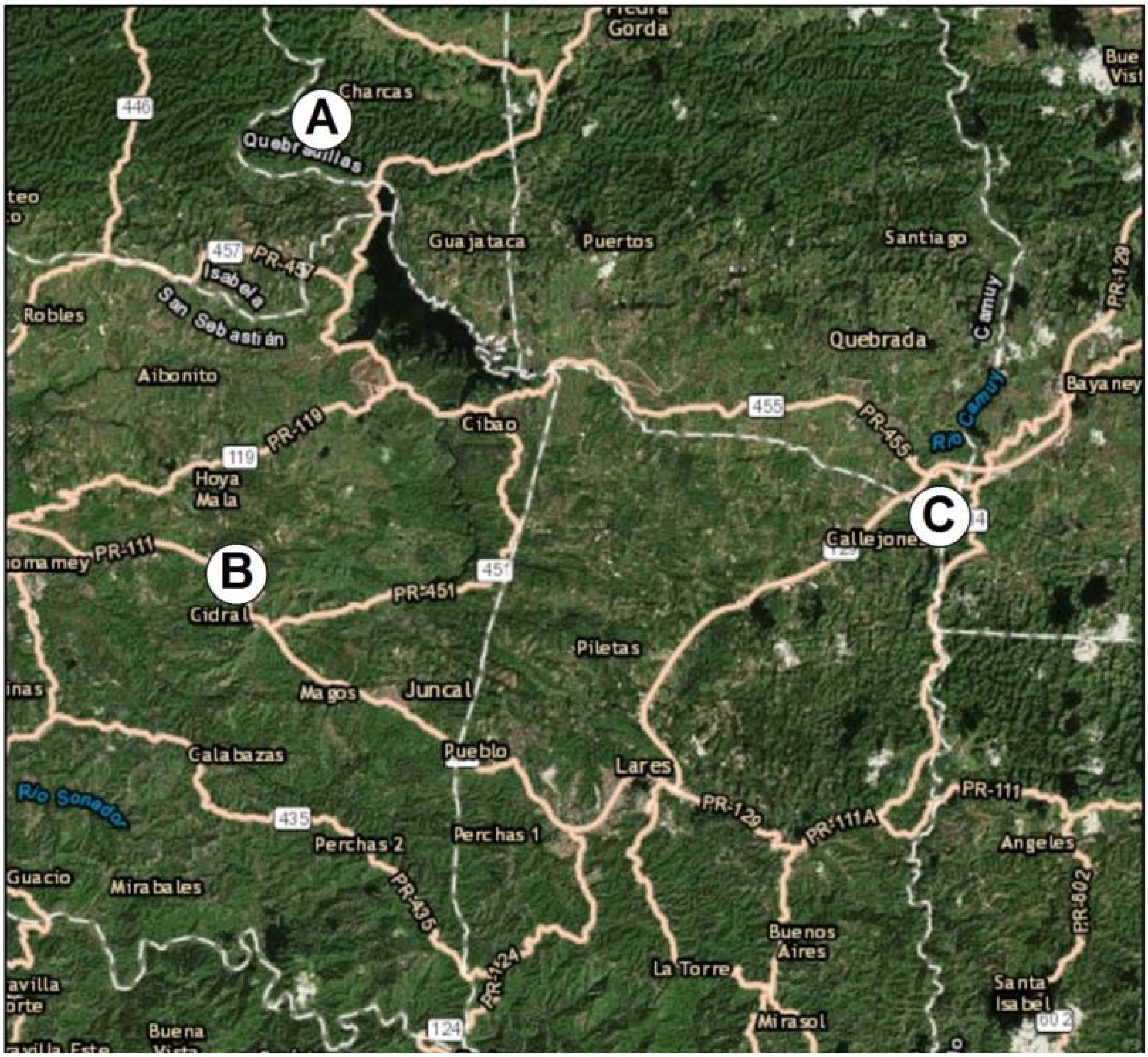
**Map 6.** *Tectaria estremerana* site west of Highway 10 in Rio Abajo Forest, monitored 11/5/14 and 1/31/16.



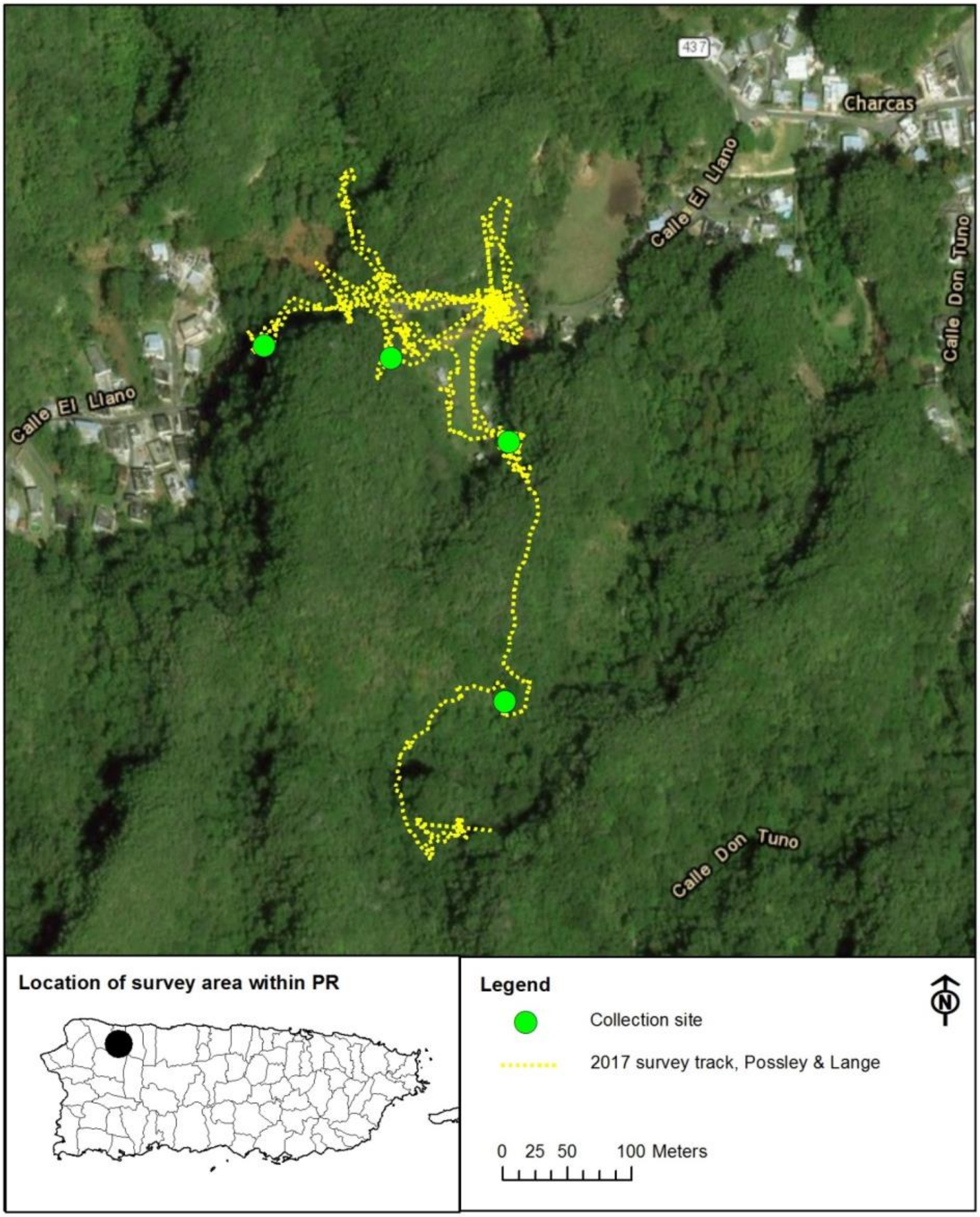
**Map 7.** *Tectaria estremerana* site near Finca Opiola, visited 11/11/14.



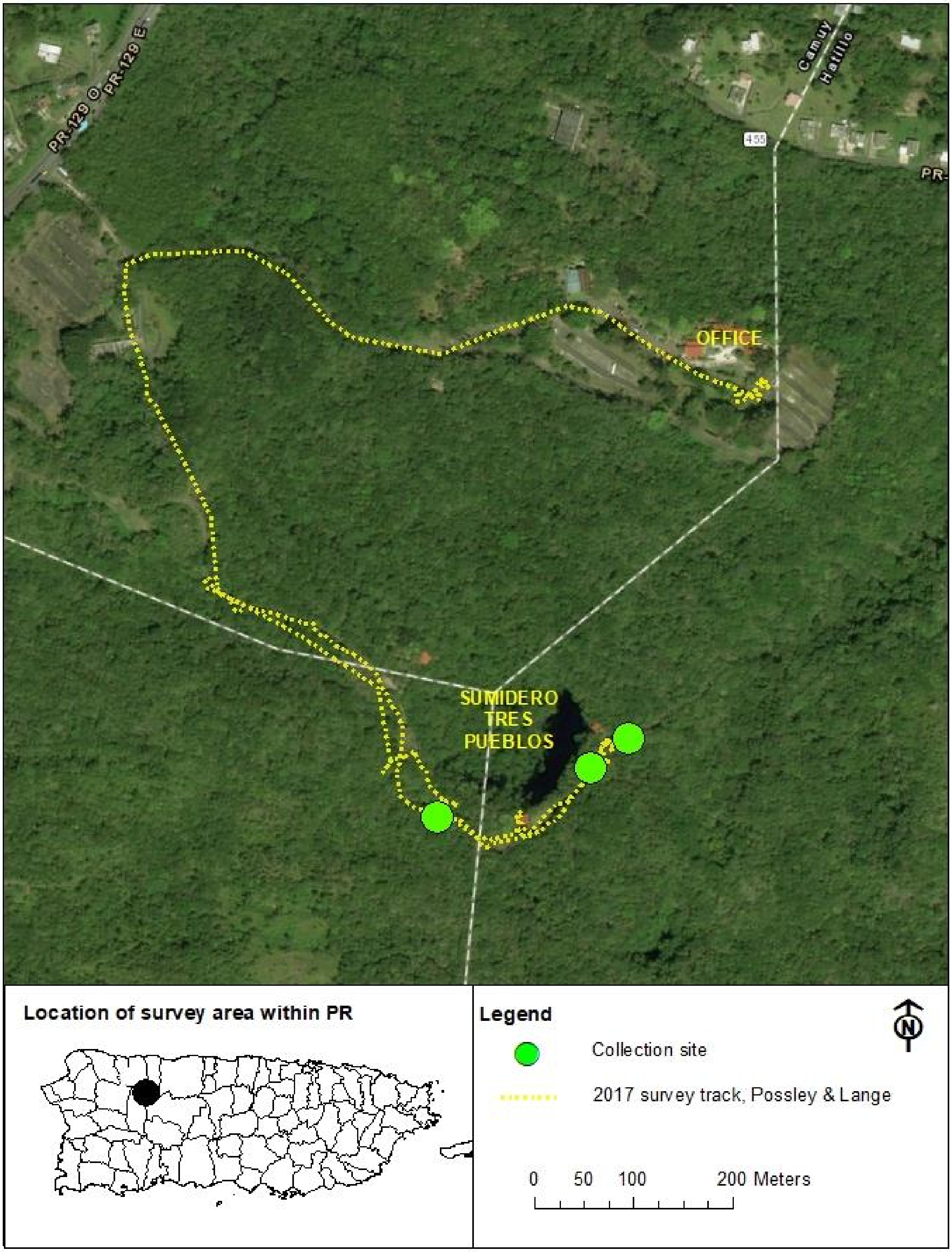
**Map 8.** *Tectaria estremerana* site near El Jobo, visited 11/11/14. This appeared to be appropriate habitat, but we did not find the taxon here.



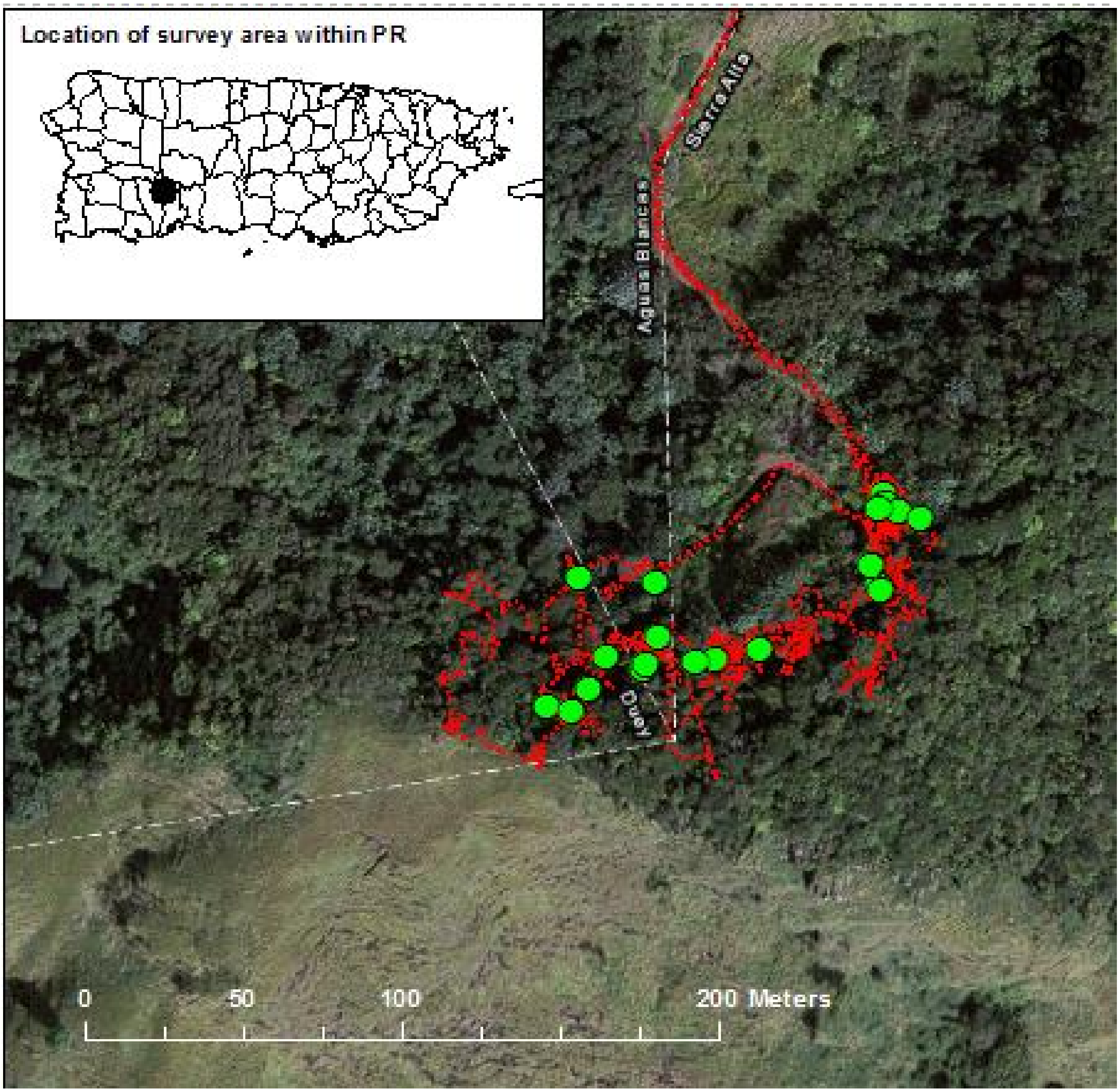
**Map 9.** *Thelypteris verecunda* survey areas visited in February 2017, shown in relation to one another and to Lake Guajataca. “**A**” is Barrio Charcas, “**B**” is Collazo Falls, and “**C**” is Camuy Caverns.



**Map 10.** *Thelypteris verecunda* survey and collection sites in Barrio Charcas.



**Map 11.** *Thelypteris verecunda* survey and collection sites in Camuy Caverns.



**Map 12.** Summit of Pico Rodadero, area surveyed on 1/29/16 (red track), and locations where we encountered what we believed was *Thelypteris yaucoensis* (but which was, in fact, *Thelypteris aplenioides*).

Appendix 2

# Fern collections

## 2014

| Taxon | Material collected | Source(s) | Purpose | Comment |
|---|---|---|---|---|
| *Polystichum calderonense* | Fertile fronds (6), buds (11) | Silla de Calderón | Germination trials, propagation trials, storage | |
| *Cyathea dryopteroides* | Fertile fronds (21) | Monte Jayuya, Toro Negro Forest, Puerto Rico | Germination trials, propagation trials, storage | |
| *Tectaria estremerana* | Fertile fronds (3) | Rio Abajo forest near Hwy 10 | Germination trials, propagation trials, storage | Spores had questionable viability. |
| *Tectaria estremerana* | Fertile frond (1) | Near Finca Opiola | Germination trials, propagation trials, storage | Spores had questionable viability. |

## 2016

| Taxon | Material collected | Source(s) | Purpose | Comment |
|---|---|---|---|---|
| *Adiantum latifolium* | DNA (5), herb specs (6) | Bo. San Antonio, Quebradillas,<br>Bo. Mayaguez Arriba, Mayaguez | *A. vivesii* genetics, GOFLAG* | |
| *Adiantum petiolatum* | DNA (4), herb specs (5) | Bo. Mayaguez Arriba, Mayaguez | *A. vivesii* genetics, GOFLAG | |
| *Adiantum pulverulentum* | herb spec (1) | Bo. Mayaguez Arriba, Mayaguez | Identification | |
| *Adiantum pyramidale* | DNA (4), herb specs (5) | Bo. San Antonio, Quebradillas | *A. vivesii* genetics, GOFLAG | |
| *Adiantum tetraphyllum* | DNA (1), herb spec (1) | Bo. San Antonio, Quebradillas | *A. vivesii* genetics, GOFLAG | |
| *Asplenium erosum* | DNA (1), herb spec (1) | Pico Rodadero, Yauco | GOFLAG | |
| *Asplenium serratum* | herb spec (1) | Bo. Mayaguez Arriba, Mayaguez | GOFLAG | |
| *Blechnum fragile* | DNA (1), herb spec (1), spores (1) | Cerro Punta, Jayuya | GOFLAG, ex situ coll. | No sporophytes resulted |
| *Campyloneurum angustifolium* | DNA (1), herb spec (1) | Pico Rodadero, Yauco | GOFLAG | |

| | | | | |
|---|---|---|---|---|
| *Danaea nodosa* | herb spec (1) | Bo. Mayaguez Arriba, Mayaguez | identification | |
| *Elaphoglossum latifolium* | DNA (1), herb spec (1), spores (1) | Cerro Punta, Jayuya | GOFLAG, ex situ coll. | |
| *Elaphoglossum maxonii* | DNA (1), herb spec (1) | Cerro Punta, Jayuya | GOFLAG, identification | |
| *Hymenophyllum tegularis* | DNA (1), herb spec (1) | Cerro Punta, Jayuya | GOFLAG | |
| *Hypoderris brownii* | DNA (1), herb spec (1) | Bo. Mayaguez Arriba, Mayaguez | GOFLAG | |
| *Microgramma heterophylla* | DNA (1), herb spec (1) | Sierra Bermeja, Cabo Rojo | GOFLAG | |
| *Microgramma pilosoloides* | DNA (1), herb spec (1) | Pico Rodadero, Yauco | GOFLAG | |
| *Neurodium lanceolatum* | DNA (1), herb spec (1), spores (1) | Cerro Punta, Jayuya | GOFLAG, ex situ coll. | |
| *Pecluma plumula* | DNA (1), herb spec (1) | Pico Rodadero, Yauco | GOFLAG | |
| *Pleopeltis polypodiodes* | DNA (1), herb spec (1) | Sierra Bermeja, Cabo Rojo | GOFLAG | |
| *Polystichum calderonense* | spores (1) | Silla de Calderon, Guilarte Forest | Propagate for ex situ | |
| *Rumohra adiantiformis* | herb spec (1) | Bo. Mayaguez Arriba, Mayaguez | identification | |

* GOFLAG is a project funded through the National Science Foundation's GoLife program. The full title of the project is: "Building a comprehensive evolutionary history of flagellate plants". Emily Sessa is a co-Principle Investigator on this project, whose goal is to improve our understanding of the history and relationships of the flagellate plants by using new sequencing technologies to produce a species-level phylogeny for these taxa that is linked to an immense and varied amount of data on fossils, phenomic characters, and geospatial distributions.

## 2017

| **Taxon** | **Material collected** | **Source(s)** | **Purpose** | **Comment** |
|---|---|---|---|---|
| *Asplenium radicans* | Frond with proliferating tip (1) | Silla de Calderón | Initially collected to identify, then for ex situ collection, but the frond did not root. | Died |
| *Cyathea dryopteroides* | Fertile fronds (4) | Monte Guilarte | Ex situ propagation and long term storage | No spores were recovered |
| *Cyathea dryopteroides* | Fertile fronds (7) | Cerro Punta | Collect spores for ex situ propagation and long term storage, but no spores were recovered. | Very few spores were recovered. One collection may have yielded a small amount. |
| *Polystichum cf. platyphyllum* | Fertile frond (1/2) | Silla de Calderón | Collect spores for ex situ propagation. | No germination |
| *Polystichum cf. polystichifolium* | Frond with proliferating tip (1) | Silla de Calderón | Initially to identify, then for ex situ collection. | Died |

| | | | | |
|---|---|---|---|---|
| *Thelypteris cf. verecunda* | Small plants (4) | Bo. Charcas | One plant—the westernmost collection site—is more consistent with Thelypteris reptans. | All survived, appear to be *T. reptans.* |
| *Thelypteris cf. verecunda* | Small plants (8) | Camuy Caverns | For ex situ collection (spore production, available for DNA) | All survived, appear to be *T. reptans.* |
| *Thelypteris hildae* | Small plant (2) | Camuy Caverns | For ex situ collection (spore production, available for DNA) | All survived |
| *Thelypteris cf. verecunda* | Small plants (6) | Collazo Falls | For ex situ collection (spore production, available for DNA) | All survived, appear to be *T. reptans.* |

Appendix 3

## Genetic data summary tables

*Tectaria* collections

| **Genus** | **Field ID** | **Genetic Results** | **DNA #** | **Ticket #** | **Locality** | **Voucher** |
|---|---|---|---|---|---|---|
| *Tectaria* | *cicutaria* | *cicutaria* | 927 | MAPR 18 | Rio Abajo | J.Possley 194 |
| *Tectaria* | *cicutaria* | ***cicutaria x incisa*** | 928 | MAPR 23 | Rio Abajo | J. Possley 199 |
| *Tectaria* | *estremerana* | ***heracleifolia x incisa*** | 929 | MAPR 17 | Rio Abajo | J. Possley 193 |
| *Tectaria* | *estremerana* | ***cicutaria x incisa*** | 930 | MAPR 25 | Rio Abajo | J. Possley 201 |
| *Tectaria* | *heracleifolia* | *herecleifolia* | 931 | MAPR 45 | Rio Abajo | J. Possley 221 |
| *Tectaria* | *herecleifolia* | *herecleifolia* | 932 | MAPR 46 | Rio Abajo | J. Possley 222 |
| *Tectaria* | *incisa* | **_cicutaria_ x _incisa_** | 933 | MAPR 24 | Rio Abajo | J. Possley 200 |
| *Tectaria* | *incisa* | *incisa* | 934 | MAPR 22 | Rio Abajo | J. Possley 198 |
| *Tectaria* | *cicutaria* | **_cicutaria_ x _incisa_** | 943 | MAPR16 | Rio Abajo | J. Possley 192 |
| *Tectaria* | *cicutaria* | *cicutaria* | 944 | MAPR 19 | Rio Abajo | J. Possley 195 |
| *Tectaria* | *cicutaria* | *cicutaria* | 945 | MAPR20 | Rio Abajo | J. Possley 196 |
| *Tectaria* | *cicutaria* | **_cicutaria_ x _incisa_** | 946 | MAPR44 | Rio Abajo | J. Possley 220 |
| *Tectaria* | *estremerana* | **_cicutaria_ x _incisa_** | 947 | MAPR 47 | Rio Abajo | J. Possley 223 |
| *Tectaria* | *incisa* | *incisa* | 948 | MAPR21 | Rio Abajo | J. Possley 197 |

*Adiantum* collections

| Genus | Field ID | Genetic Results | DNA #r | Ticket # | Locality | Voucher |
|---|---|---|---|---|---|---|
| *Adiantum* | *tetraphyllum* | *tetraphyllum* | 797 | MAPR 12 | Quebradillas | J. Possley 188 |
| *Adiantum* | *obliqum* | *latifolium* | 799 | SF 434 |  | S. Fawcett 434 |
| *Adiantum* | *cf. vivesii* | *pyramidale* | 890 | 9722138 | Quebradillas | J. Possley 249 |
| *Adiantum* | *sp.* | *pulverulentum* | 891 | 9722143 | Quebradillas | E.B. Sessa |
| *Adiantum* | *vivesii* | ***pyramidale*** **x** ***petiolatum*** | 893 | 9722140 | Quebradillas | G.J. Breckon 4625 |
| *Adiantum* | *cf. vivesii* | *tetraphyllum* | 894 | 9722141 | Quebradillas | J. Possley 248 |
| *Adiantum* | *sp.* | *tetraphyllum* | 895 | 9722142 | Quebradillas | E.B. Sessa |
| *Adiantum* | *sp.* | *tetraphyllum* | 896 | 9722144 | Quebradillas | E.B. Sessa |
| *Adiantum* | *sp.* | ***tetraphyllum*** **x** ***latifolium*** | 897 | 9722145 | Quebradillas | E.B. Sessa |
| *Adiantum* | *latifolium* | *latifolium* | 913 | 9722146 | Quebradillas | J. Possley 179 |
| *Adiantum* | *tetraphyllum* | *tetraphyllum* | 916 | 9722149 | Quebradillas | J. Possley 188 |
| *Adiantum* | *latifolium* | *latifolium* | 920 | MAPR 1 | Quebradillas | J. Possley 177 |
| *Adiantum* | *petiolatum* | *petiolatum* | 922 | MAPR 7 | Mayaguez | J. Possley 183 |
| *Adiantum* | *petiolatum* | *petiolatum* | 923 | MAPR 9 | Mayaguez | J. Possley 185 |
| *Adiantum* | *pyramidale* | *pyramidale* | 924 | MAPR 2 | Quebradillas | J. Possley 178 |
| *Adiantum* | *pyramidale* | *pyramidale* | 925 | MAPR 4 | Quebradillas | J. Possley 180 |
| *Adiantum* | *tetraphyllum* | *tetraphyllum* | 926 | MAPR 12 | Quebradillas | J. Possley 188 |
| *Adiantum* | *latifolium* | *latifolium* | 936 | MAPR 3 | Quebradillas | J. Possley 179 |
| *Adiantum* | *latifolium* | *latifolium* | 937 | MAPR 14 | Quebradillas | J. Possley 190 |
| *Adiantum* | *latifolium* | *latifolium* | 938 | MAPR 15 | Quebradillas | J. Possley 191 |
| *Adiantum* | *petiolatum* | ***latifolium*** **x** ***petiolatum*** | 939 | MAPR 6 | Mayaguez | J. Possley182 |
| *Adiantum* | *pyramidale* | *pyramidale* | 941 | MAPR 10 | Quebradillas | J. Possley 186 |
| *Adiantum* | *pyramidale* | *pyramidale* | 942 | MAPR 11 | Quebradillas | J. Possley 187 |
| *Adiantum* | *sp.* | *pulverulentum* | 1045 | 9722143 | Quebradillas | E.B. Sessa |
| *Adiantum* | *vivesii* | ***tetraphyllum*** **x** ***petiolatum*** | 1046 | 9722139 | Quebradillas | G.J. Breckon 4625 |
| *Adiantum* | *vivesii isoTYPE* | ***tetraphyllum*** **x** ***petiolatum*** | 1048 | NA | Quebradillas | Proctor 41389 |
| *Adiantum* | *obliquum* | *obliquum* | 1051 | NA | Coasta Rica | W.L. Testo 1872 |

## *Thelypteris* collections

| **Genus** | **Field ID** | **DNA #** | **Ticket #** | **Locality** | **Voucher** |
|---|---|---|---|---|---|
| *Thelypteris* | *verecunda* | 898 | 9722151 | Cavernas de Camuy | J. Possley 244 |
| *Thelypteris* | *verecunda* | 900 | 9722153 | Cavernas de Camuy | J. Possley 242 |
| *Thelypteris* | *verecunda* | 904 | 9722157 | Barrio Charcas | J. Possley 233 |
| *Thelypteris* | *verecunda* | 907 | 9722160 | Barrio Charcas | J. Possley 236 |
| *Thelypteris* | *reptans* | 909 | 9722162 | Barrio Charcas | E.B. Sessa |
| *Thelypteris* | *reptans* | 910 | 9722163 | Barrio Charcas | E.B. Sessa |
| *Thelypteris* | *abdita* | 1081 | NA | Sumidero Tres Pueblos | Fawcett 457C |
| *Thelypteris* | *abdita* | 1082 | NA | Sumidero Tres Pueblos | Fawcett 457C |
| *Thelypteris* | *yaucoensis* | 1039 | NA | Pico Rodadero | G.R. Proctor 43584 |
| *Thelypteris* | *yaucoensis* | 1032 | WPT 348 | Pico Rodadero | None |
| *Thelypteris* | *yaucoensis* | 1033 | WPT 344 | Pico Rodadero | None |

## Genbank Accessions used for phylogenetic analyses

| **Taxon** | **P.R. Fern** | **Locus** | **Genbank Accession** | **Voucher** |
|---|---|---|---|---|
| *Adiantum pedatum* | *A. vivesii* | IBR3 | KF553723.1 | Rothfels & Rushworth 4166 (DUKE) |
| *Cryptogramma acrostichoides* | *A. vivesii* | IBR3 | KF553726.1 | Rothfels & Zylinski 4088.1 (DUKE) |
| *Tectaria devexa* | *T. estremerana* | pgiC | KU605160.1 | Zhang et al. 6591 (CDBI) |
| *Tectaria griffithii* | *T. estremerana* | pgiC | KY937291.1 | L.B. Zhang et al. 8364 (CDBI) |
| *Tectaria harlandii* | *T. estremerana* | pgiC | KY937295.1 | L.B. Zhang et al. 7191 (CDBI) |
| *Tectaria heracleifolia* | *T. estremerana* | pgiC | KY937296.1 | F.Matos 08-125 (DUKE) |
| *Tectaria impressa* | *T. estremerana* | pgiC | KY937298.1 | L.B. Zhang et al. 6612 (CDBI) |
| *Tectaria kusukusensis* | *T. estremerana* | pgiC | KU605165.1 | Zhang et al. 6466 (CDBI) |
| *Tectaria mexicana* | *T. estremerana* | pgiC | KU605166.1 | Grusz 08-011 (DUKE) |
| *Tectaria morsei* | *T. estremerana* | pgiC | KY937300.1 | L.B. Zhang et al. 6584 (CDBI) |
| *Tectaria phaeocaulis* | *T. estremerana* | pgiC | KU605170.1 | Zhang et al. 6636 (CDBI) |

| | | | | |
|---|---|---|---|---|
| *Tectaria quinquefida* | *T. estremerana* | pgiC | KY937305.1 | L.B. Zhang et al. 6315 (CDBI) |
| *Tectaria setulosa* | *T. estremerana* | pgiC | KU605177.1 | Zhang et al. 7140 (CDBI) |
| *Tectaria simonsii* | *T. estremerana* | pgiC | KU605178.1 | Zhang et al. 7001 (CDBI) |
| *Tectaria vasta* | *T. estremerana* | pgiC | KY937320.1 | L.B. Zhang et al. 8254 (CDBI) |
| *Tectaria zeylanica* | *T. estremerana* | pgiC | KU605181.1 | Zhang et al. 6387 (CDBI) |
| *Triplophyllum securidiforme* | *T. estremerana* | pgiC | KU605183.1 | Jongkind 10773 (MO) |
| *Parathelypteris noveboracensis* | *T. verecunda* | pgiC | KF553811.1 | Rothfels & Rushworth 4164 (DUKE) |

Published chromosome counts

| **Taxon** | **Ploidy** | **Refrence** |
|---|---|---|
| *Adiantum tetraphyllum* | 2n and 4n | Jermy and Walker, 1985 (in Flora Mesoamericana) |
| *Tectaria incisa* | 2n and 4n | Walker, 1966 and Walker 1973 |
| *Tectaria heracleifolia* | 2n and 4n | Tryon and Tryon, 1982 |

Appendix 4

## Summary of ferns shared with other botanical institutions

Of the 474 ferns we shared with other institutions, 99 are still alive as of November 2019. Taxa include *Polystichum calderonense* (from 2014 Silla de Calderon collections), *Cyathea dryopteroides* (from 2014 Monte Jayuya collections), *Thelypteris asplenioides* (from 2017 Pico Rodadero) and *Thelypteris* "*verecunda*" (from 2017 collections at Camuy Caverns and Collazo Falls).

| **Taxon** | **Year sent** | **# of plants*** | **Size** | **Institution** | **State** | **Contact** | **Email** | **# Nov 2019** |
|---|---|---|---|---|---|---|---|---|
| PC | 2017 | 100 | sporelings | Naples Botanic Garden | FL | Chad Washburn, Deputy Director | Cwashburn@naplesgarden.org | 0 |
| PC | 2017 | 100 | sporelings | Marie Selby Botanic Gardens | FL | Dr. Sally Chambers, Karen Stewart | schambers@selby.org | 59 |
| PC | 2017 | 100 | sporelings | University of Florida | FL | Dr. Emily Sessa, Assistant Professor | emilysessa@ufl.edu | 13 |
| PC | 2018 | 100 | sporelings | Thailand Bot Garden |  | Dr. Brett Jestrow, Director of Collections (Fairchild) | bjestrow@fairchildgarden.org | unknown |
| PC | 2018 | 18 | pt, qt | Como Park Zoo & Conservatory, St. Paul MN | MN | Karen Kleber-Diggs, Horticulture Supervisor | karen.kleber.diggs@ci.stpaul.mn.us |  |
| PC | 2018 | 18 | pt, qt | Atlanta Botanical Garden | GA | Dr. Emily Coffey, Vice President of Conservation and Research | ecoffey@atlantabg.org | 12 |
| PC | 2018 | 18 | pt, qt | The Mitchell Park Horticultural Conservatory - The Domes | WI | Mary Braunreiter, Horticulturist | Mary.Braunreiter@milwaukeecountywi.gov | 0 |
| PC | 2018 | 5 | pt, plug | Missouri Botanical Garden | MO | Deborah Lalumondier | Deborah.Lalumondier@mobot.org | 5 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| PC | 2018 | 5 | pt, plug | Olbrich Botanic Garden | WI | Colten Blackburn | cblackburn@cityofmadison.com; | |
| PC | 2019 | 5 | qt | Flamingo Gardens | FL | Keith Clark, Director; Joe Parr, Horticulturist; Patrice Sonnelitter, Curator. | exec@flamingogardens.org,<br>joe.parr6@gmail.com,<br>botanical@flamingogardens.org | 5 |
| CD | 2018 | 1 | 1G | Como Park Zoo & Conservatory, St. Paul MN | MN | Karen Kleber-Diggs, Horticulture Supervisor | karen.kleber.diggs@ci.stpaul.mn.us | |
| CD | 2018 | 1 | 1G | Atlanta Botanical Garden | GA | Dr. Emily Coffey, Vice President of Conservation and Research | ecoffey@atlantabg.org | 1 |
| CD | 2018 | 1 | 1G | The Mitchell Park Horticultural Conservatory - The Domes | WI | Mary Braunreiter, Horticulturist | Mary.Braunreiter@milwaukeecountywi.gov | 0 |
| CD | 2018 | 1 | 1G | Missouri Botanical Garden | MO | Deborah Lalumondier | Deborah.Lalumondier@mobot.org | 0 |
| CD | 2018 | 1 | 1G | Olbrich Botanic Garden | WI | Colten Blackburn | cblackburn@cityofmadison.com; | |
| CD | 2019 | 1 | 3G | Marie Selby Botanic Gardens | FL | Anastasia Sallen, Karen Stewart | _ | 1 |
| CD | 2019 | 1 | 3G | Flamingo Gardens | FL | Keith Clark, Director; Joe Parr, Horticulturist; Patrice Sonnelitter, Curator. | exec@flamingogardens.org,<br>joe.parr6@gmail.com,<br>botanical@flamingogardens.org | 1 |
| TA | 2019 | 1 | pt | Como Park Zoo & Conservatory, St. Paul MN | MN | Karen Kleber-Diggs, Horticulture Supervisor | karen.kleber.diggs@ci.stpaul.mn.us | |
| TV | 2019 | 2 | 1G | Marie Selby Botanic Gardens | FL | Anastasia Sallen, Karen Stewart | karen.stewart62@verizon.net | 2 |

Appendix 5

## Summary of threats to federally listed ferns of Puerto Rico's Cordillera Central

### *Adiantum vivesii* Proctor

- Development and/or deforestation of the private land where *A. vivesii* is known to occur,
- Weed invasions from adjacent disturbed habitat,
- Inbreeding depression (this is threat is only applicable if *A. vivesii* is a true species, capable of producing viable spores and viable gametophytes),
- Environmental stochasticity, which is a threat due to the extremely small size of this population. For example, small-scale events like a rockslide or a falling tree could extinguish the population, as could large-scale events like hurricanes or drought.
- Lack of ex situ germplasm collections.

### *Cyathea dryopteroides* Maxon

- Dumping of debris from cellphone tower maintenance,
- Weed invasions from adjacent disturbed habitat (e.g.: *Gleichenella pectinata*, *Hypolepis repens*),
- Fire-- whether from electrical equipment associated with the cellphone tower or from lightning strikes,
- Genetic factors such as inbreeding depression and/or genetic swamping through hybridization with *Alsophila bryophila,* and
- Environmental stochasticity, which is a threat due to the extremely small size of this population. For example, small-scale events like a rockslide or a falling tree could extinguish the population, as could large-scale events like hurricanes or drought.
- Climate change is an additional threat to consider, as *C. dryopteroides* favors high-elevation elfin forest near the summit of mountains, and increasing global temperatures may eliminate its habitat entirely.
- There are only ex situ germplasm collections from one of three known sites, Monte Jayuya.

### *Elaphoglossum serpens* Maxon & C.V. Morton

- Dumping of debris from cellphone tower maintenance,
- Weed invasions from adjacent disturbed habitat (e.g.: *Gleichenella pectinata*, *Hypolepis repens*),
- Fire-- whether from electrical equipment associated with the tower or from lightning strikes,
- Inbreeding depression due to small population size,
- Environmental stochasticity, which is a threat due to the extremely small size of this population. For example, small-scale events like a rockslide or a falling tree could extinguish the population, as could large-scale events like hurricanes or drought.

- Climate change is an additional threat to consider, as *E. serpens* favors high-elevation elfin forest near the summit of mountains, and increasing global temperatures may eliminate its habitat entirely.
- Lack of ex situ conservation germplasm collections.
- Finally, poaching is a concern. *Elaphoglossum* spp. tend to be sought after by fern collecting enthusiasts, more so than other genera. This population's location is public on the Atlas Obscura website (www.atlasobscura.com/places/cerro-de-punta), which advertises:

> Near the summit [of Cerro de Punta] is the unique habitat of a plant known as the *Elaphoglossum serpens*, which according to the US Fish and Wildlife Service recovery plan, grows on only six moss-covered trees in the mountains forest. Like some mythical herb that can only be obtained via a heroic quest (or an endangered species that is on the razor's edge of annihilation), there are only thought to be 22 remaining specimens left in the world, all of which are found in the small summit grove.

### *Polystichum calderonense* Proctor

- Impacts from hikers such as deforestation, trampling, soil disturbance (which exacerbates invasive species), littering, intentionally set fires, and poaching,
- Weed invasions from adjacent disturbed habitat (e.g.: *Arthrostylidium* sp. and *Nephrolepis* spp.)
- Fire from lightning strikes (lightning appeared to kill one or two *P. calderonense* shortly before our 2014 visit), and
- Inbreeding depression due to small population size, and
- Environmental stochasticity, which is a threat due to the extremely small size of this population. For example, small-scale events like a rockslide or a falling tree could extinguish the population, as could large-scale events like hurricanes or drought.
- Climate change is an additional threat to consider, as *P. calderonense* favors high-elevation forest near the summit of mountains, and increasing global temperatures may eliminate its habitat entirely.

### *Tectaria estremerana* Proctor & A.M.Evans

- Development and/or deforestation on privately-owned sites,
- Weed invasions from adjacent disturbed habitat (the construction of Highway 10 has resulted in disturbed areas dominated non-native species existing within a few hundred yards of the largest known population of *T. estremerana*, which contains only three individuals),
- Inbreeding depression due to small population size,
- Changes to hydrology from road construction,
- Environmental stochasticity, which is a threat due to the extremely small size of this population. For example, small-scale events like a rockslide or a falling tree could extinguish the population, as could large-scale events like hurricanes or drought.
- Lack of ex situ conservation germplasm collections

### ***Thelypteris inabonensis*** **Proctor**

[Note: to date, we have not worked with this species.]

- Development and/or deforestation on privately-owned sites,
- Weed invasions from adjacent disturbed habitat,
- Inbreeding depression due to small population size,
- Lack of ex situ conservation germplasm collections, and
- Environmental stochasticity, which is a threat due to the extremely small size of this population. For example, small-scale events like a rockslide or a falling tree could extinguish the population, as could large-scale events like hurricanes or drought.
- Access difficulties. We have not yet visited a population of *T. inabonensis* because the only known populations are extremely remote, requiring long hikes and/or overnight camping.

### ***Thelypteris verecunda*** **Proctor**

- Development and/or deforestation on privately-owned sites,
- Weed invasions from adjacent disturbed habitat,
- Inbreeding depression due to small population size,
- Few ex situ conservation germplasm collections, and
- Environmental stochasticity, which is a threat due to the extremely small size of this population. For example, small-scale events like a rockslide or a falling tree could extinguish the population, as could large-scale events like hurricanes or drought.

### ***Thelypteris yaucoensis*** **Proctor**

- Deforestation and/or development at Pico Rodadero and at other privately-owned sites,
- Other human impacts such as ATV use, trampling, and setting of fires,
- Weed invasions from adjacent disturbed habitat,
- Fire from lightning strikes,
- Inbreeding depression due to small population size, and
- Environmental stochasticity, which is a threat due to the extremely small size of this population. For example, small-scale events like a rockslide or a falling tree could extinguish the population, as could large-scale events like hurricanes or drought.
- Little to no ex situ conservation germplasm collections, and
- Climate change is an additional threat to consider, as *T. yaucoensis* favors high-elevation forest near the summit of mountains, and increasing global temperatures may eliminate its habitat entirely.

Appendix 7:

## Article from Fairchild’s magazine, The Tropical Garden

CONSERVING

# ENCHANTED BY FERNS ON LA ISLA DEL ENCANTO

BY JENNIFER POSSLEY

PHOTOS BY JAMES LANGE AND JENNIFER POSSLEY

One of the many perks of being a botanist in subtropical South Florida is that we reside in the sweet spot between temperate and tropical zones, and much of our flora has biogeographical roots in the West Indies. If you learn to identify the native species in Miami, then you are also learning to identify many West Indian species, or at least their close relatives. This is one reason why we look forward to using our experience with Miami’s ferns to promote fern conservation throughout our region.

And so it came to pass that in early 2016, Fairchild Field Biologist Jimmy Lange and I found ourselves in western Puerto Rico for 11 days, at the invitation of U.S. Fish & Wildlife Service (USFWS) biologists. This was the South Florida Conservation Team’s second visit to the area in recent years, and we were once again dedicating half of our trip to Puerto Rico’s endemic, federally endangered ferns (the rest of our time was spent on the endemic flora of the Sierra Bermeja region, which was profiled in Dr. Joyce Maschinski’s summer 2015 article in *The Tropical Garden*).

1. *Danaea elliptica*
2. *Pleopeltis polypodioides*
3. View of mountains near Yauco
4. *Odontosoria aculeata*

Our pteridological (fern-related) goals on this excursion were many, but first and foremost was to locate federally endangered fern species and collect material for propagation. The fern species we sought included *Tectaria estremerana, Polystichum calderonense, Cyathea dryopteroides, Adiantum vivesii, Thelypteris yaucoensis* and *Elaphoglossum serpens*.

By the end of this second trip, we had succeeded in collecting spores from four of these six species. From our bounty of spores, Fairchild's horticulturist, Mike Freedman, set about developing spore propagation protocols. We reported Mike's results to USFWS, which will use them to inform its endangered species recovery plans. Some of the ferns Freedman has grown will be displayed at Fairchild; others will be kept in our nursery until they are ready to ship to our Puerto Rican colleagues. In the meantime, when these ferns reach maturity, we will collect their spores to send to long-term storage at the U.S. Department of Agriculture, further safeguarding their germplasm.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A new goal introduced for this recent trip was to collect tissue and herbarium specimens for taxonomic research. Fern taxonomy can be complicated, stemming from the fact that ferns are notorious for hybridization as well as extreme variability in form. Many of our target species have outstanding taxonomic questions whose resolution will be extremely important for their recovery. One might be a hybrid, while another might be a geographic variant of a more common species. USFWS needs to know these details so that they can best protect each species.

To untangle these taxonomic knots, we sought out two experts in fern genetics: Dr. Emily Sessa from the University of Florida, and Susan Fawcett from the University of Vermont. Lange and I may have had the fun part of the job, collecting the material, but Sessa and Fawcett's work has the potential to change Puerto Rican fern taxonomy.

In order to obtain enough material for Sessa and Fawcett, we collected pieces of fern fronds from the target species and also from close relatives (Sessa says this helps her to build a phylogeny, which shows how species are related to one another). This

**5.** *Adiantum pyramidale*
**6.** *Thelypteris hildae*
**7.** *Neurodium lanceolatum*
**8.** *Polystichum calderonense*
**9.** *Diplazium* sp.
**10.** *Dicranopteris pectinata*
**11.** *Asplenium erosum*

meant that Lange and I needed to learn to recognize a good deal more than just our six target species, which we eventually did with the help of George Proctor's book "The Ferns of Puerto Rico and the Virgin Islands," as well as help from Puerto Rican botanists, and by studying specimens in the University of Puerto Rico herbarium. By the end of our trip, we had collected 50 tissue samples for Sessa and Fawcett, learned many new (to us) species, and (most humblingly) realized how much more we still had to learn.

Working with the ferns of western Puerto Rico is especially rewarding for us, in part because we love visiting our familiar Miami ferns in a truly tropical Caribbean setting. Additionally, this work has put us in touch with collaborators from a wide range of backgrounds, all of whom share a passion for rare fern conservation. We are thrilled that we have received funding to return to western Puerto Rico in winter 2017 to continue this work. I hope that some of our photos included here convey the beauty and diversity of these plants, as well as the commitment of those of us who are working to save them.

## EDITOR'S NOTE

All collections were covered by a permit from the Puerto Rico Department of Natural and Environmental Resources; and all activities were coordinated with U.S. Fish and Wildlife Services Caribbean Ecological Services Field Office (CESFO) and the Puerto Rico Department of Natural and Environmental Resources (PRDNER). Thank you to our partners in these efforts to conserve endangered endemic ferns of western Puerto Rico, including Omar Monsegur, Xiomara Labiosa, Iván Llerandi Román, JP Segarra, Jen Valentín and José Cruz Burgos (USFWS, CESFO); Emily Sessa (University of Florida, Department of Biology); Susan Fawcett (University of Vermont, Department of Plant Biology); Jeanine Velez, Carlos Santos Flores, Benjamin Van Ee and Eugenio Santiago (University of Puerto Rico); and Jose Sustache (PRDNER).

Appendix 7

## Financial report

| | | FY2016-2017<br>ACTUAL | FY2017-2018<br>ACTUAL | BALANCE |
|---|---|---|---|---|
| **REVENUES** | | | | |
| FEDERAL GRANTS | | $ 15,000.00 | $ - | $ 15,000.00 |
| **EXPENSES** | | | | |
| WAGES & SALARIES | | $ 5,314.75 | $ - | $ (5,314.75) |
| FICA TAX | | $ 304.89 | $ - | $ (304.89) |
| UNEMPLOYMENT COMP. | | $ 11.53 | $ - | $ (11.53) |
| WORKER COMP. | | $ 223.30 | $ - | $ (223.30) |
| HEALTH INSURANCE | | $ 658.03 | $ - | $ (658.03) |
| PENSION | | $ 84.47 | $ - | $ (84.47) |
| CONSULTANTS & CONTRACTORS | | $ 1,800.00 | $ - | $ (1,800.00) |
| PROGRAM SUPPLIES | | $ 100.00 | $ - | $ (100.00) |
| MAIL SERVICE | | $ 79.44 | $ - | $ (79.44) |
| AUTOMOBILE LEASE | | $ 511.49 | $ - | $ (511.49) |
| AUTO ALLOWANCE | | $ - | $ - | $ - |
| HOTEL & MEALS | | $ 666.83 | $ - | $ (666.83) |
| TRANSP/AIRFARE | | $ 469.60 | $ - | $ (469.60) |
| INDIRECT COSTS | | $ 1,365.00 | $ 3,410.67 | $ (4,775.67) |
| | | | | |
| **EXPENSES SUBTOTAL** | | $ 11,589.33 | $ 3,410.67 | $ - |