Ecology and Evolution of the Ferns of Moorea and Tahiti, French Polynesia

Ecology and Evolution of the Ferns of Moorea and Tahiti, French Polynesia

A dissertation presented

by

Joel Hamilton Nitta

to

The Department of Organismic and Evolutionary Biology

in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

in the subject of

Biology

Harvard University

Cambridge, Massachusetts

September 2016

Dissertation Advisor: Professor Charles C. Davis

Joel Hamilton Nitta

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Abstract

Ferns are the only major lineage of land plants with haploid (gametophyte) and diploid (sporophyte) stages that can grow separately from each other for extended periods. Gametophytes, as the sexual stage, are critical to fern evolution. However, the ecology of fern gametophytes is poorly known due to their small size and cryptic morphology. In this dissertation, I use the ferns of Moorea and Tahiti, French Polynesia to investigate the relative roles of sporophytes and gametophytes in community assembly and evolution.

In Chapter 1, I use DNA sequences to identify field-collected gametophytes to species and compare community diversity between fern sporophytes and gametophytes on Moorea and Tahiti. I find that phylogenetic community diversity decreases with elevation in sporophytes, but not gametophytes. I observe several species with gametophytes that are distributed beyond the range of conspecific sporophytes, and at least one species that may lack sporophytes on the islands completely. My results suggest that the transition from gametophyte to sporophyte functions as a filter restricting phylogenetic diversity of fern communities.

In Chapter 2, I use the filmy ferns (Hymenophyllaceae) of Moorea to investigate how changes in physiology between fern gametophytes and sporophytes are correlated with ecological niche. I find that the gametophytes of filmy ferns are not more stress-

tolerant than sporophytes. Rather, they are adapted for microhabitats that are buffered relative to those of sporophytes. I suggest that the gametophytes of filmy ferns rely on asexual reproduction via gemmae to achieve dense population sizes over a wide range.

In Chapter 3, I conduct a phylogenetic investigation of epiphytic traits in the ferns of Moorea. I find that epiphytes tend to have shorter stipes, smaller rhizomes, and gametophytes with non-cordate morphotypes relative to terrestrial species. I demonstrate that epiphytic communities are clustered phylogenetically and functionally relative to terrestrial communities, supporting a scenario of extreme environmental conditions in the forest canopy acting as a filter on epiphytic ferns.

In Chapter 4, I describe a new hybrid fern species, *Microsorum* × *tohieaense*, from Moorea based on morphological and molecular phylogenetic analysis, and discuss the significance of my findings to the taxonomy of microsoroid ferns.

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Acknowledgements

I have many, many people to thank for supporting me throughout my PhD. Without their help, none of this would be possible. I will try to include everybody, but I will probably forget some. If I do, I apologize, but I am truly grateful to every one of you! As a senior graduate student told me at the beginning of all of this, it's a marathon, not a sprint. I can't believe the finish line is in sight…

First, thanks to Chuck Davis for being an amazing advisor. You've done a great job of encouraging me to explore my scientific interests while providing direction exactly when I needed it. It is always refreshing to discuss ideas with you to get a "big picture" perspective when I'm focused on the nitty-gritty details. You've gone far beyond just being a scientific mentor though—I also deeply appreciate your understanding and support of my family situation. I don't think I could have made it this far without your complete understanding that family comes first, and the rest will follow. I've enjoyed seeing Ben and Leo grow up during the course of my PhD, and look to you and Angie as mentors in that sense too.

To my other committee members: thanks to Jonathan Losos for providing an important non-plant perspective on an otherwise all-plant committee. You have taught me to be rigorous and critical in my application of important evolutionary concepts that I might otherwise take for granted. I remember well that first day of orientation when you encouraged my cohort to set high goals for ourselves. I've taken that to heart, and will continue to aim high. Thanks Missy Holbrook for helping me think like a plant physiologist. Although I ultimately want to bridge plant physiology and evolution, my background coming into this was definitely as a plant systematist. I have enjoyed learning

to think about plants from a functional perspective, which crucially informs how I now think about plant evolution. I always enjoy coming to your lab and getting to interact with plants on a more personal level. Finally, thanks to Eddie Watkins for sharing your passion for ferns, and especially fern gametophytes, with me! The OTS Costa Rica fern course had a huge impact on my scientific interests and direction. You and Don Farrar opened my eyes to the world of fern gametophytes, and how little we understand about this supremely important part of fern biology. I also truly appreciate your willingness to talk through problems scientific and otherwise on Skype, cell phone, or text, from wherever you might be. Thank you for being such a wonderful friend and mentor.

Thanks to Pamela Diggle, Elena Kramer, John Wakeley, David Haig, Andrew Knoll, and all the other OEB faculty who I've had the pleasure to learn from during the course of my PhD. I am truly fortunate to be constantly exposed to amazing research that cuts across such a diverse range of organisms and temporal and spatial scales, but is all united by evolution.

Thanks to Brent Mishler and Alan Smith for kindling my interest in ferns during my undergraduate years at UC Berkeley. Brent, you taught me the tree-thinking that continues to form the basis of my understanding of biology today. Alan, thank you for always being so generous with your time and for your willingness to share your expertise. I take great comfort in knowing that UC is always a botanical home I can return to. Thanks to Patrick O'Grady for your patience in mentoring me when I was just getting started, and seeing our *Dicranomyia* project all the way through to publication. Thanks to Motomi Ito for mentoring me during my Master's. Thanks to Atushi Ebihara for introducing me to the ferns of Japan and being a wonderful colleague and collaborator.

Thanks to my other fern mentors and colleagues including: Robbin Moran, Kathleen Pryer, Michael Windham, Dave Barrington, Michael Sundue, Eric Schuettpelz, Alejandra Vasco, Carl Rothfels, Emily Sessa, Amanda Grusz, Fay-Wei Li, Erin Sigel, Li-Yaung Kuo, Cheng-Wei Chen, Jean-Yves Dubuisson, Susan Fawcett, Weston Testo, Sally Chambers, and Jerald Pinson. There are more I would like to mention but these are just some of the fine fern folks that I have had the pleasure of learning from and working with. I am truly fortunate to be part of such a close-knit, fun, and amazingly talented community of researchers.

Thanks to members of the Davis Lab, both official and honorary, for sharing great ideas and fun times together, including: Abagail Burrus, Barnabas Daru, Liming Cai, Goia Lyra, Rafaela Jorge, Emily Meineke, Jeffrey DaCosta, Rachel Engstrand, Keith Gaddis, Lachezar Nikolov, and Wenheng Zhang. Sarah Mathews, thank you for taking the time to discuss research ideas and providing feedback on grant proposals, as well as being a role model and mentor. Laura Lagomarsino, thanks for being my desk buddy and keeping me informed about goings-on in OEB. Tallen Xi, thanks for being the go-to informatics guru of the lab, and always willing to help with analyses. Charlie Willis, thanks for listening to and reading various drafts of papers and presentations, and giving me excellent feedback. Beth Forrestel, thanks for discussions and feedback on community phylogenetic methods. Daniel Park, thanks for help with niche modeling analysis and stimulating and wide-ranging discussions about community phylogeny. Kylee Peterson, thanks for discussions how best to get DNA out of herbarium specimens. Hanno Schaefer, thanks answering many questions as I first began to dig into my project.

Simone Cappellari, in addition to our scientific discussions, thanks for sharing delicious Brazilian food and excellent advice on raising a kid in Cambridge.

Thanks to Chris Dick and the rest of your lab at University of Michigan for being such gracious and welcoming hosts during my stay in Ann Arbor. Thanks to Jordan Bemmels for kindly teaching me the ways of RAD-seq and making me feel at home in your lab.

Thanks to the staff at the Harvard University Herbaria including Michaela Schmull, Anthony Brach, Emily Wood, and Kanchi Gandhi for fruitful discussions and help with processing and storing my numerous specimens. Thanks to David Boufford for helpful discussions about Marie's research as well as my own, and for memorable and delicious meals at your home. Thanks to Dave Barrington for teaching me how to properly annotate a type specimen and helping to reorganize a entire fern collection. It was fun.

Thanks to staff also at P, UC, US, PAP, and PTBG for generously sharing specimens for my research.

Thanks to Chris Preheim for letting me know about some really, really important details for my academic survival. The welfare and sanity of myself and many other OEB graduate students has benefited greatly from your expertise. Thanks to Anna Salvato, Jason Green, Krista Carmichael, Donna Gadbois, Jeannette Everritt, and Margaret Richards for helping me navigate the financial system at Harvard and successfully submit grant applications, especially the DDIG.

I was fortunate enough to receive funding from several sources that made this work possible, including: the National Science Foundation (Doctoral Dissertation

Improvement Grant DEB-1311169), Setup Funds from Harvard University, the American Society of Plant Taxonomists, the Garden Club of America, the Harvard University Herbaria, the Society of Systematic Biologists, and the Systematics Association.

Thanks to my OEB cohort and friends for all the fun times and nerdy discussions during six years at Harvard. I feel like this is coming to a close all too quickly. You guys are absolutely amazing, and I can't wait to see where your paths take you. I know I'm definitely going to forgot some people here, and I apologize for that, but there are some in particular whom I'd like to acknowledge. Thanks to Ambika Kamath for being an awesome scientist, writing eloquent pieces for scientific and lay audiences, and for not being afraid to take a stand in your writing. I had a lot of fun with our fern interview, and am honored to have it featured in the Hindu Business Line. Thanks Kara Feilich for sharing in the ups and downs of PhD life over coffee and baked goods (and for baking some especially delicious ones yourself!). Thanks Steve Klosterman for being a good friend and neighbor. Thanks to Seth Donoughe and Christina Baik for being the social hub of our group and arranging many fun events, especially murder-mystery dinners. Thanks Alexandra Brown for being your wonderful self and helping me and other graduate students with your awesome, completely voluntary R course. Thanks to Peter Wilton for taking me birding, helping me understand at least a little of theoretical population genetics, and for being a kind soul.

Thanks to members of Ecology Journal Club and Phylogenetics Journal Club including Jack Boyle, Kadeem Gilbert, Leonora Bittleston, Allison Schultz, Bruno de Medeiros, and Cat Adams for many fun and stimulating conversations.

Thanks to the Cambridge Running Club, Harvard University Triathlon Club, and Harvard University Cycling Association for providing fun, inspiration, and much-needed stress relief during my time here. Seeing my friends strive so hard towards their goals motivates me to do the same both in endurance sports and research.

Thanks to my colleagues and field support staff in French Polynesia. Ravahere Taputuarai, I seriously owe you for all the help you provided during my three field seasons on Moorea and Tahiti. In particular, thank you so much for checking on dataloggers while I wasn't there, and letting me stay at the "Gump Annex" while I did my fieldwork on Mt. Aorai. I hope you never get stuck with a desk job again! Jean-Yves Meyer, thank you very much for letting me participate in the fern survey on Mt. Aorai and sharing your extensive botanical knowledge. Jacques Florence, thank you very much for kindly sharing your unpublished manuscript of the upcoming Pteridophyte volume of the Flora of French Polynesia. I hope to have the pleasure of meeting you in person some day soon. Thanks to Jean-François Butaud for sharing your knowledge of the ferns of the Society Islands with me. Thanks to Teriitaria Tavaearii and Juliette Sing Soi for being such kind hosts and working so hard to conserve the unique and special ecosystem on Raiatea. Suzanne Vinette, thank you so much for being a wonderful field assistant. Your resourcefulness, enthusiasm, and knowledge of ferns have been extremely helpful. I truly value our friendship, and deeply appreciate all the things you taught me during our adventures together. Thanks to Paul for letting Suzanne go traipsing in the mountains with me! Tristan Wang and Saad Amer, thanks for being quick learners and good sports, and for teaching me how to be a better mentor. Tohei Theophilus, thanks for your excellent and rapid translation of my fern key into French and for being such a cheerful

part of our team. Thank you and your family for being such gracious and welcoming hosts during our stay. Thanks to David Hembry for sharing your field notes and extensive knowledge on doing fieldwork in French Polynesia. To the staff at the UC Berkeley Richard B. Gump South Pacific Research Station, Moorea, French Polynesia: Val Brotherson, thanks for helping me make arrangements for fieldwork. Irma You Sing, thanks for keeping the station clean all these years with a smile. Tony and Jacques, thanks for fixing anything around the station that needed fixing, and Tony for taking me on the most memorable fishing trip of my life! Hinano Teavai-Murphy, thank you for helping with permits and generously sharing your culture and knowledge. Frank Murphy and Neil Davies, thanks for keeping the Gump Station running to make research like this possible. Thanks to Mike Gil, Heather Hillard, Anya Brown, Sigfrido Zimmerman, Julie Zill, Lianne Jacobson, Sammy Davis, and Stella Swanson for being awesome Gump Station buddies for several successive field seasons. One of these days we seriously need to have a Moorea Biology Conference.

Thanks to Evelyne and David Lennette for graciously hosting me three times at your beautiful field site in Costa Rica. My time at Nectandra helped me to grow as a fern systematist and botanist, and provided me with an important Neotropical perspective on my work. Thank you for your patience while I have had to focus on my thesis chapters. I look forward to publishing on the ferns of Nectandra together soon!

Thanks most especially to my family for their understanding and loving support throughout the long road to the PhD. Thanks to my grandparents, Al and Ellen, and Neil and Elaine, for working so hard, and raising loving and caring families. My parents, John and Sarah, have been the best role models I could possibly ask for. I am so grateful that

you raised us to love the outdoors. I believe that is where my interest in science and the natural world ultimately comes from, and I am so glad that I have been able to pursue this interest that you instilled in me. Alex and Colin: thanks for being my best friends as well as my brothers. You are both so talented, and it gives me great joy to know that we are all three pursuing our dreams.

Thank you Chizuko for your support of Marie and me. Thank you especially for coming all the way from Japan to help with Kei, and for taking us in during the final push at the end of our dissertations.

Thank you most of all to Marie and Kei. Each of us being in graduate programs at different institutions has been a challenge, but I am so glad that we have seen it through together. I think it is a strength for both of us that we can provide unique and different perspectives on each other's research. Thank you for the many special and fun moments that we have shared throughout this journey. Kei: you mean everything to me, and you have given me new reason to try my best in all that I do. Thank you for that inspiration.

September 8, 2016

Cambridge, Mass.

for Kei

Chapter I:

Life Cycle Matters: DNA Barcoding Reveals Contrasting Community Structure Between Fern Sporophytes and Gametophytes

1.1 Abstract

Ferns are the only major lineage of vascular plants that have nutritionally independent sporophyte (diploid) and gametophyte (haploid) life stages. However, the implications of this unique life cycle for fern community ecology have rarely been considered. To compare patterns of community structure between fern sporophytes and gametophytes, we conducted a survey of the ferns of the islands of Moorea and Tahiti (French Polynesia). We first constructed a DNA barcode library (plastid *rbcL* and *trnH–psbA*) for the two island floras including 145 fern species. We then used these DNA barcodes to identify more than 1300 field-collected gametophytes from 25 plots spanning an elevational gradient from 200 to 2000 m. We found that species richness of fern sporophytes conforms to the well-known unimodal (i.e., mid-elevation peak) pattern, reaching a maximum at ca. 1000 – 1200 m. Moreover, we found that fern sporophyte communities become increasingly phylogenetically clustered at high elevations. In contrast, species richness of fern gametophytes was consistent across sites, and gametophytes showed no correlation of phylogenetic community structure with elevation. Turnover of sporophyte and gametophyte communities was closely linked with elevation at shallow phylogenetic levels, but not at deeper nodes in the tree. Finally, we found several species for which gametophytes had broader ranges than sporophytes, including a vittarioid fern with abundant gametophytes but extremely rare sporophytes. Our study highlights the importance of including diverse life history stages in surveys of community structure, and has implications for the possible impacts of climate change on the distribution of fern diversity.

1.2 Introduction

Ferns (monilophytes) are an ancient vascular plant lineage dating back ca. 300 million years that has diversified into an astonishing array of ecological niches (Schneider et al. 2004). Ferns occupy habitats ranging from xeric to aquatic, and their morphologies span moss-like filmy ferns only a few cm in height to tree ferns reaching over 10 m. What unites this amazing diversity is a unique life cycle: the alteration of morphologically distinct diploid sporophyte and haploid gametophyte generations that are nutritionally and physically independent from each other for the majority of the life cycle. This contrasts with the life cycles of nearly all other land plants—in both bryophytes and seed plants, the two halves of the life cycle are completely overlapping due to nutritional dependence, and do not constitute distinct ecological entities. This independence of generations has important implications for fern evolution. For instance, it is possible for fern gametophytes to have broader distributions than their conspecific sporophytes, since not every gametophyte necessarily produces a sporophyte (Farrar 1990, Watkins et al. 2007b). Indeed, some fern species have reached an extreme degree of separation in this regard and no longer produce sporophytes at all, rather persisting solely in the haploid state via asexual reproduction (Farrar 1967, Raine et al. 1991, Ebihara et al. 2008, Pinson and Schuettpelz 2016)

Despite the potential for fern sporophytes and gametophytes to occur over partially disjunct ranges, there are few comprehensive studies of the ranges of gametophytes and sporophytes of the same species (Farrar et al. 2008). This is due to the cryptic nature of the fern gametophyte: these tiny (typically < 1 cm) plants are easily overlooked in the field, and generally lack morphological characters for species-level

identification (Nayar and Kaur 1971). Thus, the vast majority of fern field surveys include only the sporophyte generation, leaving an important gap in our knowledge of fern distributions (e.g., Tuomisto and Poulsen 2000, Kessler 2001, Karst et al. 2005; but see Hamilton and Lloyd 1991, Watkins et al. 2007b). However, recently developed DNA-based identification techniques (i.e., "DNA barcoding") (Hebert et al. 2003) now allow for much more accurate identification of fern gametophytes, opening new avenues for investigations into their distribution, ecology, and evolution (Schneider and Schuettpelz 2006, Li et al. 2009, de Groot et al. 2011, Chen et al. 2013). Furthermore, the DNA sequences generated by such a study can also be used to infer phylogenetic trees, thus enabling a phylogenetically informed study of community ecology (Kress et al. 2009, Muscarella et al. 2014). Despite rapid advancements at the intersection of community ecology and phylogenetic studies, comparative phylogenetic methods are just beginning to be applied to fern ecology (Kluge and Kessler 2011, Hennequin et al. 2014, Lehtonen et al. 2015). However, there has been no community phylogenetic study of ferns to date investigating both gametophytes and sporophytes.

The goals of our study are to compare the ranges of fern gametophytes and sporophytes across a well-sampled island flora and infer processes of community assembly in ferns in a phylogenetic context. We first developed a DNA barcode system for identification of fern gametophytes by assembling a reference DNA library and verifying its species discrimination potential in the context of our study system (Moorea and Tahiti, French Polynesia). We then conducted community surveys of co-occurring fern gametophytes and sporophytes, and identified the gametophytes using our DNA barcode library. We investigated the following questions: 1.) Do elevational ranges differ

between fern sporophytes and gametophytes of the same species? 2.) Are there any patterns in phylogenetic community structure along the elevational gradient, and do they differ between life stages? 3.) What are the environmental factors that determine fern community composition and turnover, and do they differ between life stages?

1.3 Methods

Study Site—Moorea and Tahiti (17.5 – 18.0° S, 149 – 150° W) belong to the Society Islands, a tropical oceanic archipelago located more than 5000 km from the nearest continental landmass (Figure 1.1A). This distance acts as a strong barrier to dispersal (Carson and Clague 1995, Dassler and Farrar 2001), which combined with the young age of the islands (Moorea ca. 1.65 million years [Ma]; Tahiti ca. 0.65 Ma; Dymond, 1975), has led to a relatively small, yet phylogenetically diverse fern flora (ca. 165 spp.; Florence, in press; 8/11 orders sensu Smith et al. 2006). Although the two islands are only 17 km apart, Moorea is smaller (134 km^2) and reaches a maximum elevation of 1207 m, whereas Tahiti is much larger (1040 km^2) and has three peaks above 2000 m (Mt. Orohena at 2241 m, Mt. Pito Hiti at 2110 m, and Mt. Aorai at 2070 m). With the exception of a few endemic taxa, the flora of Moorea is generally a subset of the flora of Tahiti. The fern flora of Moorea has been the focus of several recent papers (Murdock and Smith 2003, Ranker et al. 2005, Nitta et al. 2011) and was surveyed as part of the Moorea Biocode Project (http://mooreabiocode.org). The ferns of French Polynesia will be described in the next volume of the flora of French Polynesia (Florence, in press). We used these sources to compile a list of all ferns occurring on Moorea and Tahiti.

Community survey—Fern community diversity was sampled at a total of 25 sites established every ca. 200 m along elevational gradients from ca. 200 to 1200 m (Moorea)

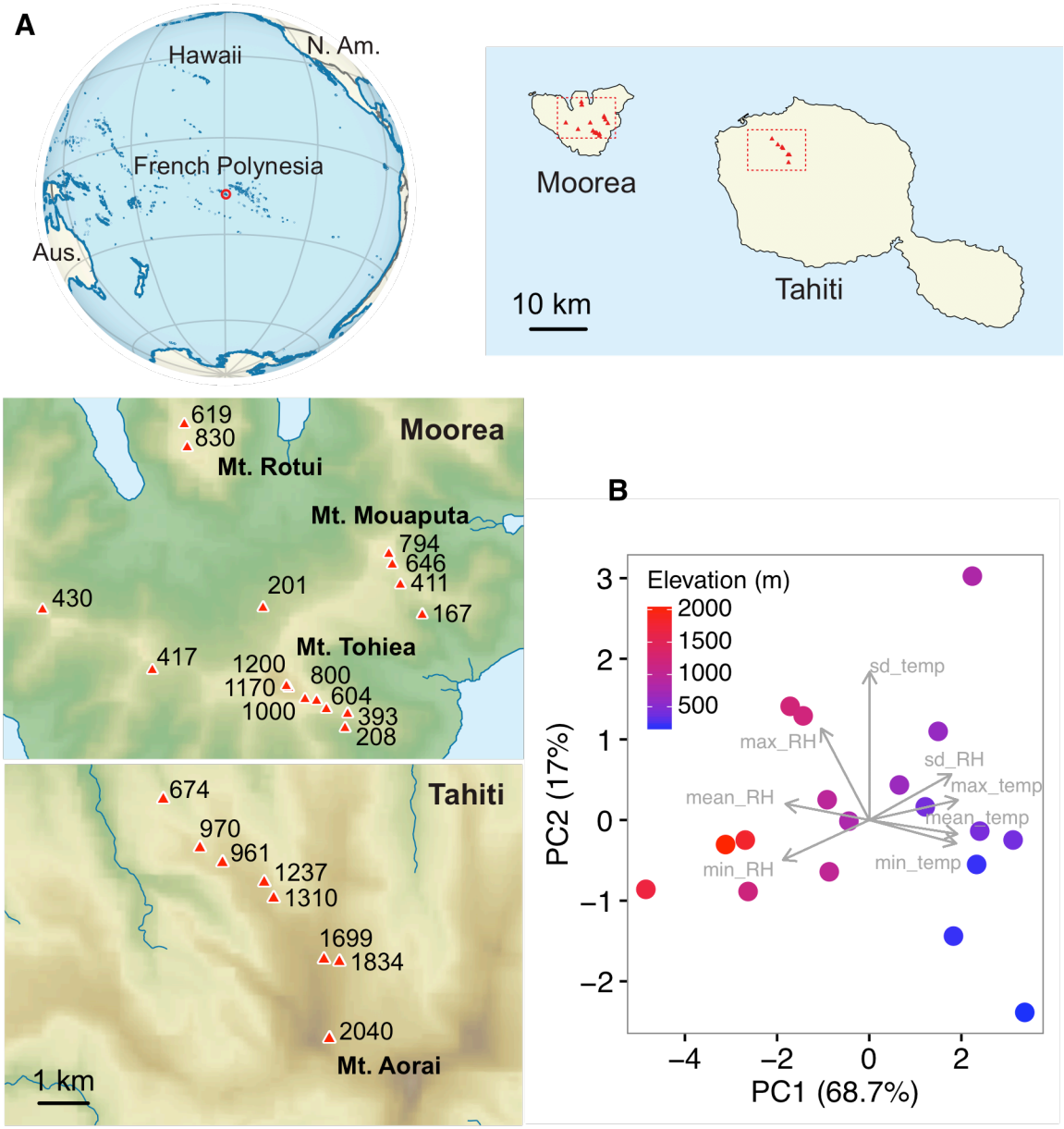

Figure 1.1. Location and environmental characteristics of study sites. (A) Maps of study area. Location of Tahiti and Moorea, French Polynesia on world map indicated by circle. Dashed boxes show locations of insets for Moorea and Tahiti (both to same scale). Study sites indicated by red triangles with elevation in m. Maps adapted from Wikimedia Commons under Creative Commons License.

Figure 1.1 (Continued). (B) Principal component analysis of climatic variables (means of daily minimum, mean, maximum, and standard deviation of temperature and relative humidity) from Nov. 12, 2013 to Jan. 17, 2014 for all sites with climatic data available (*N* = 18). Shading indicates elevation, from ca. 200 m (blue) to 2000 m (red).

and 600 to 2000 m (Tahiti) during the Austral winters (June – August) of 2012 – 2014. Moorea sites were located mainly on three mountains, each with a trail to the summit: Mt. Rotui (899 m), Mt. Mouaputa (880 m), and Mt. Tohiea (1206 m). Tahiti sites were established on the only mountain with reliable trail access to the peak, Mt. Aorai (2066 m). Two to three sites were established for each elevation point (e.g., ca. 200, 400, 600 m, etc.) to 1200 m. Above 1200 m, replication and placement of sites was restricted because of extremely steep terrain; thus, single sites were established at ca. 1300, 1700, 1800, and 2000 m (Figure 1.1A).

At each site, fern sporophytes (hereafter "sporophytes") were sampled in ca. 100 m^2 plots ("sporophyte plots"). In many cases, it was impossible to determine the exact number of sporophyte individuals because fronds growing close to each other may be either distinct individuals, or arise from a single underground rhizome. Furthermore, the complex, multilayered fern canopy including ca. 3 m tree ferns to tiny (<5 cm), colonial mat-forming filmy ferns also made assessing abundance by percent cover problematic. Therefore, we recorded presence/absence of each fern species in 2 × 2 m subplots. Plot design differed slightly between Moorea and Tahiti: on Moorea, each plot was 10 × 10 m and divided into a grid of 25 2 × 2 m subplots; on Tahiti, 24 2 × 2 m subplots were designated around the perimeter of a 14 × 14 m square. Presence/absence of species in

subplots were summed to produce an abundance ranking, where each species' abundance ranged from 0 (not observed in the plot) to 24 or 25 (observed in all subplots).

Sampling fern gametophyte (hereafter "gametophyte") diversity in the field is made difficult by two factors: most gametophytes are often tiny (typically < 1 cm^2) and difficult to locate, and cannot generally be identified to species based on morphology. We therefore conducted initial surveys to locate microsites harboring gametophytes within each sporophyte plot, then sampled terrestrial gametophytes in 50 × 50 cm subplots, each consisting of a grid of 10 × 10 cm squares (Ebihara et al. 2013). A single gametophyte, when present, was sampled from each square. In the case multiple gametophytes were present in a single square, the one closest to the center was sampled; clonal individuals occurring continuously across two or more squares were only sampled once. Epiphytic gametophytes were sampled by morphotype (at least three individuals per morphotype observed) at ca. 2 m height on trees within each sporophyte plot. The final "gametophyte plot" included all terrestrial and epiphytic gametophytes collected from a given site. We sought to collect a minimum of 50 individuals from each site by repeating this sampling procedure as necessary, but the actual number varied due to local conditions and time restrictions. To test the effect of sampling effort on species richness, we sampled two sites ("Three Pines 201 m" and "Mouaputa 646 m") each for ca. 100 individuals, and compared species accumulation and sampling completeness curves between sites at similar elevations (see *Species richness*). Photographs of fresh gametophytes were taken using a Pentax W60 camera mounted to a dissecting microscope. Each sampled gametophyte was cut in two; one half was kept as a herbarium voucher, and the other used for DNA extraction. DNA vouchers were kept in packets made from folded coffee

filters, which were then placed in sealed plastic bags with silica gel. Herbarium vouchers were fixed in FAA (50% ethanol:formaldehyde:acetic acid, 90:5:5), then transferred to 70% ethanol in 1.5 mL eppendorf tubes.

Environmental survey—Dataloggers were used to record ambient relative humidity (RH) and temperature once every 30 min. from October 12, 2013 to July 5, 2014 (Moorea) or once every hour from July 18, 2012 to February 6, 2015 (Tahiti) at 23 of the 25 sampling sites. Data were downloaded once every ca. 3 – 6 months. At each site, one datalogger was mounted at ca. 2 m on a tree trunk or pole. Dataloggers were protected with radiation shields to prevent heating due to direct solar radiation and buildup of moisture on the sensor tip. Datalogger and radiation shield models differed between Moorea and Tahiti: on Moorea, we used Hobo Pro v2 dataloggers with the RS3 Solar Radiation Shield (Onset Corporation, USA), whereas on Tahiti we used RHTemp 1000 dataloggers (MadgeTech, Warner, New Hampshire) protected with custom radiation shields made from plastic circuit boxes (Taputuarai et al. 2014). We calculated daily maximum, mean, minimum, and standard deviation for temperature and relative humidity from the raw data, then calculated the overall mean of each of these for each site. Days during which any of the dataloggers malfunctioned and failed to record were excluded from the analysis.

DNA sequencing—To produce the DNA barcode library, we sought to collect at least one individual (sporophyte phase) of each fern species from Moorea and Tahiti. Our final sampling included 99% of the known species diversity of Moorea (ca. 130 spp.) and 88% of the known species diversity of Tahiti (ca. 165 spp.). Some of the specimens from Moorea were collected as part of the Moorea Biocode Project (http://mooreabiocode.org).

Complete sampling of fern diversity at our site is made difficult by the complex topography of the islands, the rarity of some taxa, and the need for taxonomic revision in certain groups (e.g., Aspleniaceae, Hymenophyllaceae, Thelypteridaceae). However, the location of our transect on Tahiti (Mt. Aorai) has been historically well sampled, and we are confident that we have all the species known from that site, as well as nearly all of the species on Moorea, represented in our DNA barcode library. Leaf material was preserved on silica gel, and DNA extraction performed using the DNeasy Mini Kit (Qiagen, Valencia, CA) following the manufacturer's instructions or CTAB (Doyle and Doyle 1987). Polymerase chain reaction (PCR) amplification of chloroplast *rbcL* and the *trnH-psbA* intergenic spacer was performed using primers and thermocycler protocols of Schuettpelz and Pryer (2006) and Tate and Simpson (2003), respectively. Raw PCR products were sent to Genewiz Inc. (South Plainfield, NJ) for cleaning and sequencing. Forward and reverse PCR primers were used for sequencing, as well as internal primers ESRBCL654R and ESRBCL628F (Schuettpelz and Pryer 2006) for *rbcL*. ABI files were imported into Geneious v8 (Kearse et al. 2012), where they were assembled into contigs, which were then used to produce consensus sequences. All newly generated DNA sequences will be deposited in GenBank (Appendix A1).

DNA extraction and sequencing were performed similarly for gametophytes, except that the CTAB protocol was modified to 96-well plate format for high-throughput preparation and sequencing due to the large number (> 1500) of individuals (Beck et al. 2012). DNA extraction was not possible for some specimens that were lost or deemed too small to remove tissue and retain a morphological voucher. For gametophytes, forward primer ESRBCL1F and internal reverse primer ESRBCL654 were used, producing a ca.

650 bp amplicon (hereafter referred to as "*rbcL*-a"; Kress and Erickson, 2007). Only the forward primer was used for sequencing, and *trnH-psbA* was only sequenced in the case that *rbcL*-a could not be obtained (see *Species identification of gametophytes* below). ABI files were imported into Geneious and ends automatically trimmed with a 0.5% error cutoff. Due to the large number of redundant sequences, we do not deposit any gametophyte sequences in GenBank that were already represented in the DNA barcode library.

Phylogenetic analysis—Our goal here was to infer an ultrametric tree with branchlengths that are as accurate as possible to analyze phylogenetic diversity at our site and enable comparison across studies (Whitfeld et al. 2011). Therefore, rather than use only the taxon/gene sampling from Moorea and Tahiti, which would lack several clades and prevent the use of many available fossil calibration points for ferns, we first compiled a broad phylogenetic dataset including additional species and genes from GenBank. Indels are common in *trnH-psbA* and render alignment impossible at this broad phylogenetic level, so we excluded *trnH-psbA* from phylogenetic analysis. We used the PHLAWD pipeline (Smith et al. 2009) to obtain one sequence per species for all fern species (NCBI taxon "Monilioformopsis") in GenBank (release 210, October 2015) for *rbcL*, *atpA*, and *atpB*. We used our newly generated *rbcL* sequences for ferns from Moorea and Tahiti instead of the GenBank *rbcL* sequence for the same species whenever possible (144/145 spp.). Outgroup taxa were selected to represent major lycophyte and embryophyte lineages and were manually downloaded from GenBank. Each gene region was aligned separately using MAFFT (Katoh et al. 2002), then concatenated into a single Nexus alignment using Phyutility (Smith and Dunn 2008). We removed 196 sequences

that were exactly identical to others in the alignment prior to phylogenetic analysis. The final alignment (4778 bp) included 145 fern species from our study area, 3686 additional ferns, and nine outgroup species (lycophytes and seed plants; 3840 species total). We inferred phylogenetic trees using Maximum Likelihood (ML) as implemented in the parallel version of RAxML (Stamatakis 2006) using 16 threads (raxmlHPC-PTHREADS -T 16) run on the Odyssey cluster supported by the FAS Division of Science, Research Computing Group at Harvard University (http://rc.fas.harvard.edu). We first did a search using the GTR + G model of sequence evolution on 20 distinct maximum parsimony starting trees, and saved the one with the best likelihood (-m GTRGAMMA -N 20). We performed bootstrap analysis with 100 replicates under the same model, and wrote the output to the branches of the best-likelihood tree. We used treePL (Smith and O'Meara 2012) to infer molecular divergence time estimates on the best-likelihood tree after trimming the one of the outgroups (lycophytes) and 1059 other taxa identified by treePL as having extremely short branch lengths, which can interfere with divergence time estimation (Beaulieu and O'Meara 2016). We specified a fixed-age prior (i.e., minimum and maximum age the same) for the root (euphyllophytes; 411 Ma; Magallón et al. 2013), minimum age priors for three outgroup clades (seed plants, conifers, and angiosperms), and minimum age priors for 26 internal fossil calibration points from Pryer et al. (2004), Schuettpelz and Pryer (2009), and Magallón et al. (2013), and references therein (Table A1). We used random subsample and replicate cross-validation (RSRCV) over values from 0.00001 to 100 in 10-fold increments to identify the best rate smoothing parameter. We conducted an initial search with the "prime" option to identify additional optimal parameter settings, which we then used in the final analysis with the selected smoothing

value. After completion of dating analysis on the broad sampling dataset, we pruned all taxa outside of our study area using the "drop.tip" function of the "ape" package (Paradis et al. 2004) in R (R Core Team 2015) to obtain a final ultrametric tree of species from Moorea and Tahiti for use in community phylogenetic analysis.

Species identification of gametophytes—First, we tested the ability of *rbcL*-a and *trnH-psbA* to discriminate between fern species in Moorea and Tahiti by conducting local BLAST searches using the sporophyte DNA library (Ebihara et al. 2010). Separate barcode libraries for *rbcL*-a and *trnH-psbA* were constructed using the "makeblastdb" command in BLAST (Altschul et al. 1997). During the test, all *rbcL*-a and *trnH-psbA* accessions (one per accession per species) were queried against the library. Species that matched only to themselves 100% over the entire sequence length and no other accessions were considered to be successful identifications; those that matched themselves and one or more other species 100% were considered unsuccessful. We also quantified the variability of each barcode marker using smallest interspecific genetic distances (number of substitutions per site) (Meier et al. 2008, Srivathsan and Meier 2012). We found that *rbcL*-a alone could be reliably used as a barcode marker in this regional floristic context (139/145 species = 95% identification success). Therefore, we used *rbcL*-a as a primary barcode marker and *trnH-psbA* as a backup marker only in the case that *rbcL*-a could not be successfully sequenced.

After confirming the utility of these two barcode regions, sequences of field-collected gametophytes were exported from Geneious as a FASTA file and queried against the local BLAST database using the "blastn" search, retaining the top five matches (i.e., subject IDs). To maximize confident identification while accounting for

sequencing errors, we used a progressive match stringency test as follows (summarized in Table 1.1). First, any query failing to match at least a single subject ID at 99.0% similarity was counted as a failure. For queries matching at least one subject at ≥ 99.0%, the top two hits of each query were compared; in the case that the top subject ID matched the query ID at ≥ 99.0% similarity and the second best subject ID matched at < 99.0% similarity, the query was identified as the top subject ID. If the top two subject IDs both matched at ≥ 99.0% similarity, the next most stringent test was applied. During this test, if the top subject ID matched at ≥ 99.5% similarity and the second best subject ID matched at < 99.5% similarity, the query was identified as the top subject ID; if the top two subject IDs both matched at ≥ 99% similarity < 99.5%, the query was counted as a failure; if the top two subject IDs both matched at ≥ 99.5% similarity, the next most stringent test was applied in the same way at 99.7%, and finally at 99.9% similarity levels. If the top two subjects each matched at 100%, the query was counted as a failure.

We blasted unidentified sequences against the GenBank nr database to determine the cause of failure (e.g., query matches a fern species not in our reference database; query matches non-fern species thereby indicating contamination, etc.). We verified our putatively successful identifications with morphology: although fern gametophytes cannot typically be identified to species based on morphology alone, morphotypes (e.g., cordate, filamentous, and ribbon) are generally consistent within genera and/or other higher taxonomic groups (Nayar and Kaur 1971). We therefore compared observed morphotype with expected morphotype based on the barcode identification; individuals for which these did not match were excluded as possible cases of contamination.

Table 1.1 Criteria used for identifying field-collected fern gametophytes during BLAST search. The top two hits (subject 1 and subject 2) from a BLAST search querying an unknown gametophyte against the local barcode library are checked under conditions of increasing stringency (round 1 through round 5). Conditions (% identity between query and subject 1 or subject 2) resulting in either a successful ID (query is identified as subject 1) or failure to identify the query are given for each round. See 1.3 Methods for details.

Round	Subject 1	Subject 2	Result
1	< 99.0%	< 99.0%	Failure
	≥ 99.0%	< 99.0%	Subject 1
	≥ 99.0%	≥ 99.0%	Go to Round 2
2	≥ 99.0%, < 99.5%	≥ 99.0%, < 99.5%	Failure
	≥ 99.5%	< 99.5%	Subject 1
	≥ 99.5%	≥ 99.5%	Go to Round 3
3	≥ 99.5%, < 99.7%	≥ 99.5%, < 99.7%	Failure
	≥ 99.7%	< 99.7%	Subject 1
	≥ 99.7%	≥ 99.7%	Go to Round 4
4	≥ 99.7%, < 99.9%	≥ 99.7%, < 99.9%	Failure
	≥ 99.9%	< 99.9%	Subject 1
	≥ 99.9%	≥ 99.9%	Go to Round 5
5	≥ 99.9%	≥ 99.9%	Failure
	= 100%	≥ 99.9%, < 100%	Subject 1
	= 100%	= 100%	Failure

Species richness—The number of individuals and sampling protocol varied across sites and generations (sporophytes vs. gametophytes), which may influence observed species richness. We compared estimated species richness between sporophytes and gametophytes using first-order Hill numbers following Chao et al. (2014). We constructed individual-based species accumulation and sample coverage curves including bootstrap confidence intervals with a maximum sample size of 50 individuals using extrapolation / rarefaction in the "iNEXT" package (Chao et al. 2014) in R. For

gametophytes, raw individual counts were used to construct the curves; for sporophytes, the abundance ranking (number of subplots present out of 24 or 25) was used as a proxy for number of individuals.

Phylogenetic community structure—We characterized the phylogenetic community structure of co-occurring fern sporophytes and gametophytes at each site separately using two metrics. We measured Mean Nearest Taxon Distance (MNTD), which is the average phylogenetic distance between all pairs of sister taxa occurring in a plot, and Mean Phylogenetic Distance (MPD), which is the average phylogenetic distance between all possible pairwise combinations of taxa occurring in a plot (Webb 2000, Webb et al. 2002). High values of MPD or MNTD indicate phylogenetic overdispersion (i.e., greater phylogenetic diversity than expected under random community assembly), while low values indicate clustering (i.e., less phylogenetic diversity than expected under random community assembly). Mean phylogenetic distance reflects overall phylogenetic diversity, whereas MNTD is more sensitive to recent divergences. To assess statistical significance, we then compared these values to a null distribution of 1000 randomly simulated plots using the "ses.mpd" and "ses.mntd" functions in the "picante" package (Kembel et al. 2010) in R. We assume that ferns, which have tiny, light spores easily transported long distances by the wind, are not dispersal-limited at the scale of our study (< 50 km maximum distance between plots) (Tryon 1970). We therefore defined the regional pool as all fern species from Moorea and Tahiti in our phylogenetic tree (145 spp.), and selected the "phylogeny.pool" null model, which creates null communities by randomly drawing from this regional pool. We report the standard effect sizes (SES) of MPD and MNTD, which are equivalent to the negative values of the Net Relatedness

Index and Nearest Taxon Index of Webb (2000), respectively; we chose to keep the sign in the same direction between the observed value and standard effect size to make results easier to interpret. We measured the β-diversity analogs of MNTD and MPD between plots using the "comdist" and "comdistnt" functions in the R package "picante" (Kembel et al. 2010). We performed all analyses using presence/absence data only as well as weighted by relative abundances. To account for possible under-sampling of gametophyte communities that may drive differences between observed sporophyte and gametophyte community structure (see 1.5 Discussion), we ran analyses on three different datasets: the "full" dataset included all observed sporophytes and gametophytes (thus, it included some species that were only observed as sporophytes but not gametophytes and vice-versa); the "restricted" dataset included only species that were observed in each generation at least once across all plots (but not necessarily both in the same plot); the "simulated" dataset scored gametophytes as present if the sporophyte of that species was observed in the plot (even if the gametophyte was not actually observed; presence/absence only).

Correlation of phylogenetic community structure with environment—We calculated means of eight climatic variables for each site (minimum, mean, maximum, and standard deviation of daily temperature and daily relative humidity) from the raw datalogger output. We then log-transformed and scaled each variable to a mean of zero, and subjected them to a principle components analysis (PCA). We tested for spatial autocorrelation of climatic variables (PC axes 1 and 2) using Moran's *I* (Moran 1950) with the "Moran.I" function in the R package "ape." We conducted linear and second-order polynomial regression for gametophytes and sporophytes separately with species

richness, MPD, or MNTD as the response variable, and the first two environmental PC axes as explanatory variables for sites with environmental data available ($N = 18$), and with elevation as the single explanatory variable for all sites ($N = 25$). We compared models using the Akaike Information Criterion (Akaike 1973) to select the most likely model with the "dredge" function in the R package "MuMIn" (Bartoń 2011) for each combination of response and explanatory variables (Burnham and Anderson 2002).

We calculated turnover in community composition (i.e., β-diversity) using Bray-Curtis dissimilarities (species-level turnover), β-MNTD (phylogenetic turnover that is more sensitive to the tips of the tree), and β-MPD (phylogenetic turnover that is more sensitive to the overall tree) (Fine and Kembel 2011). We computed principal components of neighbor matrices (PCNM) using the latitude and longitude of each site (Borcard and Legendre 2002) using the function "pcnm." This produces a series of orthogonal eigenvectors describing spatial relationships between sites that can be used to analyze the spatial component of β-diversity (Legendre 2008). We used linear regression to partition variation (adjusted R^2) of each measure of β-diversity in turn as the response variable in relation to environmental PC1, PC2, and the PCNM eigenvectors as explanatory variables with the "varpart" function for sites with environmental data available ($N = 18$). We tested for significance with 1000 permutations using the "adonis" function. All variance partitioning was done using the R package "Vegan" (Oksanen et al. 2015).

1.4 Results

Environmental survey—Of the 23 dataloggers deployed, three failed to record for a portion of the survey time, and four failed completely. This is likely due to the

extremely high humidity at many of the sites that may cause electronic devices to fail during extended use. After excluding days that were missing data, the climatic dataset included 67 days of data (Nov. 12, 2013 – Jan. 17, 2014) for 16 sites. To obtain a maximally sampled dataset, data that were available for these days from the next year (2014 – 2015) were used in place of missing data for the Tahiti 1834 m and 2040 m sites (N = 18 sites in the final dataset). There is a linear decrease in mean daily temperature with increasing elevation (0.0052 °C per m; linear model, adjusted R^2 0.98, $P < 1.2 \times 10^{-14}$). The relationship between relative humidity (RH) and elevation is slightly different: RH increases and standard deviation of RH decreases from ca. 200 m to 1200 m; above 1200 m, the relationship reverses, and the air becomes drier with increasing elevation (Figure A1). The first two PC axes explained ca. 86% of the variation in environmental variables (minimum, mean, and maximum temperature and RH, and standard deviation of temperature and RH; Figure 1.1B). PC1 is positively correlated with minimum, mean, and maximum daily temperature and standard deviation of RH, and negatively correlated with minimum and mean daily RH; PC2 is positively correlated with standard deviation of temperature. PC1, but not PC2, is correlated with spatial distance ($P < 1 \times 10^{-6}$ and P = 0.48 respectively, Moran's I).

Evaluation of DNA barcode loci—*trnH-psbA* showed higher rates of interspecific variation than *rbcL*-a (Figure A2), but both performed similarly well in discriminatory power. In our BLAST test of *rbcL*-a and *trnH-psbA* as barcode markers, only six species were found that could not be successfully identified, i.e., matched 100% with another species, for one or both markers. These included *Asplenium gibberosum* (Forst.) Mett. and *A. shuttleworthianum* Kunze (identical *rbcL*-a and *trnH-psbA*), *Microsorum* ×

maximum (Brack.) Copel. and *M. grossum* (Langsd. & Fisch.) S.B. Andrews (identical *rbcL*-a and *trnH-psbA*), and *Prosaptia subnuda* (Mett. ex Kuhn) Copel. and *P. contigua* (G. Forst.) C. Presl (identical *rbcL*-a; identical *trnH-psbA* except for a 5 bp indel). *Asplenium shuttleworthianum* is an octoploid that may have *A. gibberosum* as a parent (Perrie and Brownsey 2005, Shepherd et al. 2008). The status of *M.* × *maximum* as a hybrid between *M. commutatum* (Blume) Copel. and *M. grossum* had been hypothesized on the basis of morphology but not previously tested with molecular data (Copeland 1932, Murdock and Smith 2003). This is the first evidence to our knowledge that the maternal parent of *M.* × *maximum* is *M. grossum*. *Prosaptia contigua* and *P. subnuda* are not known to be hybrids, and can be distinguished by location of sori (marginal on laminar protrusions in the former, on the abaxial laminar surface in the latter); it is possible that these are recently derived sister taxa. We treated each of the three species pairs with identical sequences as a single species for phylogenetic community analysis; since these are either hybrids or very closely related species, it is unlikely this treatment impacted phylogenetic diversity results. Five and four additional taxa pairs (15 species total) were > 99.5% similar for *rbcL*-a and *trnH-psbA*, respectively (Table 1.2). Given that estimates of error rates for Sanger sequences range from 0.001% to 1% (Hoff 2009), we manually inspected any matches that were between 99.5 – 99.9% to verify if the different bases appeared to be due to sequence error (e.g., low read quality at an unexpected site) or interspecific variation (e.g., high read quality at a base pair known to differ between species).

DNA barcode identification of fern gametophytes—A total of 1667 fern gametophytes was collected from all sites (25 sites; mean 60.26 ± 17.34 per site,

Table 1.2 Species >99.5% similar at barcode markers. *Prosaptia contigua* and *P. subnuda* differ in *trnH-psbA* by a single 5 bp indel, which is excluded from the calculation of genetic distance.

	Species 1	Species 2	Genetic distance (%)
rbcL-a	*Asplenium gibberosum*	*Asplenium shuttleworthianum*	0
	Microsorum grossum	*Microsorum* × *maximum*	0
	Prosaptia contigua	*Prosaptia subnuda*	0
	Nephrolepis biserrata	*Nephrolepis hirsutula*	0.18
	Hypolepis dicksonioides	*Hypolepis tenuifolia*	0.33
	Asplenium australasicum	*Asplenium nidus*	0.35
	Plesioneuron attenuatum	*Plesioneuron* sp1	0.36
	Hypolepis dicksonioides	*Hypolepis* sp1	0.49
trnH-psbA	*Asplenium gibberosum*	*Asplenium shuttleworthianum*	0
	Microsorum grossum	*Microsorum* × *maximum*	0
	Prosaptia contigua	*Prosaptia subnuda*	0
	Macrothelypteris polypodioides	*Macrothelypteris torresiana*	0.22
	Plesioneuron attenuatum	*Plesioneuron* sp1	0.23
	Asplenium affine	*Asplenium robustum*	0.24
	Humata anderssonii	*Humata pectinata*	0.39

excluding Three Pines 201 m and Mouaputa 646 m sites, which received extra sampling), of which 1632 were used for DNA extraction (summarized in Table 1.3). We were able to successfully obtain at least one of the two barcode markers (*rbcL*-a and *trnH-psbA*) from 1526 individuals, and identify 1323 individuals to species using our reference library. The small size of many gametophytes (< 0.01 mg) made DNA extraction difficult, and likely contributed to PCR failure. Thirty-nine individuals had apparently successful PCR (bands visible on an agarose gel), but could not be identified to fern species due to insufficient sequence quality. Fifty-two individuals blasted to species pairs in our reference library that were indistinguishable using *rbcL*-a, the majority of which were *Prosaptia contigua* / *Prosaptia subnuda*. Contamination by a non-fern species was

Table 1.3 Gametophyte DNA barcoding results. *: Out of 1667 individuals collected. †: Out of 1632 individuals extracted. ‡: Out of 1526 individuals with successful PCR. 1: Based on morphology, 23 are thought to be true bryophytes that were collected by mistake, and the rest contaminations. 2: Possibly independent gametophytes (see 1.5 Discussion); not including *Vaginularia paradoxa*, which was treated as a successful ID.

			No. individuals	%
Successes	DNA extracted	Total	1632	97.90*
	Successful PCR	*rbcL*-a only	1379	84.50†
		trnH-psbA only	110	6.74†
		Both	37	2.27†
		Total at least one sequence	1526	93.50†
	Successful ID	*rbcL*-a only	1225	80.28‡
		trnH-psbA only	86	5.64‡
		Both	12	0.79‡
		Total at least one sequence	1323	86.70‡
Failures	DNA extraction failed	Total	35	2.10*
	PCR failed	Total	106	6.50†
	Non-fern contamination	Bryophyte[1]	35	2.29‡
		Algae	3	0.20‡
		Angiosperm	8	0.52‡
	Fern sequences	Single sequence matches multiple fern species > 99%	52	3.41‡
		Multiple sequences from same indiviudal match multiple fern species > 99%	5	0.33‡
		Morphology doesn't match sequence	28	1.83‡
		Low quality sequence	39	2.56‡
		High quality sequence but no match in database[2]	30	1.97‡
		Pseudogene	3	0.20‡

detected in 46 individuals; contaminants included bryophytes (35 individuals), algae (3 individuals), and seed plants (8 individuals). Upon careful inspection of morphology, it

appears that 23 of the bryophyte sequences were indeed bryophytes collected by mistake. A smaller number of samples appeared to be contaminated by other fern species, as indicated by observed morphology that didn't match morphology predicted by the DNA barcode identification (28 individuals) or multiple sequences from the same individual that matched to different fern species (5 individuals). Finally, we recovered sequences from six fern species that were of high quality but were not represented in our reference library. These included three vittarioid ferns (*Antrophyum* sp., 14 individuals; *Haplopteris* sp., 2 individuals; and *Vaginularia paradoxa* (Fée) Miq. = *Monogramma paradoxa* (Fée) Bedd., 121 individuals), two bird's nest ferns (*Asplenium* sp1, 2 individuals and *Asplenium* sp2, 1 individual), and one species closely related to *Asplenium caudatum* G. Forst. (*Asplenium* sp3, 10 individuals). Although sporophytes of *V. paradoxa* from Moorea and Tahiti were not included in our reference library, we were able to identify these gametophytes to species by matching *rbcL* to an accession of *V. paradoxa* from Samoa on GenBank (accession EU024562; W.A. Sledge 1631 (L); 100% match except for a 297 bp region of missing data in the GenBank sequence).

Phylogenetic analysis—The phylogeny using ML analysis (Figure A3) was largely in agreement with previous findings (Pryer et al. 2001, 2004, Schuettpelz and Pryer 2006), and was much better resolved than a phylogeny based solely on *rbcL* of species from our study area (results not shown). We did not recover a monophyletic Lomariopsidaceae *sensu* Smith et al. (2006), and instead observed *Lomariopsis* sister to *Nephrolepis* + Tectariaceae + Davalliaceae + Oleandraceae + Polypodiaceae with 95% ML bootstrap support (Figure A3). This topology has also been recovered in other recent phylogenies (Kuo et al. 2011, Lehtonen 2011). Our divergence times estimated by treePL

Table 1.4 Comparison of sporophyte and gametophyte elevational ranges by family (families not shown for species with sporophyte range ≥ gametophyte range). The minimum observed elevation was subtracted from the maximum observed elevation to determine total elevational range of sporophytes and gametophytes separately for each species. Range sizes were compared on the basis of total elevational range, lowest elevation, highest elevation, occurrence of gametophyte without sporophyte in a given survey plot, and occurrence of gametophyte without a sporophyte match in the reference database.

Table 1.4 (Continued)

	Family	No. species	%
Sporophyte range ≥ gametophyte range	Total	88	70.97
Gametophyte range > sporophyte range	Hymenophyllaceae	4	3.23
Gametophyte lower range < sporophyte lower range	Aspleniaceae	1	0.81
	Athyriaceae	1	0.81
	Dryopteridaceae	1	0.81
	Hymenophyllaceae	8	6.45
	Polypodiaceae	2	1.61
	Pteridaceae	1	0.81
	Total	14	11.29
Gametophyte upper range > sporophyte upper range	Dennstaedtiaceae	1	0.81
	Dryopteridaceae	1	0.81
	Hymenophyllaceae	9	7.26
	Lomariopsidaceae	1	0.81
	Marattiaceae	1	0.81
	Polypodiaceae	2	1.61
	Pteridaceae	2	1.61
	Tectariaceae	1	0.81
	Total	18	14.52
Gametophyte without sporophyte in plot	Aspleniaceae	1	0.81
	Athyriaceae	2	1.61
	Dennstaedtiaceae	1	0.81
	Dryopteridaceae	3	2.42
	Hymenophyllaceae	13	10.48
	Lomariopsidaceae	1	0.81
	Marattiaceae	2	1.61
	Polypodiaceae	5	4.03
	Pteridaceae	4	3.23
	Tectariaceae	1	0.81
	Thelypteridaceae	1	0.81
	Total	34	27.42
Gametophyte without sporophyte in database	Aspleniaceae	3	2.42
	Pteridaceae	3	2.42
	Total	6	4.84

were somewhat earlier than those of Schuettpelz and Pryer (2009); for example, we found that the polygrammoid lineage began diversifying ca. 90 Ma, whereas Schuettpelz and Pryer (2009) estimate ca. 55.8 Ma (Figure A4, Table A2). There is still no consensus in the literature regarding relationships between the four earliest diverging fern lineages (marattioid ferns, ophioglossoids + whisk ferns, horsetails, and leptosporangiate ferns); our analysis recovered ophioglossoids + whisk ferns(marattioids(horsetails (leptosporangiates))), but with low support.

α-Diversity—In total, 122 species of ferns were observed across the gradient, out of ca. 165 known to occur in Tahiti and Moorea (Florence, in press). Species richness differed between sporophytes and gametophytes: 116 species were observed in the sporophyte phase across all sites (mean 23.5 ± 6.9 spp. per site), whereas only 73 species were observed in the gametophyte phase across all sites (mean 11.0 ± 2.8 spp. per site). Sixty-seven species were observed as both sporophytes and gametophytes (Figure 1.2). Sporophytes showed a mid-elevation peak in species richness at ca. 1000 – 1200 m (mean 28.2 ± 8.1 spp. per site at 1000 – 1200 m sites), with progressively less diverse communities above and below this altitude. However, no such mid-elevation peak was observed in gametophyte communities, which tended to be ca. 10 spp. per site across the gradient, except for the highest site with 18 spp. (Figures 1.2, 1.3, A5). The sites selected for intensive sampling of gametophytes (Three Pines 201 m and Mouaputa 646 m) had slightly higher observed richness than the other sites at the same elevation, but estimated sampling completeness was similar for all sites above ca. 50 individuals (Figure 1.4 A – D). Comparison of the elevational ranges of the sporophytes and gametophytes of each species revealed that most (ca. 71%) species had sporophyte ranges that equaled or

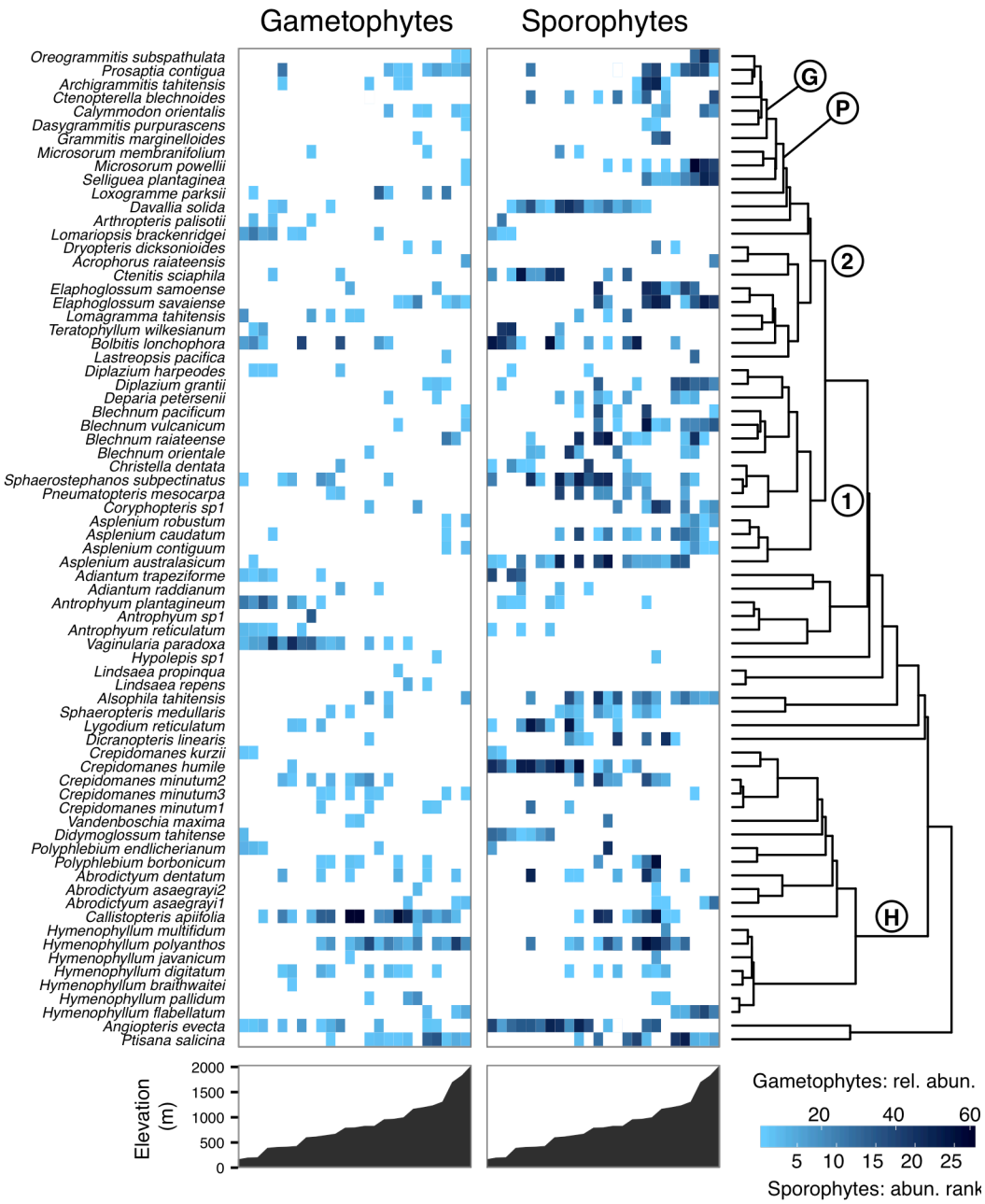

Figure 1.2. Abundance of fern species by life stage (gametophytes, left; sporophytes, right) along an elevational gradient from ca. 200 m to 2000 m on the islands of Moorea and Tahiti, French Polynesia. Each column represents one plot, with elevation shown by graph on bottom. Species not observed in a given plot in white; darker colors indicate greater abundance.

Figure 1.2 (Continued). Note different units for abundance of gametophytes and sporophytes. Phylogenetic tree inferred using maximum likelihood in RAxML. Letters in circles indicate major clades: H, Hymenophyllaceae; 1, Eupolypods I; 2, Eupolypods II; P, Polypodiaceae; G, grammitid ferns. Restricted dataset including only species that were observed in both generations at least once across all plots, except for *Vaginularia pardoxa* and *Antrophyum sp1*, which were only observed as gametophytes.

exceeded that of conspecific gametophytes (Table 1.4). In ca. 11 – 15% of species, the range of gametophytes exceeded either the upper or lower elevational limit of sporophytes. Thirty-four species (27%) were observed growing in a plot that lacked conspecific sporophytes, and six species (5%) were observed for which a sporophyte match is completely lacking in our database. Hymenophyllaceae accounted for a disproportionately large number of species with gametophytes occurring beyond the range of sporophytes (Table 1.4, Figure 1.2).

Neither collection curves for sporophytes nor gametophytes reached an asymptote, indicating that increasing the number of sites may recover additional species; however, the slope of the curve was steeper for sporophytes than gametophytes (Figure 1.4E). Gametophyte communities tended to have a more skewed abundance distribution than sporophytes, and were often dominated by one or a few species: lower elevation sites (ca. 200 – 400 m) were dominated by vittarioid ferns (*Antrophyum plantagineum* (Cav.) Kaulf. and *Vaginularia paradoxa*), whereas mid- to upper elevation sites were dominated by filmy ferns (Hymenophyllaceae), in particular *Callistopteris apiifolia*

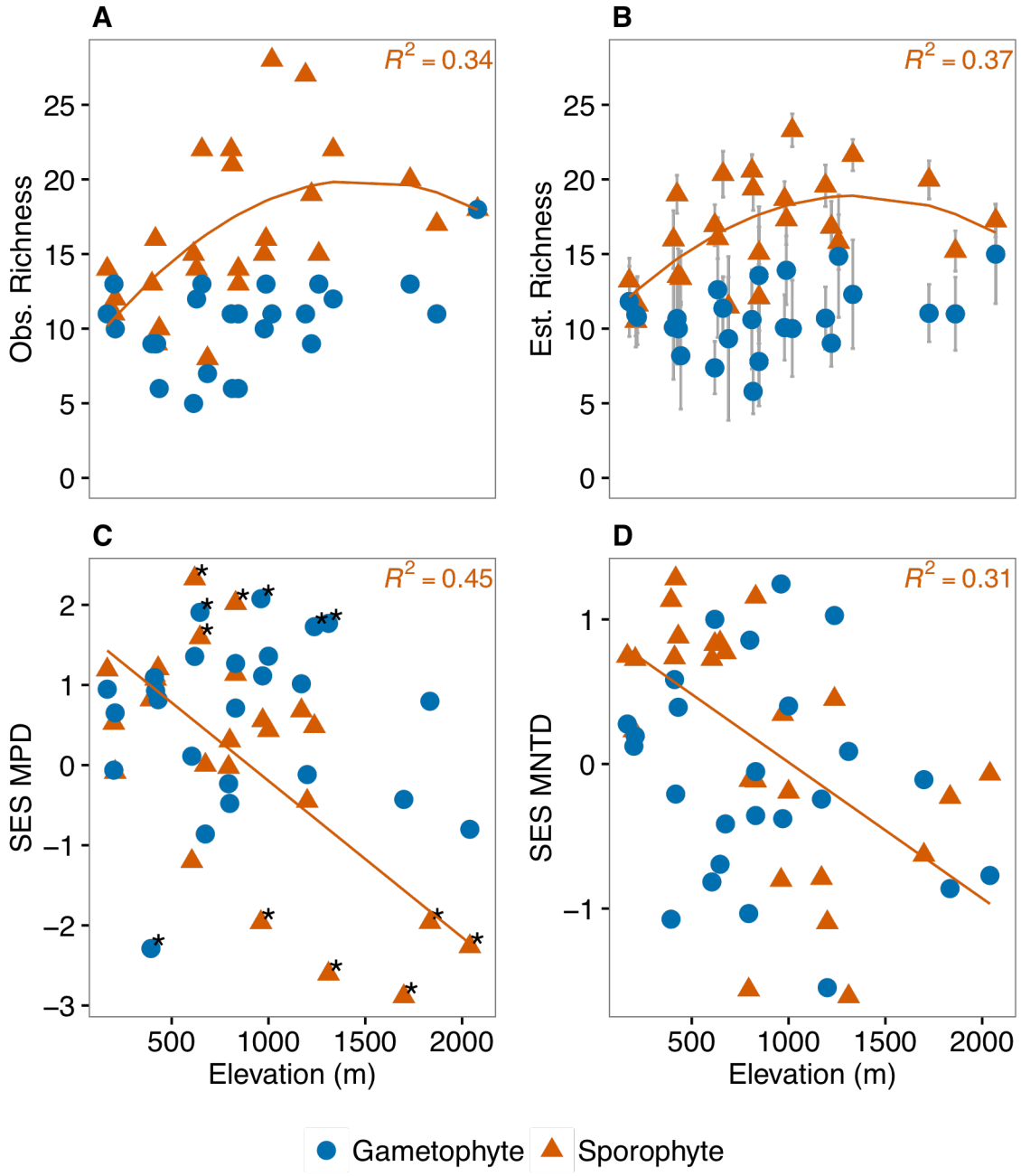

Figure. 1.3. Species richness and phylogenetic structure of fern gametophyte (blue circles) and sporophyte (red triangles) communities along an elevational gradient from ca. 200 m to 2000 m on the islands of Moorea and Tahiti, French Polynesia. **(A)** Observed species richness (restricted dataset).

Figure 1.3 (Continued). (B) Estimated species richness (zero-order Hill numbers); error bars are bootstrap 95% confidence intervals (estimated from full dataset). **(C)** Standard effect size (SES) of non-abundance weighted mean phylogenetic distance (MPD) (restricted dataset). **(D)** Standard effect size of non-abundance weighted mean nearest taxon distance (MNTD) (restricted dataset). Trendlines indicate significant ($P < 0.05$) relationship between richness or phylogenetic community structure and elevation; linear or second-order polynomial regression selected using Akaike Information Criterion. For (C) and (D), values greater than zero indicate phylogenetic overdispersion; values less than zero indicate phylogenetic clustering; asterisks indicate communities with significantly different phylogenetic structure from 999 randomly assembled null communities ($P < 0.05$, two-sided test). For results including other datasets and abundance weightings, see Appendix A.

(Presl) Copel. (ca. 600 – 1200 m) and *Hymenophyllum polyanthos* (Sw.) Sw. (ca. 1800 – 2000 m; Figure A8).

For phylogenetic community structure, we present results from the most conservative analysis (non-abundance weighted analysis of the restricted dataset), and refer the reader to Appendix A for alternate analyses/datasets. There was no significant difference in mean phylogenetic community structure (MPD or MNTD) between sporophytes and gametophytes overall (paired *t*-test, $P = 0.2007$ and 0.2794, respectively), and only a small number of communities showed phylogenetic structure that was significantly more clustered or overdispersed relative to the null model (Figures 1.3, A5). However, the trend between phylogenetic community structure and elevation differed between the two generations: we found that MPD and MNTD tend to become

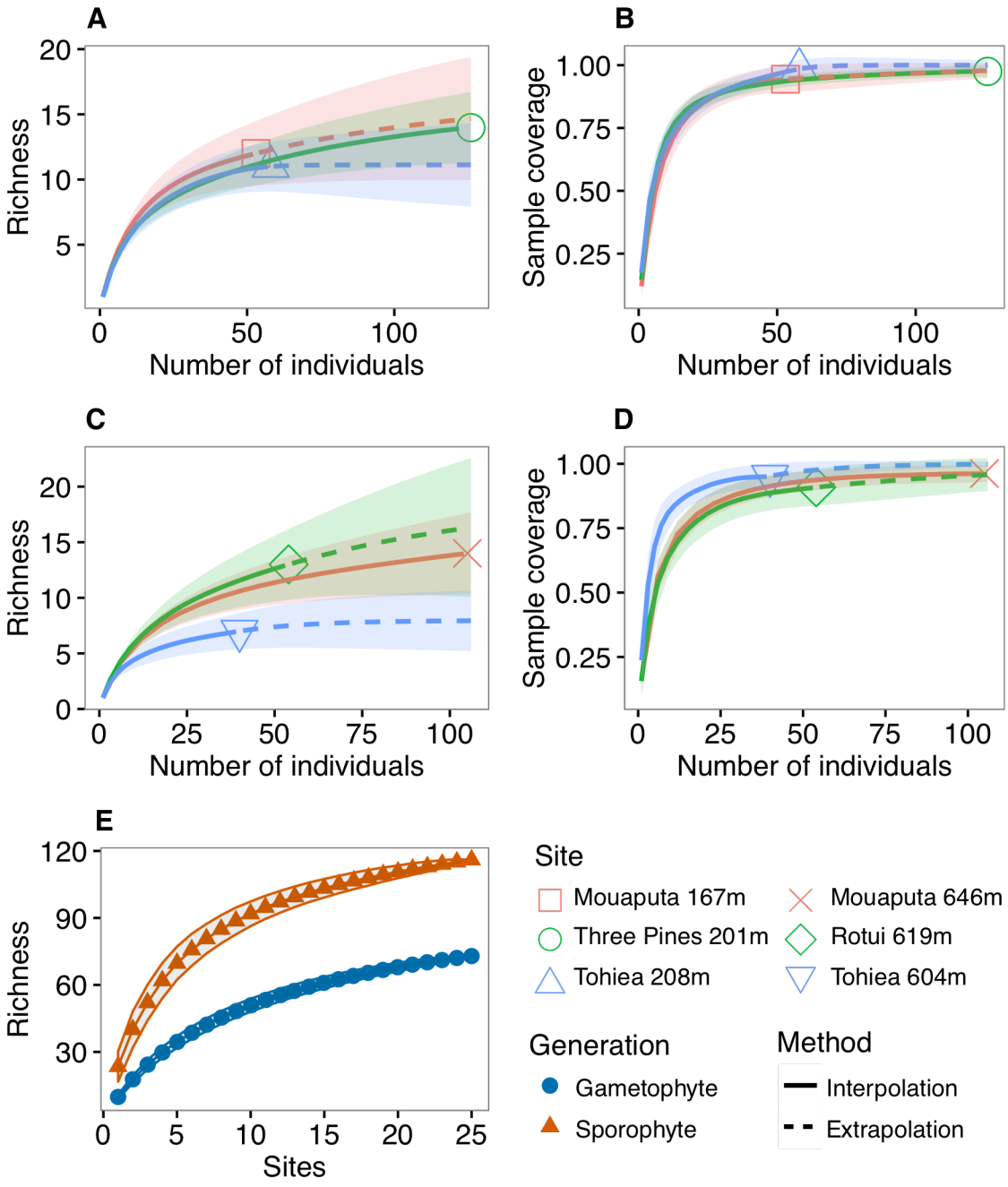

Figure 1.4. Collection curves. (A, C) Individual-based rarefaction/extrapolation curves of fern gametophytes. **(B, D)** Individual-based sample completeness curves of fern gametophytes. Symbols indicate observed values; solid lines are rarefied values; dashed lines are extrapolated values; bands indicate ± one standard error (s.e.).

Figure 1.4 (Continued). Panels divided by elevation; (A) and (B) are for ca. 200 m plots; (C) and (D) are for ca. 600 m plots. "Three Pines 201 m" and "Mouaputa 646 m" plots each received ca. twice the normal collection effort (ca. 100 individuals instead of 50). (E) Site-based collection curve for fern sporophytes and gametophytes across all plots. Band indicates ± one s.e.

more negative with increasing elevation in sporophytes, indicating that sporophyte communities become more phylogenetically clustered at high elevations. In contrast, phylogenetic community structure of gametophytes showed no correlation with elevation in most analyses (Figures 1.3, A5), and a positive correlation only in the abundance-weighted MPD analysis of the full dataset (Figure A5). For sporophytes, increased abundance of species-rich clades such as grammitid ferns at high elevations contributed to clustering; for gametophytes, the widespread occurrence of early-diverging filmy ferns contributed to overdispersion (Figure 1.2). The negative correlation between phylogenetic community structure and elevation in sporophytes was robust to dataset or abundance weighting for MPD, but not significant across all datasets for MNTD (Figure A5). Furthermore, for MPD, those sporophyte plots that were significantly overdispersed were at lower elevations, and those that were significantly clustered were at higher elevations (Figures 1.3, A5). Similarly, estimated richness, MPD, and MNTD were correlated with environmental PC1 for sporophytes, but relationships between community structure and environment was weak (MNTD, estimated richness) or non-existent (MPD, observed richness) for gametophytes (Table 1.5, Table A3).

***β-diversity*—**Sporophyte and gametophyte communities both show strong turnover with elevation (indicated by gradual change from red, or high elevation, to

Table 1.5. Second-order polynomial and linear models of fern community composition (mean phylogenetic distance [MPD], mean nearest-taxon distance [MNTD], observed species richness, and estimated species richness) in relation to environment (axes 1 and 2 of environmental PCA) for all sites with environmental data available (*N* = 18). Both linear and squared forms of each environmental PC axis were included in the global model, but we only show those that were found to be significant in at least one model. G = gametophyte, S = sporophyte. Significance of coefficients indicated by asterisks: * = $P < 0.05$; ** = $P < 0.005$; NS = not significant.

	Generation	Adjusted R^2	PC1	$PC2^2$
MPD	G	0		
	S	0.44	0.3**	
MNTD	G	0.47	0.11*	-0.14*
	S	0.52	0.23**	
Obs. Richness	G	0		
	S	0.18	-1.25 (NS)	
Est. Richness	G	0.2		0.44 (NS)
	S	0.28	-0.72*	

blue, or low elevation, across the NMDS plot from left to right) at the species (Bray-Curtis) and shallow phylogenetic (MNTD) level, but not at deeper phylogenetic levels (MPD) (Figure 1.5). Observed sporophyte and gametophyte communities differed strongly in species composition, largely due to the fact that many species were only observed growing as sporophytes. In the full dataset and the abundance-weighted analysis of the restricted dataset, sporophyte and gametophyte communities occupy distinct, non-overlapping portions of the Bray-Curtis NMDS plot (Figure A6). In the non-abundance weighted analysis of the restricted dataset, sporophyte and gametophyte communities overlap in the Bray-Curtis plot, but occupy partially non-overlapping areas in the MPD and MNTD plots (Figure 1.5).

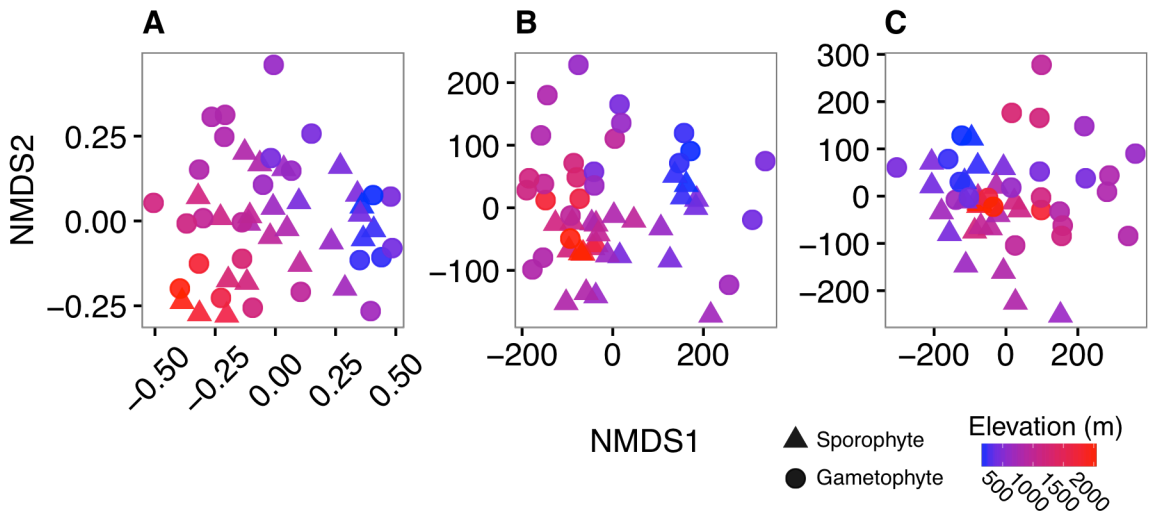

Figure 1.5. Non-metric multidimensional scaling (NMDS) of distances between fern gametophyte (circles) and sporophyte (triangles) plots. (A) Bray-Curtis (i.e., species-level) dissimilarities. **(B)** Mean nearest taxon (MNTD) distances. **(C)** Mean phylogenetic distance (MPD) distances. Non-abundance weighted analysis of restricted dataset. For results including other datasets and abundance weightings, see Appendix A.

The amount of variance in β-diversity explained by environmental and spatial components varied for different measures of β-diversity (Bray-Curtis, MNTD, and MPD) and generation (gametophyte vs. sporophyte; Figures 1.6, A7). Very little of the variation in MPD could be explained by environment or space, whereas 25 – 50% of variation in Bray-Curtis and MNTD distances were attributed to environment, with environmental PC1 much greater than PC2. Spatial variables did not account for a significant portion of variance in β-diversity. Sporophytes were slightly more structured by environmental PC1 than gametophytes.

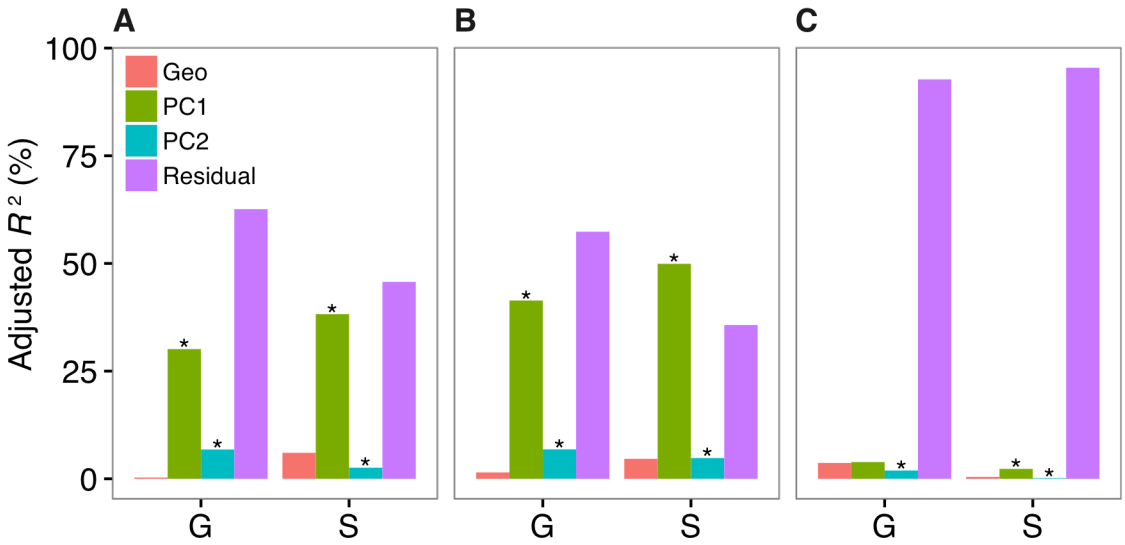

Figure 1.6. Variance partitioning of environmental and spatial components that explain turnover in fern community composition (adjusted R^2) by generation (sporophyte, "S" or gametophyte, "G"). Spatial components ("Geo") are eigenvectors produced by principal components of nearest neighbor matrices of latitude and longitude of each fern community; environmental components are the first two principal components axes ("PC1" and "PC2") of eight temperature and humidity metrics. β-diversity metrics include **(A)** Bray-Curtis (i.e., species-level) dissimilarities, **(B)** mean nearest taxon (MNTD) distances, and **(C)** mean phylogenetic distance (MPD) distances. Asterisk indicates significance at $P < 0.05$ (tested with 999 permutations using pseudo-F ratios). Non-abundance weighted analysis of restricted dataset. For results including other datasets and abundance weightings, see Appendix A.

1.5 Discussion

Our study is the first to our knowledge to investigate processes of fern community assembly at a regional scale that includes both fern gametophytes and sporophytes

identified to species. Investigations of gametophyte ecology prior to the advent of DNA sequencing were limited in scope due to insufficient ability to identify gametophytes to species, either relying on a few species with diagnostic characters, extrapolating from nearby juvenile sporophytes, or growing out sporophytes in the lab (Cousens et al. 1988, Peck et al. 1990, Watkins et al. 2007b). Recently, DNA barcoding has been increasingly applied to identify field-collected fern gametophytes, and has revealed that gametophytes do indeed occur outside of the range of sporophytes in some species (Ebihara et al. 2010, de Groot et al. 2011, Chen et al. 2013). Our study builds on these efforts by using DNA barcoding to compare patterns of community composition between fern gametophytes and sporophytes. We acknowledge that there are likely some sampling biases in our gametophyte survey due to the cryptic nature of this life stage, but we have accounted for potential artifacts whenever possible (see *Challenges of sampling a cryptic life stage* below)**.**

DNA barcoding and independent gametophytes—DNA barcoding has been the focus of interest in ecology recently because of its potential to vastly accelerate the rate of taxonomic identification in ecological surveys, especially in cases where morphological identification to species is extremely difficult (e.g., tropical insects; Gibson et al. 2014) or impossible (e.g., microorganisms, gut contents; Valentini et al. 2009). Although *rbcL*-a alone would probably not be sufficient to distinguish between closely related fern species in a global study, we found that it could distinguish between nearly all species in our study system (139/145 species = 95% identification success). This is likely because the ferns of the Society Islands contain few endemic sister species that evolved *in situ*, and rather are composed mostly of species that immigrated to the

islands independently (cf. Hennequin et al. 2014), as has been shown for several angiosperm groups (Hembry and Balukjian 2016). The few cases of failure were mostly due to hybrid taxa that could not be differentiated from their progenitors on the basis of chloroplast sequences alone. For future studies, in the case that finer distinction was required, we recommend adding a low-copy nuclear marker such as *gapCp* (Schuettpelz et al. 2008). In a similar study of the pteridophyte flora of Japan, *rbcL*-a performed slightly worse (89% success rate for sexual diploids), probably due to the fact that the Japanese fern flora includes several endemic radiations for which discriminating close relatives is difficult (Ebihara et al. 2010). We found that *trnH-psbA* had similar discriminatory power as *rbcL*, and slightly higher rates of interspecific variation overall in the context of our local flora (Figure A2). However, *trnH-psbA* has been criticized as a barcode marker in ferns due to its low rates of variation in some lineages (e.g., core leptosporangiates; Li et al. 2011). Other fern barcoding studies have used different intergenic regions, such as *trnL-F*, with success (de Groot et al. 2011, Chen et al. 2013); this could be a useful marker in future community-level studies of fern gametophytes.

Once we established the utility of our DNA barcoding library, we identified fern gametophytes to species, and compared distribution patterns between sporophytes and gametophytes. We found that for the majority (71%) of species, fern gametophytes and sporophytes did not differ in their elevational range (i.e., gametophytes were not observed growing beyond the elevational range of sporophytes; Table 1.4). Despite this, there were several cases where gametophytes were observed growing without their conspecific sporophytes in a given plot, and a few cases where gametophytes were much more widespread than conspecific sporophytes (Table 1.4). Filmy ferns

(Hymenophyllaceae) accounted for a large portion of species with gametophytes that exceeded the elevational range of sporophytes; other groups that tended to have widespread gametophytes included vittarioid ferns (Pteridaceae) and Polypodiaceae. All of these species have non-cordiform gametophytes, which is consistent with the results of Ebihara et al. (2013), who found that non-cordiform gametophytes tended to have ranges beyond sporophytes in Japan. One particularly abundant hymenophylloid gametophyte, *Callistopteris apiifolia*, exceeded the upper range of the sporophyte by 634 m and the lower range by 445 m; a similar situation has been documented in this species on Iriomote Island, Japan (Ebihara et al. 2013), as well in the closely related *Callistopteris baldwinii* (D.C. Eaton) Copel. on Hawaii (Dassler and Farrar 1997). In a more extreme case of spatial separation of life stages, the vittarioid fern *Vaginularia paradoxa* had some of the most abundant gametophytes observed in our survey (121 individuals from 14 sites), but was not observed as a sporophyte in any of our plots; indeed, sporophytes of this species are extremely rare on Tahiti (only ten collections known; the most recent in 1982) and possibly no longer occur on Moorea (only three collections known; all from ca. 1850). Furthermore, we identified gametophytes of two additional vittarioid species (*Haplopteris* sp. and *Antrophyum* sp.) and three species of *Asplenium* that were not represented in our reference library. These must either have very rare sporophytes (as in the case of *V. paradoxa*) that we failed to sample, or are "independent gametophytes," i.e., gametophyte populations that persist via asexual reproduction without producing sporophytes (Farrar et al. 2008). Distinguishing between these two scenarios requires additional, thorough sampling of closely related sporophyte-producing ferns from possible source areas. The highest such sampling priorities include rare species known

from Tahiti for which we were unable to obtain tissue samples, such as *Antrophyum subfalcatum* Brackenridge (Florence, in press), which may be a match for our *Antrophyum* sp. Similarly, *Haplopteris ensiformis* (Sw.) E. H. Crane is known from the Austral, Cook, and Pitcairn Islands (but not Tahiti; Florence, in press), and could be a match for our *Haplopteris* sp. Independent gametophytes are known from vittarioid ferns in North America (*Vittaria appalachiana*; Farrar and Mickel 1991) and Taiwan (Chen et al. 2013, Kuo et al. 2014), but have not yet been reported from the tropical Pacific to our knowledge. As with other epiphytic fern lineages, vittarioid ferns are most diverse in the tropics, and we may expect additional widespread or truly "independent" gametophytes to be found in these ferns as DNA barcoding is applied to other tropical fern floras.

Fern community structure differs between life stages—Despite the fact that gametophytes and sporophytes of most fern species occupy similar elevational ranges (Table 1.4), we detected different species composition between gametophyte and sporophyte communities (Bray-Curtis distances). This was clearest in the full dataset, which includes all observed sporophytes and gametophytes, and quantitatively similar in the abundance-weighted analysis of the restricted dataset, which includes only species observed at least once in both phases (Figure A6). This indicates that the contrast in community composition between gametophytes and sporophytes is not simply an artifact driven by "missing species" that went unsampled during our gametophyte survey (see *Challenges of sampling a cryptic life stage*). It is likely driven by gametophytes that occur without sporophytes, as well as changes in abundance between gametophytes and sporophytes. Thus, it appears that the ability of fern gametophytes to grow independently of sporophytes does indeed impact community-level diversity.

We found that the relationship between community structure (α-diversity) and elevation varied across life stages in ferns at our site: although fern gametophyte communities show no correlation in phylogenetic community structure with elevation, fern sporophyte communities become more phylogenetically clustered at high elevations, and this clustering is stronger for deep phylogenetic structure (MPD) than sister-level relationships (MNTD) (Figures 1.3, A5). This contrasts with the results of Kluge and Kessler (2011), who found no overall trend in MPD or MNTD of fern sporophyte communities along an elevational gradient from 100 to 3400 m in Costa Rica. However, Kluge and Kessler (2011) used the number of nodes separating species in a taxonomy-based tree as a proxy for phylogenetic distance rather than branch lengths derived from a time-calibrated phylogeny, which may have diminished power to detect non-random phylogenetic structure (Kress et al. 2009). Lehtonen et al. (2015) detected a negative correlation between MPD and soil fertility (i.e., rich soils tended to have increased phylogenetic clustering) in tropical lowland fern communities using a similar tree-building approach as applied here (pruning from a globally sampled molecular phylogeny), supporting our observation that fern community structure changes along environmental gradients, at least in sporophytes.

Furthermore, we observed the well-known mid-elevation peak in species richness (Colwell et al. 2004) for sporophytes, but not gametophytes (Figure 1.3A, B). A meta-analysis of 20 fern elevational transects found that a hump-shaped distribution of species richness is one of the most common patterns of fern diversity on mountains, especially in the tropics (Kessler et al. 2011); however, our study is the first to our knowledge to include gametophytes in an elevational fern transect survey. It is possible that including

the gametophyte phase may also reveal different patterns from those observed in sporophytes in other transect studies. Pouteau et al. (2016) analyzed the same Tahiti sporophyte plot data as well as island-wide diversity of fern sporophytes on Tahiti, and found that climate seems to explain the observed richness peak better than the mid-domain effect (i.e., species richness peak due to randomly distributed elevational ranges within a bounded space; Colwell & Lees, 2000).

Assuming that the peak in species richness at mid-elevations is due to optimal environmental conditions and not the mid-domain effect, the negative trend of phylogenetic diversity with elevation in sporophytes that we observed may be due to differences in the evolutionary history of traits involved in filtering at either end of the gradient (Cavender-Bares et al. 2004). This would be expected if sporophytic cold-tolerance traits (relevant at higher, cooler elevations) are evolutionarily conserved, whereas sporophytic drought-tolerance traits (relevant at lower elevations with greater vapor pressure deficit) are evolutionarily labile. Gametophytes, which potentially have wider physiological tolerances than sporophytes, may be able to exist over a wider range of habitats, but only produce sporophytes in a portion of that range (Watkins et al. 2007a, Pittermann et al. 2013). Alternatively, gametophytes may have highly specific niche requirements, but are better able to exploit widely distributed, buffered microsites (e.g., crevices in rock and bark within the boundary layer) that are not available to sporophytes due to their larger size (Dassler and Farrar 1997). The importance of microsite availability for establishment of fern gametophytes has been supported by studies in the temperate zones (Cousens et al. 1988, Peck et al. 1990, Flinn 2007) and the tropics (Watkins et al. 2007b). Either scenario is consistent with the pattern of neutral

phylogenetic community structure we observed in gametophytes relative to sporophytes. Results of desiccation tolerance experiments in one species at our site with particularly widespread gametophytes, *Callistopteris apiifolia*, suggest that both life stages of this species are sensitive to desiccation, and that its widespread gametophytes may be exploiting buffered microhabitats rather than relying on broader physiological tolerances (Nitta et al., in prep.). We are unaware of any other studies that have tested for differences in phylogenetic community structure between gametophytes and sporophytes in ferns, but studies on ontogenic shifts in community phylogenetic diversity in tropical trees have found greater clustering in adult trees relative to seedlings, consistent with a scenario of environmental filtering acting over the course of development (Webb et al. 2006, Jin et al. 2015). Common garden experiments and tests of relevant physiological parameters on additional gametophyte-sporophyte pairs will help to provide insight into the relative roles of microhabitat tracking vs. physiological niche differences between life stages in ferns.

We found that β-diversity is more strongly correlated with elevation at shallower phylogenetic levels (species level and sister taxon level) and weakly correlated at deeper phylogenetic levels (MPD) (Figures 1.5, A6). This indicates that similar lineages occur throughout the elevational gradient, but within each lineage there is turnover of closely related species with elevation, and suggests that the ecological niche of ferns is at least somewhat labile (variation within lineage but not between closely related species pairs). Comparative phylogenetic analyses of ecologically relevant traits in fern gametophytes and sporophytes are needed to determine the degree of niche conservatism in specific traits. The lack of turnover explained by distance (Figures 1.6, A7) is unsurprising, given

that our plots were located along a steep elevational gradient across small distances (median distance between plots 6.1 km, maximum distance between plots 40.3 km). Combined with the presumably high dispersal ability of ferns (Tryon 1970), it likely that differences observed in β-diversity are driven by niche preferences rather than dispersal limitation. Although sporophyte and gametophyte communities both showed turnover with elevation, they occupied different portions of some of the NMDS plots, particularly MNTD and abundance-weighted Bray-Curtis (Figures 1.5, A6). Furthermore, variance in β-diversity in sporophytes and gametophytes was partitioned similarly into spatial and environmental components (Figure 1.6). Taken together, these results suggest that fern sporophyte and gametophyte communities at a given site may differ in composition, but that they change in similar ways with elevation.

Challenges of sampling a cryptic life stage—One important caveat needs to be mentioned when interpreting the results of this study. We found that total fern sporophyte species richness was higher than that of gametophytes (116 sporophyte spp. vs. 73 gametophyte spp.; $N = 25$ plots). This is counterintuitive, given that each sporophyte individual had to have been produced by a gametophyte in the past (except in the case of asexual sporophyte reproduction, which is only known in a few species from our study area). Thus, the sporophyte species richness should in theory represent minimum gametophyte richness, which could possibly be higher due to species that occur as gametophytes but do not produce sporophytes. There are several possible reasons why we did not observe this at our site. One explanation is that we did not sample across all seasons. Our surveys were conducted during the Austral winter (dry season), from June to August. It is possible that only a subset of species were sporulating and germinating as

gametophytes at this time. A second explanation is that the lifespan of sporophytes vs. gametophytes can differ greatly. Although long-term demographic data for tropical fern species are few, fern sporophytes tend to be perennial, whereas fern gametophytes of most species have short life spans (< 1 yr; but epiphytic gametophytes can be long-lived) (Watkins et al. 2007b, Farrar et al. 2008). Thus, the sporophytes at a particular plot represent all species that successfully recruited there over a multiple year window, whereas the gametophytes growing at a given time point may change throughout the year. Finally, a third explanation is that our collection efforts were insufficient. It is relatively simple for an experienced botanist to visually check for presence of all fern sporophytes or seed plant species in a plot; however, this is impossible for fern gametophytes, as they generally cannot be identified to species by morphology (with the exception of a few species that have unique characters or character combinations). Gametophyte individuals must be collected and sequenced in order to determine species, which is time-consuming. Due to the limited amount of time available for fieldwork and the scope of this study that includes many plots over an elevational gradient, we could not sample each site exhaustively for all gametophyte individuals, and had to use a semi-randomized sampling approach. Furthermore, many plots were dominated by one or a few very abundant species, with the rest relatively rare (Figure A8). This made random collection of rare species very difficult. Increasing sampling intensity by a factor of two (ca. 100 individuals instead of 50) at the Three Pines 201 m and Mouaputa 646 m site resulted in an increase of species richness by 2 – 3 spp. relative to other sites at similar elevations; however, collection curves and sampling coverage were similar between sites regardless of sampling intensity, indicating that our target of 50 individuals per plot was sufficient

(Figure 1.4 A – D). In a similar study comparing fern gametophyte and sporophyte composition carried out over a full year with exhaustive sampling of gametophytes every two months at a single plot in Taiwan, higher species richness was indeed found for gametophytes relative to sporophytes (Kuo et al. 2014).

Despite this caveat, we feel our dataset can reveal patterns in β-diversity that are impossible to address in an exhaustive study confined to a single (or a few) sites such as Kuo et al. (2014). By sampling consistently during the Austral winter over three field seasons, we avoided confounding the effects of seasonality and elevation on species richness. Although rare species do contribute to community structure, we have captured the ecologically dominant species in each plot. Finally, we verified observed patterns whenever applicable with both a restricted dataset including only those species that were observed both as sporophytes and gametophytes ($N = 66$ spp.), as well as a simulated dataset that considered "missing" species observed as sporophytes but not gametophytes to be present in the gametophyte phase, thus controlling for the effect of under-sampling of gametophytes.

Conclusion—DNA barcoding of ferns is an efficient tool not only for identification of fern gametophytes, but also to produce DNA sequence data that can be used to infer phylogenetic trees and allow phylogenetically informed ecological analysis. Because of the local nature of our study, even a single marker provided enough resolution to distinguish species in 95% of cases. We found that, just as fern sporophytes and gametophytes may have partially non-overlapping ranges on a species basis, sporophyte and gametophyte communities also differ in species composition and phylogenetic structure. On Moorea and Tahiti, fern sporophytes become more phylogenetically

clustered at higher elevations, but fern gametophytes do not follow this trend. Above ca. 1200 m, the air became increasingly cool and dry, which may act as a filter to invasion by tropical species that evolved at lower, warmer elevations. Thus, our results are consistent with the hypothesis that fern sporophytes are more subject to environmental filtering than fern gametophytes, which have greater amplitude of ecological tolerances. It has been suggested that the ability of gametophytes to explore new habitats beyond the range of sporophytes may enable ferns to hedge against the impacts of climate change, especially in species with long-lived gametophytes (Farrar 2016). It should be possible to gain greater insight into the mechanisms structuring fern communities and how these will be affected by climate change by coupling community diversity surveys with field and greenhouse experiments on the physiological tolerances of fern sporophytes and gametophytes.

1.6 Acknowledgements

We are grateful to members of the Davis Lab, N. Michele Holbrook, Jonathan B. Losos, Brent D. Mishler, Alan R. Smith, James E. Watkins, Jr., Campbell O. Webb, and Charles G. Willis for helpful discussion and comments on drafts. Saad Amer, Tohei Theophilus, Suzanne Vinette, and Tristan Wang assisted with fieldwork. The “Moveclim” (Montane Vegetation as Listening Posts for Climate Change) biodiversity project funded by the Net-Biome research program for the Outermost Territories and Countries of Europe (www.netbiome.org) and by the Agence National de la Recherche (France) supported the survey on Mt. Aorai. Jacques Florence kindly shared his unpublished manuscript of the upcoming pteridophyte volume of the Flora of French Polynesia. Alan R. Smith helped with species identification of fern sporophytes. Staff at the University of California

Berkeley's Richard B. Gump South Pacific Research Station, Moorea, French Polynesia, in particular Valentine Brotherson, Neil Davies, Hinano Teavai-Murphy, and Frank Murphy provided logistical support for fieldwork. Funding provided in part by National Science Foundation (Doctoral Dissertation Improvement Grant DEB-1311169 to JHN and CCD), Setup Funds from Harvard University to CCD, the American Society of Plant Taxonomists (Research Grant for Graduate Students to JHN), the Garden Club of America (Award in Tropical Botany to JHN), the Harvard University Herbaria (Fernald Fieldwork Fellowship to JHN), the Society of Systematic Biologists (Graduate Student Research Award to JHN), and the Systematics Association (Systematics Research Fund to JHN).

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Chapter II:

Intergenerational Niche Differentiation in Filmy Ferns (Hymenophyllaceae)

2.1 Abstract

Desiccation tolerance was a key trait allowing plants to colonize land. However, it is rare among extant tracheophytes, and little is known about the transition from desiccation tolerant non-vascular plants to desiccation sensitive vascular ones. Filmy ferns (Hymenophyllaceae) present a unique system to study how water-stress strategies differ between non-vascular and vascular stages within a single organism, as they have vascularized sporophytes and nonvascular gametophytes that are each capable of varying degrees of desiccation tolerance. We surveyed sporophytes and gametophytes of 22 species of filmy ferns along an elevational gradient from ca. 200 to 1200 m on the island of Moorea (French Polynesia). We used chlorophyll fluorescence to measure light responses and desiccation tolerance. We sequenced plastid *rbcL* and *trnHpsbA* to identify field-collected gametophytes, and conducted phylogenetically informed analyses to identify differences in physiology between life stages and growth habits. Epiphytes were more tolerant of desiccation than terrestrial species. For most species, gametophytes had similar or less desiccation tolerance and lower photosynthetic optima than sporophytes. Despite the lack of broader physiological tolerances in gametophytes relative to sporophytes, gametophytes of several species occurred over a wider elevational range than conspecific sporophytes. It is likely that filmy fern gametophytes are exploiting protected microhabitats rather than greater physiological tolerance to occur beyond the range of sporophytes. Our results show that filmy fern gametophytes and sporophytes differ in their physiology and niche requirements, and point to the importance of microhabitat in shaping the evolution of water-use strategies in vascular plants.

2.2 Introduction

The movement of plants from water to land was one of the most important evolutionary events in the history of the earth, fundamentally altering the global carbon and water cycle and setting the stage for modern terrestrial ecosystems (Graham 1993, Kenrick and Crane 1997). The transition from aquatic to terrestrial growth was complex, requiring the evolution of a suite of adaptations to survive in dry habitats bombarded with radiation (Waters 2003). One particularly important early adaptation that enabled plants to move onto land was desiccation tolerance (DT), the ability for an organism to lose water until it reaches equilibrium with atmospheric humidity, and recover upon rewetting (Mishler and Churchill 1985).

Compared with the ubiquitous DT among the earliest land plants, the occurrence of DT varies across extant land plant lineages and life stages. Many bryophytes have retained vegetative DT in the gametophyte stage (Oliver et al., 2004 and references therein). In extant vascular plants (tracheophytes), DT occurs in certain non-vegetative stages (e.g., seeds and pollen), but has largely been lost in vegetative tissues (with some notable exceptions, the so-called “resurrection plants”). Instead, tracheophytes avoid desiccation of vegetative tissues with adaptations including a waxy cuticle, stomata, and an extensive vascular system. Correlated with these differences in functional strategies to cope with water stress is a major difference in life cycle: in most vascular plants (including all seed plants) the long-lived, dominant phase of the life cycle is the sporophyte (diploid phase), whereas in bryophytes it is the gametophyte (haploid phase). Little is known about the details of the transition from desiccation tolerance to sensitivity, nor how this took place in conjunction with the switch from a gametophyte to a

sporophyte-dominated life cycle; yet, these two steps underpin the radiation of plants onto dry land. Studies of bryophytes have greatly advanced our understanding of the evolution and ecology of DT. However, bryophytes are distantly related to seed plants and have a number of unique functional attributes. Thus, comparisons between extant seed plants and bryophytes can only provide limited insight in our quest to understand the role of DT in the evolution of land plants.

Ferns represent an important, understudied group of tracheophytes for investigating the transition from desiccation tolerance to desiccation avoidance in land plants. Ferns are the only major lineage among land plants with sporophytes and gametophytes that are capable of growing independently from each other. These two life stages differ drastically in their morphology and physiology—fern sporophytes have true vascular tissue and regulate their internal water content (i.e., they are homoiohydric), whereas fern gametophytes are much smaller (often < 1 cm), lack vascular tissue, and allow their internal water content to reach equilibrium with atmospheric humidity (i.e., they are poikilohydric). Recent studies indicate that the gametophytes of many fern species are desiccation tolerant, while sporophytes of the same species are thought to lack DT (Watkins et al. 2007, Pittermann et al. 2013). Thus, ferns present a unique opportunity to observe the transition from desiccation tolerance to desiccation avoidance *across* independently growing life phases *within* a single organism.

Filmy ferns (Hymenophyllaceae) represent a particularly interesting case for studying the ecological and evolutionary significance of vegetative DT across life stages in ferns. Filmy ferns are a large family (ca. 600 spp.) of primarily tropical, leptosporangiate ferns named for their remarkably thin leaf laminae, which are typically

Figure 2.1. Examples of filmy fern diversity from French Polynesia. A – **D** are sporophytes; **E** – **H** are gametophytes. **A)** *Crepidomanes humile*, a low elevation epiphyte. *Nitta 3392* (GH). **B)** *Hymenophyllum multifidum*, a high elevation epiphyte growing in association with leafy liverworts and mosses. *Nitta 1257* (GH). **C)** *Polyphlebium endlicherianum*, a saxicolous (i.e., growing on rocks) species found at low to middle elevations. *Nitta 629* (GH). **D)** *Callistopteris apiifolia*, a terrestrial species with sporophytes occurring only in humid cloud forests. *Nitta 3990* (GH). **E)** *Callistopteris apiifolia* (ribbon morphotype). Arrow indicates gemmae (asexual propagules) at tip of lobe. *Nitta 2565* (GH). **F)** *Hymenophyllum polyanthos* (ribbon morphotype). *Nitta 3862* (GH). **G)** Filamentous gametophytes of an unidentified species of Hymenophyllaceae. *Nitta 4036* (GH). **H)** *Callistopteris apiifolia*, showing detail of gemmifers (attachment points of gemmae, example indicated by arrow) and branched gemmae. *Nitta 3887* (GH). Scalebars: A – C, 1.0 cm; D, 5.0 cm; E, G, H, 1.0 mm; F, 2.0 mm. Photographs by J. H. Nitta.

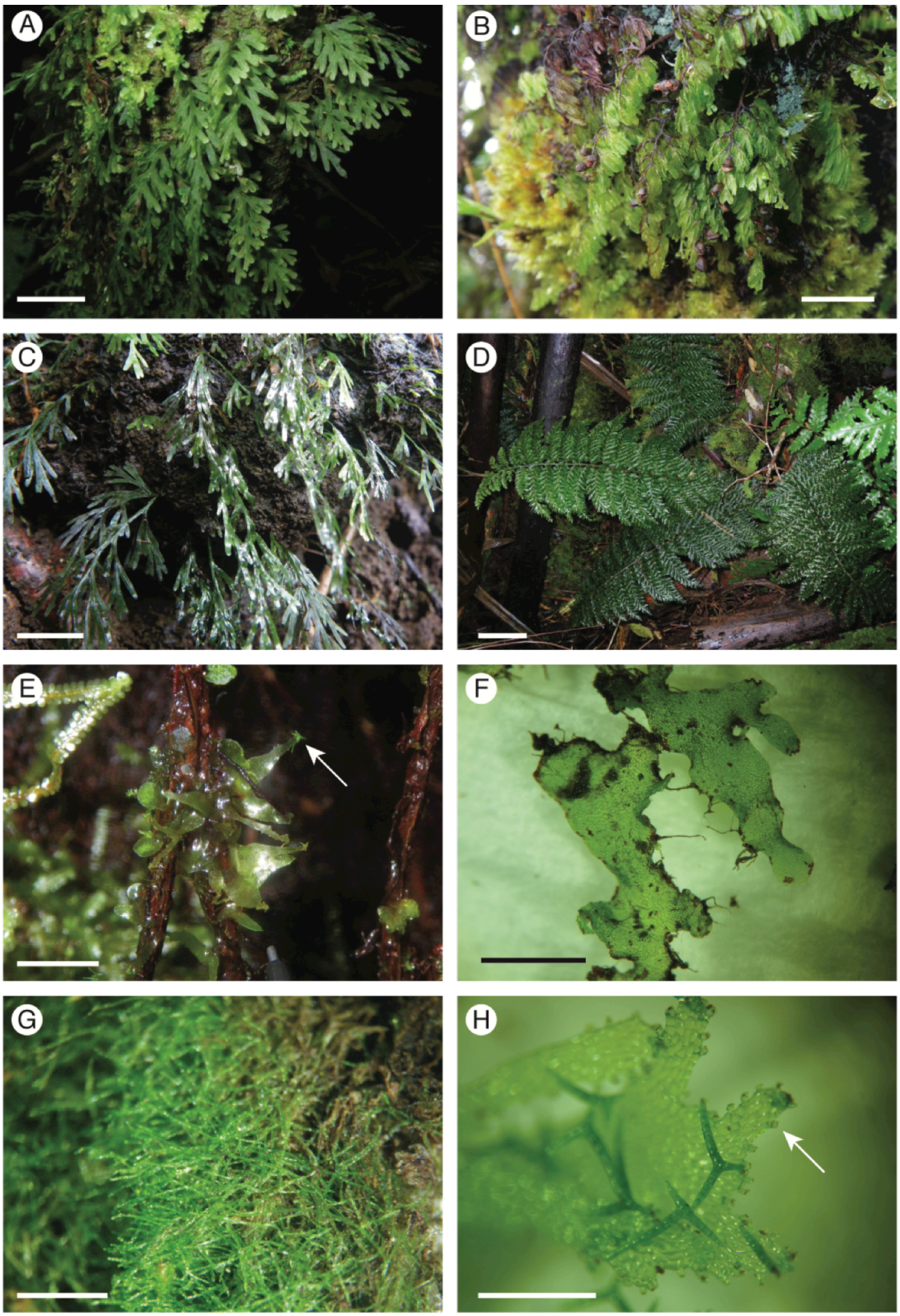

Figure 2.1 (Continued).

only a single cell layer in thickness, and lack cuticle and stomata (Figure 2.1). Filmy fern sporophytes are an exception to the "typical" fern sporophyte described above because they are poikilohydric: their thin leaf laminae allow for passive, rapid uptake and loss of moisture, thereby maintaining nearly constant equilibrium with atmospheric humidity. Many species of epiphytic filmy ferns have extremely reduced root systems (Schneider 2000), relying completely upon absorption through the laminar surface for water (Shreve 1911), although terrestrial species possess true roots and rely on ground water for hydration (Dubuisson et al. 2011). As would be expected from their poikilohydric nature, DT of varying degrees has been reported in sporophytes of many filmy fern species (see references below). Filmy fern sporophytes encompass a broad diversity of growth forms (terrestrial, saxicolous, hemi-epiphytic, and holoepiphytic) and morphologies, with rhizome and leaf sizes varying over several orders of magnitude (Hennequin et al. 2008, Nitta and Epps 2009, Dubuisson et al. 2013). Filmy fern gametophytes are similarly diverse, with morphotypes including filamentous, ribbon, and a combination of these two types (Figure 2.1). While there are no quantitative data about DT in filmy fern gametophytes available, DT has been reported in gametophytes from several other fern lineages (Watkins et al. 2007, Watkins and Cardelús 2012, Pittermann et al. 2013), and may be expected to occur in filmy ferns as well. The possibility for varying degrees of vegetative DT in both stages of the life cycle of filmy ferns makes them a potentially useful system to examine how DT changes across haploid, non-vascular to diploid, vascular life stages. Furthermore, the diversity of growth forms and habitats in filmy ferns make them an ideal group for a comparative study of water-stress strategies (i.e.,

desiccation tolerance vs. desiccation avoidance) and the correlation of these strategies with ecological niche.

The degree of DT in filmy fern sporophytes occupying different niches has been relatively well studied. In a series of elegant experiments on Jamaican filmy ferns, Shreve (1911) demonstrated that water-use strategies differ between species occupying contrasting habitats (i.e., terrestrial, low elevation epiphyte, and high elevation epiphyte). Proctor (2003, 2012) investigated water relations and the ability to recover from desiccation in sporophytes of several filmy ferns, and found a general correlation between high desiccation tolerance and adaptation for high light levels in widespread species vs. low desiccation tolerance and adaptation to low light levels in shade-adapted species. In the only study to our knowledge including *in situ* physiological observations of hymenophyllaceous gametophytes, Johnson et al. (2000) found that gametophytes of *Vandenboschia speciosa* (Willd.) G. Kunkel (= *Trichomanes speciosum* Willd.) are adapted for extremely low light environments, and operate at lower light levels than conspecific sporophytes. Parra et al. (2009) and Saldaña et al. (2013) investigated the relationship between physiology (photosynthetic rates and water relations) and vertical stratification in several epiphytic filmy fern species (primarily genus *Hymenophyllum*) along tree trunks in Chile. They found that species occurring at greater heights in the trees tended to have greater desiccation tolerance, higher photosynthetic capacity (A_{max}), and lower rates of evapotranspiration. A recent study using two species of *Hymenophyllum* demonstrated that desiccation tolerance in filmy ferns likely operates via a constitutive mechanism, which is more similar to bryophytes than other drought-

tolerant vascular plants, thus allowing filmy ferns to adjust to dry conditions extremely rapidly (Garcés Cea et al. 2014).

Despite this considerable amount of interest in filmy fern ecophysiology, with the sole exception of Johnson et al. (2000), no studies have compared the physiology of the gametophyte and sporophyte phases, and we are aware of none that have done so across multiple species. Furthermore, to our knowledge, no physiological studies of filmy ferns have accounted for their phylogenetic relationships, and we lack an understanding of how various physiological strategies have evolved in this clade. In addition to DT, photosynthetic optima (adaptation to different light levels) are likely to be important in determining the niches of life stages and species of filmy ferns. For example, terrestrial species experience very different levels of both photosynthetic radiation and desiccation relative to epiphytes, and light levels vary with height along the host plant in epiphytes (Watkins and Cardelús 2009, Sanger and Kirkpatrick 2014). In a recent study (Testo et al., unpublished), the combined effect of desiccation and light stress together was found to have a greater impact on filmy fern physiological performance than either of these stressors alone, suggesting the need to integrate studies of DT with tests of photosynthetic optima to gain a full picture of physiological adaptation in filmy ferns. Here, we leverage a recently developed DNA barcoding system (Nitta et al., in review) to test several hypotheses related to DT and photosynthesis in a comparative phylogenetic framework including both filmy fern sporophytes and gametophytes: 1.) Filmy fern sporophytes and gametophytes share similar levels of DT and photosynthetic optima when they co-occur. 2.) For filmy ferns with gametophytes that occur over a wider range than sporophytes, the gametophyte has greater DT and broader photosynthetic optima than the sporophyte. 3.)

Epiphytic filmy ferns have a greater degree of DT and increased photosynthetic optima than non-epiphytic species, and these similar physiologies have evolved multiple times independently. 4.) Amongst epiphytic filmy ferns, those occurring at high elevation are more desiccation tolerant and adapted for higher light levels than those at low elevation.

2.3 Materials and Methods

Study system—We selected the island Moorea, French Polynesia (17°30'S, 149°50'W) for our survey of filmy fern ecophysiology (Figure 2.2). Moorea is a small

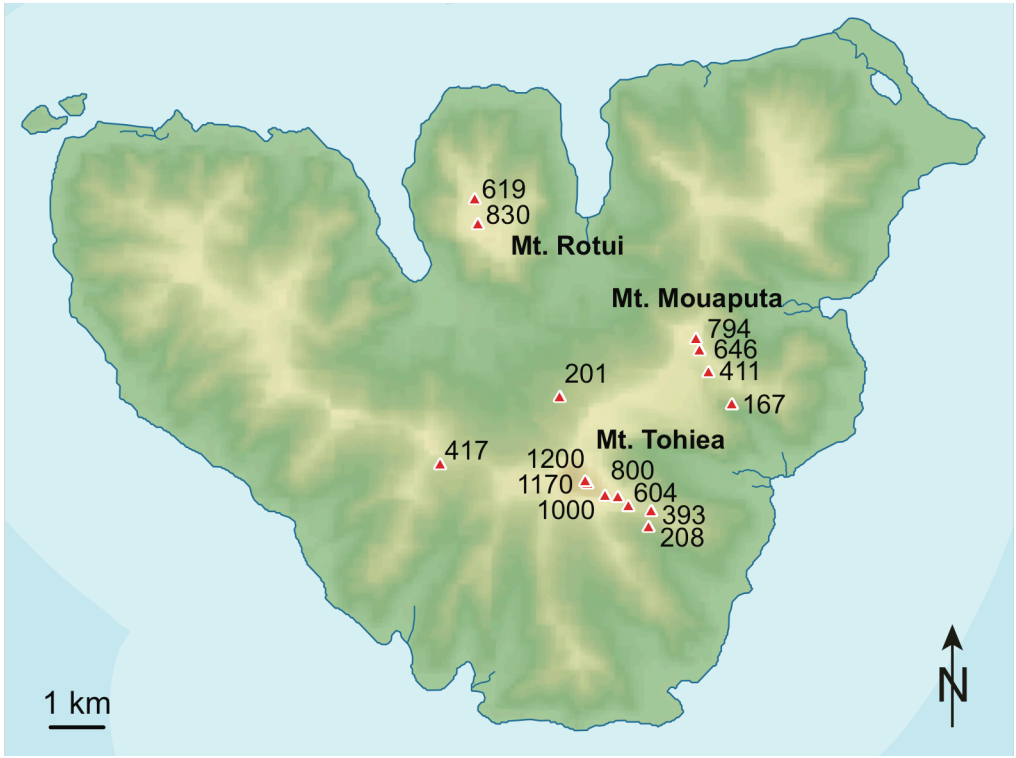

Figure 2.2. Map of Moorea, French Polynesia, showing location and elevation (m) of datalogger sites (red triangles) and names of peaks surveyed for filmy ferns. Map adapted from Wikimedia Commons under Creative Commons License.

(135 km^2; maximum elevation 1206 m) tropical oceanic island, located about 15 km NW of its larger sister island Tahiti. Despite its small size, it hosts a range of habitats

including coastal strand, low elevation rainforest, and high elevation cloud forest. At the time of the study, a total of 18 filmy fern species were known from Moorea (Murdock and Smith 2003, Ranker et al. 2005, Nitta et al. 2011b; Florence, in press), and include multiple terrestrial, saxicolous, and epiphytic species representing both hymenophylloid and trichomanoid lineages (Pryer et al. 2001). We follow the taxonomic system of Ebihara et al. (2006).

Field survey—We surveyed filmy ferns along trails to the summits of three large peaks on Moorea: Mt. Rotui (899 m), Mt. Mouaputa (880 m), and Mt. Tohiea (1206 m) (Figure 2.2). Although many low elevation sites are readily accessible on the island, several species (e.g., *Hymenophyllum* spp., *Callistopteris apiifolia* (Presl) Copel., *Polyphlebium endlicherianum* (C. Presl) Ebihara & K. Iwats.) are restricted to high elevation cloud forests, and these three mountains are the only ones on the island with trails allowing access to cloud forest. Epiphytes were sampled to a maximum height of ca. 2 m on their host plants, or from fallen tree branches. Unlike many other ferns, filmy fern gametophytes have unique morphologies that allow them to be distinguished from most other ferns at our field site (e.g., filamentous, ribbon with round gemmae). This allowed us to specifically collect filmy fern gametophytes, which we later identified to species using DNA barcoding (see below). We characterized the elevational range of each species based on the results of the field survey; any species found with gametophytes occurring > 200 m above or below the maximal observed elevational range of sporophytes were considered "widespread" (i.e., facultatively independent *sensu* Rumsey and Sheffield [1996]).

We characterized the microenvironment experienced by filmy ferns by logging relative humidity (RH) and temperature every 15 min from July 7, 2013 to July 5, 2014 at 16 sites spanning an elevational gradient from ca. 200 m to 1200 m (Figure 2.2). To do this we used Hobo ProV2 dataloggers (Onset Computer Corp, Massachusetts, USA) with external sensors outfitted with radiation shields (RS1 or RS3, Onset Computer Corp, Massachusetts, USA) to prevent direct contact with precipitation and solar radiation. To compare between terrestrial and epiphytic habitats, dataloggers were placed in pairs at each site (one "epiphytic" datalogger at 1.5 m on a tree, one "terrestrial" datalogger at 10 cm next to the tree). During the study period, some dataloggers malfunctioned, presumably due to prolonged exposure to high humidity. We excluded any days during which one or more dataloggers failed from the final dataset, and some sites are missing data completely for one or both dataloggers. One terrestrial datalogger (Mt. Tohiea 393 m site) was lacking data for more than half of the survey period, but had data available for the same period from the previous year (2012–2013). We used the previous year's data for this site instead. The final dataset included 201 days of data from 15 sites (26 dataloggers). We tested for differences in climatic variables between epiphytic and terrestrial habitats using analysis of covariance (ANCOVA) with each climatic variable in turn as the response variable and elevation as the explanatory variable with habitat (terrestrial vs. epiphytic) as a cofactor.

Molecular methods—We used a DNA-barcoding approach to identify filmy fern gametophytes to species as described in Nitta et. al (in review). Briefly, DNA extraction was performed using modified CTAB or the Plant Mini DNEasy kit (Qiagen, Valencia, CA) following the manufacturer's protocol. Chloroplast *rbcL* and *trnH-psbA* were

amplified using primers and protocol following Nitta et al. (in review). For each marker, a reference library was constructed from sporophyte sequences using the "makeblastDB" command in BLAST (Altschul et al. 1997). Field-collected gametophytes were identified by a local BLAST query against the reference library. Gametophytes matching > 99.5% with a single reference sequence and no others were identified as that species; those that did not match any reference sequence or matched > 99.5% with multiple sequences were not identified and excluded from further analysis. We derived the phylogenetic tree used in this study by extracting Hymenophyllaceae from the tree of Nitta et al. (in review) with the "extract.clade" function of the "ape" package in R (Paradis et al. 2004).

Desiccation tolerance—Samples were collected in the field and stored in plastic bags with a small amount of water to keep them fresh during transportation to the lab. For sporophytes, 8–12 individuals (one individual = single whole frond including a ca. 2 cm section of rhizome) were used per treatment, and all individuals for each species came from a single population. We did not attempt to differentiate genotypes between fronds in species with long-creeping rhizomes, which often form clonal mats; hence, in some cases, individuals may be genetically identical ramets. All experiments were initiated within 48 h of collection. Pre-treatment chlorophyll fluorescence (F_v/F_m) was measured in fresh plants after a 10 min period of dark-adaptation using a portable mini-PAM fluorometer (Walz Gmbh, Effeltrich, Germany). Plants were then transferred to desiccation chambers containing saturated salts at three different desiccation intensities or a control treatment with moist tissues (100% RH), and water withheld for either a short (2 d) or long (15 d) interval (Testo and Watkins 2013). Conditions inside the desiccation chambers were monitored during the experiment using Hobo ProV2 dataloggers. The desiccation

chambers were kept in an air-conditioned room, and temperature inside the chambers ranged from 22 to 25°C. Salts used for desiccation and their corresponding mean water potentials are as follows: LiCl (-282 MPa), $Mg(NO_3)_2$ (-86 MPa), and NaCl (-38 MPa). Because field-collected gametophytes could not be identified to species prior to DNA extraction, no planned replication by species was possible; we therefore used the same treatment for all gametophytes (2 d at -86 MPa), and verified species of each individual after the experiment using the DNA barcode approach described above. Thus, experiments involving gametophytes were sampled many times for some species whereas other species were not. Following the desiccation treatment, plants were rewetted using water from a local stream. Plants were allowed to rehydrate for 24 h, and chlorophyll fluorescence was again measured at 0.5 h, 24 h, and 48 h following rewetting. Ability to recover from desiccation was quantified by comparing pre- and post-treatment chlorophyll fluorescence yield (Watkins et al. 2007).

Light responses—Rapid light response curves were constructed for each species by measuring photosynthetic yield at gradually increasing levels of photosynthetically active actinic light (400 – 700 nm) with the "Light Curve" function of the mini-PAM portable chlorophyll fluorometer as described by the manufacturer. Briefly, the maximum yield (F_m) was first measured in the absence of actinic light. Next, plants were equilibrated to the actinic light for 30 s, and photosynthetic yield ($\Delta F/F_m$) measured with a 0.8 s saturating pulse. This was repeated for each photosynthetic photon flux density (PPFD) level, up to ca. 500 μmol m^{-2} s^{-1}. Response curves were fitted using the equation $y = A(1 - e^{-kx})$, where y is relative electron transport rate of photosystem II (RETR), x is PPFD, A is the asymptote of the curve, and k is a slope parameter; outliers at high PPFD

likely to distort the curve were discarded (Proctor 2012). The PPFD at 95% saturation of RETR ($PPFD_{95\%}$) and maximum RETR (ETR_{max}) were then calculated from the curve. Light responses were measured in eight field-collected replicates for each sporophyte species, and on single gametophyte individuals in the field, which were later identified to species using the DNA barcodes.

Statistical analysis—We first quantified the degree of phylogenetic signal in physiological traits using Blomberg's *K* (Blomberg et al. 2003) and Pagel's λ (Pagel 1999, Freckleton et al. 2002) as implemented with the "phylosig" function in the "phytools" package in R (Revell 2012). Both measures test the hypothesis that the trait of interest is evolving according to Brownian Motion (BM). λ is a scaling parameter that ranges from zero to one: λ near zero indicates random distribution of trait values across the tree (i.e., a star phylogeny), and λ near one indicates evolution of traits along the phylogeny following BM. For *K,* values near one indicate evolution of traits following BM; $K > 1$ indicate traits more conserved than expected under BM, and $K < 1$ indicates that traits have less phylogenetic signal than expected under BM. We tested the significance of *K* by comparing the observed value against values from a null distribution of 1000 phylogenies with the traits randomly shuffled across the tips. We tested the significance of λ with a log-likelihood test comparing the likelihood of the observed value of λ vs. $\lambda = 0$. For all further analyses, we used methods that account for phylogenetic history for physiological traits that showed significant phylogenetic signal, and standard methods otherwise.

We used general linear mixed models (GLMMs) to investigate the effect of growth habit, range size, and life stage on DT (% recovery of F_v/F_m after 48 h recovery

from desiccation at -86 MPa for 2 d) and photosynthetic parameters (ETR_{max} and $PPFD_{95\%}$) using the "MCMCglmm" package in R (Hadfield 2010). For each of the three response variables (DT, ETR_{max} and $PPFD_{95\%}$), we used growth habit (epiphyte vs. non-epiphyte) or range size (widespread vs. not widespread) and generation (sporophyte vs. gametophyte) and their interactions as fixed effects. We accounted for intraspecific variation and phylogeny (only for response variables with significant phylogenetic signal) by including species (within generation) and an inverse phylogenetic variance-covariance matrix as random effects, respectively (Hadfield 2010). We used an inverse-Gamma distribution for all priors, and ran analyses for 500000 iterations, with burnin after 1000 iterations and thinning every 500 iterations. Comparisons of DT, ETR_{max}, and $PPFD_{95\%}$ between gametophytes and sporophytes of the same species do not involve phylogeny, so we used a *t*-test for these.

To test for correlation between environmental water potential and DT, we first derived a single minimum water potential value for each species by taking the average of the minimum daily water potential at all sites where that species was present. We used minimum values because these may represent a climatic extreme that limits the occurrence of species. We used data from the "epiphytic" dataloggers mounted at 1.5 m on trees for epiphytic species and data from the "terrestrial" dataloggers on the ground for non-epiphytic (i.e., terrestrial or saxicolous) species. Data from the Mt. Rotui 830 m site were excluded because this site was much more exposed than the other high elevation sites, and appears as a significant outlier in the microclimate data (see 2.4 Results). This may be due to its location; unlike other mountains on Moorea, Mt. Rotui is completely isolated with no connecting ridges to other peaks (Figure 2.2). Since DT showed

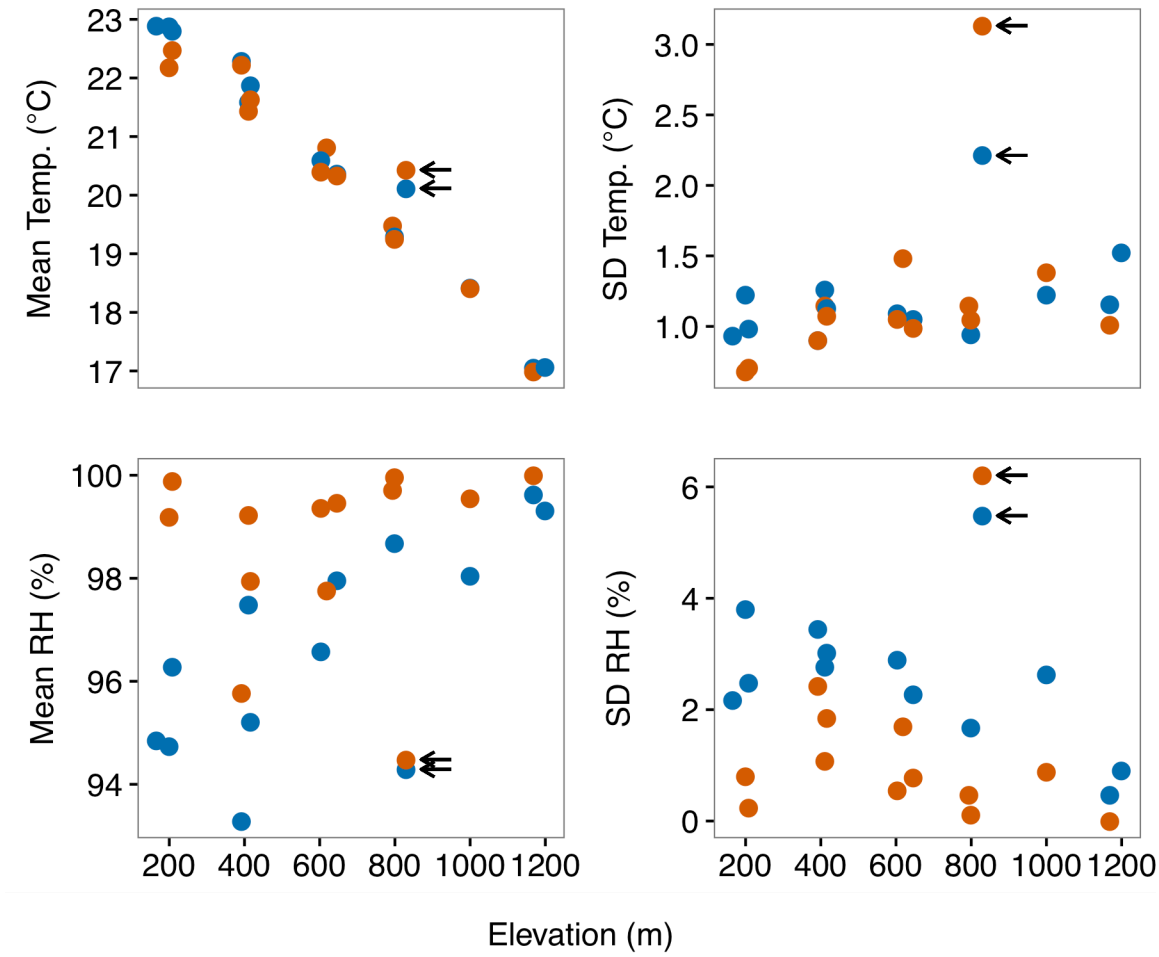

Figure 2.3. Mean and standard deviation (SD) of daily mean temperature and relative humidity (RH) for 15 sites on Moorea, French Polynesia recorded by dataloggers from July 7, 2013 to July 5, 2014. Blue dots indicate "epiphytic" dataloggers mounted at ca. 1.5 m on trees; red dots indicate "terrestrial" dataloggers positioned on the ground next to tree. Arrows indicate Mt. Rotui 830 m site, which was much more exposed than other high elevation sites.

significant phylogenetic signal (see 2.4 Results), we used phylogenetic generalized least squares (PGLS) to test for the relationship between environmental water potential and DT

while taking phylogeny into account, using species' means. We ran PGLS using the "caper" package in R (Orme et al. 2013).

2.4 Results

Mean daily temperature was strongly correlated with elevation, and dropped ca. 6°C from the lowest surveyed site (201 m) to the highest (1206 m) (Figure 2.3). Although epiphytic and terrestrial habitats were very similar in temperature, epiphytic habitats tended to be drier (lower daily mean RH), and had greater daily variation in RH than terrestrial ones (ANCOVA, Table B1). This difference was greatest at low elevations, and smallest at high elevations. One significant departure from these trends was observed at the Mt. Rotui 830 m site (indicated by arrows in Figure 2.3). This site is much more exposed than the other cloud forest sites (Figure 2.2), and many of the plants on the N facing slope are stunted (J. Nitta, pers. obs.). Daily variation in temperature and RH was much greater, and mean RH much lower, at this site than other high elevation sites.

Twenty-two filmy fern taxa (including one new record and two species that were recognized as multiple varieties) were observed as sporophytes, and 19 as gametophytes on Moorea in total (Table 2.1). *Hymenophyllum braithwaitei* (Ebihara & K. Iwats.), only observed near the peak of Mt. Tohiea (1206 m), is a new record for Moorea and French Polynesia. It was previously known only from Vanuatu and New Caledonia (Ebihara et al. 2010). Three taxa with distinct *rbcL* sequences, morphology, and elevational ranges could be distinguished within the *Crepidomanes minutum* (Blume) K. Iwats. species complex, but species status of these taxa remains uncertain (Nitta et al. 2011a). We refer to these here as *C. minutum* var. 1, 2, and 3. Similarly, two members of the *Abrodictyum asae-grayi* (Bosch) Ebihara & K. Iwats. complex were observed on the basis of *rbcL* and morphology, but as taxonomic treatment is beyond the scope of the current study we refer

Table 2.1. Elevational ranges of sporophytes and gametophytes of selected Moorean filmy ferns. Growth habit: T, terrestrial; S, saxicolous; HE, high elevation epiphyte; LE, low elevation epiphyte. Range status: N, gametophytes not widespread beyond sporophytes; Y, gametophytes widespread beyond sporophytes (more than 200 m above or below maximum sporophyte elevational range).

Table 2.1 (Continued).

	Growth Habit	Range Status	Gametophyte Min. El. (m)	Gametophyte Max. El. (m)	Sporophyte Min. El. (m)	Sporophyte Max. El. (m)	Range of Gametophyte Beyond Sporophyte Below (m)	Range of Gametophyte Beyond Sporophyte Above (m)
Abrodictyum asaegrayi var 1	T	N	1170	1170	1170	1200	-	-
Abrodictyum asaegrayi var 2	T	N	1200	1200	1170	1170	-	30
Abrodictyum caudatum	LE	N	not observed		411	830	-	-
Abrodictyum dentatum	T	N	411	1000	411	1170	-	-
Callistopteris apiifolia	T	Y	201	1200	646	1200	445	-
Crepidomanes bipunctatum	LE	N	not observed		201	604	-	-
Crepidomanes humile	LE	N	417	417	167	800	-	-
Crepidomanes kurzii	S	N	167	201	167	201	-	-
Crepidomanes minutum var 1	LE	N	619	619	411	1170	-	-
Crepidomanes minutum var 2	HE	Y	411	830	646	1200	235	-
Crepidomanes minutum var 3	HE	Y	619	830	1000	1000	381	-
Didymoglossum tahitense	LE	N	167	201	167	646	-	-
Hymenophyllum braithwaitei	HE	N	not observed		1200	1200	-	-
Hymenophyllum digitatum	HE	Y	411	1170	619	1200	208	-
Hymenophyllum flabellatum	HE	N	not observed		823	1007	-	-
Hymenophyllum javanicum	HE	Y	794	794	1170	1170	376	-
Hymenophyllum multifidum	HE	N	1200	1200	1200	1200	-	-
Hymenophyllum pallidum	HE	Y	830	1200	1170	1200	340	-
Hymenophyllum polyanthos	HE	N	619	1200	411	1200	-	-
Polyphlebium borbonicum	S	N	619	800	794	1170	175	-
Polyphlebium endlicherianum	HE	N	167	208	167	800	-	-
Vandenboschia maxima	S	N	794	800	800	800	-	-

to them as *A. asae-grayi* var. 1 and 2. The inferred phylogeny is well-supported and generally in accord with previously published, more densely sampled phylogenies (Pryer et al. 2001, Dubuisson et al. 2003, Hennequin et al. 2006, Ebihara et al. 2007; Figure 2.4). Desiccation tolerance data were collected for sporophytes of 15 species and gametophytes of 14 species, including 12 species with both life phases represented (Table B2). Photosynthetic optima were measured for sporophytes of 19 species and gametophytes of 11 species, including 10 species with both life phases (Table B3).

In several species, gametophytes exceeded the maximum or minimum elevational range of sporophytes (Table 2.1). The species with the most widely distributed gametophytes was *C. apiifolia*. Gametophytes for this species were observed from ca. 200 to 1200 m, but its sporophytes are confined to humid cloud forests from ca. 600 to 1200 m.

Desiccation tolerance of sporophytes largely reflected habitat (Figure 2.5). Both terrestrial species (*A. dentatum* and *C. apiifolia*) lacked even moderate DT, and failed to recover from the gentlest treatment of 2 d at -38 MPa (more stringent tests were not conducted on these species after confirming their lack of DT at this level). Saxicolous species (*Crepidomanes kurzii* (Bedd.) Tagawa & K. Iwats., *P. endlicherianum*, and *Vandenboschia maxima* (Blume) Copel.) also had low values of DT; only *C. kurzii* was able to recover from desiccation up to -86 Mpa for 2 d but not more. All epiphytic species were capable of recovering from desiccation to a certain degree, but ability varied between species. Most low elevation epiphytes could not tolerate long periods (15 d) of desiccation; only *Crepidomanes bipunctatum* (Poir.) Copel. was able to recover from desiccation greater than 2 d. Most of the high elevation epiphytes could recover from 2 d

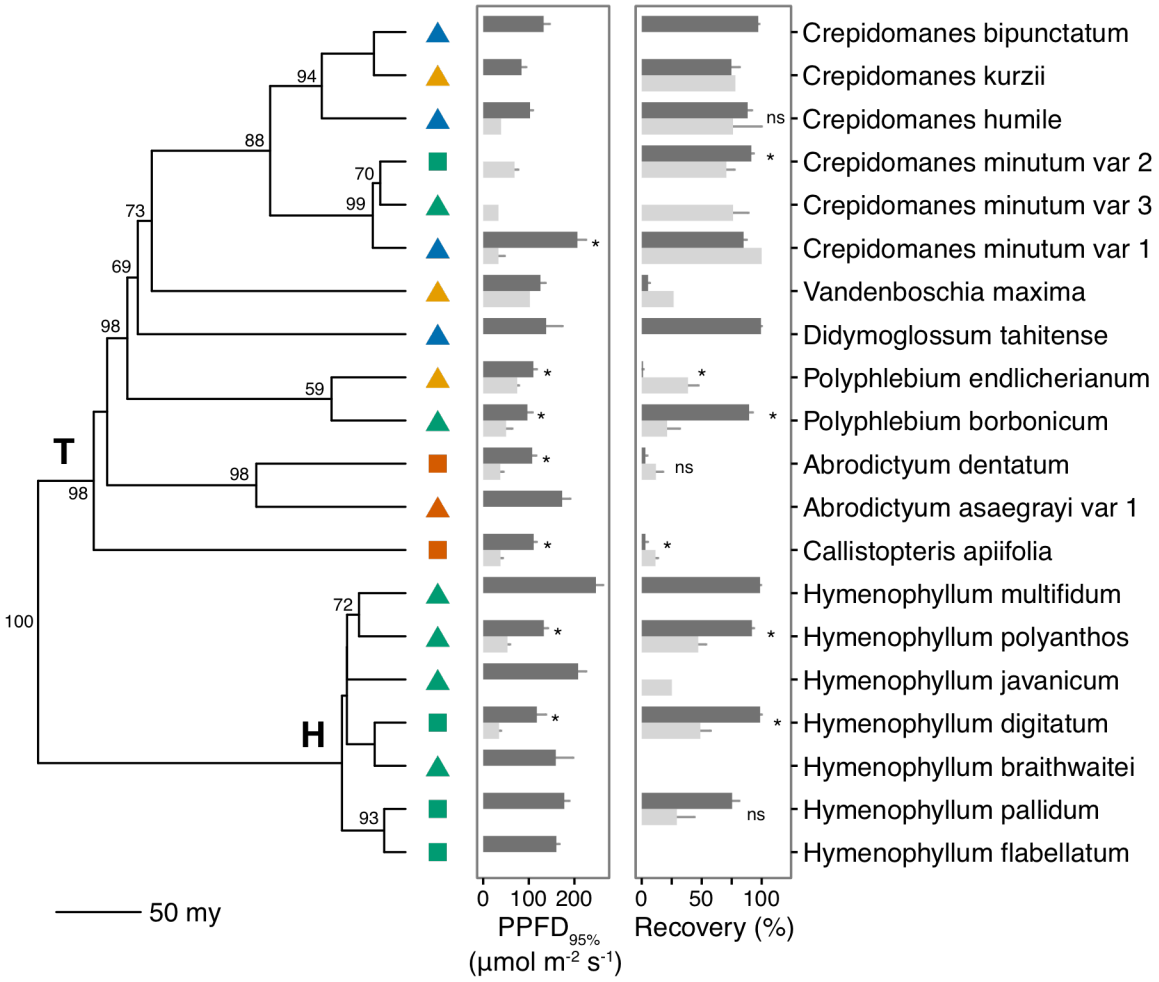

Figure 2.4. Phylogeny of Moorean filmy ferns with comparison of physiological parameters (mean ± one standard error) between sporophytes (dark grey bars) and gametophytes (light grey bars). Phylogeny adapted from Nitta *et al.* (in review). Bootstrap support > 50% from 100 ML bootstraps indicated at nodes. Bold letters indicate major clades ("H" = hymenophylloid clade, "T" = trichomanoid clade). Growth habit indicated by colored symbols (blue = low elevation epiphyte, green = high elevation epiphyte, yellow = saxicolous, red = terrestrial); range type indicated by symbol shape (square = gametophytes widespread; triangle = gametophytes not widespread). Asterisk indicates significant difference in means ($P < 0.05$) between generations for a particular species (*t*-test); "ns" indicates no significant difference; lack of "ns" or asterisk means

Figure 2.4 (Continued) not enough observations available for that species for *t*-test. Right bar plot shows recovery (%) of chlorophyll fluorescence (F_v/F_m) following 2 d desiccation at -86 MPa (two terrestrial sporophytes, *C. apiifolia* and *A. dentatum*, were desiccated at -38 Mpa instead of -86 MPa). Left bar plot shows light level (μmol m^{-2} s^{-1}) at which 95% of maximum relative electron transport rate is reached ($PPFD_{95\%}$). The other photosynthetic parameter measured, maximum relative electron transport rate (ETR_{max}), was qualitatively very similar to $PPFD_{95\%}$ and not shown.

treatments regardless of desiccation intensity (except for *Hymenophyllum pallidum* (Blume) Ebihara & K. Iwats. and *Polyphlebium borbonicum* (Bosch) Ebihara & Dubuisson, which failed to recover from 2 d at -38 MPa), and many could withstand desiccation for 15 d. Interestingly, *C. minutum* var. 2, *Hymenophyllum multifidum* (G. Forst.) Sw., and *Hymenophyllum polyanthos* (Sw.) Sw. could recover from desiccation at -86 or -282 MPa for 15 d, but not -38 MPa.

Results of the tests for phylogenetic signal (Blomberg's *K* and Pagel's λ) were similar for sporophytes and gametophytes, revealing significant phylogenetic signal for DT (gametophytes *K* and λ both near 1, $P < 0.05$; sporophytes $K = 0.78$, $P = 0.03$ and $\lambda = 0.96$, $P = 0.11$), but no signal for $PPFD_{95\%}$ or ETR_{max} (Table 2.2). The GLMM showed that both generation and the interaction of generation and growth habit, but not growth habit alone, have a significant effect on DT while controlling for phylogeny (Table 2.3). Gametophytes of low elevation epiphytes had the highest DT (ca. 80% recovery), followed by high elevation epiphytes and saxicolous species (both with recovery from ca. 25% to 75%), with terrestrial species essentially non-tolerant (less than 20% recovery; Figure 2.4). There was no significant difference in DT between widespread and non-

Figure 2.5. Recovery of F_v/F_m (%) following desiccation treatment for filmy fern sporophytes by recovery time. Species means ± one standard error (s.e.); N = 8–16 individuals per species. Length of desiccation period indicated by line (dashed line = 2 d, solid line = 15 d). Intensity of desiccation indicated by shape (circles = -38 MPa, triangles = -86 Mpa, diamonds = -282 MPa). Growth habit of species indicated by background color of species name (green = high elevation epiphyte, blue = low elevation epiphyte, yellow = saxicolous, red = terrestrial).

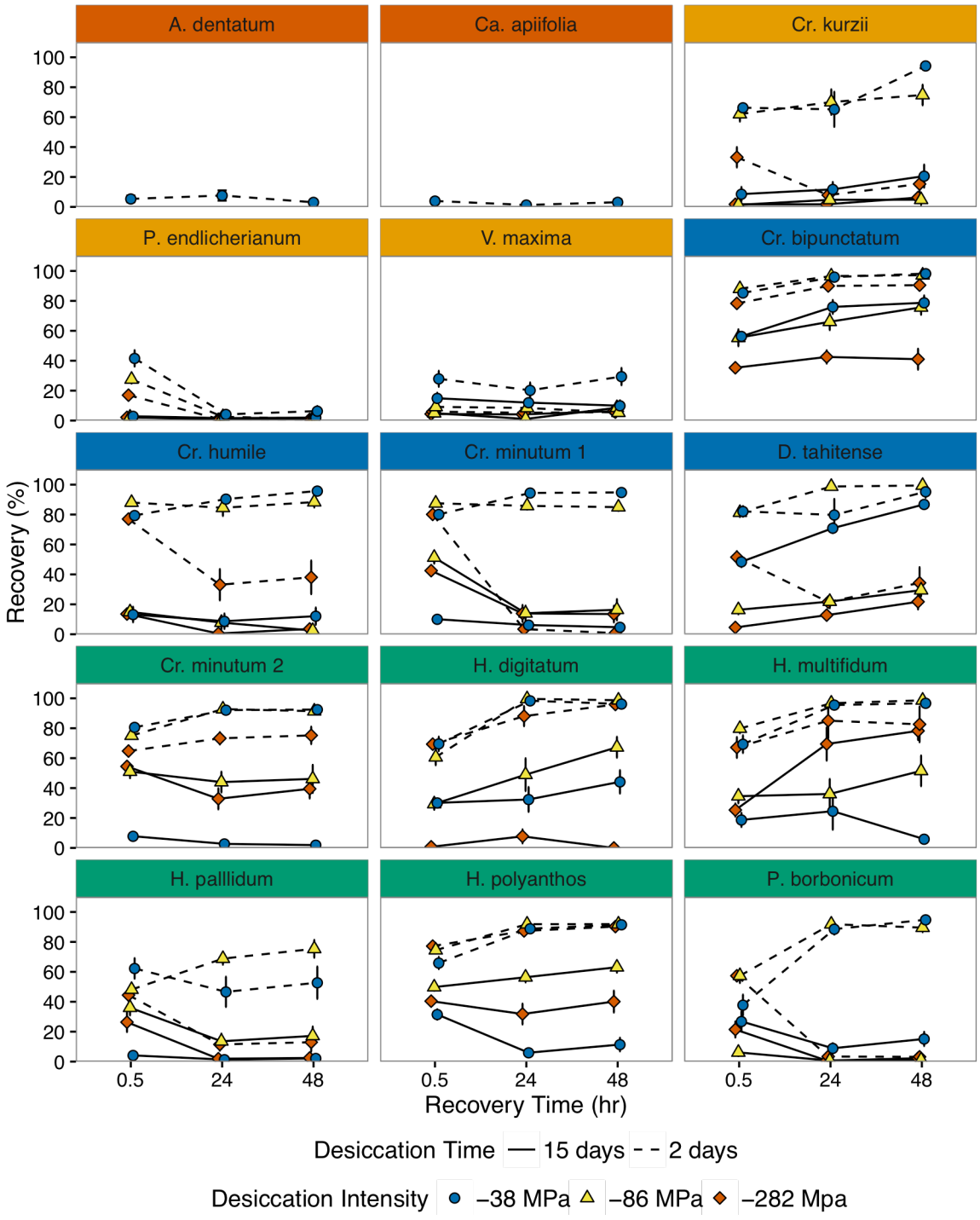

Figure 2.5 (Continued).

Table 2.2. Results of tests for phylogenetic signal (Blomberg's *K* and Pagel's λ) in desiccation tolerance (DT) and photosynthetic parameters of Moorean filmy ferns by generation. ETR_{max}, maximum relative electron transport rate. $PPFD_{95\%}$, light level at which 95% of ETR_{max} is reached.

Generation	Trait	λ	*K*
Sporophyte	DT	0.96 ($P = 0.11$)	0.78 ($P = 0.03$)
	ETR_{max}	0 ($P = 1$)	0.19 ($P = 0.87$)
	$PPFD_{95\%}$	0.17 ($P = 0.39$)	0.33 ($P = 0.25$)
Gametophyte	DT	0.9 ($P = 0.01$)	1.13 ($P < 0.001$)
	ETR_{max}	0 ($P = 1$)	0.19 ($P = 0.89$)
	$PPFD_{95\%}$	0 ($P = 1$)	0.33 ($P = 0.5$)

widespread gametophytes (Table 2.3). DT of gametophytes did not significantly exceed that of sporophytes for any species except for the saxicolous *P. endlicherianum* (Figure 2.4; *t*-test), and was lower in gametophytes than sporophytes of some high elevation epiphytic species (e.g., *C. minutum* var. 2, *Hymenophyllum digitatum* (Sw.) Fosberg, *H. polyanthos*; Figure 2.4). Photosynthetic optima tended to vary between life phases, but not across growth habits or range type: sporophytes are adapted for higher light levels, whereas gametophytes are adapted for lower light levels (Figure 2.4, Table 2.3).

Desiccation tolerance tends to become greater with decreasing environmental water potential for both gametophytes and sporophytes (Figure 2.6). However, these trends were not significant when analyzed using phylogenetic generalized least squares (sporophytes $P = 0.057$; gametophytes $P = 0.521$).

2.5 Discussion

Vegetative desiccation tolerance was a key innovation linked to the rise of early land plants, but is largely limited to non-vascular groups such as bryophytes and algae in

Table 2.3 Phylogenetic general linear mixed models for desiccation tolerance (DT) and photosynthetic parameters of Moorean filmy ferns. Fixed effects included growth habit (epiphyte or terrestrial) or range type (widespread or not widespread) and their interaction with generation (sporophyte vs. gametophyte). Random effects included species (within generation) and phylogeny. Significant effects in bold. ETR_{max}, maximum relative electron transport rate. $PPFD_{95\%}$, light level at which 95% of ETR_{max} is reached.

Table 2.3 (Continued).

		Estimate	Lower 95% CI	Upper 95% CI	*P*	Effect
Growth Habit	DT	46.2	19.145	68.1	0.006	(Intercept)
		-12.5	-44.395	15	0.397	Habit (Terrestrial)
		36.7	**22.292**	**51.7**	**0.001**	**Generation (Sporophyte)**
		-49.9	**-75.979**	**-24.9**	**0.004**	**Habit (Terrestrial) × Generation (Sporophyte)**
	ETR_{max}	**4.1**	**2.513**	**5.6**	**0.001**	**(Intercept)**
		-0.446	-3.053	2.37	0.725	Habit (Terrestrial)
		4.97	**3.281**	**6.94**	**0.001**	**Generation (Sporophyte)**
		-0.642	-3.979	2.77	0.695	Habit (Terrestrial) × Generation (Sporophyte)
	$PPFD_{95\%}$	**46.9**	**14.436**	**74.2**	**0.001**	**(Intercept)**
		8.5	-37.583	65.8	0.754	Habit (Terrestrial)
		110	**70.765**	**145**	**0.001**	**Generation (Sporophyte)**
		-48.7	-115.873	14.9	0.144	Habit (Terrestrial) × Generation (Sporophyte)
Range Type	DT	31.6	-8.229	70.1	0.102	(Intercept)
		14.3	-20.925	48.5	0.385	Range (Not Widespread)
		19.8	-11.467	52.4	0.202	Generation (Sporophyte)
		-2.18	-42.462	35.5	0.898	Range (Not Widespread) × Generation (Sporophyte)
	ETR_{max}	**3.56**	**1.779**	**5.57**	**0.001**	**(Intercept)**
		0.645	-1.785	3.21	0.625	Range (Not Widespread)
		4.27	**1.91**	**7.12**	**0.002**	**Generation (Sporophyte)**
		0.546	-2.597	3.94	0.776	Range (Not Widespread) × Generation (Sporophyte)
	$PPFD_{95\%}$	44.4	4.138	82.3	0.032	(Intercept)
		10.4	-40.518	65.4	0.685	Range (Not Widespread)
		90	**35.845**	**144**	**0.004**	**Generation (Sporophyte)**
		2.33	-67.498	67	0.93	Range (Not Widespread) × Generation (Sporophyte)

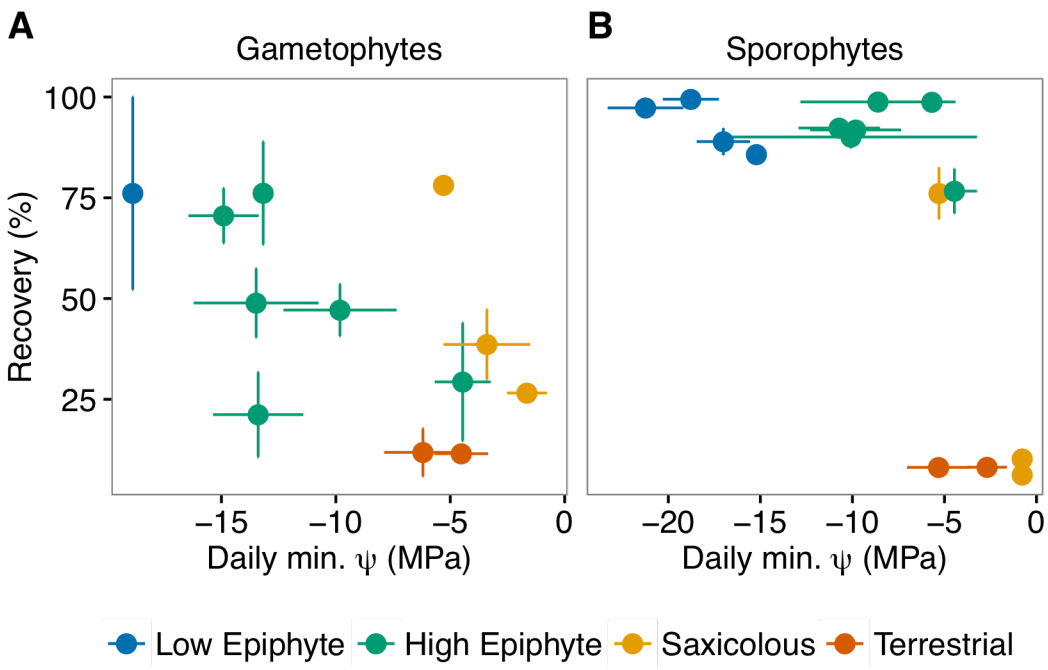

Figure 2.6 Recovery of F_v/F_m (%) following 2 d desiccation at -86 MPa plotted against water potential (MPa) by generation. (A) Gametophytes. **(B)** Sporophytes. Species means ± s.e. are shown. Two terrestrial sporophytes, *C. apiifolia* and *A. dentatum*, were desiccated at -38 Mpa instead of -86 MPa.

modern plants. Filmy ferns have received considerable attention over the years for their remarkable reversion to vegetative DT in the sporophyte phase. Yet, no previous study to our knowledge has evaluated parallel physiology of sporophytes and gametophytes of ferns in relation to DT. Here, we present the first comparisons of physiological parameters between generations within filmy ferns in a phylogenetic comparative framework.

Filmy fern sporophytes have a wide range of DT—We found a wide variation of DT in filmy fern sporophytes on Moorea, from no tolerance in terrestrial species to extreme tolerance of water potentials well below -200 MPa in some epiphytes. Levels of

similarly extreme DT have also been reported in other species of *Hymenophyllum* (Proctor 2003, 2012) and a general correlation of habitat with DT level in filmy ferns has been previously established (Shreve 1911, Proctor 2003, 2012, Parra et al. 2009, Saldaña et al. 2013, Testo et al., unpublished), but no other studies to our knowledge have investigated DT in terrestrial filmy ferns, or documented a similar range of values in species from a single site. One may wonder then, what is the adaptive significance of being able to withstand drying at -200 MPa vs. -100 MPa when the ambient water potential is typically > -5 MPa and only rarely, if ever, drops below -50 MPa. The recent finding of a constitutive mechanism for DT in filmy ferns (Garcés Cea et al. 2014) supports the need for a relatively wide "safety margin", and similar (or even more extreme) levels of constitutive DT are well known in bryophytes (Oliver et al., 1993). The conservative behavior of fern stomata, which lack a response to the plant stress hormone ABA, is well documented (Brodribb and Mcadam 2011, Mcadam and Brodribb 2012, 2013). It is possible that filmy ferns also behave conservatively with respect to water-stress. We suggest that the variation in DT observed here is relevant because it is correlated with habitat, and likely reflects overall physiological tolerance, not just artificial extremes. For example, sporophytes of some high elevation epiphytes that we surveyed (*C. minutum* var. 2, *H. digitatum*, *H. multifidum*, *H. polyanthos*) seem to be able to better withstand intense vs. moderate desiccation, with higher recovery values at -282 and -86 MPa vs. -35 MPa when dry for 15 d. This may be an adaptation to cloud forest conditions; although cloud forests are frequently moist due to constant fog, dry periods are intense during the times when there is no cloud cover. The complete lack of DT in terrestrial species is not surprising, considering they have much more direct access to

water than epiphytes and therefore substantially less selective pressure to maintain DT. Since controls kept at 100% RH maintained F_v/F_m values near pre-treatment levels in all sporophytes (Table B2), it is unlikely that the lack of tolerance seen in terrestrial species is due solely to removal from the soil. Comparison of DT with the minimum water potential envelope of each species (Figure 2.6) indicates that sporophytes of non-tolerant terrestrial and saxicolous species may not be able to occur in habitats with minimum water potential exceeding ca. -7 MPa. In contrast, habitats with minimum water potential below -7 MPa are nearly all epiphytic, and the filmy fern sporophytes occurring there are all capable of DT (Figure 2.6).

Filmy fern gametophytes are not more stress-tolerant than sporophytes—Contrary to our hypotheses, gametophytes of filmy ferns are in general not more tolerant of the abiotic environment (light levels and water potential) than sporophytes. This differs from other fern groups with desiccation tolerant gametophytes but desiccation sensitive sporophytes (Watkins et al. 2007). In the filmy ferns we studied, DT levels of both sporophytes and gametophytes largely reflected habitat; in general, epiphytic species tended to have higher DT than terrestrial or saxicolous species, regardless of life stage. Interestingly, this echoes much earlier field observations (not experimental data) by Holloway (1930) on filmy fern gametophytes in New Zealand: he reported apparent DT in gametophytes of *Hymenophyllum rarum* R. Br. and *H. villosum* Colenso, both high elevation epiphytes with desiccation tolerant sporophytes, and lack of DT in terrestrial filamentous gametophytes of *Polyphlebium colensoi* (Hook.) Ebihara & K. Iwats., *Abrodictyum strictum* (Menzies ex Hook. & Grev.) Ebihara & K. Iwats., and *A. elongatum* (A. Cunn.) Ebihara & K. Iwats., occupying moist, protected sites on the forest

floor. Watkins et al. (2007) also found that DT of fern gametophytes tended to match species habitat, with terrestrial species having lower DT than epiphytes. However, we found that within high elevation epiphytes, gametophytes had lower DT than sporophytes, and only one species (saxicolous *P. endlicherianum*) with gametophytes that had significantly greater DT than sporophytes (Figures 2.4, 2.6). At high elevation sites (i.e., cloud forest), epiphytic ferns often occur in a dense matrix of bryophytes (Figure 2.1B). It is possible that this bryophyte cover helps to retain moisture during brief periods of drought and thus protect the epiphytic fern gametophytes that grow amongst them. More detailed studies of the high elevation epiphytic environment and interactions between co-occurring gametophytes of ferns and bryophytes are needed to test this hypothesis.

Although we predicted that species with gametophytes that occur beyond the range of their sporophytes would have higher DT and be adapted for higher light levels, this was not the case. This is perhaps best illustrated by *C. apiifolia* (Figure 2.1D, E, H). Sporophytes of this species are restricted to moist cloud forest sites from ca. 600–1200 m, but we collected gametophytes across a much wider range, down to 200 m. A similar distribution pattern has been reported in the closely related *Callistopteris baldwinii* (D. C. Eaton) Copel. from Hawaii (Dassler and Farrar 1997). Since the gametophytes of *C. apiifolia* are common and have a unique morphology, we were able to collect enough material of this species for additional tests of DT at higher humidity levels (48 h at 75% and 100% RH; results not shown). However, it still failed to recover from even these gentle treatments. This suggests that the widespread gametophytes of *C. apiifolia* do not rely on DT to attain their distribution, but instead must do so via some other mechanism. Although we measured terrestrial microhabitats by positioning sensors a few cm above

the ground, these sensors are still outside of the boundary layer. It is likely that the gametophytes of *C. apiifolia* do not actually experience the conditions measured with our "terrestrial" dataloggers, but instead grow within a much more consistently moist boundary layer. Gametophytes of *C. apiifolia* produce gemmae (Figure 2.1E, H), and often form dense clonal mats. In the case that their microhabitats do become too dry, they may suffer loss at the edge of the population, and then recover by clonal growth. Furthermore, the complex surface of clonal mats has been shown to increase the boundary layer and decrease rates of evaporative water loss in mosses (Rice et al. 2001), and may function similarly in *C. apiifolia*. Other filmy fern species also produce gemmae, but they are much more frequent in *C. apiifolia*, and the large size of the clonal mats formed by *C. apiifolia* indicate that it may have a high growth rate (J. Nitta, pers. obs.). It is possible that there is a tradeoff between growth rate and DT, such that species capable of rapid asexual growth like *C. apiifolia* are unable to tolerate desiccation, but slower growing epiphytes such as *Hymenophyllum* spp. can better tolerate it (Oliver et al. 2000). Growth rates of tolerant and non-tolerant gametophytes including quantification of gemmae production should be investigated in these species to test this hypothesis.

Gametophytes are adapted for lower light levels than sporophytes—Unlike DT, photosynthetic parameters did not vary significantly between species from different habitats. Rather, we observed a clear intergenerational difference, with gametophytes consistently adapted for lower light levels than sporophytes (Figure 2.4). In the only other study to our knowledge that compared photosynthetic rates between gametophytes and sporophytes of a filmy fern, Johnson et al. (2000) also found that gametophytes of *V. speciosa* reached maximum ETR at lower levels than sporophytes: in gametophytes of

this species, photosynthesis became saturated at a PPFD of 4 – 5 μmol $m^{-2}s^{-1}$ in the field or 30 – 50 μmol $m^{-2}s^{-1}$ in the lab vs. 30 – 50 μmol $m^{-2}s^{-1}$ for the sporophyte (lab measurement only). Although we found similar differences in direction between gametophytes and sporophytes, the measurements of Johnson et al. (2000) are considerably lower than what we observed in the field for both Moorean filmy fern gametophytes ($PPFD_{95\%}$ ca. 40 – 60 μmol $m^{-2}s^{-1}$) and sporophytes ($PPFD_{95\%}$ ca. 130 – 160 μmol $m^{-2}s^{-1}$). This makes sense in light of the fact that *V. speciosa* is an extremely deep-shade plant, with gametophytes occupying protected rock crevices that receive less than 1 μmol $m^{-2}s^{-1}$ PPFD for most of the day (Johnson et al. 2000); many of the gametophytes sampled in our study occur in much more exposed conditions. It is surprising, however, that we did not observe any differences between species in different habitats, given that light levels should vary between tree trunks and the forest floor, at least on a ca. 1 m scale. It is possible that the actual light levels experienced by gametophytes growing in these habitats may not vary significantly if they exploit protected microsites such as bryophyte mats or crevices in rock or bark.

Influence of phylogeny on traits—Previous studies of filmy fern ecophysiology also found a link between habitat and DT and/or photosynthetic rates (Proctor 2003, 2012, Parra et al. 2009, Saldaña et al. 2013); however, ours is the first to our knowledge to investigate this pattern while taking phylogeny into account. We detected significant phylogenetic signal in DT, suggesting that phylogeny should be considered when interpreting differences in DT between species (Table 2.2). Although we did find a significant relationship between degree of DT and environmental water potential using standard linear regression (results not shown), the relationship is not significant when

correcting for phylogeny with PGLS (Figure 2.5). Of the two main filmy fern clades, the hymenophylloid clade (i.e., *Hymenophyllum*) is much more uniform in morphology and habitat (mostly small, high elevation epiphytes) and has shorter divergence times between species than the trichomanoid clade (Figure 2.4) (Schuettpelz & Pryer, 2006; Hennequin *et al.*, 2008). It is likely that the short divergence times of *Hymenophyllum* decreased their influence in comparisons of DT between species once phylogeny was taken into account. In a more densely sampled study of morphological evolution in the trichomanoid clade, Dubuisson et al. (2013) identified several morphological changes including reduced stele and root systems associated with the evolution of epiphytic growth within the HE subclade (comprising the genera *Polyphlebium*, *Didymoglossum*, *Crepidomanes*, and *Vandenboschia*). It is possible that both reduced morphology and increased DT are important parts of an integrated adaptive strategy for epiphytic growth within this subclade of filmy ferns, and have evolved together repeatedly. However, Dubuisson et al. (2013) were unable to determine if these morphological changes had occurred independently, or if there was a single origin at the origin of the clade followed by multiple losses. Increased sampling of both morphological and physiological traits of additional trichomanoid taxa is needed to distinguish between these scenarios.

Concluding remarks and future directions—In filmy ferns, both sporophyte and gametophyte generations are poikilohydric; however, we have found here that this does not mean they necessarily share similar physiological optima. Although filmy fern sporophytes are often of small stature, elevating a leaf even a few cm above the substrate surface means escape from the boundary layer and exposure to substantially greater evapotranspiration. It is likely that even in this clade, wherein the physiological divide

between sporophyte and gametophyte is probably the smallest amongst ferns, the two generations experience substantially different selective pressures. Fossils indicate that some of the earliest vascular plants may have had sporophytes and gametophytes that were both branched and small (Kenrick and Crane 1997), but it is unclear at what point selective pressures began to diverge to favor large, complex sporophytes and small, simple gametophytes. The relevance of plant size to water relations is supported by a recent study showing increased DT in smaller size classes of the epiphytic fern *Asplenium auritum* Sw. (Testo and Watkins 2012). Future studies of DT in filmy ferns should include juvenile stages of the sporophyte, which are expected to experience similar microhabitats as gametophytes, to better determine the role of microhabitat vs. life stage (and plant size) on DT. Finally, more detailed analyses of the strategies used by each generation to cope with desiccation stress, such as transcriptomics, are needed to better understand the ecology and evolution of filmy ferns as well as the transition from desiccation tolerance to avoidance in vascular plants.

2.6 Acknowledgements

We thank the members of the Davis Lab for helpful discussions and comments on drafts. Saad Amer, Tohei Theophilus, and Suzanne Vinnette provided assistance with fieldwork and experiments. Staff at the University of California Berkeley's Richard B. Gump South Pacific Research Station, Moorea, French Polynesia, in particular Valentine Brotherson, Neil Davies, Hinano Teavai-Murphy, and Frank Murphy provided logistical support for fieldwork. Funding provided in part by National Science Foundation (Doctoral Dissertation Improvement Grant DEB-1311169 to JHN and CCD), Setup Funds from Harvard University to CCD, the American Society of Plant Taxonomists (Research Grant

for Graduate Students to JHN), the Garden Club of America (Award in Tropical Botany to JHN), the Harvard University Herbaria (Fernald Fieldwork Fellowship to JHN), the Society of Systematic Biologists (Graduate Student Research Award to JHN), and the Systematics Association (Systematics Research Fund to JHN).

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Chapter III:

Life in the Canopy: Comparative and Community Phylogenetic Analyses of Epiphytic Growth in Ferns

3.1 Abstract

Transitions from terrestrial to epiphytic growth have occurred in at least five large fern clades, and are associated with increased rates of diversification possibly coinciding with the appearance of angiosperm-dominated forests during the late Cretaceous and early Cenozoic. Despite the significance of epiphytic growth for fern evolution, however, few studies have investigated traits related to epiphytism in ferns while accounting for phylogeny. We investigate evolutionary patterns in traits related to epiphytic growth using a broadly sampled phylogeny that includes representatives of all five Cretaceous epiphytic fern radiations focusing on the ferns of Moorea, French Polynesia. We analyze traits at the species and community level, and recover evidence for strong phylogenetic conservatism in multiple traits related to epiphytic growth in both fern sporophytes and gametophytes. We find that epiphytes tend to have shorter stipes, smaller rhizomes, and a higher frequency of non-cordate gametophytes relative to terrestrial species, and that these correlations occur in multiple epiphytic clades independently. Consistent with a scenario of the epiphytic environment acting as a strong filter due to its low humidity and frequent drying events, we find that epiphytic communities are clustered both phylogenetically and functionally. Our results provide an important phylogenetic perspective on the role of functional traits in ferns, and pave the way for more detailed studies examining how niche space is filled within terrestrial and epiphytic habitats.

3.2 Introduction

The widespread appearance of angiosperm-dominated forests in the late Cretaceous and early Cenozoic had an immense effect on terrestrial ecosystems (Niklas et al. 1983, Kenrick and Crane 1997, Lloyd et al. 2008). The heterogeneous niche space of the

angiosperm canopy comprising stratified light and humidity levels and a range of substrates for growth became rapidly filled by a variety of epiphytic plants, including mosses (Fiz-Palacios et al. 2011), leafy liverworts (Feldberg et al. 2014), orchids (Givnish et al. 2015), bromeliads (Givnish et al. 2014), and ferns (Schneider et al. 2004). Diversification rates in some of these groups may have further increased during the Paleocene / Eocene thermal maximum, a period of globally warmer and wetter climates that may have enabled movement into the canopy (Schuettpelz and Pryer 2009). The transition to epiphytic growth has been particularly important for fern diversity, with ca. 29% of all species exhibiting this growth form relative to ca. 10% across all vascular plants (Kress 1986). It is thought that major transitions to epiphytism occurred at least five times during the evolution of ferns, each followed by explosive diversification during the late Cretaceous or early Cenozoic (Schuettpelz and Pryer 2009).

Despite the ecological opportunities afforded by life in the canopy, including escape from disturbance (Page 2002) and abundant light for photosynthesis (Benzing 2000), epiphytes must contend with greater abiotic stress in the form of increased evaporation and more extreme oscillations in temperature relative to terrestrial plants (Zotz and Hietz 2001, Lowman and Schowalter 2012). Associated with this change, epiphytic ferns have evolved various morphological adaptations that minimize water loss from their leaves, including shorter stipes (Watkins et al. 2010), thicker leaves (Kluge and Kessler 2007, Watkins et al. 2007c), and protection with scales or hairs (Watkins et al. 2006b). Some lineages have specialized adaptations to epiphytic growth, such as humus-collection (e.g., *Asplenium*, Benzing 1990; *Drynaria*, Janssen and Schneider 2005), vegetative desiccation tolerance (e.g., Hymenophyllaceae, Shreve 1911;

Pleopeltis, Pessin 1925), CAM photosynthesis (e.g., *Pyrrosia*, Wong and Hew 1976), and symbiotic relationships with ants (e.g., *Antrophyum*, Watkins et al. 2008; *Lecanopteris*, Gay 1993). Despite the evidence for such adaptations in multiple lineages (Dubuisson et al. 2009), no studies to our knowledge have analyzed the evolution of such traits across the fern evolutionary tree including representatives of all five Cretaceous epiphytic radiations *sensu* Schuettpelz and Pryer (2009). Furthermore, few studies comparing traits of epiphytic and terrestrial ferns have included traits of fern gametophytes, which grow independently of sporophytes. Fern gametophytes and sporophytes differ markedly in anatomy, morphology, and physiology, so it is expected that these life cycle stages will each experience unique selective pressures. For example, desiccation tolerance, a key trait for non-vascular plants living in water-limited environments, is prevalent in gametophytes of multiple lineages of epiphytic ferns, but is rare in sporophytes (Watkins et al. 2007a).

The majority of studies investigating adaptations to epiphytism have utilized a univariate, cross-species approach, analyzing traits individually between terrestrial and epiphytic species (e.g., Watkins et al. 2007c, 2010). However, given the evolutionary history of ferns with multiple radiations within the epiphytic niche (Schuettpelz and Pryer 2009), it is likely that traits related to epiphytism carry at least some degree of phylogenetic signal (i.e., the tendency for closely related species to resemble each other). Therefore, a comparative approach incorporating phylogenetic relationships (and if possible, branch lengths) is appropriate (Felsenstein 1985). However, such approaches have only recently been applied to studies of epiphytism in ferns, and at relatively limited phylogenetic scales (e.g., Zhang et al. 2014).

Furthermore, it should be possible to gain greater insight into the selective strength exerted by the epiphytic habitat by utilizing a community-based approach. If the relatively harsh abiotic conditions of the canopy act as a filter, we would expect to find lower functional richness at a community level, which should also result in decreased phylogenetic diversity under a scenario of phylogenetic trait conservatism (Webb et al. 2002). However, the relative strength of this environmental filter may also depend on overall abiotic conditions; for example, relative humidity and temperature are expected to vary not only between epiphytic and terrestrial environments, but also with elevation (Cardelús et al. 2006). Elevation is a strong predictor of fern species richness, with maximal richness tending to occur at mid-elevations, especially in the tropics (Kessler et al. 2011). Functional diversity is also correlated with species richness (as few species tend to have exactly the same functional traits; Schleuter et al. 2010). Thus, environmental variation along elevational gradients is expected to impact the functional, phylogenetic, and species diversity of epiphytic and terrestrial fern communities.

Here, we investigate the evolution and role in community assembly of functional traits related to epiphytic growth in fern gametophytes and sporophytes on Moorea, French Polynesia. Moorea is an isolated tropical oceanic island with a high phylogenetic diversity of ferns distributed over a steep elevational gradient, making it ideal for a community phylogenetic analysis of functional traits. Specifically, we test the following hypotheses related to epiphytic growth in ferns: 1). The epiphytic environment experiences greater extremes in temperature and humidity than the terrestrial environment, thus acting as a filter on the traits of plants that grow there. 2). Epiphytic ferns have converged upon similar morphologies multiple times in both sporophyte and

gametophyte phases. 3). Epiphytic fern communities have lower functional and phylogenetic diversity relative to terrestrial communities due to environmental filtering, and the strength of this filtering varies with changes in environment.

3.3 Methods

Study Site—Moorea, French Polynesia (17°30'S, 149°50'W) is a small (135 km^2) tropical oceanic island located more than 5000 km from the nearest continental landmass. This distance acts as a strong barrier to dispersal (Carson and Clague 1995, Dassler and Farrar 2001), which combined with the young age of the island (ca. 1.5 Ma; Duncan and McDougall 1976) has led to a relatively small, yet phylogenetically diverse fern flora (ca. 130 spp., 8/11 orders *sensu* Smith et al. 2006). The ferns of Moorea include representatives of all five of the Cretaceous epiphytic radiations described by Schuettpelz and Pryer (2009) and their terrestrial relatives, making them suitable for a comparative study of traits related to the evolution of epiphytic growth in ferns.

Community Survey—A fern community survey was carried out on Moorea as part of a separate study including 17, 10 × 10 m plots spanning an elevational gradient from 200 to 2000 m, with plots placed ca. every 200 m (Nitta et al., in review). Most plots were located along trails leading to the three main peaks on the island, Mt. Rotui (899 m), Mt. Mouaputa (890 m), and Mt. Tohiea (1206 m, highest point on Moorea). Each plot was divided into 25, 2 × 2 m subplots. Presence/absence of all fern sporophytes in each subplot was scored and summed to produce an abundance ranking for each species per plot from zero (not observed in any subplots) to 25 (observed in all subplots). Epiphytic ferns were either sampled by hand to ca. 2 m on tree trunks or confirmed visually from the ground. Species were categorized as either epiphytic or terrestrial based

on field observations (see Trait Selection and Measurement below). The list of observed species in each plot was split into epiphytic and terrestrial groups and treated separately during data analysis, hereafter referred to as epiphytic and terrestrial plots. Voucher specimens were deposited at GH and UC.

Environmental Survey—Temperature and relative humidity (RH) of epiphytic and terrestrial habitats were measured using Hobo Pro v2 dataloggers with the RS3 Solar Radiation Shield (Onset Corporation, USA). A pair of dataloggers was installed for each plot, one mounted at ca. 2 m on a tree ("epiphytic datalogger"), and one at ground level ("terrestrial datalogger"). Temperature and RH were logged once every 15 min during two survey periods: a preliminary survey from July 18, 2012 to July 7, 2013 at a subset of sites, and a final survey from July 7, 2013 to July 5, 2014 at all sites. We used the 2013–2014 data for the final analysis, with one exception: the terrestrial datalogger at the Mt. Tohiea 393 m site failed near the start of the final survey period, so we used data from the preliminary survey period for this datalogger instead. Days missing data for any datalogger were excluded from the final dataset. The final dataset included 201 days of data for 13 epiphytic plots and 13 terrestrial plots ($N = 26$ plots total).

Trait Selection and Measurements—We selected several traits that have been hypothesized to be important for epiphytic growth, summarized in Table 3.1 and Figure 3.1. We preferred morphological traits over physiological traits for our study because data for morphological traits are much easier to gather at the scale of our study (ca. 130 spp. distributed over an elevational gradient from 200 to 1200 m). In addition, plant morphology and physiology are closely linked, and physiological traits such as rates of photosynthesis and water use efficiency are known to be correlated with morphological

Figure 3.1 Examples of traits used in this study. A) Sporophyte traits. All sporophyte traits were quantitative, including frond length and width, stipe length, rhizome dia., number of pinna pairs (in this example, nine) and degree of lamina dissection (in this example, once-pinnate). Specific leaf area (ratio of area to mass of leaf lamina) not depicted. **B)** Gametophyte traits. Morphotype was defined as a binary trait, either cordate or non-cordate. Examples of non-cordate morphotypes include ribbon (thallus elongate and two-dimensional) and filamentous (thallus single lines of cells). Other gametophyte traits (gemmae, hairs, and glands) were scored as present or absent. Arrows point out instances of each binary trait. Scalebars 1 mm except for glands, which is 0.1 mm. Photographs by J. H. Nitta.

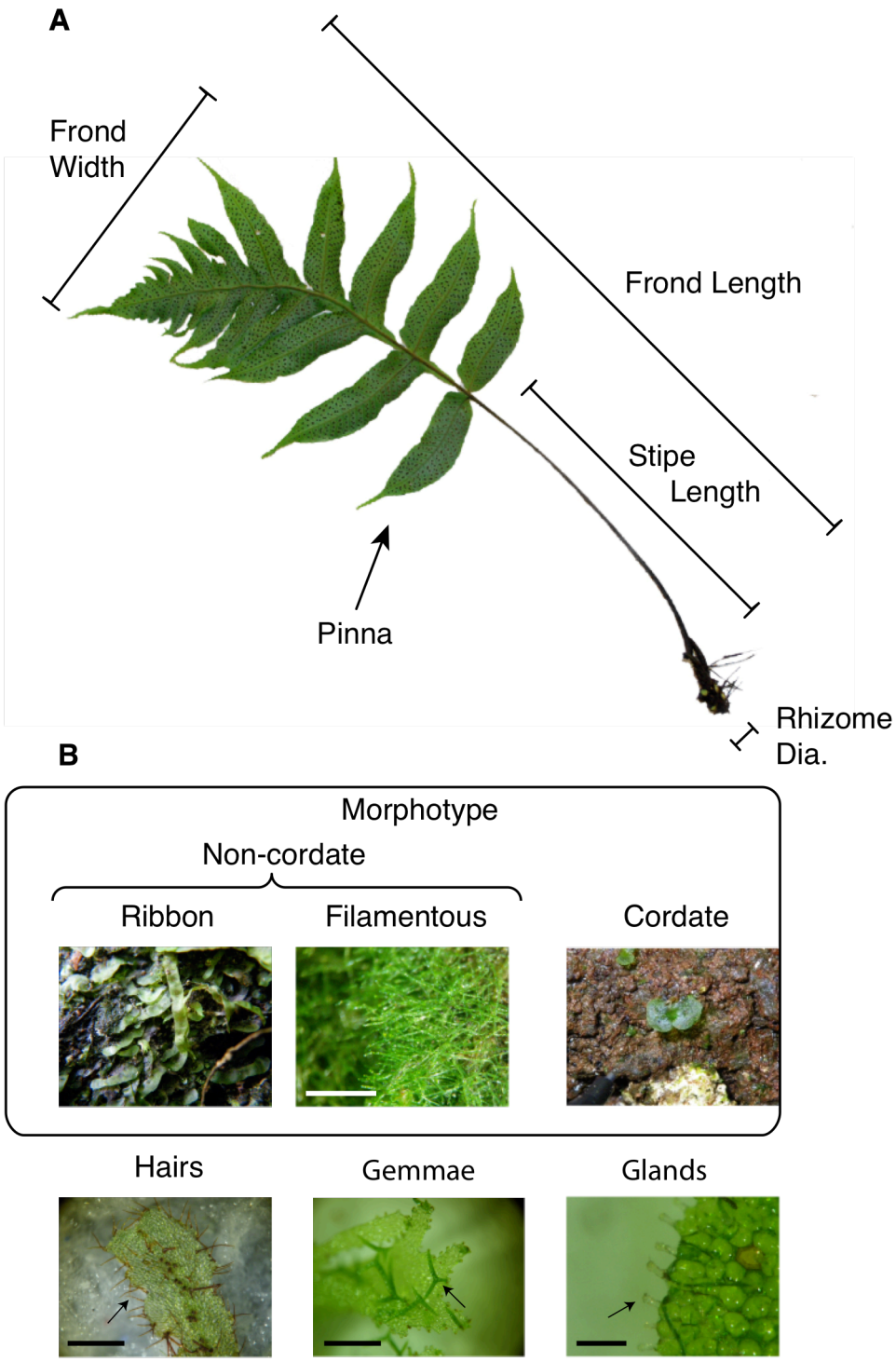

Figure 3.1 (Continued).

traits such as stipe length in ferns (Watkins et al. 2010). Studying the relationships between ecology and morphological traits is also useful from a paleobotanical and climate change perspective, as trends in morphological trait variation in the fossil record can be used to gain insight into past climatic conditions (Chaloner and McElwain 1997, McElwain and Punyasena 2007, Mcelwain et al. 2007, Royer et al. 2008).

Morphological traits of sporophytes were measured from herbarium specimens for most species, or obtained from the literature when plant material was unavailable. Measurements were made directly on plants in the field for a few species with very large fronds. A single leaf or rhizome per individual was measured on one to three separately collected individuals per species, and mean values were calculated for each species. Although trait values have been found to vary within fern species with elevation (Kessler et al. 2007), our study includes a broad phylogenetic sampling across all ferns, so we assume intraspecific variation is greatly outweighed by interspecies differences. To measure specific leaf area (SLA), ca. 10 – 12, 2 or 4 mm dia. punches were taken from leaf lamina of a single leaf per individual between primary veins using biopsy punches, dried at 60˚C for 24 h, and weighed. SLA was calculated as the ratio of area (m^2) to mass (kg) of each leaf punch, and mean values used for each species. For species with narrow laminae (less than 2 mm between primary veins), leaf fragments not including primary veins were obtained by dissection, dried at 60˚C for 24 h, weighed, and scanned. ImageJ software (http://imagej.nih.gov/ij) was used to measure area per fragment, and SLA calculated in the same way as leaf punches.

Morphological traits of gametophytes were all qualitative (either categorical or binary; Table 3.1). Trait states were scored by observing gametophytes that were either

Table 3.1 Traits used in this study. Noncordate gametophyte morphotypes include ribbon, strap and filamentous.

	Trait	Data type (unit)	Ecological Significance
Sporophyte Traits	Stipe Length	Continuous (cm)	Shorter stipes compensate for low conductivity (Watkins et al. 2010)
	Frond Length	Continuous (cm)	Smaller leaf size reduces evapotranspiration (Vogel 1968)
	Frond Width	Continuous (cm)	Smaller leaf size reduces evapotranspiration (Vogel 1968)
	Rhizome Diameter	Continuous (cm)	Scales with overall plant size (Creese et al. 2011)
	Frond Dissection	Ordinal	Simple laminae reduce evapotranspiration (Kluge and Kessler 2007)
	Pinna number	Integer	Scales with overall plant size (Creese et al. 2011)
	Specific Leaf Area	Continuous (m^2 kg^{-1})	Species in more stressful environments invest in thicker leaves (Wright et al. 2004)
Gametophyte Traits	Morphotype	Binary (cordate vs. noncordate)	Noncordate morphology reduces drying rates by holding external water in crevices and folds (Watkins et al. 2007a, Pittermann et al. 2013)
	Gemmae	Binary (present/absent)	Enable asexual reproduction, thereby overcoming local extinction due to drying (Farrar et al. 2008)
	Glands	Binary (present/absent)	Lipophilic exudates produced by glands may reduce transpiration rates (Wollenweber and Schneider 2000)
	Hairs	Binary (present/absent)	Hairs reduce drying rates by holding external water and increasing boundary layer thickness (Watkins et al. 2007a)

collected in the field and identified using DNA barcoding (Nitta et al. in review) or grown from spores of known species in the lab, or taken from the literature. For comparative analysis, gametophyte morphotypes were aggregated into two categories: either cordate (i.e., heart-shaped) or non-cordate. Non-cordate morphotypes include elongate (strap or ribbon *sensu* Farrar et al. [2008]) and filamentous forms (Figure 3.1B). Gametophytes were cultivated on Bold's media (Bold 1957) supplemented with Nitch's micronutrients (Nitsch 1951).

Growth habit was coded as a binary trait (epiphytic or terrestrial) based on field observations. Some species do not fall clearly into either category (e.g., hemi-epiphytes; Benzing 1990, Dubuisson et al. 2003, Nitta and Epps 2009). We used connection to the soil as the criterion for defining binary growth habit; thus, epipetric plants were treated as epiphytic, and hemi-epiphytes and climbing plants were treated as terrestrial. For the purposes of this study, we treated growth habits as a fixed trait and used these categories to define epiphytic and terrestrial communities. Other studies have demonstrated that exceptional epiphytic growth of terrestrial fern species (and vice-versa) is extremely rare (Cardelús et al. 2006, Kluge and Kessler 2006, Watkins and Cardelús 2009), and we do not believe that such rare exceptions would affect our analysis.

Statistical Analysis—We calculated mean daily maximum, mean, minimum, and standard variation of temperature and RH from the raw climate data, then tested for differences in these summary statistics between epiphytic and terrestrial plots using analysis of covariance (Scheffers et al. 2013). We used daily mean values of temperature and RH each in turn as the response variable, and constructed linear models with growth habit as the factor and elevation as the covariate, and with their interaction to test for

differences in response of climatic variables to elevation between epiphytic and terrestrial plots. We also calculated the number of drying events at various minimum RH values, as well as daily mean values for water potential, but found that these were both highly correlated with mean RH and did not analyze them further (data not shown).

For comparative analyses, we used the dated phylogenetic tree constructed in Nitta et al. (in review), which includes all species from Moorea as well as some from the neighboring island of Tahiti. We trimmed the tree to species occurring only on Moorea (N = 130 spp.) as appropriate during the analysis using the "drop.tip" function of the "ape" package (Paradis et al. 2004) in R (R Core Team 2015). We tested for phylogenetic signal in quantitative traits using two metrics that relate the observed trait values to those expected under a model of trait evolution following Brownian motion (BM), Blomberg's K (Blomberg et al. 2003) and Pagel's λ (Pagel 1999), with the function "phylosig" in the "phytools" R package (Revell 2012). λ is a scaling parameter that ranges from 0 (traits evolving randomly, as on a star phylogeny) to 1 (traits evolving along branches according to Brownian motion). K describes the ratio between the amount of observed variance in traits vs. the amount of variance expected under a BM model of trait evolution: when K = 1, traits are evolving according to BM; when $K > 1$, traits are more conserved than expected under BM; when $K < 1$, traits have less phylogenetic signal than expected under BM (Blomberg et al. 2003). We tested for statistical significance in K by comparing observed values of K against a null distribution of 1000 trees with trait values shuffled randomly across the tips, and in λ by conducting a likelihood ratio test comparing the log likelihoods of the observed value of λ vs. λ = 0 (no phylogenetic signal). We tested for phylogenetic signal in qualitative traits using Fritz and Purvis' D (Fritz and Purvis 2010),

which is designed to test for signal in binary traits. Values of *D* range from 0 under a model of trait evolution by BM, and 1 for random distribution of traits; negative values (greater trait conservation than BM) and values greater than 1 (trait overdispersion) are possible. We analyzed *D* using the "phylo.d" function in the "caper" R package (Orme et al. 2013), which also conducts a significance test by comparing the observed value of *D* with distributions of simulated values produced under one of two models: random shuffling of traits on the tree, or simulation of a binary trait under a BM threshold model. The probability of obtaining the observed value of *D* under either model is then calculated.

We used a multivariate approach to investigate differences in traits between epiphytes and terrestrial species. We used only quantitative traits, and included only species that had no missing data for any traits ($N = 104$ spp.). We first log-transformed traits as necessary to attain normality, then scaled each trait by its range. We subjected the transformed, scaled traits to a Principal Components Analysis (PCA) to reduce their dimensionality. We conducted both a standard and a phylogenetically corrected PCA, using the "PCA" and "phyl.pca" functions in the "FactoMineR" (Lê et al. 2008) and "phytools" R packages, respectively. The position of each species was plotted in the multi-dimensional trait space using the first two PC axes, then color-coded according to growth habit (epiphytic vs. terrestrial).

We detected significant phylogenetic signal in most traits, so we tested for differences between traits of epiphytic vs. terrestrial species using methods that can test for significant differences between traits associated with a binary character while accounting for phylogeny. For quantitative traits, we used the "brunch" function in the R

package “caper” (Orme et al. 2013), which calculates phylogenetically independent contrasts in a quantitative character between alternative states of a binary trait. For qualitative (binary) traits, we used Pagel’s test of correlated evolution (Pagel 1994) as implemented with the “fitPagel” function in the R package “phytools” (Revell 2012). This method compares the likelihood between two alternative models for a pair of binary traits. The first model tests the null hypothesis that rates of evolution of the two traits are independent. The second model tests the alternative hypothesis that the rates of evolution of one trait depends on the other. The likelihoods of obtaining the observed data are calculated for each model, then compared using a log likelihood test. A significantly better fit of the dependent model indicates that evolution of the two traits is correlated.

To verify the effect of analysis method on our results, we performed alternative tests for correlation of growth habit with functional traits using phylogenetic generalized linear mixed models (PGLMMs). Models were constructed with each trait as the response variable in turn dependent on growth habit, with phylogeny as a random effect. For binary (gametophyte) traits we used the “binaryGLMM” function in the “ape” R package (Paradis et al. 2004). For quantitative (sporophyte) traits, we used the “MCMCglmm” function in the R package of the same name (Hadfield 2010). Priors were set to an inverse-Gamma distribution with shape and scale parameters equal to 0.01 (Hadfield and Nakagawa 2010). The MCMC algorithm was run for 5,000,000 iterations with a burnin of 1000 and thinning every 500 iterations.

We analyzed community phylogenetic diversity using the Mean Phylogenetic Distance (MPD) and Mean Nearest Taxon Distance (MNTD) indices of Webb (2000). MPD is the mean phylogenetic distance of each species in a community to all other

species in the same community; MNTD is the mean phylogenetic distance of each species in a community to its sister species in the same community. Communities with low MPD or MNTD are "phylogenetically clustered" (co-occurring species are closely related to each other), whereas those with high MPD or MNTD are "phylogenetically overdispersed" (co-occurring species are distantly related to each other). MPD therefore provides a measure of phylogenetic diversity across the whole tree, while MNTD is more sensitive to distances towards the tips. Alone, MPD and MNTD are not very informative; to assess their statistical significance, we calculated their standard effect sizes (SES) by comparing observed values to a distribution of 1000 randomized communities (equivalent to the Net Relatedness Index and Nearest Taxon Index, respectively, of Webb [2000] with the sign reversed). Observed values above 95% or less than 5% of the null distribution were considered significant. Selection of the randomization procedure can strongly bias outcomes (Gotelli and McGill 2006, Ulrich et al. 2012). Our null hypothesis is that fern spores are easily capable of dispersal between terrestrial and epiphytic habitats, and across the relatively short distances separating our plots. We therefore constructed null communities by drawing species randomly from a regional pool including terrestrial and epiphytic taxa. The regional pool included all known species of ferns from Moorea, as well as all species from the neighboring island of Tahiti with genetic data available (N = 146 spp. total; Nitta et al., in review). We used the "ses.mpd" and "ses.mntd" functions in the R package "picante" (Kembel et al. 2010) to calculate community phylogenetic diversity metrics.

We analyzed community functional trait diversity using the "dbFD" function in the "FD" R package (Laliberté and Shipley 2011). This function takes as input values any

number of traits for species in a given community, plots them in a multidimensional trait space using PCA, and calculates several summary statistics. Functional richness (FRic) is equivalent to convex hull volume containing all observed trait values plotted in multidimensional trait space (Cornwell et al. 2006), functional evenness (FEve) is the average distance to the center of all the traits, and functional dispersion (FDis) is the mean distance between traits (Villéger et al. 2008). We chose these three metrics because they are statistically independent and can each provide different information about the structure of community trait diversity (Savage and Cavender-Bares 2012). Functional richness does not use abundance data, but functional evenness and divergence can be weighted by abundance, so we preformed analyses for these metrics with abundance weighting to correct for the effect of rare species with unusual morphologies. Selection, transformation, and scaling of traits was performed similarly as for the PCA, except that the method used to calculate distance (Gower 1971) can accommodate missing data, so we included some species with observations missing for some traits (N = 126 spp. total). We calculated each metric separately for epiphytic and terrestrial communities, and compared results between the two. Under a scenario of environmental filtering (as hypothesized for epiphytic communities), we expect relatively lower values for the three metrics of functional diversity (Savage and Cavender-Bares 2012). We also investigated relationships between functional trait diversity and environment with linear models by testing for dependence of each functional trait diversity metric against elevation and daily minimum RH. We chose these independent variables because we found that overall climate tended to change with elevation, and to test if minimum RH specifically had a filtering effect on diversity along the gradient.

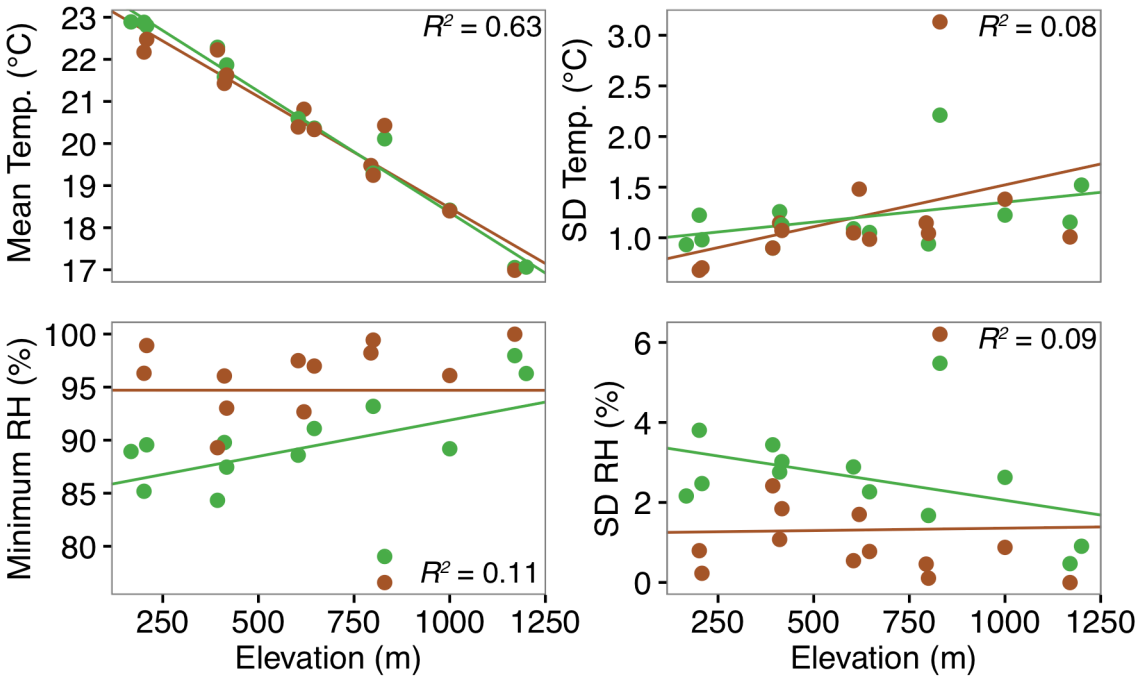

Figure 3.2 Selected microclimatic variables of study plots along an elevational gradient from 200 to 1200 m on Moorea, French Polynesia. Relative humidity (RH) and temperature were recorded every 15 min with dataloggers from July 7, 2013 to July 5, 2014, and overall means calculated for daily mean, minimum, maximum, and standard deviation (SD). Color indicates growth habit: epiphytic plots in green, terrestrial plots in brown. Trendlines fitted using linear models with an interaction between growth habit and elevation (see 3.3 Methods); all trendlines significant at $P < 0.05$.

3.4 Results

Environmental survey—There were significant interaction effects between growth habit and elevation on all microclimatic variables except for minimum temperature (Table C1). Slope and intercept for variables related to temperature were very similar between epiphytic and terrestrial plots, but differed more strongly for those related to humidity (Table C1, Figure 3.2). Minimum RH increased 0.68% per 100 m

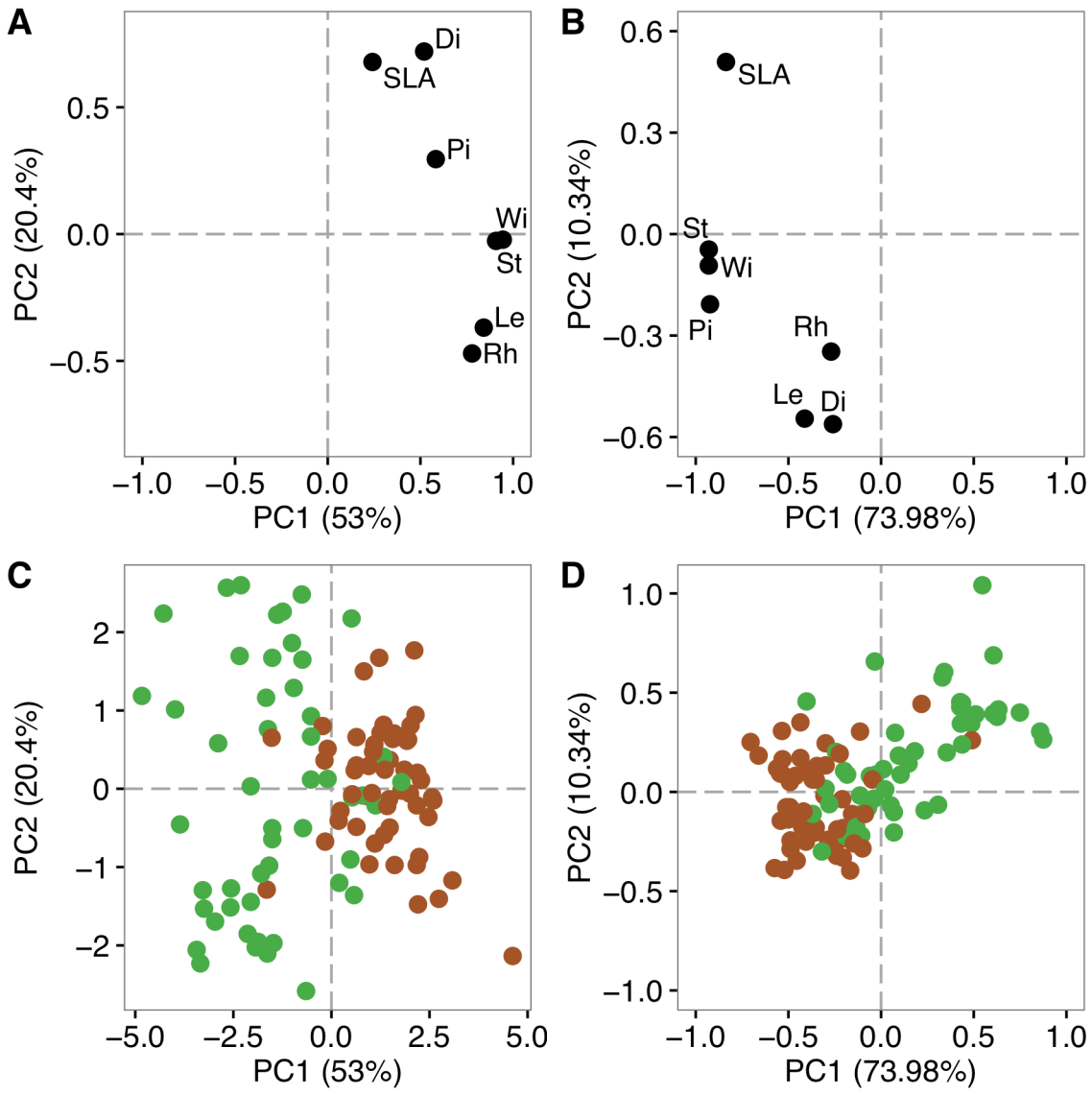

Figure 3.3 Principal components analysis (PCA) of traits related to epiphytic growth in ferns from Moorea, French Polynesia. A–B) PC loadings. **C–D)** species scores. A and C are standard PCA, B and D are phylogenetic PCA. Epiphytes in green, terrestrial species in brown. Quantitative traits only, including only species with no missing observations for any traits (N = 104 spp.). Abbreviations for traits as follows: Di, degree of frond dissection; Le, frond length; Pi, number of pinna pairs; Rh, rhizome diameter; SLA, specific leaf area; St, stipe length; Wi, frond width.

Figure 3.3 (Continued) For description of traits used in analysis, see Table 3.1. PC loadings for each trait summarized in Table C2.

elevation for epiphytes, but was nearly flat (decrease of 0.001% per 100 m) for terrestrial plots (linear model, R^2 =0.114, $P < 0.001$). The Mt. Rotui 830 m site was a clear outlier, with greater mean temperature and lower minimum RH relative to other sites at similar elevation (outliers in Figure 3.2 at ca. 800 m). Mt. Rotui is a solitary peak located between Cook's Bay and Oponohu Bay. It is more isolated with greater exposure than the other two mountains where the rest of the plots were established (J. Nitta, pers. obs.), which are both part of a mountain complex on the interior of the island.

Principal components analysis of trait data—Terrestrial and epiphytic species are partly differentiated in trait space, occupying mostly distinct areas but overlapping in the middle regardless of method used (standard or phylogenetic PCA) (Figure 3.3C, D). The first two PC axes explained 73.4% and 84.3% of the variation in the trait data when analyzed using standard and phylogenetic PCA, respectively. In both analyses, stipe length and frond width were loaded on PC1, and SLA, degree of frond dissection, and frond length were loaded on PC2 (Figure 3.3A, B, Appendix C2). Results for number of pinna pairs differed between the two analyses, tending toward PC2 in the standard PCA and PC1 in the phylogenetic PCA (Appendix C2).

Phylogenetic signal—Most measured traits showed some degree of phylogenetic signal, but the strength varied across traits (Table 3.2, Figure 3.4), and for quantitative traits, different results were obtained for λ and *K*. When measured with λ, most sporophyte traits show phylogenetic signal (as expected under a BM model), with values of λ close to 1; only number of pinna pairs had λ close to zero. However, when measured

Table 3.2 Results of phylogenetic signal analysis for growth habit and related traits. Values of λ or *K* >= 1 indicate that traits are evolving according to Brownian Motion (BM); values of λ or *K* near 0 indicate random distribution of traits. For the *D* statistic, values of 0 or less indicate evolution according to BM; values near 1 indicate random trait distribution. PRnd, probability of random distribution of traits; PBM, probability of traits evolving under BM. Number of asterisks indicates statistical significance: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$.

	λ	*K*	*D*
Growth Habit	-	-	-0.21 (PRnd = 0, PBM = 0.769)
Stipe Length	0.74***	0.22**	-
Frond Length	0.91***	0.48**	-
Frond Width	0.98***	0.63**	-
Rhizome Diameter	1***	1.31**	-
Frond Dissection	0.9***	0.42**	-
Pinna number	0.2*	0.17*	-
Specific Leaf Area	0.72**	0.1 (NS)	-
Glands	-	-	-0.66 (PRnd = 0, PBM = 0.971)
Hairs	-	-	-0.15 (PRnd = 0.004, PBM = 0.616)
Gemmae	-	-	-1.26 (PRnd = 0, PBM = 0.998)
Morphotype	-	-	-0.64 (PRnd = 0, PBM = 0.975)

with *K*, only rhizome diameter showed strong phylogenetic signal (more than expected under BM); other traits had values of $K < 1$. All binary gametophyte traits showed significant phylogenetic signal, either similar to (hairs, $D = -0.15$) or more conserved (glands, $D = -0.66$; morphotype, $D = -0.64$) than expected under a scenario of evolution by BM (Table 3.2). Phylogenetic generalized linear mixed models (PGLMMs) also indicated significant signal in all gametophyte traits (Table C3). Growth habit showed phylogenetic signal close to that expected under BM ($D = -0.2$).

Table 3.3 Results of phylogenetically corrected analyses of traits related to epiphytic growth. A) Phylogenetically independent contrasts analysis of quantitative (sporophyte) traits. All trait contrasts were made by subtracting epiphytic values from terrestrial values; thus, a positive contrast indicates greater values for terrestrial clades. Traits with significant differences between epiphytes and terrestrial clades shown in bold. **B)** Pagel's (1994) test of correlated evolution for quantitative (gametophyte) traits. Each gametophyte trait was coded as a binary trait and tested for correlated evolution with growth habit. Traits with a significantly lower log likelihood for the dependent model are significantly correlated with growth habit, and shown in bold.

Table 3.3 (Continued).

A) Quantitative (sporophyte) traits

Trait	Number total contrasts	Number positive contrasts	*t*-value	*P*
Stipe Length	**21**	**20**	**3.9535**	**8.00E-04**
Frond Length	**21**	**15**	**2.4015**	**0.0262**
Frond Width	**21**	**18**	**2.7209**	**0.0132**
Rhizome Diameter	**20**	**18**	**2.7963**	**0.0115**
Frond Dissection	**20**	**16**	**3.3479**	**0.0034**
Pinna number	21	15	1.7345	0.0982
Specific Leaf Area	21	16	1.9552	0.0647

B) Binary (gametophyte) traits

Trait	Log likelihood (independent model)	Log likelihood (dependent model)	Likelihood ratio	*P*
Morphotype	**-106**	**-96.5**	**18.2**	**0.00111**
Glands	-89.7	-108	-35.8	1
Hairs	-74.1	-71	6.28	0.18
Gemmae	-70.2	-66.7	6.96	0.138

Figure 3.4 Time-calibrated phylogenetic tree of ferns from Moorea, French Polynesia with growth habit and associated traits mapped on the tips. Relative value of quantitative (sporophyte) traits in greyscale: low values are lighter, high values are darker. States of qualitative (gametophyte) states and growth habit indicated by colors in key. Slashes indicate missing data or non-applicable trait states. Trends for three leaf size traits (stipe length, frond length, and frond with) were quantitatively very similar, so of these, we only present stipe length. Species missing data for six or more traits not shown. For a summary of traits, see Table 3.1. Abbreviations for clades of interest are as follows. Major Cretaceous epiphytic radiations *sensu* Schuettpelz and Pryer (2009) in green: H, hymenophylloids; T, trichomanoids, V, vittarioids; A, asplenioids; E, elaphoglossoids; P, polygrammoids (trichomanoids diversified prior to the Cretaceous but are included as an important extant epiphytic clade). Other major clades: EI, eupolypods I; EII, eupolypods II.

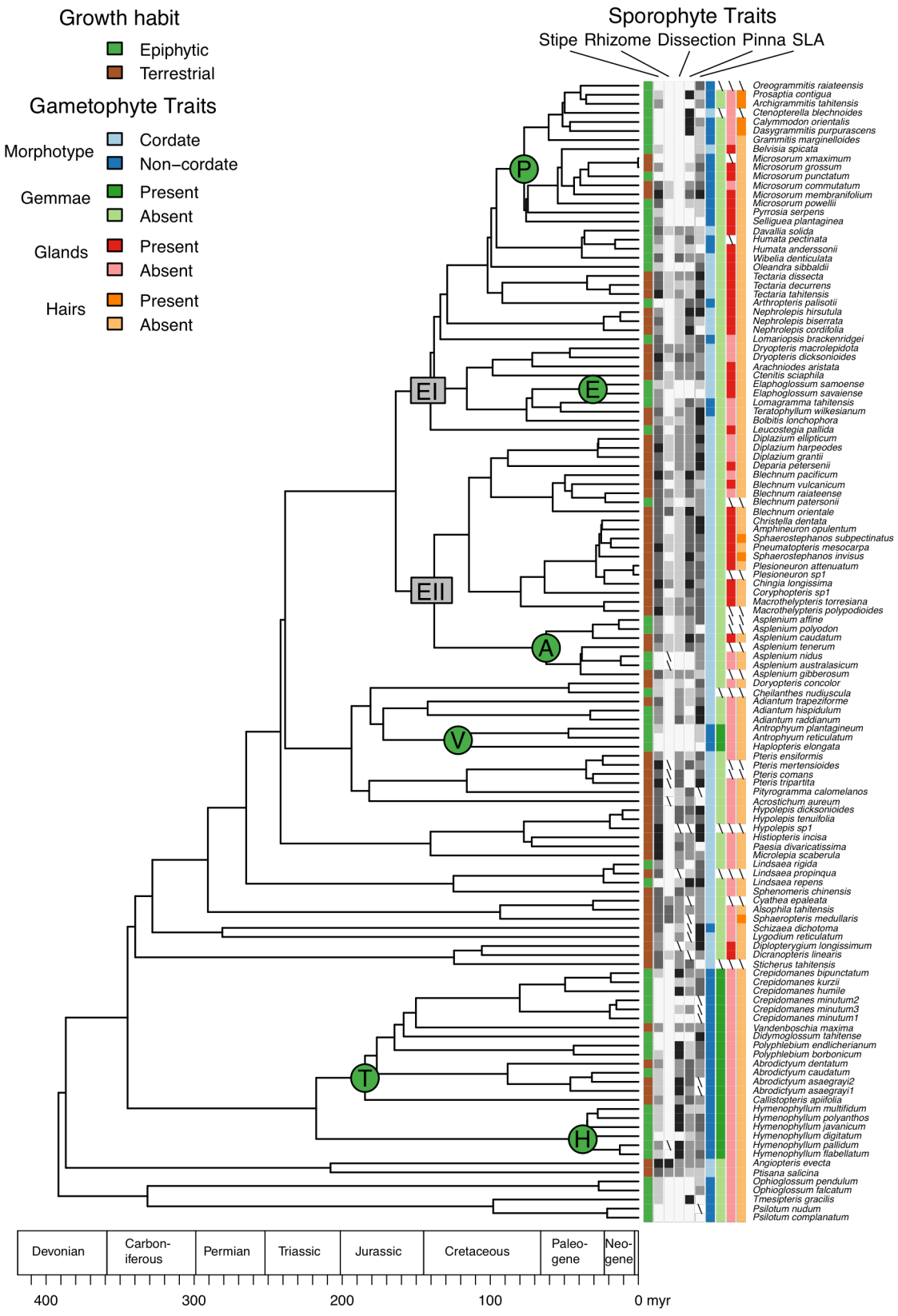

Figure 3.4 (Continued).

Correlation of traits with growth habit—Most sporophyte traits, including all those related to plant size, are significantly correlated with growth habit while taking phylogeny into account (Table 3.3). Epiphytic species tend to have smaller values for all traits (Table 3.3; contrasts were made by subtracting epiphytic from terrestrial values). Short stipe length in particular appears to be strongly correlated with epiphytic growth (phylogenetically independent contrasts, $P < 0.001$), whereas SLA was marginally non-significant (P = 0.0647) and number of pinna pairs showed no correlation (P = 0.0982). For the gametophyte traits, only morphotype (non-cordate vs. cordate) was significantly correlated with growth habit (Pagel's test of correlated evolution, $P = 0.0011$; Table 3.3). Quantitatively similar results were obtained using PGLMMs, except that no significant relationship was observed between rhizome diameter and growth habit, whereas SLA did show a significant relationship with growth habit (Table C3).

Functional and phylogenetic diversity—On a single-trait basis, distribution of community-weighted mean values (CWMs) showed significant correlation with elevation for some traits, but no significant relationship was detected for any traits with minimum RH (Figure 3.5A). SLA decreased with elevation for epiphytes (linear model, $R^2 = 0.6787$, $P < 0.001$) and terrestrial (linear model, $R^2 = 0.8118$, $P < 0.001$) communities. Degree of lamina dissection decreased with elevation in epiphytes (linear model, $R^2 = 0.3634$, $P = 0.0104$), but not terrestrial communities. Stipe length showed a very weak, but significant, increase with elevation for epiphytic communities (linear model, $R^2 = 0.2721$, $P = 0.0317$). Rhizome diameter decreased with elevation for terrestrial communities (linear model, $R^2 = 0.238$, $P = 0.047$), but increased slightly in epiphytes (linear model, $R^2 = 0.3525$, $P = 0.0120$).

Figure 3.5 Mean values of selected functional morphological traits of epiphytic and terrestrial ferns on Moorea, French Polynesia. Epiphytic values in green, terrestrial values in brown. **A)** Community-weighted mean (CWM) values for each trait plotted against either minimum relative humidity ($N = 13$ communities each for epiphytes and terrestrial species) or elevation ($N = 17$ communities each). Trendlines indicate significant relationships determined by a linear model at $P < 0.05$. **B)** Mean values for all species by growth habit for each trait. Numbers of species with observations available differed for each trait and are shown at bottom of each barplot. Asterisks indicate statistical significance of one-sided *t*-test; *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$. Trends for three leaf size traits (stipe length, frond length, and frond with) were quantitatively very similar, so of these we only present stipe length.

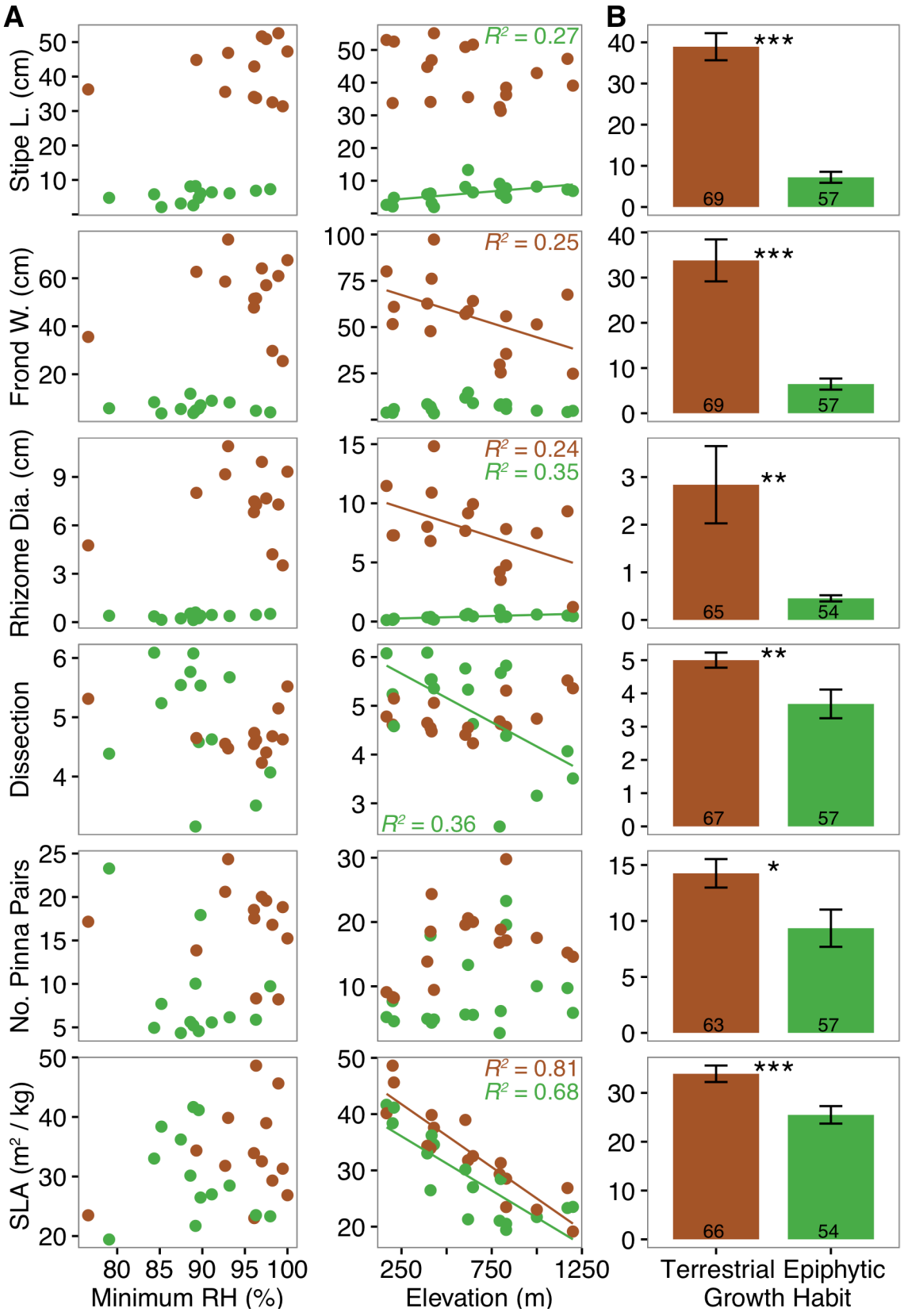

Figure 3.5 (Continued).

Figure 3.6 Functional and phylogenetic diversity of epiphytic and terrestrial fern communities on Moorea, French Polynesia. Epiphytic communities in green, terrestrial communities in brown. Response variable abbreviations as follows: MPD, standard effect size (SES) of mean phylogenetic distance; MNTD, SES of mean nearest taxon distance; FRic, functional richness; FEve, functional evenness; FDiv, functional diversity. **A)** Response variables plotted against either minimum relative humidity ($N = 13$ communities each for epiphytes and terrestrial species) or elevation ($N = 17$ communities each). Trendlines indicate significant relationships determined by a linear model at $P < 0.05$. **B)** Median values (box plots) of response variables by growth habit (epiphytic or terrestrial) across all communities (epiphytic communities, $N = 17$; terrestrial communities, $N = 17$). Medians shown with bold lines, lower and upper hinges correspond to first and third quartiles, and whiskers extend to values within 1.5 × the interquartile range. Asterisks indicate statistical significance of one-sided *t*-test; *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

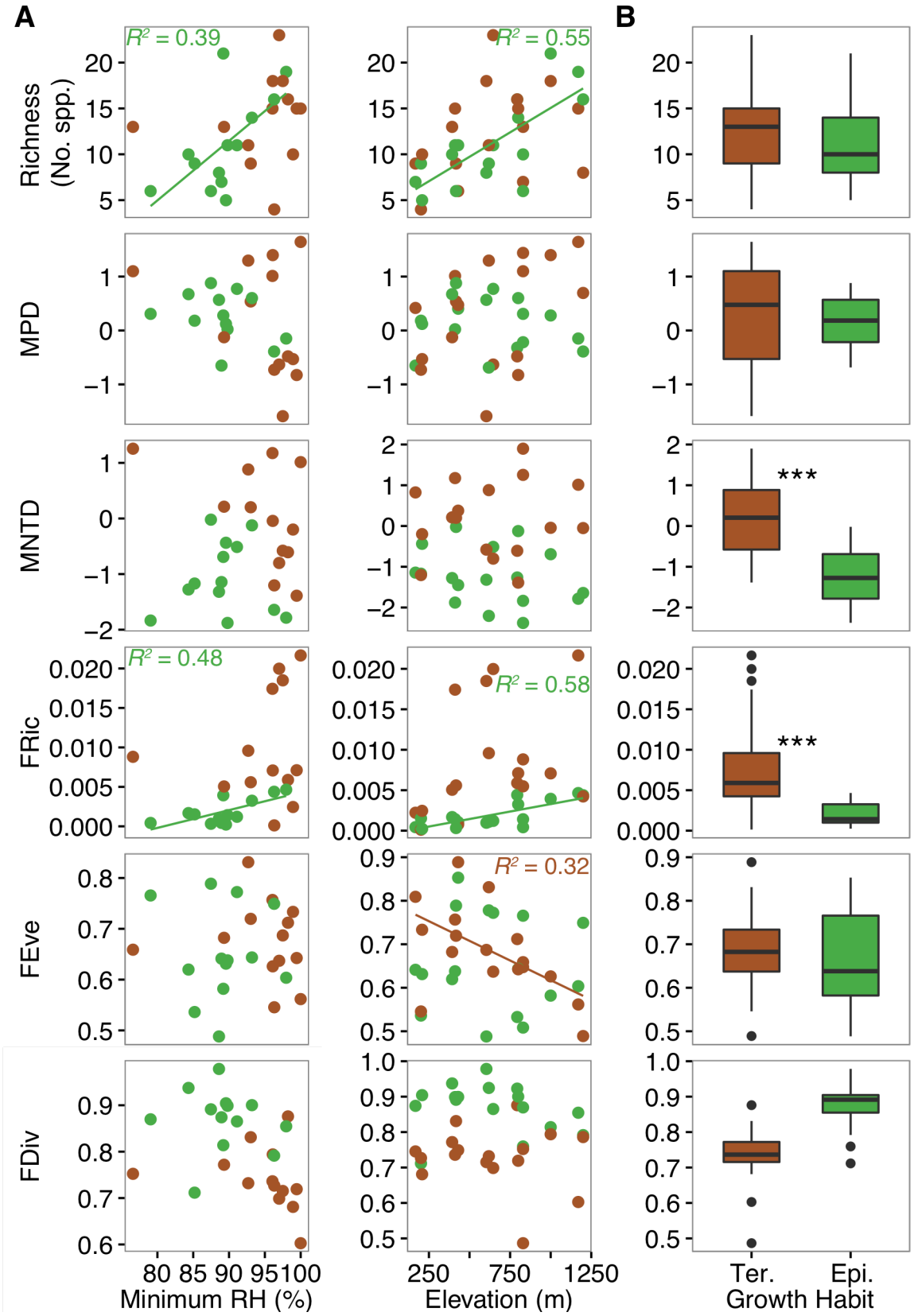

Figure 3.6 (Continued).

We observed a total of 104 species across all plots, including 48 epiphytic and 56 terrestrial species. Mean species richness was 11.12 ± 4.6 spp. per plot for epiphytes and 12.35 ± 5.0 spp. per plot for terrestrial species (not significantly different; one sided *t*-test, $P = 0.23$; Figure 3.6A). Species richness increased linearly with elevation and minimum RH for epiphytes, but not terrestrial communities (Figure 3.6B). Trait richness (FRic) was lower in epiphytic vs. terrestrial communities overall (one sided *t*-test, $P < 0.001$), whereas functional evenness (FEve) and diversity (FDiv) did not differ between the two groups. FEve decreased with elevation, but not minimum RH, in terrestrial communities (Figure 3.6A). Epiphytic communities showed a trend of increasing trait richness along the environmental gradient, with greater FRic at high elevations (linear model, $R^2 = 0.5836$, $P < 0.001$) and sites with greater minimum RH (linear model, $R^2 = 0.6626$, $P = 0.009$). Epiphytic communities were more phylogenetically clustered than terrestrial communities overall when measured using MNTD (one sided *t*-test, $P < 0.001$) but not MPD ($P = 0.30$; Figure 3.6B). No significant trend was detected in phylogenetic community structure with environment in either terrestrial or epiphytic communities (Figure 3.6A).

3.5 Discussion

Here, we show for the first time using a broadly sampled phylogenetic dataset that morphological traits related to epiphytic growth are strongly evolutionarily conserved, appearing consistently in multiple unrelated groups. The canalized morphology of epiphytic ferns suggests that this habitat strongly filters for a specialized growth form. We find that communities of epiphytic ferns are more clustered functionally and phylogenetically relative to terrestrial species, again supporting the role of environmental

filtering in determining epiphytic fern community structure. Our results are similar between gametophyte and sporophyte traits, suggesting that morphology of each life stage is important for epiphytic growth.

Effect of phylogeny on analysis of epiphytic traits—Although several other studies have addressed the relationship of functional traits with growth habit in ferns (Hietz and Briones 1998, Kluge and Kessler 2007, Watkins et al. 2010), relatively few have accounted for evolutionary history. We find that growth habit shows phylogenetic signal, as do several of the traits associated with it. In one of the few previous studies to analyze phylogenetic signal in functional traits of ferns, Zhang et. al (2014) found that only one of 16 traits showed significant phylogenetic signal. The only trait in common between the current study and that of Zhang et al. (2014) is SLA, which did not show phylogenetic signal in either study. Given the high degree of phylogenetic signal we observe in most traits and the fact that at least some of the traits between our two studies are likely to be correlated, it is rather surprising that Zhang et al. (2014) did not detect phylogenetic signal in more traits. However, Zhang et al. (2014) only used *K*, which tended to indicate less phylogenetic signal than λ in our study. In simulation studies, λ has been shown to have lower error (both Type I and Type II) than *K*, which can be highly inconsistent unless the strength of BM is very strong (Münkemüller et al. 2012). Our results indicate the importance of including a phylogenetic component, and including multiple measures of phylogenetic signal, when analyzing traits related to epiphytic growth in ferns.

Sporophyte traits—The sporophyte traits that differed most strongly between epiphytic and terrestrial taxa were those related to overall size, including stipe and frond

length, frond width, and rhizome diameter. Stipe length in particular differ strongly between epiphytes and terrestrial species (Figure 3.5B, Table 3.3). Stipe length has been posited as a critical trait controlling whole leaf hydraulic conductance; since ferns have highly resistive stipes in general, species growing in water-limited habitats are expected to decrease stipe length to minimize resistance (Watkins et al. 2010). Our results support previous non-phylogenetically controlled studies that also found a prevalence of short stipes in epiphytic ferns (Watkins et al. 2010, Creese et al. 2011). However, given that many of these size-related traits scale together in ferns (Arcand et al. 2008, Creese et al. 2011), careful experimental manipulations are needed to distinguish the role (or lack thereof) of each in epiphytic growth.

Surprisingly, SLA was only slightly lower in epiphytes relative to terrestrial species (Table 3.3, Figure 3.5). SLA is thought to be an important part of the leaf economic spectrum, which posits that leaf traits including SLA, N and C content, and leaf life span vary along well-defined axes such that plants generally fall into one of two categories: fast-growing species with high SLA, low nutrient content, and short life spans vs. slow-growing species with low SLA, high nutrient content, and long life spans (Wright et al. 2004). We therefore hypothesized that epiphytes would have lower SLA, given their more extreme growing conditions. Indeed, the species with the lowest SLA values were epiphytes (e.g., *Selliguea plantaginea* Brack., 8.7 $m^2 kg^{-1}$; *Humata anderrsonii* (Mett. ex Kuhn) C. Chr., 8.43 $m^2 kg^{-1}$), but this relationship was not significant across the dataset (Table 3.3, Figure 3.5B). The tradeoffs posited in the leaf economics spectrum have been most thoroughly documented in global studies including the widest possible range of taxa and environments, and do not always hold up in local

studies (Petter et al. 2016). For example, Zhu et al. (2016) did not find a significant relationship between SLA and leaf life span in a survey of 16 ferns across a disturbance (light) gradient in China. It is possible that other small-scale effects (e.g., microclimate), obscure this pattern in local studies such as ours and that of Zhu et al. (2016). This is supported by the strong, parallel relationship in CWM values of SLA with elevation in both epiphytes and terrestrial species (Figure 3.5A).

Gametophyte traits—Of the gametophyte traits we investigated, only morphotype was found to have a significant association with epiphytic growth. Morphotype has previously been observed to correlate with life history and habitat in ferns: terrestrial species tend to have short-lived (< 1 yr), cordate gametophytes that establish following disturbance and rapidly produce sporophytes, whereas epiphytic species tend to have non-cordate (e.g., elongate or filamentous) gametophytes that persist over multiple growing seasons and produce sporophytes more slowly (Watkins et al. 2007b, Farrar et al. 2008). The complex, three-dimensional structure of non-cordate gametophytes slows their drying rates, and may be an adaptation to drought-prone epiphytic habitats (Watkins et al. 2007a, Pittermann et al. 2013), as well as promote out-crossing (Farrar et al. 2008). Our study is the first to our knowledge to demonstrate that the correlation between non-cordate morphology and epiphytism in ferns is significant while controlling for phylogeny.

We did not find that gemmae production was linked with epiphytism. Gemmae are asexual propagules that allow gametophytes to persist over multiple growing seasons and attain large sizes (Farrar et al. 2008). Gemmae production should be a useful trait in an epiphytic context because it may allow gametophytes to increase their surface area and

hedge against population loss due to extreme drying events (Farrar et al. 2008). It is possible that some, but not all, epiphytic ferns rely on such strategies, and that they are not under similarly strong selection as other traits such as stipe length and gametophyte morphotype. Interestingly, when we classified "widespread" species as those with gametophytes occurring more than 200 m above or below the minimum or maximum elevational range of conspecific sporophytes (data not shown), we found that gemmae production is correlated with widespread growth (log likelihood dependent model = -54.5, log likelihood independent model = -63.6, $P = 0.001$, Pagel's test of correlated evolution). Thus, it seems that gemmae do function significantly in long-term gametophyte persistence, but may not be required for epiphytic growth.

Presence of hairs and glands has strong phylogenetic signal (Tables 3.2, C3), and these traits have been previously observed to correlate with certain taxonomic groups (Stokey 1951, Nayar and Kaur 1971). Although hairs have been hypothesized to reduce rates of drying by increasing the boundary layer and holding external water (Watkins et al. 2007a), we did not detect a correlation between hair production and epiphytic growth. Similarly, production of glands does not appear to be correlated with epiphytic growth. Wax-secreting glands are prevalent in both sporophytes and gametophytes of many notholaenid ferns, a xeric-adapted clade, but their functional significance has not been established (Johnson et al. 2012). Further tests are needed to determine if such glands serve a functional role in preventing water loss in desert ferns as well as epiphytes.

Environmental filtering—We observed the predicted trend of decreased trait richness (FRic) in epiphytic relative to terrestrial communities overall, as well as a significant positive correlation of FRic with humidity in epiphytes (i.e., decreased FRic at

drier, low elevation sites and increased FRic at more humid, high elevation sites; Figure 3.6). However, we did not observe similar patterns in the other two measures of functional diversity, evenness (FEve) and divergence (FDiv) (Figure 3.6B). Indeed, FEve decreased with elevation (but not minimum RH) in terrestrial communities (Figure 3.6A). Overall, the lack of a clear pattern in functional evenness and divergence suggests that within each trait pool, there is no significant difference in spacing of traits between epiphytes and terrestrial species. This could be maintained by niche partitioning within the different habitats. Epiphytic habitats are highly heterogeneous, and microclimatic conditions vary with height along the host and distance from the trunk (Hietz and Hietz-Seifert 1995). The vertical stratification of epiphytic species into different zones along height gradients in host trees is well documented (Zotz 2007). In a study comparing terrestrial and epiphytic ferns along an elevational gradient from 30 to 3000 m in Costa Rica, Watkins et al. (2006) found that within epiphytes, species tended to sort into low-trunk and canopy habitats, with only 18% found in both, and no species that overlapped between canopy and terrestrial habitats. It is possible that epiphytic growth per se limits overall morphological variation in epiphytic ferns, but that species with different traits are then sorted into different niches.

In addition to their decreased functional diversity, we also found that epiphytic communities are more phylogenetically clustered relative to terrestrial communities overall (Figure 3.6B). However, we did not detect any significant trends in phylogenetic community structure of epiphytic and terrestrial communities along the elevational gradient (Figure 3.6A). It is possible that the scale of the current study (from 200 to 1200 m) prevented the detection of such trends. When the plots in this study were analyzed

together with additional plots from Tahiti reaching a maximum altitude of just over 2000 m, Nitta et al. (in review) detected increasing phylogenetic clustering in fern sporophyte communities (epiphytes and terrestrial species together) with elevation. Kluge and Kessler (2011) also compared phylogenetic and trait diversity between epiphytic and terrestrial ferns over an elevational gradient from 100 to 3400 m in Costa Rica, and found trait overdispersion at mid-elevations for epiphytes but no trends in phylogenetic diversity for either group. They accounted this to evolutionary plasticity in traits, but did not quantify the degree of phylogenetic signal in the traits they studied.

***Conclusion and future directions*—**We have demonstrated that growth habit itself, as well as several traits associated with it, show significant phylogenetic signal in ferns. Furthermore, we find that epiphytes have restricted morphologies both on a per-species basis and at the community level, and that epiphytic communities are phylogenetically clustered relative to terrestrial ones. Finally, we observed drier conditions in the canopy relative to terrestrial habitats. Taken together, our results support a scenario of environmental filtering acting on conserved traits to limit diversity in epiphytic ferns. The generality of this pattern could be further tested by investigating the functional traits of other groups with large epiphytic radiations such as orchids and bromeliads. Future studies in ferns should focus on determining in more detail how niche space is partitioned within epiphytic vs. terrestrial habitats, which is clearly a major dividing line for ferns.

3.6 Acknowledgements

We thank members of the Davis Lab, N. Michele Holbrook, and Jonathan Losos for helpful discussion and comments on drafts. Suzanne Vinette, Saad Amer, Tristan Wang,

and Tohei Theophilus helped with community surveys and measurement of traits. Staff at the University of California, Berkeley Richard B. Gump South Pacific Research Station, Moorea, French Polynesia, in particular Valentine Brotherson, Hinano Teavai-Murphy, Frank Murphy, and Neil Davies, provided support with obtaining research and collection permits, laboratory space, and logistics. Funding provided in part by National Science Foundation (Doctoral Dissertation Improvement Grant DEB-1311169 to JHN and CCD), Setup Funds from Harvard University to CCD, the American Society of Plant Taxonomists (Research Grant for Graduate Students to JHN), the Garden Club of America (Award in Tropical Botany to JHN), the Harvard University Herbaria (Fernald Fieldwork Fellowship to JHN), the Society of Systematic Biologists (Graduate Student Research Award to JHN), and the Systematics Association (Systematics Research Fund to JHN).

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Chapter IV:

Microsorum × *tohieaense* (Polypodiaceae), a new hybrid fern species from French Polynesia, with implications for the taxonomy of *Microsorum*

4.1 Abstract

A new hybrid species of microsoroid fern, ***Microsorum* × *tohieaense*** (*Microsorum commutatum* × *Microsorum membranifolium*) (J. H. Nitta, hyb. sp. nov.) from Moorea, French Polynesia is described based on morphology and molecular phylogenetic analysis. *Microsorum* × *tohieaense* can be distinguished from other French Polynesian *Microsorum* by the combination of sori distributed more or less in a single line between the costae and margins and sweet smell. Genetic evidence is also presented for the first time supporting the hybrid origin of *Microsorum* × *maximum* (*Microsorum grossum* × *Microsorum punctatum*), and possibly indicating a hybrid origin of Hawaiian endemic *Microsorum spectrum*. The implications of hybridization for the taxonomy of microsoroid ferns is discussed.

4.2 Introduction

Hybridization, the process of interbreeding between species, plays an important role in evolutionary diversification (Anderson 1949, Stebbins 1959). Hybridization can increase species diversity through reinforcement if hybrids have reduced fitness relative to parents (Barton and Hewitt 1985) or by the generation of completely new taxa if hybrids are fertile (Chapman and Burke 2007). Alternatively, hybridization may also decrease diversity by allowing gene flow between previously separated lineages (Mayr 1966). Hybridization is particularly significant to the diversification of ferns, which have relative few prezygotic barriers because of their reliance on passive transport of sperm by water for fertilization (Haufler 2002). This contrasts strongly with the complicated pollen selection mechanisms of flowering plants (e.g., pollinator specificity, pollen/stigma interactions). Furthermore, ferns have high rates of both polyploidy and apogamy (i.e., asexual reproduction via unreduced spores), which allows them to overcome hybrid

sterility with relative ease (Barrington et al. 1989). Hybridization has been documented in a wide range of fern lineages (e.g., Wagner 1954, Barrington 1990, Beck et al. 2010, Rothfels et al. 2014). However, many reported cases of fern hybrids are based solely on morphological evidence. Morphological variation within taxa may arise through various processes including phenotypic plasticity and intraspecific variation. Thus, morphological traits alone, although informative, are not sufficient for confidently identifying hybrid taxa (Zhang et al. 2013).

Here, we investigate a putative case of hybridization between species of microsoroid ferns (Polypodiaceae), a large and diverse (ca. 160 spp.) clade of epiphytic or epilithic ferns distributed mainly in the paleotropics (Schneider et al. 2004b). Generic taxonomy of the microsoroid ferns is in flux. Some of the segregate genera are monophyletic with clearly defined apomorphies, such as *Lecanopteris*, which forms symbiotic associations with ants (Haufler et al. 2003), and *Belvisia*, with simple leaves and coenosori restricted to a reduced apical lamina segment (Wang et al. 2010a). However, several other microsoroid genera including *Microsorum*, *Lepisorus*, and *Neocheiropteris* defined on the basis of morphology (Bosman 1991, Nooteboom 1997) have been shown to be polyphyletic in molecular investigations (Schneider et al. 2004a, 2004b, Kreier et al. 2008, Wang et al. 2010a, 2010b). Species placed in *Microsorum* in particular are distributed throughout the microsoroid clade, and badly in need of taxonomic revision (Schneider et al. 2004b, Kreier et al. 2008). Studies of hybridization may provide evidence for the genetic distinctness of taxa to help inform delimitation of genera. Although various hybrid taxa have been described previously in the microsoroid ferns (Nooteboom 1997), we are unaware of any cases that have been verified genetically.

As part of another study (Nitta et al., in review), we recently conducted a field survey of the ferns of Moorea and Tahiti, French Polynesia. There are seven species of microsoroid ferns known from French Polynesia: *Belvisia spicata* (L.f.) Mirbel, *Microsorum commutatum* (Blume) Copel., *M. grossum* (Langsd. & Fisch.) Brownlie, *M. membranifolium* (R. Br.) Ching, *M. powellii* (Baker) Copel., *M. punctatum* (L.) Copel., and *M.* × *maximum* (Brack.) Copel. (a supposed hybrid between *M. punctatum* and *M. grossum*) (Copeland 1932, Murdock and Smith 2003, Nitta et al. 2011, Florence in press). During our field survey, we encountered a population of *Microsorum* that did not match any these species in gross morphology but that we suspected could be a hybrid based on the observation of malformed spores. Furthermore, the relationships of these ferns has never been tested in a global context, so it is unknown if the French Polynesian microsoroid ferns represent diversification from a single introduction, or are the result of multiple colonization events.

Here, we present results of our morphological and molecular analyses that support recognition of this plant as a new hybrid species, *Microsorum* × *tohieaense* between *M. commutatum* and *M. membranifolium*. Furthermore, we include samples of endemic *Microsorum spectrum* (Kaulf.) Copel. from Hawaii, and discuss the taxonomic implications of our results for *Microsorum*.

4.3 Methods

We use microsoroid ferns in the broad sense following Kreier et al. (2008); this includes the goniophlebioid, lepisoroid, lecanopteroid, and microsoroid (*sensu stricto*) clades of Schneider et al. (2004). We observed morphological characters in all microsoroid ferns from our study area (Moorea and Tahiti; Figure 4.1) in herbarium specimens or fresh

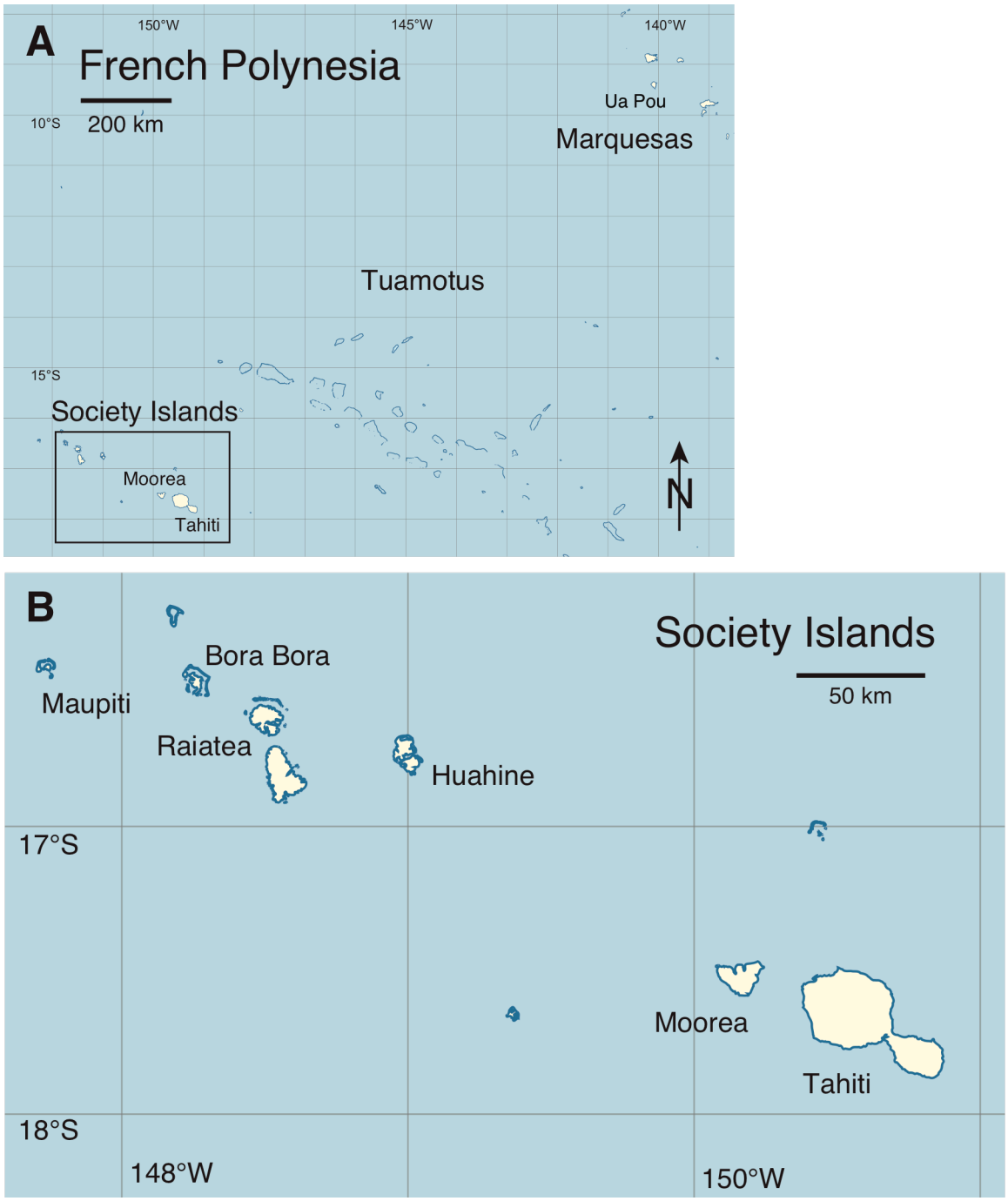

Figure 4.1 Map of the study area. A) French Polynesia, showing the locations of Moorea (Society Islands) and Ua Pou (Marquesas Islands). Location of inset (B) indicated by rectangle. **B)** Society Islands. Maps adapted from Wikimedia Commons under Creative Commons License.

material. Morphological traits include: arrangement of sori, immersion of sori in lamina, spore color, rhizome scales, rhizome diameter, frond length, frond width, stipe length, specific leaf area (SLA), degree of lamina dissection, number of pinna pairs, and presence or absence of a sweet fragrance. SLA was measured by taking leaf samples with a 4 mm diameter biopsy punch, which were dried for 24 h at 60°C and weighed. SLA was calculated as area (m^2) per mass (kg) of each punch. Eight to ten punches were taken from a single leaf for each specimen and the mean SLA per specimen was recorded. Other quantitative leaf traits were measured on the single largest leaf per specimen, and rhizome diameter measured at the widest point of the rhizome. We also examined spores of the unknown species using a compound microscope (photographs taken with Olympus DP70 digital microscope camera). Voucher specimens were deposited in GH and UC.

For our phylogenetic analysis, we used both plastid and nuclear loci. Plastid *rbcL* and *trnLF* have been used in previous studies of microsoroid ferns including a broad taxonomic sampling (Schneider et al. 2004b, Kreier et al. 2008), and are therefore useful to place the French Polynesian microsoroid ferns in a broader global context. However, it is impossible to detect hybridization events using only plastid loci as these are maternally inherited in almost all known cases in ferns (Gastony and Yatskievych 1992, Vogel et al. 1998b). We therefore sequenced two nuclear markers, the "long" and "short" copies of *gapCp* (Schuettpelz et al. 2008) (hereafter *gapCp long* and *gapCp short*) in multiple specimens from all microsoroid ferns from our study area, as well as two specimens of *M. membranifolium* from the Marquesas Islands, one specimen of *M. spectrum* (Kaulf.) Copel. var. *pentadactylum* (Hillebr.) D. D. Palmer from Hawaii, and one specimen of *Leptochilus ellipticus* var. *pothifolius* (Buch.-Ham. ex D. Don) X.C. Zhang (= *Colysis*

pothifolia (Hamilt. Ex D. Don) Presl) from Okinawa. *Microsorum spectrum* was included to investigate the phylogenetic affinities of this unusual Hawaiian endemic species to other Pacific *Microsorum*, and *L. ellipticus* was included to test the monophyly of *Microsorum* with nuclear data. Outgroup species were selected from other polygrammoid taxa (*gapCp long* and *short*) or davallioids (*gapCp short* only). We included all *rbcL* and *trnLF* sequences sampled in Kreier et al. (2008), as well as additional sequences from GenBank of these two markers for species closely related to French Polynesian microsoroid ferns (summarized in Table D1).

DNA extraction was performed on leaf tissue preserved on silica gel either by modified CTAB (Doyle and Doyle 1987) or using the DNEasy kit (Qiagen, Valencia, CA, United States) following the manufacturer's instructions. PCR amplification was performed using TRNLF-f (forward) and FernL 1Ir1 (reverse) (Li et al. 2009) for *trnLF*, ESRBCL1F and ESRBCL1379R (Schuettpelz and Pryer 2007) for *rbcL*, and ESGAPCP8F1 and ESGAPCP11R1 (Schuettpelz et al. 2008) for *gapCp*. PCR protocols and thermocycler settings followed those of Schuettpelz and Pryer (2007) for *rbcL* and *trnLF* (except for annealing temperature set to 56°C) and Schuettpelz et al. (2008) for *gapCp*. Amplification success was visually inspected by gel electrophoresis including 3 uL PCR product per lane on a 1% agarose gel, with a current of 80V applied for 1 hr. Successful plastid PCR products were sent without further modification for enzymatic cleaning and Sanger sequencing to GENEWIZ (South Plainfield, NJ, United States; http://genewiz.com). The *gapCp* primers used here typically amplify both *gapCp long* (ca. 900 bp) and *short* (ca. 600 bp.), in addition to shorter bacterial fragments lacking introns, in a single PCR reaction (Schuettpelz et al. 2008). We separated the two *gapCp* copies by

gel electrophoresis as follows: 5 uL of 6× dye was added to each 25 uL PCR product, loaded on a 1.25% agarose gel in TE buffer, and a 60 V current applied for 2 hr. This allowed the bands to be visually distinguished under UV light and excised using a clean razor blade. Excised bands were purified using the Montage gel extraction kit (Millipore, USA) following the manufacturer's instructions. Next, alleles within each *gapCp* copy were separated by cloning using the TOPO TA kit (Thermo Fisher Scientific, Waltham, USA) following the manufacturer's instructions. 12-16 colonies were selected per region (*gapCp short* or *long*) per species, and amplified using vector-based primers M13F and M13R. Verification of amplification success and sequencing was performed in the same way as for plastid regions, except that we only used primer M13R for sequencing to reduce costs. Typically, at least one colony per allele was obtained in the reverse orientation and obviated the need for sequencing in the reverse direction; if this was not the case, we selected a single PCR sample of known genotype (from the first sequencing reaction) to sequence using M13F. AB1 files were imported into Geneious v 9.1.3 (Kearse et al. 2012), and automatically trimmed with an error cutoff of 4.0% per base. Chimeras and other sequence errors were identified using a similar approach to that of Grusz et al. (2009): for each *GapCp* copy per specimen, all trimmed AB1 files were aligned using MAFFT (Katoh et al. 2002) as implemented within Geneious, sequencing and PCR primer regions were trimmed from the alignment, and a phylogenetic analysis was performed using RAxML (Stamatakis 2006) as implemented within Geneious. This generally resulted in a phylogenetic tree with 2 – 4 clades containing most of the sequences, with chimeric sequences (i.e., artifacts consisting of fragments of distinct sequences that became fused together during PCR) either at the base of one clade or

occupying intermediate positions between clades. Obviously chimeric sequences were excluded, and monophyletic groups containing sequences each with less than 5 bp difference from the consensus per sequence were considered to be alleles. Lists of sequences representing putative alleles were then selected, exported from the alignment, and assembled into contigs. Consensus sequences were obtained from the resulting contigs and used as the final alleles for phylogenetic analysis.

Phylogenetic analysis was conducted using maximum likelihood as implemented in RAxML (Stamatakis 2006) with a GTR + G model of sequence evolution on the Odyssey cluster supported by the FAS Division of Science, Research Computing Group at Harvard University (http://rc.fas.harvard.edu). We searched for the best likelihood tree from among 20 distinct maximum parsimony starting trees, conducted 1000 bootstrap replicates, and wrote the results of the bootstrap analysis to the best likelihood tree using the rapid analysis option (-f a).

During our initial phylogenetic analysis, we observed an unexpectedly high number of alleles in three putatively non-hybrid specimens (*M. membranifolium Nitta 573*, *M. punctatum Nitta 1399*, and *M. punctatum Nitta 3818*) that matched other, non-hybrid species ("rogue alleles"). We believe these are artifacts due to the protocol we used (cloning), which is highly sensitive to even small amounts of contaminating DNA (Ruecker et al. 2011). There are several lines of evidence that support this conclusion: each rogue allele was recovered only for *gapCp long* or *short* (never for both); plastid sequences of these specimens matched others of the same species exactly; nothing about the morphology of these specimens indicate hybrid origin; and other accessions of the same species do not show such a pattern. We therefore excluded these specimens from

Table 4.1. Morphological traits and habitat of microsoroid ferns from French Polynesia. For quantitative traits, means ± SD are given. Species with simple fronds were not considered to have any pinna pairs.

	Stipe Length (cm)	Frond Length (cm)	Frond Width (cm)	Rhizome Dia. (cm)	Pinna Pairs	SLA (m^2 kg^{-1})
Belvisia spicata	2.95 ± 1.35 (N = 5)	26.92 ± 4.75 (N = 5)	1.53 ± 0.61 (N = 5)	0.5 ± 0.2 (N = 5)	n/a	17.4 ± 6.01 (N = 5)
Microsorum commutatum	39.38 ± 10.41 (N = 6)	114.39 ± 29.69 (N = 6)	28.23 ± 5.01 (N = 6)	0.92 ± 0.4 (N = 6)	15.33 ± 5.69 (N = 6)	33.3 ± 8.04 (N = 2)
Microsorum grossum	25.4 ± 9.07 (N = 4)	56.15 ± 11.88 (N = 4)	20.71 ± 4.12 (N = 4)	0.61 ± 0.23 (N = 4)	3.75 ± 0.5 (N = 4)	37.37 ± 12.9 (N = 3)
Microsorum membranifolium	81.89 ± 26.48 (N = 3)	200.27 ± 84.42 (N = 3)	42.59 ± 19.52 (N = 3)	0.9 ± 0.52 (N = 3)	15.33 ± 4.51 (N = 3)	53.49 ± 8.39 (N = 2)
Microsorum powellii	20.6 ± 11.33 (N = 4)	43.15 ± 17.83 (N = 4)	18.91 ± 7.94 (N = 4)	0.71 ± 0.24 (N = 4)	5 ± 3.37 (N = 4)	15.12 (N = 1)
Microsorum punctatum	0 ± 0 (N = 5)	72.85 ± 22.34 (N = 5)	7.15 ± 1.28 (N = 5)	0.68 ± 0.21 (N = 5)	n/a	22.96 ± 2.1 (N = 2)
Microsorum × maximum	13.41 ± 21.3 (N = 3)	86.86 ± 61.2 (N = 3)	18.58 ± 13.2 (N = 3)	0.76 ± 0.14 (N = 3)	2.0 ± 1.73 (N = 3)	20.42 ± 8.5 (N = 3)
Microsorum × tohieanse	21.2 ± 8.98 (N = 3)	47.26 ± 14.44 (N = 3)	14.58 ± 2.39 (N = 3)	0.45 ± 0.05 (N = 3)	5.67 ± 0.58 (N = 3)	34.33 ± 9.08 (N = 2)

Table 4.1 (Continued).

	Habitat	Dissection	Soral Arrangment	Soral Immersion	Fragrance	Spore Color	Rhizome Scales
Belvisia spicata	epiphytic	simple	coenosorus	none	no	yellow	ovate-lanceolate, clathrate, margins denticulate
Microsorum commutatum	terrestrial, mesic	pinnatifid	scattered	none	yes	yellow	round, weakly clathrate, dark in center, entire margins
Microsorum grossum	terrestrial, xeric	pinnatifid	regular	moderate	no	yellow	ovate-lanceolate, strongly clathrate, margins denticulate
Microsorum membranifolium	terrestrial, mesic	pinnatifid	regular	deep	no	yellow	irregularly round, non-clathrate, light-brown, margins entire
Microsorum powellii	epiphytic	pinnatifid	regular	deep	no	yellow	ovate-lanceolate, strongly clathrate, margins denticulate
Microsorum punctatum	epiphytic	simple	scattered	none	no	yellow	ovate-lanceolate, denticulate, not clathrate, lustrous
Microsorum × *maximum*	terrestrial or epipetric	irregularly pinnatifid	scattered	none	no	clear	ovate-lanceolate, clathrate, margins denticulate
Microsorum × *tohieanse*	terrestrial, mesic	pinnatifid	mostly regular	moderate	yes	clear	round, dark in the center, margins entire

our final analysis, as we could not be confident of their homology. For our initial phylogenetic analyses including specimens with rogue alleles see the appendix (Figures D1 – D3).

4.4 Results

The morphology of the unknown species does not match any previously described microsoroid ferns from French Polynesia (Table 4.1, Figures 4.2, 4.3). It is close in overall size to *M. grossum* and *M. commutatum*, but has much thinner laminae than *M. grossum*, and the arrangement of sori differs strongly from *M. commutatum* (generally one line of sori on either side of the midvein in the unknown species, but with irregularities, vs. many scattered sori in *M. commutatum*). It matches well with *M. membranifolium* in laminae thinness and texture, but is much smaller with fewer pinna pairs than the former. It shares in common a sweet smell with *M. commutatum*, a trait not observed in any other microsoroid fern from French Polynesia.

None of the non-hybrid French Polynesian microsoroid ferns form an exclusive clade in the plastid tree, suggesting that each represents an independent colonization event of the islands (Figure 4.4). Rather, French Polynesian microsoroid ferns occupy a range of positions in the plastid tree. *Microsorum grossum*, *M. punctatum,* and *M.* × *maximum* are closely related and belong to a strongly supported clade containing only other *Microsorum* species (BP = 98), which is in turn weakly supported as sister to *M. commutatum* (BP = 58). Most *M.* × *maximum* plastid sequences match those of *M. grosssum*, but one specimen from Maupiti matches with *M. punctatum*. The *grossum-punctatum-commutatum* clade is in turn sister to a clade containing *Leptochilus*, *M. membranifolium*, the unidentified *Microsorum* species, and *M. spectrum* from Hawaii

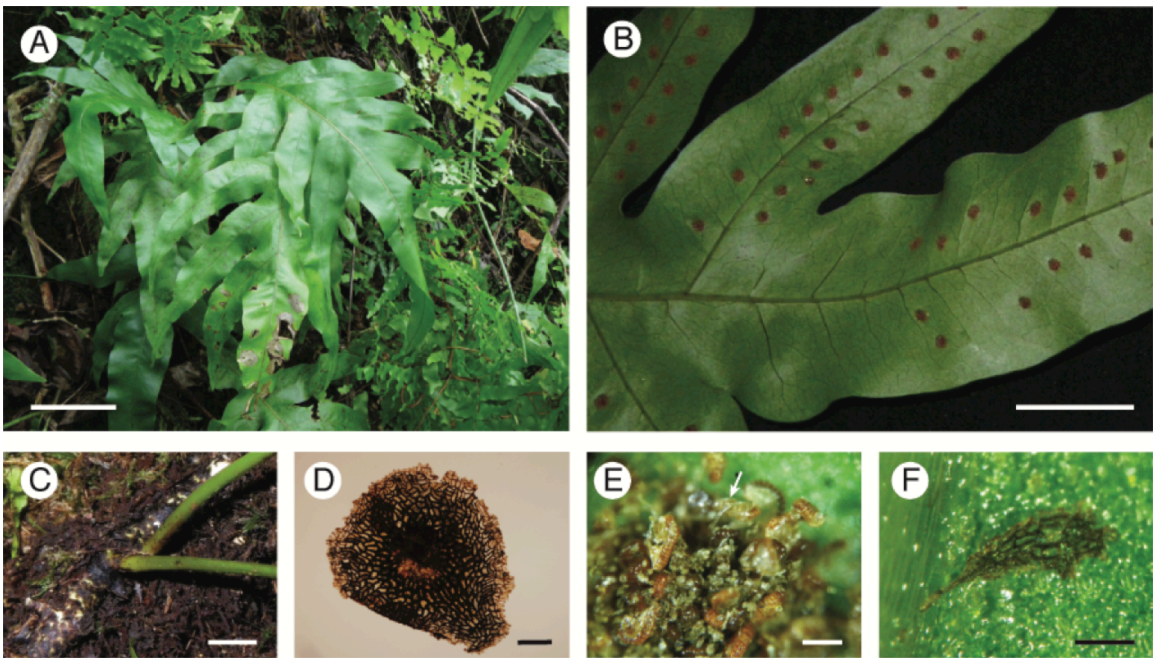

Figure 4.2 Photographs of *Microsorum* × *tohieaense*. A) Whole plant *in situ*. **B)** Abaxial surface of lamina. **C)** Rhizome *in situ*. **D)** Rhizome scale. **E)** Sorus. Arrow indicates acicular paraphysis. Note clear spores. **F)** Costal scale. Scalebars: A, 5 cm; B – C, 2 cm; D – E, 250 µm; F, 100 µm. A – C, E – F: *Nitta 3929* (GH); D: *Nitta 1040* (GH). Photographs by J. H. Nitta.

(BP = 58). The unidentified *Microsorum* species from Moorea is nested within a clade of *M. membranifolium* including specimens from the Marquesas (Ua Pou), the Society Islands (Moorea, Huahine, and Tahiti), and another unidentified *Microsorum* species from Laos (BP = 99). *Microsorum spectrum* is nested within a clade containing *Microsorum cuspidatum* (D. Don) Tagawa and *Microsorum hainanense* Noot (BP = 54). The other two microsoroid ferns, *B. spicata* and *M. powellii*, are more distantly related, and nested within clades containing *Lepisorus*, *Lemmaphyllum*, and *Neocheiropteris* in

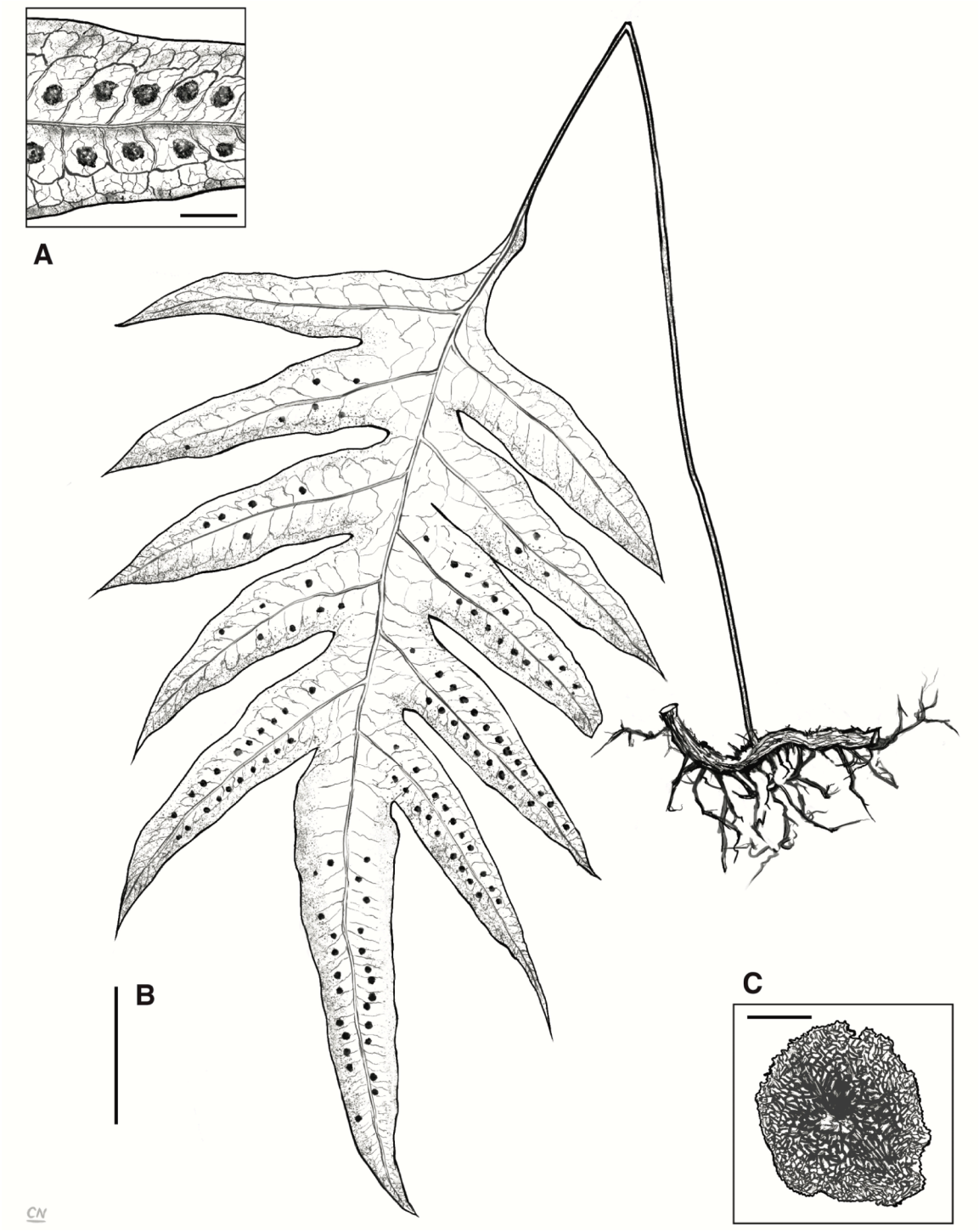

Figure 4.3 Illustration of *Microsorum* × *tohieaense*. A) Detail of abaxial surface showing arrangement of sori and veins. **B)** Whole plant. **C)** Rhizome scale. Scalebars: A, 1 cm; B, 5 cm; C, 500 µm. A, B: *Nitta 3929*. C: *Nitta 1040*. Illustrations by C. C. Nitta.

Figure 4.4 Maximum-likelihood phylogenetic tree inferred using plastid markers (*rbcL* and *trnLF*) including French Polynesian microsoroid ferns and related taxa. Dataset including all species with either *rbcL* (*N* = 138 spp.) or *trnLF* (*N* = 137 spp.), *N* = 151 spp. total. Names of taxa from French Polynesia colored by species. Diamonds indicate monophyletic genera that have been collapsed for plotting; number of collapsed species indicated in parenthesis. Bootstrap values > 50 shown at nodes. Scalebar indicates expected number of changes per site. Outgroup species (*Thylacopteris papillosa*, *Pleopeltis munchii*, and *Platycerium stemaria* var. *laurentii*) not shown.

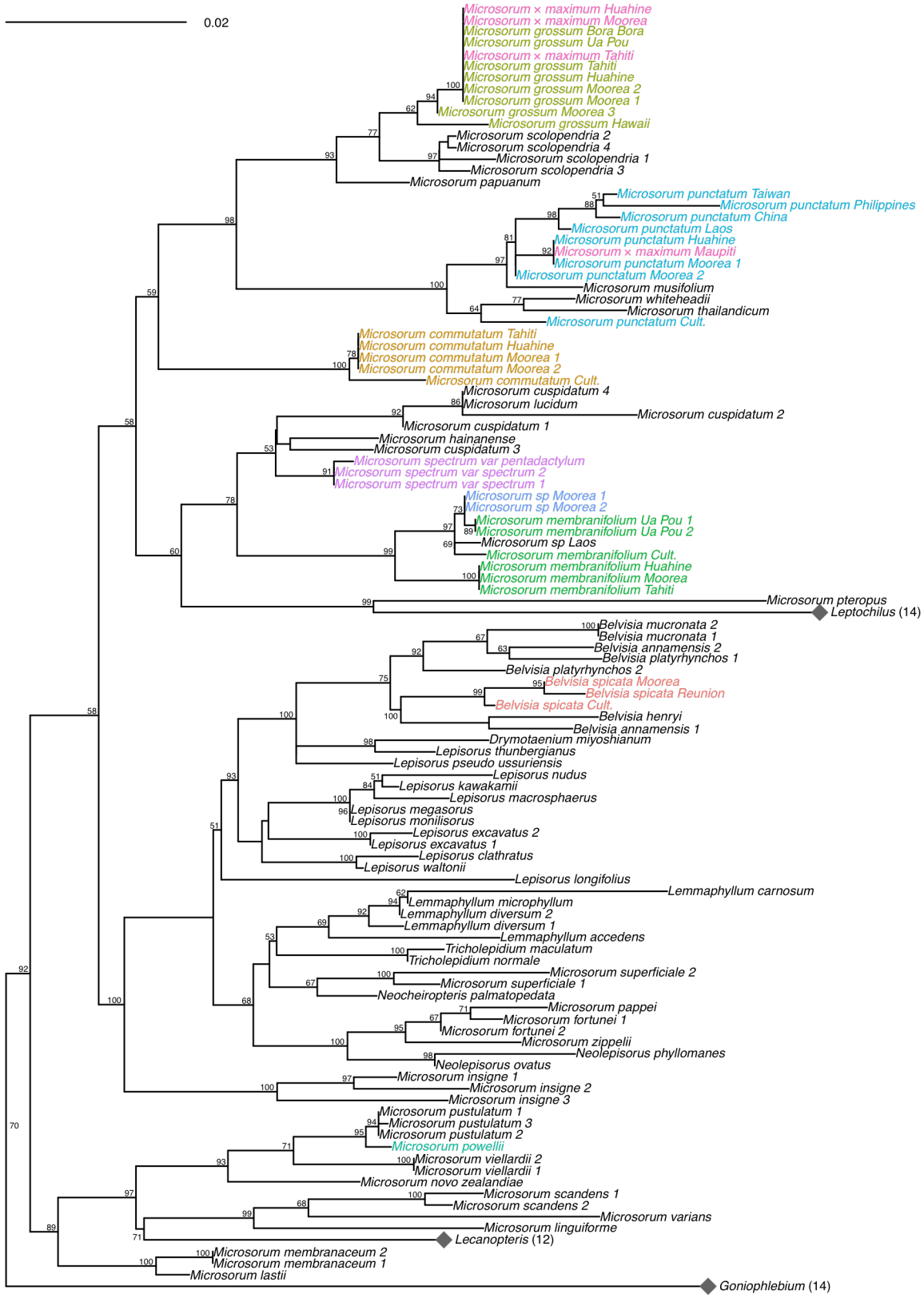

Figure 4.4 (Continued).

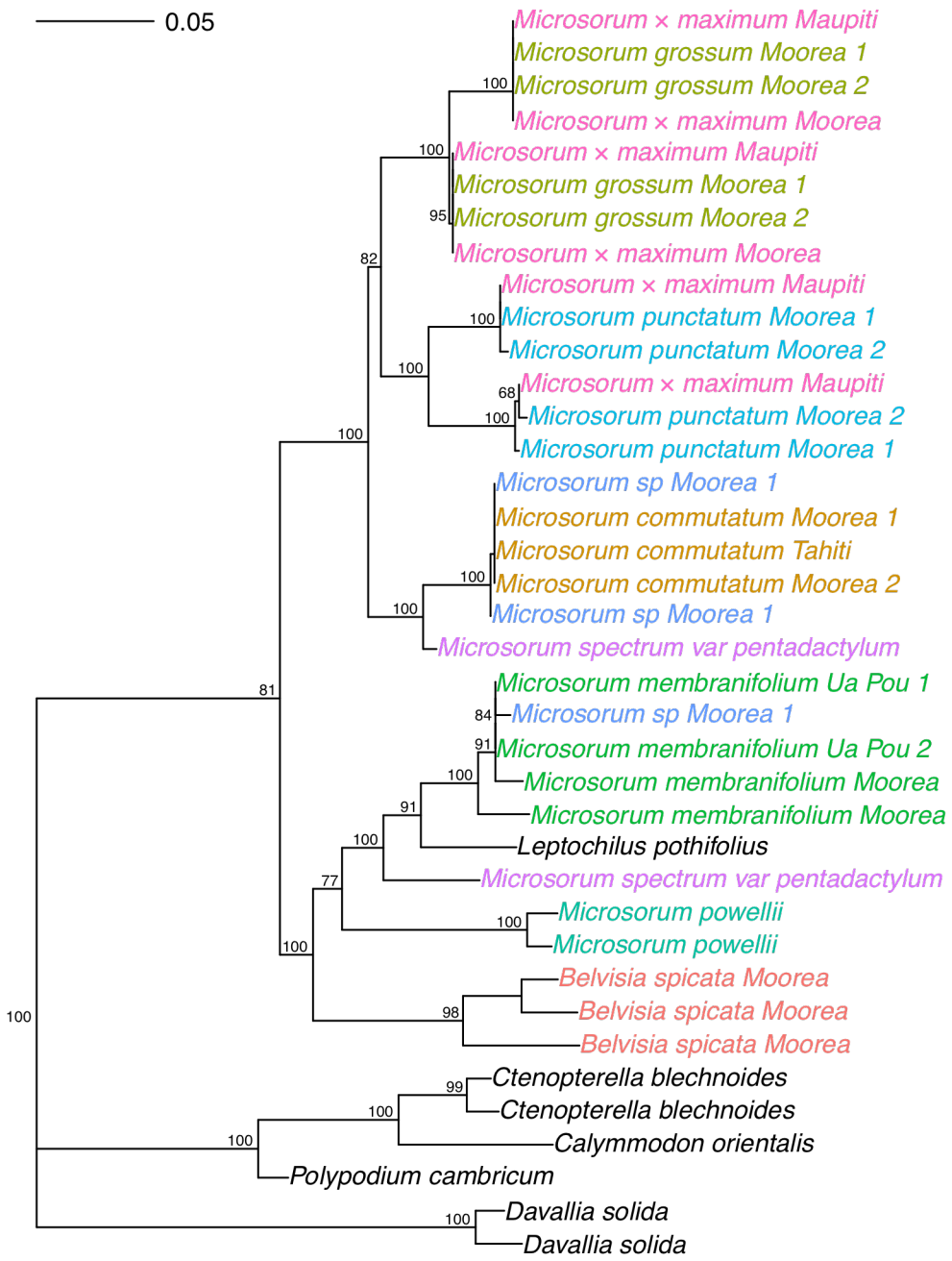

Figure 4.5 Maximum-likelihood phylogenetic tree inferred using nuclear *gapCp short* including French Polynesian microsoroid ferns and related taxa. Names of taxa from French Polynesia colored by species. Bootstrap values > 50 shown at nodes. Scalebar indicates expected number of changes per site.

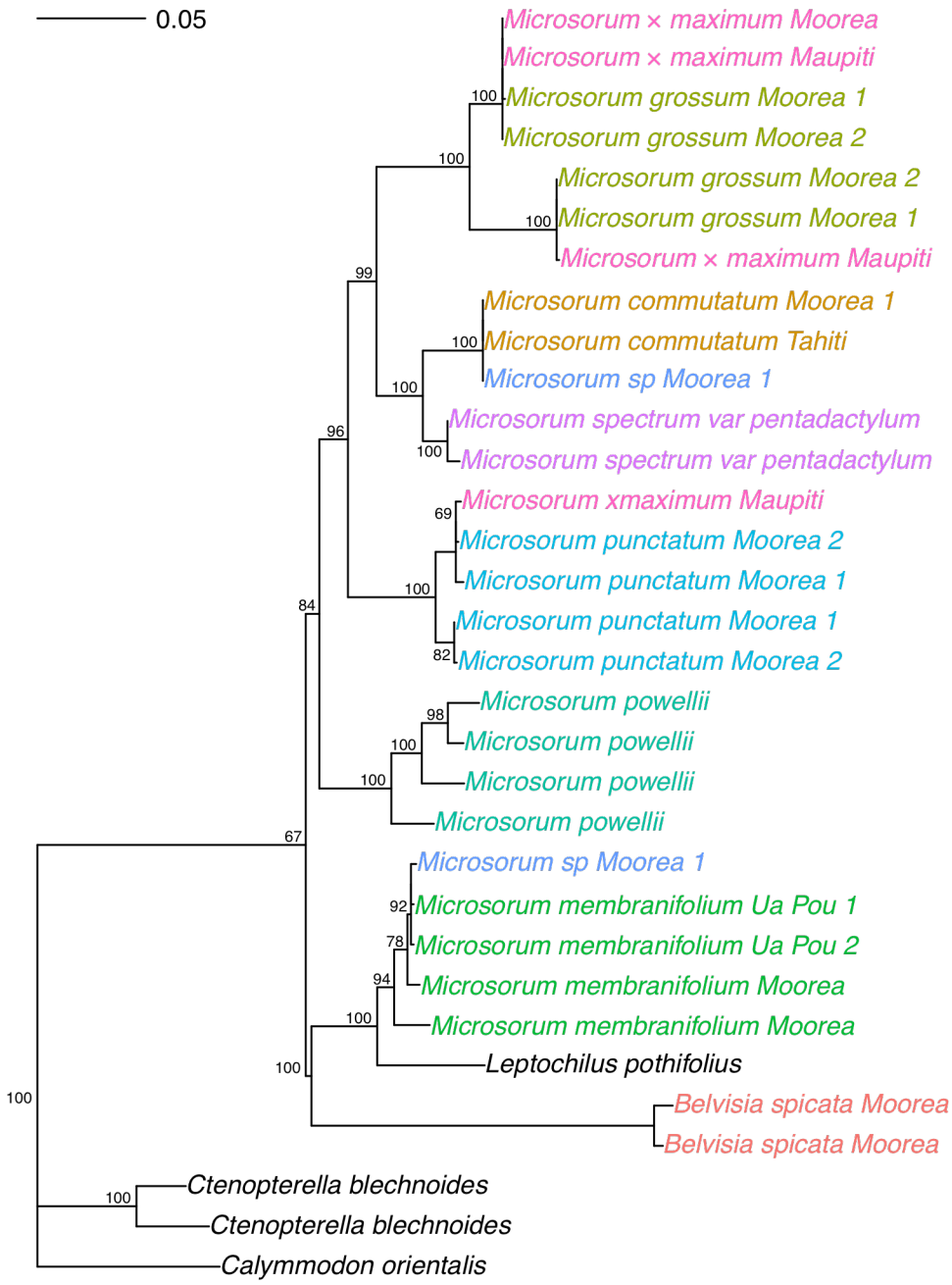

Figure 4.6 Maximum-likelihood phylogenetic tree inferred using nuclear *gapCp long* including French Polynesian microsoroid ferns and related taxa. Names of taxa from French Polynesia colored by species. Bootstrap values > 50 shown at nodes. Scalebar indicates expected number of changes per site.

the case of the former, and *Lecanopteris* and other taxa named *Microsorum* mostly from New Zealand, New Caledonia, and Australia in the latter.

We detected some of the same deeper divergences observed in the plastid tree in the nuclear trees. These split the non-hybrid species into two main clades: one including *M. grossum*, *M. punctatum*, and *M. commutatum,* and the other including *M. membranifolium* and *B. spicata* (Figures 4.5, 4.6). However, the structure of some internal nodes varies between the two copies of *gapCp*. Considering only the putatively non-hybrid taxa, in *gapCp long*, *M. grossum* is sister to *M. commutatum* (BP = 99), which together are sister to *M. punctatum* (BP = 96). In *gapCp short*, *M. grossum* is sister to *M. punctatum* (BP = 82), which together are sister to *M. commutatum* (BP = 100). The placement of *M. powellii* also varies between the two phylogenies: in *gapCp long*, it is sister to the *punctatum-commutatum-grossum* clade (BP = 84), whereas in *gapCp short*, it is sister to the clade containing *M. membranifolium* (BP = 77).

Alleles from *M.* × *maximum* match exactly with those of *M. grossum* and *M. punctatum*, and those from the unknown species match closely to *M. membranifolium* and exactly (*gapCp long* and *short*) or very closely (*gapCp short*) to *M. commutatum*, supporting the hypotheses for hybrid origins of both of these taxa. Furthermore, multiple divergent alleles were also recovered for *M. spectrum*, one sister to *M. commutatum* (*gapCp long* and *short*) and one nested within the clade including *M. membranifolium,* and *B. spicata* (observed in *gapCp short* only). *Microsorum grossum* and *M. punctatum* each contain two distinct alleles for *gapCp short* and *gapCp long*, and these both appear in the accession of *M* × *maximum* from Maupiti for *gapCp short*. Multiple alleles were recovered within some other non-hybrid species as well (e.g., *M. powellii*, *B. spicata*) but

it is unclear if these represent allelic diversity in diploid populations, PCR-introduced error, or additional duplications of *gapCp*.

4.5 Taxonomic Treatment

Microsorum × tohieaense J. H. Nitta, hyb. sp. nov.—TYPE: FRENCH POLYNESIA. Moorea: Mt. Tohiea, 393 m, 12 July 2012, *J. H. Nitta 1040* (holotype: GH!; isotype: TI! UC! P!).

Plants terrestrial or lithophytic; rhizomes long creeping, 5 – 7 mm dia., pale green, phyllopodia distinct, moderately to densely scaly; rhizome scales round, 1 – 1.5 mm dia., peltate, clathrate, light in center, dark brown between center and margins, becoming lighter towards the margins, margins entire; fronds 60 – 70 cm long, 2 – 5 cm apart; stipes 28 – 32 cm long, stramineous; laminae 32 – 37 × 17 – 20 cm, ovate, deeply pinnatifid, apically subconform, with a slightly sweet fragrance; pinnae 9 – 11 × 2.5 cm, 5 – 6 (7) pairs, linear-lanceolate, slightly ascending, entire; apical pinnae 16 × 3 cm, slightly larger than lateral pinnae, with primary veins more evident, slightly narrowing at the base; rachises and costae stramineous, with few scattered scales; costal scales 0.5 × 0.1 – 0.2 mm, clathrate, ovate, apex acuminate; laminae tissue glabrous; veins reticulate, main lateral veins distinct, spaced 5 – 6 mm, connecting veins forming one row of large areoles parallel to the costa, smaller veins variously anastomosing; sori round, 1.5 – 2 mm diameter, located usually in a single row between costa and pinna margins, occasionally irregularly placed, slightly immersed, appearing raised adaxially, with uniseriate paraphyses; spores clear, occasionally misshapen, presumably not fertile. Figures 4.2, 4.3.

Ecology and Distribution—*Microsorum* × *tohieaense* is known from a single population located at ca. 400 m on the slope of Mt. Tohiea, Moorea, French Polynesia. Plants were observed growing terrestrially, but on a steep and rocky surface, so they are considered both terrestrial and epipetric.

Etymology—The new hybrid species is named after the type locality.

4.6 Discussion

***Patterns of hybridization in* Microsorum—**The results of our morphological and phylogenetic analyses strongly support the status of the unknown species as a hybrid between *M. membranifolium* and *M. commutatum*, which we describe as **Microsorum × tohieaense**, **hyb. sp. nov**. Furthermore, we provide the first genetic evidence that *Microsorum* × *maximum* is indeed a hybrid between *M. punctatum* and *M. grossum*, and that the Hawaiian endemic *M. spectrum* may also be a hybrid, although elucidating the progenitor taxa requires further investigation. We describe each of these cases in turn below, then discuss the implications of hybrid species for the taxonomy of *Microsorum*.

Microsorum × tohieaense: The phylogenetic placement of sequences obtained from *M.* × *tohieaense* provides more insight into the details of its origins. We detected an exact match between one set of *gapCp short* and *long* alleles recovered from *M.* × *tohieaense* with those of *M. commutatum* from the Society Islands, suggesting that *M. commutatum* from nearby the type population directly contributed to formation of the hybrid. Furthermore, we observed a close match between plastid genes and the other set of *gapCp* alleles of *M.* × *tohieaense* with *M. membranifolium*, suggesting that *M. membranifolium* is the mother, and *M. commutatum* is the father, of *M.* × *tohieaense.* However, none of the plastid or nuclear sequences from *M.* × *tohieaense* match exactly

with sequences of *M. membranifolium* from the Society Islands. Rather, they appear more closely related to (but not exactly the same as) accessions of *M. membranifolium* from the Marquesas Islands, ca. 1380 km distant (Figure 4.1A). This pattern has several conceivable explanations. First, it is possible that there are additional genotypes of *M. membranifolium* currently present in the Society Islands near the type location of *M.* × *tohieaense* that contributed to hybrid formation that we failed to sample. However, our plastid sequences of *M. membranifolium* from Moorea, Tahiti, and Huahine are all identical, indicating a single genotype of *M. membranifolium* in the Society Islands. Alternatively, there could have been a more widely distributed genotype of *M. membranifolium* in the past, which contributed to hybrid formation and subsequently became extirpated. Finally, the hybrid could be the result of recent long-distance spore dispersal, possibly from a source nearby the Marquesas Islands. The inclusion of another unidentified *Microsorum* accession from Laos in the *M. membranifolium Ua Pou – Microsorum sp. Moorea* clade supports a scenario of two independent colonizations of Pacific islands by *M. membranifolium* from Asia. Additional sequencing of specimens from throughout the South Pacific region and Asia is needed to distinguish between these scenarios.

Microsorum × maximum: This species was previously considered to be a hybrid on the basis of its intermediate morphology between *M. punctatum* (simple laminae) and *M. grossum* (deeply pinnatifid laminae). The laminae of *M.* × *maximum* are more or less simple, but often have irregularly shaped lobes, and irregularly scattered sori (Copeland 1932; Table 4.1). Our phylogenetic analysis of nuclear *gapCp* shows that *M.* × *maximum* contains alleles from both putative parents, confirming its status as a hybrid species.

Furthermore, of the four samples included in our analysis, three (from Huahine, Moorea, and Tahiti) have plastid sequences matching *M. grossum*, while one from Maupiti has plastid sequences matching the other parent, *M. punctatum* (Figure 4.4). We also recovered one additional *M.* × *maximum* specimen from Moorea (*Vinette 34*, UC) not included in the current phylogenetic analysis with plastid sequences matching *M. grossum* (data not shown). Thus, there appears to be a bias in parentage of *M.* × *maximum*, a phenomenon that has been observed in several other hybrid fern species (Vogel et al. 1998a, Xiang et al. 2000, Zhang et al. 2013, Testo et al. 2015). Maupiti, the site of the sole *M.* × *maximum* with a *M. grossum* plastid genotype, is the westernmost and smallest of the main Society Islands, with a lower maximum altitude (380 m), drier climate, and less diverse flora than the other islands (Fosberg and Sachet 1987; Figure 4.1B). Geographical and/or ecological processes could be involved in structuring the hybridization bias we observed (Sigel et al. 2014), but our sample size is insufficient to test such hypotheses. Phylogeographic splits between the Leeward (Huahine, Raiatea, Bora Bora, and Maupiti) and Windward (Moorea, Tahiti) Society Islands have been observed in diverse organisms including insects, birds, and plants (Hembry and Balukjian 2016). Interestingly, *M. punctatum* and *M. grossum* themselves each have two distinct copies of *gapCp long* and *short* that appear to be the result of duplication, and *M.* × *maximum* has inherited both of these (Figures 4.5 and 4.6). Similar apparent duplications of *gapCp* within particular lineages in ferns have been observed in *Adiantum* (Rothfels and Schuettpelz 2014), *Astrolepis* (Beck et al. 2010), the common ancestor of *Culcita* and *Plagiogyria* (Rothfels et al. 2013), and within Lindsaceae (Rothfels et al. 2013).

Microsorum spectrum: Unlike *M.* × *maximum*, we are unaware of any previous suggestions that Hawaiian endemic *M. spectrum* may be a hybrid species. We detected two distinct *gapCp* alleles in *M. spectrum*, one closely related to *M. commutatum* (observed in *gapCp short* only), and one that forms a clade with *M. membranifolium* and *L. pothifolius* (observed in both *gapCp short* and *long*). Plastid sequences of *M. spectrum* were nested within a clade containing *Microsorum cuspidatum* and *Microsorum hainanense*, which is sister to the *M. membranifolium* clade. It is therefore possible that *M. spectrum* is a hybrid with a mother from the *membranifolium-cuspidatum-Leptochilus* clade and a father closely related to *M. commutatum,* but we lack exact matches for either of these putative parents. *Microsorum spectrum* is a morphologically variable taxon, and has been treated as two varieties (var. *spectrum* and var. *pentadactylum*; Palmer 2003, Vernon and Ranker 2013). It is possible that some of this variation is due to its hybrid origins. Our nuclear sampling only includes one specimen of *M. spectrum* (var. *pentadactylum*), but additional sampling including multiple morphotypes may help clarify if any of the morphological variation is correlated with genetic diversity in this taxon. Furthermore, *M. spectrum* is the only native *Microsorum* occurring on Hawaii (Palmer 2003, Vernon and Ranker 2013), and the results of our phylogenetic analyses indicate that it is not derived from any of the other Pacific *Microsorum*. Additional sampling in Hawaii and elsewhere is needed to confirm whether this taxon is indeed a hybrid, and if so, where its parents occur.

***Taxonomic implications for* Microsorum**—The results of our phylogenetic analysis agree with previous studies showing that *Microsorum* is polyphyletic (Schneider et al. 2004a, 2004b, 2006, Kreier et al. 2008). Although our sampling is not sufficient to

allow for taxonomic revision of the genus, the patterns of hybridization we observe here may be informative for future taxonomic studies. If *Microsorum* were expanded to include all species with this name, it would involve sinking several distinct monophyletic genera (e.g., *Leptochilus*, *Lecanopteris*, *Lepisorus s.l.*) into synonymy, which does not seem warranted. An alternative option is to restrict *Microsorum* to only *M. punctatum* (the type species of *Microsorum*) and closely related species including *M. musifolium*, *M. thailandicum*, and *M. whiteheadii*. *Phymatosorus* (type *M. scolopendrium*) could be applied for the species *M. grossum*, *M. scolopendrium*, and *M. papuanum* (Kreier et al. 2008). However, our results showing that hybrids (*M.* × *maximum*) between *M. punctatum* and *M. grossum* occur frequently and reciprocally (i.e., either species is capable of acting as the mother or father) indicate that these two clades are genetically similar, and at least lack pre-zygotic mating barriers. Furthermore, all of these species are nested within a clade comprising other species of *Microsorum* and a monophyletic *Leptochilus*, corresponding to the "Microsoroid s.s. clade" of Kreier et al. (2008). The other hybridization events we detect (*M.* × *tohieaense*, possibly *M. spectrum*) also occur within this clade, again indicating the genetic affinity of the species involved. Hybridization events between fern genera are not unheard of, but they are extremely rare (Rothfels et al. 2015). Were *Microsorum* split to recognize *Phymatosorus* and *Leptochilus*, it would result in at least two additional inter-generic hybrids.

Our study is the first to our knowledge to show genetic evidence of hybridization in microsoroid ferns. Nooteboom (1997) lists nine other putative hybrid species on the basis of morphology in this group. Inclusion of these taxa in future studies may help clarify the distinctness of proposed genera. Detailed studies into the dynamics of

hybridization, including effect of antheridiogens, sperm and archegonia neck sizes, and the degree of niche overlap between progenitor taxa should also provide further insight into the maintenance of barriers to gene flow between microsoroid ferns (Sigel et al. 2014, Testo et al. 2015) and guide appropriate generic delimitation.

***Evolution of nuclear genomes in* Microsorum**—In addition to the clear evidence we recover for hybrid origins of *M.* × *maximum* and *M.* × *tohieaense*, our dataset also indicates the complicated nature of nuclear genome evolution in this group. We observed multiple well-supported, yet conflicting topologies between the two *gapCp* phylogenies: *M. grossum* and *M. punctatum* form a clade, which are then sister to *M. commutatum*, in the *gapCp short* (BP = 82) and plastid (BP = 98) phylogenies, whereas *M. grossum* is sister to *M. commutatum* + *M. spectrum* (BP = 99) in the *gapCp long* phylogeny. Furthermore, the position of *M. powellii* also varies between phylogenies, appearing amongst other early-diverging groups in the plastid and *gapCp short* phylogenies, but sister to "core *Microsorum*" (i.e., *M. punctatum*, *M. grossum*, and *M. commutatum*) (BP = 84) in the *gapCp long* tree. Such conflict between nuclear genes at internal nodes (as opposed to at the tips as in hybrids) may indicate processes of incomplete lineage sorting or introgression. It is therefore possible that hybridization has played an important role in the evolution of microsoroid ferns over long time scales. The method used here, cloning, is labor-intensive and cannot accommodate a large number of samples / loci. Application of recently developed next-generation DNA sequencing methods for polyploid species complexes (Rothfels et al. 2016) that can produce datasets for a much larger number of unlinked loci, combined with species-tree methods (Edwards 2009), should provide better insight into the complex evolutionary history of this group.

4.7 Acknowledgements

We are grateful to Alan R. Smith and Jean-François Butaud for helpful discussions on the possible origins of *M.* × *tohieaense*. Atsushi Ebihara kindly provided use of the microscope for observations of rhizome scales and spores. Colin C. Nitta provided illustrations. Suzanne Vinette, Tohei Theophilus, and Tristan Wang assisted with fieldwork. Staff at the University of California, Berkeley Richard B. Gump South Pacific Research Station, station provided logistical support for fieldwork. Funding for this project provided in part by National Science Foundation (Doctoral Dissertation Improvement Grant DEB-1311169 to JHN and CCD), Setup Funds from Harvard University to CCD, the American Society of Plant Taxonomists (Research Grant for Graduate Students to JHN), the Garden Club of America (Award in Tropical Botany to JHN), the Harvard University Herbaria (Fernald Fieldwork Fellowship to JHN), the Society of Systematic Biologists (Graduate Student Research Award to JHN), and the Systematics Association (Systematics Research Fund to JHN).

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Appendix A:

Supporting material for Chapter I

Figure A1. Climatic variables for 18 sites along an elevational gradient on Moorea and Tahiti, French Polynesia from Nov. 12, 2013 to Jan. 17, 2014. Black arrows indicate Mt. Rotui 830 m site, which was much more exposed than other sites. All variables are means of daily values.

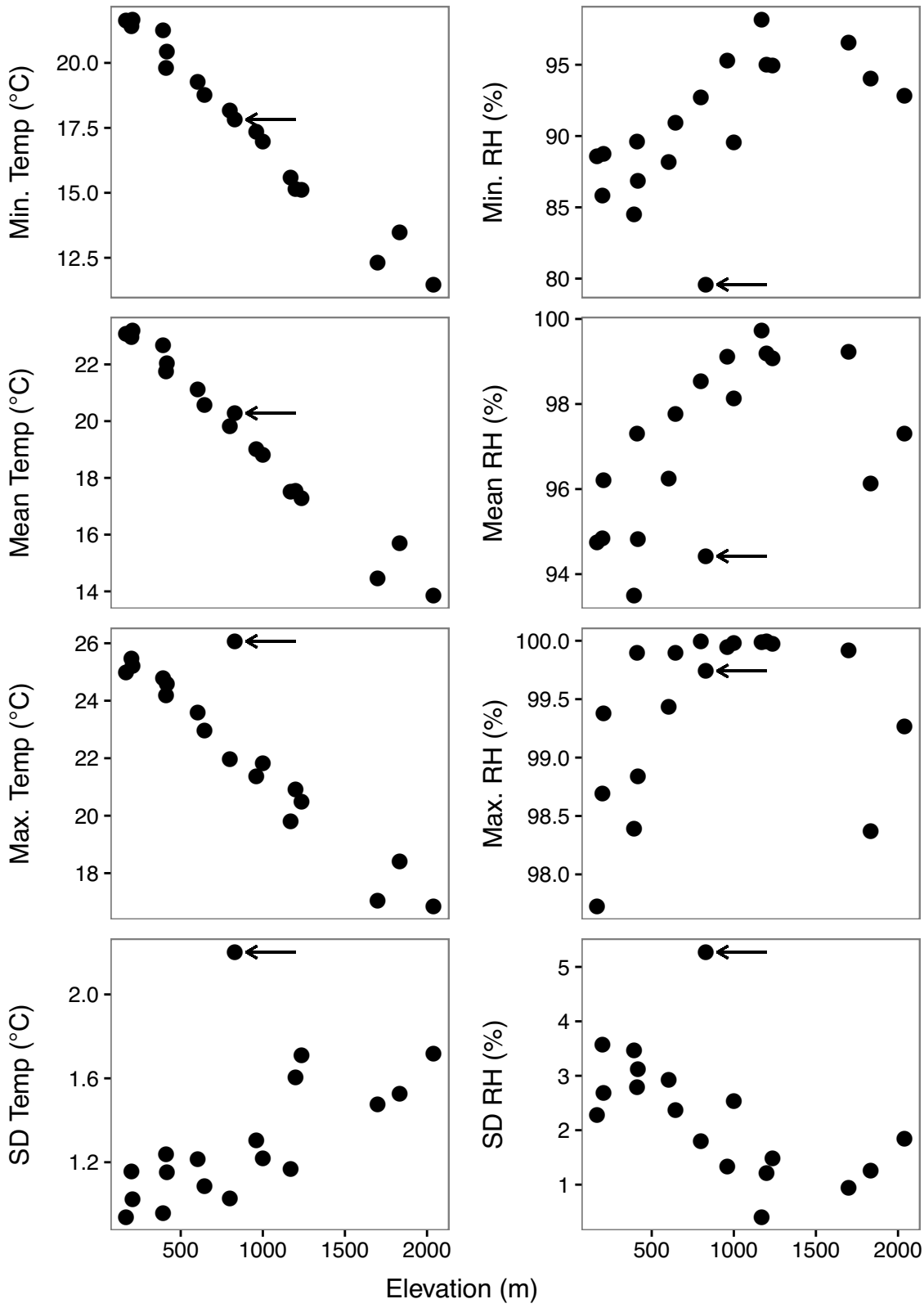


Figure A1 (Continued).

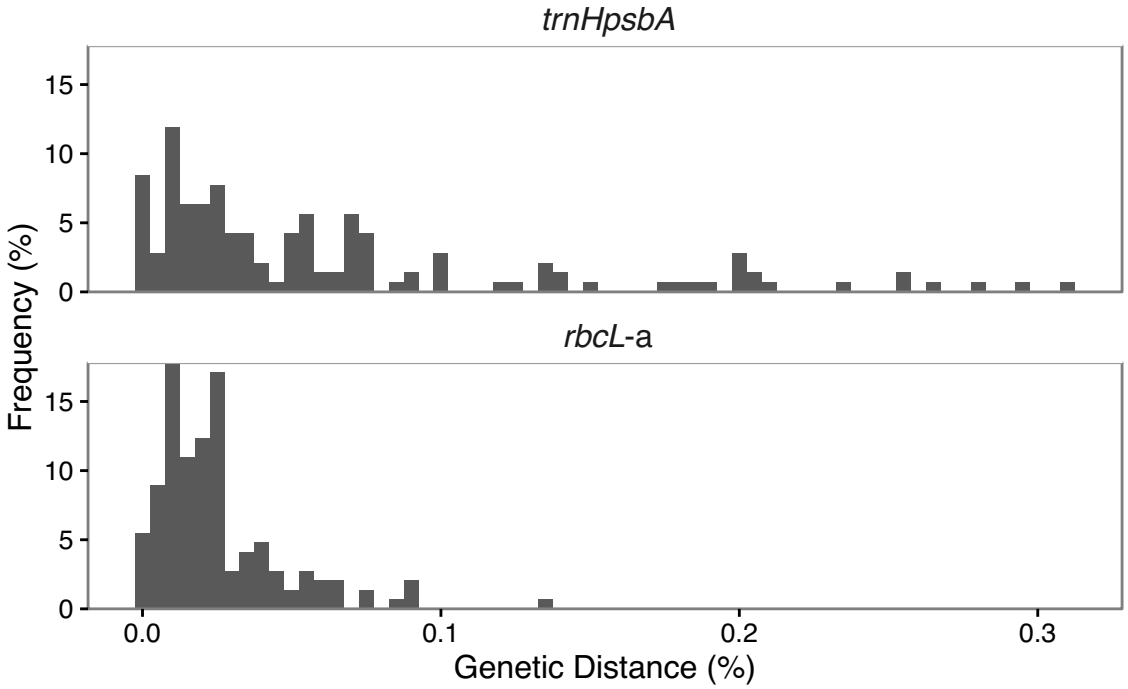
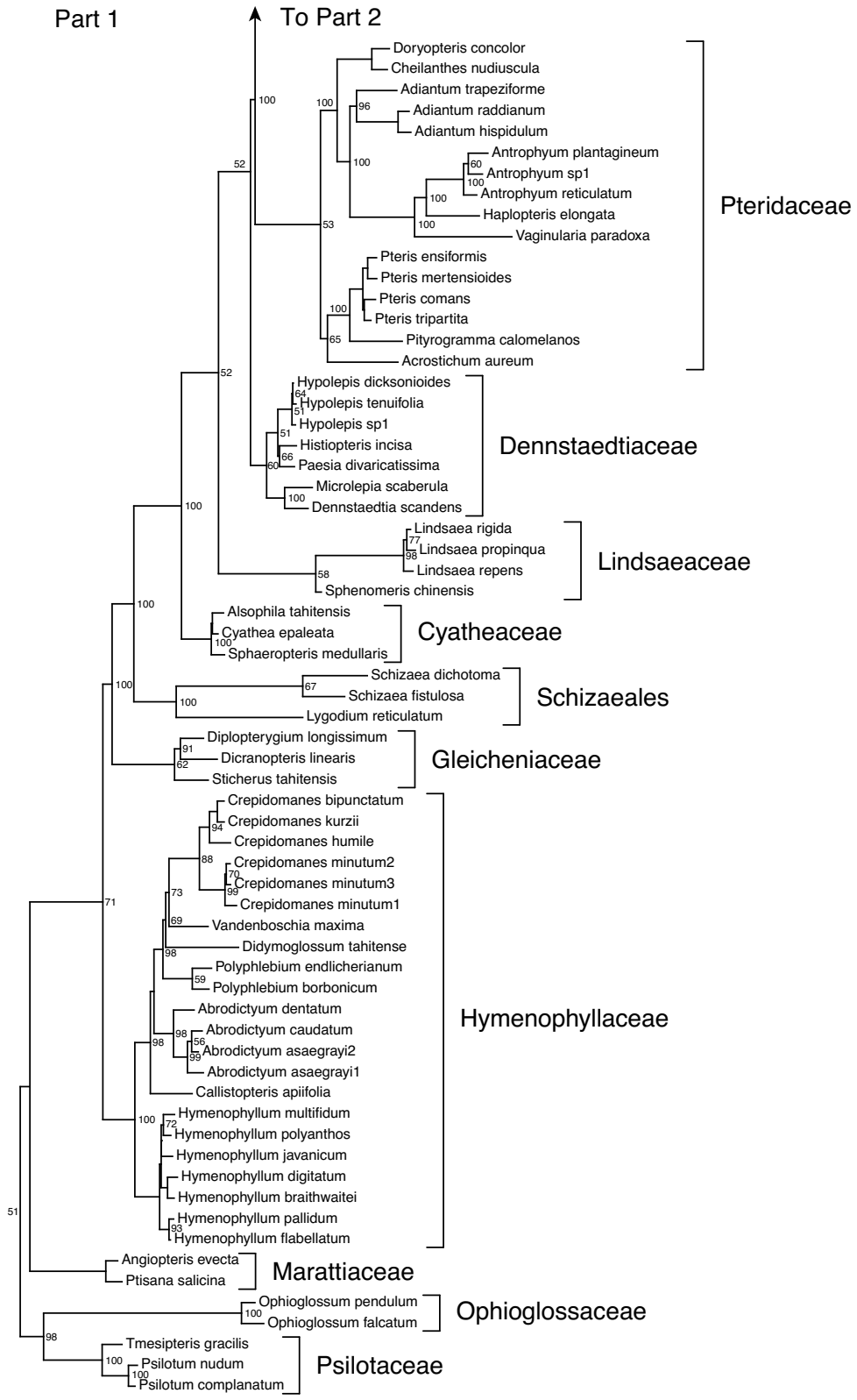


Figure A2. Interspecific genetic distances for *rbcL*-a (*N* = 146 spp.) and *trnH-psbA* (*N* = 143 spp.). The single, closest interspecific distance was calculated for each species for each marker.

Figure A3. Maximum likelihood phylogenetic tree of fern species from Moorea and Tahiti inferred from chloroplast *rbcL*, *atpA*, and *atbB*. The original tree (not shown) was inferred using a broader sampling of species from GenBank (3840 spp.), then trimmed to species from Moorea and Tahiti only (145 spp.; see 1.3 Methods). Taxonomy follows Pteridophyte Phylogeny Group (http://botany.si.edu/PPG). Bootstrap support > 50% from 100 ML bootstraps shown at nodes.



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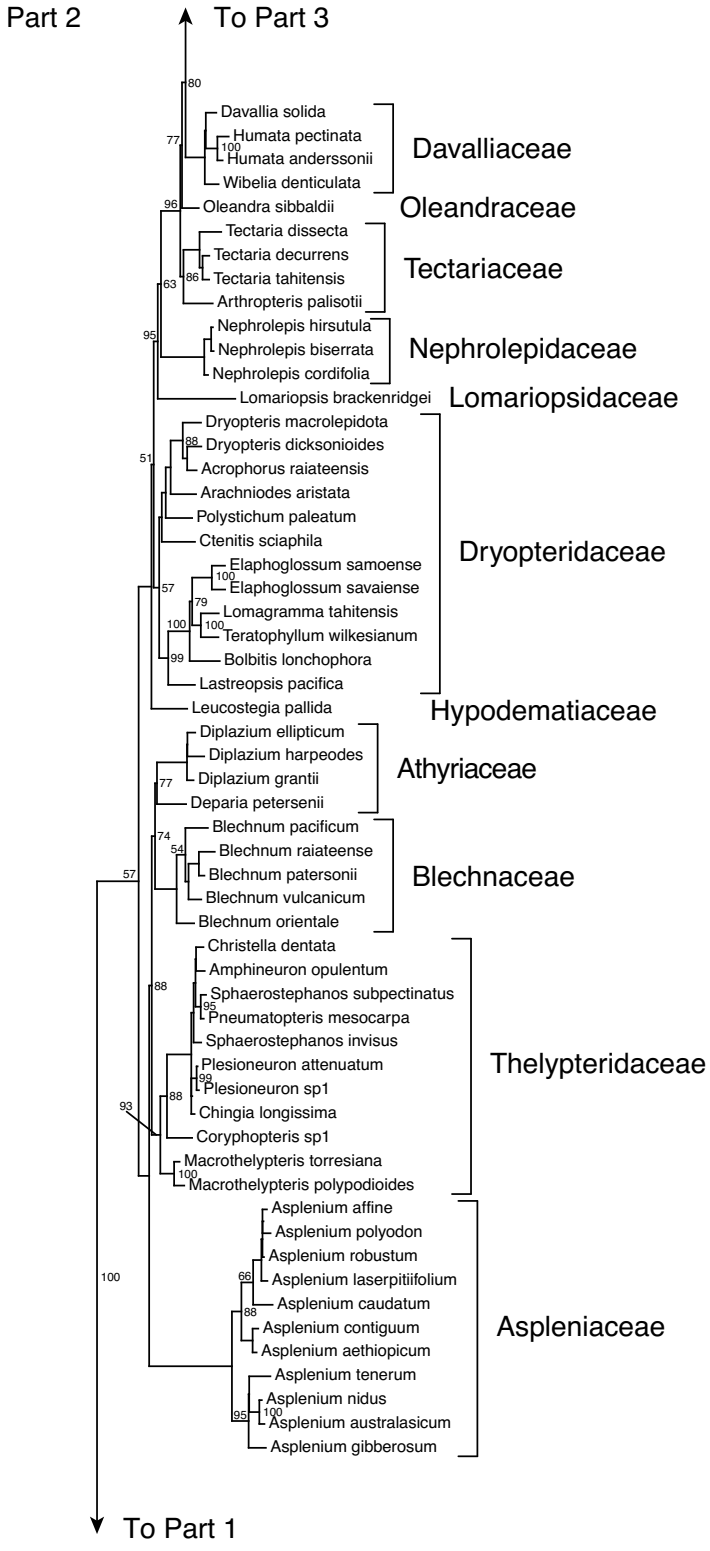

Figure A3 (Continued).

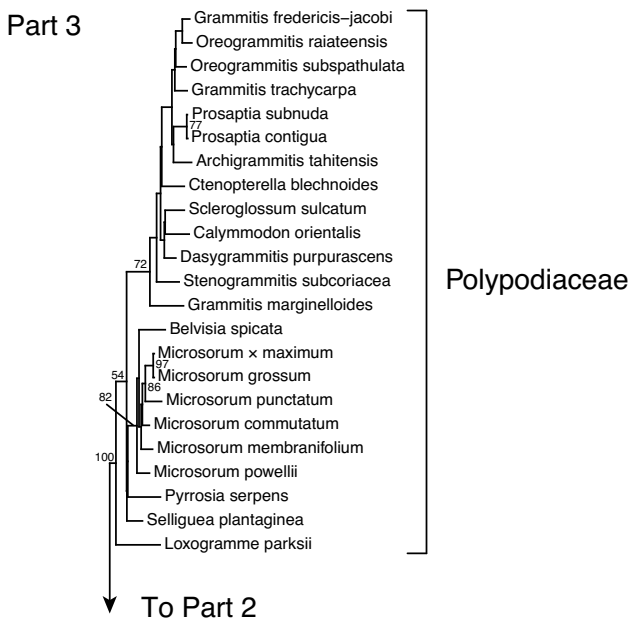

Figure A3 (Continued).

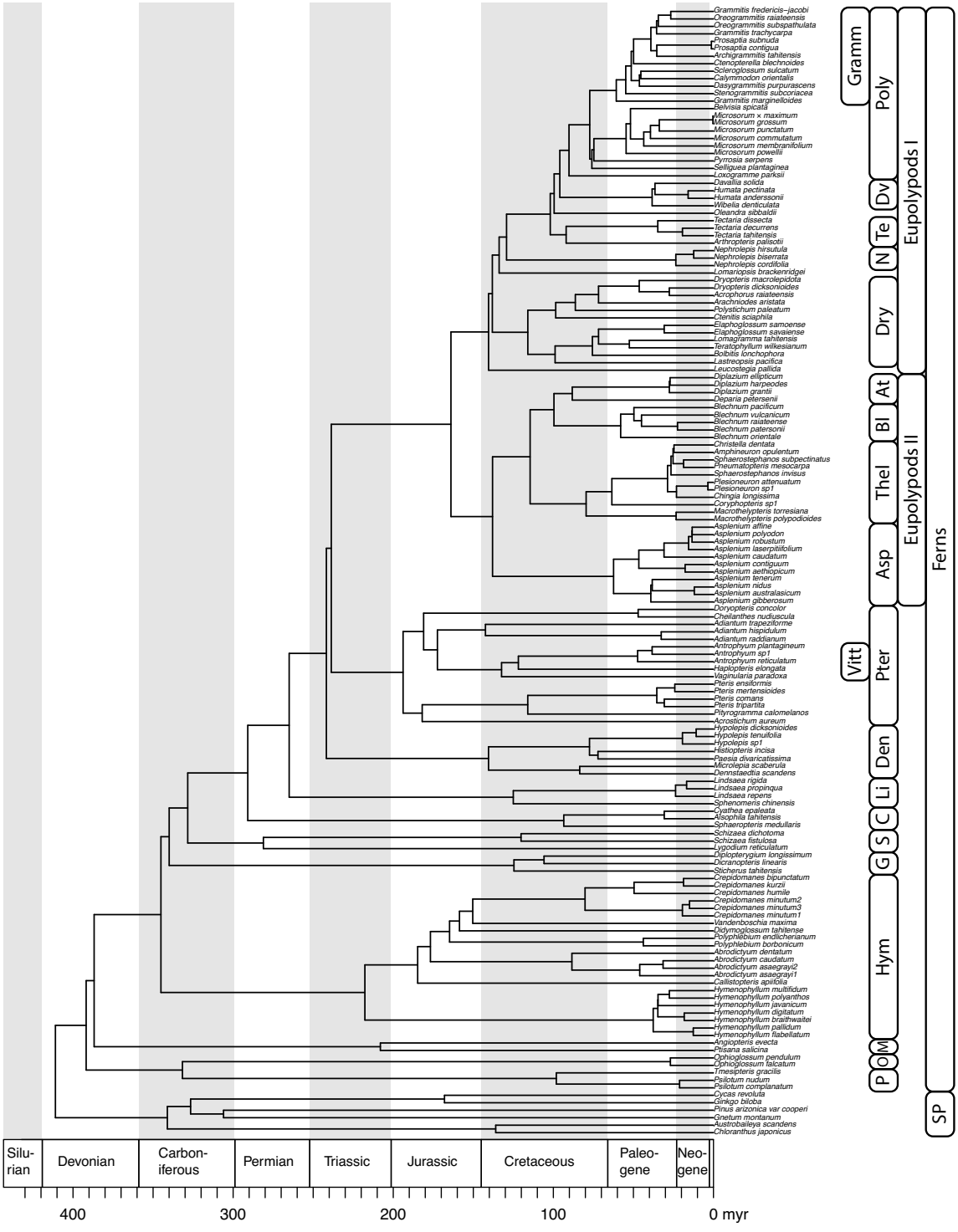

Figure A4. Time-calibrated ultrametric tree of fern species from Moorea and Tahiti plus seed plants (outgroup).

Figure A4 (Continued). Rates estimated using treePL. Taxonomy follows Pteridophyte Phylogeny Group (http://botany.si.edu/PPG). Clade names not shown for some clades with only one species each in our sampling (*Oleandra sibbaldii* in Oleandraceae, *Leucostegia pallida* in Hypodematiaceae, *Lomariopsis brackenridgei* in Lomariopsidaceae). Abbreviations: SP, seed plants; P, Psilotaceae; O, Ophioglossaceae; M, Marattiaceae; Hym, Hymenophyllaceae; G, Gleicheniaceae; S; Schizaeales; C, Cyatheaceae; Li, Lindsaceae; Den, Dennstaedtiaceae; Pter, Pteridaceae; Asp, Aspleniaceae; Thel, Thelypteridaceae; Bl, Blechnaceae; At; Athyriaceae; Dry, Dryopteridaceae; N, Nephrolepidaceae; Te, Tectariaceae; Dv, Davalliaceae; Poly, Polypodiaceae; Vitt, vittarioids; Gramm, grammitids.

Figure A5. Phylogenetic structure of fern gametophyte (blue circles) and sporophyte (red triangles) communities along an elevational gradient by abundance weighting (analysis abundance-weighted or not) and dataset (full, restricted, or simulated; simulated dataset does not include abundance; see 1.3 Methods). (A) Standard effect size (SES) of mean phylogenetic distance (MPD). **(B)** Standard effect size of mean nearest taxon distance (MNTD). **(C)** Observed richness. Trendlines indicate significant ($P < 0.05$) linear or second-order polynomial relationships between phylogenetic community structure or species richness and elevation. For (A) and (B), values greater than zero indicate phylogenetic overdispersion; those less than zero indicate phylogenetic clustering; asterisks indicate communities with significantly different phylogenetic structure from 999 randomly assembled null communities ($P < 0.05$, two-sided test).

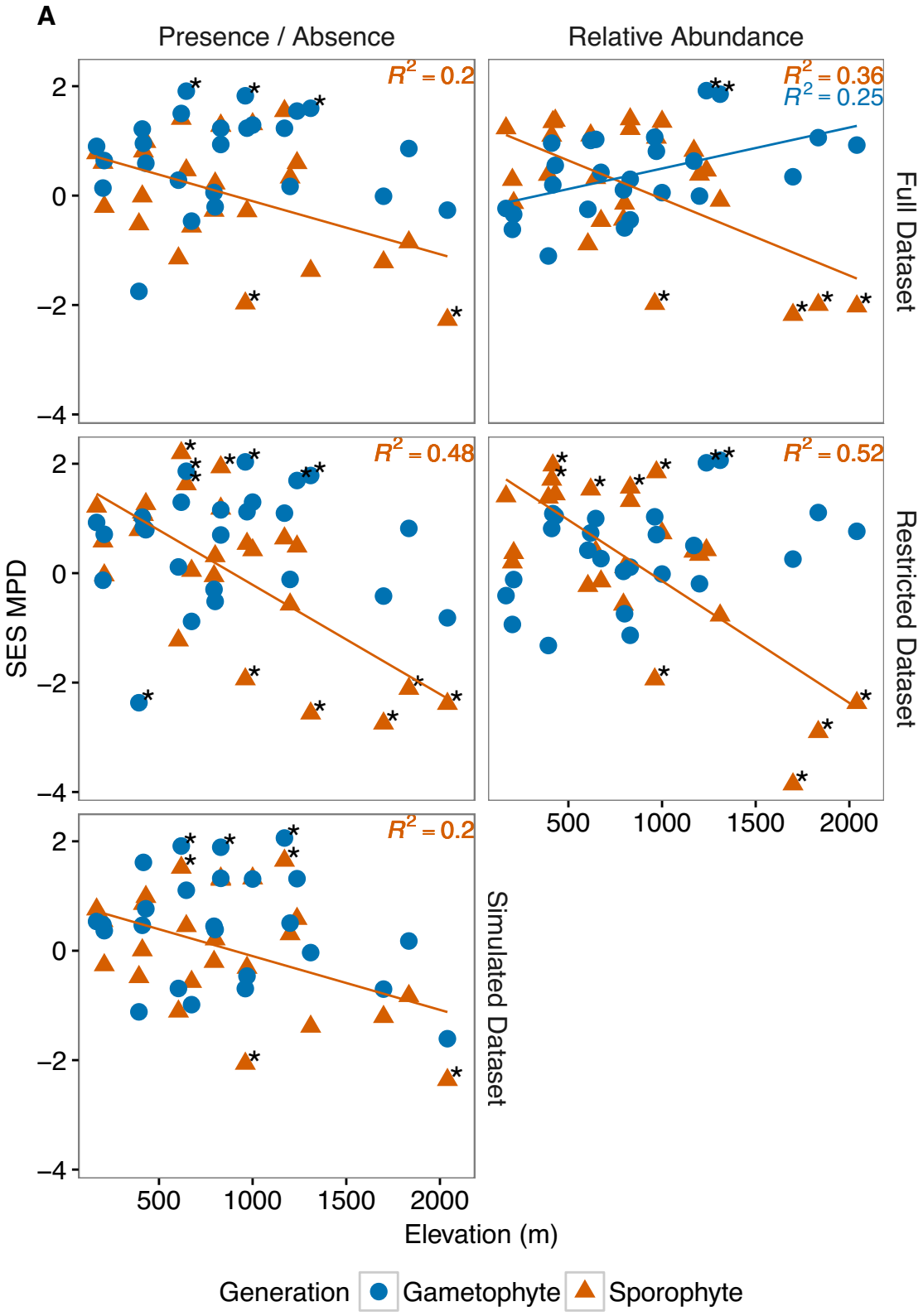

Figure A5 (Continued).

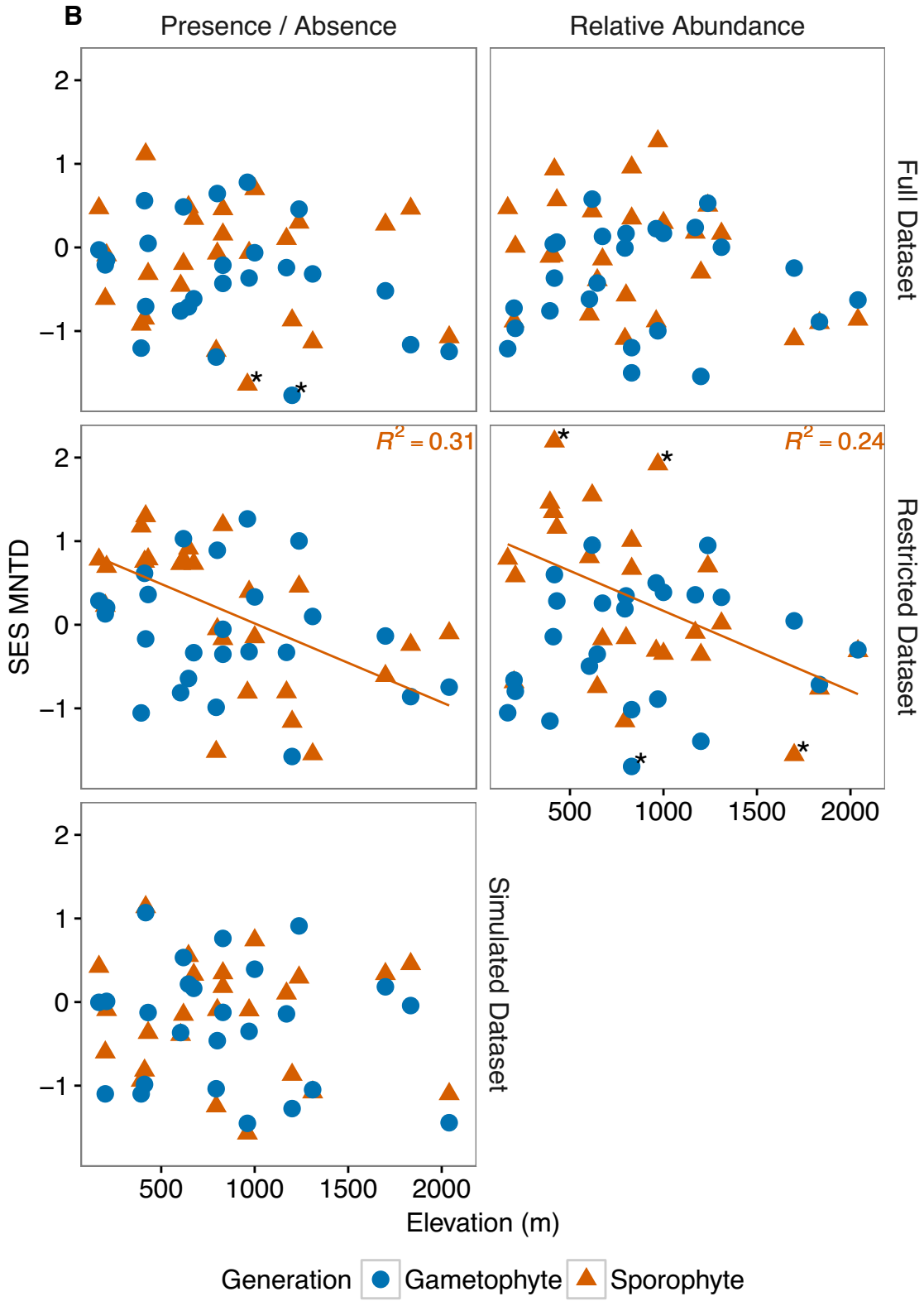

Figure A5 (Continued).

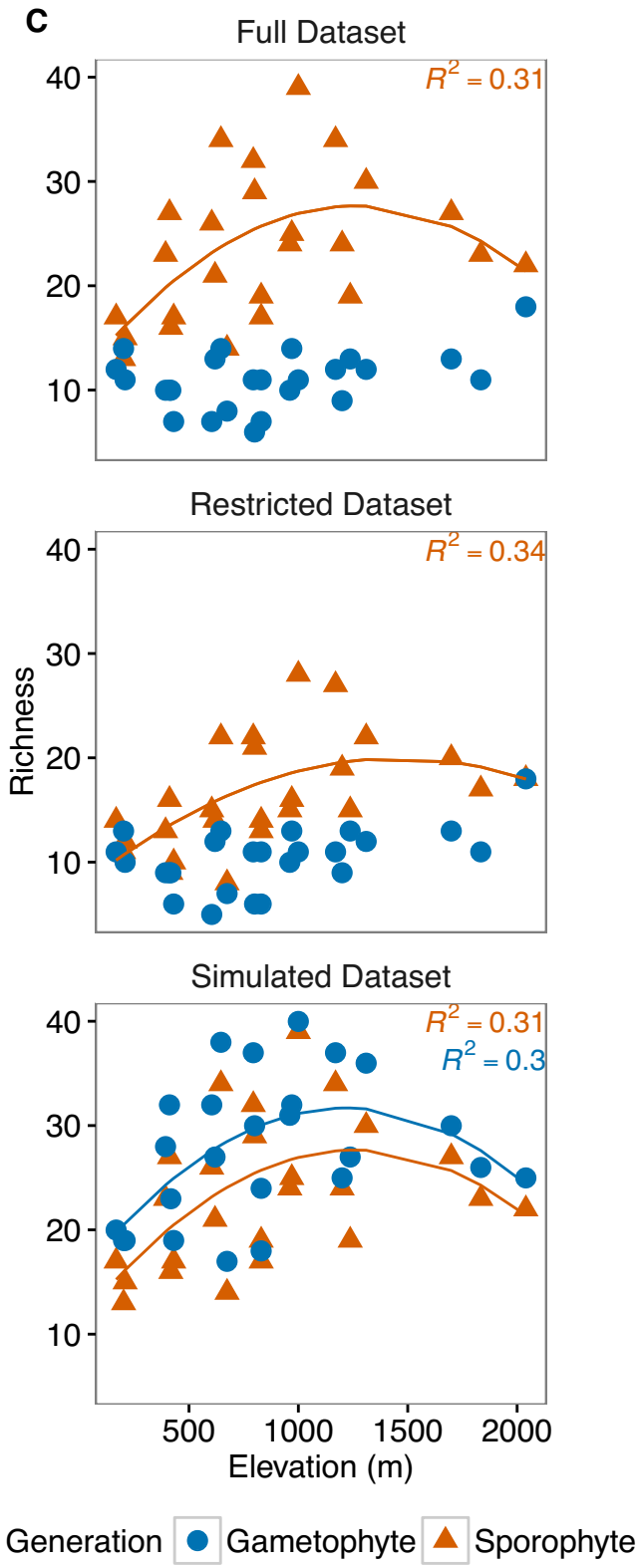

Figure A5 (Continued).

Figure A6. Non-metric multidimensional scaling (NMDS) of distances between fern sporophyte and gametophyte plots by abundance weighting (analysis abundance-weighted or not) and dataset (full, restricted, or simulated; simulated dataset does not include abundance; see 1.3 Methods). (A) Bray-Curtis (i.e., species-level) dissimilarities. **(B)** Mean nearest taxon (MNTD) distances. **(C)** Mean phylogenetic distance (MPD) distances. Circles indicate gametophyte plots; triangles indicate sporophyte plots; color indicates elevation.

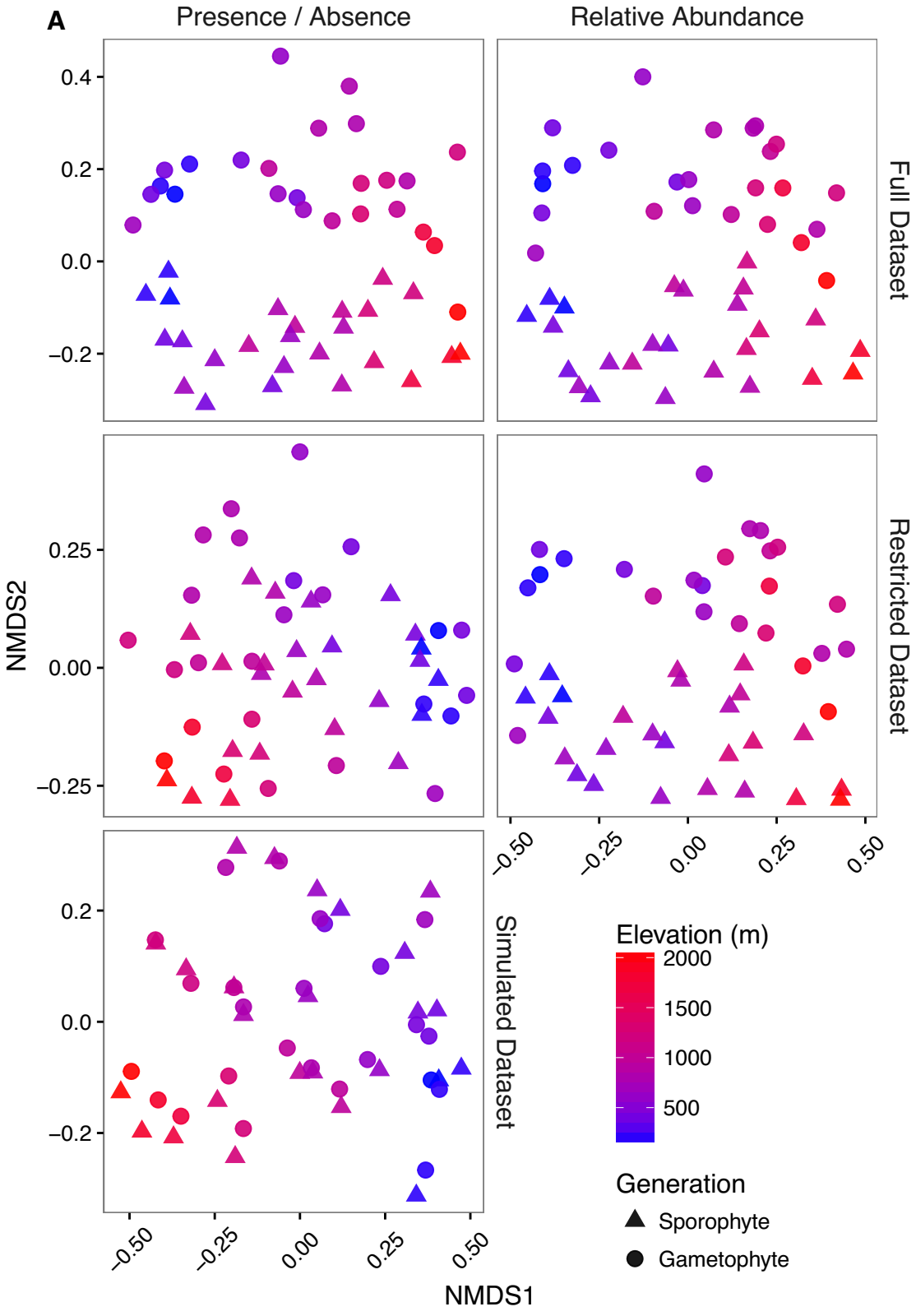

Figure A6 (Continued).

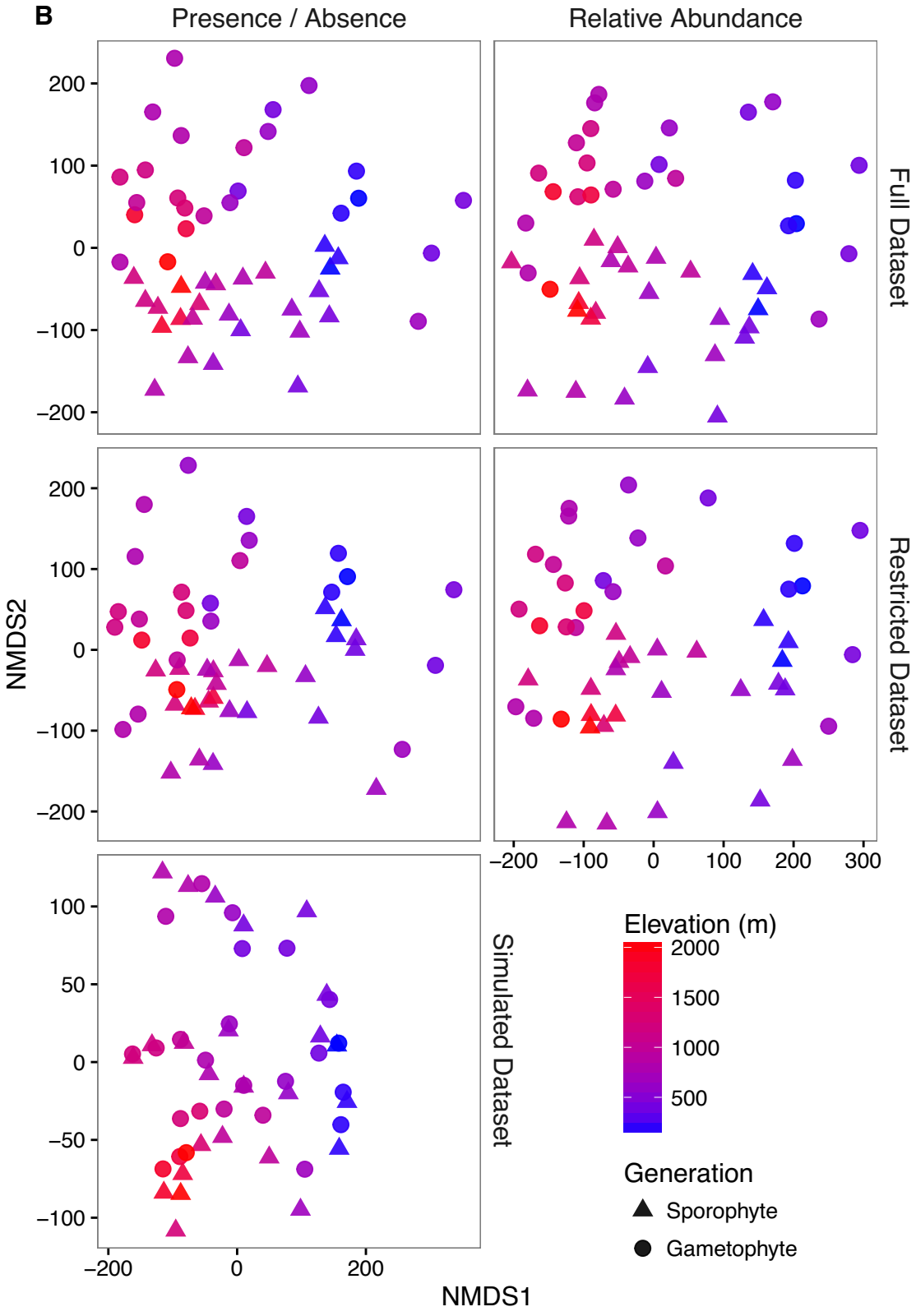

Figure A6 (Continued).

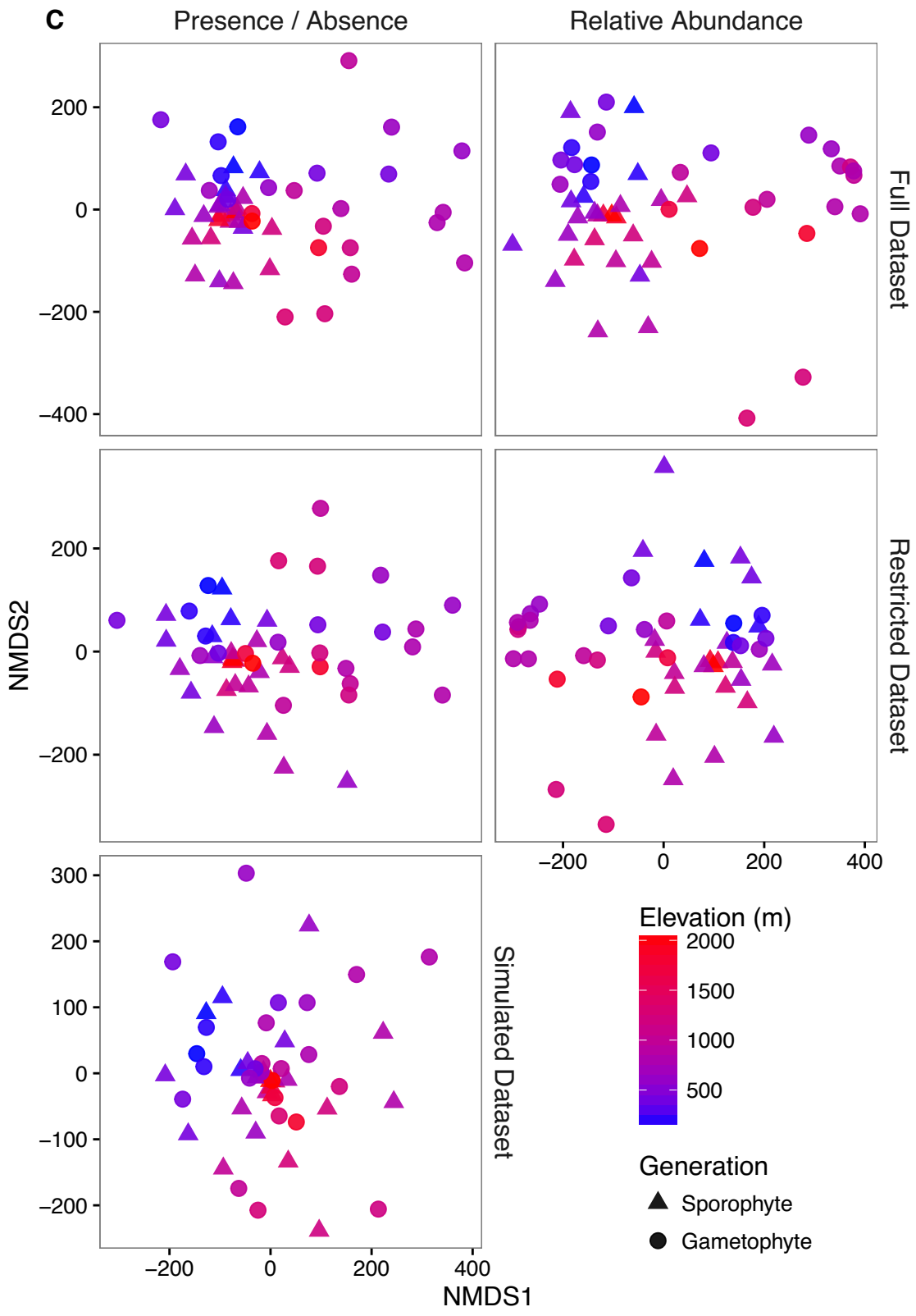

Figure A6 (Continued).

Figure A7. Variance partitioning on environmental and spatial components that explain turnover in fern community composition (adjusted R^2) by generation (sporophyte or gametophyte) for all combinations of abundance weighting (analysis abundance-weighted or not) and dataset (full, restricted, or simulated; simulated dataset does not include abundance; see 1.3 Methods). Spatial components (Geo) are eigenvectors produced by principal components of nearest neighbor matrices of latitude and longitude of each fern community; environmental components are the first two principal components axes (PC1 and PC2) of eight temperature and humidity metrics. β-diversity metrics include **(A)** Bray-Curtis (i.e., species-level) dissimilarities, **(B)** mean nearest taxon (MNTD) distances, and **(C)** mean phylogenetic distance (MPD) distances. Asterisk indicates significance at $P < 0.05$ (tested with 999 permutations using pseudo-F ratios).

(A) Bray–Curtis

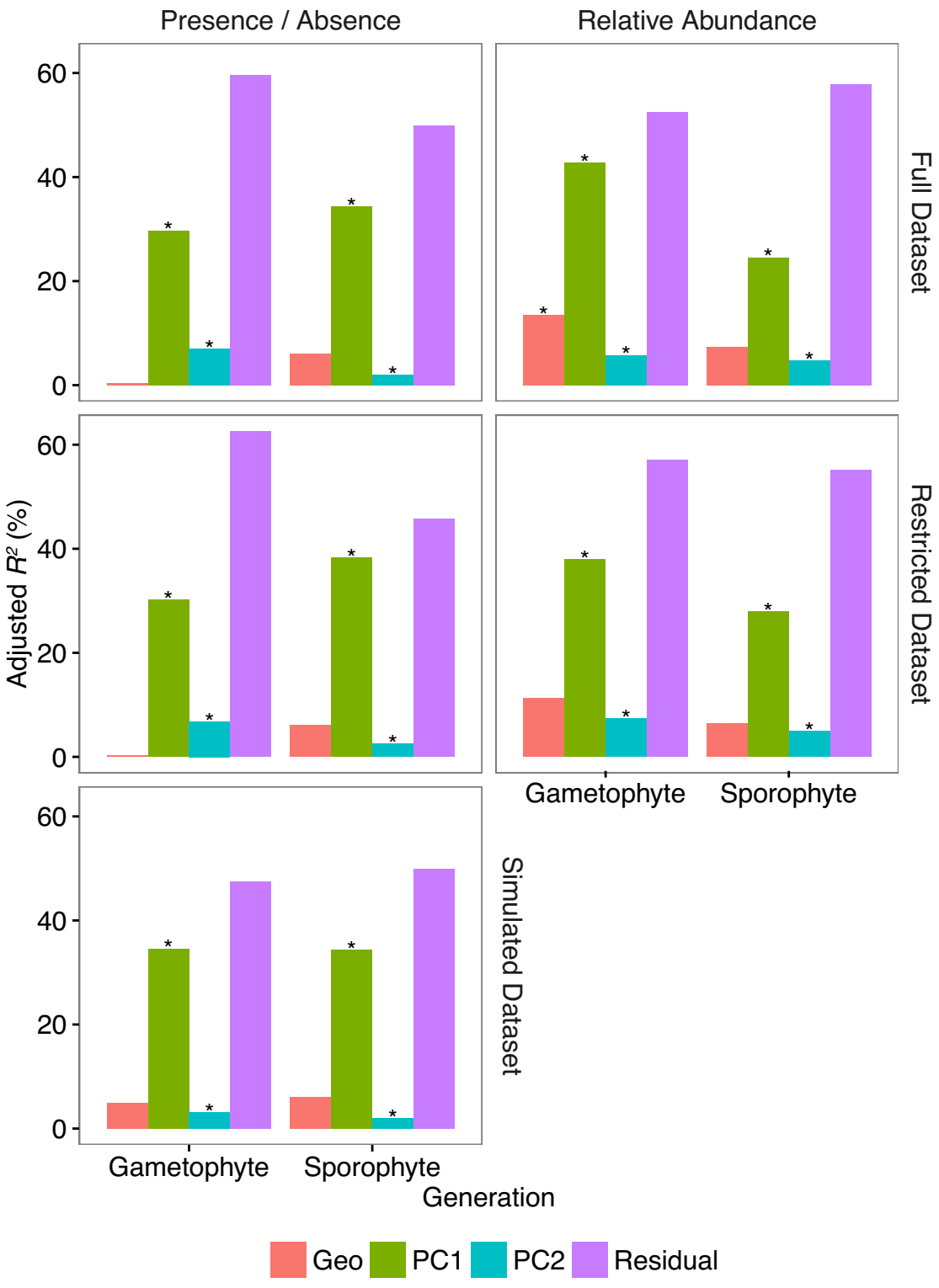

Figure A7 (Continued).

(B) MNTD

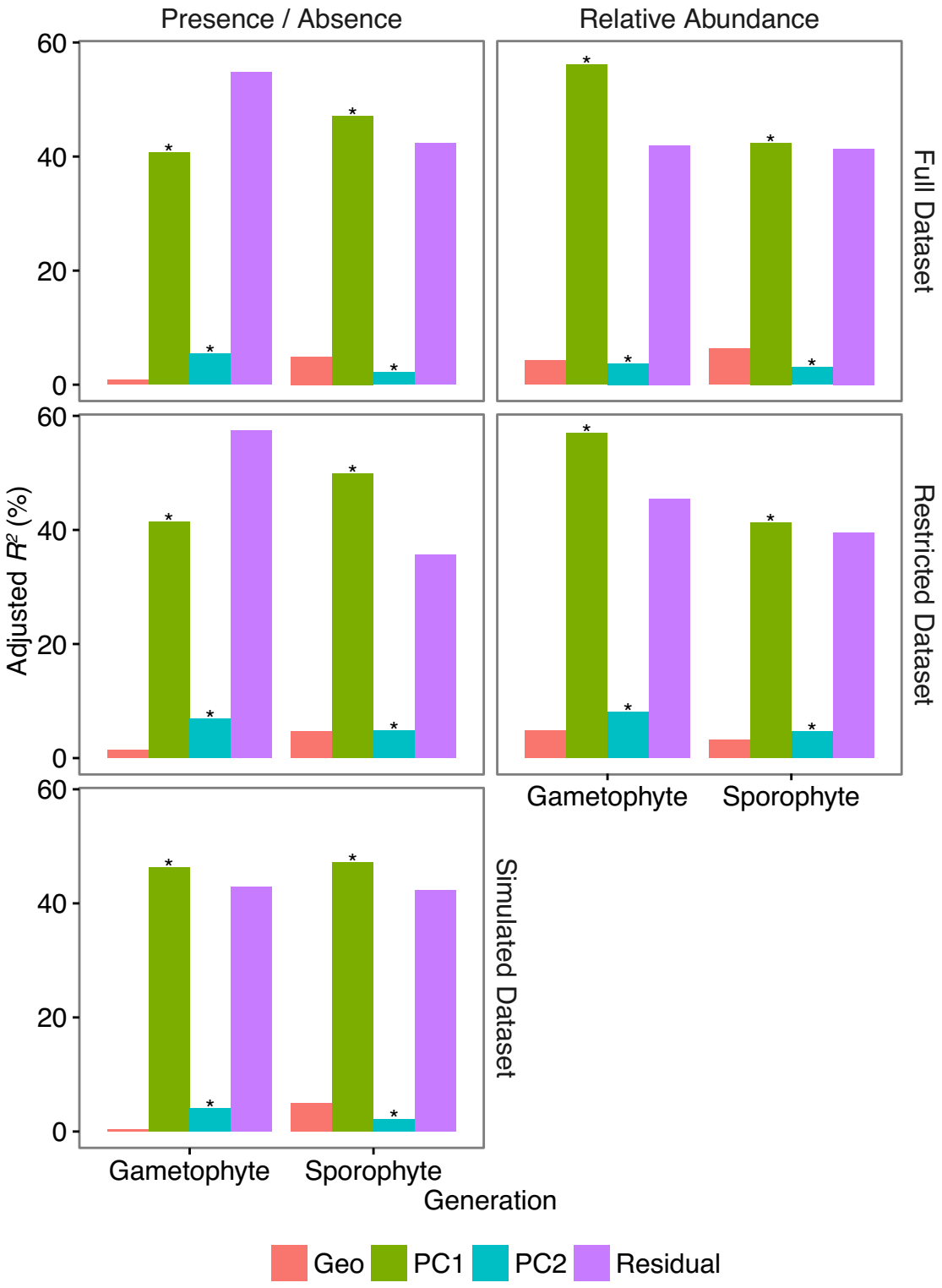

Figure A7 (Continued).

(C) MPD

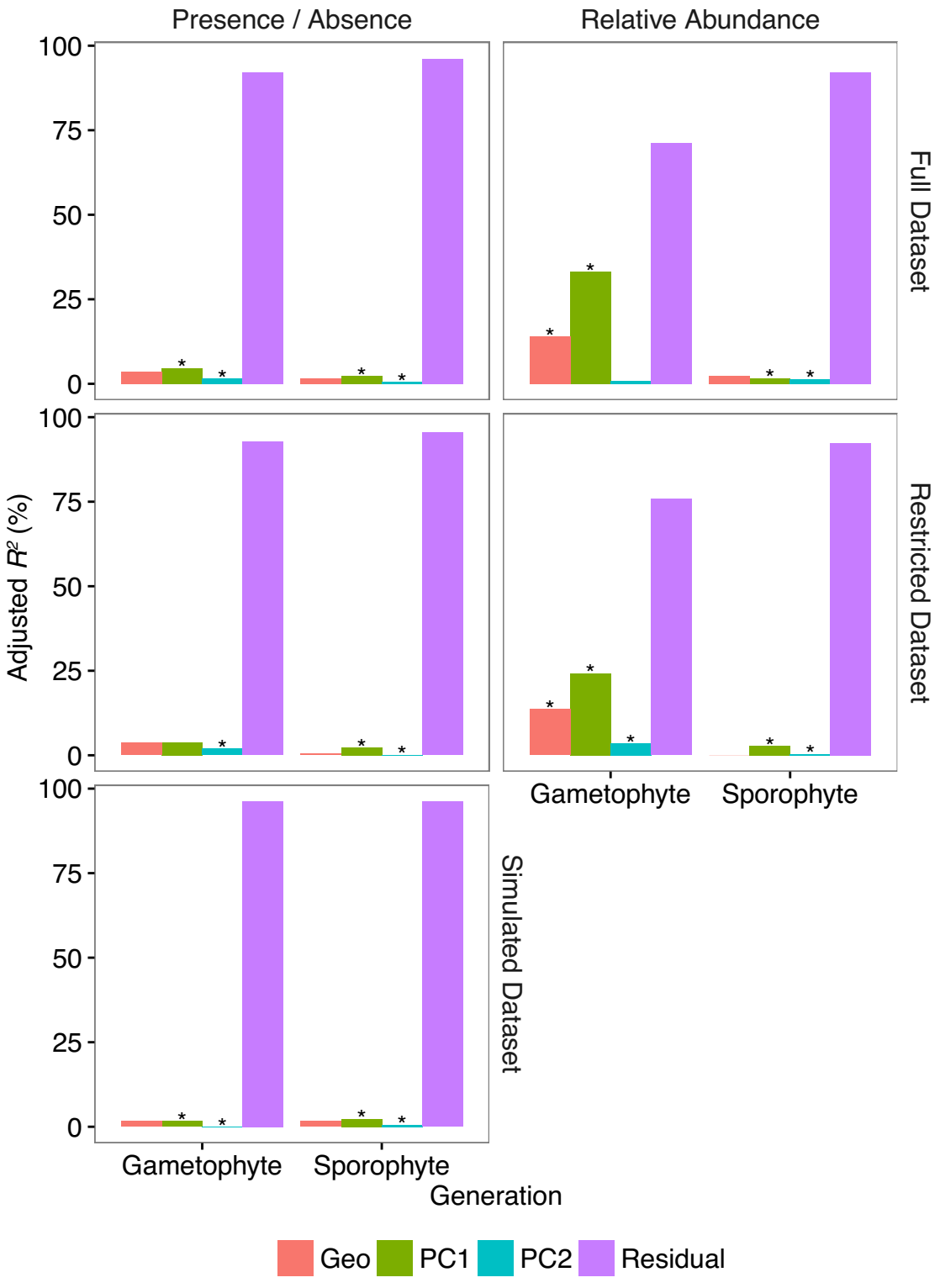

Figure A7 (Continued).

Figure A8. Rank species abundance curves by elevation. Elevations rounded to the nearest 100 m, and occurrences pooled together for 200, 400, 600, 800, 1000, and 1200 m plots. Two most abundant taxa labeled in each plot. **(A)** gametophytes. **(B)** sporophytes.

(A) Gametophytes

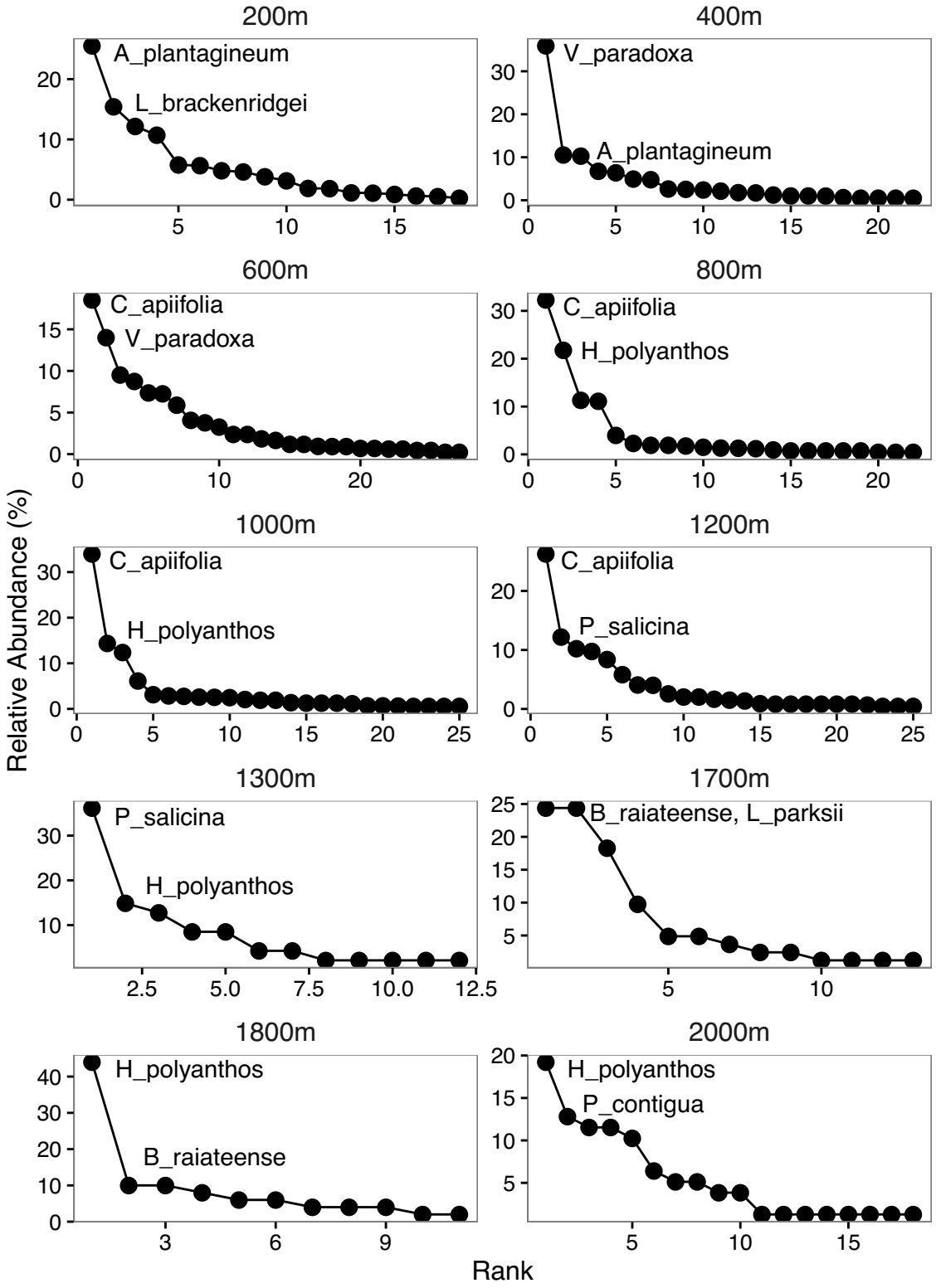

Figure A8 (Continued).

(B) Sporophytes

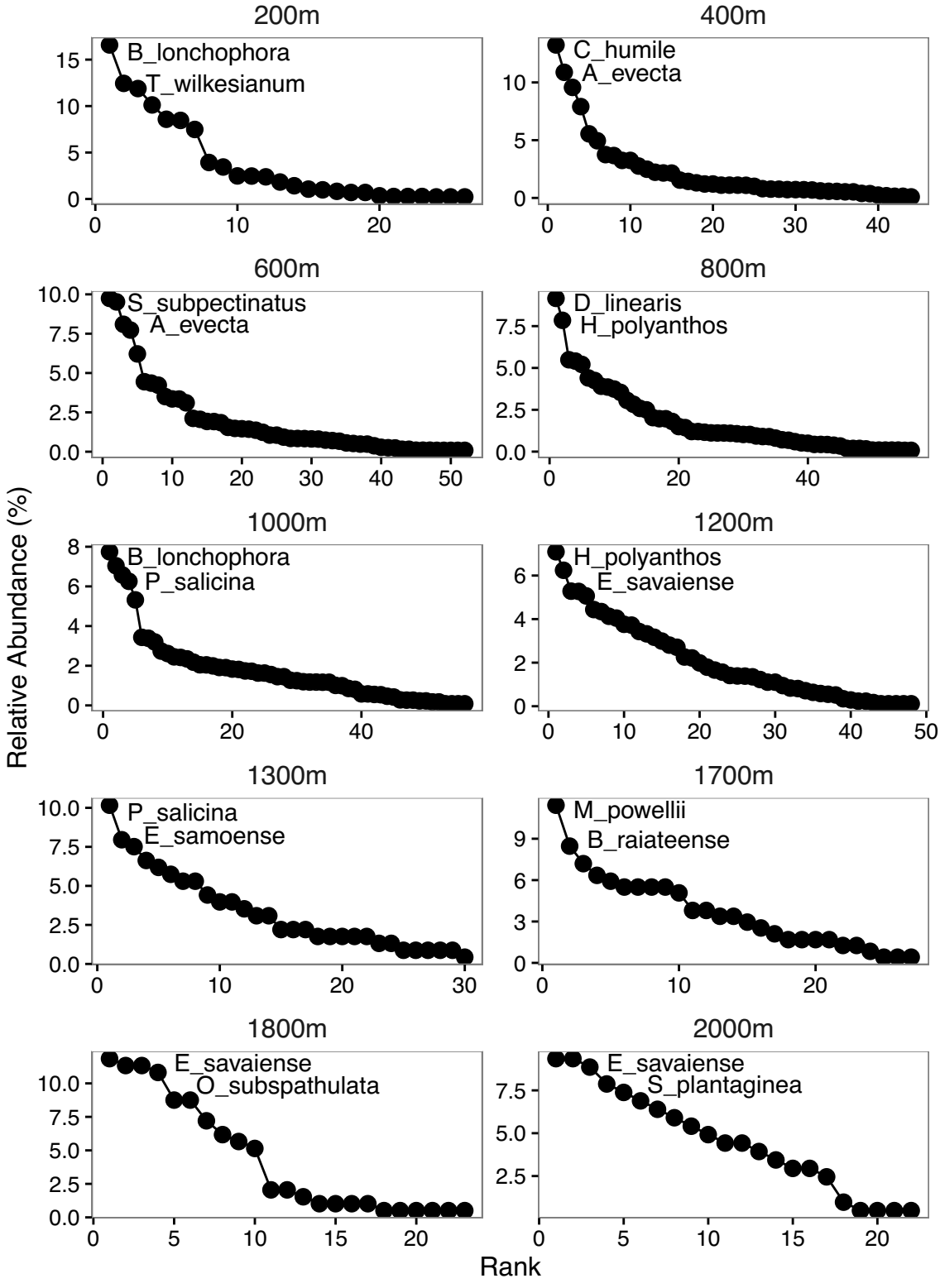

Figure A8 (Continued).

Table A1. Fossil calibration points used for divergence dating analysis with treePL.

References: 1, Magallón et al. (2013); 2, Pryer et al. (2004) 3, Schuettpelz and Pryer (2009).

Clade	Calibration Type	Age (Ma)	Reference
Euphyllophytes	fixed	411	1
Seed plants	minimum	318	1
Conifers	minimum	306	1
Angiosperms	minimum	136	1
Horsetails	minimum	385	1
Marattia	minimum	206	2
Osmundaceae	minimum	299	3
Osmunda	minimum	199.6	3
Matoniaceae	minimum	228	3
Gleicheniaceae subclade	minimum	99.6	3
Stromatopteris	minimum	89.3	3
Lygodium	minimum	167.7	3
Anemia	minimum	136.4	3
Marsileaceae	minimum	140.2	3
Azolla	minimum	83.5	3
Pilularia	minimum	83.5	3
Scaly Tree Ferns	minimum	145.5	3
Lophosoria	minimum	112	3
Loxomataceae	minimum	112	3
Cyathea plus *Alsophila*	minimum	93.5	3
Lindsaeoids	minimum	99.6	3
Dennstaeceae subclade	minimum	70.6	3
Ceratopteris plus *Acrostichum*	minimum	65.5	3
Ceratopteris	minimum	37.2	3
Pteroids	minimum	93.5	3
Cyclosoroids	minimum	33.9	3
Athyrioids	minimum	37.2	3
Onoclea	minimum	55.8	3
Woodwordia	minimum	55.8	3
Polygrammoids	minimum	33.9	3

Table A2. Divergence times for selected clades estimated with treePL. Clade names follow Schuettpelz and Pryer (2009).

Clade	Age (Ma)
Monilophytes	391.81
Whisk ferns	98.15
Ophioglossoid ferns	213.68
Marattioids	218.98
Horsetails	60.09
Leptosporangiates	362.64
Osmundaceous ferns (Osmundales)	199.60
Filmy ferns (Hymenophyllales)	217.72
Trichomanoids	184.75
Hymenophylloids	50.61
Gleichenioids (Gleicheniales)	312.72
Schizaeoids (Schizaeales)	281.11
Core leptosporangiates	300.72
Heterosporous ferns (Salviniales)	246.31
Tree ferns (Cyatheales)	157.49
Scaly tree ferns	93.50
Polypods (Polypodiales)	265.00
Lindsaeoids	125.03
Dennstaedtioids	160.46
Pteroids	197.60
Pteridoids	101.24
Cheilanthoids	162.53
Adiantoids	142.42
Vittarioids	132.35
Eupolypods	163.97
Eupolypods II	140.68
Asplenioids	92.94
Thelypteroids	79.53
Blechnoids	61.47
Athyrioids	88.20
Eupolypods I	141.91
Dryopteroids	115.94
Elaphoglossoids	52.23
Tectarioids	38.20
Davallioids	63.03
Polygrammoids	90.23
Grammitids	61.56

Table A3. Second-order polynomial and linear models of fern community composition (mean phylogenetic distance, mean nearest-taxon distance, observed species richness, and estimated species richness) in relation to environment (axes 1 and 2 of environmental PCA) for all sites with environmental data available (*N* = 18), including full, restricted, and simulated datasets. G = gametophyte, S = sporophyte. pa = non-abundance-weighted analysis; rel = abundance-weighted analysis. Significance of coefficients indicated by asterisks: * = $P < 0.05$; ** = $P < 0.005$; *** = $P < 0.0005$; NS = not significant.

Table A3 (Continued).

Dataset	Abundance	Response	Generation	Adjusted R^2	Intercept	PC1	$PC1^2$	PC2	$PC2^2$
full	pa	MPD	G	0.21	0.48*			-0.33 (NS)	
full	pa	MPD	S	0.47	-0.3 (NS)	0.25**			
full	pa	MNTD	G	0.46	-0.6***	0.11 (NS)		-0.27*	
full	pa	MNTD	S	0.44	-0.37*	0.22*			
full	pa	Obs. Richness	G	0	11.33***				
full	pa	Obs. Richness	S	0.18	23.94***	-1.25 (NS)			
full	pa	Est. Richness	G	0.2	10.29***				0.44 (NS)
full	pa	Est. Richness	S	0.28	16.78***	-0.72*			
restricted	pa	MPD	G	0	0.18 (NS)				
restricted	pa	MPD	S	0.44	-0.33 (NS)	0.3**			
restricted	pa	MNTD	G	0.47	-0.37*	0.11*			-0.14*
restricted	pa	MNTD	S	0.52	-0.43*	0.23**			
restricted	pa	Obs. Richness	G	0	11.33***				
restricted	pa	Obs. Richness	S	0.18	23.94***	-1.25 (NS)			
restricted	pa	Est. Richness	G	0.2	10.29***				0.44 (NS)
restricted	pa	Est. Richness	S	0.28	16.78***	-0.72*			
full	rel	MPD	G	0.27	0.23 (NS)			-0.46*	
full	rel	MPD	S	0.86	-0.32 (NS)	0.53***		-0.36*	
full	rel	MNTD	G	0.2	-0.48*			-0.27 (NS)	
full	rel	MNTD	S	0.87	-0.43*	0.5***	0.08**		-0.16*
full	rel	Obs. Richness	G	0	10.78***				
full	rel	Obs. Richness	S	0.35	17***	-1.26*			
full	rel	Est. Richness	G	0.2	10.29***				0.44 (NS)
full	rel	Est. Richness	S	0.28	16.78***	-0.72*			
restricted	rel	MPD	G	0	0.05 (NS)				
restricted	rel	MPD	S	0.72	-0.27 (NS)	0.54***			
restricted	rel	MNTD	G	0	-0.56*				

Table A3 (Continued).

Dataset	Abundance	Response	Generation	Adjusted R^2	Intercept	PC1	$PC1^2$	PC2	$PC2^2$
restricted	rel	MNTD	S	0.54	-0.13 (NS)	0.37**			
restricted	rel	Obs. Richness	G	0	10.78***				
restricted	rel	Obs. Richness	S	0.35	17***	-1.26*			
restricted	rel	Est. Richness	G	0.2	10.29***				0.44 (NS)
restricted	rel	Est. Richness	S	0.28	16.78***	-0.72*			
simulated	pa	MPD	G	0.43	0.2 (NS)	0.26**			
simulated	pa	MPD	S	0.46	-0.31 (NS)	0.25**			
simulated	pa	MNTD	G	0.44	-0.52*	0.25**			
simulated	pa	MNTD	S	0.43	-0.37*	0.22*			
simulated	pa	Obs. Richness	G	0	28.22***				
simulated	pa	Obs. Richness	S	0.18	23.94***	-1.25 (NS)			
simulated	pa	Est. Richness	G	0.2	10.29***				0.44 (NS)
simulated	pa	Est. Richness	S	0.28	16.78***	-0.72*			

Table A4. Voucher specimens for sequences newly generated for this study. –

indicates no sequence available.

Table A4 (Continued).

Species	rbcL	trnHpsbA
Abrodictyum asaegrayi (Bosch) Ebihara & K. Iwats. var 1	Nitta 262 (UC)	Nitta 1547 (GH)
Abrodictyum asaegrayi (Bosch) Ebihara & K. Iwats. var 2	Nitta 272 (UC)	Nitta 1581A (GH)
Abrodictyum caudatum (Brack.) Ebihara & K. Iwats.	Nitta 39 (UC)	Nitta 31 (UC)
Abrodictyum dentatum (Bosch) Ebihara & K. Iwats.	Nitta 44 (UC)	Nitta 56 (UC)
Acrophorus raiateensis J.W.Moore	Nitta 3334 (GH)	Nitta 3334 (GH)
Acrostichum aureum L.	Cousteau 8 (UC)	Cousteau 8 (UC)
Adiantum hispidulum Sw.	Vinette 9 (UC)	Vinette 9 (UC)
Adiantum raddianum C. Presl	Vinette 11 (UC)	Vinette 11 (UC)
Adiantum trapeziforme L.	Vinette 4 (UC)	Ranker 1942 (COLO)
Alsophila tahitensis Brack.	Nitta 611 (GH)	Nitta 611 (GH)
Amphineuron opulentum (Kaulf.) Holttum	Nitta 300 (UC)	Nitta 300 (UC)
Angiopteris evecta (G. Forst.) Hoffm.	Cousteau 3 (UC)	Cousteau 3 (UC)
Antrophyum plantagineum (Cav.) Kaulf.	Nitta 2901 (GH)	Ranker 1949 (COLO)
Antrophyum reticulatum (G. Forst.) Kaulf.	Nitta 2900 (GH)	Vinette 26 (UC)
Antrophyum sp.	Nitta 1473 (GH)	Nitta 1473 (GH)
Arachniodes aristata (G. Forst.) Tindale	Nitta 1179 (GH)	-
Archigrammitis tahitensis (C.Chr.) Parris	Nitta 656 (GH)	Meyer 3122 (UC)
Arthropteris palisotii (Desv.) Alston	Nitta 618 (GH)	Nitta 618 (GH)
Asplenium aethiopicum (Burm.f.) Bech.	Meyer s.n. 5 (GH)	Meyer s.n. 5 (GH)
Asplenium affine Sw.	Nitta 1535 (GH)	Nitta 1535 (GH)
Asplenium australasicum Hook.	Cousteau 5 (UC)	Cousteau 5 (UC)
Asplenium caudatum G. Forst.	Nitta 570 (GH)	Nitta 570 (GH)
Asplenium contiguum Kaulf.	Meyer s.n. 8 (GH)	Nitta 1862 (GH)
Asplenium gibberosum (G. Forst.) Mett.	Vinette 25 (UC)	Vinette 25 (UC)
Asplenium laserpitiifolium Bedd.	Nitta 3720 (GH)	Nitta 3720 (GH)
Asplenium nidus L.	Nitta 298 (UC)	Nitta 298 (UC)
Asplenium polyodon G. Forst.	Nitta 331 (UC)	Vinette 19 (UC)
Asplenium robustum Blume	Meyer s.n. 7 (GH)	Meyer s.n. 7 (GH)
Asplenium shuttleworthianum Kunze	Meyer s.n. 6 (GH)	Nitta 1857 (GH)
Asplenium sp. 1	Nitta 1619 (GH)	-
Asplenium sp. 2	Nitta 1701 (GH)	-
Asplenium sp. 3	Nitta 4130 (GH)	-
Asplenium tenerum G. Forst.	Cousteau 17 (UC)	Cousteau 17 (UC)
Belvisia spicata (L.f.) Mirbel ex Copel.	Nitta 218 (UC)	Nitta 218 (UC)
Blechnum orientale L.	Vinette 28 (UC)	Ranker 1950 (COLO)
Blechnum pacificum Lorence & A.R. Sm.	Nitta 616 (GH)	Nitta 616 (GH)
Blechnum patersonii (R. Br.) Mett.	Nitta 288 (UC)	Nitta 288 (UC)
Blechnum raiateense J. W. Moore	Nitta 698 (GH)	Nitta 698 (GH)
Blechnum vulcanicum (Blume) Kuhn	Nitta 271 (UC)	Nitta 271 (UC)
Bolbitis lonchophora (Fée) C. Chr.	Nitta 203 (UC)	Nitta 203 (UC)
Callistopteris apiifolia (C. Presl) Copel.	Nitta 68 (UC)	Nitta 36 (UC)
Calymmodon orientalis Copel.	Nitta 682 (GH)	Meyer 3124 (UC)
Cheilanthes nudiuscula (R. Brown) T. Moore	Nitta 619 (GH)	Nitta 619 (GH)
Chingia longissima (Brack.) Holttum	Nitta 620 (GH)	Nitta 620 (GH)
Christella dentata (Forssk.) Brownsey & Jermy	Nitta 624 (GH)	Nitta 624 (GH)
Coryphopteris sp.	Nitta 279 (UC)	Nitta 279 (UC)
Crepidomanes bipunctatum (Poir.) Copel.	Nitta 61 (UC)	Nitta 61 (UC)
Crepidomanes humile (G. Forst.) Bosch.	Nitta 60 (UC)	Nitta 22 (UC)
Crepidomanes kurzii (Bedd.) Tagawa & K. Iwats.	Nitta 21 (UC)	Nitta 21 (UC)
Crepidomanes minutum1 (Blume) K. Iwats. var 1	Nitta 250A (UC)	Nitta 86 (UC)
Crepidomanes minutum2 (Blume) K. Iwats. var 2	Nitta 28 (UC)	Nitta 28 (UC)
Crepidomanes minutum3 (Blume) K. Iwats. var 3	Nitta 1200 (GH)	Nitta 1200 (GH)

Table A4 (Continued).

Species	rbcL	trnHpsbA
Ctenitis sciaphila (Mxon) Ching	Nitta 1154 (GH)	Nitta 1154 (GH)
Ctenopterella blechnoides (Grev.) Parris	Nitta 658 (GH)	Nitta 658 (GH)
Cyathea epaleata (Holttum) Holttum	Nitta 703 (GH)	Nitta 703 (GH)
Dasygrammitis purpurascens (Nadeaud) Parris	Nitta 694 (GH)	Meyer 3129 (UC)
Davallia solida (G. Forst.) Sw.	Ranker 1935 (COLO)	Ranker 1974 (COLO)
Dennstaedtia scandens (Bloom) T. Moore	Nitta 3079 (GH)	-
Deparia petersenii (Kunze) Kato	Nitta 592 (GH)	Nitta 699 (GH)
Dicranopteris linearis (Burm.f.) Underw.	Vinette 3 (UC)	Vinette 3 (UC)
Didymoglossum tahitense (Nadeaud) Ebihara & K. Iwats.	Nitta 5 (UC)	Nitta 5 (UC)
Diplazium ellipticum (Copel.) C. Chr.	Nitta 2783 (GH)	Nitta 1194 (GH)
Diplazium grantii (Copel.) C. Chr.	Nitta 3176 (GH)	Nitta 3176 (GH)
Diplazium harpeodes T.Moore	Nitta 2812 (GH)	Nitta 1411 (GH)
Diplopterygium longissimum (Blume) Nakai	Nitta 667 (GH)	Nitta 667 (GH)
Doryopteris concolor (Langsd. & Fisch.) Kuhn	Ranker 1939 (COLO)	Ranker 1939 (COLO)
Dryopteris dicksonioides (Mett. ex Kuhn) Copel.	Nitta 1524 (GH)	Nitta 1524 (GH)
Dryopteris macrolepidota Copel.	Nitta 655 (GH)	Nitta 655 (GH)
Elaphoglossum samoense Brack.	Nitta 664 (GH)	Nitta 1250 (GH)
Elaphoglossum savaiense (Baker) Diels	Nitta 605 (GH)	Nitta 605 (GH)
Grammitis fredericis-jacobi ined.	Meyer s.n. 1 (GH)	-
Grammitis marginelloides (J.W. Moore) Copel.	Nitta 666 (GH)	Meyer 3121 (UC)
Grammitis trachycarpa (Mett. ex Kuhn) Copel.	Nitta 3975 (GH)	Nitta 3975 (GH)
Haplopteris elongata (Sw.) E. H. Crane	Vinette 18 (UC)	Ranker 1931 (COLO)
Haplopteris sp.	Nitta 3362 (GH)	-
Histiopteris incisa (Thunb.) J. Sm.	Nitta 679 (GH)	Nitta 679 (GH)
Humata anderssonii Mett.	Nitta 606 (GH)	Meyer 3127 (UC)
Humata pectinata (Sm.) Desv.	Vinette 5 (UC)	Vinette 5 (UC)
Hymenophyllum braithwaitei Ebihara & K. Iwats.	Nitta 2564 (GH)	Nitta 2564 (GH)
Hymenophyllum digitatum (Sw.) Fosberg	Nitta 27 (UC)	Nitta 37 (UC)
Hymenophyllum flabellatum Labill.	Nitta 665 (GH)	Nitta 665 (GH)
Hymenophyllum javanicum A. Spreng	Nitta 675 (GH)	Nitta 675 (GH)
Hymenophyllum multifidum (G. Forst.) Sw.	Nitta 696 (GH)	Nitta 696 (GH)
Hymenophyllum pallidum Ebihara & K. Iwats.	Nitta 71 (UC)	Nitta 72 (UC)
Hymenophyllum polyanthos (Sw.) Sw.	Nitta 34 (UC)	Nitta 34 (UC)
Hypolepis dicksonioides (Endl.) Hook.	Nitta 260 (UC)	Nitta 260 (UC)
Hypolepis sp.	Nitta 1523 (GH)	Nitta 1523 (GH)
Hypolepis tenuifolia (G. Forst.) Bernh.	Nitta 306 (UC)	Nitta 306 (UC)
Lastreopsis pacifica Tindale	Nitta 3189 (GH)	Nitta 3189 (GH)
Leucostegia pallida (Mett.) Copel.	-	Nitta 651 (GH)
Lindsaea propinqua Hook.	Nitta 1308 (GH)	Nitta 1308 (GH)
Lindsaea repens (Bory) Thwaites	Nitta 308 (UC)	Nitta 308 (UC)
Lindsaea rigida J. Sm.	Nitta 284 (UC)	Nitta 284 (UC)
Lomagramma tahitensis Holttum	Nitta 222 (UC)	Vinette 16 (UC)
Lomariopsis brackenridgei Carruth.	Vinette 15 (UC)	Ranker 1944 (COLO)
Loxogramme parksii Copel.	Nitta 3194 (GH)	Nitta 3194 (GH)
Lygodium reticulatum Schkuhr	Cousteau 2 (UC)	Cousteau 2 (UC)
Macrothelypteris polypodioides (Hook.) Holttum	Nitta 580 (GH)	Sanchez-Baracaldo 167 (UC)
Macrothelypteris torresiana (Gaudich.) Ching	Nitta 569 (GH)	Nitta 569 (GH)
Microlepia scaberula Mett.; Kuhn	Nitta 1010 (GH)	Nitta 1010 (GH)
Microsorum commutatum (Blume) Copel.	Vinette 31 (UC)	Vinette 31 (UC)
Microsorum grossum (Langsd. & Fisch.) S. B. Andrews	Vinette 33 (UC)	Hinkle 107 (UC)
Microsorum membranifolium (R. Br.) Ching	Nitta 573 (GH)	Nitta 573 (GH)
Microsorum powellii (Baker) Copel.	Nitta 1468 (GH)	Nitta 654 (GH)

Table A4 (Continued).

Species	rbcL	trnHpsbA
Microsorum punctatum Copel.	Vinette 32 (UC)	Sanchez-Baracaldo 151 (UC)
Microsorum x maximum (Brack.) Copel.	Hinkle 106 (UC)	Hinkle 106 (UC)
Nephrolepis biserrata (Sw.) Schott	Nitta 215 (UC)	Nitta 215 (UC)
Nephrolepis cordifolia (L.) C. Presl	Nitta 285 (UC)	Nitta 285 (UC)
Nephrolepis hirsutula (G. Forst.) C. Presl	Vinette 2 (UC)	Vinette 2 (UC)
Oleandra sibbaldii Grev.	Nitta 670 (GH)	Nitta 670 (GH)
Ophioglossum falcatum (Presl) Fowler	Vinette 27 (UC)	Vinette 27 (UC)
Ophioglossum pendulum L.	Ranker 1932 (COLO)	Ranker 1932 (COLO)
Oreogrammitis raiateensis (J.W. Moore) Parris	Nitta 681 (GH)	Meyer 3123 (UC)
Oreogrammitis subspathulata (Brack.) Parris	Nitta 3187 (GH)	Nitta 3187 (GH)
Paesia divaricatissima (Dryand.) Copel.	Nitta 594 (GH)	Nitta 594 (GH)
Pityrogramma calomelanos (L.) Link	Vinette 1 (UC)	Ranker 1945 (COLO)
Plesioneuron attenuatum (Brack.) Holttum	Vinette 20 (UC)	Vinette 20 (UC)
Plesioneuron sp.	Nitta 281 (UC)	Nitta 3032 (GH)
Pneumatopteris mesocarpa (Copel.) Holttum	Nitta 586 (GH)	Nitta 586 (GH)
Polyphlebium borbonicum (Bosch) Ebihara & Dubuisson	Nitta 73 (UC)	Nitta 73 (UC)
Polyphlebium endlicherianum (C. Presl) Ebihara & K. Iwats.	Nitta 653 (GH)	Nitta 653 (GH)
Polystichum paleatum Copel.	Nitta 3196 (GH)	Nitta 3196 (GH)
Prosaptia contigua (G. Forst.) C. Presl	Nitta 310 (UC)	Nitta 310 (UC)
Prosaptia subnuda Copel.	Nitta 662 (GH)	Nitta 662 (GH)
Psilotum complanatum Sw.	Nitta 642 (GH)	Nitta 642 (GH)
Psilotum nudum (L.) P. Beauv.	Cousteau 6 (UC)	Cousteau 6 (UC)
Pteris comans G. Forst.	Cousteau 11 (UC)	Cousteau 11 (UC)
Pteris ensiformis Burm. f.	Vinette 10 (UC)	Vinette 10 (UC)
Pteris mertensioides Willd.	Nitta 325 (UC)	-
Pteris tripartita Sw.	Nitta 1470 (GH)	Vinette 10 (UC)
Ptisana salicina (Sm.) Murdock	Nitta 229 (UC)	Nitta 229 (UC)
Pyrrosia serpens (G. Forst.) Ching	Nitta 199 (UC)	Nitta 199 (UC)
Schizaea dichotoma (L.) Sm.	Vinette 29 (UC)	Nitta 198 (UC)
Schizaea fistulosa Labill.	Nitta 3736 (GH)	Nitta 3736 (GH)
Scleroglossum sulcatum (Kuhn) Alderw.	Nitta 3695 (GH)	Nitta 3695 (GH)
Selliguea plantaginea Brack.	Nitta 657 (GH)	Meyer 3128 (UC)
Sphaeropteris medullaris (G. Forst.) Bernh.	Nitta 328 (UC)	Nitta 328 (UC)
Sphaerostephanos invisus (G. Forst.) Holttum	Vinette 13 (UC)	Vinette 13 (UC)
Sphaerostephanos subpectinatus (Copel.) Holttum	Nitta 1165 (GH)	Meyer 3130 (UC)
Sphenomeris chinensis (L.) Maxon	Ranker 1963 (COLO)	Ranker 1963 (COLO)
Stenogrammitis subcoriacea (Copel.) Labiak	Nitta 3316 (GH)	Nitta 3316 (GH)
Sticherus tahitensis (Copel.) St. John	Nitta 1221 (GH)	Nitta 1221 (GH)
Tectaria decurrens (C. Presl) Copel.	Cousteau 9 (UC)	Nitta 1793 (GH)
Tectaria dissecta (G. Forst.) Lellinger	Nitta 603 (GH)	Nitta 603 (GH)
Tectaria tahitensis Maxon	Vinette 23 (UC)	Vinette 23 (UC)
Teratophyllum wilkesianum (Brack.) Holttum	Vinette 6 (UC)	Ranker 1937 (COLO)
Tmesipteris gracilis Chinnock	Nitta 683 (GH)	Nitta 683 (GH)
Vaginularia paradoxa (Fée) Miq.	Nitta 2447 (GH)	Nitta 3686 (GH)
Vandenboschia maxima (Blume) Copel.	Nitta 1397 (GH)	Nitta 1689 (GH)
Wibelia denticulata (Burm.f.) M. Kato & Tsutsumi	Nitta 302 (UC)	Nitta 302 (UC)

Appendix B:

Supporting material for Chapter II

Table B1. Results of analysis of covariance (ANCOVA) for climatic variables.

Climatic variables are grand means of daily minimum, mean, maximum, and standard deviation in temperature and relative humidity (RH). Temperature and RH measured by dataloggers every 15 m from July 7, 2013 to July 5, 2014 along an elevational gradient from ca. 200 to 1200 m on Moorea, French Polynesia (N = 17). Two linear models were compared for each climatic variable: one with the same slope but different intercepts (y ~ growth habit + elevation), and one with different slopes and intercepts (y ~ growth habit × elevation), and compared using the "aov" function in R.

	Intercept			Slope		
	df	*F*	*P*	df	*F*	*P*
Min. Temperature	2, 5223	4676.06	0.6	3, 5222	3116.8	0.87
Mean Temperature	2, 5223	4416.28	0.05	3, 5222	2957.56	< 0.001
Max. Temperature	2, 5223	771.46	0.3	3, 5222	534.3	< 0.001
SD Temperature	2, 5223	187.91	0.7	3, 5222	144.39	< 0.001
Min. Rel. Hum.	2, 5223	295.31	< 0.001	3, 5222	224.38	< 0.001
Mean Rel. Hum.	2, 5223	416.11	< 0.001	3, 5222	334.03	< 0.001
Max Rel. Hum.	2, 5223	267.25	< 0.001	3, 5222	235.11	< 0.001
SD Rel. Hum.	2, 5223	229.84	< 0.001	3, 5222	173.94	< 0.001

	Estimates (epiphytic)		Estimates (terrestrial)	
	slope	intercept	slope	intercept
Min. Temperature	-0.0064	22.74	-0.0064	22.74
Mean Temperature	-0.0058	24.12	-0.0053	23.76
Max. Temperature	-0.0048	26.11	-0.0033	25.13
SD Temperature	0.0004	0.96	0.0008	0.7
Min. Rel. Hum.	0.0068	85.06	-1.06E-05	94.71
Mean Rel. Hum.	0.004	94.13	0.0006	98.29
Max Rel. Hum.	0.0015	98.5	0.0002	99.69
SD Rel. Hum.	-0.0015	3.53	0.0001	1.24

Table B2. Recovery of F_v/F_m (%) of selected Moorean filmy ferns following desiccation treatment. Species means ± s.e.; $N = 8$ unless otherwise indicated.

Table B2 (Continued).

Species	Recovery Time	Sporophytes, 2 day desiccation			
		-39 MPa	-87 Mpa	-285 Mpa	control (100% RH)
	30min	5.4 ± 2.2	-	-	100.0 ± 0.0 (N=4)
	24hr	7.6 ± 3.4	-	-	-
Abrodictyum dentatum	48hr	3.1 ± 1.6	-	-	100.0 ± 0.0 (N=4)
	30min	4.3 ± 1.4	-	-	98.4 ± 1.2
	24hr	1.3 ± 1.2	-	-	98.1 ± 1.6
Callistopteris apiifolia	48hr	3.1 ± 2.0	-	-	97.5 ± 1.5
	30min	85.2 ± 1.2 (N=10)	88.1 ± 1.5 (N=11)	78.3 ± 2.2 (N=10)	99.2 ± 0.3 (N=10)
	24hr	95.9 ± 1.0 (N=10)	96.5 ± 0.6 (N=10)	90.0 ± 2.6 (N=10)	98.8 ± 0.3 (N=10)
Crepidomanes bipunctatum	48hr	98.4 ± 0.6 (N=10)	97.2 ± 0.9 (N=11)	90.5 ± 2.1 (N=10)	99.2 ± 0.3 (N=10)
	30min	79.4 ± 1.5	88.0 ± 0.9	77.0 ± 2.2	99.4 ± 0.2
	24hr	90.4 ± 1.3	84.5 ± 5.4	33.1 ± 10.4	99.6 ± 0.3
Crepidomanes humile	48hr	95.7 ± 0.8	88.3 ± 3.6	38.1 ± 11.2	99.6 ± 0.3
	30min	66.3 ± 2.8	62.0 ± 5.0	33.2 ± 6.8	-
	24hr	65.2 ± 11.7	70.1 ± 8.5	7.9 ± 3.4	-
Crepidomanes kurzii	48hr	94.3 ± 2.0	74.8 ± 6.8	15.3 ± 6.0	-
	30min	80.1 ± 2.3	87.5 ± 1.0	81.2 ± 2.2	99.6 ± 0.2
	24hr	94.4 ± 1.7	85.8 ± 2.4	62.9 ± 9.6	98.9 ± 0.5
Crepidomanes minutum var 1	48hr	94.8 ± 1.7	85.0 ± 2.5	-	99.2 ± 0.3
	30min	80.6 ± 2.9	75.1 ± 2.1	64.8 ± 2.1	91.9 ± 1.7
	24hr	92.1 ± 2.6	92.8 ± 1.8	73.3 ± 4.1	91.4 ± 1.8
Crepidomanes minutum var 2	48hr	92.5 ± 2.2	91.4 ± 1.9	75.2 ± 5.8	90.9 ± 1.6
	30min	-	-	-	-
	24hr	-	-	-	-
Crepidomanes minutum var 3	48hr	-	-	-	-
	30min	82.1 ± 3.5	81.3 ± 2.9	51.5 ± 4.0	-
	24hr	77.5 ± 11.8	98.7 ± 0.6	21.5 ± 3.4	-
Didymoglossum tahitense	48hr	95.6 ± 3.5	99.4 ± 0.6	34.3 ± 10.4	-
	30min	69.6 ± 4.7	60.6 ± 5.3	69.4 ± 3.6	96.7 ± 1.2 (N=7)

Table B2 (Continued).

Species	Recovery Time	Sporophytes, 2 day desiccation			
		-39 MPa	-87 Mpa	-285 Mpa	control (100% RH)
	24hr	98.4 ± 0.7	99.6 ± 0.3	88.1 ± 6.7	98.5 ± 1.2
Hymenophyllum digitatum	48hr	96.2 ± 1.0	98.7 ± 1.3	95.9 ± 2.6	99.5 ± 0.3
	30min	-	-	-	-
	24hr	-	-	-	-
Hymenophyllum javanicum	48hr	-	-	-	-
	30min	69.4 ± 5.8	79.8 ± 3.1	67.1 ± 6.9	98.0 ± 1.0
	24hr	95.5 ± 2.4	96.7 ± 2.3	85.0 ± 11.9	97.4 ± 1.9
Hymenophyllum multifidum	48hr	96.7 ± 1.0	98.7 ± 0.7	82.6 ± 11.9	98.9 ± 1.0
	30min	62.3 ± 6.8	48.2 ± 3.1	44.4 ± 4.0	90.2 ± 2.5
	24hr	46.6 ± 10.1	68.8 ± 4.0	11.5 ± 4.1	87.2 ± 2.5
Hymenophyllum pallidum	48hr	52.7 ± 10.8	75.4 ± 5.9	12.9 ± 6.2	90.6 ± 2.1
	30min	65.9 ± 4.0 (N=16)	74.5 ± 2.2 (N=16)	77.2 ± 2.6 (N=16)	94.4 ± 1.9 (N=15)
	24hr	88.8 ± 2.0 (N=16)	91.9 ± 1.4 (N=16)	87.3 ± 1.8 (N=16)	98.2 ± 0.7
Hymenophyllum polyanthos	48hr	91.5 ± 1.4 (N=16)	91.9 ± 1.6 (N=16)	90.2 ± 1.5 (N=16)	91.5 ± 2.4 (N=15)
	30min	37.8 ± 7.0	57.4 ± 4.8	57.4 ± 3.4	97.3 ± 0.8
	24hr	88.5 ± 3.6	91.8 ± 2.1	3.4 ± 0.9	93.4 ± 2.3
Polyphlebium borbonicum	48hr	94.8 ± 2.4	89.5 ± 3.0	3.2 ± 1.1	95.5 ± 1.6
	30min	41.6 ± 5.4 (N=7)	27.5 ± 2.8	17.0 ± 1.5	97.7 ± 0.7
	24hr	4.1 ± 1.4	2.5 ± 0.7	0.9 ± 0.6	97.0 ± 0.8
Polyphlebium endlicherianum	48hr	6.3 ± 3.3	1.1 ± 0.4	0.5 ± 0.5	98.1 ± 0.6
	30min	27.9 ± 5.3	9.1 ± 2.2	6.0 ± 0.9	95.3 ± 1.6
	24hr	20.2 ± 5.2	8.4 ± 2.5	5.2 ± 1.3	93.6 ± 1.7
Vandenboschia maxima	48hr	29.4 ± 5.7	5.3 ± 1.4	5.7 ± 2.1	93.5 ± 1.3

Table B2 (Continued).

Species	Recovery Time	Sporophytes, 15 day desiccation				Gametophytes, 2d day desiccation
		-39 MPa	-87 Mpa	-285 Mpa	control (100% RH)	-87 Mpa
	30min	-	-	-	-	1.9 ± 1.0 (N=3)
	24hr	-	-	-	-	4.9 ± 1.8 (N=3)
Abrodictyum dentatum	48hr	-	-	-	-	11.8 ± 5.9 (N=3)
	30min	-	-	-	-	5.0 ± 1.5 (N=79)
	24hr	-	-	-	-	8.6 ± 1.8 (N=79)
Callistopteris apiifolia	48hr	-	-	-	-	11.5 ± 2.0 (N=79)
	30min	56.3 ± 2.9 (N=12)	55.3 ± 5.6 (N=12)	35.3 ± 2.6 (N=12)	98.4 ± 0.5	-
	24hr	76.0 ± 4.6 (N=12)	66.1 ± 5.5 (N=12)	42.5 ± 4.4 (N=11)	99.2 ± 0.3 (N=10)	-
Crepidomanes bipunctatum	48hr	78.8 ± 4.8 (N=12)	75.7 ± 4.9 (N=12)	41.0 ± 7.0 (N=11)	98.0 ± 0.8 (N=10)	-
	30min	13.2 ± 5.1	14.9 ± 3.7	13.5 ± 2.6	99.6 ± 0.2	47.5 ± 35.9 (N=2)
	24hr	8.7 ± 5.1	7.8 ± 4.6	0.5 ± 0.2	99.9 ± 0.1	66.7 ± 33.3 (N=2)
Crepidomanes humile	48hr	12.1 ± 5.7	2.5 ± 0.7	3.6 ± 1.3	99.7 ± 0.1	76.1 ± 23.9 (N=2)
	30min	8.5 ± 4.8	1.4 ± 1.1	1.7 ± 0.6	-	42.0 (N=1)
	24hr	11.7 ± 4.9	4.7 ± 2.4	1.7 ± 1.1	-	71.2 (N=1)
Crepidomanes kurzii	48hr	20.6 ± 7.6	4.8 ± 3.4	6.2 ± 1.9	-	78.1 (N=1)
	30min	8.9 ± 1.6 (N=7)	51.3 ± 4.1	42.7 ± 3.2 (N=7)	99.5 ± 0.2	64.5 (N=1)
	24hr	4.8 ± 1.0 (N=7)	14.0 ± 5.0	14.6 ± 6.4 (N=7)	99.4 ± 0.4	97.0 (N=1)
Crepidomanes minutum var 1	48hr	4.0 ± 1.7 (N=7)	16.4 ± 7.1	14.3 ± 6.3 (N=7)	98.6 ± 0.7	100.0 (N=1)
	30min	7.8 ± 2.0	51.0 ± 4.4	54.5 ± 3.1	96.9 ± 1.6	57.2 ± 5.8 (N=15)
	24hr	2.7 ± 0.8	44.0 ± 6.9	32.8 ± 7.0	96.2 ± 1.7	66.5 ± 7.3 (N=15)
Crepidomanes minutum var 2	48hr	1.9 ± 0.8	46.1 ± 9.3	39.5 ± 6.4	95.1 ± 1.5	70.6 ± 6.8 (N=15)
	30min	-	-	-	-	76.6 ± 11.7 (N=3)
	24hr	-	-	-	-	78.9 ± 11.6 (N=3)
Crepidomanes minutum var 3	48hr	-	-	-	-	76.2 ± 12.8 (N=3)
	30min	48.5 ± 3.6	16.3 ± 3.0	4.5 ± 1.2	-	-
	24hr	70.9 ± 3.3	21.9 ± 4.4	12.8 ± 2.9	-	-
Didymoglossum tahitense	48hr	86.8 ± 2.2	29.5 ± 4.1	21.7 ± 5.1	-	-

Table B2 (Continued).

Species	Recovery Time	Sporophytes, 15 day desiccation				Gametophytes, 2d day desiccation
		-39 MPa	-87 Mpa	-285 Mpa	control (100% RH)	-87 Mpa
	30min	30.1 ± 1.1	29.4 ± 3.9	0.8 ± 0.6	-	32.9 ± 7.1 (N=15)
	24hr	32.4 ± 8.3	49.0 ± 10.9	7.7 ± 4.6	92.8 ± 6.1	44.8 ± 8.9 (N=15)
Hymenophyllum digitatum	48hr	44.2 ± 7.8	67.3 ± 6.9	0.0 ± 0.0	88.7 ± 10.2	48.9 ± 8.5 (N=15)
	30min	-	-	-	-	0.0 (N=1)
	24hr	-	-	-	-	5.4 (N=1)
Hymenophyllum javanicum	48hr	-	-	-	-	25.1 (N=1)
	30min	18.7 ± 4.7	34.6 ± 4.7	25.3 ± 4.4	100.0 (N=1)	-
	24hr	24.6 ± 12.4	36.1 ± 10.0	69.5 ± 11.1	99.8 ± 0.2	-
Hymenophyllum multifidum	48hr	5.9 ± 1.5	51.5 ± 10.2	78.2 ± 6.0	93.9 ± 5.5	-
	30min	4.2 ± 1.1	36.1 ± 5.1	26.3 ± 6.5	98.5 ± 1.4	26.5 ± 7.3 (N=3)
	24hr	1.3 ± 0.6	13.6 ± 5.0	1.9 ± 0.7	95.5 ± 2.4	33.5 ± 16.8 (N=3)
Hymenophyllum pallidum	48hr	2.2 ± 0.8	17.2 ± 6.2	2.4 ± 0.9	97.2 ± 1.7	29.3 ± 14.7 (N=3)
	30min	31.5 ± 3.4 (N=16)	49.8 ± 2.4 (N=16)	40.4 ± 2.3 (N=16)	99.0 ± 0.4	39.8 ± 4.7 (N=34)
	24hr	6.9 ± 1.8 (N=16)	56.4 ± 3.4 (N=16)	31.8 ± 6.9 (N=16)	98.9 ± 0.4	41.8 ± 6.2 (N=34)
Hymenophyllum polyanthos	48hr	11.4 ± 4.5 (N=16)	63.1 ± 3.6 (N=16)	40.1 ± 7.3 (N=16)	97.6 ± 0.9	47.1 ± 6.5 (N=34)
	30min	26.8 ± 6.8	6.1 ± 2.4	21.4 ± 5.3	-	13.4 ± 8.1 (N=3)
	24hr	8.9 ± 3.3	0.7 ± 0.5	0.7 ± 0.4	-	9.0 ± 5.5 (N=3)
Polyphlebium borbonicum	48hr	15.2 ± 4.7	1.1 ± 0.4	2.3 ± 1.1	-	21.2 ± 10.5 (N=3)
	30min	2.9 ± 0.4	2.2 ± 0.8	2.1 ± 0.6	99.3 ± 0.3	16.3 ± 3.7 (N=5)
	24hr	1.6 ± 0.7	1.0 ± 0.4	0.9 ± 0.4	99.6 ± 0.3	37.5 ± 10.4 (N=5)
Polyphlebium endlicherianum	48hr	2.0 ± 0.7	1.2 ± 0.3	0.8 ± 0.5	98.1 ± 0.6	38.6 ± 8.7 (N=5)
	30min	14.9 ± 3.6	5.0 ± 2.1	4.4 ± 2.3	92.3 ± 1.7	12.3 (N=1)
	24hr	12.0 ± 2.8	1.1 ± 1.0	4.1 ± 1.4	96.8 ± 1.1	20.9 (N=1)
Vandenboschia maxima	48hr	9.9 ± 3.6	8.6 ± 2.7	6.3 ± 2.2	97.7 ± 0.8	26.6 (N=1)

Table B3. Photosynthetic parameters of selected Moorean filmy ferns. Species means ± s.e.; N = 8 unless otherwise indicated.

Species	Sporophytes		Gametophytes	
	ETR_{max}	$PPFD_{95\%}$	ETR_{max}	$PPFD_{95\%}$
Abrodictyum asaegrayi var 1	9.60 ± 0.92	173.20 ± 17.64	-	-
Abrodictyum dentatum	7.79 ± 0.97 (N=13)	107.48 ± 8.80 (N=13)	3.04 ± 0.54 (N=5)	37.57 ± 6.91 (N=5)
Callistopteris apiifolia	8.44 ± 0.55 (N=11)	110.78 ± 6.34 (N=11)	2.99 ± 0.21 (N=48)	38.07 ± 4.87 (N=48)
Crepidomanes bipunctatum	9.81 ± 0.60 (N=14)	132.56 ± 13.25 (N=14)	-	-
Crepidomanes humile	7.16 ± 0.71 (N=18)	102.74 ± 6.19 (N=18)	3.80 (N=1)	39.35 (N=1)
Crepidomanes kurzii	6.74 ± 0.78 (N=11)	84.09 ± 10.28 (N=11)	-	-
Crepidomanes minutum var 1	13.32 ± 1.03 (N=16)	206.62 ± 18.83 (N=16)	2.90 ± 0.60 (N=2)	33.54 ± 13.46 (N=2)
Crepidomanes minutum var 2	-	-	5.31 ± 0.88 (N=7)	68.81 ± 7.97 (N=7)
Crepidomanes minutum var 3	-	-	3.60 (N=1)	33.64 (N=1)
Didymoglossum tahitense	8.37 ± 2.52 (N=3)	138.07 ± 35.73 (N=3)	-	-
Hymenophyllum digitatum	6.49 ± 0.66 (N=11)	117.67 ± 20.08 (N=11)	3.09 ± 0.44	34.81 ± 4.22
Hymenophyllum flabellatum	5.50 ± 0.25	160.50 ± 6.73	-	-
Hymenophyllum javanicum	9.28 ± 0.37 (N=9)	208.16 ± 17.56 (N=9)	-	-
Hymenophyllum multifidum	12.88 ± 0.81 (N=9)	247.18 ± 15.74 (N=9)	-	-
Hymenophyllum pallidum	10.88 ± 1.05	177.88 ± 11.08	-	-
Hymenophyllum polyanthos	10.01 ± 0.63 (N=11)	133.01 ± 9.06 (N=11)	5.01 ± 0.41 (N=16)	53.42 ± 5.41 (N=16)
Hymenophyllum braithwaitei	8.15 ± 1.50 (N=6)	159.26 ± 38.09 (N=6)	-	-
Polyphlebium borbonicum	6.60 ± 0.49 (N=11)	96.99 ± 11.24 (N=11)	4.17 ± 1.03 (N=3)	50.25 ± 13.53 (N=3)
Polyphlebium endlicherianum	6.54 ± 0.29	110.13 ± 7.33	5.30 ± 0.90 (N=2)	74.73 ± 3.61 (N=2)
Vandenboschia maxima	8.76 ± 0.55	125.60 ± 10.99	3.90 (N=1)	102.59 (N=1)

Appendix C:

Supporting material for Chapter III

Table C1. Results of analysis of covariance (ANCOVA) for microclimatic variables between epiphytic and terrestrial plots along an elevational gradient on Moorea, French Polynesia. Linear models were constructed for each response variable with growth habit as a fixed effect and elevation as a co-factor. Differences in intercept between growth habits was tested with models of the form y ~ growth habit + elevation. Differences in slope between growth habits were tested with models of the form y ~ growth habit × elevation. Models were compared using the "aov" function in R.

	Intercept			Slope		
	df	*F*	*P*	df	*F*	*P*
Min. Temperature	2, 5223	4676.06	0.6	3, 5222	3116.8	0.87
Mean Temperature	2, 5223	4416.28	0.05	3, 5222	2957.56	< 0.001
Max. Temperature	2, 5223	771.46	0.3	3, 5222	534.3	< 0.001
SD Temperature	2, 5223	187.91	0.7	3, 5222	144.39	< 0.001
Min. Rel. Hum.	2, 5223	295.31	< 0.001	3, 5222	224.38	< 0.001
Mean Rel. Hum.	2, 5223	416.11	< 0.001	3, 5222	334.03	< 0.001
Max Rel. Hum.	2, 5223	267.25	< 0.001	3, 5222	235.11	< 0.001
SD Rel. Hum.	2, 5223	229.84	< 0.001	3, 5222	173.94	< 0.001

	Estimates (epiphytic plots)		Estimates (terrestrial plots)	
	slope	intercept	slope	intercept
Min. Temperature	-0.0064	22.74	-0.0064	22.74
Mean Temperature	-0.0058	24.12	-0.0053	23.76
Max. Temperature	-0.0048	26.11	-0.0033	25.13
SD Temperature	0.0004	0.96	0.0008	0.7
Min. Rel. Hum.	0.0068	85.06	-1.06E-05	94.71
Mean Rel. Hum.	0.004	94.13	0.0006	98.29
Max Rel. Hum.	0.0015	98.5	0.0002	99.69
SD Rel. Hum.	-0.0015	3.53	0.0001	1.24

Table C2. PC loadings for principal components analyses (PCA) of traits related to epiphytic growth in ferns from Moorea, French Polynesia. For each PC, values of the three traits with the greatest loadings (absolute values) shown in bold.

	Standard PCA		Phylogenetic PCA	
	PC 1	PC 2	PC 1	PC 2
Frond Width	**0.95**	-0.02	**-0.93**	-0.09
Stipe Length	**0.91**	-0.03	**-0.93**	-0.04
Frond Length	**0.84**	-0.37	-0.41	**-0.54**
Rhizome Dia.	0.78	**-0.47**	-0.27	-0.35
No. Pinna Pairs	0.58	0.3	**-0.92**	-0.21
Frond Dissection	0.52	**0.72**	-0.26	**-0.56**
SLA	0.24	**0.68**	-0.83	**0.51**
Total Variance (%)	53.02	20.40	73.98	10.34
Cumulative Variance (%)	53.02	73.42	73.98	84.32

Table C3. Phylogenetic generalized linear mixed models (PGLMMs) for traits related to epiphytic growth. A) Quantitative (sporophyte) traits. **B)** Binary (gametophyte) traits. The response of each trait was modeled against growth habit using a PGLMM with phylogeny as a random effect. For binary traits, σ^2 is a measure of phylogenetic signal calculated from the variance in the PGLMM. Significance of σ^2 calculated using an approximate likelihood ratio test between a model with the observed value of σ^2 against a null model with $\sigma^2 = 0$. Traits significantly correlated with growth habit in bold.

A) Quantiative (sporophyte) traits

	Parameter estimate	Lower 95% CI	Upper 95% CI	Effective sample size	*P*
Stipe Length	**-23.68**	**-32.7**	**-14.3**	**9417.27**	**< 0.001**
Frond Length	**-37.38**	**-66.06**	**-10.34**	**9998**	**0.011**
Frond Width	**-11.99**	**-20.95**	**-2.98**	**9998**	**0.008**
Rhizome Diameter	-0.91	-2.17	0.45	9998	0.173
Frond Dissection	**-1.56**	**-2.44**	**-0.62**	**9998**	**0.002**
Pinna number	-4.23	-8.58	0.17	9998	0.06
Specific Leaf Area	**-8.15**	**-13.91**	**-2.68**	**10360.65**	**0.006**

B) Binary (gametophyte) traits

	σ^2	Z score	*P*
Morphotype	**5.577 (*P* < 0.001)**	**-2.623**	**0.009**
Glands	5.868 (*P* < 0.001)	-0.768	0.443
Hairs	4.114 (*P* < 0.001)	0.69	0.49
Gemmae	7.649 (*P* < 0.001)	1.566	0.117

Table C4. List of all ferns from Moorea, French Polynesia with observed trait values and growth habit. For continuous trait values, means ± s.d. shown. For growth habit, E = epiphytic, T = terrestrial. For frond dissection, 1 = simple, 2 = pinnatifid or pinnatisect, 3 = 1-pinnate, 4 = 1- pinnate-pinnatifid, 5 = 2-pinnate, 6 = 2-pinnate-pinnatifid, 7 = 3-pinnate, 8 = 3-pinnate-pinnatifid, 9 = > 3-pinnate-pinnatifid, 10 = binpinnatifid or tripinnatifid.

Table C4 (Continued).

Species	Growth Habit	Morphotype	Glands	Hairs	Gemmae
Abrodictyum asaegrayi var 1 (Bosch) Ebihara & K. Iwats.	T	filament	0	0	1
Abrodictyum asaegrayi var 2 (Bosch) Ebihara & K. Iwats.	T	filament	0	0	1
Abrodictyum caudatum (Brack.) Ebihara & K. Iwats.	E	filament	0	0	1
Abrodictyum dentatum (Bosch) Ebihara & K. Iwats.	T	filament	0	0	1
Acrostichum aureum L.	T	cordate	0	0	0
Adiantum hispidulum Sw.	E	cordate	0	0	0
Adiantum raddianum C. Presl	E	cordate	0	0	0
Adiantum trapeziforme L.	T	cordate	0	0	0
Alsophila tahitensis Brack.	T	cordate	0	0	0
Amphineuron opulentum (Kaulf.) Holttum	T	cordate	1	0	0
Angiopteris evecta (G. Forst.) Hoffm.	T	cordate	0	0	0
Antrophyum plantagineum (Cav.) Kaulf.	E	ribbon	0	0	1
Antrophyum reticulatum (G. Forst.) Kaulf.	E	ribbon	0	0	1
Arachniodes aristata (G. Forst.) Tindale	T	cordate	1	0	0
Archigrammitis tahitensis ined.	E	strap	0	1	0
Arthropteris palisotii (Desv.) Alston	E	strap	1	0	0
Asplenium affine Sw.	E	cordate	NA	NA	0
Asplenium australasicum Hook.	E	cordate	0	0	0
Asplenium caudatum G. Forst.	T	cordate	1	0	0
Asplenium gibberosum (G. Forst.) Mett.	T	cordate	NA	NA	0
Asplenium nidus L.	E	cordate	0	0	0
Asplenium polyodon G. Forst.	E	cordate	NA	NA	0
Asplenium tenerum G. Forst.	T	cordate	NA	NA	0
Belvisia spicata (L.f.) Mirbel ex Copel.	E	cordate	1	0	0
Blechnum orientale L.	T	cordate	1	0	0
Blechnum pacificum Lorence & A.R. Sm.	T	cordate	0	0	0
Blechnum patersonii (R. Br.) Mett.	E	cordate	NA	NA	0
Blechnum raiateense J. W. Moore	T	cordate	0	0	0
Blechnum vulcanicum (Blume) Kuhn	T	cordate	1	0	0
Bolbitis lonchophora (Fée) C. Chr.	T	cordate	0	0	0
Callistopteris apiifolia (C. Presl) Copel.	T	ribbon	0	0	1
Calymmodon orientalis Copel.	E	ribbon	0	1	0
Cheilanthes nudiuscula (R. Brown) T. Moore	E	cordate	NA	NA	NA
Chingia longissima (Brack.) Holttum	T	cordate	1	0	0
Christella dentata (Forssk.) Brownsey & Jermy	T	cordate	1	0	0
Coryphopteris sp.	T	cordate	1	0	0
Crepidomanes bipunctatum (Poir.) Copel.	E	filament	0	0	1
Crepidomanes humile (G. Forst.) Bosch.	E	filament	0	0	1
Crepidomanes kurzii (Bedd.) Tagawa & K. Iwats.	E	filament	0	0	1
Crepidomanes minutum var 1 (Blume) K. Iwats.	E	filament	0	0	1
Crepidomanes minutum var 2 (Blume) K. Iwats.	E	filament	0	0	1
Crepidomanes minutum var 3 (Blume) K. Iwats.	E	filament	0	0	1
Ctenitis sciaphila (Mxon) Ching	T	cordate	1	0	0
Ctenopterella blechnoides (Grev.) Parris	E	cordate	0	NA	NA
Cyathea epaleata (Holttum) Holttum	T	cordate	NA	NA	0
Dasygrammitis purpurascens (Nadeaud) Parris	E	strap	0	1	0
Davallia solida (G. Forst.) Sw.	E	cordate	1	0	0
Deparia petersenii (Kunze) Kato	T	cordate	1	0	0
Dicranopteris linearis (Burm.f.) Underw.	T	cordate	1	0	0
Didymoglossum tahitense (Nadeaud) Ebihara & K. Iwats.	E	filament	0	0	1
Diplazium ellipticum (Copel.) C. Chr.	T	cordate	0	0	0
Diplazium grantii (Copel.) C. Chr.	T	cordate	0	0	0
Diplazium harpeodes T.Moore	T	cordate	0	0	0
Diplopterygium longissimum (Blume) Nakai	T	cordate	1	0	0
Doryopteris concolor (Langsd. & Fisch.) Kuhn	T	cordate	0	0	0
Dryopteris dicksonioides (Mett. ex Kuhn) Copel.	T	cordate	0	0	0
Dryopteris macrolepidota Copel.	T	cordate	0	0	0
Elaphoglossum samoense Brack.	E	cordate	1	0	0
Elaphoglossum savaiense (Baker) Diels	E	cordate	1	0	0
Grammitis marginelloides (J.W. Moore) Copel.	E	ribbon	0	0	0

Table C4 (Continued).

Species	Growth Habit	Morphotype	Glands	Hairs	Gemmae
Haplopteris elongata (Sw.) E. H. Crane	E	ribbon	0	0	1
Histiopteris incisa (Thunb.) J. Sm.	T	cordate	0	0	0
Humata anderssonii Mett.	E	ribbon	1	0	0
Humata pectinata (Sm.) Desv.	E	ribbon	NA	0	0
Hymenophyllum braithwaitei Ebihara & K. Iwats.	E	ribbon	NA	NA	NA
Hymenophyllum digitatum (Sw.) Fosberg	E	ribbon	0	0	1
Hymenophyllum flabellatum Labill.	E	ribbon	0	0	1
Hymenophyllum javanicum A. Spreng	E	ribbon	0	0	1
Hymenophyllum multifidum (G. Forst.) Sw.	E	ribbon	0	0	1
Hymenophyllum pallidum Ebihara & K. Iwats.	E	ribbon	0	0	1
Hymenophyllum polyanthos (Sw.) Sw.	E	ribbon	0	0	1
Hypolepis dicksonioides (Endl.) Hook.	T	cordate	0	0	0
Hypolepis sp.	T	cordate	NA	NA	NA
Hypolepis tenuifolia (G. Forst.) Bernh.	T	cordate	0	0	0
Leucostegia pallida (Mett.) Copel.	E	cordate	1	0	0
Lindsaea propinqua Hook.	T	cordate	NA	NA	NA
Lindsaea repens (Bory) Thwaites	E	cordate	0	0	0
Lindsaea rigida J. Sm.	E	cordate	0	0	0
Lomagramma tahitensis Holttum	E	strap	0	0	0
Lomariopsis brackenridgei Carruth.	E	strap	0	0	0
Lygodium reticulatum Schkuhr	T	cordate	0	0	0
Macrothelypteris polypodioides (Hook.) Holttum	T	cordate	NA	NA	0
Macrothelypteris torresiana (Gaudich.) Ching	T	cordate	1	0	0
Microlepia scaberula Mett.; Kuhn	T	cordate	0	0	0
Microsorum commutatum (Blume) Copel.	T	strap	0	0	0
Microsorum grossum (Langsd. & Fisch.) S. B. Andrews	T	strap	1	0	0
Microsorum membranifolium (R. Br.) Ching	T	strap	1	0	0
Microsorum powellii (Baker) Copel.	E	strap	1	0	0
Microsorum punctatum Copel.	E	strap	1	0	0
Microsorum × tohieaense ined.	T	strap	NA	NA	NA
Microsorum × maximum (Brack.) Copel.	T	strap	NA	0	0
Nephrolepis biserrata (Sw.) Schott	T	cordate	1	0	0
Nephrolepis cordifolia (L.) C. Presl	T	cordate	1	0	0
Nephrolepis exaltata (L.) Schott	T	cordate	1	0	0
Nephrolepis hirsutula (G. Forst.) C. Presl	T	cordate	1	0	0
Oleandra sibbaldii Grev.	E	cordate	1	0	0
Ophioglossum falcatum (Presl) Fowler	E	tuber	0	0	0
Ophioglossum pendulum L.	E	tuber	0	0	0
Oreogrammitis raiateensis (J.W. Moore) Parris	E	strap	NA	NA	NA
Paesia divaricatissima (Dryand.) Copel.	T	cordate	0	0	0
Pityrogramma calomelanos (L.) Link	T	cordate	0	0	0
Plesioneuron attenuatum (Brack.) Holttum	T	cordate	1	0	0
Plesioneuron sp.	T	cordate	NA	NA	0
Pneumatopteris mesocarpa (Copel.) Holttum	T	cordate	1	0	0
Polyphlebium borbonicum (Bosch) Ebihara & Dubuisson	E	filament	0	0	1
Polyphlebium endlicherianum (C. Presl) Ebihara & K. Iwats.	E	filament	0	0	1
Prosaptia contigua (G. Forst.) C. Presl	E	strap	0	1	0
Psilotum complanatum Sw.	E	tuber	0	0	0
Psilotum nudum (L.) P. Beauv.	E	tuber	0	0	0
Pteris comans G. Forst.	T	cordate	NA	NA	0
Pteris ensiformis Burm. f.	T	cordate	0	0	0
Pteris mertensioides Willd.	T	cordate	NA	NA	0
Pteris tripartita Sw.	T	cordate	0	0	0
Ptisana salicina (Sm.) Murdock	T	cordate	0	0	0
Pyrrosia serpens (G. Forst.) Ching	E	strap	1	0	0
Schizaea dichotoma (L.) Sm.	T	filament	0	0	0
Selliguea plantaginea Brack.	E	strap	1	0	0
Sphaeropteris medullaris (G. Forst.) Bernh.	T	cordate	0	1	0
Sphaerostephanos invisus (G. Forst.) Holttum	T	cordate	1	1	0
Sphaerostephanos subpectinatus (Copel.) Holttum	T	cordate	1	1	0

Table C4 (Continued).

Species	Growth Habit	Morphotype	Glands	Hairs	Gemmae
Sphenomeris chinensis (L.) Maxon	T	cordate	0	0	0
Sticherus tahitensis (Copel.) St. John	T	cordate	NA	NA	NA
Tectaria decurrens (C. Presl) Copel.	T	cordate	1	0	0
Tectaria dissecta (G. Forst.) Lellinger	T	cordate	1	0	0
Tectaria tahitensis Maxon	T	cordate	1	0	0
Teratophyllum wilkesianum (Brack.) Holttum	T	strap	0	0	0
Tmesipteris gracilis Chinnock	E	tuber	0	0	0
Vandenboschia maxima (Blume) Copel.	T	filament	0	0	1
Wibelia denticulata (Burm.f.) M. Kato & Tsutsumi	E	cordate	1	0	0

Table C4 (Continued).

Species	Frond Dissection	Stipe Length (cm)	Frond Length (cm)	Frond Width (cm)
Abrodictyum asaegrayi var 1	10	4.07 +/- 1.62 (N = 2)	9.35 +/- 1.44 (N = 2)	2.09 +/- 0.72 (N = 2)
Abrodictyum asaegrayi var 2	10	3.7 +/- 0.42 (N = 2)	10.24 +/- 0.89 (N = 2)	1.99 +/- 0.03 (N = 2)
Abrodictyum caudatum	6	2.75 (N = 1)	28.01 (N = 1)	5.07 (N = 1)
Abrodictyum dentatum	6	8.63 (N = 1)	19.97 (N = 1)	6.95 (N = 1)
Acrostichum aureum	3	40 (N = 1)	130 (N = 1)	27 (N = 1)
Adiantum hispidulum	5	8.75 (N = 1)	20.64 (N = 1)	6.13 (N = 1)
Adiantum raddianum	7	18.06 (N = 1)	30.3 (N = 1)	11.13 (N = 1)
Adiantum trapeziforme	5	48 (N = 2)	88 (N = 2)	33 (N = 2)
Alsophila tahitensis	6	23.5 +/- 4.95 (N = 3)	90 +/- 7.07 (N = 3)	51 (N = 3)
Amphineuron opulentum	4	47.95 (N = 1)	92.38 (N = 1)	26.72 (N = 1)
Angiopteris evecta	5	150 (N = 1)	700 (N = 1)	300 (N = 1)
Antrophyum plantagineum	1	4.67 +/- 4.2 (N = 5)	11.44 +/- 4.49 (N = 5)	1.74 +/- 0.7 (N = 5)
Antrophyum reticulatum	1	0 +/- 0 (N = 2)	19.61 +/- 0.51 (N = 2)	3.24 +/- 0.49 (N = 2)
Arachniodes aristata	6	35.86 (N = 1)	55.93 (N = 1)	16.31 (N = 1)
Archigrammitis tahitensis	1	16.13 +/- 30.9 (N = 4)	10.3 +/- 2.64 (N = 4)	0.83 +/- 0.24 (N = 4)
Arthropteris palisotii	3	0.99 +/- 0.61 (N = 3)	17.05 +/- 8.77 (N = 3)	3.37 +/- 0.52 (N = 3)
Asplenium affine	3	12.96 +/- 5.51 (N = 2)	32.02 +/- 3.55 (N = 2)	5.55 +/- 0.18 (N = 2)
Asplenium australasicum	1	0 (N = 1)	70 (N = 1)	9 (N = 1)
Asplenium caudatum	3	26.74 +/- 8.32 (N = 2)	112.82 +/- 12.58 (N = 2)	13.05 +/- 0.21 (N = 2)
Asplenium gibberosum	6	30 +/- 4.86 (N = 2)	78.77 +/- 0.94 (N = 2)	25.45 +/- 3.61 (N = 2)
Asplenium nidus	1	0 +/- 0 (N = 3)	105.59 +/- 23.6 (N = 3)	12.36 +/- 5.27 (N = 3)
Asplenium polyodon	3	20.05 +/- 7.14 (N = 2)	48.03 +/- 25.5 (N = 2)	19.55 (N = 2)
Asplenium tenerum	3	13.61 +/- 2.05 (N = 2)	31.87 +/- 0.35 (N = 2)	4.47 (N = 2)
Belvisia spicata	1	3.24 (N = 1)	33.59 (N = 1)	2.54 (N = 1)
Blechnum orientale	3	30 (N = 2)	160 +/- 56.57 (N = 2)	53 +/- 18.38 (N = 2)
Blechnum pacificum	3	56.4 (N = 1)	117.8 (N = 1)	27.21 (N = 1)
Blechnum patersonii	2	27.65 +/- 17.21 (N = 2)	49.05 +/- 21.49 (N = 2)	14.74 +/- 2.29 (N = 2)
Blechnum raiateense	3	29.21 (N = 1)	82.29 (N = 1)	15.38 (N = 1)
Blechnum vulcanicum	3	37.5 +/- 17.68 (N = 2)	72.5 +/- 31.82 (N = 2)	20.4 +/- 6.51 (N = 2)
Bolbitis lonchophora	4	12.56 (N = 1)	34.85 (N = 1)	14.66 (N = 1)
Callistopteris apiifolia	6	12.5 +/- 4.67 (N = 2)	37.06 +/- 5.89 (N = 2)	10.83 +/- 3.99 (N = 2)
Calymmodon orientalis	2	0.25 (N = 1)	8.34 (N = 1)	0.44 (N = 1)
Cheilanthes nudiuscula	6	2.79 (N = 1)	6.35 (N = 1)	2.38 (N = 1)
Chingia longissima	4	102.21 +/- 8.55 (N = 2)	194.22 +/- 36.2 (N = 2)	49.13 +/- 23.75 (N = 2)
Christella dentata	4	49.07 +/- 22.59 (N = 3)	102.03 +/- 39.19 (N = 3)	25.15 +/- 6.65 (N = 3)
Coryphopteris sp.	4	20.29 +/- 2.16 (N = 3)	46.86 +/- 3.06 (N = 3)	12.28 +/- 4.1 (N = 3)
Crepidomanes bipunctatum	10	2.05 (N = 1)	11.6 (N = 1)	2.84 (N = 1)
Crepidomanes humile	10	0.64 +/- 0.01 (N = 2)	4.9 +/- 0.67 (N = 2)	1.86 +/- 0.63 (N = 2)
Crepidomanes kurzii	3	0.2 (N = 1)	1 (N = 1)	0.8 (N = 1)
Crepidomanes minutum var 1	2	0.6 (N = 1)	1.6 (N = 1)	1.2 (N = 1)
Crepidomanes minutum var 2	2	0.88 (N = 1)	2.48 (N = 1)	0.74 (N = 1)
Crepidomanes minutum var 3	3	0.28 (N = 1)	3.96 (N = 1)	0.97 (N = 1)
Ctenitis sciaphila	6	20.43 (N = 1)	43.4 (N = 1)	23.28 (N = 1)
Ctenopterella blechnoides	2	0.72 +/- 0.02 (N = 2)	19.29 +/- 3.56 (N = 2)	1.83 +/- 0.24 (N = 2)
Cyathea epaleata	6	33.5 (N = 1)	NA	62 (N = 1)
Dasygrammitis purpurascens	2	1.75 +/- 1.05 (N = 3)	13.42 +/- 1.82 (N = 3)	1.24 +/- 0.07 (N = 3)
Davallia solida	6	27.15 (N = 1)	48.69 (N = 1)	32.01 (N = 1)
Deparia petersenii	6	26.63 +/- 8.65 (N = 4)	58.04 +/- 15.1 (N = 4)	12.71 +/- 4.88 (N = 4)
Dicranopteris linearis	5	35 (N = 1)	NA	36 (N = 1)
Didymoglossum tahitense	1	0 +/- 0 (N = 2)	1.19 +/- 0.62 (N = 2)	1.05 +/- 0.19 (N = 2)
Diplazium ellipticum	6	33.19 +/- 7.44 (N = 4)	75.07 +/- 17.21 (N = 4)	33.27 +/- 8.59 (N = 4)
Diplazium grantii	6	21.06 (N = 1)	60.31 (N = 1)	19.11 (N = 1)
Diplazium harpeodes	6	76.4 +/- 26 (N = 2)	165.27 +/- 60.38 (N = 2)	37.66 +/- 10.3 (N = 2)
Diplopterygium longissimum	NA	20 (N = 2)	34 (N = 2)	81 +/- 55.15 (N = 2)
Doryopteris concolor	4	5.71 (N = 1)	9.13 (N = 1)	3.59 (N = 1)
Dryopteris dicksonioides	8	84.83 +/- 35.59 (N = 3)	159.53 +/- 85.51 (N = 3)	63.34 +/- 41.63 (N = 3)
Dryopteris macrolepidota	6	48.79 +/- 2.12 (N = 2)	109.65 +/- 19.21 (N = 2)	49.32 +/- 20.23 (N = 2)
Elaphoglossum samoense	1	4.92 (N = 1)	16.02 (N = 1)	2.25 (N = 1)
Elaphoglossum savaiense	1	15.53 (N = 1)	64.46 (N = 1)	9.67 (N = 1)
Grammitis marginelloides	1	1.78 +/- 1.54 (N = 3)	13.96 +/- 5.52 (N = 3)	0.77 +/- 0.12 (N = 3)

Table C4 (Continued).

Species	Frond Dissection	Stipe Length (cm)	Frond Length (cm)	Frond Width (cm)
Haplopteris elongata	1	0 (N = 1)	58.02 (N = 1)	0.63 (N = 1)
Histiopteris incisa	6	56.24 (N = 2)	130.69 (N = 2)	54.71 +/- 6.66 (N = 2)
Humata anderssonii	4	3.74 (N = 1)	6.95 (N = 1)	3.23 (N = 1)
Humata pectinata	4	11.39 +/- 1.06 (N = 2)	23.4 +/- 1.06 (N = 2)	4.48 +/- 0.61 (N = 2)
Hymenophyllum braithwaitei	NA	NA	NA	NA
Hymenophyllum digitatum	2	0.52 +/- 0.2 (N = 2)	2.98 +/- 0.53 (N = 2)	1.5 +/- 0.29 (N = 2)
Hymenophyllum flabellatum	10	2.92 +/- 0.13 (N = 2)	20.21 +/- 13.61 (N = 2)	3.46 +/- 1 (N = 2)
Hymenophyllum javanicum	10	3.92 +/- 0.5 (N = 3)	18.88 +/- 2.57 (N = 3)	6.83 +/- 1.84 (N = 3)
Hymenophyllum multifidum	10	2.67 +/- 1.32 (N = 4)	5.78 +/- 1.48 (N = 4)	1.97 +/- 0.49 (N = 4)
Hymenophyllum pallidum	10	7.34 (N = 1)	14.29 (N = 1)	2.77 (N = 1)
Hymenophyllum polyanthos	10	3.4 +/- 0.44 (N = 2)	14.31 +/- 1.58 (N = 2)	3.16 +/- 1.38 (N = 2)
Hypolepis dicksonioides	8	40 (N = 2)	90 (N = 2)	44.5 +/- 10.61 (N = 2)
Hypolepis sp.	NA	80 (N = 1)	200 (N = 1)	87.6 (N = 1)
Hypolepis tenuifolia	8	46 (N = 2)	107 (N = 2)	51.5 +/- 26.16 (N = 2)
Leucostegia pallida	6	45.31 +/- 29.26 (N = 2)	49.31 (N = 2)	25.12 +/- 9.34 (N = 2)
Lindsaea propinqua	NA	21.91 +/- 0.72 (N = 2)	34.65 +/- 3.1 (N = 2)	13.61 +/- 2.41 (N = 2)
Lindsaea repens	3	1.06 +/- 0.23 (N = 2)	32 +/- 15.51 (N = 2)	2.66 +/- 0.13 (N = 2)
Lindsaea rigida	5	19.18 +/- 0.96 (N = 2)	36.23 +/- 2.7 (N = 2)	12.58 (N = 2)
Lomagramma tahitensis	3	12.75 (N = 1)	47.43 (N = 1)	14.88 (N = 1)
Lomariopsis brackenridgei	3	22.99 (N = 1)	65.1 (N = 1)	22.9 (N = 1)
Lygodium reticulatum	5	15 (N = 1)	NA	30 (N = 1)
Macrothelypteris polypodioides	6	79.99 +/- 31.52 (N = 2)	143.03 +/- 62.16 (N = 2)	31.72 +/- 1.13 (N = 2)
Macrothelypteris torresiana	6	44.56 +/- 16.38 (N = 2)	109.45 (N = 2)	33.3 (N = 2)
Microlepia scaberula	6	63.53 +/- 37.43 (N = 2)	133.79 +/- 93.63 (N = 2)	43.08 +/- 23.93 (N = 2)
Microsorum commutatum	2	34.54 (N = 2)	79.56 (N = 2)	29.96 +/- 7.12 (N = 2)
Microsorum grossum	2	13.58 (N = 1)	42.6 (N = 1)	19.83 (N = 1)
Microsorum membranifolium	2	87.66 (N = 1)	278.81 (N = 1)	64.76 (N = 1)
Microsorum powellii	2	20.2 +/- 6.83 (N = 2)	44.8 +/- 16.07 (N = 2)	20.31 +/- 9.83 (N = 2)
Microsorum punctatum	1	0 +/- 0 (N = 3)	68.8 +/- 25.5 (N = 3)	7.2 +/- 1.56 (N = 3)
Microsorum × tohieaense	NA	NA	NA	NA
Microsorum × maximum	2	1.22 (N = 1)	36.58 (N = 1)	3.74 (N = 1)
Nephrolepis biserrata	3	20.3 +/- 0.43 (N = 2)	98.6 +/- 51.48 (N = 2)	14.44 +/- 3.63 (N = 2)
Nephrolepis cordifolia	3	8.3 (N = 1)	38.8 (N = 1)	4.63 (N = 1)
Nephrolepis exaltata	3	NA	NA	NA
Nephrolepis hirsutula	3	19.65 +/- 10.08 (N = 3)	70.52 +/- 25.75 (N = 3)	9.49 +/- 6.7 (N = 3)
Oleandra sibbaldii	1	3.92 +/- 0.27 (N = 2)	34.9 +/- 5.28 (N = 2)	3.18 +/- 0.81 (N = 2)
Ophioglossum falcatum	1	3.68 +/- 6.27 (N = 4)	36.24 +/- 17.21 (N = 4)	2.39 +/- 0.54 (N = 4)
Ophioglossum pendulum	1	4.24 +/- 3.91 (N = 3)	91.06 +/- 77.76 (N = 3)	3.26 +/- 1.89 (N = 3)
Oreogrammitis raiateensis	1	0.46 +/- 0.15 (N = 2)	5.18 +/- 1.05 (N = 2)	0.54 +/- 0.09 (N = 2)
Paesia divaricatissima	8	76.06 +/- 44.04 (N = 4)	56.1 (N = 4)	35.73 +/- 8.38 (N = 4)
Pityrogramma calomelanos	4	24.98 (N = 1)	68.21 (N = 1)	11.81 (N = 1)
Plesioneuron attenuatum	4	53.67 (N = 1)	128.79 (N = 1)	27.84 (N = 1)
Plesioneuron sp.	4	20.56 (N = 1)	49.76 (N = 1)	10.81 (N = 1)
Pneumatopteris mesocarpa	4	76.53 +/- 14.15 (N = 3)	144.1 +/- 18.19 (N = 3)	25.92 +/- 3.9 (N = 3)
Polyphlebium borbonicum	10	3.25 +/- 1 (N = 2)	9.17 +/- 1.81 (N = 2)	4.17 +/- 0.34 (N = 2)
Polyphlebium endlicherianum	10	0.88 +/- 0.12 (N = 2)	4.98 +/- 0.73 (N = 2)	1.2 +/- 0.3 (N = 2)
Prosaptia contigua	2	4.36 +/- 2.44 (N = 3)	26.34 +/- 6.11 (N = 3)	2.82 +/- 0.58 (N = 3)
Psilotum complanatum	1	0 +/- 0 (N = 2)	155.33 +/- 134.1 (N = 2)	0.42 +/- 0.22 (N = 2)
Psilotum nudum	1	0 (N = 1)	30 (N = 1)	0.2 (N = 1)
Pteris comans	7	50 (N = 1)	100 (N = 1)	40 (N = 1)
Pteris ensiformis	5	11.07 (N = 1)	27.61 (N = 1)	6.68 (N = 1)
Pteris mertensioides	5	80 (N = 1)	200 (N = 1)	48 (N = 1)
Pteris tripartita	8	79.28 (N = 1)	132.04 (N = 1)	58.61 (N = 1)
Ptisana salicina	5	47 (N = 1)	100 (N = 1)	83 (N = 1)
Pyrrosia serpens	1	2.58 (N = 1)	11.03 (N = 1)	0.92 (N = 1)
Schizaea dichotoma	3	32.75 +/- 0.14 (N = 2)	42.84 +/- 0.07 (N = 2)	12.1 +/- 0 (N = 2)
Selliguea plantaginea	1	10.47 +/- 4.49 (N = 2)	26.49 +/- 6.75 (N = 2)	5.53 +/- 0.09 (N = 2)
Sphaeropteris medullaris	6	23.5 +/- 4.95 (N = 2)	147.5 +/- 31.82 (N = 2)	68 (N = 2)
Sphaerostephanos invisus	4	31.62 +/- 7.02 (N = 2)	100.54 +/- 48.9 (N = 2)	24.43 +/- 10.89 (N = 2)
Sphaerostephanos subpectinatus	4	22.51 (N = 1)	49.49 (N = 1)	13.36 (N = 1)

Table C4 (Continued).

Species	Frond Dissection	Stipe Length (cm)	Frond Length (cm)	Frond Width (cm)
Sphenomeris chinensis	8	29.69 (N = 1)	87.19 (N = 1)	14.72 (N = 1)
Sticherus tahitensis	5	33.58 +/- 10.61 (N = 2)	46.54 +/- 10.93 (N = 2)	21.69 +/- 6.84 (N = 2)
Tectaria decurrens	3	38.37 +/- 16.87 (N = 2)	79.26 +/- 37.91 (N = 2)	31.64 +/- 18.98 (N = 2)
Tectaria dissecta	6	43.65 +/- 3.23 (N = 2)	88.76 +/- 8.82 (N = 2)	38.25 +/- 11.35 (N = 2)
Tectaria tahitensis	6	60.5 (N = 1)	99.48 (N = 1)	39.33 (N = 1)
Teratophyllum wilkesianum	5	20.77 (N = 1)	46.44 (N = 1)	15.6 (N = 1)
Tmesipteris gracilis	1	4.2 (N = 1)	22.9 (N = 1)	2.48 (N = 1)
Vandenboschia maxima	6	10.9 +/- 6.08 (N = 4)	29.17 +/- 11.92 (N = 4)	10.54 +/- 4.93 (N = 4)
Wibelia denticulata	8	40 (N = 2)	100 (N = 2)	52 +/- 14.14 (N = 2)

Table C4 (Continued).

Species	Rhizome Dia. (cm)	No. Pinna Pairs	SLA (m^2 kg^{-1})
Abrodictyum asaegrayi var 1	0.34 +/- 0.1 (N = 2)	7.5 +/- 2.12 (N = 2)	NA
Abrodictyum asaegrayi var 2	0.29 +/- 0.02 (N = 2)	14 +/- 1.41 (N = 2)	NA
Abrodictyum caudatum	0.3 (N = 1)	20 (N = 1)	27.07 (N = 1)
Abrodictyum dentatum	0.4 (N = 1)	15 (N = 1)	25.75 +/- 8.35 (N = 3)
Acrostichum aureum	NA	13 (N = 1)	9.18 (N = 1)
Adiantum hispidulum	0.34 (N = 1)	1 (N = 1)	62.7 +/- 13.2 (N = 2)
Adiantum raddianum	0.5 (N = 1)	5 (N = 1)	66.22 (N = 1)
Adiantum trapeziforme	1.05 (N = 2)	5 (N = 2)	42.73 +/- 0.83 (N = 2)
Alsophila tahitensis	10 (N = 3)	10 (N = 3)	22.35 +/- 2.93 (N = 3)
Amphineuron opulentum	0.86 (N = 1)	14 (N = 1)	48.86 +/- 5.63 (N = 2)
Angiopteris evecta	50 (N = 1)	10 (N = 1)	22.17 +/- 5.27 (N = 5)
Antrophyum plantagineum	0.4 +/- 0.07 (N = 5)	0 +/- 0 (N = 5)	15.72 (N = 1)
Antrophyum reticulatum	0.3 +/- 0.14 (N = 2)	0 +/- 0 (N = 2)	15.77 +/- 3.38 (N = 3)
Arachniodes aristata	0.88 (N = 1)	8 (N = 1)	20.59 (N = 1)
Archigrammitis tahitensis	0.34 +/- 0.07 (N = 4)	0 +/- 0 (N = 4)	19.95 +/- 5.42 (N = 2)
Arthropteris palisotii	0.16 +/- 0.05 (N = 3)	16.67 +/- 5.77 (N = 3)	29.84 +/- 5.5 (N = 2)
Asplenium affine	0.7 +/- 0.14 (N = 2)	12.5 +/- 0.71 (N = 2)	31.79 (N = 1)
Asplenium australasicum	NA	0 (N = 1)	24.78 +/- 9.84 (N = 5)
Asplenium caudatum	1.5 (N = 2)	41 +/- 1.41 (N = 2)	34.04 +/- 20.44 (N = 4)
Asplenium gibberosum	1.91 +/- 0.83 (N = 2)	12.5 +/- 3.54 (N = 2)	16.92 (N = 1)
Asplenium nidus	NA	0 (N = 3)	22.65 (N = 1)
Asplenium polyodon	0.8 (N = 2)	12 (N = 2)	11.28 (N = 1)
Asplenium tenerum	1.87 (N = 2)	20 (N = 2)	26.75 (N = 1)
Belvisia spicata	0.8 (N = 1)	0 (N = 1)	17.4 +/- 6.01 (N = 5)
Blechnum orientale	13.58 (N = 2)	39 (N = 2)	22.86 +/- 5.04 (N = 3)
Blechnum pacificum	4.5 (N = 1)	37 (N = 1)	23.29 +/- 3.46 (N = 3)
Blechnum patersonii	2.8 +/- 2.12 (N = 2)	4.5 +/- 3.54 (N = 2)	23.18 (N = 1)
Blechnum raiateense	5.31 (N = 1)	25 (N = 1)	23.63 +/- 2.26 (N = 2)
Blechnum vulcanicum	1 (N = 2)	15 (N = 2)	13.34 (N = 1)
Bolbitis lonchophora	1.42 (N = 1)	8 (N = 1)	47.24 +/- 3.78 (N = 2)
Callistopteris apiifolia	1.15 +/- 0.01 (N = 2)	15 (N = 2)	24.2 +/- 6.94 (N = 3)
Calymmodon orientalis	0.22 (N = 1)	35 (N = 1)	26.42 (N = 1)
Cheilanthes nudiuscula	0.3 (N = 1)	6 (N = 1)	28.56 (N = 1)
Chingia longissima	8.99 +/- 1.22 (N = 2)	41 +/- 5.66 (N = 2)	20.94 (N = 1)
Christella dentata	0.67 +/- 0.12 (N = 3)	17.67 +/- 3.21 (N = 3)	44.88 (N = 1)
Coryphopteris sp.	0.71 (N = 3)	15.67 +/- 1.15 (N = 3)	32.01 +/- 4.98 (N = 6)
Crepidomanes bipunctatum	0.11 (N = 1)	9 (N = 1)	24.95 +/- 3.87 (N = 2)
Crepidomanes humile	0.09 +/- 0.02 (N = 2)	7 (N = 2)	40.68 +/- 13.3 (N = 2)
Crepidomanes kurzii	0.05 (N = 1)	4 (N = 1)	44.08 +/- 19.08 (N = 2)
Crepidomanes minutum var 1	0.03 (N = 1)	0 (N = 1)	NA
Crepidomanes minutum var 2	0.02 (N = 1)	0 (N = 1)	NA
Crepidomanes minutum var 3	0.04 (N = 1)	9 (N = 1)	NA
Ctenitis sciaphila	1.7 (N = 1)	10 (N = 1)	42.3 +/- 3.64 (N = 2)
Ctenopterella blechnoides	0.61 (N = 2)	52.5 +/- 3.54 (N = 2)	8.66 (N = 1)
Cyathea epaleata	6 (N = 1)	NA	27.01 (N = 1)
Dasygrammitis purpurascens	0.88 +/- 0.57 (N = 3)	31.33 +/- 7.09 (N = 3)	15.11 (N = 1)
Davallia solida	1.24 (N = 1)	8 (N = 1)	19.6 +/- 6.07 (N = 2)
Deparia petersenii	0.88 +/- 0.07 (N = 4)	11.5 +/- 2.52 (N = 4)	61.74 +/- 1.55 (N = 2)
Dicranopteris linearis	3 (N = 1)	NA	24.8 +/- 2.98 (N = 2)
Didymoglossum tahitense	0.08 +/- 0.04 (N = 2)	0 +/- 0 (N = 2)	53.09 +/- 6.36 (N = 3)
Diplazium ellipticum	2.15 +/- 0.42 (N = 4)	12.75 +/- 1.71 (N = 4)	45.89 +/- 3.92 (N = 5)
Diplazium grantii	1.54 (N = 1)	10 (N = 1)	46.27 (N = 1)
Diplazium harpeodes	1.6 +/- 0.57 (N = 2)	13 +/- 4.24 (N = 2)	35.35 (N = 1)
Diplopterygium longissimum	0.8 (N = 2)	3 (N = 2)	52.13 (N = 1)
Doryopteris concolor	0.5 (N = 1)	0 (N = 1)	40.28 (N = 1)
Dryopteris dicksonioides	2 (N = 3)	14.5 +/- 3.54 (N = 3)	24.17 (N = 1)
Dryopteris macrolepidota	6 (N = 2)	12 +/- 0 (N = 2)	31.39 +/- 9.41 (N = 3)
Elaphoglossum samoense	1.43 (N = 1)	0 (N = 1)	15.29 +/- 0.35 (N = 2)
Elaphoglossum savaiense	1.21 (N = 1)	0 (N = 1)	10.5 (N = 1)
Grammitis marginelloides	0.54 +/- 0.06 (N = 3)	0 +/- 0 (N = 3)	14.4 +/- 2.85 (N = 2)

Table C4 (Continued).

Species	Rhizome Dia. (cm)	No. Pinna Pairs	SLA (m^2 kg^{-1})
Haplopteris elongata	0.33 (N = 1)	0 (N = 1)	10.45 +/- 1.98 (N = 3)
Histiopteris incisa	0.9 +/- 0.14 (N = 2)	10 (N = 2)	51.01 (N = 1)
Humata anderssonii	0.18 (N = 1)	4 (N = 1)	8.43 (N = 1)
Humata pectinata	0.29 +/- 0.02 (N = 2)	22 +/- 2.83 (N = 2)	14.12 +/- 5.72 (N = 4)
Hymenophyllum braithwaitei	NA	NA	25.17 (N = 1)
Hymenophyllum digitatum	0.02 +/- 0.01 (N = 2)	3 +/- 0 (N = 2)	26.8 (N = 1)
Hymenophyllum flabellatum	0.1 +/- 0.06 (N = 2)	12.5 +/- 7.78 (N = 2)	31.14 (N = 1)
Hymenophyllum javanicum	0.07 +/- 0.01 (N = 3)	10.33 +/- 1.53 (N = 3)	44.31 +/- 8.22 (N = 2)
Hymenophyllum multifidum	0.05 +/- 0 (N = 4)	4.75 +/- 1.5 (N = 4)	17.73 +/- 0.85 (N = 2)
Hymenophyllum pallidum	NA	8 (N = 1)	21.06 +/- 0.89 (N = 2)
Hymenophyllum polyanthos	0.06 +/- 0.03 (N = 2)	13.5 +/- 2.12 (N = 2)	34.72 +/- 2.56 (N = 2)
Hypolepis dicksonioides	0.89 +/- 0.42 (N = 2)	14 +/- 8.49 (N = 2)	60.67 (N = 1)
Hypolepis sp.	0.4 (N = 1)	NA	53.58 (N = 1)
Hypolepis tenuifolia	0.85 +/- 0.21 (N = 2)	12.5 +/- 0.71 (N = 2)	38.9 (N = 1)
Leucostegia pallida	1.01 +/- 0.37 (N = 2)	12.5 +/- 0.71 (N = 2)	30.93 (N = 1)
Lindsaea propinqua	0.18 +/- 0.04 (N = 2)	4 +/- 1.41 (N = 2)	40.67 +/- 8.08 (N = 2)
Lindsaea repens	0.23 +/- 0.01 (N = 2)	40 +/- 14.14 (N = 2)	44.92 (N = 1)
Lindsaea rigida	0.16 +/- 0.02 (N = 2)	1 +/- 1.41 (N = 2)	37.37 +/- 19.21 (N = 5)
Lomagramma tahitensis	0.74 (N = 1)	22 (N = 1)	26.66 (N = 1)
Lomariopsis brackenridgei	0.6 (N = 1)	13 (N = 1)	30.57 +/- 6.29 (N = 2)
Lygodium reticulatum	0.32 (N = 1)	NA	45.27 +/- 9.89 (N = 4)
Macrothelypteris polypodioides	1.45 +/- 0.21 (N = 2)	15.5 +/- 3.54 (N = 2)	34.91 (N = 1)
Macrothelypteris torresiana	1.4 +/- 0.48 (N = 2)	12 (N = 2)	34.91 (N = 1)
Microlepia scaberula	0.7 +/- 0.28 (N = 2)	10 (N = 2)	25.84 (N = 1)
Microsorum commutatum	1.3 +/- 0.56 (N = 2)	9 (N = 2)	33.3 +/- 8.04 (N = 2)
Microsorum grossum	0.92 (N = 1)	4 (N = 1)	37.37 +/- 12.9 (N = 3)
Microsorum membranifolium	1.5 (N = 1)	20 (N = 1)	53.49 +/- 8.39 (N = 2)
Microsorum powellii	0.82 +/- 0.21 (N = 2)	6.5 +/- 4.95 (N = 2)	15.12 (N = 1)
Microsorum punctatum	0.7 +/- 0.28 (N = 3)	0 +/- 0 (N = 3)	22.96 +/- 2.1 (N = 2)
Microsorum × tohieaense	NA	NA	NA
Microsorum × maximum	0.88 (N = 1)	0 (N = 1)	20.42 +/- 8.5 (N = 3)
Nephrolepis biserrata	0.4 (N = 2)	20 (N = 2)	18.32 (N = 1)
Nephrolepis cordifolia	0.75 (N = 1)	30 (N = 1)	9.67 (N = 1)
Nephrolepis exaltata	NA	NA	NA
Nephrolepis hirsutula	1.08 +/- 0.57 (N = 3)	43.33 +/- 7.64 (N = 3)	44.99 +/- 10.42 (N = 5)
Oleandra sibbaldii	0.41 +/- 0.01 (N = 2)	0 (N = 2)	33.99 +/- 30.99 (N = 2)
Ophioglossum falcatum	0.32 +/- 0.04 (N = 4)	0 +/- 0 (N = 4)	23.53 +/- 6.25 (N = 3)
Ophioglossum pendulum	0.45 +/- 0.07 (N = 3)	0 +/- 0 (N = 3)	19.57 +/- 0.33 (N = 2)
Oreogrammitis raiateensis	0.29 (N = 2)	0 (N = 2)	32.79 +/- 22.75 (N = 2)
Paesia divaricatissima	0.31 +/- 0.12 (N = 4)	7 +/- 4.24 (N = 4)	8.92 (N = 1)
Pityrogramma calomelanos	1.14 (N = 1)	18 (N = 1)	NA
Plesioneuron attenuatum	1.79 (N = 1)	22 (N = 1)	35.64 +/- 8.84 (N = 3)
Plesioneuron sp.	1.7 (N = 1)	15 (N = 1)	38.96 +/- 0.43 (N = 2)
Pneumatopteris mesocarpa	1.98 +/- 0.31 (N = 3)	27 +/- 3.61 (N = 3)	32.7 +/- 0.24 (N = 2)
Polyphlebium borbonicum	0.09 +/- 0.01 (N = 2)	6.5 +/- 0.71 (N = 2)	36.96 +/- 7.19 (N = 3)
Polyphlebium endlicherianum	0.05 +/- 0 (N = 2)	4.5 +/- 2.12 (N = 2)	38.44 +/- 6.76 (N = 4)
Prosaptia contigua	0.55 +/- 0.07 (N = 3)	55 +/- 0 (N = 3)	13.8 (N = 1)
Psilotum complanatum	0.21 +/- 0.02 (N = 2)	0 +/- 0 (N = 2)	9.51 (N = 1)
Psilotum nudum	0.7 (N = 1)	0 (N = 1)	NA
Pteris comans	NA	2 (N = 1)	39.19 (N = 1)
Pteris ensiformis	0.7 (N = 1)	5 (N = 1)	30.72 (N = 1)
Pteris mertensioides	NA	14 (N = 1)	26.72 (N = 1)
Pteris tripartita	NA	0 (N = 1)	54.92 +/- 12.27 (N = 2)
Ptisana salicina	8 (N = 1)	4 (N = 1)	15.31 +/- 4.44 (N = 4)
Pyrrosia serpens	0.16 (N = 1)	0 (N = 1)	13.61 +/- 6.8 (N = 4)
Schizaea dichotoma	0.3 (N = 2)	NA	57.76 (N = 1)
Selliguea plantaginea	0.47 +/- 0.03 (N = 2)	0 +/- 0 (N = 2)	8.77 (N = 1)
Sphaeropteris medullaris	11 (N = 2)	NA	22.96 +/- 9.04 (N = 4)
Sphaerostephanos invisus	0.8 (N = 2)	30.5 +/- 6.36 (N = 2)	22.44 (N = 1)
Sphaerostephanos subpectinatus	1.61 (N = 1)	15 (N = 1)	42.89 +/- 7.94 (N = 5)

Table C4 (Continued).

Species	Rhizome Dia. (cm)	No. Pinna Pairs	SLA (m^2 kg^{-1})
Sphenomeris chinensis	1.23 (N = 1)	12 (N = 1)	26.18 (N = 1)
Sticherus tahitensis	0.25 +/- 0 (N = 2)	16 +/- 0 (N = 2)	10.85 (N = 1)
Tectaria decurrens	2.75 +/- 0.41 (N = 2)	3.5 +/- 2.12 (N = 2)	38.2 +/- 6.64 (N = 2)
Tectaria dissecta	1.77 +/- 0.39 (N = 2)	8.5 +/- 2.12 (N = 2)	46.11 +/- 7.3 (N = 2)
Tectaria tahitensis	1.72 (N = 1)	5 (N = 1)	49.72 +/- 1.04 (N = 2)
Teratophyllum wilkesianum	0.61 (N = 1)	8 (N = 1)	58.71 +/- 15.77 (N = 2)
Tmesipteris gracilis	0.21 (N = 1)	28 (N = 1)	11.66 (N = 1)
Vandenboschia maxima	0.34 +/- 0.05 (N = 4)	8.25 +/- 1.71 (N = 4)	23.09 +/- 3.78 (N = 2)
Wibelia denticulata	0.92 +/- 0.59 (N = 2)	10.5 +/- 0.71 (N = 2)	22.25 (N = 1)

Appendix D:

Supporting material for Chapter IV

Figure D1. Maximum-likelihood phylogenetic tree inferred using plastid markers (*rbcL* and *trnLF*) including specimens with rogue alleles. Dataset including all species with either *rbcL* (*N* = 141 spp.) or *trnLF* (*N* = 140 spp.), *N* = 154 spp. total. For a description of specimens with rogue alleles (*N* = 3), see 4.3 Methods. Names of taxa from French Polynesia colored by species. Diamonds indicate monophyletic genera that have been collapsed for plotting; number of collapsed species indicated in parenthesis. Bootstrap values > 50 shown at nodes. Scalebar indicates expected number of changes per site. Asterisks indicate specimens with rogue alleles. Outgroup species (*Thylacopteris papillosa*, *Pleopeltis munchii*, and *Platycerium stemaria* var. *laurentii*) not shown.

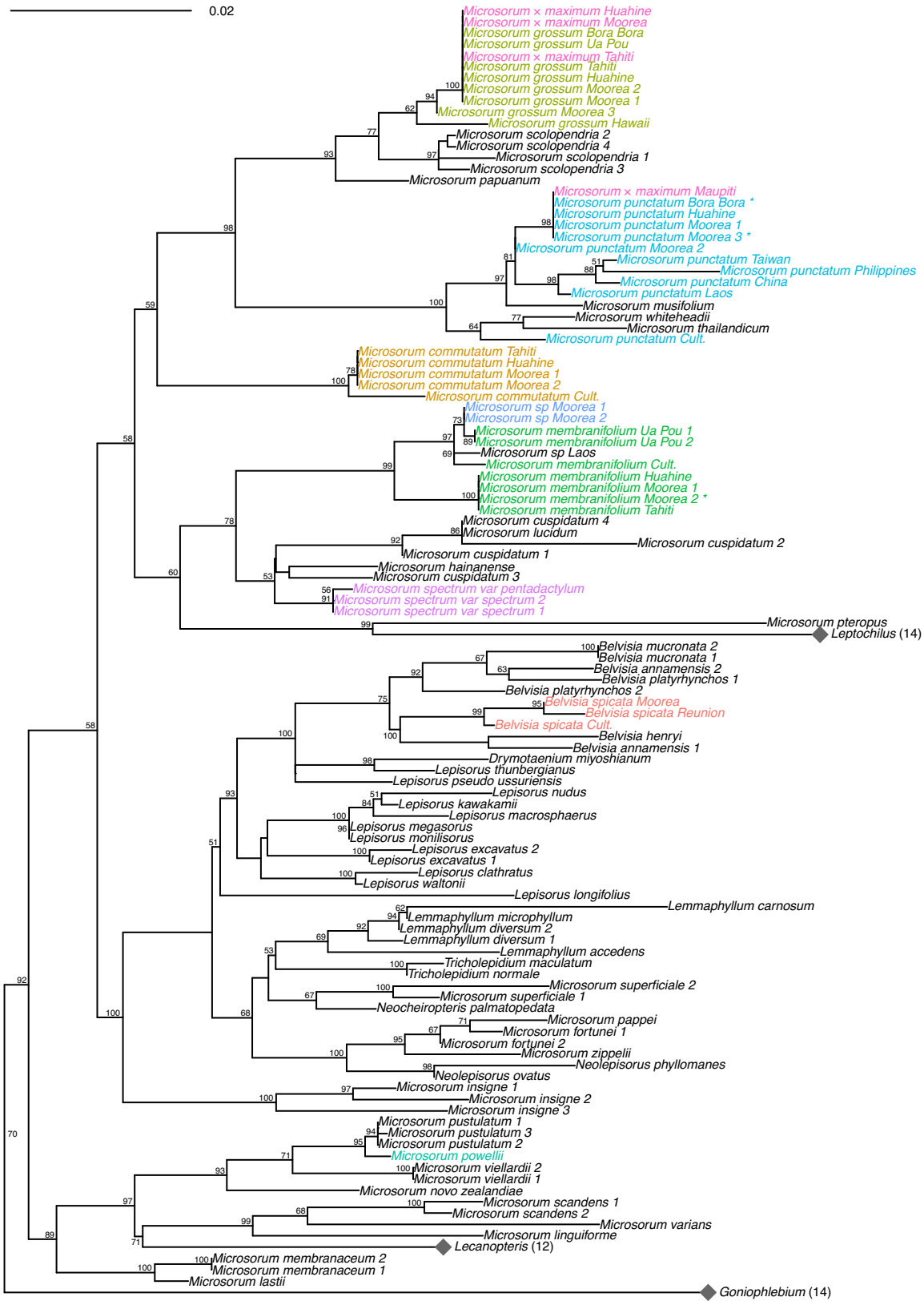

Figure D1 (Continued).

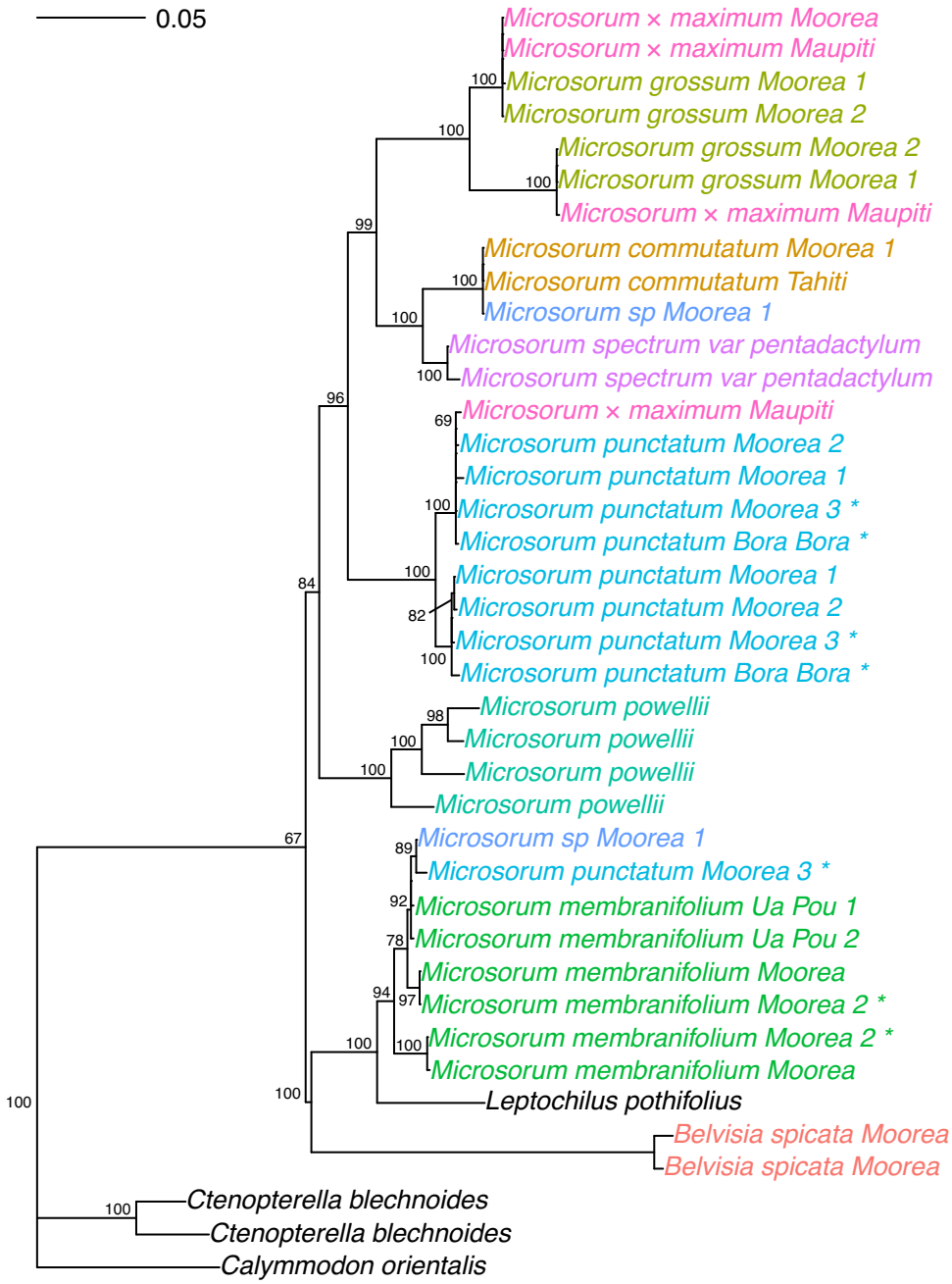

Figure D2. Maximum-likelihood phylogenetic tree inferred using nuclear *gapCp long* including specimens with rogue alleles. For a description of specimens with rogue alleles ($N = 3$), see 4.3 Methods. Names of taxa from French Polynesia colored by species.

Figure D2 (Continued). Bootstrap values > 50 shown at nodes. Scalebar indicates expected number of changes per site. Asterisks indicate specimens with rogue alleles.

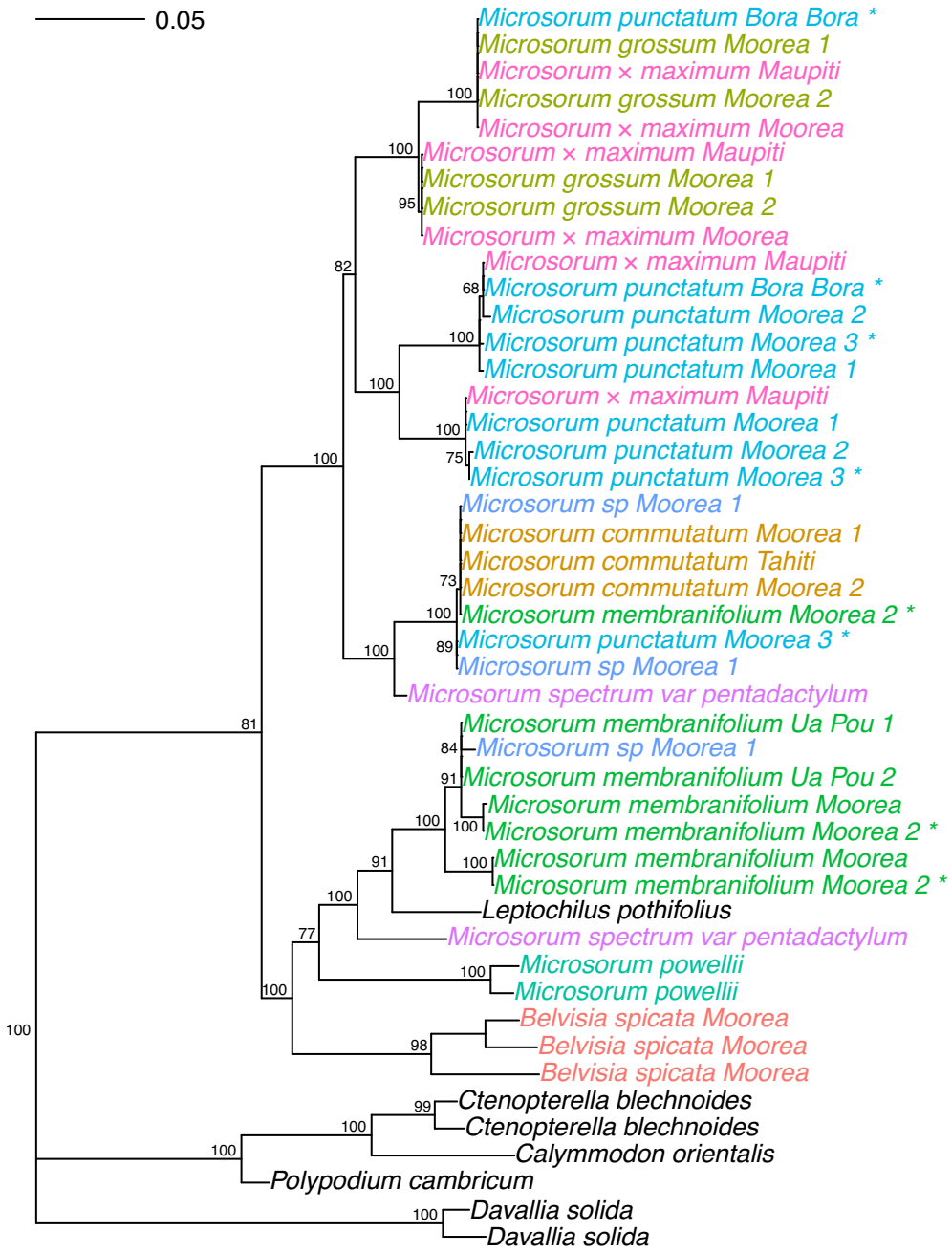

Figure D3. Maximum-likelihood phylogenetic tree inferred using nuclear *gapCp short* including specimens with rogue alleles. For a description of specimens with rogue alleles ($N = 3$), see 4.3 Methods. Names of taxa from French Polynesia colored by species. Bootstrap values > 50 shown at nodes.

Figure D3 (Continued). Scalebar indicates expected number of changes per site. Asterisks indicate specimens with rogue alleles.

Table D1. List of voucher specimens and GenBank accessions used in this study. Newly sequenced accessions not yet deposited in GenBank indicated with the number 1 (GenBank accession numbers will be assigned upon publication). Unknown sources indicated with question mark. Abbreviations of botanical garden names follows Kreier et al. (2008): ASG, Private Garden of Alan R. Smith (Berkeley); BGB, Botanical Garden Berlin-Dahlem; BGBO, Botanical Garden Bogor; BGG, Old Botanical Garden Göttingen; BGH, Botanical Garden Heidelberg; BGL, Botanical Garden Leiden; BGU, Botanical Garden Utrecht; BGUB, Botanical Garden University of California at Berkeley; BGZ, Botanical Garden Zurich; CAG, Garden of Charles Alford (Florida); NYBG, Botanical Garden New York; RBGE, Royal Botanic Garden Edinburgh; and RBGK, Royal Botanic Garden Kew.

Table D1 (Continued).

Taxon	Indiviudal Code	*rbcL*	*trnLF*	*rps4*
Belvisia annamensis (C. Chr.) S.H. Fu	1	GQ256252	GQ256166	
Belvisia annamensis (C. Chr.) S.H. Fu	2	EU482931	EU483025	EU482976
Belvisia henryi (Hieron. ex C. Chr.) Raymond		GQ256253	GQ256167	
Belvisia mucronata (Fée) Copel.	1		GQ256168	
Belvisia mucronata (Fée) Copel.	2	AY362562	DQ642232	AY362629
Belvisia platyrhynchos (Kunze) Copel.	1		GQ256169	
Belvisia platyrhynchos (Kunze) Copel.	2	DQ642152	DQ642233	DQ642190
Belvisia spicata (L. f.) Mirb.	Moorea	1	1	
Belvisia spicata (L. f.) Mirb.	Cult.	DQ642153	DQ642234	DQ642191
Belvisia spicata (L. f.) Mirb.	Reunion	KF992443		
Calymmodon orientalis Copel.				
Ctenopterella blechnoides (Grev.) Parris				
Davallia solida (G. Forst.) Sw.				
Drymotaenium miyoshianum (Makino) Makino		AY362563	DQ179639	AY362630
Goniophlebium amoenum var. chinense (Christ) Rödl-Linder		DQ078630		
Goniophlebium argutum (Wall. ex Hook.) J. Sm. ex Hook.		DQ164442	DQ164505	DQ164473
Goniophlebium formosanum (Baker) Rödl-Linder		AB043100	DQ642235	AY096224
Goniophlebium manmeiense (H. Christ) Rödl-Linder		DQ078628		DQ078631
Goniophlebium mehibitense (C. Chr.) Parris		EU482932	EU483026	EU482977
Goniophlebium mengtzeense (H. Christ) Rödl-Linder	1	AY362560		
Goniophlebium mengtzeense (H. Christ) Rödl-Linder	2	DQ078624		DQ078633
Goniophlebium microrhizomum (Clarke ex Baker) Clarke ex		DQ078627		DQ078632
Goniophlebium niponicum (Mett.) Bedd.	1	AB043098	EU483027	AY362626
Goniophlebium niponicum var. wattii (Bedd.) Bedd.	2	DQ078625		DQ078634
Goniophlebium percussum (Cav.) Wagner & Grether		AY362561		AY362628
Goniophlebium persicifolium (Desv.) Bedd.	1	EU482933	EU483028	
Goniophlebium pseudocommutatum (Copel.) Copel.		EU482934	EU483029	Eu482978
Goniophlebium subauriculatum (Blume) C.Presl		AF470342	AY083645	DQ168812
Lecanopteris balgoyii Hennipman		AF470328	AY083631	EU482980
Lecanopteris carnosa Blume		AF470322	AY083625	AY096227
Lecanopteris celebica Hennipman		AF470323	AY083626	EU482981
Lecanopteris crustcea Copel.		AF470329	AY083632	EU482982
Lecanopteris deparioides (Ces.) Baker		AF470324	AY083627	
Lecanopteris lomarioides (Kunze ex Mett.) Copel.		AF470326	AY083629	
Lecanopteris luzonensis Hennipman		AF470325	AY083628	EU482983
Lecanopteris mirabilis (C. Chr.) Copel.		AF470330	AY083633	EU482984
Lecanopteris pumila Blume		AF470331	AY083634	
Lecanopteris sarcopus (Teijsm. & Binn.) Copel.		EU482935	EU483030	EU482985
Lecanopteris sinuosa (Hook.) Copel.		AF470321	AY083624	AY362634
Lecanopteris spinosa Jermy & Walker		AF470327	AY083630	
Lemmaphyllum accedens Donk ex. Holttum		EU482936	EU483031	EU482986
Lemmaphyllum carnosum (Wall. ex J. Sm.) C. Presl		AF470332	AY083635	AY362631
Lemmaphyllum diversum (Rosenst.) Tagawa	2	EU482937	EU483032	EU482987
Lemmaphyllum diversum (Rosenst.) Tagawa	1	EU482939	EU483034	EU482989
Lemmaphyllum microphyllum C. Presl		EU482938	EU483033	EU482988
Lepisorus clathratus (C.B. Clarke) Ching		DQ642154	DQ642236	DQ642192
Lepisorus excavatus (Bory ex Willd.) Ching	1	DQ642155	DQ642237	DQ642193
Lepisorus excavatus (Bory ex Willd.) Ching	2	DQ642156	DQ642238	DQ642194
Lepisorus kawakamii (Hayata) Tagawa		EU482940	EU483035	EU482990
Lepisorus longifolius (Bl.) Holtt.		DQ642157	DQ642239	DQ642195
Lepisorus macrosphaerus (Baker) Ching		EU482941	EU483036	EU482991
Lepisorus megasorus (C.Chr.) Ching		DQ642158	DQ642240	DQ642196
Lepisorus monilisorus (Hayata) Tagawa		EU482942	EU483037	EU482992
Lepisorus nudus (Hook.) Ching		AY362564		
Lepisorus pseudo-ussuriensis Tagawa		EU482943	EU483038	EU482993
Lepisorus thunbergianus (Kaulf.) Ching		U05629	DQ642241	AY096226
Lepisorus waltonii (Ching) S.L. Yu		EU482944	EU483039	EU482994
Leptochilus axillaris (Cav.) Kaulf.			EU483040	
Leptochilus cantoniensis (Baker) Ching	1		EU483041	EU482995

Table D1 (Continued).

Taxon	Indiviudal Code	*rbcL*	*trnLF*	*rps4*
Leptochilus cantoniensis (Baker) Ching	2	EU482945	EU483042	EU482996
Leptochilus decurens Blume		EU482946	DQ179640	AY096228
Leptochilus digitatus (Baker) Noot.	2	EU482947	EU483044	EU482998
Leptochilus digitatus (Baker) Noot.	1	AY096203	EU483043	EU482997
Leptochilus ellipticus (Thunb. ex Murray) Noot.		EU482948	EU483045	EU482999
Leptochilus ellipticus var. pothifolius (Buch.-Ham. ex D. Don)		1	1	
Leptochilus hemionitideus (C. Presl) Noot.	1	EU482949		
Leptochilus hemionitideus (C. Presl) Noot.	2	U05612	EU503045	EU503044
Leptochilus hemitomus (Hance) Noot.		EU482951	EU483047	EU483001
Leptochilus henryi (Baker) Ching		EU482952	EU483048	EU483002
Leptochilus macrophyllus var. wrightii (Hook. & Baker) Noot.		EU482954	EU483050	EU483004
Leptochilus simplifrons (H. Christ) Tagawa		EU482953	EU483049	EU483003
Microsorum × maximum (Brack.) Copel.	Moorea	1	1	
Microsorum × maximum (Brack.) Copel.	Tahiti	1	1	
Microsorum × maximum (Brack.) Copel.	Maupiti	1	1	
Microsorum × maximum (Brack.) Copel.	Huahine	1	1	
Microsorum × tohieaense J. H. Nitta	Moorea 2	1	1	
Microsorum × tohieaense J. H. Nitta	Moorea 1	1	1	
Microsorum commutatum (Bl.) Copel.	Cult.	AY362571	EU483051	EU483005
Microsorum commutatum (Bl.) Copel.	Tahiti	1	1	
Microsorum commutatum (Bl.) Copel.	Moorea 2	1		
Microsorum commutatum (Bl.) Copel.	Huahine	1	1	
Microsorum commutatum (Bl.) Copel.	Moorea 1	1	1	
Microsorum cuspidatum (D. Don) Tagawa	1		HQ597021	
Microsorum cuspidatum (D. Don) Tagawa	3	AF470335		AY096230
Microsorum cuspidatum (D. Don) Tagawa	4		JX103791	
Microsorum cuspidatum (D. Don) Tagawa	2		AY083638	
Microsorum fortunei (T.Moore) Ching	1	DQ642159	DQ642242	DQ642197
Microsorum fortunei (T.Moore) Ching	2	EU482955	EU483052	EU483006
Microsorum grossum (Langsd. & Fisch.) S.B. Andrews	Tahiti	1	1	
Microsorum grossum (Langsd. & Fisch.) S.B. Andrews	Huahine	1	1	
Microsorum grossum (Langsd. & Fisch.) S.B. Andrews	Hawaii	EU482956	EU483053	EU483007
Microsorum grossum (Langsd. & Fisch.) S.B. Andrews	Ua Pou	1	1	
Microsorum grossum (Langsd. & Fisch.) S.B. Andrews	Bora Bora	1	1	
Microsorum grossum (Langsd. & Fisch.) S.B. Andrews	Moorea 3	DQ179633	DQ179642	DQ179636
Microsorum grossum (Langsd. & Fisch.) S.B. Andrews	Moorea 1	1	1	
Microsorum grossum (Langsd. & Fisch.) S.B. Andrews	Moorea 2	1	1	
Microsorum hainanense Noot.		EU482960	EU483057	EU483011
Microsorum insigne (Blume) Copel.	1	EU482957	EU483054	EU483008
Microsorum insigne (Blume) Copel.	2	EU482958	EU483055	EU483009
Microsorum insigne (Blume) Copel.	3	EU482959	EU483056	EU483010
Microsorum lastii (Baker) Tardieu		EU482961	EU483058	EU483012
Microsorum linguiforme Copel.		AF470334	AY083637	AY362635
Microsorum lucidum (Roxb.) Copel.			JX103810	
Microsorum membranaceum (D. Don) Ching	2	EU482963	DQ642244	DQ642198
Microsorum membranaceum (D. Don) Ching	1	EU482962	EU483059	EU483013
Microsorum membranifolium (R.Br.) Ching	Tahiti	1	1	
Microsorum membranifolium (R.Br.) Ching	Ua Pou 1	1	1	
Microsorum membranifolium (R.Br.) Ching	Ua Pou 2	1	1	
Microsorum membranifolium (R.Br.) Ching	Moorea	1	1	
Microsorum membranifolium (R.Br.) Ching	Huahine	1	1	
Microsorum membranifolium (R.Br.) Ching	Moorea 2*	1	1	
Microsorum membranifolium (R.Br.) Ching	Cult.	DQ642161	DQ642245	DQ642200
Microsorum musifolium Copel.		AF470333	AY083636	AY362636
Microsorum novo-zealandiae (Baker) Copel.		DQ401116	DQ401121	DQ401126
Microsorum pappei (Mett. ex Kuhn) Tardieu		AF470336	AY083639	
Microsorum papuanum (Baker) Parris		DQ642162	DQ642246	EU483015
Microsorum powellii (Baker) Copel.		1	1	

Table D1 (Continued).

Taxon	Indiviudal Code	*rbcL*	*trnLF*	*rps4*
Microsorum pteropus (Blume) Copel.		EU482965	EU483061	EU483016
Microsorum punctatum (L.) Copel.	Moorea 2	1		
Microsorum punctatum (L.) Copel.	Moorea 3*	1	1	
Microsorum punctatum (L.) Copel.	Bora Bora*	1	1	
Microsorum punctatum (L.) Copel.	Huahine		1	
Microsorum punctatum (L.) Copel.	Taiwan	EU482966	EU483063	EU483017
Microsorum punctatum (L.) Copel.	Philippines	AF470337	AY083640	
Microsorum punctatum (L.) Copel.	Cult.	DQ164444	DQ164508	DQ164475
Microsorum punctatum (L.) Copel.	Moorea 1	1	1	
Microsorum punctatum (L.) Copel.	Laos	JX103705	JX103789	
Microsorum punctatum (L.) Copel.	China	GQ256316	GQ256244	
Microsorum pustulatum (G. Forst.) Copel.	1		KF591321	
Microsorum pustulatum (G. Forst.) Copel.	2		KF591329	
Microsorum pustulatum (G. Forst.) Copel.	3	DQ401117	DQ401122	DQ401127
Microsorum scandens (G. Forst.) Tindale	2	DQ212057	DQ179641	DQ212058
Microsorum scandens (G. Forst.) Tindale	1	DQ401118	DQ401123	DQ401128
Microsorum scolopendria (Burm. f.) Copel.	3	DQ642164	DQ642248	DQ642202
Microsorum scolopendria (Burm. f.) Copel.	2	GQ256317	GQ256245	
Microsorum scolopendria (Burm. f.) Copel.	4	DQ642163	DQ642247	DQ642201
Microsorum scolopendria (Burm. f.) Copel.	1	AB575281		
Microsorum sp.	Laos		JX103792	
Microsorum spectrum (Kaulf.) Copel. var. pentadactylum		1	1	
Microsorum spectrum var. spectrum (Kaulf.) Copel.	1	EU482968	EU483065	EU483019
Microsorum spectrum var. spectrum (Kaulf.) Copel.	2	EU482967	EU483064	EU483018
Microsorum superficiale (Blume) Bosman	1	EU482971	EU483062	EU483022
Microsorum thailandicum T. Booknerd & Noot.		EU482969	EU483066	EU483020
Microsorum varians (Mett.) Hennipman & Hett.		AY362566	DQ179643	AY362638
Microsorum viellardii (Mett.) Copel.	2	DQ179635	DQ179645	DQ179638
Microsorum viellardii (Mett.) Copel.	1		DQ179644	
Microsorum whiteheadii A.R. Sm. & Hoshiz.		EU482970	EU483067	EU483021
Microsorum zippelii (Blume) Ching		AB232411	DQ642249	DQ642203
Neocheiropteris palmatopedata (Baker) H.Christ		AY362567	DQ212059	AY362640
Neocheiropteris superficiale (Blume) Bosman	2	AY725055	AY725049	AY725048
Neolepisorus ovatus (Wall. ex Bedd.) Ching		EU482972	EU483068	EU483024
Neolepisorus phyllomanes (H. Christ) Ching		EU482973	EU483069	EU483024
Platycerium stemaria var. laurentii (P. Beauv.) Desv.		DQ164458	DQ164522	
Pleopeltis munchii (Christ) A.R. Smith		AY362615	EU650074	
Polypodium cambricum L.				
Thylacopteris papillosa (Blume) Krause ex J.Sm.		AY459174	AY459183	AY459188
Tricholepidium maculatum (H.Christ) Ching		EU482974	EU483070	
Tricholepidium normale (D.Don) Ching		EU482975	EU483071	

Table D1 (Continued).

Taxon	Individual Code	*gapcp long*	*gapcp short*	Country	Voucher
Belvisia annamensis	1			China	D Li 873
Belvisia annamensis	2			Indonesia (East Kalimantan)	Hovenkamp 05-277 (L)
Belvisia henryi				China	Shui 80679
Belvisia mucronata	1			Malaysia	Jaman 5891
Belvisia mucronata	2			Cult. BGZ	Kreier s.n. (GOET)
Belvisia platyrhynchos	1			Cult.	Kreier s.n.
Belvisia platyrhynchos	2			Cult. BGZ	Kreier s.n. (GOET)
Belvisia spicata	Moorea	1	1	French Polynesia (Moorea)	Nitta 323 (GH)
Belvisia spicata	Cult.			Cult. BGG	Schneider s.n. (GOET)
Belvisia spicata	Reunion			Reunion	T. Janssen 2681 (P, REU)
Calymmodon orientalis		1	1	French Polynesia (Moorea)	Nitta 682 (GH)
Ctenopterella blechnoides		1	1	French Polynesia (Moorea)	Nitta 658 (GH)
Davallia solida			1	French Polynesia (Moorea)	Ranker 1935 (UC)
Drymotaenium miyoshianum				Taiwan	Cranfill TW087 (UC)
Goniophlebium amoenum var. chinense				China (Yunnan)	SG Lu X14 (PYU)
Goniophlebium argutum				Taiwan	Cranfill TW075 (UC)
Goniophlebium formosanum				Taiwan	Cranfill TW043 (UC)
Goniophlebium manmeiense				China (Yunnan)	SG Lu K4 (PYU)
Goniophlebium mehibitense				Indonesia (East Kalimantan)	Hovenkamp 05-278 (L)
Goniophlebium mengtzeense	1			China (Yunnan)	Barrington 2085a (VT)
Goniophlebium mengtzeense	2			China (Yunnan)	SG Lu K9 (PYU)
Goniophlebium microrhizomum				China (Yunnan)	SG Lu K8 (PYU)
Goniophlebium niponicum	1			Japan	Kato s.n. (TI)
Goniophlebium niponicum var. wattii	2			China (Yunnan)	SG Lu (PYU)
Goniophlebium percussum				Cult. ASG	Smith s.n. (UC)
Goniophlebium persicifolium	1			Cult. BGB	239-12-90-33 (B)
Goniophlebium pseudocommutatum				Cult. BGB	239-36-90-30 (B)
Goniophlebium subauriculatum				Cult. BGBO	Smith s.n. (UC)
Lecanopteris balgoyii				Sulawesi	Hennipman s.n. (L)
Lecanopteris carnosa				Cult. RBGK	Cranfill 153 (UC)
Lecanopteris celebica				Cult. BGG	Schneider s.n. (GOET)
Lecanopteris crustcea				Cult. CAG	A.R. Smith s.n. (UC)
Lecanopteris deparioides				Cult. BGU	Hennipman 7865 (U)
Lecanopteris lomarioides				Cult. BGU	Hennipman s.n. (U)
Lecanopteris luzonensis				Cult. BGG	Schneider s.n. (GOET)
Lecanopteris mirabilis				Cult. BGU	Hennipman s.n. (U)
Lecanopteris pumila				Cult. BGU	Hennipman s.n. (UC)
Lecanopteris sarcopus				Cult. RBGE	Ridl 171 (E)
Lecanopteris sinuosa				Cult. BGU	Hennipman 7821 (L)
Lecanopteris spinosa				Cult. BGU	Hennipman s.n. (U)
Lemmaphyllum accedens				Indonesia (East Kalimantan)	Hovenkamp 05-298 (L)
Lemmaphyllum carnosum				Cult. BGUB	A.R. Smith s.n. (UC)
Lemmaphyllum diversum	2			Taiwan	Ranker 2079 (COLO)
Lemmaphyllum diversum	1			China	Zhang 1854 (PE)
Lemmaphyllum microphyllum				Cult. BGZ	Schneieder s.n. (GOET)
Lepisorus clathratus				Tibet	Dickoré 12430 (GOET)
Lepisorus excavatus	1			Tanzania	Hemp 3561 (DSM)
Lepisorus excavatus	2			Comoros (Grande Comore)	Rakotondrainibe 6785 (P)
Lepisorus kawakamii				Taiwan	Ranker 2051 (COLO)
Lepisorus longifolius				Cult. BGM	Schneider s.n. (GOET)
Lepisorus macrosphaerus				Taiwan	Cranfill TW018 (UC)
Lepisorus megasorus				Taiwan	Cranfill TW069 (UC)
Lepisorus monilisorus				Taiwan	Cranfill TW012 (UC)
Lepisorus nudus				Cult. UCGB	Smith s.n. (UC)
Lepisorus pseudo-ussuriensis				Taiwan	Cranfill TW093 (UC)
Lepisorus thunbergianus				Cult. BGZ	Kreier s.n (GOET)

Table D1 (Continued).

Taxon	Indiviudal Code	*gapcp long*	*gapcp short*	Country	Voucher
Lepisorus waltonii				China	Cranfill 94-266-29 (UC)
Leptochilus axillaris				Java	Walker 11557 (BM)
Leptochilus cantoniensis		1		China	Dong 172 (PE)
Leptochilus cantoniensis		2		China	Dong 743 (PE)
Leptochilus decurens				Cult. BGUB	Douglas 28 (UC)
Leptochilus digitatus		2		Vietnam	A.R. Smith 00-036 (UC)
Leptochilus digitatus		1		China	Zhang 3509 (PE)
Leptochilus ellipticus				China	Zhang 1923 (PE)
Leptochilus ellipticus var. pothifolius			1	1 Japan (Okinawa)	Nitta 377 (GH)
Leptochilus hemionitideus		1		Japan	Hasebe 26551 (TI)
Leptochilus hemionitideus		2		Cult. NYBG	Moran s.n. (NY)
Leptochilus hemitomus				China	Zhang 3302 (PE)
Leptochilus henryi				China	Zhang 2541 (PE)
Leptochilus macrophyllus var. wrightii				Japan	Tsutsumi 1067 (CT)
Leptochilus simplifrons				Cult. JNU	Zhang 3800 (PE)
Microsorum × maximum	Moorea		1	1 French Polynesia (Moorea)	Hinkle 106 (UC)
Microsorum × maximum	Tahiti			French Polynesia (Tahiti)	Nitta 1863 (GH)
Microsorum × maximum	Maupiti		1	1 French Polynesia (Maupiti)	Nitta 3674 (GH)
Microsorum × maximum	Huahine			French Polynesia (Huahine)	Nitta 3972 (GH)
Microsorum × tohieaense	Moorea 2			French Polynesia (Moorea)	Nitta 1040 (GH)
Microsorum × tohieaense	Moorea 1		1	1 French Polynesia (Moorea)	Nitta 3929 (GH)
Microsorum commutatum	Cult.			Cult. Whitehead	A.R. Smith 2901 (UC)
Microsorum commutatum	Tahiti		1	1 French Polynesia (Tahiti)	Amer 13 (GH)
Microsorum commutatum	Moorea 2			1 French Polynesia (Moorea)	Gulamhussein 2 (UC)
Microsorum commutatum	Huahine			French Polynesia (Huahine)	Nitta 4046 (GH)
Microsorum commutatum	Moorea 1		1	1 French Polynesia (Moorea)	Sanchez-Baracaldo 175 (UC)
Microsorum cuspidatum		1		?	?
Microsorum cuspidatum		3		Cult. NYBG	A.R. Smith 1738194 (UC)
Microsorum cuspidatum		4		Cult.	Kim 2012-6 (KUN)
Microsorum cuspidatum		2		?	LBG 3560, collector unknown
Microsorum fortunei		1		Taiwan	Ranker 2087 (COLO)
Microsorum fortunei		2		China	Zhang 3446 (PE)
Microsorum grossum	Tahiti			French Polynesia (Tahiti)	Amer 14 (GH)
Microsorum grossum	Huahine			French Polynesia (Huahine)	Dunn 504 (PTBG)
Microsorum grossum	Hawaii			Hawaii	Lorence 9155 (DL)
Microsorum grossum	Ua Pou			French Polynesia (Ua Pou)	Lorence 9155 (PTBG)
Microsorum grossum	Bora Bora			French Polynesia (Bora Bora)	Nitta 3837 (GH)
Microsorum grossum	Moorea 3			French Polynesia (Moorea)	Ranker 1941 (COLO)
Microsorum grossum	Moorea 1		1	1 French Polynesia (Moorea)	Sanchez-Baracaldo 170 (UC)
Microsorum grossum	Moorea 2		1	1 French Polynesia (Moorea)	Vinette 33.3 (UC)
Microsorum hainanense				Cult. SCIB	Wang 1348 (PE)
Microsorum insigne		1		China	Liu 204 (PE)
Microsorum insigne		2		China	Liu 214 (PE)
Microsorum insigne		3		China	Zhang 3510 (PE)
Microsorum lastii				?	Perier 7937 (P)
Microsorum linguiforme				New Guinea	Ranker 1176 (UC)
Microsorum lucidum				Cult.	Kim 2012-14 (KUN)
Microsorum membranaceum		2		Taiwan	Cranfill TW042 (UC)
Microsorum membranaceum		1		Cult. Xishuanbanna	Li 95 (PE)
Microsorum membranifolium	Tahiti			French Polynesia (Tahiti)	Amer 15 (GH)
Microsorum membranifolium	Ua Pou 1		1	1 French Polynesia (Ua Pou)	Dunn 250 (PTBG)
Microsorum membranifolium	Ua Pou 2		1	1 French Polynesia (Ua Pou)	Dunn 458 (PTBG)
Microsorum membranifolium	Moorea		1	1 French Polynesia (Moorea)	Nitta 1145 (GH)
Microsorum membranifolium	Huahine			French Polynesia (Huahine)	Nitta 4073 (GH)
Microsorum membranifolium	Moorea 2*		1	1 French Polynesia (Moorea)	Nitta 573 (GH)
Microsorum membranifolium	Cult.			Cult. BGG	Schneider s.n. (GOET)
Microsorum musifolium				Java	UCBG 58.0649
Microsorum novo-zealandiae				New Zealand	Perrie WELT P20873
Microsorum pappei				Cult. BGL	901812 (L)

Table D1 (Continued).

Taxon	Indiviudal Code	*gapcp long*	*gapcp short*	Country	Voucher
Microsorum papuanum				Cult. BGB	Schuettpelz 603 (GOET)
Microsorum powellii		1	1	French Polynesia (Moorea)	Nitta 654 (GH)
Microsorum pteropus				Cult. BGG	Kreier s.n. (GOET)
Microsorum punctatum	Moorea 2	1	1	French Polynesia (Moorea)	Baltrushes s.n. (UC)
Microsorum punctatum	Moorea 3*	1	1	French Polynesia (Moorea)	Nitta 1399 (GH)
Microsorum punctatum	Bora Bora*	1	1	French Polynesia (Bora Bora)	Nitta 3818 (GH)
Microsorum punctatum	Huahine			French Polynesia (Huahine)	Nitta 4045 (GH)
Microsorum punctatum	Taiwan			Taiwan	Ranker 2096 (COLO)
Microsorum punctatum	Philippines			Philippines	Ridsdale s.n.
Microsorum punctatum	Cult.			Cult. BGH	Schneider s.n. (GOET)
Microsorum punctatum	Moorea 1	1	1	French Polynesia (Moorea)	Vinette 32.2 (UC)
Microsorum punctatum	Laos			Laos	Wu 2506 (KUN)
Microsorum punctatum	China			China	Zhang 4194
Microsorum pustulatum	1			New Zealand	Allan Herbarium CHR 630381
Microsorum pustulatum	2			New Zealand	Allan Herbarium CHR 630387
Microsorum pustulatum	3			New Zealand	Perrie WELT P20874
Microsorum scandens	2			Cult. BGG	Kreier s.n. (GOET)
Microsorum scandens	1			New Zealand	Perrie WELT P20875
Microsorum scolopendria	3			Mayotte	Rakotondrainibe 6601 (P)
Microsorum scolopendria	2			Mayotte	Rakotondrainibe et al. 6601
Microsorum scolopendria	4			Cult. BGG	Schneider s.n. (GOET)
Microsorum scolopendria	1			Japan	TNS: 764387
Microsorum sp.	Laos			Laos	Wu 2367 (KUN)
Microsorum spectrum var. pentadactylum		1	1	Hawaii (Kauai)	Wood 15756 (PTBG)
Microsorum spectrum var. spectrum	1			Hawaii (Oahu)	Hoshizaki 1350 (UC)
Microsorum spectrum var. spectrum	2			Hawaii (Kauai)	Wood 10936 (PTBG)
Microsorum superficiale	1			Taiwan	Cranfill 030 (UC)
Microsorum thailandicum				Cult. BGG	Schwertfeger s.n. (GOET)
Microsorum varians				Cult. BGG	Schneider s.n. (GOET)
Microsorum viellardii	2			Cult. BGD	Schneider s.n. (GOET)
Microsorum viellardii	1			Cult. C. Alford. Bot. Gard.	Smith s.n (UC)
Microsorum whiteheadii				Sumatra	Whitehead s.n. (UC)
Microsorum zippelii				Indonesia	Tsutsumi IN112 (TI)
Neocheiropteris palmatopedata				Cult. BGZ	Schneider s.n. (GOET)
Neocheiropteris superficiale	2			Taiwan	Cranfill TW073 (UC)
Neolepisorus ovatus				China	Zhang 728/1 (PE)
Neolepisorus phyllomanes				Cult. RBGE	Nicholson s.n. (E)
Platycerium stemaria var. laurentii				Cult. BGG	Kreier GG0411
Pleopeltis munchii				Mexico	Diggs & Corcoran 210 (UC)
Polypodium cambricum			KJ748235	Mexico	Diggs & Corcoran 210 (UC)
Thylacopteris papillosa				Java	Gravendeel 559 (L)
Tricholepidium maculatum				China	Zhang 3100 (PE)
Tricholepidium normale				China	Shen S4-1 (PE)